Revista
Brasileira de
Ciência do Solo

Division - Soil Use and Management | Commission - Soil and Water Management and Conservation

Contribution of *Eucalyptus* Harvest Residues and Nitrogen Fertilization to Carbon Stabilization in Ultisols of Southern Bahia

Fernanda Cristina Caparelli Oliveira(1)*, Ivo Ribeiro Silva(2), Gabriel William Dias Ferreira(3), Emanuelle Mercês Barros Soares(2), Sérgio Ricardo Silva(4) and Eulene Francisco Silva(5)

(1) Universidade Federal de Sergipe, Programa de Pós-Graduação em Desenvolvimento e Meio Ambiente, São Cristovão, Sergipe, Brasil.
(2) Universidade Federal de Viçosa, Departamento de Solos, Viçosa, Minas Gerais, Brasil.
(3) Universidade Federal de Viçosa, Departamento de Solos, Programa de Pós-Graduação em Solos e Nutrição de Plantas, Viçosa, Minas Gerais, Brasil.
(4) Empresa Brasileira de Pesquisa Agropecuária, Embrapa Trigo, Passo Fundo, Rio Grande do Sul, Brasil.
(5) Universidade Federal Rural do Semi-Árido, Departamento de Ciências Agronômicas e Florestais, Mossoró, Rio Grande do Norte, Brasil.

ABSTRACT: *Eucalyptus* forests in southern Bahia (BA) are planted in soils with a sandy surface layer and humid tropical climate, conditions that lead to soil carbon (C) decomposition. Recent studies have shown that nitrogen (N) may be important for soil C stabilization. The aim of this study was to evaluate the contribution of *Eucalyptus* harvest residues and nitrogen fertilization to C stabilization in Ultisols of southern BA. The experiment was conducted in *Eucalyptus* clonal plantations cultivated in two regions of Eunápolis, BA, Brazil, with different clay content: southern region (140 g kg^{-1} of clay) and western region (310 g kg^{-1} of clay). Five treatments were evaluated: one control (CTR), without *Eucalyptus* harvest residues and N fertilization, and four treatments with harvest residues combined with four rates of N fertilization: 0, 25, 50, and 100 kg ha^{-1}. Soil samples were collected from the 0.00-0.10, 0.10-0.20, 0.20-0.40, and 0.40-0.60 m layers at the beginning and the end of the experiment (36 months). The amount of C and N and the C and N isotopic ratio ($\delta^{13}C$ and $\delta^{15}N$) of particulate organic matter (POM) and mineral-associated organic matter (MAOM) were determined. In the southern region after 36 months, the C-MAOM stocks in the 0.00-0.10 m layer of the CTR decreased by 33 %. The addition of harvest residue followed by 100 kg ha^{-1} N increased C-POM and N-POM stocks (0.00-0.10 m) compared to the CTR, and the final N-POM stocks and residue-C recovery in the surface soil layer were positively correlated with the increase in N fertilization rates. In the western region, residue maintenance resulted in increased C-MAOM stocks (0.00-0.10 m) compared to the CTR, but an increase in N availability reduced this increment. The increase in N fertilization rates did not alter C stocks, but reduced N stocks of POM and MAOM in the upper soil layer. At the end of the experiment, N fertilizer recovery (0.00-0.60 m) was similar among the regions evaluated. In soil with lower clay content, higher N availability led to higher C and N stocks in the particulate fraction. In soils with high clay content, physical and chemical protections are more important than N fertilization for soil C stabilization, and just maintaining harvest residues may suffice to increase C and N in the more stable SOM fraction.

Keywords: planted forests, harvest residue management, soil organic matter, stable isotope.

*** Corresponding author:**
E-mail: nandacaparelli@gmail.com

Received: June 20, 2016
Approved: May 25, 2017

How to cite: Oliveira FCC, Silva IR, Ferreira GWD, Soares EMB, Silva SR, Silva EF. Contribution of *Eucalyptus* harvest residues and nitrogen fertilization to carbon stabilization in Ultisols of southern Bahia. Rev Bras Cienc Solo. 2018;42:e0160340.
https://doi.org/10.1590/18069657rbcs20160340

INTRODUCTION

Currently, global climate change and particularly the contribution of greenhouse gases (GHG) to global warming and the need to develop strategies to mitigate GHG are among the major concerns of society. In this context, carbon (C) storage in soil organic matter (SOM) fractions is capable of reducing GHG emissions and soil degradation (Graves et al., 2015). However, to better understand soil C storage potential, it is necessary to assess the C input and decomposition, and mechanisms of C stabilization.

Carbon input into the soil originates predominantly from plant biomass via litterfall, roots, and root exudation. In planted forests, such as those of the *Eucalyptus* genus, cultivated specifically for pulp and timber production, C stocks exported at harvest reach, on average, 60 t ha^{-1}, and harvest residues generated may contain up to half of the stem wood C content (Gatto et al., 2010). Therefore, keeping harvest residues in the field makes a crucial C contribution to soils with *Eucalyptus* plantation.

Harvesting operations influence the quantity and quality of the residues. In Brazil, two mechanized systems are commonly employed: conservation system (roots, bark, branches, and leaves are left in the field) and conventional system (cutting and de-barking operations occur outside the forest stand area and the harvest residues are used for energy production). The most suitable harvesting operation should be based not only on economic feasibility, but also on the sustainability of current and future forest productivity.

The composition (nutrients and labile C amounts) and content of phenolic constituents influence the decomposition rate of plant litter (Berg, 2014). This author proposes that the litter decomposition rate during the initial stage increases rapidly in response to increasing N content, in contrast with that observed in the latter phases, in which lignified materials predominate. However, despite some prior studies reporting N as an important factor for soil C dynamics (Stockmann et al., 2013; Du et al., 2014), in tropical regions, a complete understanding of the role played by this nutrient in decomposition of plant residue (Cusack et al., 2011; Ferreira et al., 2016) and soil C stabilization (Prescott, 2010) remains unclear. Probably, the effect of N on the decomposition process may be linked to the response of the microbial community to changes in the C/N and lignin/N ratio (Cotrufo et al., 2013). Furthermore, the consequence of increasing N availability on soil C stabilization depends upon the various chemical changes that occur in each soil organic matter (SOM) fraction (Grandy et al., 2008). For instance, the addition of mineral N induced higher C accumulation in recalcitrant SOM fraction and reduced the soil labile C:recalcitrant C ratio of temperate forests (Du et al., 2014). In tropical rain forests, however, the mineral-associated organic matter (MAOM) fraction appears to react more quickly to changes in N availability (Cusack et al., 2010). Frequently, the lack of consensus regarding N dynamics in soil-plant systems arises from the difficulty of defining the origin of the different N sources. Thus, isotopic techniques, using ^{15}N tracer, allow mineral N or plant residue N to be traced, which leads to better understanding of N dynamics and the role of N in soil C stabilization (Bird et al., 2008; Dail et al., 2009).

Soil C stabilization in pools with longer residence times relies on the interaction of soil, climatic and biological factors (Leckie et al., 2004). Physical protection of SOM within soil aggregates (Dieckow et al., 2009; Dungait et al., 2012) and chemical adsorption of C onto mineral surfaces (Six et al., 2002) favor soil C accretion and are strongly related to soil texture. However, climatic factors such as temperature may alter soil C stabilization capacity by influencing the binding energy between SOM and soil minerals (Conant et al., 2011). However, the climate effect during soil C stabilization is weaker than the influence of geochemical soil properties (Doetterl et al., 2015).

In tropical soils, management practices should provide for maintenance and accretion of soil C, since SOM is responsible for most of the cation exchange capacity (CEC) of these soils, and SOM also influences soil physical properties, favoring higher water retention and aggregation stability (Six and Paustian, 2014). Moreover, in planted forests, concerns over maintenance of soil C stocks are driven by the relationship of these stocks to the long-term productivity of the forests (Versini et al., 2013). Therefore, the conversion of residue C to stable soil C may ensure sustainability of planted forest in the long-term.

We therefore hypothesize: removal of *Eucalyptus* harvest residues reduces soil C stocks and the effect is stronger in soils with lower clay content; and stabilization of residue derived C is greater in soils with higher N availability.

