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Agrosilvopastoral Systems and Well-Managed Pastures Increase Soil Carbon Stocks in the Brazilian Cerrado☆

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ABSTRACT

Agrosilvopastoral systems have been promoted as sustainable models that combine crops, livestock grazing, and forestry in the same area. We hypothesize that agrosilvopastoral systems can improve soil C and N stocks over time. Therefore, in this study, we aimed to evaluate the changes in soil C and N stocks after conversion of low-productivity pasture into well-managed pasture and agrosilvopastoral land in the Brazilian Cerrado. Soil samples were collected in 2016 and 2018 at 0–5, 5–10, 10–20, and 20–30 cm depths from the following areas: integrated crop-livestock-forest (ICLF), marandu grass *(Brachiaria brizantha)* monoculture (MAR), low productivity pasture of signal grass *(Brachiaria decumbens)* monoculture (PAST), and native vegetation (NV; "Cerrado"). The C and N content and stocks, ^{13}C natural abundance, and C contents in the physical and chemical fractions of soil organic matter (SOM) were measured. The ICLF and MAR systems promoted faster recovery of soil C and N stocks at all soil depths compared with PAST. The C content of the free light fraction of SOM under the ICLF and MAR systems increased, reaching values similar to NV up to 20 cm. The C content was higher in the humin fraction at all depths in all areas, and for this measurement, the ICLF system performed remarkably compared with PAST at a depth of 5–10 cm. Our findings support the hypothesis that conversion of low-productivity pasture into agrosilvopastoral and actively managed pasture systems leads to improvements in soil quality and C and N stocks in the Brazilian Cerrado.

Introduction

Inadequate soil use and a lack of conservation practices cause an imbalance in soil function, jeopardizing conservation efforts and food production required to meet global needs. The current agricultural production models that are characterized by a low diversity of plant species and intense tilling have caused physical, chemical, and biological degradation of soils. Conservation approaches such as those focused on agrosilvopastoral systems, also known as integrated crop-livestock-forest (ICLF), have been introduced as alternatives to current production models. These aim to increase biodiversity and the sustainability of crops, livestock, and forestry (Cordeiro et al. 2015). The integration of these three components is carried out in rotation, consortium, or succession in the same area (Balbino et al. 2011).

Soil organic matter (SOM) contents are directly influenced by type of soil use. SOM is a key component of soil quality and is responsible for a number of ecosystem services, such as improvement of physical, chemical, and biological qualities of soil, and mitigation of greenhouse gas (GHG) emissions (Lal 2015). The quantification of soil organic carbon (SOC) and N content and stocks,

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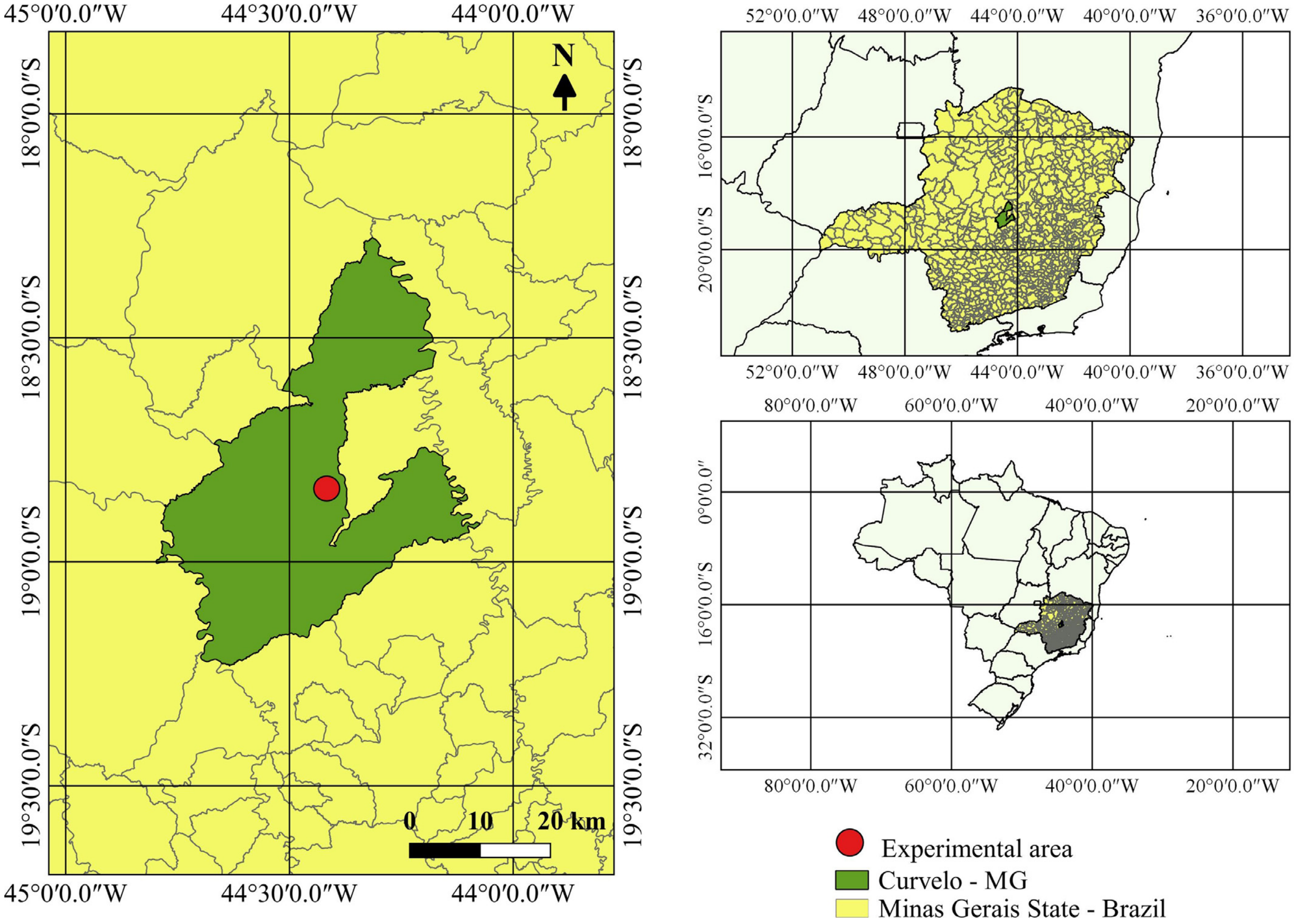

Fig. 1. Location map of the study area in the Moura Farm at municipality of Curvelo, Minas Gerais State, Brazil.

which are important constituents of SOM, allow us to understand the impacts of adopting different systems of land use and management (Rangel and Silva 2007; Baldotto et al. 2015).

Physical fractionation of SOM also has an important role in identifying the level of soil conservation. This process allows the evaluation of SOM dynamics, as each fraction has a specific function and a distinct response to changes in the soil (Carmo et al. 2012; Conceição et al. 2014; Kunde et al. 2016). Another parameter used to evaluate soil quality is based on the fractionation of humic substances, which are sensitive to variation in land use. This can be an alternative method to diagnose the anthropogenic impact on agrosystems (Rosa et al. 2003).

