

## Article

# Soil Carbon and Nitrogen Stocks under Agrosilvopastoral Systems with Different Arrangements in a Transition Area between Cerrado and Caatinga Biomes in Brazil

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**Abstract:** Production systems that promote the accumulation of soil organic matter (SOM) must be implemented to maintain the sustainability of agriculture, livestock, and forestry. Since increases in MOS content contribute to improving the chemical, physical, and biological quality of the soil, as well as helping to reduce carbon emissions to mitigate climate change. Therefore, the objective of this study was to evaluate soil organic carbon (SOC) and nitrogen (N) stocks after the implementation of agrosilvopastoral (ASP) systems in a Cerrado-Caatinga transition zone in Brazil. Native vegetation of Cerrado-Caatinga (NV), regenerating stratum of Cerrado-Caatinga (RS), two arrangements of ASP systems cultivating *Cenchrus ciliaris* L. intercropped with *Eucalyptus camaldulensis* Dehnh. × *Eucalyptus tereticornis* Sm. hybrid (ASP1 and ASP2), and intercropped with *Eucalyptus urophylla* S.T. Blake × *Eucalyptus grandis* W. Mill ex Maiden hybrid (ASP3 and ASP4) were evaluated. Soil C and N stocks and the C content in the humic fractions of SOM were evaluated at 0–10, 10–20, and 20–30 cm soil depths. The introduction of ASP2, ASP3, and ASP4 systems in an area previously occupied by low productivity pasture increased and restored SOC stocks to levels found in NV, at a depth of 0–30 cm. N stocks were higher in ASP systems, regardless of the arrangement studied. As a result, the ASP systems provided accumulations that ranged from 1.0 to 4.31 Mg SOC ha<sup>-1</sup> yr<sup>-1</sup> and from 0.33 to 0.36 Mg N ha<sup>-1</sup> yr<sup>-1</sup>. The carbon contents in humic fractions remained higher in NV. The hierarchical grouping and principal component analysis showed that the implementation of the ASP systems was efficient in increasing soil C and N stocks over time. In conclusion, the present study identified that integrated production systems can support land use intensification strategies based on sustainable and low-carbon agriculture in a transition area between the Cerrado and Caatinga biomes in Brazil.

**Keywords:** agroforestry systems; humification index; soil organic matter; soil quality

## 1. Introduction

The adoption of sustainable agricultural systems has been increasing due to the need for the recovery of degraded lands and the evaluation of ecosystem services in agriculture. The productivity of arable land can be reduced due to the loss of soil organic matter more intensely in regions with low rainfall (SOM) [1]. In this sense, alternative farming systems integrate forests, agriculture, and livestock as a strategy for restoring the soil's physical, chemical, and biological properties [2].

The agrosilvopastoral (ASP) systems are production models that include the cultivation of annual crops, forage grass, and tree species in the same area, in rotation, consortium or succession, resulting in a sustainable intensification and diversification of the production [3]. Studies have reported that integrated production systems can be adopted as a

strategy for mitigating climate change, enabling the atmospheric carbon dioxide (CO<sub>2</sub>) sequestration [4,5], and improving soil quality [6–13].

The contents and stocks of soil organic carbon (SOC) and nitrogen (N) and the content of humic substances are considered indicators of soil quality that can be used to assess the effects of implementing ASP systems [6]. The humin fraction has greater recalcitrance, which increases the stocks and residence time of soil C compared to humic acid, while the fulvic acid has greater lability and mobility in the soil, and is used as an energy source by microorganisms [14–16]. The humification process improves the soil physical and chemical attributes [17,18], and also favors the soil C sequestration [2]. Increase in water retention, cation exchange capacity and immobilization capacity of heavy metals and xenobiotics (such as pesticides) and greater availability of phosphorus are examples of benefits that can be promoted by humic substances, providing improved fertility and soil structure [2,17]. These processes are intensified by the presence of functional groups of carboxylic and phenolic acids in humic substances [2].

The Cerrado is the second most extensive biome in Brazil, occupying about 2 million km<sup>2</sup>, which represents 24% of the country's territory [19,20]. Pasture cultivation covers approximately 28% of the Brazilian Cerrado. Of these pastures, 39% show some level of degradation [21]. Degraded pastures intensify SOM losses and, consequently, contribute to increased greenhouse gas emissions [22]. Studies have shown that integrated production systems can increase SOM content in the Brazilian Cerrado [6,9,10].

