

Grass vs. tree origin of soil organic carbon under different land-use systems in the Brazilian Cerrado

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Abstract

Background and Aims Silvopastoral management of tree plantations is becoming popular in Brazil. The impact of this practice on carbon dynamics of these soils is unknown, and predicting it is difficult because historical land-use records of the region do not exist. The objective of the study was to quantify the relative soil organic carbon (SOC) contributions of C3 and C4 plants in different land-use system.

Methods We evaluated total $\delta^{13}\text{C}$, the contribution of C4 and C3-derived soil organic C in three fraction-size classes of soils in six land-use systems (Eucalyptus hybrid plantations established in 1985 and 2005; a native forest; silvopasture stands of *Brachiaria brizantha* under eucalyptus established in 1994 and 2004; and an open pasture), and undertook carbon dating (^{14}C) for three of those systems, an Oxisol in Minas Gerais, Brazil. From each

system, soil samples were collected from four depths (0–10, 10–20, 20–50, and 50–100 cm); samples were fractionated into 250–2000, 53–250, and <53 μm size classes, and their $\delta^{13}\text{C}$ determined. Carbon dating (^{14}C analysis) was done for whole soil samples from 0 to 10 cm and 50–100 cm depth classes of three land-use systems. **Results** The $\delta^{13}\text{C}$ values increased (showing increase in C contribution by C4 plants, i.e., grasses) with soil depth at all sites and all size-classes. The older systems showed a higher contribution of C4-derived SOC at all depths. Carbon dating indicated that the area had been cleared about 300 years ago.

Conclusions Silvopastoral and pasture systems can be considered as good carbon sinks. We infer that the study site was grassland with high proportion of C4 plants in the past, not a forest as it is today.

Keywords Agroforestry · *Brachiaria brizantha* · Carbon dating · *Eucalyptus* spp. · Soil fractionation · Stable isotope ratio analysis

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Introduction

Agroforestry systems that combine trees and/or shrubs with crops and/or livestock production are planned and managed agroecosystems (Sharrow et al. 2009). Increasing the overall productivity and efficiency of the land-use system (LUS) are major goals of agroforestry (Nair 2013). Agroforestry systems have the potential to enhance carbon (C) sequestration in soil compared with treeless (agricultural) systems (Nair et al. 2010; Zomer

et al. 2016). The underlying premise for such claims is that tree components in agroforestry systems (Saha et al. 2009) and diversity of grassland species and specific plant functional traits (Steinbeiss et al. 2008) can be significant sinks of atmospheric C. Functional consequences of integration of trees into grass-dominated vegetation include changes of above- and below ground productivity (Schwendenmann and Pendall 2006), modifications to rooting depth and distribution (Joslin et al. 2006), and changes in the quantity and quality of litter inputs (Sayer et al. 2011).

Soil organic matter (SOM) is extremely vulnerable to land-use changes (IPCC 2013), as well as to intensification of agricultural practices (Alcântara et al. 2016). Thus, in order to quantify the strength and longevity of the C sink in tree-based pasture systems, it is important to understand the mechanisms and processes associated with C transformation and storage. The nature and extent of turnover of soil organic carbon (SOC) depend on soil aggregate-size fractions as well as to soil structure and extent of aggregation (Liu et al. 2014). Different components of SOC have different residence time, ranging from labile to stable forms (Yoo et al. 2011). Thus, correct evaluation of changes in SOC dynamics is necessary to understand the functionally soil organic matter. Soil size fractionation helps to differentiate these different SOC fractions. It is based on the premise that SOC associated with sand-size aggregates (or macroorganic matter >250 μm) is often more labile than SOC in the clay and silt fractions (Gunina and Kuzyakov 2014).

Stable isotopic-ratio analysis in SOC studies emerged as a tool to trace the source of SOC to C3 and C4 components in vegetation. Numerous studies (Haile et al. 2010; Gunina and Kuzyakov 2014; Vicente et al. 2016) have been successful in applying $\delta^{13}\text{C}$ to understand plant–soil carbon dynamics, making stable isotopic analysis a useful technique. When one type of vegetation is replaced with another, $\delta^{13}\text{C}$ values can be used to identify SOC derived from residues in the native vegetation and the new vegetation based on discrimination between C3 and C4 plants. The reported $\delta^{13}\text{C}$ values range from -9 to -19‰ for C4 plants and -20 to -35‰ for C3 plants (Biedenbender et al. 2004). When a C4 plant is introduced to a system that had previously been under a C3 plant (or vice versa), the relative contribution of new vs. old SOC can be quantified using the mass balance of stable isotope contents based on the change in ^{13}C signature of SOC (Dawson et al. 2002). In a combined tree and grass land-use

system, C3 inputs are dominated by either woody shrubs or tree and C4 inputs are dominated by grass (Monroe et al. 2016). In eucalyptus-based silvopastoral systems where C3 and C4 plants are grown simultaneously, such studies that use natural abundance of $\delta^{13}\text{C}$ to understand the C dynamics are rare or absent.