Several studies have found that integrated production systems contribute to the improvement of the soil chemical, physical, and biological quality when compared with monoculture and low-productivity grass systems (Freitas et al. 2012; Assis et al. 2015; Baldotto et al. 2015; Silva et al. 2016). However, given the dynamics and complexity of soil management required when integrating different production components, it is necessary to perform regionalized research that is specifically focused on different developmental stages in these systems (Balbino et al. 2011).

A higher input of litter into the topsoil and the increased root volume in integrated systems, along with the adoption of other conservation practices, can contribute to an increase in soil quality. Several studies have shown an increase in SOC caused by the ICLF system management in the Cerrado biome of Brazil (Tonucci et al. 2011; Wendling et al. 2011; Baldotto et al. 2015).

Given this information, we hypothesized that the introduction of integrated systems and well-managed pasture can improve soil quality and increase soil C and N stocks over time in the Cerrado biome. Therefore, the aim of this study was to compare C and N contents and stocks and ^{13}C natural abundance from a typical dystrophic Red Latosol (Rhodic Ferralsol) under pasture, an agrosilvopastoral system, and native vegetation in Minas Gerais State, Brazil. Through our investigation, we hoped to determine the respective contribution of C derived from C3 and C4 species, as well as to evaluate C content in different physical and chemical fractions of SOM, under different land use strategies.

Materials and methods

Study location and soil characterization

The experimental field site was located at Moura Experimental Farm (18°44′52.03″S and 44°26′53.56″W) in a sector of the Federal University of Vales do Jequitinhonha e Mucuri, in the municipality of Curvelo, Minas Gerais State, Brazil (Fig. 1). The study was conducted between December 2014 and July 2018. Elevation of the area was approximately 644 m, with flat topography and “Cerrado” biome type.

The Köppen climate classification of the region is Aw, tropical savanna, with dry winters (May to September) and rainfall concentrated in the summer (October to April). The average annual precipitation in the municipality over the past 15 yr was 1 064 mm, with an average temperature of 22°C (Instituto Nacional de Meteorologia [INMET] 2016).

Historic land use and treatments

The experimental area consisted of signal grass pasture (*Brachiaria decumbens* Stapf; PAST), which, for approximately 20 yr, was used without defined management practices such as maintenance, reseeding, and fertilization of pastures. It also had no defined stocking rate since the animals grazed primarily in the rainy season. The site was characterized by exposed soil and weeds. The soil was classified as typical clayey dystrophic Red Latosol (Rhodic Ferralsol), with a granulometric composition at a 0–30 cm depth of 94.8, 249.2, and 656.0 g kg^{-1} for sand, silt, and clay, respectively (Embrapa 1997). According to Alvarez et al. (1999), this soil had a low pH value (5.32), an average Al^{3+} value of 0.67 cmolc dm^{-3}, a sum of bases of 2.30 cmolc dm^{-3}, potential acidity of 2.88 cmolc dm^{-3}, an effective cation exchange capacity of 2.97 cmolc dm^{-3}, a base saturation of 41.08%, and SOC of 19.62 g kg^{-1}.

Once the soil exhibited low pH and base saturation indices, limestone was applied ≈90 d before implementation of the treatments, using the previously obtained chemical characterization of the soil as a reference. After application of limestone, conventional tillage of the soil was performed with plowing and harrowing up to 30 cm depth.

The experimental design was completely randomized with four land managements (treatments) described as follows as a chronosequence:

(i) Native vegetation (NV): a reference area characterized by a typical phytophysiognomy of Cerrado known as “Cerradão.” A previous survey verified that the NV system had not suffered anthropogenic intervention and, thus, was used as control treatment. According to Otoni’s (2011) characterization, the study area has tortuous individuals with xeromorphic attributes and erect individuals. Species of up to 12 m tall were found in the area, but an average of 4.5 m tall was observed, clearly defined stands of vegetation types. The main tree species observed in the area were *Magonia pubescens, Qualea grandiflora, Tachigali subvelutina, Terminalia argentea, Q. parviflora, Kielmeyera coriacea*, and *Protium heptaphyllum*. These species comprised 1 221 individuals of the 2 424 identified in a sample area of 1 ha.

(ii) PAST: the system introduced after removal of native vegetation in 1994, consisting of unmanaged signal grass pasture *(B. decumbens).* The area was previously used by dairy and beef cattle and left without defined management, maintenance, or fertilization for ≈20 yr. The dry matter production potential of such a habitat is up to 15 t ha^{-1} yr^{-1} (Alvim et al. 2002). However, this pasture was characterized by low productivity, exposed soil, and weed infestation.

(iii) ICLF: an agrosilvopastoral system introduced in December 2014 in a location previously occupied by PAST. This area was cultivated with eucalyptus (144 seedlings of *Eucalyptus urograndis* hybrid [*E. grandis* × *E. urophylla*] clones) intercropped with maize (*Zea mays* hybrid [SHS 7920]) and *B. brizantha* cv. Marandu (used as forage). During planting, fertilization with 56 kg ha^{-1} of reactive phosphate and 35 kg ha^{-1} of NPK (8-28-16) was used. Transplanting of eucalyptus was done simultaneously with sowing of maize and forage. In the eucalyptus plantation, a 12 m × 3 m spacing was adopted, with a 1.5-m strip separating the intercropped maize and forage from the seedlings. The cover fertilization of eucalyptus was applied 60 d after transplanting and comprised 35 kg ha^{-1} of potassium chloride, 14 kg ha^{-1} of ammonium sulfate, 3 kg ha^{-1} of sodium borate, and 1.5 kg ha^{-1} of zinc sulfate. Maize was planted with 7 seeds/linear m and spaced 0.8 m between rows, totaling 12 rows of maize between 2 rows of eucalyptus. Forage seeds were mixed with planting fertilizer and intercropped with maize, using 4 kg ha^{-1} of viable pure seeds and 0.4 m spacing between rows. In the fertilization of these crops during planting, 400 kg ha^{-1} NPK (8–28–16) was used, whereas 100 kg ha^{-1} N (urea and ammonium sulfate) was applied for the cover fertilization. The marandu forage was only intercropped with maize during the first yr of cultivation, after which only the grass remained between the original rows.

(iv) Well-managed pasture of *B. brizantha* cv. Marandu (MAR): a system also introduced in December 2014 in an area previously occupied by PAST. This used the same planting recommendations and cultivation treatments as previously described for forage grass in the ICLF system. The dry matter production potential of this habitat is up to 23 t ha^{-1} yr^{-1} (Alvim et al. 2002).