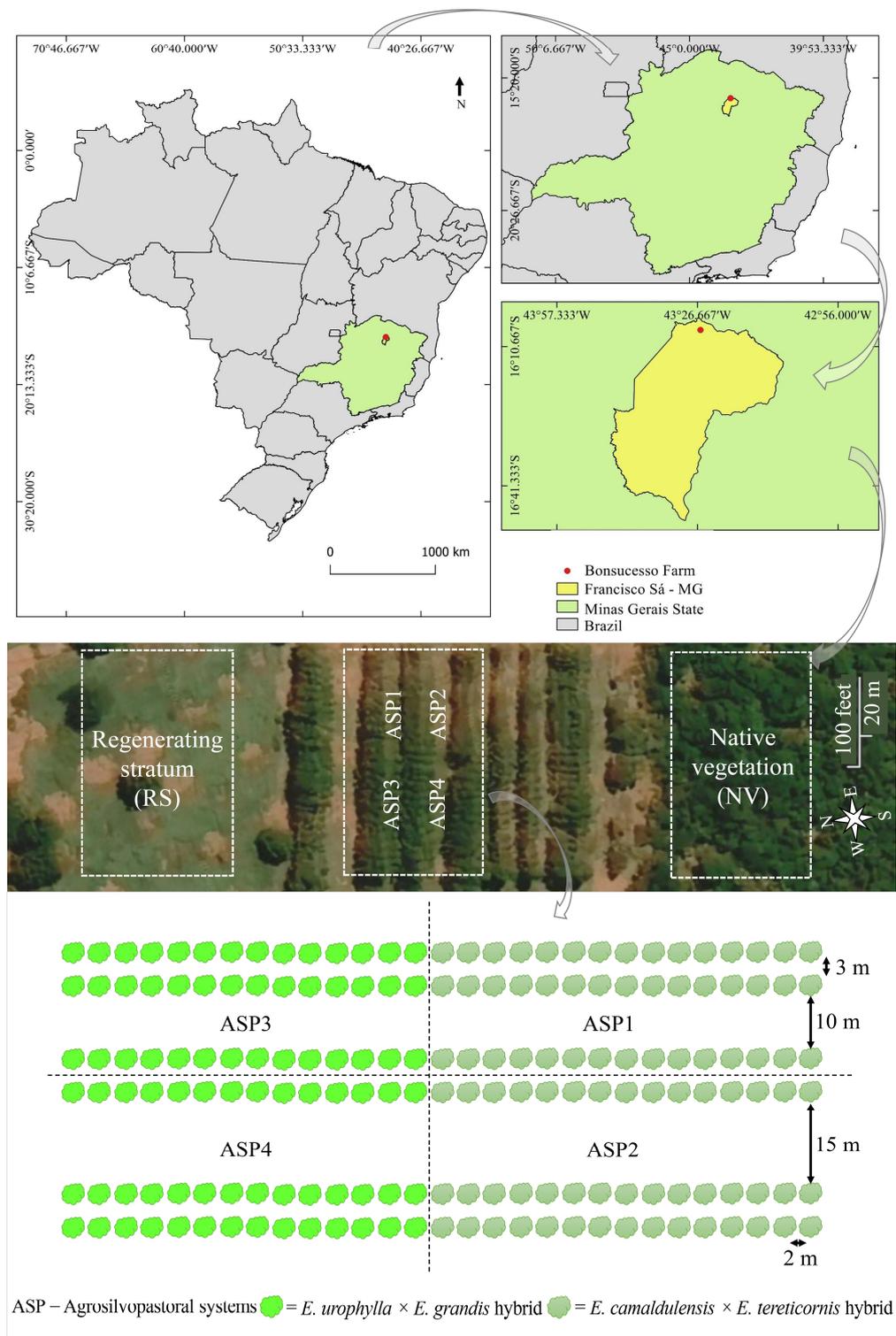
In the reported condition, increases in SOC and N stocks were observed after four years of the conversion of degraded pasture in integrated production systems located in the transition region between Cerrado and Semideciduous Seasonal Forest, however they showed lower values than those found in native vegetation [6]. ASP systems located in the Cerrado biome, introduced eight years ago in a low-productivity pasture area, showed SOC stocks equal to those found in native vegetation, with values of approximately 60 Mg ha<sup>-1</sup> in the 0–20 cm layer [9]. Stocks of approximately 72 and 4.7 Mg ha<sup>-1</sup> of SOC and N up to 30 cm deep, respectively, were found in ASP systems also located in the Brazilian Cerrado, demonstrating integrated production systems as strategies to recover SOC and N stocks from the ground [10]. However, studies in a transition region between the Cerrado and Caatinga biomes, where agricultural production is more sensitive to climate fluctuations, are still scarce. Additionally, the management complexity of agrosilvopastoral systems highlight the need to conduct studies to better understand the feasibility of adoption according to regional characteristics, environmental and social conditions, and different arrangements that can be used [23,24].

In this context, our hypothesis is that the ASP systems increase stocks and promote significant accumulations of C and N in the soil in a transition region between the Cerrado and Caatinga biomes in Brazil, consisting of strategies to obtain a sustainable production that can contribute to the recovery of soil quality. Given the above, this study aimed to evaluate SOC and N stocks 75 months after the conversion of low productivity pasture in ASP systems with different arrangements in a transition region between the Cerrado and Caatinga biomes in Brazil.