Considering that *Eucalyptus* forests in southern Bahia are predominantly planted in Ultisols (upper soil layer with sandy texture) and established in regions with high mean annual temperature and rainfall, the aim of this study is to quantify the effects of harvest residue removal on SOM C and N stocks; and to determine the contribution of increasing mineral N fertilization to C stabilization in Ultisols with different clay contents.

MATERIALS AND METHODS

Site description

The study was set up in commercial *Eucalyptus urograndis* hybrid stands in two areas of the municipality of Eunápolis (southern Bahia, Brazil): one in the south (16° 42’ 04” S, 39° 18’ 40” W) and the other in the west (16° 13’ 17” S, 39° 44’ 31” W), hereinafter called the South region and West region, respectively.

Both regions are within the Atlantic Forest Biome, under a climate transition area between tropical without dry season (Af) and tropical monsoon (Am), according to Köppen’s classification system (Alvares et al., 2013). During the experiment, the monthly average temperature was 22.5 °C in both regions, and annual average rainfall was 1,634 and 1,370 mm in the South and West region, respectively. Soil in the South region was classified as an *Argissolo Amarelo Distrófico abrúptico* and in the West region as an *Argissolo Amarelo Distrófico típico*, according to the Brazilian Soil Classification System (Santos et al., 2013), and as Ultisols for both regions by the Soil Taxonomy system (Soil Survey Staff, 2014). The initial soil properties are shown in table 1.

The first forestry companies arrived in southern Bahia at the beginning of 1980, encouraged by soil and climate conditions, land availability and price, availability of labor, and feasibility of forest products distribution (Almeida et al., 2008). Specifically, in the regions selected, land use change occurred primarily from native forest being replaced by grassland cultivation. Grasslands were managed for 15 years, until 1999, when short rotation *Eucalyptus* forests were introduced (Silva, 2008). In 2014, average forest productivity in the state of Bahia exceeded mean Brazilian productivity by 8 % (39 m³ ha^{-1} year^{-1}) and contributed 20 % of Brazilian cellulose production, mainly through the high productivity achieved from planted forests in southern Bahia (Abaf, 2015).

Table 1. Chemical and physical properties of a Typic Hapludults located in two regions of the municipality of Eunápolis, BA, Brazil

Region/Soil[(1)]	Soil layer	pH(H_2O)	Ca^{2+}	Mg^{2+}	H+Al	P	K	Sand	Silt	Clay
	m			$cmol_c$ dm^{-3}		mg dm^{-3}			g kg^{-1}	
South/*Argissolo Amarelo Distrófico abrúptico*	0.00-0.10	4.5	1.1	0.5	4.6	7	93	830	30	140
	0.10-0.20	4.7	0.9	0.5	4.3	4	85	780	30	190
	0.20-0.40	5.0	0.9	0.4	4.3	1	64	680	40	280
	0.40-0.60	5.0	0.9	0.3	4.0	1	61	620	40	340
West/*Argissolo Amarelo Distrófico típico*	0.00-0.10	4.4	1.6	0.6	4.5	4	90	670	20	310
	0.10-0.20	4.6	1.5	0.5	3.6	3	75	670	40	290
	0.20-0.40	4.6	1.2	0.4	3.1	2	58	580	40	380
	0.40-0.60	4.5	1.0	0.3	3.3	1	45	540	40	420

(1) Brazilian soil classification system (Santos et al., 2013). pH in water at a ratio of 1:2.5 v/v; Ca^{2+} and Mg^{2+} extracted by KCl 1 mol L^{-1}; H+Al extracted by calcium acetate 0.5 mol L^{-1}, buffer at pH 7.0; P and K extracted with Mehlich-1 solution; sand, silt, and clay determined by the pipette method (Ruiz, 2005).

Experimental design and treatments

This experiment began in 2006, at the end of the first *Eucalyptus* rotation (7 years) in both areas. The experiment was set up after harvest and was conducted at the same time as the second *Eucalyptus* rotation.

Five treatments were set up in a randomized block design with five replications. Treatments were arranged in a split-plot in time design (0 and 36 months) and consisted of a control (CTR), in which harvest residues were removed and no mineral-N fertilization was used; and treatments in which harvest residues was maintained, followed by four rates of mineral N fertilization: 0; 25; 50; and 100 kg ha^{-1}.

The biomass dry weight (DB) of each harvest component [leaves (DBL), thin twigs (DBtT), thick twigs (DBTT), and bark (DBB)] was calculated, based on mean annual increment of the former rotation, through NUTRICALC® software (Barros et al., 1995). As a result, the following were added to each experimental unit (EU) of 1 m²: 253 g of DBL, 339 g of DBtT, 337 g of DBTT, and 1,296 g of DBB. Prior to setting up the experiment, total organic matter on the EU surface was manually removed. Afterwards, except for the CTR treatment, harvest residues were placed in direct contact with the soil surface. Residues were kept similarly as their occurrence in the field; thus twigs and bark were only chopped (0.15-0.20 m) when their size exceed the EU limits.

Nitrogen fertilizer (NH_4NO_3) was diluted in 5 L of water, and the solution was uniformly distributed in the EU. The same water volume was applied to the treatments without N fertilization (CTR and 0 kg ha^{-1} of N) to maintain the same initial soil moisture condition. Labelled ammonium sulfate [$(^{15}NH_4)_2SO_4$, 98 atom% excess] was added in 5 % of the 50 kg ha^{-1} N rate to trace the fate of N fertilizer in different SOM fractions. The aim of N labelling was only to clarify mineral N dynamics in the soil, so it was not used in all the N fertilization rates evaluated.

Finally, the EU was covered with a 4 mm² mesh net with dimensions of 1.20 × 1.20 m, fixed with metal rods, to prevent external litterfall input.

Residue and soil analyses

At the beginning and end of the experiment, the residue remaining in the EU was manually sampled, dried at 60 °C in a forced-air circulation oven until constant weight, weighed, and ground (<500 μm). Carbon and N content and natural ^{13}C ($^{13}C/^{12}C$) and ^{15}N ($^{15}N/^{14}N$) abundance were measured using a continuous flow isotope ratio mass spectrometer (IRMS) (20-20 ANCA-GLS, Sercon, Crewe, UK). The isotopic C ($\delta^{13}C$) and N ($\delta^{15}N$) ratio were calculated from natural ^{13}C and ^{15}N abundance and measured against standard Pee Dee Belemnite and the air, respectively.

In both sampling times, soil was sampled in the 0.00-0.10 m layer for all treatments and in particular for the ^{15}N-labeled 50 kg ha^{-1} N treatment, samples were taken from the 0.10-0.20, 0.20-0.40, and 0.40-0.60 m soil layers. Soil samples were obtained with a cylindrical auger and were collected as single samples in triplicate to form a composite sample per treatment. Soil samples were air dried and passed through a 2-mm mesh sieve. Afterwards, the samples were dispersed with sodium hexametaphosphate (5 g L^{-1}) for 15 h to obtain two SOM pools: the particulate organic matter (POM, 53-2000 μm) and mineral-associated organic matter (MAOM, <53 μm) fractions (Cambardella and Elliott, 1992). The SOM fractions were dried and ground to pass through a 100 mesh (149 μm) sieve prior to analyses. Total C and N content and $\delta^{13}C$ and $\delta^{15}N$ were determined by IRMS analysis. In each region, at the end of the full *Eucalyptus* rotation and prior to the machine harvesting operation, five undisturbed soil sample cores were collected from the 0.00-0.10 m layer to obtain soil bulk density (Claessen, 1997).

C and N stocks and residue-C and mineral-N recovery

In each region, C and N stocks in the 0.00-0.10 m layer were calculated using their contents (g kg^{-1}) and their respective density.

The residue-derived C contained in POM or MAOM (f_c) was estimated through the equation 1 adapted from Moran et al. (2005):

$$f_c = \frac{\delta^{13}C_{final}-\delta^{13}C_{ref}}{\delta^{13}C_{residue}-\delta^{13}C_{ref}} \qquad \text{Eq. 1}$$

in which $\delta^{13}C_{final}$ is the $\delta^{13}C$ of POM or MAOM at the end of the experiment; $\delta^{13}C_{ref}$ is the $\delta^{13}C$-POM or $\delta^{13}C$-MAOM of the CTR treatment at the end of the experiment; and $\delta^{13}C_{residue}$ is the $\delta^{13}C$ weighted average of the harvest residues components added.