Soil sampling and preparation

The inclusion of a pretreatment baseline in tillage studies is essential to monitor over time the different tillage and cropping systems effects on the SOC (Olson et al. 2014). Therefore, soil sampling was performed in January 2016 and March 2018, 13 and 40 mo after the implementation of MAR and ICLF systems, and 12 mo after the last fertilization in these areas. One cropping cycle of maize in ICLF occurred before soil sampling in 2016. Soil composite samples ($n = 4$) were randomly collected in four minitrenches per treatment at 0–5, 5–10, 10–20, and 20–30 cm depths, as suggested by Olson and Al-Kaisi (2015), totaling 64 samples in each soil sampling. Soil bulk density (Olson 2013) was determined using the core method (stainless steel rings—Ø 5 cm) for all samples.

After collection, the soil samples were air-dried. The roots were then removed from the samples and passed through 2-mm sieves to assess the granulometric and humic fractions of SOM (Baldotto et al. 2015). After this initial processing, the samples were ground and passed through 0.150-mm sieves to determine SOC and N content and ^{13}C natural abundance.

SOC and N content and stocks and ^{13}C natural abundance

SOC and N content were determined by dry combustion with a LECO Tru-Spec CN analyzer (Leco Corp., St. Joseph, MI) using, respectively, infrared absorption and thermal conductivity. The SOC and N stocks in the soil were determined from the multiplication of C or N content by the bulk density and soil depth layer. C and N stocks were corrected for the original equivalent soil mass (Lee et al. 2009), allowing more precise and accurate quantitative comparison among the evaluated systems (Olson 2013). In 2018, we also determined the ^{13}C natural abundance in soil samples using a Finnigan Delta Plus mass spectrometer coupled with a Carlo Erba/CNH-1110 analyzer. The natural abundance of ^{13}C is related to the predominant vegetation. Therefore, the calculation to determine the soil carbon origin (C3 or C4 species) was based on the study of Martinelli et al. (1996).

The granulometric fractions of SOM were produced using a methodology adapted from Christensen (1985; 1992). To separate the fractions, 20 g of soil was passed through 2-mm sieves and then packed in glass vials, where 70 mL of water per vial were added. These vials were then refrigerated at 4°C and later sonicated (Sonics Vibra Cell) for 25 min, with amplitude of 53% (corresponding to 1028.57 J mL^{-1}), then manually stirred with a glass rod every 5 min. After dispersion, the samples were sifted through 0.053-mm sieves into glass refractory containers (samples corresponding to the silt + clay fraction [$F < 0.053$ mm]). The material

Table 1
Soil bulk density under different land-use systems in the Brazilian Cerrado.

Systems[1]	Soil depth (cm)							
	0-5 cm		5-10 cm		10-20 cm		20-30 cm	
	2016	2018	2016	2018	2016	2018	2016	2018
PAST	1.20 aA[2]	1.14 aA	1.23 aA	1.16 aB	1.26 abA	1.23 aA	1.21 aA	1.19 aA
MAR	1.02 bA	0.82 abB	1.10 aA	1.05 abA	1.17 abA	1.11 aA	1.20 aA	1.15 aA
ICLF	1.10 abA	0.84 abB	1.18 aA	1.03 abB	1.27 aA	1.11 aB	1.22 aA	1.15 aA
NV	1.02 bA	0.80 ba	1.14 aA	1.02 bA	1.08 bA	1.08 aA	1.19 aA	1.19 aA

[1] PAST indicates pasture with low productivity of *Brachiaria decumbens;* MAR, monoculture of *Brachiaria brizhanta* cv. Marandu; ICLF, integrated crop-livestock-forest system; NV, native vegetation.

[2] Values represent the mean ($n = 4$). Means in small letter within each column and capital letter within each line followed by the same letter are not significantly different from Kruskal-Wallis ($P < 0.05$).

retained in the sieve (free light fraction [FLF] and heavy fraction [F-sand]) was transferred to a porcelain crucible. Water was added to the crucible and, using panning movements, the differences in density separated FLF from F-sand. The resulting SOM fractions were dried in an air circulation furnace at 65°C for ≈24 h (FLF and F-sand fractions) and 96 h (F $<$ 0.053 mm or F-silt + clay). After drying, the fractions were weighed and crushed at 0.150 mm in a porcelain crucible. C content was obtained through dry combustion of the resulting dry fractions by infrared absorption in a LECO Tru-Spec CN analyzer.

The humic fractions of SOM were measured according to methodology proposed by the International Society of Humic Substances (Swift 1996) and adapted by Mendonça and Matos (2017). The humic fractions were separated in fulvic acid (FAF), humic acid (HAF), and humin (HUM), according to differences in solubility in acid and alkaline media. For this, 10.0 mL of 0.1 mol L^{-1} NaOH was added to 0.5 g of air-dried soil and shaken on a horizontal shaker table for 30 min at 150 rpm. The material was then allowed to stand for 12 h. The alkaline extract (AE [FAF + HAF]) was separated from the residue (HUM) by centrifugation at 3 000 *g* for 20 min. The extract, obtained earlier, was washed twice with this solution, resulting in a final volume of ≈30 mL. HUM was kept in an oven at 45°C until completely dry. The pH of the AE was adjusted to 2.0 (±0.1) with 20% H_2SO_4, followed by 14 h of decantation. The precipitate (HAF) was separated from the soluble fraction (FAF) by centrifugation at 3 000 *g* for 6 min. The supernatant (FAF) was transferred to a 50.0 mL flask, and its volume was adjusted with distilled water. The precipitate trapped in the tube (HAF) was diluted with 0.1 mol L^{-1} NaOH, and the final volume was then adjusted to 50.0 mL with the same solution.

Quantification of C content in the FAF and HAF was performed with aliquots containing 5.0 mL of extract, 2.0 mL 0.033 mol L^{-1} $K_2Cr_2O_7$, and 2.0 mL H_2SO_4 in a digestion block at 170°C for 30 min. H_3PO_4 (1.0 mL, PA) was then added, followed by titration with 0.03 mol L^{-1} ammonia ferrous sulfate in the presence of diphenylamine. For HUM, the methodology proposed by Yeomans and Bremner (1988) was followed. Following the determination of C contents in different humic fractions, HAF/FAF and AE/HUM ratios were calculated.

Statistical analyses

The Lilliefors test was used to verify if the values of each variable satisfied a normal distribution, while the Cochran and Bartlett test was used to assess the results for homogeneity of variances. As the evaluated treatments were arranged laterally (without randomization) and the hypotheses of normality and homogeneity were not validated, we chose the use of nonparametric statistics for all variables. Thus, the differences between treatments were compared using the Kruskal-Wallis multiple comparison test ($P < 0.05$), using R statistical software (R Development Core Team 2016).

Results

Soil bulk density and C and N content

On the basis of the evaluations carried out in 2016 and 2018, it was possible to verify that the soil bulk density decreased in the ICLF and MAR treatments (Table 1), possibly due to the management interventions performed in these 2 systems. Comparing the 2 evaluations, the soil density (up to a depth of 20 cm) in the ICLF system was statistically higher in 2016 than 2018. In contrast, only the first layer (0–5 cm) in the MAR system showed any difference between evaluations.

