

# Effect of Mineral Nitrogen on Transfer of $^{13}\text{C}$ -Carbon from *Eucalyptus* Harvest Residue Components to Soil Organic Matter Fractions

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**ABSTRACT:** The amount of harvest residues retained in *Eucalyptus* plantations strongly influences soil organic matter (SOM), but the efficiency of conversion to SOM may vary according to the type of residue. This study evaluated the recovery of C from *Eucalyptus* residue components - leaves, bark, branches, roots, and a mix of all residues - in different SOM fractions with or without mineral-N supplementation (200 mg kg<sup>-1</sup> of N). Variation in natural  $^{13}\text{C}$  abundance was used to trace the destination of residue-derived C in the soil. The C content of the light fraction (LF) and heavy fraction (HF) of SOM increased over a 240-days decomposition period in response to incorporation of *Eucalyptus* residues in the soil. Bark and leaf residues showed the best results. Bark residues increased the C content of the HF by 45 % over the initial condition. Leaf residues made the largest contribution to LF-C, increasing it by 8.6 times. Leaf residues also led to the highest N contents in the LF and HF, whereas branches, roots, and the mixture of residues caused significant net transfers of N from the HF. Mineral-N supplementation had no effect on stabilization of organic C in the HF of SOM, in which the C could be maintained for longer periods due to physical/colloidal protection against microbial decomposition. These results highlight the importance of keeping *Eucalyptus* harvest residues in the planted area, especially the bark, which is the most abundant harvest residue component under field conditions, for maintenance of SOM.

**Keywords:** C cycling, N cycling, SOM, stable isotopes.

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## INTRODUCTION

The increase in atmospheric CO<sub>2</sub> concentration in recent decades has imposed the need for agricultural practices that favor removal of CO<sub>2</sub> from the atmosphere through C sequestration in more stable fractions of soil organic matter (SOM). Plants have a major role in C cycling in soils through deposition of organic C either above ground (shoot litter) or below ground (root litter and rhizodepositions). The impact of this plant-derived C on SOM will depend on the composition of the inputs and their use by components of the soil food web (Chabbi and Rumpel, 2009).

Decomposition of organic residues in soils is controlled by three main variables: the composition of the decomposing community, including macro- and microorganisms (Tian et al., 1992; Grandy and Neff, 2008); the quality of the organic material (Tian et al., 1992; Wang et al., 2004; Vane et al., 2006); and the physico-chemical characteristics of the environment, which are affected by the climate, soil properties, and, especially, N availability (Wang et al., 2004; Manzoni et al., 2008; Stockmann et al., 2013). All these variables work together to determine organic matter dynamics in soils so that the persistence or decomposition of organic residues can be seen as an ecosystem property (Grandy and Neff, 2008; Schmidt et al., 2011).

Carbon cycling can be manipulated to favor C stabilization in soils. The most significant factor in plantations is the amount and quality of plant residue inputs. In short-rotation *Eucalyptus* plantations, which currently occupy 5.1 million hectares of land in Brazil (Abraf, 2013), minimum tillage practices are being introduced in order to increase wood yield while minimizing deleterious effects on the environment. Under such practices, *Eucalyptus* harvest residues - such as leaves, bark, branches, and roots - are left on the soil surface in the plantation area. Some of these residues, such as bark and branches, are more recalcitrant due to the presence of phenolic compounds, especially lignin, tannin, and suberin, which decrease the residue decomposition rate in soils (Wang et al., 2004; Vane et al., 2006). Given the importance of residue quality in C cycling, would *Eucalyptus* residue fractions be selectively left or removed from the stand area in order to maximize C stabilization in SOM?

Decomposition of organic residues is highly affected by N availability. Low N availability can increase CO<sub>2</sub>-C loss during litter decomposition as microorganisms use labile residues to acquire N from recalcitrant substrates (Craine et al., 2007). Thus, N availability controls the C-use efficiency of decomposers such that substrates with low initial C:N ratio increase efficiency, and N-poor substrates (high C:N) lead to a less-efficient use of C (Manzoni et al., 2008). The CO<sub>2</sub>-C loss from increased heterotrophic respiration under low C-use efficiency can be alleviated by high soil N supply (Craine et al., 2007), with consequent gains in organic C stabilization in SOM (Bird et al., 2003; Moran et al., 2005; Kirkby et al., 2013; Kirkby et al., 2014). An increase in C-use efficiency by microorganisms can favor the contributions of microbial residues to the SOM fractions associated with minerals (Cotrufo, 2013), assumed to be the fractions of greater stability in SOM. Stable isotopes are excellent tracers of organic matter input and transfer (Peterson, 1999), allowing the destination of C and N applied to soils to be tracked.