The plant community in most of the silvopastoral systems in the Brazilian Cerrado is composed of eucalyptus (*Eucalyptus* spp.) a C3 plant with a $\delta^{13}\text{C} \approx -27.8\text{‰}$ and a grass normally *Brachiaria brizantha* a C4 plant with $\delta^{13}\text{C} \approx -12.6\text{‰}$. The $\delta^{13}\text{C}$ value ranges of C3 and C4 plants do not overlap. Therefore, differences in isotope ratio can be used to quantify the relative contribution of plants of each photosynthetic pathway to SOC (Balesdent et al. 1988). As a naturally occurring radioisotope, ^{14}C has been used as a useful tool to study carbon dynamics and cycling in different LUS in tropical soils (Pessenda et al. 1998b), and provide information about soil chronology in paleo-environmental studies (Biedenbender et al. 2004).

With this background, the main objective of the present study was to assess the impact of different land-use systems on the SOC content and SOC fraction size, and quantify the relative SOC contribution of C3 and C4 in each LUS. Furthermore, given the lack of any record on the vegetation history of the area, carbon dating was undertaken to elicit information on the past vegetation-composition of the site.

Materials and methods

Study area and site description

The study was conducted on a large farm (over 80,000 ha) in Paracatu, Minas Gerais, Brazil, located in the Cerrado biome (Latitude $17^{\circ}36'09''\text{S}$ and Longitude $46^{\circ}42'02''\text{W}$). Climatic characteristics of the region include an average annual rainfall of 1350 mm (mainly during summer: Nov to Mar), annual average temperature of $22\text{ }^{\circ}\text{C}$, and air humidity around 72.5%. The Cerrado includes different physiognomic features, ranging from completely open Cerrado (open field dominated by grass) to the closed and dense Cerrado (high Cerrado), which is a closed canopy forest. Intermediate Cerrado, an “open arboreal savanna,” is the dominant type (also called Cerrado *sensu stricto*). The site characteristics, land-use systems included in the study, and soil sampling procedures have been described by Tonucci et al. (2011). Brief overviews of those are given

below, followed by details of the additional studies reported in this paper.

Soil samples were taken from six different land-use sites located on Oxisols:

1. A native forest that belongs to the intermediate Cerrado; dominant tree species include *Dalbergia* spp., *Machaerium scleroxylon* and *Vateria* spp.
2. An “Old Eucalyptus Forest” (OEF): A eucalyptus forest planted in 1985 by slash-and-burn procedure after burning the native forest and removing the debris from the site; tree density: 1090 plants ha⁻¹.
3. A “New Eucalyptus Forest” (NEF) a eucalyptus plantation established in 2005, after felling the large trees with a chain saw and removing economically important wood, without using the slash-and-burn procedure; tree density: 1650 plants ha⁻¹; the soil was plowed 20 cm-deep.
4. An open pasture field without any trees, established in 1965 using slash-and-burn after harvesting economically important wood trees, with the grass species *Brachiaria decumbens* and *B. brizantha* cv. Marandú. The animal stocking rate ranged from 0.5 animal ha⁻¹ in the dry season to 2.5 ha⁻¹ in the wet season.
5. An “Old Agroforestry” site (OAF), established in 1994.
6. A “New Agroforestry” site (NAF), established in 2004.

Both OAF and NAF were established the same way. It consisted of removal of natural forest including chain-saw removal of large trees and burning of remaining vegetation, and planting *Eucalyptus* hybrids in East–West rows 10 m apart with 4 m between plants in a row at a tree density of 250 plants ha⁻¹. In the first year, a crop of rice (*Oryza sativa*) was raised by sowing seeds between eucalyptus rows in the beginning of the rainy season (Oct–Nov). After harvesting the rice crop, soybean (*Glycine max*) was planted in the second year without tilling in the interspaces between trees, and was harvested mechanically when mature. Grass species (*Brachiaria brizantha* cv. Marandú) was then sown at the end of rainy season in year 3 in between the eucalyptus rows.

Soil Sampling

Four soil samples each were collected from each of the land-use systems, in 2008. In all systems with planted

trees (OAF, NAF, OEF and NEF), samples were collected at two positions each: near the tree (0.5 m from the tree trunk) and away from the tree (5 m and 1.5 m from the trunk in the silvopasture and eucalyptus stands, respectively), giving a total of 32 samples in these four LUS (4 LUS × 2 positions × 4 replications). The eucalyptus and pasture systems had no sampling at definite horizontal distance from trees (sampling by position), because the pastures had no trees and in the forest stand, the trees were not in any regular spacing pattern; thus, these two systems had four (replicate) samples each. In each sampling plot, soil was collected from four depths (0–10; 10–20; 20–50 and 50–100 cm). Thus, there was a total of (32 + 8 = 40) × 4 = 160 samples. Each of the 160 samples was a composite of four subsamples; the subsamples were collected from four random spots representing the specific location and depth, mixed well, and a composite sample drawn.

Soil bulk density for each depth interval had been measured by the core method as reported by (Tonucci et al. 2011). All samples were air-dried and sieved (2 mm) at the Federal University of Viçosa, Viçosa, MG, Brazil soils laboratory, bagged and sent to the University of Florida, Gainesville, FL, USA, for further analyses.