The recovery of residue-derived C in POM or MAOM was estimated according to equation 2:

$$C_{rec}\ (\%) = \frac{f_c \times T \times 100}{C_{ad}} \qquad \text{Eq. 2}$$

in which T is the C content (g kg^{-1}) in the 0.00-0.10 m layer; and C_{ad} is the C content of residue added in the EU (g kg^{-1}).

The fraction of fertilizer-derived N in each SOM fraction (fn) was calculated according to equation 3:

$$fn(\%) = \frac{PAEx_t}{PAEx_{fert}} \times 100 \qquad \text{Eq. 3}$$

in which $PAEx$ is the ^{15}N enrichment in each fraction at the beginning or end of the experiment (t) and the ^{15}N enrichment of the mineral N fertilizer ($fert$). The recovery of N fertilizer in POM and MAOM was estimated through the equation 4:

$$N_{rec} = \frac{fn \times T \text{ x } 100}{Nad} \qquad \text{Eq. 4}$$

in which T is the N content (g kg^{-1}) of SOM in each layer and Nad is the mineral N fertilizer content (g kg^{-1}).

Statistical analyses

Data normality was assessed using the Shapiro-Wilk test and log or Box-Cox transformations were used when normality was not met. The C and N stocks and $\delta^{13}C$ and $\delta^{15}N$ values from each region were subjected to analyses of variance (Anova). Contrasts were used to test differences between treatments (N fertilization rates) and the CTR treatment through the Dunnett test. In each region, the N *vs.* time interaction inside Anova was decomposed and linear equations were fitted to estimate C and N stocks, C recovery rate, and $\delta^{13}C$ values as a function of N fertilization rates. To select the best-fitting models, the significance of regression deviation and equation parameters were checked by the F-test, and the coefficient of determination (R^2) of the model was observed. The C and N contents and N-fertilizer recovery in the 0.00-0.10, 0.10-0.20, 0.20-0.40, and 0.40-0.60 m soil layers were evaluated by confidence intervals. All statistical analyses were performed using SISVAR® software (Ferreira, 2011).

RESULTS

Harvest residues management and soil C stocks

At the end of the experiment, C and N stocks of the CTR treatments in the South region were significantly different from the initial values ($p<0.05$) (Table 2). In this region, harvest residues removal reduced the C and N stocks of both SOM fractions, except for C-POM; the C and N stocks of the MAOM fraction decreased 35 and 15 %, respectively, and the N-POM stock was 50 % lower. In contrast, the $\delta^{13}C$ of SOM was not altered. In the West region, C and N stocks of the SOM fractions were not altered by harvest residues removal, although differences in δ^{13}C values were noted: the δ^{13}C POM were higher (less negative) and δ^{13}C MAOM were lower (more negative) at the end of the experiment.

At 36 months, in both regions, harvest residues decomposition reached 85 % of the initial dry biomass (data not shown). Contrasts between the CTR and treatments with residue and N fertilization (0; 25; 50; and 100 kg ha^{-1} of N) revealed the distinct SOM fractions behavior in the regions studied (Figure 1). In the South region, increasing N availability did not affect C MAOM stocks in comparison to the CTR ($p>0.05$). Nevertheless, maintenance of harvest residues without N fertilization (0 kg ha^{-1} N) reduced the C-POM stock, whereas addition of 100 kg ha^{-1} of N increased the C and N-POM stocks. In the West region, N fertilization rates had no effect on the C-POM stock. In this region, maintenance of harvest residues without N fertilization (0 kg ha^{-1} N) resulted in the highest C-MAOM stock increment (5.3 Mg ha^{-1}). Maintaining harvest residues in the planted area increased N stocks of both SOM fractions in this region compared to the CTR, whether followed by N fertilization or not, except for application of 100 kg ha^{-1} N (Figure 1).

N effects on soil C stocks

Overall, increasing N availability had little effect on topsoil (0.00-0.10 m) C-POM and C-MAOM contents in the South and West regions (Figure 2). In both regions, C-POM reached 1.7 Mg ha^{-1} on average at the end of the experiment. In the South region, in comparison to the 0 kg ha^{-1} N rate, C-MAOM increased 11.9 % with the addition of 25 kg ha^{-1} of N. In this region, differences in the C-MAOM stocks among the 25, 50, and 100 kg ha^{-1} N fertilization rates reached 20 %, and the highest N level resulted in the highest N-POM stock (0.28 Mg ha^{-1}). In the West region, there was a negative linear relationship between N fertilization rates and N-POM and N-MAOM stocks.

Nitrogen effects on isotopic ratio and residue-C recovery

The δ^{13}C-POM values in both regions were around -24 ‰ at the end of the experiment (Figure 3). In the South region, the δ^{13}C-MAOM ranged from -21 ‰ in the treatment with harvest residues and 0 kg ha^{-1} N to -18 ‰ with increasing N fertilization rates. In this region, N addition enhanced residue-C recovery (Crec) in the POM fraction, contrary to what was observed in MAOM. In the West region, the δ^{13}C MAOM was also -21 ‰ in the treatment with harvest residues and 0 kg ha^{-1} N, and slight decreases were observed at the higher N fertilization rates. In this region, increasing N rates did not affect Crec in the POM fraction, and a 13 % recovery was observed on average. The highest Crec in the MAOM fraction (51.8 %) in the West region was achieved with the addition of 26.3 kg ha^{-1} N (Figure 3).

Table 2. Carbon (C) and nitrogen (N) stocks of particulate organic matter (POM) and mineral-associated organic matter (MAOM) and carbon isotopic ratio of soil organic matter (SOM) fractions (δ^{13}C POM and δ^{13}C MAOM) in the 0.00-0.10 m layer of the treatment without harvest residues and nitrogen fertilization (CTR)

Soil property ⁽¹⁾	South region			West region		
	Initial	Final	p-value	Initial	Final	p-value
C-POM (Mg ha^{-1})	1.4 ±0.02	1.8 ±0.1	0.058	1.5 ±0.1	1.4 ±0.1	0.530
C-MAOM (Mg ha^{-1})	21.7 ±0.6	14.6 ±1.5	0.006	16.7 ±0.6	15.6 ±0.5	0.056
N-POM (Mg ha^{-1})	0.4 ±0.02	0.2 ±0.02	0.001	0.2 ±0.01	0.2 ±0.01	0.883
N-MAOM (Mg ha^{-1})	1.9 ±0.1	1.4 ±0.1	0.003	1.1 ±0.1	1.4 ±0.05	0.276
δ^{13}C-POM (‰)	-24 ±1.2	-20 ±1.6	0.181	-24 ±0.5	-18 ±0.5	0.001
δ^{13}C- MAOM (‰)	-17 ±0.2	-18 ±0.7	0.112	-20 ±0.5	-22 ±0.7	0.005

Values shown are means with standard errors (n = 5). ⁽¹⁾ POM and MAOM determined by Cambardella and Elliott (1992) fractionation method; C, N contents and δ^{13}C values determined using a continuous flow isotope ratio mass spectrometer (IRMS).