SOC and N content decrease with increasing depth (Fig. 2). At 0–5 cm depth, C and N content were lower in the PAST system. We found SOC and N content to be similar between PAST, MAR, and ICLF systems at 0–5, 5–10, and 10–20 cm depths. Measurements were also similar between MAR, ICLF, and NV treatments, showing improvement in soil quality over time.

Comparing the results obtained in 2016 and 2018 (see Fig. 2), it was possible to verify an increase in SOC and N levels in the MAR and ICLF systems, while in PAST, a decrease was observed for the same period. The average C/N ratio was variable among treatments, ranging from 12.9 (NV) to 17.0 (ICLF).

SOC and N stocks and ^{13}C natural abundance

We found similar patterns for SOC and N stocks and content to those previously described. The PAST system showed lower soil C stocks compared with NV at 0–5, 5–10, and 10–20 cm depths and across the 0–30 cm profile (Table 2). Three yr after introducing the ICLF and MAR systems (2018), soil C stocks increased in these areas and did not differ significantly from NV at any analyzed depth (see Table 2). The results for N stocks (see Table 2) followed a similar pattern to the C stocks ($P < 0.05$) for all analyzed depths and across the soil profile (0–30 cm). Between 2016 and 2018, C stocks in the MAR system increased by 0.32 Mg ha^{-1} yr^{-1}, while those in the ICLF system increased by 1.72 Mg ha^{-1} yr^{-1}.

The conversion of NV to PAST, and later to ICLF and MAR, contributed to an increase in ^{13}C natural abundance in the soil (Fig. 3). We found an increase in soil C3 and C4 stocks at all depths after conversion of PAST to MAR and ICLF (Table 3). The ICLF system showed a higher contribution of C4 species to soil C stocks between 0 cm and 30 cm (47.63 Mg ha^{-1}) compared with MAR (39.83 Mg ha^{-1}), a difference of 7.80 Mg ha^{-1} (see Table 3).

C associated with granulometric and humic fractions of SOM

The highest percentage of C was found in F-silt + clay, and the lowest percentage was in F-sand (Fig. 4). In addition, the percentage of C with increasing depth generally increased in F-silt + clay

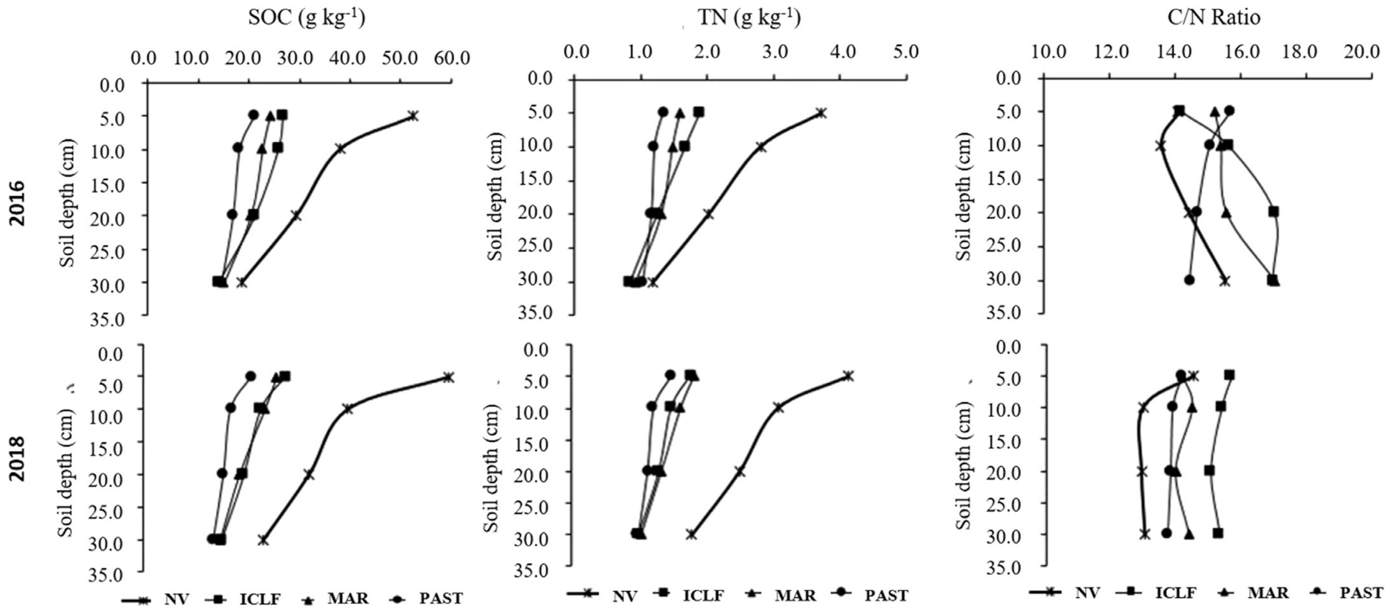

Fig. 2. Soil C and N content, and C/N ratios under different land-use systems in the Brazilian Cerrado.

Table 2
Soil C and N stocks under different land-use systems in the Brazilian Cerrado.

System[1]	Soil depth (cm)				
	0-5	5-10	10-20	20-30	0-30
2016					
C stocks (Mg ha^{-1})					
PAST	10.74 ± 0.85 b[2]	10.36 ± 0.62 c	18.35 ± 1.51 b	17.50 ± 1.14 b	56.94 ± 3.43 b
MAR	12.36 ± 0.28 b	12.91 ± 0.28 b	22.09 ± 1.78 ab	17.85 ± 0.57 b	65.21 ± 2.33 ab
ICLF	13.75 ± 1.65 b	14.76 ± 1.68 ab	23.11 ± 3.43 ab	16.78 ± 1.00 b	68.40 ± 5.91 ab
NV	26.98 ± 5.31 a	21.41 ± 3.51 a	31.82 ± 6.73 a	22.04 ± 0.89 a	102.25 ± 15.70 a
N stocks (Mg ha^{-1})					
PAST	0.68 ± 0.05 c	0.69 ± 0.05 c	1.25 ± 0.10 b	1.21 ± 0.03 b	3.84 ± 0.16 b
MAR	0.81 ± 0.03 bc	0.84 ± 0.05 b	1.42 ± 0.11 ab	1.09 ± 0.06 bc	4.17 ± 0.18 b
ICLF	0.97 ± 0.08 b	0.95 ± 0.07 b	1.36 ± 0.17 ab	0.99 ± 0.09 c	4.26 ± 0.24 b
NV	1.90 ± 0.28 a	1.58 ± 0.29 a	2.21 ± 0.45 a	1.42 ± 0.07 a	7.11 ± 0.96 a
2018					
C stocks (Mg ha^{-1})					
PAST	12.74 ± 0.58 b	10.54 ± 0.41 b	19.76 ± 0.63 b	16.23 ± 0.98 b	59.27 ± 1.02 b
MAR	13.27 ± 0.54 ab	12.90 ± 1.30 ab	21.97 ± 1.50 ab	17.70 ± 1.65 ab	65.85 ± 3.05 ab
ICLF	14.87 ± 1.78 ab	13.65 ± 1.0 ab	25.19 ± 1.44 ab	18.13 ± 1.51 ab	71.84 ± 1.42 ab
NV	30.58 ± 2.44 a	22.92 ± 1.64 a	35.16 ± 3.52 a	27.57 ± 3.47 a	116.23 ± 4.31 a
N stocks (Mg ha^{-1})					
PAST	0.90 ± 0.04 b	0.76 ± 0.03 b	1.43 ± 0.05 b	1.18 ± 0.06 b	4.27 ± 0.04 b
MAR	0.93 ± 0.03 ab	0.89 ± 0.08 ab	1.57 ± 0.11 ab	1.23 ± 0.06 ab	4.62 ± 0.12 ab
ICLF	0.95 ± 0.12 ab	0.89 ± 0.05 ab	1.67 ± 0.09 ab	1.19 ± 0.07 ab	4.69 ± 0.10 ab
NV	2.10 ± 0.19 a	1.77 ± 0.15 a	2.73 ± 0.26 a	2.11 ± 0.28 a	8.72 ± 0.30 a