Soil Fractionation

The soil samples were manually fractionated into three aggregate size classes (250–2000 μm, 53–250 μm, <53 μm) at the Soil and Water Sciences Department laboratory, University of Florida, according to a procedure from Elliott and Coleman (1988) adapted by Haile et al. (2008, 2010) as described by Tonucci et al. (2011). The overall procedure yielded a water-stable, macro-sized fraction 250–2000 μm; a micro-sized fraction 53–250 μm; and silt + clay size-fraction <53 μm. The overall average recovery mass percentage of soil fractions after the sieving procedure ranged from 96% to 99% of the initial soil mass.

Soil Analysis

For chemical analysis, whole and oven-dried fractionated soils were ground to fine powder using a QM-3A High Speed Vibrating Ball Mill (MTI Corp., Richmond, CA) for 10 min. Total SOC was determined for whole soil and for fractionated soil samples by dry combustion using an automated C and N analyzer (Thermo

Finnegan Flash EA 1112 NC; Thermo Fisher Scientific Inc., Waltham, MA).

For stable C isotope determinations, oven-dried whole- and fractionated soils were analyzed for C and for $\delta^{13}\text{C}$ values using a Carlo Erba EA-1108 (CE Elantech, Lakewood, NJ) interfaced with a Delta Plus (Thermo Finnigan, San Jose, CA) isotope ratio mass spectrometer operating in continuous flow mode. Carbon isotope ratios are presented in δ -notation:

$$\delta^{13}\text{C} = \left(\frac{[R_{\text{sample}} - R_{\text{STD}}]}{R_{\text{STD}}} \right) \times 10^3 \quad (1)$$

where, R_{sample} is the $^{13}\text{C}/^{12}\text{C}$ ratio of the sample, and R_{STD} is the $^{13}\text{C}/^{12}\text{C}$ ratio of the Vienna Pee Dee Belemnite (VPDB) standard (Coplen 1996). Precision of duplicate was 0.1‰ and none of the samples contained CaCO_3 or any other form of inorganic C. The percentage of SOC derived from the *Brachiaria* spp., a C4 plant, or from the eucalyptus or native forest, C3 plants, was estimated based on the equations (Balesdent and Mariotti 1996):

$$\% \text{C4-derived SOC} = (\delta - \delta_{\text{T}}) / (\delta_{\text{G}} - \delta_{\text{T}}) \times 100, \quad (2)$$

$$\% \text{C3-derived SOC} = 100 - \% \text{C4-derived SOC}, \quad (3)$$

where δ is the $\delta^{13}\text{C}$ of a given samples, δ_{T} a composite sample of the C3 plant (eucalyptus) and δ_{G} is a composite sample of pasture grass, C4 plant.

Based on eqs. 2 and 3, C content in each soil sample was calculated, and the contributions of C3 and C4 species to SOC C were derived, as follows:

$$\begin{aligned} \text{C3-derived SOC (Mg ha}^{-1}\text{)} \\ = (\% \text{C3-derived SOC}) \times (\text{SOC content, Mg ha}^{-1}), \end{aligned} \quad (4)$$

$$\begin{aligned} \text{C4-derived SOC (Mg ha}^{-1}\text{)} \\ = (\% \text{C4-derived SOC}) \times (\text{SOC content, Mg ha}^{-1}). \end{aligned} \quad (5)$$

^{14}C Analysis

Three out of the six LUS treatments were chosen for ^{14}C analysis based on their relevance to research goals, and whole soil samples from the uppermost and lowermost

depth classes (0–10 cm and 50–100 cm) were chosen. For the size fractions, the OAF treatment was chosen for aging inquiry. Radiocarbon concentrations were reported as fractions of the Modern standard, $\Delta^{14}\text{C}$, and conventional radiocarbon age, following the conventions of Stuiver and Polach (1977), and the values were adjusted against measurements of ^{14}C -free coal. All results were corrected for isotopic fractionation according to the conventions of Stuiver and Polach (1977), with $\delta^{13}\text{C}$ values measured on prepared graphite using the AMS spectrometer.

Statistical Analysis

A completely randomized design with land-use as a factor was used. Planned-comparison ANOVA with Tukey's studentized range test was used to compare the mean differences between land management practices on $\delta^{13}\text{C}$, C3-derived SOC, and C4-derived SOC in whole soil, macro-sized, micro-sized, and silt + clay-sized fractions at all six sites. Statistical analysis were performed separately with SAS, and differences were considered significant at $p < 0.05$. The ^{14}C data were not analyzed statistically because, unlike the other C analyses, the ^{14}C analysis was not done for all replicated samples owing to cost considerations; moreover, the relatively short timeframe (of a few years) of LUS treatments was not expected to have any impact on the C-dating data referring to the situation of hundreds of years ago.

Results

Natural abundance of ^{13}C soil organic carbon in whole soil

At all soil depths, pasture showed the highest $\delta^{13}\text{C}$ values, with an average of -14.45% , whereas the NEC (new eucalyptus plantation) had the lowest $\delta^{13}\text{C}$ values, with an average of -25.52% . Both agroforestry systems showed intermediary values ranging from -17.21% , at the 50–100 cm depth of OAF, to -22.65% , at the surface (0–0 cm) of the NAF (Table 1). Native forest $\delta^{13}\text{C}$ values increased with the depth from -26.82% (0–10 cm) to -18.24% (50–100 cm) depth. Overall, there was a trend of increase in $\delta^{13}\text{C}$ values with soil depth.