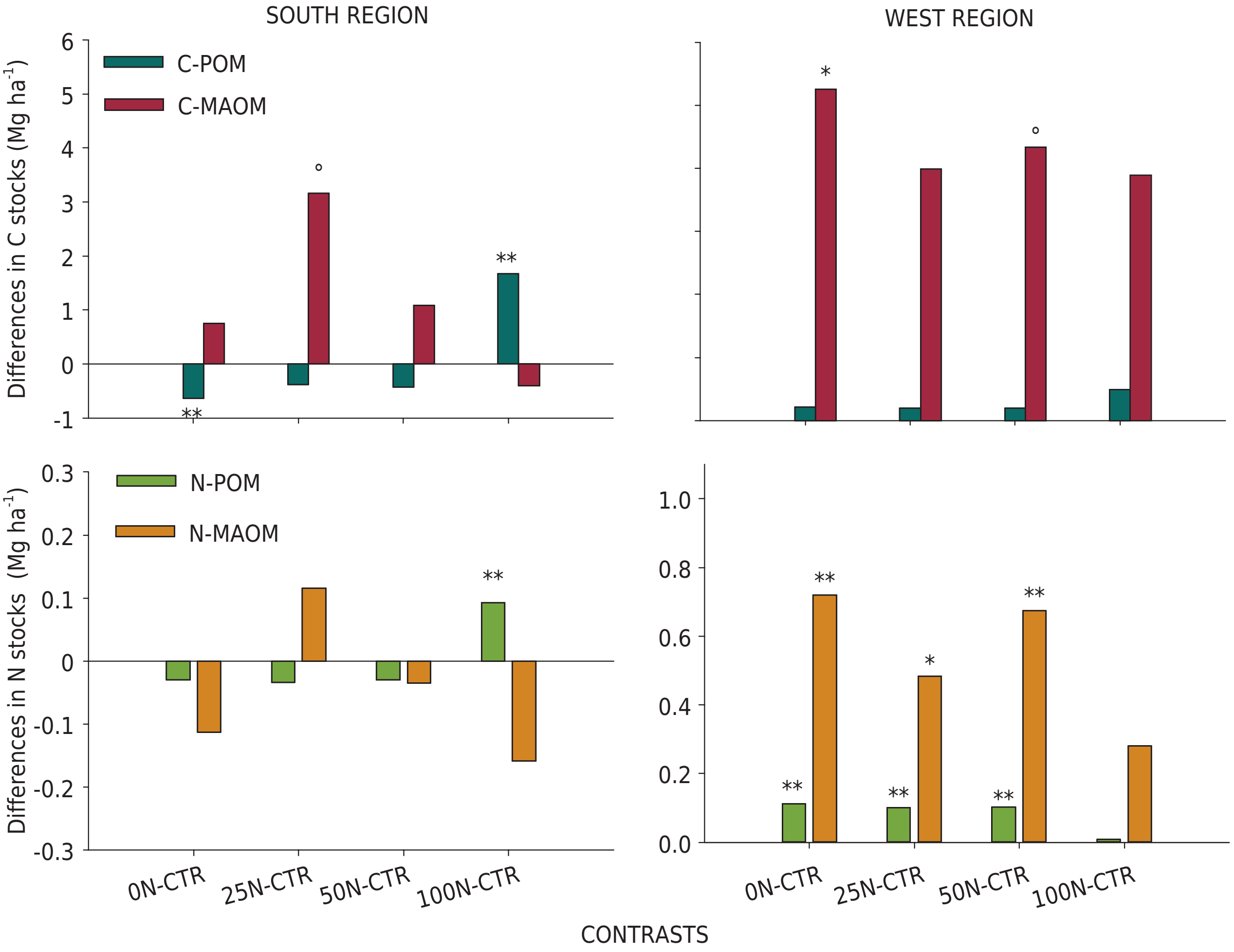

Figure 1. Contrasts of final C and N stocks (Mg ha^{-1}) in particulate organic matter (POM) and mineral-associated organic matter (MAOM) in the 0.00-0.10 m soil layer. 0N-CTR: effect of residue addition; 25N-CTR: effect of residue and 25 kg ha^{-1} of N addition; 50N-CTR: effect of residue and 50 kg ha^{-1} of N addition; 100N-CTR: effect of residue and 100 kg ha^{-1} of N addition. °, *, and **: significant contrasts at 10, 5, and 1 %, respectively, by the Dunnett test.

C and N contents in subsurface layers

The C and N contents of the 50 kg ha^{-1} N treatment in the beginning and end of the experiment are shown in figure 4. At the beginning of the experiment, the C and N contents were higher in the surface layers (0.00-0.10 and 0.10-0.20 m) than in the deeper ones for both regions (Figure 4). However, in the South region, some of the differences initially observed in the C and N content in the MAOM fraction could not be seen at the end, due to an increase in C and N contents in deeper layers. In the West region, 1.8 and 0.47 g kg^{-1} increases in the C-MAOM and N-MAOM contents, respectively, were observed in the surface layer (0.00-0.10 m) at the end of the experiment. The C-MAOM and N-POM contents were also higher in deeper layers (0.40-0.60 m) than initially recorded in the western region.

Mineral-N recovery

Immediately after application of the treatments, i.e., at the beginning of the experiment, 8 and 4 % of fertilizer-N were associated with the harvest residues in the South and West region, respectively. In both regions and samplings, the highest mineral-N recoveries were observed in the MAOM fraction (Figure 5). In the South region, at the beginning of the experiment, the N recovered in the MAOM fraction was 33 % in the 0.00-0.10 m layer, which was roughly twice the N recovered in the 0.10-0.20 m layer and almost ninefold the N recovered in the deepest layer evaluated (0.40-0.60 m). In the West region, however, N recovered in the 0.00-0.10 m layer was 19 %, which was sevenfold the N recovered in the deepest layer. At the end of the experiment, approximately 6 % of the added N was recovered in the MAOM fraction in both regions.

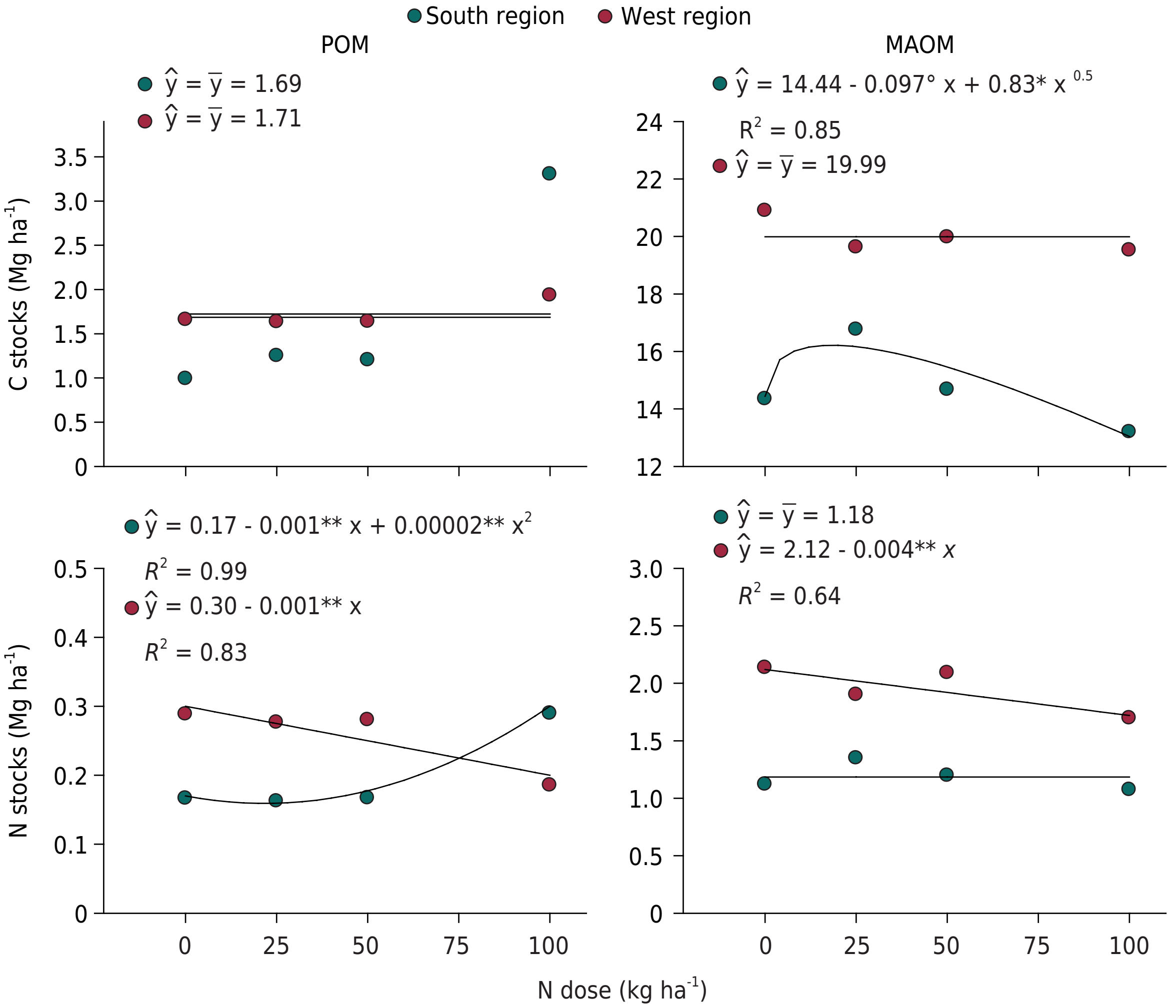

Figure 2. Carbon (C) and nitrogen (N) stocks of particulate organic matter (POM) and mineral-associated soil organic matter (MAOM) as a function of N fertilization rates. °, *, and ** indicate equation parameters significant at 10, 5, and 1 %, respectively.