We hypothesized that less labile *Eucalyptus* residue components have a greater contribution to the SOM when there is higher soil N availability. Using the natural abundance of <sup>13</sup>C, we aimed to evaluate recovery of C from different *Eucalyptus* residues into SOM fractions. In addition, we studied the effect of mineral-N supplementation on C recovery from *Eucalyptus* residues.

## MATERIALS AND METHODS

### Soil

The soil used in the incubation experiment was obtained from the 0.00-0.20 m layer of an Hapludox (*Latossolo Amarelo Distrófico*) situated in the southeastern part of the state of Minas Gerais, Brazil (20° 52' 31.1" S, 42° 58' 21.6" W). The soil had been cultivated with

*Brachiaria brizantha* for over 20 years, so the SOM was predominantly derived from this C4 photosynthesis plant, resulting in a  $\delta^{13}\text{C}_{\text{PDB}}$  value of 16.75 ‰. We were then able to determine the destination of C derived from *Eucalyptus* residue into SOM fractions. However, soil collected from a distinct previous land use may have a significant effect on how the existing microbial communities deal with the newly added *Eucalyptus* residues. Previous studies have shown that substituting pastures for *Eucalyptus* plantations led to reduction in SOM (Mendham et al., 2002), but the reverse was found by Lima et al. (2006), indicating that not only changes in microbial communities and litter quality affects the stabilization of residue C into SOM, but also the soil C saturation deficit (Castellano et al., 2015). Thus, absolute residue C decomposition values presented in this study must be viewed with due caution.

The C and N concentrations in different fractions of the organic matter of the soil used in the experiment were as follows: Total (C: 25.4 and N: 1.4 g kg<sup>-1</sup>); physical procedure: light fraction of SOM - LF (C: 0.79 and N: 0.03 g kg<sup>-1</sup>) and heavy fraction of SOM - HF (C: 21.80 and N: 1.39 g kg<sup>-1</sup>); and chemical separation: humin (C: 14.33 and N: 0.44 g kg<sup>-1</sup>), fulvic acid (C: 0.24 and N: 0.02 g kg<sup>-1</sup>), and humic acid (C: 0.88 and N: 0.04 g kg<sup>-1</sup>).

Before use of the soil in the incubation experiment, coarse plant debris visible to the naked eye was manually removed; the soil was ground, sieved to 2 mm, and kept at the moisture content found under field conditions at the time of sampling (~12 % w/w). Soil subsamples were taken for physical and chemical characterization. The soil had a sandy clay loam texture (340 g kg<sup>-1</sup> clay, 110 g kg<sup>-1</sup> silt, and 550 g kg<sup>-1</sup> sand).

### ***Eucalyptus* residues**

The harvest residues used in the experiment were collected from a 4-year-old clonal plantation of a *Eucalyptus grandis* × *E. urophylla* hybrid. The plant residues were separated into leaf, bark, branch ( $\emptyset > 20$  mm), and root components. The fine ( $\emptyset < 2$  mm), medium ( $\emptyset 2$  to 10 mm), and coarse ( $\emptyset > 10$  mm) roots were collected through manual soil excavation and sieving in the field. Afterwards, in the lab, they were washed with deionized water to remove attached soil particles. The *Eucalyptus* residues were dried and ground to pass through a 1-mm sieve using a Willey mill. Plant residue subsamples were taken for chemical characterization (Table 1). A mix of all residue components (leaves, bark, branches, and roots) at the same relative proportion found in the field was also prepared (12.9, 26.4, 12.7, and 49 % of leaf, bark, branch, and root residues, respectively). Just before application in the experiment, the residues were moistened to 60 % of humidity (dry basis) with distilled water to avoid soil desiccation upon their incorporation.

### **Experimental set up and incubation conditions**

The experiment combined five *Eucalyptus* residues with two levels of mineral N (0 or 200 mg kg<sup>-1</sup> of N); a control (no residue) was also used. Each *Eucalyptus* residue (including the mixture of all residues) was mixed with 100 g of soil at a rate equivalent to 20 g kg<sup>-1</sup> of C. Mineral N was applied (when appropriate) as an aqueous 50:50 mixture of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and NH<sub>4</sub>NO<sub>3</sub> (based on N content).

Soil moisture was adjusted to 80 % of the water holding capacity, and the moist *Eucalyptus* residues were then applied. The soils with distinct treatments were placed in sealed 350-mL glass jars in an incubation room with temperature controlled at 25±1 °C for 240 days.