Table 1 $\delta^{13}\text{C}$ (‰) values of soil organic carbon (SOC) in whole soil in different depths of six different land-use systems in the Brazilian Cerrado, Minas Gerais, Brazil

Depth (cm)	$\delta^{13}\text{C}$ values of SOC (‰)					
	Site					
	Pasture	OEC	NEC	OAF	NAF	Forest
0–10	–14.16 a	–24.07 c	–26.53 d	–21.87 b	–22.65 bc	–26.82 d
10–20	–14.32 a	–20.05 b	–26.13 d	–20.10 b	–22.45 c	–25.06 d
20–50	–14.53 a	–16.50 b	–25.45 e	–18.30 c	–21.96 d	–22.20 d
50–100	–14.82 a	–15.85 ab	–23.98 d	–17.21 abc	–19.64 c	–18.24 bc

Lowercase letters in the same row indicate significant differences in SOC at a given depth and site. OEC, old eucalyptus; NEC, new eucalyptus; OAF, old agroforestry; NAF, new agroforestry

The C4-derived SOC showed higher values in pasture than any other LUS at all soil depth classes. At any given depth, the new eucalyptus had the lowest SOC value among C4 plants, while the two agroforestry systems had intermediary mean values of 2.70 and 2.15 Mg C ha⁻¹ for the old and new agroforestry systems, respectively (Table 2). The C4-derived SOC was highest in the native forest in the 50–100 cm depth (2.56 Mg ha⁻¹), but it was comparatively low at all other depth classes of the native forest system. A reverse trend was observed in the C3-derived SOC, where NEC showed higher values and pasture showed lower, with 4.28 Mg ha⁻¹ in the NEC 10–20 cm, and 0.35 Mg ha⁻¹ for pasture 0–10 cm depth (Table 2); however, the maximum C3-derived SOC (4.82 Mg ha⁻¹) was in the 0–10 cm depth for the native forest.

Natural abundance of ¹³C soil organic carbon in soil fraction sizes

Soil organic carbon $\delta^{13}\text{C}$ followed the same trend in all fractions with values being higher for pasture and lower for NEC. In macro-sized fraction (250–2000 μm), pasture and NEC showed an average of –14.95‰ and –25.52‰, respectively. Within the agroforestry systems, OAF always showed a higher $\delta^{13}\text{C}$ values than NAF, at any given depth (Table 3). Native forest $\delta^{13}\text{C}$ values were considerably low, ranging from –26.81‰, in the 0–10 cm depth to –21.40‰, in the 50–100 cm depth.

The same trend was observed in the micro-sized fraction (250–53 μm) where pasture had the highest $\delta^{13}\text{C}$ value (–14.90‰) and NEC the lowest (–26.50‰). The OAF and NAF showed similar $\delta^{13}\text{C}$ values for this

Table 2 Soil organic carbon derived from C3 or C4 plants (SOC-derived) in the whole soil at different depths of six different land-use systems in the Brazilian Cerrado, Minas Gerais, Brazil

LUS	Depth (cm)							
	0–10		10–20		20–50		50–100	
	SOC derived carbon (Mg ha ⁻¹)							
	C3	C4	C3	C4	C3	C4	C3	C4
Pasture	0.35 ^d	5.50 ^a	0.38 ^d	4.99 ^a	0.41 ^d	4.61 ^a	0.40 ^c	3.65 ^a
OEC	3.61 ^b	1.83 ^c	1.72 ^c	2.39 ^b	0.82 ^d	3.17 ^b	0.58 ^c	2.98 ^{ab}
NEC	3.78 ^b	0.86 ^d	4.28 ^a	1.14 ^c	3.08 ^a	1.04 ^d	2.50 ^a	1.28 ^c
OAF	2.81 ^c	2.48 ^b	1.89 ^c	2.59 ^b	1.34 ^c	2.96 ^b	0.88 ^{bc}	2.73 ^b
NAF	2.71 ^c	1.98 ^{bc}	2.90 ^b	2.21 ^b	2.46 ^b	2.13 ^c	1.51 ^b	2.29 ^b
Forest	4.82 ^a	0.97 ^d	3.75 ^a	1.43 ^c	2.20 ^b	1.78 ^c	1.31 ^{bc}	2.56 ^b

Lowercase letters in the same column indicate significant differences in SOC-derived carbon at any given depth and site. OEC, old eucalyptus; NEC, new eucalyptus; OAF, old agroforestry; NAF, new agroforestry

Table 3 Differences in $\delta^{13}\text{C}$ (‰) of soil organic carbon (SOC) in macro-sized (250–2000 μm), micro-sized (<53 μm) fractions in six different land-use systems at four different depths in the Brazilian Cerrado, Minas Gerais, Brazil