DISCUSSION

After three years, the removal of harvest residues induced a loss of 7 Mg ha^{-1} in the C stock of the stable SOM fraction in the lower clay content region (Table 2). The losses recorded in this region were greater than those reported by Xiong et al. (2008) and Gentile et al. (2010). In central Kenya, with an average annual rainfall of 1,200 mm and in a soil with 650 g kg^{-1} of clay, removal of *Eucalyptus* harvest residues decreased the content of C-rich macroaggregates by 25 % compared to the content in the area where residues were maintained in the field (Epron et al., 2015). Similarly, in New Zealand pine forests, harvest residues removal caused a decline in C and N stocks (3.07 Mg ha^{-1} C and 0.10 Mg ha^{-1} N) as well as a 15 % decline in the basal area (m^2 ha^{-1}) of the planted forest (Zhang et al., 2006). In our experiment, the combined climatic, soil, and biological factors might explain the greater reduction in the C stock in the South region. High temperatures and the lack of long dry periods during the experiment may favor high soil enzyme activity - for instance, Β-glucosidase and alkaline and acid phosphatase (Waring et al., 2014), which may have induced more intense SOM mineralization. In addition, the lower clay content in the soil surface layer reduces C physical-chemical protection, which increases access of the microbial community to soil C and favors SOM decomposition process (Dungait et al., 2012). Corroborating our findings, in the Cerrado region, a 49 % difference in the clay content between two soils was responsible for a 46 % reduction in soil organic carbon (SOC) losses when the native vegetation was converted to conventional tillage systems (Dieckow et al., 2009).

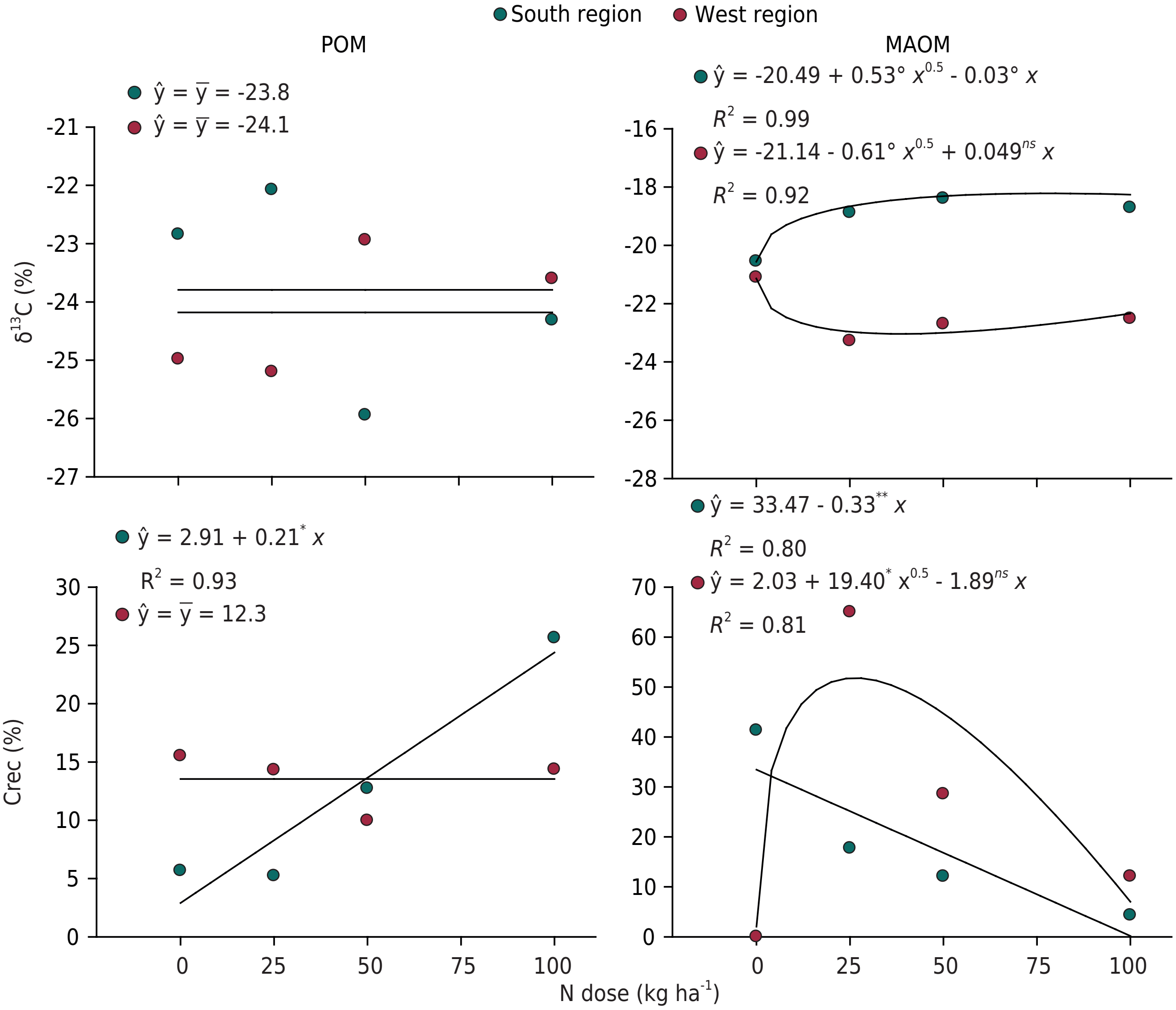

Figure 3. Recovery of residue-derived carbon (Crec) and the isotopic C ratio of SOM fractions ($\delta\ ^{13}C$) as a function of N fertilization rates. °, *, and ** indicate equation parameters significant at 10, 5, and 1 %, respectively. ns: not significant.

The lower aggregation capacity, specific surface, and charge density in sandy soils make soil C biochemistry properties of fundamental importance to SOC preservation over time (Sleutel et al., 2010). Remarkably, after three years of residue removal, $\delta^{13}C$-POM was less negative than initially recorded in the West region (Table 2), indicating a selective preservation of C derived from C_4 plants. Historically, prior to the introduction of planted clonal *Eucalyptus* forests, these regions had been used for grazing and the pasture management often involved the use of fire. The cycling of pyrogenic C produced by incomplete vegetation biomass combustion is estimated at more than 100 years (Singh et al., 2012), and also offers higher resistance to decomposition than plant biomass (Lehmann, 2007). In the lack of litter input to the soil, similar to the treatment without residue (CTR), microorganisms could preferentially degrade C_3-derived C instead of the C_4 pyrogenic C. This may have resulted in the higher $\delta^{13}C$ values, i.e., less negative, observed in the particulate fraction.

Maintaining harvest residues in the field was an important C input for the soil, particularly for soils with higher clay content in the surface layers (Figure 1). In the West region, increases in the C stock of the most stable SOM fraction were greater (Figure 1) than C stocks losses due to residue removal (CTR) (Table 2). In other words, maintaining residue resulted in higher soil C stock than that observed at beginning of the experiment. In the South, however, residue maintenance without N fertilization induced practically no change in C-MAOM stocks (Figure 1), and we argue that this