### **Carbon and nitrogen contents of SOM fractions**

We used a method adapted from the procedure of Anderson and Ingram (1993) and Liao et al. (2006) for physical separation of the uncomplexed SOM fraction and mineral-associated organic matter. In summary, air-dried soil samples (15 g) were shaken in a rotary shaker with 30 mL of distilled water for 16 h in plastic centrifuge tubes with glass marbles to facilitate disaggregation. Then, these samples were immediately centrifuged (630 g for 15 min) and the supernatant was separated, thus

**Table 1.** Characteristics of the residues from the hybrid *Eucalyptus grandis* × *E. urophylla*

Characteristic	Leaves	Bark	Branches	Roots	Mix <sup>(1)</sup>
C (g kg <sup>-1</sup> )	512.70	441.70	471.80	474.00	474.93
$\delta^{13}\text{C}$	-28.75	-28.81	-27.67	-27.45	-28.28
N (g kg <sup>-1</sup> )	19.50	2.40	1.80	3.40	5.04
Soluble lignin (g kg <sup>-1</sup> )	105.50	47.10	45.10	27.50	45.25
Insoluble lignin (g kg <sup>-1</sup> )	250.20	191.40	221.60	281.90	249.08
Solvent-extractable C (g kg <sup>-1</sup> )	181.10	20.10	11.40	9.70	34.87
C:N ratio	30.80	214.70	305.80	162.70	94.16
Lignin:N ratio	12.80	79.80	123.10	82.90	49.38

<sup>(1)</sup> Mixture of leaves, bark, branches, and roots at the relative proportions of 12.9, 26.4, 12.7, and 49 %, respectively. Values are given by the weighted mean of the residues.

obtaining the light fraction (LF) released from inter- and intra-aggregates. The organic matter associated with soil minerals that sedimented to the bottom of the tube was considered the heavy fraction (HF). The energy applied might not have been enough to break all levels of aggregation; and by using a lower density liquid to separate the LF, a fraction of the unprocessed harvest residue might have been sedimented during the separation phase. However, the advantages of using water are low cost and lower generation of chemical artifacts (we were interested in carrying out spectroscopic analysis of the physical fractions). The use of water as a separation agent has been advocated elsewhere (Olk and Gregorich, 2006).

The SOM fractions thus obtained were oven dried at 72 °C for 72 h, ground in a ball mill, and sieved to 0.149 mm (100 mesh). The HF was further fractionated chemically into operationally-defined humic substances (humins, humic acids, and fulvic acids) based on the method of the International Humic Substances Society (Swift, 1996). After fractionation, the humin fraction was oven dried, ground in a ball mill, and sieved to 0.149 mm. For the humic and fulvic acids, 500 µL of each fraction were added to tin capsules and oven dried at 40 °C for subsequent spectrometric analysis.

The content of C and N and the isotopic ratio of  $^{13}\text{C}/^{12}\text{C}$  of the SOM fractions were determined using a continuous flow isotope ratio mass spectrometer (IRMS, 20-20, ANCA GSL, Sercon, Crewe, UK). The abundance of  $^{13}\text{C}$  was expressed as  $\delta^{13}\text{C}$  (‰ values in relation to the V-PDB standard).

For each SOM fraction, an isotope-mixing model was used to determine the C derived from residues (Equation 1).

$$f_c = \frac{\delta_{\text{sample}} - \delta_{\text{soil}}}{\delta_{\text{residue}} - \delta_{\text{soil}}} \quad \text{Eq. 1}$$

in which  $f_c$  is the fraction of residue-derived C,  $\delta_{\text{sample}}$  is the  $\delta^{13}\text{C}$  value of SOM fractions in the treatments,  $\delta_{\text{soil}}$  is the  $\delta^{13}\text{C}$  value of the soil used in the experiment (-16.75 ‰), and  $\delta_{\text{residue}}$  is the  $\delta^{13}\text{C}$  value of the *Eucalyptus* residues (Table 1).

The C recovered from residues in each SOM fraction was calculated according to equation 2:

$$C_{\text{recovered}} (\%) = \frac{f_c \times C_{\text{fraction}}}{C_{\text{input}}} \times 100 \quad \text{Eq. 2}$$

in which  $C_{\text{fraction}}$  is the total amount of C in each SOM fraction, and  $C_{\text{input}}$  is the total C input via residue (20 g kg<sup>-1</sup> of C).