Site	0–10		10–20		20–50		50–100	
	250–2000	<53	250–2000	<53	250–2000	<53	250–2000	<53
Pasture	-14.47 ^a	-14.90 ^a	-14.51 ^a	-15.04 ^a	-15.35 ^a	-15.20 ^a	-15.47 ^a	-16.20 ^a
OEC	-24.14 ^b	-24.30 ^c	-19.82 ^b	-20.53 ^b	-16.83 ^{ab}	-16.39 ^a	-16.27 ^a	-15.46 ^a
NEC	-26.66 ^c	-26.50 ^d	-26.03 ^c	-25.74 ^c	-25.50 ^d	-25.18 ^d	-23.60 ^c	-23.13 ^c
OAF	-22.25 ^b	-21.45 ^b	-20.30 ^b	-20.58 ^b	-18.24 ^b	-18.32 ^b	-17.60 ^a	-16.44 ^a
NAF	-22.47 ^b	-22.72 ^{bc}	-22.25 ^b	-22.25 ^b	-22.38 ^c	-21.91 ^c	-20.08 ^b	-19.14 ^b
Forest	-26.81 ^c	-26.76 ^d	-26.33 ^c	-25.91 ^d	-23.70 ^{cd}	-23.02 ^c	-21.40 ^{bc}	-21.30 ^b

Lowercase letters in the same column indicate significant differences in SOC at a given depth and site

OEC, old eucalyptus; NEC, new eucalyptus; OAF, old agroforestry; NAF, new agroforestry

fraction at most soil depths, except for the 20–50 cm and the 50–100 cm depths where the OAF had higher values. In the silt + clay fraction (<53 μm), pasture had the highest and NEC the lowest $\delta^{13}\text{C}$ values, at any given depth. At the 0–10 and 10–20 cm depths, pasture showed the highest (-14.95‰) $\delta^{13}\text{C}$ value; OEC, OAF and NAF showed intermediary and, NEC and native forest the lowest values (-26.33‰). This trend changed in the bottom layers (mid-lower and lower) where pasture and OEC had the highest values; OAF, NAF and native forest had intermediary and, NEC the lowest (-23.72‰).

All sites showed an increase in $\delta^{13}\text{C}$ values at any given depth with increase in depth. Although comparisons among depth classes in the same site were not analyzed in this study, results appeared to indicate that $\delta^{13}\text{C}$ values in OEC, compared with pasture, increased sharply with increasing depth (Table 3).

SOC derived from C3 vs. C4 plants

The amount of C3 and C4-derived SOC in the soil fractions were significantly different among sites and depths. For example, in the NEC site 85%, 87%, 75% and, 62% of SOC were C3-derived in the 0–10 cm, 10–20 cm, 20–50 cm and the 50–100 cm depth, respectively, whereas the corresponding C3 values for the pasture site were 6% 7%, 8% and, 11%. In the macro-sized fraction (250–2000 μm) under pasture showed more C4-derived SOC than any other site, at any given depth. Agroforestry systems showed intermediary values of C4-derived SOC with values ranging from 1.75 Mg ha⁻¹ to 0.97 Mg ha⁻¹, in the OAF at 20–50 cm depth and NAF at the 50–100 cm depth, respectively (Fig. 1). Native forest showed intermediary to lower C4-derived SOC values, and these values tended to be steady with soil depth. New eucalyptus had the lowest C4-derived SOC at any given depth. Except for pasture in this fraction, the C4-derived values for other sites were somewhat uniform at different depths (Table 2).

Micro-sized fraction (250–53 μm) followed the same trend as the macro-sized fraction. Pasture had higher C4-derived SOC at all depths with values ranging from 1.17 to 1.07 Mg ha⁻¹ (Fig. 1). A reverse trend was observed in micro-sized fraction regarding site behavior with depth with all sites, except pasture, showing increases in C4-derived SOC with increases in soil depth. Pasture also showed the highest values at any given depth for the silt + clay fraction (<53 μm). In the 0–10 cm depth agroforestry systems showed intermediary