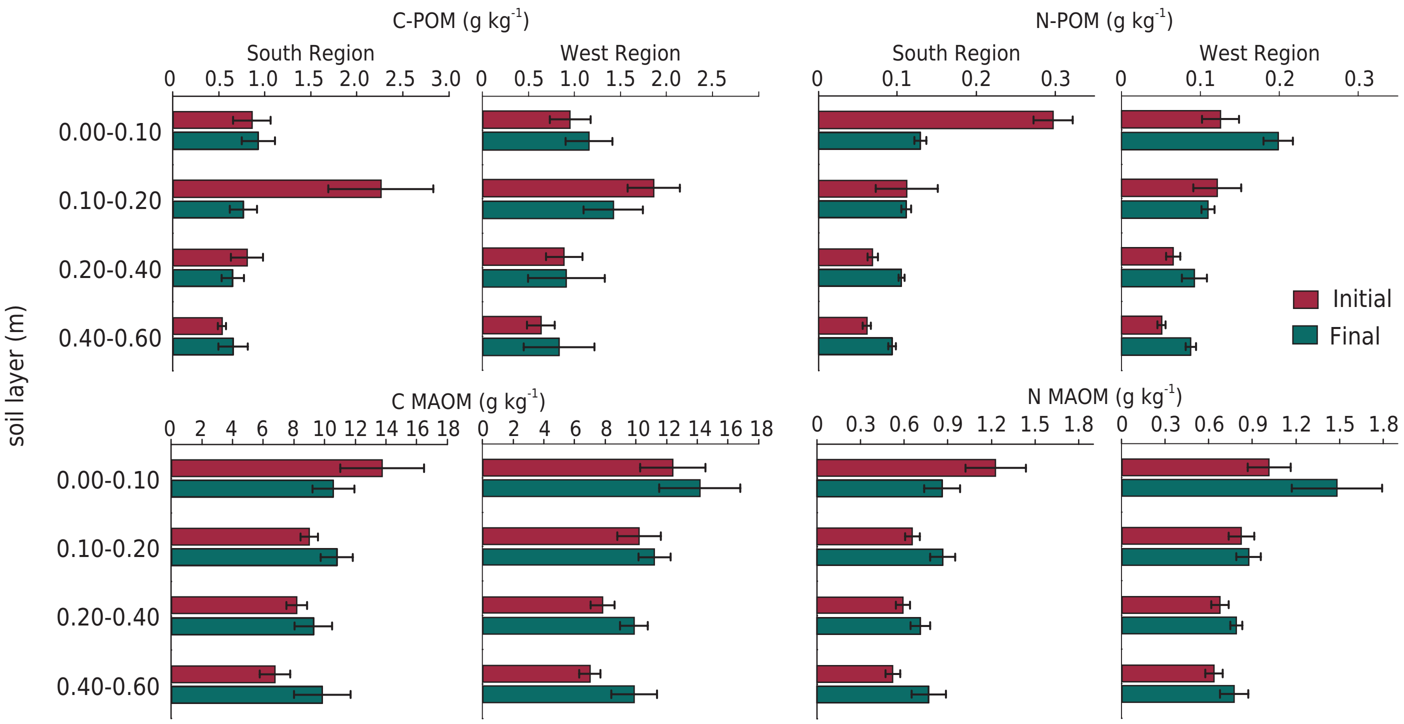

Figure 4. Carbon (C) and nitrogen (N) content in particulate organic matter (POM) and mineral-associated remover soil organic matter (MAOM) soon after application of 50 kg ha^{-1} N (initial) and at 36 months (final). Bars in each column represent standard deviation of the means.

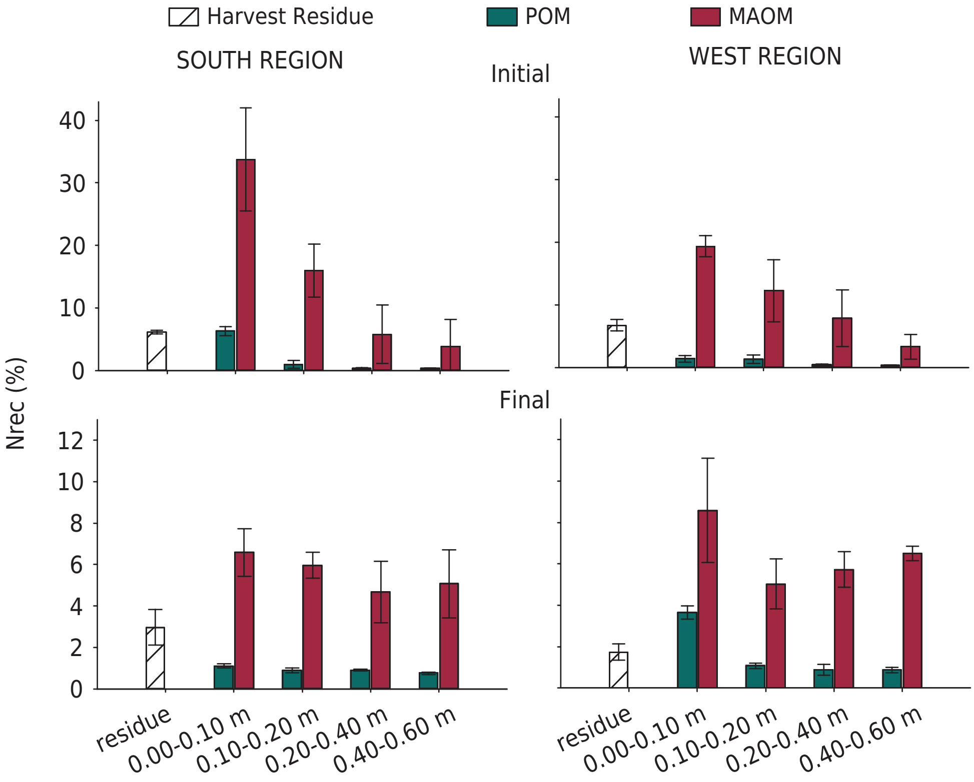

Figure 5. Recovery of nitrogen fertilizer (Nrec) in harvest residues, particulate organic matter (POM), and mineral-associated organic matter (MAOM) soon after application of 50 kg ha^{-1} (initial) and at 36 months (final). Bars in each column represent standard deviation of the means.

distinct behavior was caused by the lower clay content in this area. According to Stewart et al. (2007) and Doetterl et al. (2015), C soil stabilization relies on the presence of mineral surfaces for C adsorption and on physical protection, especially in soil microaggregates (Six et al., 2002). In this context, the soil textural difference between the regions was a fundamental factor for greater residue C storage in the higher clay content soil.

Some previous studies have indicated the importance of N on C dynamics in forest systems (Cusack et al., 2011; Jones et al., 2011) and tropical regions (Cusack et al., 2011; Huang et al., 2011), as well as on microorganism activities (Keeler et al., 2009; Janssens et al., 2010). If, on the one hand, N addition has been related to an increase in the formation of microbial products with longer soil residence time (Cotrufo et al., 2013), on the other hand, it has been related to higher SOM mineralization rates (Khan et al., 2007). Here, two distinct situations were observed in response to increasing N rates (Figure 2). In the South, the highest N fertilization rate resulted in higher C-residue recovery in the POM fraction (Figure 3), greater N-POM stock (Figure 2), and C-POM amount accretion (compared to the CTR) (Figure 1). Grandy et al. (2008) reported that the increased N availability in lignin-rich litter could lead to a higher soil C content, mainly due to the accumulation of lignin-derived compounds in the POM fraction. The greater C accrual in this fraction may have further implications for SOM stabilization. First, because there is an increase in C mineralization of particulate fractions with increased rainfall (Muñoz et al., 2016), and second, because transfer of organic compounds between SOM fractions may occur (Six et al., 2002; Oliveira, 2015). Although the addition of 25 kg ha^{-1} of N resulted in C-POM accretion in the South region, the same behavior was not observed for N-POM stocks and, moreover, an increase in the δ^{13}C value was noted in comparison to the treatment with residues and without N addition. This indicates that the effect observed from the addition of 25 kg ha^{-1} may have been random.

In the West region, in turn, although the higher clay and silt content could have offered more surface sites for chemical adsorption of cations and anions (Pozza et al., 2007), and thus minimize the dependence of N fertilization on soil C dynamics, our findings did not show higher N-fertilizer recovery (Figure 5). For this region, the higher N availability reduced the positive effects on soil C due to maintenance of harvest residues (Figure 1). In fact, N fertilization may have enhanced the activity of heterotrophic microorganisms responsible for SOM degradation (Khan et al., 2007). The losses observed in N stocks with increasing N fertilization rates, even if minimal, is a matter of concern as they occur in a system characterized by low N supply through fertilizer and in soils characterized by low CEC and poor natural fertility (Table 1), and this may compromise the sustainability of forest productivity in the future.