## 2. Materials and Methods

### 2.1. Study Site and Characterization of the Experimental Area

The experimental area is located at Bonsucesso Farm, in the municipality of Francisco Sá (16°07'22" S, 43°26'10" W, altitude 591 m), Minas Gerais State, Brazil, in a transition area between the Cerrado and Caatinga biomes (Figure 1). The Köppen climate classification of the studied region is Aw, tropical savanna, with dry winters (May to September) and rainfall concentrated in the summer (October to April). The average annual rainfall is 981 mm, and the average temperature is 22.3 °C. The soil is classified as Acrisol according to FAO classification system [25], with clayey texture.



**Figure 1.** Location of the study area and schematic representation of agrosilvopastoral systems in the municipality of Francisco Sá, Minas Gerais, Brazil.

## 2.2. Treatments

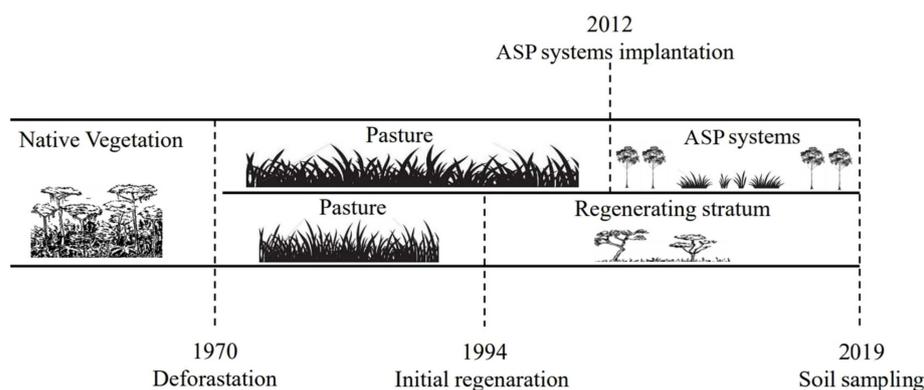
The experimental area consisted of six systems, using a completely randomized design (CRD) with 5 replication ( $n = 5$ ), totaling 30 experimental units. The treatments were: Native vegetation of Cerrado-Caatinga (NV); Regenerating stratum of Cerrado-Caatinga (RS) previously cultivated with pasture; two ASP systems cultivated with *Eucalyptus*

*camaldulensis* Dehnh. × *Eucalyptus tereticornis* Sm. hibrid and *Cenchrus ciliaris* L. using two arrangements [2 × 3 + 10 m (ASP1) and 2 × 3 + 15 m (ASP2)]; two ASP systems with *Eucalyptus urophylla* S. T. Blake × *Eucalyptus grandis* W. Mill ex Maiden hybrid and *Cenchrus ciliaris* using two arrangements [2 × 3 + 10 m (ASP3) and 2 × 3 + 15 m (ASP4)] (Figure 1).

Eucalyptus and buffel grass are exotic species in Brazil that were chosen because of their good adaptability (selected eucalyptus hybrids and grass species used) to local conditions, such as tolerance to low rainfall and rainfall concentration in a relatively short period of the year (see Section 2.1), which allows adequate productions to be obtained in the study region [26,27] that can contribute to the sustainability of the systems.

### 2.3. Land Use History

The native vegetation (NV) was designated as a transitional area between Cerrado and Caatinga biomes [28], of variable size, with a canopy generally less than 25 m. The regenerating stratum area (RS), on the other hand, was extremely uncharacterized. The native vegetation that previously existed in the RS system was removed in the 1970s. After deforestation, management was carried out exclusively with pasture for livestock use, without fertilization at the establishment or replacement of nutrients by chemical or organic fertilization, with only mechanical brush cutting until 1994. Since then, spontaneous plants and native species are in the process of reestablishment (Figure 2).



**Figure 2.** Schematic representation of the land use history at Bonsucesso farm in Minas Gerais State, Brazil.

The main native species observed in the local vegetation, whose phytophysiognomy was characterized as Seasonal Deciduous Forest, were: *Acacia glomerosa* Benth., *Myracrodruon urundeuva* Allemão, *Piptadenia viridiflora* (Kunth) Benth., *Ziziphus* sp., *Enterolobium timbouva* Mart., *Schinopsis brasiliensis* Engl., *Combretum duarteianum* Cambess., *Lonchocarpus campestris* Mart. ex Benth., *Stryphnodendron* sp., *Vernonia discolor* (Spreng.) Less., *Myrcia splendens* (Sw.) DC., *Jatropha mollissima* (Pohl) Baill., *Anadenanthera peregrina* (L.) Speg., *Patagonula bahiensis* Moric., *Centrolobium sclerophyllum* H.C. Lima, *Goniorrhachis marginata* Taub., *Banisteriopsis argyrophylla* (A. Juss.) B. Gates, *Galipea ciliata* Taub., *Anadenanthera colubrina* (Vell.) Brenan, *Ziziphus joazeiro* Mart., *Callisthene minor* Mart., *Cereus jamacaru* DC., *Cassia ferrugínea* (Schrad.) Schrader ex DC., *Vatairea macrocarpa* (Benth.) Ducke and *Zeyheria montana* Mart. The nomenclature of the mentioned plants was obtained from the database provided by the Missouri Botanical Garden [29].