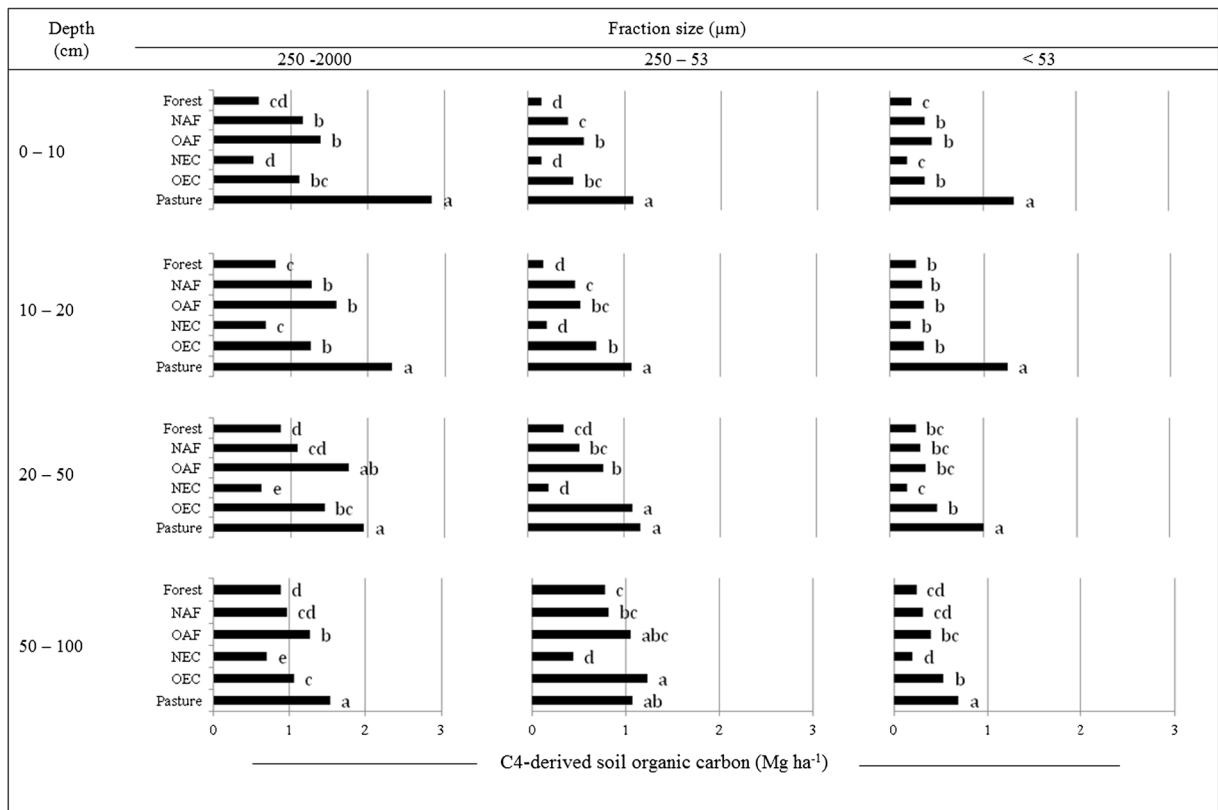


Fig. 1 C4-derived soil organic carbon (Mg ha⁻¹) in macro-sized (250–2000 μm), micro-sized (250–53 μm), and silt + clay (<53 μm) fractions in six different land-use systems at four different depths in the Brazilian Cerrado, MG. Lowercase letters

indicate significant differences in SOC at a given depth, fraction, and site. OEC, old eucalyptus; NEC, new eucalyptus; OAF, old agroforestry; NAF, new agroforestry

values and, forest lower (0.23 Mg ha⁻¹). In the 20–50 depth OAF, NAF and native forest did not show significant differences among each other and NEC had the lowest values (0.18 Mg ha⁻¹). In this fraction size the percentage of C4-derived SOC was 89%, 89% 88% and, 78% in pasture at 0–10 cm, 10–20 cm, 20–50 cm and, 50–100 cm depths, respectively. Corresponding values for the native forest were 20%, 27%, 40% and, 51%, respectively. The site that showed the lowest values for C4-derived SOC was NEC with values of 22% of C4 SOC origin in the 0–10 cm depth. All sites tended to show an increase in the percentage of C4-derived SOC with increase in soil depth, in all sized fractions.

As expected, C3-derived SOC showed a reverse trend compared to the C4-derived. New eucalyptus showed the highest C3 values for all fractions and depths. Both agroforestry systems showed intermediary to low C3-derived SOC, with higher values in the 0–10 cm and 10–20 cm depths. Forest soils showed intermediary values of C3-derived SOC.

Age of Carbon (Δ¹⁴C)

The ¹⁴C age profiles for SOC in the whole soil showed that C at lower depths was “older” (Table 4). In general, C in soils under pasture was the oldest compared with that under other LUS; the oldest age being 2390 years in the lower depths. Carbon aging in the fraction indicated that silt + clay fraction (finest) had older C and the macro-sized fractions had more recent carbon. Comparison between the finer fraction (<53 μm) and the coarsest fraction (250–2000) showed an age difference of over 1000 years.

Discussion

SOM derived from C3 and C4 plants in whole soil

The δ¹³C values are used to identify SOM derived from residues in the native vegetation and the new vegetation

Table 4 Radiocarbon dating of total soil at different depth of three LUS

Site	Fraction (μm)	Depth (cm)	$\Delta^{14}\text{C}$ (‰)	^{14}C age (BP) ^a
Pasture	Whole soil	0–10	-45.5 ± 1.2	315
		50–100	-262.5 ± 1.0	2390
Forest	Whole soil	0–10	73.0 ± 1.4	Modern ^b
		50–100	-251.3 ± 0.9	2270
OAF ^c	Whole soil	0–10	0.5 ± 1.2	Modern
		50–100	-234.3 ± 0.9	2085
OAF	250–2000	50–100	-270.8 ± 1.0	2480
	250–53	50–100	-257.2 ± 0.9	2330
	<53	50–100	-348.3 ± 0.8	3385