It should be noted that variation in the  $\delta^{13}\text{C}$  in a sample after incorporation of the residues may be due to two different reasons: incorporation of the residue components (with different contents of sugar, lignins, lipids, peptides, and N-compounds that

had different  $\delta$  values), and alteration of the  $\delta^{13}\text{C}$  value of the original SOM caused by discrimination against the heavier isotope promoted by heterotrophic organisms. However, microbial degradation processes have only a minor influence on the natural distribution of stable C isotopes of respired  $\text{CO}_2\text{-C}$  (Ekblad et al., 2002).

### Statistical analyses

The experiment was carried out under a randomized block design with four replicates. The data were subjected to multivariate analysis of variance (Manova) with two factors (residue type and the addition of mineral N) and 12 dependent variables (see Results). Pillai's Trace was adopted to assess the overall difference between treatment groups ( $\alpha = 0.05$ ), and then separate univariate tests were employed to address the individual differences for each dependent variable. The alpha level for these univariate tests was adjusted by Bonferroni inequality [adjusted  $\alpha = (\text{overall } \alpha)/(\text{number of tests})$ ], resulting in a significance level of 0.004 (Hair Jr et al., 2009). The treatments were compared for each dependent variable using Tukey's test ( $p < 0.004$ ).

## RESULTS

Multivariate analysis showed significant differences among the *Eucalyptus* residues (Pillai's Trace = 3.33,  $p < 0.001$ ) and treatments with or without the addition of mineral N (Pillai's Trace = 0.84,  $p = 0.044$ ) when all the variables were considered jointly. However, the interaction of the two factors was not significant.

### Carbon changes in SOM fractions

At the end of 240 days, the highest C recovery in the LF was obtained from leaves, branches, roots, and the mixture of residues (Figure 1a). The addition of mineral N decreased C recovery from leaves, whereas it improved recovery from the other residues. These changes were reflected in the C content of the LF measured 240 days after application of the residues (Table 2). The highest C content was measured in the treatment with leaf residues without the addition of mineral N, which caused a variation ( $\Delta\text{C}$ ) of  $6.77 \text{ g kg}^{-1}$  of C compared to the initial condition, i.e., the C content was 8.6 times greater. When mineral N was added to the soil, the LF-C content was an average of 6 times greater with the application of bark, branches, roots, or the mixture of residues.

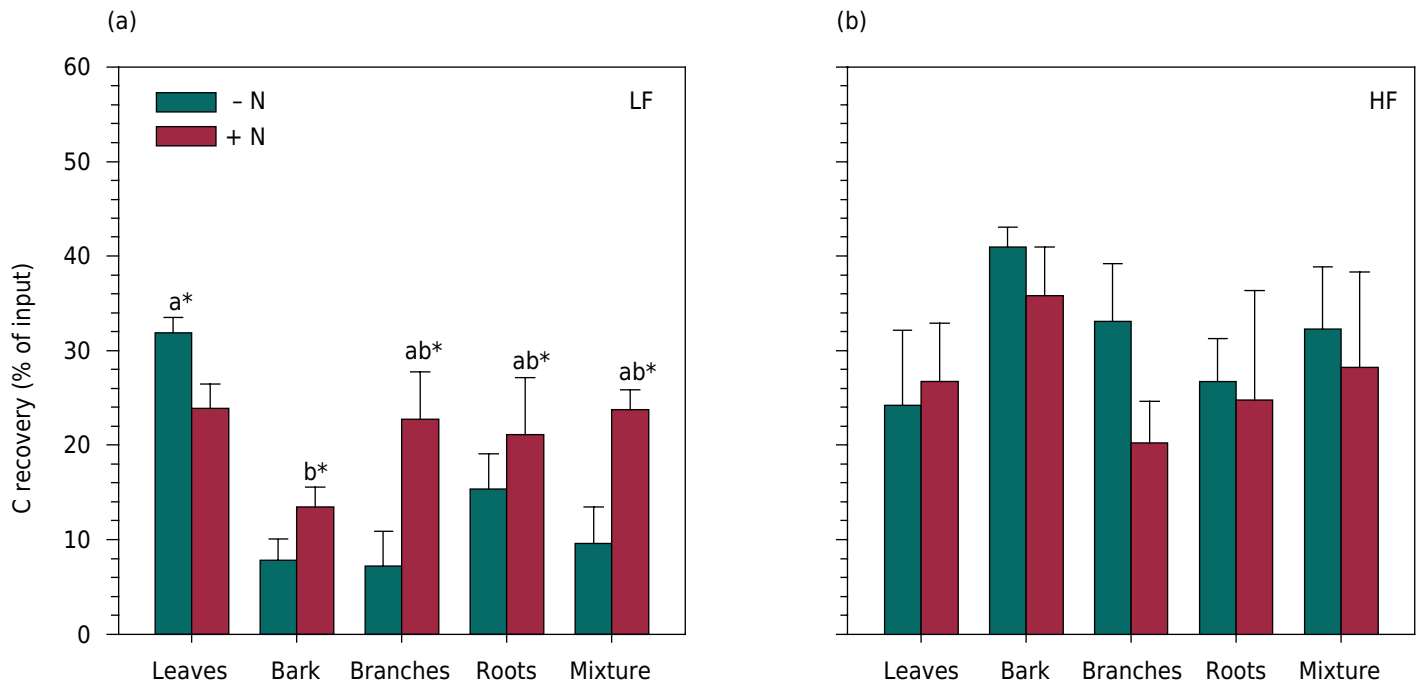
The recovery of residue-derived C in the HF was, on average, 28 % of input, but no significant difference was observed among the residues (Figure 1b). The soils receiving bark, leaves, and the mixture of residues had the highest C content in the HF at the end of 240 days, representing an increase of up to 45 % in the C content compared to the initial condition (Table 2). The addition of mineral N had no effect on residue-C recovery in the HF (Figure 1b) and hence in the C content of this fraction (Table 2).