[1] PAST indicates pasture with low productivity of *Brachiaria decumbens;* MAR, monoculture of *Brachiaria brizhanta* cv. Marandu; ICLF, integrated crop-livestock-forest system; NV, native vegetation.

[2] Values represent the mean ($n = 4$) ± standard deviation. Means within each column of the same parameter followed by the same letter are not significantly different from Kruskal-Wallis ($P < 0.05$).

and decreased in FLF, whereas for F-sand the percentages of C were less affected by soil depth. The C values of FLF were higher in NV than in PAST up to a depth of 20 cm, while the ICLF and MAR values did not differ from NV values (Table 4). C values in the treatments PAST, ICLF, and MAR did not differ among themselves.

The C contents associated with F-sand were similar between depth layers and similar between the ICLF and MAR systems and NV. However, the values in NV were greater than in PAST up to a depth of 20 cm. As observed in F-sand, the C values associated with F-silt + clay were similar in ICLF, MAR, and NV.

Regardless of treatment area or soil depth, HUM displayed the highest levels of C, followed by HAF (Table 5). NV had a higher FAF C content than ICLF in the 0–5 cm layer. In the 10–20 cm soil layer, NV had a lower C content than MAR, while in the 20–30 cm layer NV had a lower C content than both MAR and ICLF.

The NV treatment displayed higher C levels in HAF compared with the PAS until 20-cm soil depth, while it was higher than in ICLF in the 10–20 cm soil layer (see Table 5). However, for this same layer, no difference was observed between PAST, MAR, and ICLF study areas.

We found a higher HAF/FAF ratio in NV than in PAST (5–10 cm), ICLF (10–20 cm), and MAR (20–30 cm; see Table 5). The HAF/FAF ratio was higher (>1.0) for all treatments and soil layers, except for PAST at a depth of 0–5 cm, and MAR at a depth of 20–30 cm. In

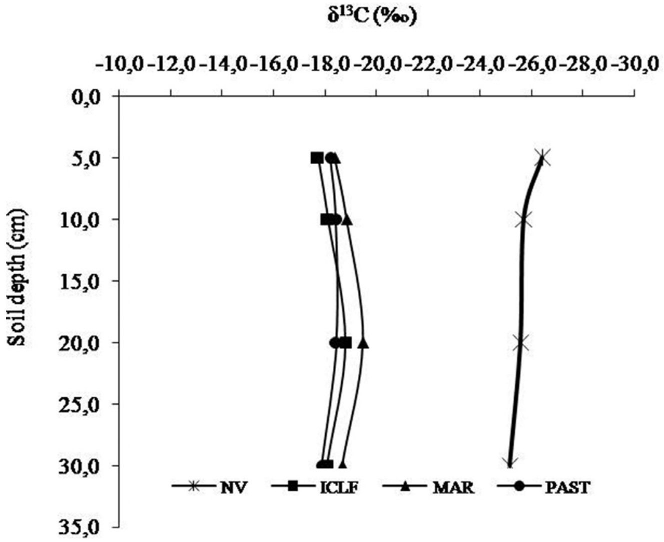

Fig. 3. ^{13}C abundance after conversion of native vegetation to different land-use systems in the Brazilian Cerrado.

Table 3
^{13}C abundance and carbon derived from C3 and C4 species in land-use systems in the Brazilian Cerrado.

Systems[1]	Soil depth	Soil C stocks	δ^{13}C	C$_3$	C$_4$	C$_3$	C$_4$
	cm	Mg ha^{-1}	‰	Mg ha^{-1}		%	
ICLF	0-5	14.87	−17.71	4.01	10.86	26.95	73.05
	5-10	13.65	−18.10	4.38	9.27	32.09	67.91
	10-20	25.19	−18.78	9.74	15.45	38.67	61.33
	20-30	18.13	−18.08	6.09	12.05	33.56	66.44
MAR	0-5	13.27	−18.35	4.29	8.98	32.31	67.69
	5-10	12.90	−18.83	4.98	7.93	38.58	61.42
	10-20	21.97	−19.46	9.83	12.14	44.76	55.24
	20-30	17.70	−18.67	6.92	10.78	39.09	60.91
PAST	0-5	12.74	−18.19	3.95	8.80	30.97	69.03
	5-10	10.54	−18.40	3.67	6.87	34.82	65.18
	10-20	19.76	−18.42	7.00	12.76	35.42	64.58
	20-30	16.23	−17.86	5.12	11.11	31.54	68.46

[1] PAST indicates pasture with low productivity of *Brachiaria decumbens;* MAR, monoculture of *Brachiaria brizhanta* cv. Marandu; ICLF, integrated crop-livestock-forest system.

general, the C content of HUM was higher in NV when compared with the PAST system (see Table 4). In the 10–20 cm soil depth layer, there was no statistical difference between NV and the MAR system. The AE/HUM ratio ranged from 0.22 to 0.48 for all treatments (see Table 5). At 20–30 cm soil layer, the AE/HUM ratio in ICLF was higher than in NV.

Discussion

Although some studies have shown that agrosilvopastoral systems improve soil quality over time (Freitas et al. 2012; Assis et al. 2015; Baldotto et al. 2015; Silva et al. 2016), the conversion of overgrazing pasture into integrated systems is still poorly understood in the various regions of Brazil. We therefore hypothesized that the introduction of integrated systems and well-managed pasture can improve soil quality and increase soil C and N stocks in Cerrado biome. Our findings confirmed our hypotheses, since we observed improvement in the protection of soil organic matter and increase in soil C and N stocks under cultivation of marandu grass and agrosilvopastoral systems.

Soil bulk density and C and N content

Our results showed that conversion of PAST to MAR or ICLF contributed to a decrease in soil bulk density (see Table 1). These results suggest that good soil management practices contribute to the root development of trees and forage grasses, improving soil density. Similar results were found by Santana et al. (2016) after conversion of degraded pasture into an ICLF system. The presence of trees in the production system improved the physical properties of the soil, contributing to the reduction of soil density, which reached values close to that of native vegetation. As the complexity of soil physical structure is related to the quantity, quality, and diversity of the phytomass available to the system (Assis et al. 2015), crop diversity in the agrosilvopastoral system can contribute to improving physical qualities of soil (Aryal et al. 2019).