^a Conventional radiocarbon ages (years BP); ^b Chronological samples is reported as MODERN when the reservoir corrected, conventional radiocarbon age is less than 200 yrs.; ^c OAF, old agroforestry

based on discrimination between C3 and C4 plants. The higher $\delta^{13}\text{C}$ values under pastures in this study (Table 1) were expected since C4 photosynthesis discriminates less against $^{13}\text{CO}_2$ and, therefore, have larger $\delta^{13}\text{C}$ values than C3 plants. These differences are probably due to differences in stomatal diffusion and carboxylation of phosphoenol pyruvate, the two photosynthesis steps that are significant for isotope fractionation (O’Leary 1988). On the same basis, the establishment of pasture as an understory in tree stands could have contributed to the increasing share of C4-derived SOC in the two agroforestry systems (OAF and NAF), which explains their intermediary $\delta^{13}\text{C}$ values. Trouve et al. (1994) studying SOC dynamics under eucalyptus and pines planted on savannas in Congo observed that in the upper 50 cm depth of the savanna soil, the $\delta^{13}\text{C}$ were close to those of C4 plants resulting from the mixing of old organic matter of tree origin with more recent organic matter derived from the present savanna plants. On the other hand, Alcântara et al. (2004) observed that converting the savanna to a planted forest caused a decrease in signature of $\delta^{13}\text{C}$ values by 3.40‰ on average.

The increase in $\delta^{13}\text{C}$ values with depth that was noted in this study has been reported by many authors (Guareschi et al. 2014; Miranda et al. 2016) and it seems to be characteristic of all biologically active soils. Such increases, as large as 3‰ to 4‰ within the upper 1 m in tropical forest soils, may be a result from a preferential decomposition and removal of ^{13}C -impoverished

components or molecules. In some cases, this could be due to the selective mitigation and re-deposition of clay-humic material with ^{13}C content higher than that of the whole SOM. Jobbágy and Jackson (2000) working with a database of soils varying in texture, land-use, environmental and climate conditions and their relations with vertical distribution of SOC highlighted that the relative distribution of SOC with depth was lightly correlated with climate and more with vegetation and soil types.

The $\delta^{13}\text{C}$ values reported in literature range from -9 to -19 ‰ for C4 plants and -20 to -35 ‰ for C3 plants (Biedenbender et al. 2004). In this study, the values ranged from -26.53 ‰ (NEC, 0–10 cm) to -14.16 ‰ (pasture, 0–10 cm) (Table 1). This relatively wide range of $\delta^{13}\text{C}$ values observed suggests a predominance of C4 vegetation that likely represents the middle Holocene, and showed a process of changing to a vegetation community consisting predominantly of mixed C4 plants to a single plant of C4 origin (Table 4). Similar $\delta^{13}\text{C}$ profiles have been reported by Schwartz et al. (1986), in Africa, and Desjardins et al. (1996) and Pessenda et al. (1996, 1998b), in Brazil.

Soil particle sized fractions

The $\delta^{13}\text{C}$ values of organic carbon associated with particle sized fractions differed by 12‰ in the 0–10 cm depth and by 8.2‰ in the 50–100 cm depth (Table 3). The greater difference at the surface is probably due to higher pasture and eucalyptus fine roots contribution (Joslin et al. 2006), at the lower depth the decrease may be associated with stabilization of the organic matter turnover (Castellano et al. 2015), or a greater contribution of C4-derived SOC in the vegetation that originally existed. Desjardins et al. (2004) studying the effects of forest conversion to pasture on soil carbon content and dynamics in the Brazilian Amazon found that the establishment and increasing age of pasture resulted in a clear increase in $\delta^{13}\text{C}$ in the whole soil and particle-sized fractions. This $\delta^{13}\text{C}$ increase with depth was correlated with increasing decay of soil organic carbon (Balesdent and Mariotti 1996).

Isotopic methods confirm that all particle-size fractions were affected by inputs of C4-derived carbon (Fig. 1). Usually, faster substitution of C3-derived carbon by C4-derived carbon was observed in the coarse SOM fractions than in the fine ones (Feller and Beare 1997), the difference of SOM turnover among fractions being more pronounced in temperate soils than in tropical soils

(Desjardins et al. 1994). Desjardins et al. (2004) working on soils with two different textures in the Brazilian Amazon found that in a 15-year old pasture, 38 and 42% of the carbon in the clay fraction was derived from pasture, confirming that pasture-derived C is quickly incorporated in the finer fraction, irrespective of the soil texture. Balesdent and Mariotti (1996) reported an increase in $\delta^{13}\text{C}$ in soil size fractions in maize fields cultivated for over 13 years and the residence time of C in each fraction. Turnover times were 4 ± 1.5 years for the 200–2000 μm fraction, and 13 ± 2 years for the 50–200 μm fraction.

Old established systems showed a higher contribution of C4 SOC than new systems, in all depths. This trend might be due to a faster turnover of C4-derived SOC when compared to C3-derived SOC, C4-derived C being more rapidly incorporated into soil than C3-derived C. It could also be due to management practices (such as choice of graminaceous species, weed control, fertilizer use, and control of stocking rate), which might have an influence on the pasture (C4) derived input. Pasture management is reported to have an important role in C accumulation or loss (Fearnside and Barbosa 1998). A high level of replacement of C3 C by C4 C following conversion of native forest into pasture has been reported (Stahl et al. 2016), especially during the early years of such conversions (Moraes et al. 1996). Roscoe et al. (2001) also reported that the replacement of C3 with C4 followed a gradual trend with depth; their data also showed a strong decrease in forest-derived C below 5 cm with grass-derived SOC being still dominant. That study, however, was limited to the upper 20 cm of the soil, just as in the case of most such studies.