Chemical fractionation of the HF showed that the highest portion of the C is contained in the operationally defined humin fraction (Table 2). The data indicate that the C content in this fraction significantly increased through application of bark, branches, and the mixture of residues. Conversely, the operationally defined humic acid decreased in soils receiving bark and branches, while it showed no significant variation ( $\Delta\text{C}$ ) with the other residues. Finally, the operationally defined fulvic acid showed a small increase in soils receiving leaf and bark residues.

### Nitrogen changes in SOM fractions

As observed for C, leaf residues led to the highest N content in the LF of SOM, which was 15 times greater than the initial condition (Table 3). Except for leaf residues, the LF-N content was higher when mineral N was added. However, a significant net increase over the control soil ( $\Delta\text{N}$ ) was observed only for leaf and mixed residues.

The highest N content in the HF of SOM was obtained with application of leaf residues (Table 3). This was the only residue type able to promote net accumulation of N ( $\Delta N$ ) in the HF. Conversely, branches, roots, and the mixture of all residues caused net losses of N from this SOM fraction. Moreover, supplementation with mineral N had no effect on HF-N content.



**Figure 1.** Recovery of residue-derived C (based on natural abundance of C isotopes) in the (a) light fraction (LF) and (b) heavy fraction (HF) of organic matter in soil with (+ N) or without (- N) the addition of mineral N (200 mg kg<sup>-1</sup> of N) after 240 days of incubation. Mixture = 12.9, 26.4, 12.7, and 49 % of leaves, bark, branches, and roots residues, respectively. Residues with the same letter are not significantly different (Tukey test,  $p < 0.004$ ). Asterisks indicate a significant effect from the addition of mineral N ( $p < 0.004$ ). No significant differences among treatments were detected for the HF. Error bars denote mean standard deviation ( $n = 4$ ).

**Table 2.** Changes in the carbon content of soil organic matter fractions 240 days after application of different residues of *Eucalyptus* with or without the addition of mineral nitrogen (200 mg kg<sup>-1</sup> of N)

Residue	LF		HF		Humins		Humic acid		Fulvic acid	
	240 d <sup>(1)</sup>	$\Delta C$ <sup>(2)</sup>	240 d	$\Delta C$	240 d	$\Delta C$	240 d	$\Delta C$	240 d	$\Delta C$
g kg <sup>-1</sup> of C										
- N										
Leaves	7.56 aA	6.77*	27.16 abA	5.36*	21.86 aA	7.53	0.74 aA	-0.14	0.36 aA	0.12*
Bark	2.53 bB	1.74*	32.39 aA	10.59*	27.72 aA	13.39*	0.58 aA	-0.30*	0.32 aA	0.08*
Branches	1.71 abB	0.92*	27.88 bA	6.08*	26.18 aA	11.85*	0.57 aA	-0.31*	0.32 aA	0.08
Roots	4.19 abB	3.40*	27.16 bA	5.36*	21.37 aA	7.04	0.82 aA	-0.06	0.32 aA	0.08
Mix <sup>(3)</sup>	2.29 abB	1.50*	28.33 abA	6.53*	24.10 aA	9.77*	0.67 aA	-0.21	0.30 aA	0.06
+ N										
Leaves	5.67 aB	4.88*	28.84 abA	7.04*	19.65 aA	5.32	0.76 aA	-0.12	0.38 aA	0.14*
Bark	4.21 bA	3.42*	30.65 aA	8.85*	24.68 aA	10.35*	0.61 aA	-0.27*	0.34 aA	0.10*
Branches	5.00 abA	4.21*	25.72 bA	3.92*	25.68 aA	11.35*	0.60 aA	-0.28*	0.31 aA	0.07
Roots	5.29 abA	4.50*	25.90 bA	4.10*	19.55 aA	5.22	0.76 aA	-0.12	0.29 aA	0.05
Mix	5.48 abA	4.69*	26.07 abA	4.27*	25.16 aA	10.83*	0.67 aA	-0.21	0.30 aA	0.06