The ASP systems were implemented in an area where deforestation occurred in the 1970s (Figure 2). After deforestation, the site was managed exclusively with pasture for livestock use with mechanical brush cutting until 2011, when the degradation process of the pasture was observed. The introduction of the ASP systems occurred in August 2012, firstly with a subsoiling operation followed by the opening of planting furrows in an east-west direction. The experimental area used for the introduction of the ASP was 26.400 m<sup>2</sup>. After that, in September 2012, the eucalyptus (*E. camaldulensis* × *E. tereticornis*

and *E. urophylla* × *E. grandis* hybrids) seedlings were transplanted in the experimental plots.

Sorghum (*Sorghum bicolor*, cultivar BRS 655) was planted in the 2012/2013 and 2013/2014 seasons between the eucalyptus planting lines in the different arrangements of ASP systems studied. The planting spacing of 70 cm between lines and 10 cm between plants was adopted, with a distance of 1 m from the eucalyptus lines. During the 2016/2017 season, sorghum was planted in consortium with buffel grass (*Cenchrus ciliaris*). However, the formation of pasture was not possible due to the prolonged drought that occurred in this period. Subsequently, in 2017 the remaining seeds of the buffel grass emerged and the pasture was formed, and in February 2018, there was grazing with cattle. In each of the three sorghum crops, 400 kg ha<sup>-1</sup> of formulated fertilizer NPK (4-30-10) were applied at planting and 200 kg ha<sup>-1</sup> of NPK (20-00-20) in topdressing. The fertilization and management practices adopted were described in detail in the studies by Albuquerque [26,30].

#### 2.4. Soil Sampling

Soil sampling was performed in December 2018 and the soil samples were randomly collected in five mini-trenches (replicates), with 40 × 40 cm in dimension per treatment at 0–5, 5–10, 10–20 and 20–30 cm soil depths, totaling 90 soil samples. In all evaluated systems, including native vegetation, soil samples were taken from the entire area covered by the plants, including root zones.

The mini-trenches were randomly distributed in areas of 40 × 10 m (ASP1 and ASP3) and in areas of 40 × 15 m (ASP2 and ASP4). In the NV systems (located to the north of the ASP systems) and RS (located to the south of the ASP systems), which bordered the ASP systems, areas of 1.000 m<sup>2</sup> were delimited for the opening of mini-trenches (randomly distributed).

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 content of total organic carbon, total nitrogen, and humic substances.

#### 2.5. Soil Organic Carbon (SOC) and Nitrogen (N) Contents and Stocks

After the initial processing, the soil samples were ground and passed through 0.150 mm sieves to determine soil SOC and N contents. Subsequently, SOC was determined by wet oxidation according to the methodology proposed by Yeomans [31]. Soil N was determined by sulfuric digestion followed by distillation, according to the Kjeldhal method [32,33]. With the values obtained, the C/N ratios were calculated. Soil bulk density was determined using the core method (stainless steel rings—Ø 5 cm) for all samples [34]. The soil C and N stocks were determined from the multiplication of C or N content by the bulk density and soil depth layer. Subsequently, the C and N stocks were corrected according to the original equivalent soil mass method, using native vegetation as a reference [35].

The annual SOC and N accumulation rates were also calculated by subtracting the SOC and N stocks of the ASP systems by the stocks of the RS system, the result being divided by the implementation time of the ASP systems (75 months) over RS (baseline).

#### 2.6. C Contents in the Humic Fractions of SOM

The humic fractions of SOM were separated in fulvic acid (FAF), humic acid (HAF), and humin (HUF) based on differences in solubility in acid (20% H<sub>2</sub>SO<sub>4</sub> solution) and alkaline (NaOH 0.1 mol L<sup>-1</sup>) media, according to methodology proposed by the International Society of Humic Substances [36] and adapted by Mendonça [37]. Sulfuric acid was purchased from CRQ Produtos Químicos Eireli (Diadema, SP, Brazil) and sodium hydroxide was purchased from F. Marques de Sá (São Paulo, SP, Brazil). The procedure started with the addition of 10.0 mL of NaOH (0.1 mol L<sup>-1</sup>) in 0.5 g of soil. Subsequently, the resulting material was placed on a horizontal shaker table model SL-180/D (SOLAB, Piracicaba, SP, Brazil) for 30 min at 150 rpm. After stirring, the sample was left for a 12 h rest period. The separation between the FAF and HAF (whose sum corresponds to the alkaline extract (AE))

and the residue (HUF) occurred by centrifugation ( $3000\times g$ , for 20 min) with a centrifuge model BMC (Benfer, São Paulo, SP, Brazil). The supernatant was then transferred to another container, in which another 10.0 mL of NaOH was added, leaving it again to rest and performing a new centrifugation, whose supernatant was added to the previous one. The procedure was repeated again, adding the extract to that obtained previously. The HUF was placed in an SSDcr-480 L model oven (SolidSteel, Piracicaba, SP, Brazil) at a temperature of  $45^{\circ}\text{C}$  until reaching constant mass. The pH of the AE was corrected to  $2 (\pm 0.1)$  using 20%  $\text{H}_2\text{SO}_4$ . After pH adjustment, the material underwent a settling period of 12 h. Finally, the precipitated fraction (HAF) was separated from the soluble fraction (FAF) by centrifugation ( $3000\times g$ , for 5 min). The C contents in humic fractions were determined by wet oxidation according to Yeomans [31].