An interesting finding of this study is concerning the contribution of C4-derived C to total SOC. While there

was no significant difference in percentage of C4-derived C to total SOC at various depth classes under pasture, the percentages increased with soil depth under all other LUSs (Tables 2 and 5), even in tree-dominated systems such as Forest and NEC. In clayey soils near Manaus, Brazil, Choné et al. (1991) found higher inputs of C4-derived C in carbon stock of the 0–3 cm upper layer after 2 years, and up to 68% after 8 years. In coarse-textured soil in Amazonia, Stahl et al. (2016) found that 20 years after deforestation 54% of the total carbon stock of the 0–20 cm upper layer was derived from pasture. In the present study, however, C4-derived C accounted for more than 90% of total SOC in all depth classes in the pasture field. While that is understandable in the top layer of soil, it is intriguing that the trend persisted even at 50–100 cm layer. In a previous paper from the same study site, Tonucci et al. (2011) attributed the high stock of soil C at lower depths in the pasture plot to extensive root system of the grasses, even in the lower layers (Fisher et al. 1994; Hipondoka et al. 2003; February and Higgins 2010). But that argument needs to be re-evaluated in the light of the current results that even in the forest stand and other tree-dominated systems such as OEC and OAF, C4-derived C accounted for more than 75% SOC in the 50–100 soil layer (Table 5). Thus, while several observations on the pattern of $\delta^{13}\text{C}$ values in the study are along expected lines reported in literature (Haile et al. 2010; Monroe et al. 2016; Vicente et al. 2016), the increase in C4-derived C noted in lower soil depths, even in treatments dominated by C3 plants such as forests, is a major deviation from previous results. This raises questions about some unknown factors that might have contributed to such a situation. The results of the C dating study throws some light on this issue.

Table 5 Ratios of stock of C3-derived to C4-derived C, and the percentages of C4-derived C in SOC (whole soil) at different soil depths of six different land-use systems in the Brazilian Cerrado, Minas Gerais, Brazil

LUS	C Ratio (C3-derived:C4-derived) at various soil depths (cm)				C% (C4-derived C as % total SOC) at various soil depths (cm)			
	0–10	10–20	20–50	50–100	0–10	10–20	20–50	50–100
Pasture	0.063	0.076	0.089	0.109	94.02	92.92	91.83	90.12
OEC	1.973	0.720	0.259	0.195	33.57	58.75	63.53	83.71
NEC	4.395	3.754	2.961	1.953	18.53	21.03	25.24	33.86
OAF	1.133	0.730	0.453	0.322	23.59	57.68	68.83	75.62
NAF	1.369	1.312	1.155	0.660	42.22	43.25	46.40	60.26
Forest	4.970	2.451	1.236	0.512	16.75	27.61	44.72	66.15

Based on data in Table 2

Age of Carbon

The age of Oxisols is reported to increase consistently with depth (Pessenda et al. 1998a; Pessenda et al. 2001). Different studies in Brazil have reported a range of variations between 2440 and 3700 years at 75 cm in Cerrado native vegetation (Pessenda et al. 2001) to 4550–5500 years for the average at 110 cm in São Paulo soils (Gouveia and Pessenda 2000). Since SOC is a mixture of a recent and old carbon pool (Pessenda et al. 1998a), the average carbon age in the sites of 2248 years should correspond to the minimum age of carbon present in these profiles. Other studies have indicated that finer fractions are older than coarse fractions (Gonzalez and Laird 2003; Doick et al. 2005).

Based on the aging of surface soil results (0–10 cm: Table 4), it appears that the forested land of this study site was cleared about 300 years ago. It is likely that a natural calamity such as a massive fire might have destroyed the grass-dominated ecosystem, which then was inhabited by a tree-dominated system, which is the typical vegetation of the Cerrado at present in the study region. If this speculation is proven correct, it is conceivable that the Cerrado contains large quantities of stored carbon, and opening it up for cultivation as is being done now might lead to large-scale release of stored carbon into the atmosphere. One way of addressing this potential problem (other than, of course, avoiding indiscriminate agricultural expansion into the vast region), as highlighted by Silva et al. (2016), would be to adopt environmentally sustainable land-use practices such as agroforestry and other tree-farming operations that could help store stable C in fine (silt + clay) soil fractions. The conjecture on the land-use history of the region, that the Cerrado biome was not forested, but was grassland with high proportion of C4 plants, is also worth investigating further in more detail.

Conclusions

The $\delta^{13}\text{C}$ values in the whole soil as well as the soil size-fractions were different for the land-use systems in the Cerrado biome that we studied, indicating the differences in soil carbon sequestration potential of the systems. Silvopastoral agroforestry and pasture systems can be considered as good carbon sinks given that a major part of SOC are found in the more stable soil fractions (silt + clay) in those systems. We also infer,

based on the data of carbon dating that the study site of the Cerrado biome was a grassland with high proportion of C4 plants in the past, not a forest as it is today, and opening up these soils for agricultural purposes could have environmental consequences resulting from oxidation and release of stored carbon.

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