<sup>(1)</sup> For each SOM fraction, lowercase letters compare different *Eucalyptus* residues and uppercase letters compare the effect of addition of mineral N according to the Tukey test ( $p < 0.004$ ). Values are means of four replicates. <sup>(2)</sup> Variation in the amount of each SOM fraction 240 days after setting up the experiment ( $\Delta C = C_{\text{final}} - C_{\text{initial}}$ ). The values marked with an asterisk represent a significant increase or decrease compared to the control soil ( $p < 0.004$ ). <sup>(3)</sup> Mixture of leaves, bark, branches, and roots at the relative proportions of 12.9, 26.4, 12.7, and 49 %, respectively. LF: light fraction of SOM, HF: heavy fraction of SOM.

**Table 3.** Changes in the nitrogen content of soil organic matter fractions 240 days after application of different residues of *Eucalyptus* with or without the addition of mineral nitrogen (200 mg kg<sup>-1</sup> of N)

Residue	LF		HF		Humin		Humic acid		Fulvic acid	
	240 d <sup>(1)</sup>	ΔN <sup>(2)</sup>	240 d	ΔN	240 d	ΔN	240 d	ΔN	240 d	ΔN
mg kg <sup>-1</sup> of N										
- N										
Leaves	491.7 aA	461.7*	1470.3 aA	80.3*	1125.3 aA	685.3*	39.2 aA	-0.8	24.9 aA	4.9
Bark	47.4 bB	17.4	1218.3 bA	-171.7	628.7 bA	188.7*	25.3 aA	-14.7	31.9 aA	11.9
Branches	21.7 bB	-8.3	1146.0 bA	-244*	567.6 bA	127.6	26.6 aA	-13.4	19.2 aA	-0.8
Roots	64.1 bB	34.1	830.4 bA	-559.6*	514.0 bA	74.0	47.5 aA	7.5	22.4 aA	2.4
Mix <sup>(3)</sup>	80.4 bB	50.4*	982.5 bA	-407.5*	602.9 bA	162.9	39.2 aA	-0.8	16.6 aA	-3.4
+ N										
Leaves	483.5 aB	453.5*	1658.0 aA	268.0*	993.3 aA	553.3*	39.2 aA	-0.8	16.7 aA	-3.3
Bark	124.7 bA	94.7	1266.0 bA	-124.0	689.2 bA	249.2*	27.3 aA	-12.7	25.7 aA	5.7
Branches	79.3 bA	49.3	1080.5 bA	-309.5*	679.0 bA	239.0	29.2 aA	-10.8	18.3 aA	-1.7
Roots	113.7 bA	83.7	1193.3 bA	-196.7*	510.4 bA	70.4	36.8 aA	-3.2	17.9 aA	-2.1
Mix	157.6 bA	127.6*	958.8 bA	-431.2*	660.2 bA	220.2	34.9 aA	-5.1	18.2 aA	-1.8

<sup>(1)</sup> For each SOM fraction, lowercase letters compare different *Eucalyptus* residues and uppercase letters compare the effect of addition of mineral N according to the Tukey test ( $p < 0.004$ ). Values are means of four replicates. <sup>(2)</sup> Variation in the amount of each SOM fraction 240 days after setting up the experiment ( $\Delta N = N_{\text{final}} - N_{\text{initial}}$ ). The values marked with an asterisk represent a significant increase or decrease compared to the control soil ( $p < 0.004$ ). <sup>(3)</sup> Mixture of leaves, bark, branches, and roots at the relative proportions of 12.9, 26.4, 12.7, and 49 %, respectively. LF: light fraction of SOM, HF: heavy fraction of SOM.