The C-HAF/C-FAF and alkaline extract  $([\text{C-FAF} + \text{C-HAF}]/\text{C-HUF})$  ratios and the humification index  $(\text{IH} = [(\text{C-FAF} + \text{C-HAF} + \text{C-HUF})/\text{SOC}] \times 100)$  were calculated. Methodology details can be found in Freitas [10].

### 2.7. Statistical Analyses

The data were submitted to the Shapiro-Wilk test to verify the occurrence of normal distributions. Bartlett's test was also applied to verify the homogeneity of variances. After that the analysis of variance (ANOVA) was performed and, subsequently, the means were compared by the Scott-Knott test ( $p < 0.05$ ). Cluster analysis was also performed using a hierarchical method to verify the similarities between the treatments studied. Mahalanobis distance was used as a measure of similarity between the records and Ward's method was applied as a clustering strategy. Principal component analysis (PCA) was also performed as a complementary analysis. The statistical procedures were performed using software R (version 3.6.2, Vienna, Austria) [38].

## 3. Results

The SOC levels were different between the land use and management systems in all soil depth layers (Table 1), and the higher SOC content was found in the NV ( $25.63 \text{ g kg}^{-1}$ ) at 0–10 cm. The ASP2, ASP3, and ASP4 systems showed intermediate values (between  $16.85$  and  $17.57 \text{ g kg}^{-1}$ ), while the ASP1 and RS showed the lowest values ( $14.20$  and  $13.02 \text{ g kg}^{-1}$ , respectively). In the deep layers (10–20 and 20–30 cm) the SOC contents in the ASP systems were similar to the NV, except for the ASP1, but this system showed a potential for improving the SOC contents when compared to the RS at 20–30 cm soil depth.

A significant increase in N contents was observed in all ASP systems when compared to the RS (Table 1). In the 0–10 and 10–20 cm soil depths the values were similar between the NV (reference system) and the ASP systems, varying from  $1.51$  to  $1.66 \text{ g kg}^{-1}$  and from  $1.33$  to  $1.55 \text{ g kg}^{-1}$ , respectively. In the 20–30 cm soil depth, the N content was higher in the ASP systems when compared to the RS and NV. Evaluating the soil profile (0–30 cm) we found a similarity between the ASP and NV systems and soil N contents higher than in the RS system.

The C/N ratio, considering all treatments and soil depths evaluated, ranged from 8.03 to 15.39. The highest value along the soil profile was found in the NV (15.10) due the high SOC contents in this system (Table 1).

The C contents in the humic fractions of the SOM differed between the evaluated systems (Table 2). In addition, there was a higher proportion of C present in the HUF followed by the HAF and FAF, regardless of the evaluated system. The highest C-FAF, C-HAF, and C-HUF contents along the soil profile (0–30 cm) were also found in the NV and difference was no found between the ASP and RS systems. The C-HAF/C-FAF ratios were similar between the systems, while the  $(\text{C-HAF} + \text{C-FAF})/\text{C-HUF}$  ratios were higher in NV followed by RS system.

**Table 1.** Soil organic C and N contents and C/N ratio under different land use and management systems at Bonsucesso farm in Minas Gerais State, Brazil. Systems: *E. camaldulensis* × *E. tereticornis* hybrid intercropped with buffel grass using the arrangements 2 × 3 + 10 m (ASP1) and 2 × 3 + 15 m (ASP2); *E. urophylla* × *E. grandis* hybrid intercropped with buffel grass using the arrangements 2 × 3 + 10 m (ASP3) and 2 × 3 + 15 m (ASP4); Regeneration stratum (RS); and native vegetation (NV).