Soon after N application, fertilizer tracer was found in the 0.40-0.60 m layer (Figure 5), likely indicating fertilizer leaching. Regardless of the region, an average of 6 % of the N fertilizer was recovered in MAOM in the 0.00-0.10 m layer by the time the experiment ended. In the particulate fraction, in turn, fertilizer recovery in the same layer was 3.6 % on average in the West and 1.1 % in the South region. Given the high mean annual rainfall in these regions, the sandy texture of the surface layer and probable N uptake by the plant roots growing near the experimental plots, the observed recovery rate of the fertilizer N is a good indicator of the long-term interactions between soil C and N. Such interactions between the mineral N and organic soil compounds may result in formation of N-linked aromatic compounds, which exhibit longer soil residence time (Gillespie et al., 2014). Carbon residence time in tropical soils may also be longer in deeper soil layers as a result of constraints on SOM mineralization in these layers (Stahl et al., 2016). Indeed, an increase in C and N contents in the most stable SOM fraction was observed in the 0.40-0.60 m layer in both regions by the end of the experiment (Figure 4). In forests that are N fertilized, an increase in C stocks in subsurface layers may result from increased dissolved organic C content, root cycling (Cusack et al., 2011), and specific soil root C-preservation mechanisms (Rasse et al., 2005).

CONCLUSIONS

The removal of *Eucalyptus* harvest residues results in a decrease in SOM C and N contents, particularly in low clay soils. Nitrogen fertilization effects on the stabilization of C derived from harvest residues in the Ultisols of southern Bahia depend on the N fertilization rate, but these effects are limited by the specific natural capacity of soil to stabilize C. In soil with lower clay content, increasing N availability favors recovery of residue-C in the SOM particulate fraction and enhances C and N stocks in this fraction. In contrast, in higher clay content soil, chemical and physical protections are more important than N input for residue-C stabilization in surface layers. In this latter condition, only the maintenance of *Eucalyptus* harvest residues may be sufficient to increase C and N stocks in the more stable SOM fraction.

ACKNOWLEDGMENTS

We are thankful to the Nutree group and Veracel Celulose S.A. for providing access to the experimental area, financial support, and help in fieldwork; we also thank the financial support from CNPq - *Conselho Nacional de Pesquisa e Desenvolvimento Tecnológico* to I.R.S. and *Fundação de Amparo à Pesquisa do Estado de Minas Gerais* (Fapemig) for the scholarship during this study as part of the first author master's dissertation. We would also like to thank the two anonymous reviewers for their valuable suggestions in earlier versions of this manuscript.

REFERENCES

Almeida TM, Moreau AMSS, Moreau MS, Pires MM, Fontes EO, Góes LM. Reorganização socioeconômica no extremo sul da Bahia decorrente da introdução da cultura do eucalipto. Soc & Nat. 2008;20:5-18. https://doi.org/10.1590/S1982-45132008000200001

Alvares CA, Stape JL, Sentelhas PC, Gonçalves JLM, Sparovek G. Köppen's climate classification map for Brazil. Meteorol Z. 2013;22:711-28. https://doi.org/10.1127/0941-2948/2013/0507

Associação Baiana das Empresas de Base Florestal - Abaf. Bahia Florestal. Salvador: Relatório ABAF; 2015. (Informativo Digital, agosto de 2015).

Barros NF, Novais RF, Teixeira JL, Fernandes Filho EI. Sistema para cálculo del balance nutricional y recomendación de fertilizantes para el cultivo de eucalipto. Bosque. 1995;16:129-31.

Berg B. Decomposition patterns for foliar litter - A theory for influencing factors. Soil Biol Biochem. 2014;78:222-32. https://doi.org/10.1016/j.soilbio.2014.08.005

Bird JA, Kleber M, Torn MS. ^{13}C and ^{15}N stabilization dynamics in soil organic matter fractions during needle and fine root decomposition. Org Geochem. 2008;39:465-77. https://doi.org/10.1016/j.orggeochem.2007.12.003

Cambardella CA, Elliott ET. Particulate soil organic-matter changes across a grassland cultivation sequence. Soil Sci Soc Am J. 1992;56:777-83. https://doi.org/10.2136/sssaj1992.03615995005600030017x

Claessen MEC, organizador. Manual de métodos de análise de solo. 2a ed. Rio de Janeiro: Centro Nacional de Pesquisa de Solos; 1997.

Conant RT, Ryan MG, Ågren GI, Birge HE, Davidson EA, Eliasson PE, Evans SE, Frey SD, Giardina CP, Hopkins FM, Hyvönen R, Kirschbaum MUF, Lavallee JM, Leifeld J, Parton WJ, Steinweg JM, Wallenstein MD, Wetterstedt JÅM, Bradford MA. Temperature and soil organic matter decomposition rates - synthesis of current knowledge and a way forward. Glob Change Biol. 2011;17:3392-404. https://doi.org/10.1111/j.1365-2486.2011.02496.x

Cotrufo MF, Wallenstein MD, Boot CM, Denef K, Paul E. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? Glob Change Biol. 2013;19:988-95. https://doi.org/10.1111/gcb.12113

Cusack DF, Silver WL, Torn MS, McDowell WH. Effects of nitrogen additions on above- and belowground carbon dynamics in two tropical forests. Biogeochemistry. 2011;104:203-25. https://doi.org/10.1007/s10533-010-9496-4

Cusack DF, Torn MS, McDowell WH, Silver WL. The response of heterotrophic activity and carbon cycling to nitrogen additions and warming in two tropical soils. Glob Change Biol. 2010;16:2555-72. https://doi.org/10.1111/j.1365-2486.2009.02131.x

Dail DB, Hollinger DY, Davidson EA, Fernandez I, Sievering HC, Scott NA, Gaige E. Distribution of nitrogen-15 tracers applied to the canopy of a mature spruce-hemlock stand, Howland, Maine, USA. Oecologia. 2009;160:589-99. https://doi.org/10.1007/s00442-009-1325-x

Dieckow J, Bayer C, Conceição PC, Zanatta JA, Martin-Neto L, Milori DBM, Salton JC, Macedo MM, Mielniczuk J, Hernani LC. Land use, tillage, texture and organic matter stock and composition in tropical and subtropical Brazilian soils. Eur J Soil Sci. 2009;60:240-9. https://doi.org/10.1111/j.1365-2389.2008.01101.x

Doetterl S, Stevens A, Six J, Merckx R, van Oost K, Pinto MC, Casanova-Katny A, Muñoz C, Boudin M, Venegas EZ, Boeckx P. Soil carbon storage controlled by interactions between geochemistry and climate. Nat Geosci. 2015;8:780-3. https://doi.org/10.1038/ngeo2516

Du Y, Guo P, Liu J, Wang C, Yang N, Jiao Z. Different types of nitrogen deposition show variable effects on the soil carbon cycle process of temperate forests. Glob Change Biol. 2014;20:3222-8. https://doi.org/10.1111/gcb.12555

Dungait JAJ, Hopkins DW, Gregory AS, Whitmore AP. Soil organic matter turnover is governed by accessibility not recalcitrance. Glob Change Biol. 2012;18:1781-96. https://doi.org/10.1111/j.1365-2486.2012.02665.x

Epron D, Mouanda C, Mareschal L, Koutika L-S. Impacts of organic residue management on the soil C dynamics in a tropical eucalypt plantation on a nutrient-poor sandy soil after three rotations. Soil Biol Biochem. 2015;85:183-9. https://doi.org/10.1016/j.soilbio.2015.03.010

Ferreira DF. Sisvar: a computer statistical analysis system. Cienc agrotec. 2011;35:1039-42. https://doi.org/10.1590/S1413-70542011000600001

Ferreira GWD, Soares EMB, Oliveira FCC, Silva IR, Dungait JAJ, Souza IF, Vergütz L. Nutrient release from decomposing *Eucalyptus* harvest residues following simulated management practices in multiple sites in Brazil. Forest Ecol Manag. 2016;370:1-11. https://doi.org/10.1016/j.foreco.2016.03.047

Gatto A, Barros NF, Novais RF, Silva IR, Leite HG, Leite FP, Villani EMA. Estoques de carbono no solo e na biomassa em plantações de eucalipto. Rev Bras Cienc do Solo. 2010;34:1069-79. https://doi.org/10.1590/S0100-06832010000400007

Gentile R, Vanlauwe B, Kavoo A, Chivenge P, Six J. Residue quality and N fertilizer do not influence aggregate stabilization of C and N in two tropical soils with contrasting texture. Nutr Cycl Agroecosys. 2010;88:121-31. https://doi.org/10.1007/s10705-008-9216-9