When the HF was chemically fractionated, most N was recovered in the humin fraction, as was observed for C (Table 3). The highest N content in the humin fraction was observed in the treatment with leaf residues. Some significant net N increases were detected in the soils receiving leaf and bark residues. Humic and fulvic acids showed low N content values and no significant variation compared to the control soil.

## DISCUSSION

In this study, C content of the LF and HF of SOM increased over a 240-days period through incorporation of *Eucalyptus* residues in the soil. Among the residues evaluated - namely leaves, bark, branches, roots, and the mixture of all residues - bark and leaf residues stood out. Bark and leaf residues showed the highest increases in the HF-C, increasing C content by up to 45 %. In turn, leaf residues caused the highest increases in the LF-C, LF-N, and HF-N.

The incorporation of *Eucalyptus* residues in the soil caused changes in the C content of SOM fractions. The LF of SOM is composed mainly of partially decomposed macroscopic organic residues, generally more closely related to the original material (Demolinari et al., 2008). Curiously, leaf residue led to a high amount of C recovered in the LF 240 days after application of the residues (Figure 1a). This was unexpected, since leaves are labile residues that are normally decomposed quickly by the soil microbiota. The fast degradation of material may promote the secretion of specific polysaccharides by the microorganisms and some of these compounds have adhesive properties. The production of adhesive compounds may contribute to particle aggregation (mineral and organo-mineral particles) around the plant material that is being attacked by the organisms. In fact, it has been shown that plant litter with a greater content of more easily decomposable soluble-C was more favorable to the overall microbial community, which in turn increased water-stable aggregate formation as a result of the concerted action of several binding agents produced during plant litter decomposition, including microbial-released polysaccharides and lipids, and even adhesion of microbial cells to soil particles (Le Guillou et al., 2012). Most importantly, the LF-C content was 8.6 times

greater with incorporation of leaf residues (Table 2). In fact, all the *Eucalyptus* residues led to net increases in the LF-C content.

All the *Eucalyptus* residues increased the C content in the HF of SOM, with an average C recovery of 28 % of input. Bark, leaves, and the mixture of residues led to the highest increases; the HF-C was up to 45 % higher (Table 2). These results highlight the significance of maintaining harvest residue in the area, given the net increase in the soil organic C observed, especially in the mineral associated fraction (HF), which represents a more stable C stock of SOM (Grandy and Neff, 2008). Nevertheless, the contribution of root material to SOM, especially fine roots, is underestimated in the present study, because the direct contribution by root exudation and turnover, as well the indirect effect of roots on the microbial communities in the rhizosphere (Rasse et al., 2005), were not taken into account. It is also important to note that in the current incubation experiment, the residues used were ground. Under field conditions, the residues show very different characteristics, which can influence their degradation rate.

Residue-derived N had a different destination than that of C in the SOM fractions, as observed in a previous study on incorporation of rice straw in soil (Bird et al., 2003). Net increases in N content were observed in the LF of soil receiving leaves and the mixture of residues (Table 3), probably because of the high N content of these residues (Table 1). The rest of the residues had no effect on the LF-N content. The HF showed the most highly contrasting characteristics. While the HF-C increased through application of the residues (Table 2), significant net N transfer (immobilization) from the HF was observed in the soils receiving branches, roots, and the mixture of residues (Table 3). Except for leaves, the residues contained low amounts of N and, hence, high C:N ratios (Table 1). Thus, it is likely that part of the original soil organic N was taken up and immobilized in the microbial biomass during decomposition of those N-poor residues (Bird et al., 2003; Craine et al., 2007; Manzoni et al., 2008).

While supplementation with mineral N has previously been reported to improve the incorporation of plant residues to soils (Bird et al., 2003; Moran et al., 2005; Kirkby et al., 2013; Kirkby et al., 2014), our data showed that the addition of mineral N had a minor effect on C recovery from *Eucalyptus* residues (Figure 1, Table 2). Mineral-N supplementation of N-poor residues can increase C recovery by improving the efficiency of microbial biomass production, decreasing losses of CO<sub>2</sub>-C, and, especially, by increasing deposition of microbial products and debris, which are the main sources of stable SOM associated with mineral particles (Craine et al., 2007; Grandy and Neff, 2008; Manzoni et al., 2008; Vogel et al., 2014). Nevertheless, in this study, the addition of mineral N affected residue-C recovery only in the less stable SOM fraction (LF) (Figure 1a). These results suggest that N availability did not hinder SOM stabilization in our experiment. The C:N ratios of the *Eucalyptus* residues (Table 1) are higher than that of the rice straw (144) used by Moran et al. (2005). Thus, N supplementation would be expected to have more pronounced effect in our experiment, which did not occur. Two factors could explain the lack of effect from mineral-N supplementation. First, there are the intrinsic characteristics of the residues that confer recalcitrance, such as lignin content (Urquiaga et al., 1998). Second, and most important, there are the ecosystem properties, including the abiotic and biotic characteristics (Schmidt et al., 2011), e.g., a microbiota adapted to deal with N-poor residues (Manzoni et al., 2008).