Soil Depth (cm)	Land Use and Management Systems					
	ASP1	ASP2	ASP3	ASP4	RS	NV
	SOC (g kg <sup>-1</sup> )					
0–10	14.20 ± 0.69 <sup>*c</sup>	17.58 ± 1.22 b	16.85 ± 1.14 b	17.57 ± 0.90 b	13.02 ± 2.13 c	25.63 ± 1.53 a
10–20	12.96 ± 0.72 b	15.01 ± 1.48 a	15.84 ± 0.79 a	16.66 ± 0.50 a	11.37 ± 1.49 b	19.77 ± 2.20 a
20–30	11.20 ± 0.77 b	14.82 ± 0.71 a	15.12 ± 0.52 a	14.50 ± 0.25 a	8.81 ± 1.32 c	15.67 ± 0.68 a
0–30	12.79 ± 0.29 c	15.80 ± 1.12 b	15.94 ± 0.66 b	16.24 ± 0.43 b	11.06 ± 1.05 c	20.36 ± 1.34 a
	N (g kg <sup>-1</sup> )					
0–10	1.63 ± 0.05 a	1.61 ± 0.05 a	1.51 ± 0.09 a	1.59 ± 0.09 a	1.11 ± 0.10 b	1.66 ± 0.08 a
10–20	1.48 ± 0.09 a	1.55 ± 0.06 a	1.38 ± 0.08 a	1.53 ± 0.06 a	1.03 ± 0.08 b	1.33 ± 0.11 a
20–30	1.39 ± 0.06 a	1.22 ± 0.13 a	1.27 ± 0.12 a	1.35 ± 0.03 a	0.82 ± 0.02 b	1.05 ± 0.06 b
0–30	1.50 ± 0.06 a	1.46 ± 0.07 a	1.39 ± 0.09 a	1.49 ± 0.06 a	0.99 ± 0.06 b	1.35 ± 0.08 a
	C/N					
0–10	8.75 ± 0.50 b	10.97 ± 0.91 b	11.44 ± 1.30 b	11.11 ± 0.53 b	11.38 ± 1.08 b	15.39 ± 0.50 a
10–20	8.82 ± 0.60 b	9.85 ± 1.23 b	11.69 ± 0.99 b	10.96 ± 0.46 b	10.96 ± 1.18 b	14.87 ± 1.04 a
20–30	8.03 ± 0.33 b	12.95 ± 1.93 a	12.49 ± 1.80 a	10.74 ± 0.15 b	10.69 ± 1.62 b	15.02 ± 0.65 a
0–30	8.53 ± 0.32 c	11.26 ± 1.27 b	11.87 ± 1.33 b	10.94 ± 0.28 b	11.01 ± 0.53 b	15.10 ± 0.49 a

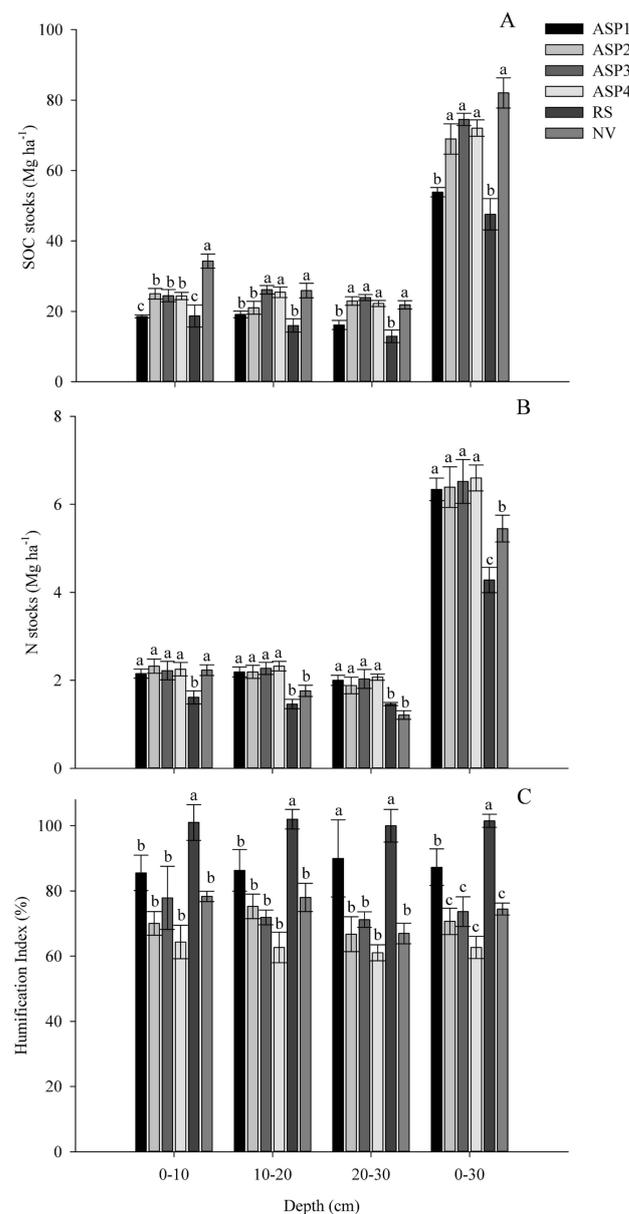
Means followed by the same letter for the same line (horizontal) or depth did not differ by the Scott-Knott test ( $p < 0.05$ ). \* Mean value ± standard error of the mean.

**Table 2.** Carbon contents in the humic fractions of SOM under different land use and management systems at Bonsucesso farm in Minas Gerais State, Brazil. Systems: *E. camaldulensis* × *E. tereticornis* hybrid intercropped with buffel grass using the arrangements 2 × 3 + 10 m (ASP1) and 2 × 3 + 15 m (ASP2); *E. urophylla* × *E. grandis* hybrid intercropped with buffel grass using the arrangements 2 × 3 + 10 m (ASP3) and 2 × 3 + 15 m (ASP4); Regeneration stratum (RS); and native vegetation (NV).