The PAST system had a lower C and N content in relation to NV (see Fig. 2), mainly due to the reduction in organic material transferred to the soil over time. According to Pinheiro (2007) and Calonego et al. (2012), the conversion of a natural ecosystem to agricultural fields promotes changes in the rates of addition and decomposition of SOM, with decomposition intensified through the destruction of macroaggregates. This disaggregation is caused

Table 4
C content in the free light (FLF), sand (F-sand), and silt + clay (F-silt + clay) fractions of soil organic matter (SOM) under different land-use systems in the Brazilian Cerrado.

Soil depth (cm)	PAST[1]	MAR	ICLF	NV
C FLF (g kg^{-1})				
0-5	1.32 ± 0.33[2] a	1.88 ± 0.20 a	2.75 ± 1.02 a	7.53 ± 3.64 a
5-10	0.72 ± 0.12 c	1.38 ± 0.20 b	2.25 ± 1.42 ab	3.03 ± 0.67 a
10-20	0.50 ± 0.09 b	1.16 ± 0.39 ab	1.19 ± 0.45 ab	2.16 ± 0.55 a
20-30	0.39 ± 0.11 b	0.37 ± 0.08 b	0.31 ± 0.15 b	0.87 ± 0.15 a
C F-sand (g kg^{-1})				
0-5	0.24 ± 0.06 b	0.25 ± 0.02 b	0.49 ± 0.50 ab	0.51 ± 0.04 a
5-10	0.18 ± 0.01 b	0.24 ± 0.02 b	0.58 ± 0.68 ab	0.46 ± 0.05 a
10-20	0.18 ± 0.01 b	0.21 ± 0.02 b	0.72 ± 1.00 ab	0.46 ± 0.07 a
20-30	0.18 ± 0.03 b	0.20 ± 0.01 b	0.23 ± 0.09 ab	0.35 ± 0.03 a
C F-silt + clay (g kg^{-1})				
0-5	20.18 ± 1.33 b	22.90 ± 0.44 b	23.86 ± 3.47 b	45.00 ± 5.59 a
5-10	18.52 ± 0.72 b	22.44 ± 0.42 ab	24.85 ± 2.26 ab	35.40 ± 5.26 a
10-20	19.03 ± 2.07 a	20.86 ± 1.26 a	19.95 ± 1.56 a	26.77 ± 4.77 a
20-30	16.14 ± 1.16 ab	16.36 ± 0.05 b	15.58 ± 1.03 ab	19.15 ± 1.38 a

[1] PAST indicates pasture with low productivity of *Brachiaria decumbens;* MAR, monoculture of *Brachiaria brizhanta* cv. Marandu; ICLF, integrated crop-livestock-forest system; NV, native vegetation.

[2] Values represent the mean ($n = 4$) ± standard deviation. Means within each row followed by the same letter are not significantly different from Kruskal-Wallis ($P < 0.05$).

Table 5
C content in the fulvic acid (FAF), humic acid (HAF) humin (HUM) fractions, HAF/FAF, and AE/HUM ratios of soil organic matter (SOM) under different land-use systems in the Brazilian Cerrado.

System[1]	g kg^{-1}				
	C-FAF	C-HAF	C-HUM	HAF/FAF	AE/HUM
0-5 cm					
PAST	2.21 ± 0.39[2] b	2.02 ± 0.91 b	14.22 ± 1.97 b	0.89 ± 0.34 a	0.31 ± 0.12 a
MAR	2.68 ± 0.64 ab	4.50 ± 0.62 a	16.87 ± 0.45 b	1.81 ± 0.75 a	0.42 ± 0.03 a
ICLF	2.05 ± 0.46 b	3.33 ± 1.37 ab	18.75 ± 3.14 b	1.56 ± 0.39 a	0.29 ± 0.11 a
NV	4.25 ± 0.46 a	5.84 ± 0.65 a	35.42 ± 3.65 a	1.37 ± 0.02 a	0.29 ± 0.04 a
5-10 cm					
PAST	1.61 ± 0.31 a	2.71 ± 0.31 b	13.39 ± 0.90 c	1.72 ± 0.28 b	0.32 ± 0.03 ab
MAR	2.27 ± 0.26 a	4.18 ± 0.31 a	15.37 ± 1.49 bc	1.86 ± 0.33 b	0.42 ± 0.03 a
ICLF	1.64 ± 0.39 a	3.15 ± 1.35 ab	18.64 ± 1.49 ab	1.83 ± 0.46 b	0.27 ± 0.10 ab
NV	1.59 ± 0.29 a	4.78 ± 0.65 a	25.85 ± 4.46 a	3.13 ± 0.27 a	0.25 ± 0.04 b
10-20 cm					
PAST	1.52 ± 0.28 a	2.07 ± 0.31 b	11.05 ± 1.77 b	1.42 ± 0.44 b	0.33 ± 0.04 a
MAR	2.18 ± 0.31 a	3.18 ± 0.54 ab	13.40 ± 1.70 ab	1.49 ± 0.35 b	0.40 ± 0.03 a
ICLF	1.55 ± 0.38 a	2.19 ± 0.73 b	14.69 ± 2.81 ab	1.35 ± 0.41 b	0.27 ± 0.10 a
NV	1.15 ± 0.53 a	5.04 ± 0.88 a	19.04 ± 2.87 a	5.47 ± 1.03 a	0.34 ± 0.07 a
20-30 cm					
PAST	1.47 ± 0.15 a	1.75 ± 0.38 a	8.91 ± 1.05 a	1.19 ± 0.27 b	0.36 ± 0.07 ab
MAR	1.77 ± 0.45 a	1.68 ± 0.37 a	8.53 ± 0.71 a	0.96 ± 0.05 b	0.40 ± 0.08 ab
ICLF	1.92 ± 0.48 a	2.87 ± 1.18 a	10.05 ± 1.52 a	1.74 ± 0.84 b	0.48 ± 0.13 a
NV	0.62 ± 0.21 b	2.39 ± 1.17 a	13.75 ± 2.88 a	4.86 ± 0.87 a	0.22 ± 0.08 b

[1] PAST indicates pasture with low productivity of *Brachiaria decumbens;* MAR, monoculture of *Brachiaria brizhanta* cv. Marandu; ICLF, integrated crop-livestock-forest system; NV, native vegetation.

[2] Values represent the mean ($n = 4$) ± standard deviation. Means within each row followed by the same letter are not significantly different from Kruskal-Wallis ($P < 0.05$).

mainly by tilling. Thus, SOM, previously protected by macroaggregates, is exposed to oxidation and microbial action, reducing its content in soil and simultaneously increasing CO_2 emissions. The lack of pasture management, including reseeding and fertilization, leads to a reduction in SOM content (Cardoso et al. 2010).