The effect of mineral-N supplementation would be expected to be more pronounced in the N content of SOM fractions. However, the addition of mineral N increased the N content only in the LF of soils receiving bark, branches, roots, and the mixture of residues (Table 3). The added mineral N could have been incorporated into organic compounds, probably by the action of microorganisms (Grandy and Neff, 2008; Vogel et al., 2014), or interacted directly with charges on the surface of soil particles. Accordingly, Moran et al. (2005) reported that most of the mineral N added to soil remained in inorganic form after



incubation of soil samples with or without rice straw. In the present study, we were not able to distinguish these different destinations of mineral N through the fractionating procedure used.

Most of the C and N contained in the HF were found to be associated with the operationally defined humin fraction. Indeed, the formation of organo-mineral complexes is evolving as a more important mechanism of soil C stabilization (Mikutta et al., 2006; Marschner et al., 2008; Doetterl et al., 2015). The incorporation of residue-derived C (Table 2) and N (Table 3) in the humin fraction in a relatively short period (240 days) may be evidence of rapid modifications in this previously considered refractory fraction. Recent studies have shown that microorganisms play an essential role in the stabilization of SOM through the deposition of compounds and cell debris on the surface of organo-mineral soil particles (Grandy and Neff, 2008; Cotrufo et al., 2013; Vogel et al., 2014; Throchmorton et al., 2015). Moreover, microorganisms can, under favorable environmental conditions, rapidly decompose molecules previously thought to be highly recalcitrant (Fontaine et al., 2007). However, the incorporation of residue-derived C in the humin fraction is not necessarily evidence of the occurrence of rapid changes through heterotrophic activity. Nonpolar substances from residues may be degraded less than hydrophilic compounds and, hence, be incorporated directly in the humin fraction without being biologically or chemically transformed (Song et al., 2011). Furthermore, aliphatic compounds abundant in plant material are not extractable in acid/alkali (e.g., suberin, cutin, and homogeneous alkyl chains or those with a low degree of hydrophilic substitution), and tend to remain in the humin fraction (Deshmukh et al., 2005; Kelleher and Simpson, 2006; Song et al., 2011).

This study highlights the significance of maintaining harvest residues in *Eucalyptus* plantations. All residues led to significant increases in soil organic C, especially in the SOM fraction associated with mineral particles (HF), which can stabilize organic compounds by protection against degradation (Kleber and Johnson, 2010). Maintaining harvest residues, associated with benefits of cultivation of *Eucalyptus* on soil C stocks (Lima et al., 2006), is an advantageous way to improve the SOM in *Eucalyptus* plantations, with consequent gains in system productivity. Moreover, other nutrients are added with the residues and the cumulative effect of this should be studied under natural conditions. However, the effect of residue addition on soil organic N was negative in some cases, namely for branch and root residues, where net N losses were detected. This N was probably immobilized in the microbial biomass during decomposition of residues and would be available after mineralization of this biomass. Supplementation with external mineral N aiming to improve residue stabilization in SOM proved to be inefficient under the conditions of the experiment at the application rate tested. Nutrients other than N (Hobbie, 2015; Ferreira et al., 2016) may have limited residue decomposition and C stabilization into SOM. Finally, attention should be directed to the efficiency of leaf and bark residues, which led to the greatest net C increases in the LF and HF. Because of this, maintenance of these *Eucalyptus* harvest residues in the plantation area is highly recommended.

## CONCLUSIONS

Addition of *Eucalyptus* residues produced a net increase in soil organic C content over a 240-days period.

Bark, leaves, and the mixture of residues provided the greatest gains in the HF of SOM, increasing C content by up to 45 %. The C and N content of the LF increased through incorporation of leaf residues. Leaf residues also increased N content in the HF. By contrast, branches, roots, and the mixture of residues caused significant losses in N from the HF.

External mineral-N supplementation had no effect on C and N accumulation in the most stable fraction of the SOM, i.e., the heavy fraction.

The results obtained highlight the importance of keeping harvest residues in the planted area, especially the bark - the largest residue component under field conditions, for maintenance of SOM.

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