Soil Depth (cm)	Land Use and Management Systems					
	ASP1	ASP2	ASP3	ASP4	RS	NV
	C-FAF (g kg <sup>-1</sup> )					
0–10	1.26 ± 0.08 *b	1.23 ± 0.10 b	0.99 ± 0.09 b	0.79 ± 0.11 b	1.34 ± 0.33 b	1.95 ± 0.09 a
10–20	1.15 ± 0.06 b	1.07 ± 0.11 b	0.77 ± 0.05 b	0.73 ± 0.05 b	1.30 ± 0.22 a	1.74 ± 0.29 a
20–30	1.00 ± 0.06 b	0.86 ± 0.09 b	0.84 ± 0.07 b	0.62 ± 0.07 b	1.04 ± 0.22 b	1.37 ± 0.13 a
0–30	1.14 ± 0.05 b	1.05 ± 0.08 b	0.87 ± 0.06 b	0.72 ± 0.07 b	1.22 ± 0.25 b	1.68 ± 0.15 a
	C-HAF (g kg <sup>-1</sup> )					
0–10	2.13 ± 0.18 b	2.05 ± 0.33 b	2.37 ± 0.28 b	2.17 ± 0.20 b	2.55 ± 0.24 b	4.11 ± 0.38 a
10–20	2.54 ± 0.48 b	1.80 ± 0.39 b	2.23 ± 0.34 b	1.96 ± 0.33 b	2.72 ± 0.39 b	3.88 ± 0.31 a
20–30	2.07 ± 0.45 a	1.65 ± 0.23 a	2.15 ± 0.29 a	1.34 ± 0.25 a	1.95 ± 0.16 a	2.63 ± 0.24 a
0–30	2.25 ± 0.27 b	1.83 ± 0.29 b	2.25 ± 0.27 b	1.82 ± 0.23 b	2.41 ± 0.24 b	3.54 ± 0.31 a
	C-HUF (g kg <sup>-1</sup> )					
0–10	8.66 ± 0.46 b	9.02 ± 0.66 b	9.54 ± 1.20 b	8.22 ± 0.37 b	9.67 ± 0.56 b	13.92 ± 0.67 a
10–20	7.40 ± 0.35 a	8.44 ± 0.84 a	8.38 ± 0.39 a	7.76 ± 0.51 a	8.26 ± 0.63 a	9.76 ± 1.19 a
20–30	6.70 ± 0.31 a	7.40 ± 0.76 a	7.80 ± 0.42 a	6.90 ± 0.27 a	6.20 ± 0.32 a	6.57 ± 0.61 a
0–30	7.58 ± 0.30 b	8.29 ± 0.73 b	8.57 ± 0.60 b	7.63 ± 0.32 b	8.04 ± 0.45 b	10.08 ± 0.67 a
	C-HAF/C-FAF					
0–10	1.73 ± 0.19 a	1.65 ± 0.23 a	2.44 ± 0.30 a	2.94 ± 0.40 a	2.98 ± 1.28 a	2.12 ± 0.20 a
10–20	2.27 ± 0.53 a	1.67 ± 0.37 a	3.01 ± 0.63 a	2.64 ± 0.35 a	2.38 ± 0.56 a	2.44 ± 0.37 a
20–30	2.07 ± 0.42 a	1.89 ± 0.15 a	2.70 ± 0.55 a	2.13 ± 0.27 a	2.46 ± 0.74 a	1.95 ± 0.13 a
0–30	2.02 ± 0.27 a	1.74 ± 0.24 a	2.72 ± 0.48 a	2.57 ± 0.13 a	2.61 ± 0.70 a	2.17 ± 0.13 a
	(C-HAF+C-FAF)/C-HUF					
0–10	0.39 ± 0.01 a	0.36 ± 0.03 a	0.36 ± 0.01 a	0.36 ± 0.02 a	0.40 ± 0.04 a	0.44 ± 0.03 a
10–20	0.50 ± 0.06 a	0.33 ± 0.03 b	0.36 ± 0.04 b	0.34 ± 0.02 b	0.49 ± 0.05 a	0.58 ± 0.01 a
20–30	0.46 ± 0.07 b	0.34 ± 0.03 c	0.38 ± 0.02 c	0.28 ± 0.04 c	0.48 ± 0.04 b	0.61 ± 0.02 a
0–30	0.45 ± 0.03 b	0.34 ± 0.03 c	0.36 ± 0.02 c	0.33 ± 0.03 c	0.46 ± 0.04 b	0.54 ± 0.01 a

Means followed by the same letter for the same line (horizontal) or depth did not differ by the Scott-Knott test ( $p < 0.05$ ). \* Mean value ± standard error of the mean.