The increase in C and N content in the MAR and ICLF treatments at 0–5, 5–10, and 10–20 cm depths (see Fig. 2) is due to litter inputs on the soil surface and the presence of greater microorganism biodiversity in the soil, which increases nutrient cycling and influences C and N transformations. In addition, the use of fertilizers and correctives contributes to maintaining the productive capacity of both the soil and the cultivated species. Significant development, along the soil profile, of the grass roots used in these systems was also noteworthy, as it encourages the aggregation process through a structuring and cementing action. The roots release polysaccharide-rich exudates, protecting the organic matter of the soil from microbial action by increasing formation and stabilization of aggregates (Santos et al. 2007; Salton et al. 2008).

Comparing our results in 2016 and 2018 (see Fig. 2), it can be inferred that conversion of PAST into MAR or ICLF and the continuous proper management of these production systems can increase C and N content over time. Several studies have shown that integrated production systems promote an increase in soil C content (Carvalho et al. 2010; Lorenz and Lal 2014; Lal 2018). However, this increase is dependent on the species used, type of soil in the area, climate, and time the system is implemented. Wendling et al. (2011) reported a reduction in C and N content in the early years after agrosilvopastoral system introduction, but they observed a significant increase after 10 yr of cultivation.

The increase in soil C contents in the ICLF treatment was possibly due to the increased biodiversity resulting from the integration of marandu grass, corn, and eucalyptus. As reported by Tonucci et al. (2011), the introduction of integrated crop-livestock-forest systems in the Cerrado biome can enhance carbon sequestration due to the presence of different components that increase root volume and promote a greater production of vegetal biomass. Other studies have suggested that converting conventional systems into conservationist systems using tree species increases C content (Don et al. 2011; Shi et al. 2013) and preserves soil C and N, reducing CO_2 emissions to the atmosphere (Cubbage et al. 2012).

The lower C/N ratio in NV suggests that more SOM cycling occurs in the native ecosystem due to the greater plant diversity in relation to cultivated areas, favoring the mineralization and release of N in the soil (Machado et al. 2014). The high number of plant species in native forest systems (including N–fixing plants) and the establishment of symbiotic associations among plants, mycorrhizal fungi, and diazotrophic bacteria contribute to the increase of C and N fixation in the soil (Braghirolli et al. 2012).

C and N stocks and ^{13}C abundance

The introduction of good management practices via ICLF and MAR systems increased soil C and N stocks after 3 yr of its establishment (see Table 2). On the other hand, soil C stocks were lower in the PAST system than in NV (see Table 2) due to the reduction of litter inputs and loss of physical protection of SOM. This reduction is a result of tilling under ideal temperature and humidity conditions, determined by the action of the soil microbiota (Corazza et al. 1999; Souto et al. 2005; Silva et al. 2008; Calonego et al. 2012; Caldeira et al. 2013). After the conversion of forests to agrosystems, Fontana et al. (2011) also reported a reduction of soil C content and stocks due to an increase in microbial activity from the decrease in organic residue input to the soil surface. Vergutz et al. (2010) found that introduction of agrosilvopastoral system to a dystrophic oxisol only restored the soil C stocks 12 yr after adoption of good management practices.

This increase in soil C stocks between 2016 and 2018 (see Table 2) can be explained by the by the consortium of grasses and trees, which are known to increase C stocks in soil. Therefore, we recommend the continuous monitoring of ICLF and MAR systems for accurately defining the potential of C and N accumulation over time. As observed in this study, Neves et al. (2004) also found an increase in C stocks after 3 yr of implementing an agrosilvopastoral system in an oxisol in Minas Gerais State, Brazil. Six yr after implementation of a silvopastoral system in Michoacán, Mexico, López-Santiago et al. (2019) also found higher C stocks than

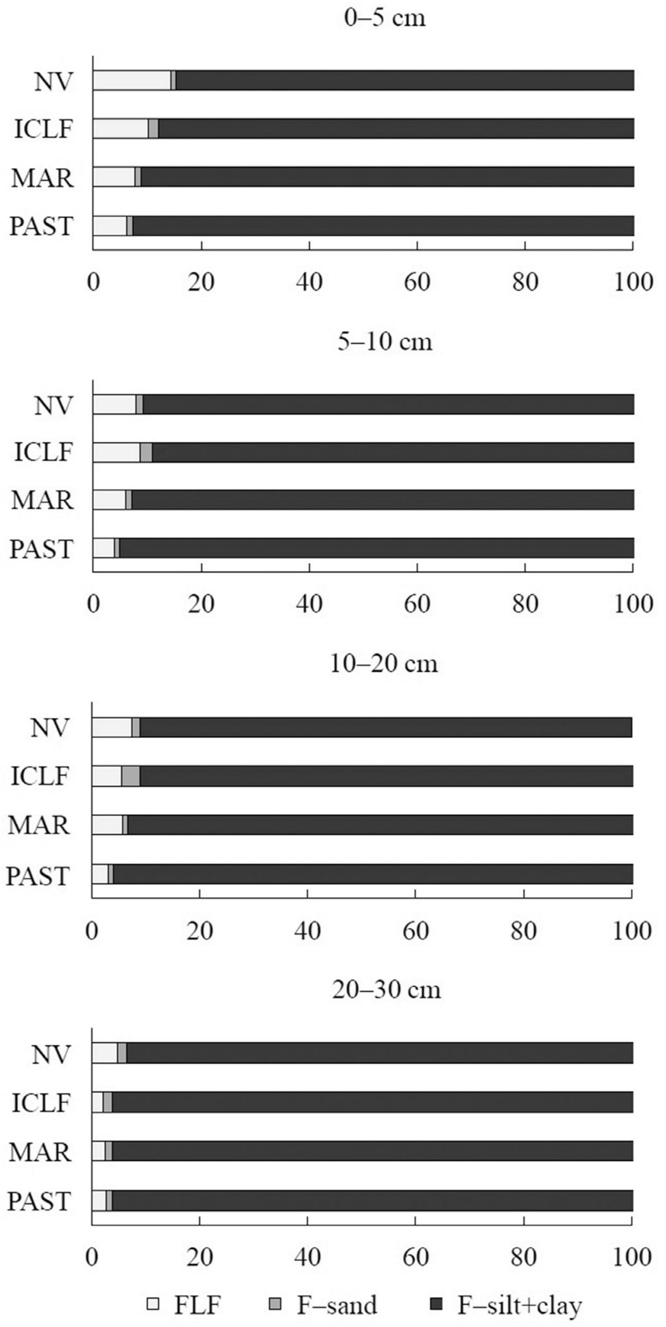

Fig. 4. Contribution (%) of different physical fractions [free light (FLF), sand (F-sand) and silt+clay (F-silt+clay)] of SOM in the soil organic C under different land-use systems in the Brazilian Cerrado. PAST = Pasture with low productivity of *Brachiaria decumbens*; MAR = Monoculture of *Brachiaria brizhanta* cv. Marandu; ICLF = Integrated crop-livestock-forest system; NV = Native vegetation.

in a monoculture of pasture, similar to that of native forest. These findings support our hypothesis that integrated production systems have the potential to recover C stocks over time. The same conclusion can be applied to soil N stocks, agreeing with Silva et al. (2011) that found similar N stocks between native vegetation and an integrated production system after 8 yr of cultivation in an oxisol in Mato Grosso do Sul State, Brazil.