Gillespie AW, Diochon A, Ma BL, Morrison MJ, Kellman L, Walley FL, Regier TZ, Chevrier D, Dynes JJ, Gregorich EG. Nitrogen input quality changes the biochemical composition of soil organic matter stabilized in the fine fraction: a long-term study. Biogeochemistry. 2014;117:337-50. https://doi.org/10.1007/s10533-013-9871-z

Grandy AS, Sinsabaugh RL, Neff JC, Stursova M, Zak DR. Nitrogen deposition effects on soil organic matter chemistry are linked to variation in enzymes, ecosystems and size fractions. Biogeochemistry. 2008;91:37-49. https://doi.org/10.1007/s10533-008-9257-9

Graves AR, Morris J, Deeks LK, Rickson RJ, Kibblewhite MG, Harris JA, Farewell TS, Truckle I. The total costs of soil degradation in England and Wales. Ecol Econ. 2015;119:399-413. https://doi.org/10.1016/j.ecolecon.2015.07.026

Huang Z, Clinton PW, Baisden WT, Davis MR. Long-term nitrogen additions increased surface soil carbon concentration in a forest plantation despite elevated decomposition. Soil Biol Biochem. 2011;43:302-7. https://doi.org/10.1016/j.soilbio.2010.10.015

Janssens IA, Dieleman W, Luyssaert S, Subke J-A, Reichstein M, Ceulemans R, Ciais P, Dolman AJ, Grace J, Matteucci G, Papale D, Piao SL, Schulze E-D, Tang J, Law BE. Reduction of forest soil respiration in response to nitrogen deposition. Nat Geosci. 2010;3:315-22. https://doi.org/10.1038/ngeo844

Jones HS, Beets PN, Kimberley MO, Garrett LG. Harvest residue management and fertilisation effects on soil carbon and nitrogen in a 15-year-old *Pinus radiata* plantation forest. Forest Ecol Manag. 2011;262:339-47. https://doi.org/10.1016/j.foreco.2011.03.040

Keeler BL, Hobbie SE, Kellogg LE. Effects of long-term nitrogen addition on microbial enzyme activity in eight forested and grassland sites: implications for litter and soil organic matter decomposition. Ecosystems. 2009;12:1-15. https://doi.org/10.1007/s10021-008-9199-z

Khan SA, Mulvaney RL, Ellsworth TR, Boast CW. The myth of nitrogen fertilization for soil carbon sequestration. J Environ Qual. 2007;36:1821-32. https://doi.org/10.2134/jeq2007.0099

Leckie SE, Prescott CE, Grayston SJ, Neufeld JD, Mohn WW. Characterization of humus microbial communities in adjacent forest types that differ in nitrogen availability. Microb Ecol. 2004;48:29-40. https://doi.org/10.1007/s00248-003-1020-0

Lehmann J. A handful of carbon. Nature. 2007;447:143-4. https://doi.org/10.1038/447143a

Moran KK, Six J, Horwath WR, van Kessel C. Role of mineral-nitrogen in residue decomposition and stable soil organic matter formation. Soil Sci Soc Am J. 2005;69:1730-6. https://doi.org/10.2136/sssaj2004.0301

Muñoz C, Cruz B, Rojo F, Campos J, Casanova M, Doetterl S, Boeckx P, Zagal E. Temperature sensitivity of carbon decomposition in soil aggregates along a climatic gradient. J Soil Sci Plant Nut. 2016;16:461-76. https://doi.org/10.4067/S0718-95162016005000039

Oliveira FCC. Emissão de gases de efeito estufa e dinâmica da população microbiana e do carbono no solo em povoamento de eucalipto implantando no bioma Pampa [tese]. Viçosa, MG: Universidade Federal de Viçosa; 2015.

Pozza AAA, Curi N, Costa ETS, Guilherme LRG, Marques JJGSM, Motta PEF. Retenção e dessorção competitivas de ânions inorgânicos em gibbsita natural de solo. Pesq Agropec Bras. 2007;42:1627-33. https://doi.org/10.1590/S0100-204X2007001100015

Prescott CE. Litter decomposition: what controls it and how can we alter it to sequester more carbon in forest soils? Biogeochemistry. 2010;101:133-49. https://doi.org/10.1007/s10533-010-9439-0

Rasse DP, Rumpel C, Dignac M-F. Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. Plant Soil. 2005;269:341-56. https://doi.org/10.1007/s11104-004-0907-y

Ruiz HA. Incremento da exatidão da análise granulométrica do solo por meio da coleta da suspensão (silte + argila). Rev Bras Cienc do Solo. 2005;29:297-300. https://doi.org/10.1590/S0100-06832005000200015

Santos HG, Jacomine PKT, Anjos LHC, Oliveira VA, Oliveira JB, Coelho MR, Lumbreras JF, Cunha TJF. Sistema brasileiro de classificação de solos. 3a ed. Rio de Janeiro: Embrapa Solos; 2013.

Silva EF. Frações da matéria orgânica e decomposição de resíduos da colheita de eucalipto em solo de Tabuleiros Costeiros da Bahia [tese]. Viçosa, MG: Universidade Federal de Viçosa; 2008.

Singh N, Abiven S, Torn MS, Schmidt MWI. Fire-derived organic carbon in soil turns over on a centennial scale. Biogeosciences. 2012;9:2847-57. https://doi.org/10.5194/bg-9-2847-2012

Six J, Conant RT, Paul EA, Paustian K. Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. Plant Soil. 2002;241:155-76. https://doi.org/10.1023/A:1016125726789

Six J, Paustian K. Aggregate-associated soil organic matter as an ecosystem property and a measurement tool. Soil Biol Biochem. 2014;68:A4-9. https://doi.org/10.1016/j.soilbio.2013.06.014

Sleutel S, Kader MA, Begum SA, Neve S. Soil-organic-matter stability in sandy cropland soils is related to land-use history. J Plant Nutr Soil Sci. 2010;173:19-29. https://doi.org/10.1002/jpln.200900062

Soil Survey Staff. Keys to soil taxonomy. 12th ed. Washington, DC: United States Department of Agriculture, Natural Resources Conservation Service; 2014.

Stahl C, Freycon V, Fontaine S, Dezécache C, Ponchant L, Picon-Cochard C, Klumpp K, Soussana J-F, Blanfort V. Soil carbon stocks after conversion of Amazonian tropical forest to grazed pasture: importance of deep soil layers. Reg Environ Change. 2016;16:2059-69. https://doi.org/10.1007/s10113-016-0936-0

Stewart CE, Paustian K, Conant RT, Plante AF, Six J. Soil carbon saturation: concept, evidence and evaluation. Biogeochemistry. 2007;86:19-31. https://doi.org/10.1007/s10533-007-9140-0

Stockmann U, Adams MA, Crawford JW, Field DJ, Henakaarchchi N, Jenkins M, Minasny B, McBratney AB, Courcelles VR, Singh K, Wheeler I, Abbott L, Angers DA, Baldock J, Bird M, Brookes PC, Chenu C, Jastrow JD, Lal R, Lehmann J, O'Donnell AG, Parton WJ, Whitehead D, Zimmermann M. The knowns, known unknowns and unknowns of sequestration of soil organic carbon. Agric Ecosyst Environ. 2013;164:80-99. https://doi.org/10.1016/j.agee.2012.10.001

Versini A, Nouvellon Y, Laclau J-P, Kinana A, Mareschal L, Zeller B, Ranger J, Epron D. The manipulation of organic residues affects tree growth and heterotrophic CO_2 efflux in a tropical *Eucalyptus* plantation. Forest Ecol Manag. 2013;301:79-88. https://doi.org/10.1016/j.foreco.2012.07.045

Waring BG, Weintraub SR, Sinsabaugh RL. Ecoenzymatic stoichiometry of microbial nutrient acquisition in tropical soils. Biogeochemistry. 2014;117:101-13. https://doi.org/10.1007/s10533-013-9849-x

Xiong Y, Xia H, Li Z, Cai X, Fu S. Impacts of litter and understory removal on soil properties in a subtropical *Acacia mangium* plantation in China. Plant Soil. 2008;304:179-88. https://doi.org/10.1007/s11104-007-9536-6

Zhang G, Jiang H, Niu G, Liu X, Peng S. Simulating the dynamics of carbon and nitrogen in litter-removed pine forest. Ecol Model. 2006;195:363-76. https://doi.org/10.1016/j.ecolmodel.2005.11.041