According to Macedo (2009), the use of integrated production systems leads to C storage in soil being equal to or greater than in native systems, also due to the use of tropical grasses that promote good soil cover, straw production, and soil structuring. Torres et al. (2014) found that a greater number of tree or shrub species per unit area promotes greater C storage and consequent GHG mitigation. Litter production is one of the factors leading to higher values of C stock in these areas, but the soil organic C accumulation in the deeper horizon plays an important role in its sequestration (Olson and Al-Kaisi 2015).

We found that the ^{13}C natural abundance increased in ICLF and MAR systems (see Fig. 3) and this result can be related to the introduction of forage grasses that have a C4 photosynthetic cycle. Santos et al. (2019) also reported an increase in ^{13}C abundance after conversion of a native forest to *B. brizantha* cv. Marandu monoculture. The same pattern was observed by Oliveira et al. (2018) in the soil surface of an ICLF system with central establishment of marandu grass in the system arrangement, away from the trees.

Our findings in the ICLF system also may be explained by the greater diversity of C4 species (cultivation of corn and marandu grass) and the shade effect of trees, providing a more favorable environment for root growth and C uptake by soil microorganisms. Tonucci et al. (2017) reported similar results after conversion of monoculture of C3 species (rice and soybean) into ICLF with marandu grass as a forage component. Furthermore, Sant-Anna et al. (2017) also found greater ^{13}C abundance in the topsoil and a difference in ^{13}C with increasing depth, after conversion of the Cerrado area to an integrated system with pasture and agricultural crops.

C associated with granulometric and humic fractions of SOM

Regardless of the evaluated systems, we found the highest percentage of C was associated with F-clay + silt (see Fig. 4), once it displayed a highest level of decomposition among the physical fractions, with a longer residence time in the soil and lower carbon mineralization potential, due to the presence of more humified compounds (Roscoe and Machado 2002). According to these authors, F-clay + silt stores the majority of C due to the high specific surface area and surface charge density of the clay; F-sand has an intermediate potential for mineralization, whereas FLF is easily decomposed by soil microorganisms.

Our results regarding granulometric fractions of SOM indicate improvement of the soil quality in the MAR and ICLF treatments (see Table 4), inferring that the adoption of adequate management of agricultural systems can contribute to the protection of C in soil aggregates. Also, among the fractions assessed, FLF appears the most susceptible to use and management changes since it is highly labile and influenced by the degradation of compounds by soil microorganisms (Conceição et al. 2014; Signor et al. 2014; Kunde et al. 2016). Vergutz et al. (2010) found that an agrosilvopastoral system particularly promoted the reduction of FLF and observed a tendency to recover over the course of extended periods after implementation. Santos et al. (2013) observed that SOC and FLF values in the superficial soil layer of an oxisol under agrosilvopastoral system were similar to those in the natural grassland used as reference, indicating it harbored potential for the maintenance of soil quality. Additionally, Santos et al. (2011) observed similar results for C and the coarse fraction of SOM when comparing an agrosilvopastoral system with NV.

As Roscoe and Machado (2002) argue, the increase in F-sand observed in ICLF and MAR (see Table 4) possibly originates from the organic matter recently deposited in the soil, with the potential for intermediate decomposition driven by soil microorganisms. Thus, we may infer that despite the short time it was allowed to recover, the system has contributed to the increase of the most sensitive fractions to management changes, probably due to greater biodiversity, provided by the integration of different components and abundant root systems of *B. brizantha*.

In relation to the protection of C in the HUM fraction (see Table 5), other studies in tropical soils also found similar results when comparing fractions (Rossi et al. 2011; Silva et al. 2011; Loss et al. 2014; Gazolla et al. 2015). This behavior of fractions is due to the strong interaction between organic matter and the mineral fraction of the soil, forming highly stable organomineral complexes at the soil surface and leaving the majority of the organic matter insoluble (Majzik and Tombácz 2007).

The C associated with the FAF decrease in ICLF and MAR (see Table 5) is due to soil management in agricultural systems, once the conversion of natural vegetation to agriculture favors the decomposition of the more soluble fractions of SOM. Soil tillage in areas subject to anthropogenic influences promotes the increase in C content of FAF in subsurface layers, as this fraction has greater mobility in the soil than other fractions (Loss et al. 2010). On the other hand, the low C content of the HAF in PAST can be explained by the exposition of the soil and low input of vegetal material in the superficial layers of soil in this system. According to Loss et al. (2010), the increase in microbial activity and decrease in residues added to the soil surface alters SOM dynamics, reducing the C content in HUM.

The high HAF/FAF ratio in NV (see Table 5) indicates a selective loss of FAF due to its greater mobility in the soil (Nascimento et al. 2010). Thus, C dominates in HAF in relation to FAF, indicating the presence of preserved soils, possibly due to conservationist soil management (Canellas et al. 2003). As suggested in this study, Fontana et al. (2001) infer that a higher concentration of humic acids indicates the existence of more stable organic material.

We found higher C contents associated to HUM at a 10–20 cm depth in NV than in PAST (see Table 5). Gazolla et al. (2015) attributed this pattern to a greater input of litter and the absence of anthropogenic action in native areas. No difference in the C values of HUM was found among NV, MAR, and ICLF. Higher levels of HUM in integrated systems can be attributed to higher crop residues with greater C/N and lignin/N ratios, resulting in slower decomposition and favoring an increase in recalcitrant soil fractions (Silva and Mendonça 2007; Gazolla et al. 2015). The formation of more stable compounds (HUM and HAF) suggests that beneficial, conservationist management promotes improvement in soil fertility (Loss et al. 2007).

In this study, we found AE/HUM ratios of < 1.0, in line with the study performed by Rosset et al. (2016) in oxisols under conservationist management systems in the southern Brazil. According to Fontana et al. (2014), the strong interaction between organic matter and the mineral matrix through the formation of highly stable organomineral complexes contributes to low AE/HUM ratios. Silva and Mendonça (2007) found that the predominance of lignin-poor plant residues reduces the AE/HUM ratio. Therefore, species with a lignin-rich chemical composition that also contribute a great amount of vegetal material to the soil tend to increase the AE/HUM ratio.

Conclusions

Our findings demonstrated that conversion of low-productivity pasture to agrosilvopastoral and well-managed pasture systems promoted soil C and N stocks recovery at 0–30 cm depths after 3 yr of establishment in the Cerrado biome. In addition, the diversity of C4 species for grain and forage production in agrosilvopastoral systems can be used to enhance GHG mitigation since it leads to increases in soil C and N stocks over time. Our results also confirmed the improvement in SOM protection under conservationist management systems, as C content increased in the recalcitrant fractions of SOM under agrosilvopastoral and well-managed pasture treatments.

Declaration of Competing Interest

None.

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