The results for the SOC and N stocks were similar those found for the SOC and N contents in all the evaluated systems. The values in the ASP2, ASP3, and ASP4 systems were 68.96, 74.53, and 72.02 Mg ha<sup>-1</sup>, respectively, indicating the SOC accumulation after the adoption of the ASP systems, since the SOC stocks were higher than in RS and similar to the NV system (Figure 3A). The lowest SOC stocks were found in the ASP1 and RS systems, with values of 53.85 and 47.58 Mg ha<sup>-1</sup>, respectively, showing losses of SOC stocks at 28.20 and 34.47 Mg ha<sup>-1</sup> up to 0–30 cm after the deforestation process and the establishment of these systems. Compared to the RS system, the ASP systems contributed with an annual accumulation rate of SOC ranging from 1.00 and 4.31 Mg ha<sup>-1</sup> after the establishment (Table 3).



**Figure 3.** Soil organic C (SOC) (A) and N (B) stocks and humification index (C) under different land use and management systems at Bonsucesso farm in Minas Gerais State, Brazil. Systems: *E. camaldulensis* × *E. tereticornis* hybrid intercropped with buffel grass using the arrangements 2 × 3 + 10 m (ASP1) and 2 × 3 + 15 m (ASP2); *E. urophylla* × *E. grandis* hybrid intercropped with buffel grass using the arrangements 2 × 3 + 10 m (ASP3) and 2 × 3 + 15 m (ASP4); Regeneration stratum (RS); and native vegetation (NV). Means followed by the same letter for each soil depth layer did not differ in the Scott-Knott test ( $p < 0.05$ ). Error bars indicate the standard error of the mean.

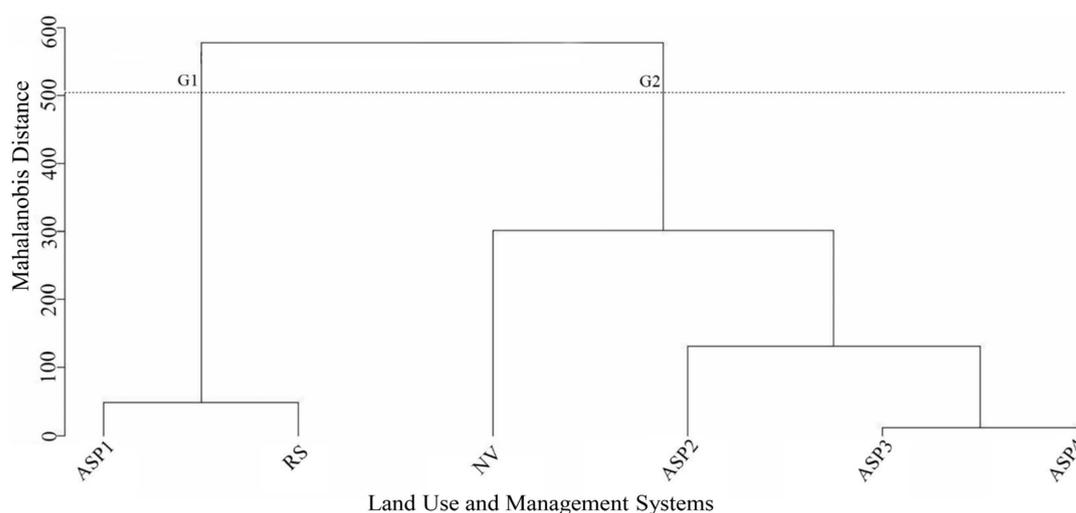
**Table 3.** Annual accumulation rate of SOC and N in the ASP systems compared to RS systems at 0–30 cm soil depth at Bonsucesso Farm, Minas Gerais State, Brazil. Systems: *E. camaldulensis* × *E. tereticornis* hybrid intercropped with buffel grass using the arrangements 2 × 3 + 10 m (ASP1) and 2 × 3 + 15 m (ASP2); *E. urophylla* × *E. grandis* hybrid intercropped with buffel grass using the arrangements 2 × 3 + 10 m (ASP3) and 2 × 3 + 15 m (ASP4).

System	Annual Accumulation Rate (Mg ha <sup>-1</sup> Year <sup>-1</sup> )	
	SOC	N
ASP1	1.00	0.33
ASP2	3.42	0.34
ASP3	4.31	0.36
ASP4	3.91	0.36

The integrated production systems were effective in recovering and increasing the soil N stocks (Figure 3B). The values for the ASP1 (6.34 Mg ha<sup>-1</sup>), ASP2 (6.39 Mg ha<sup>-1</sup>), ASP3 (6.52 Mg ha<sup>-1</sup>), and ASP4 (6.60 Mg ha<sup>-1</sup>) were higher than the NV (5.45 Mg ha<sup>-1</sup>) and RS (4.28 Mg ha<sup>-1</sup>) in the evaluated soil profile (0–30 cm). When compared to the RS system, the ASP systems showed an annual accumulation rate of N ranging from 0.33 and 0.36 Mg ha<sup>-1</sup> (Table 3).

The humification index (HI) was different between the evaluated systems (Figure 3C). In the 0–10, 10–20, and 20–30 cm soil depths, the highest HI were obtained in the ASP1 and RS systems, compared to other ASP systems and the NV. Evaluating the soil profile (0–30 cm), we found that 100% of the SOM was humidified in the RS system, while in the other systems, except for the ASP1, the HI ranged between 60 and 80%.

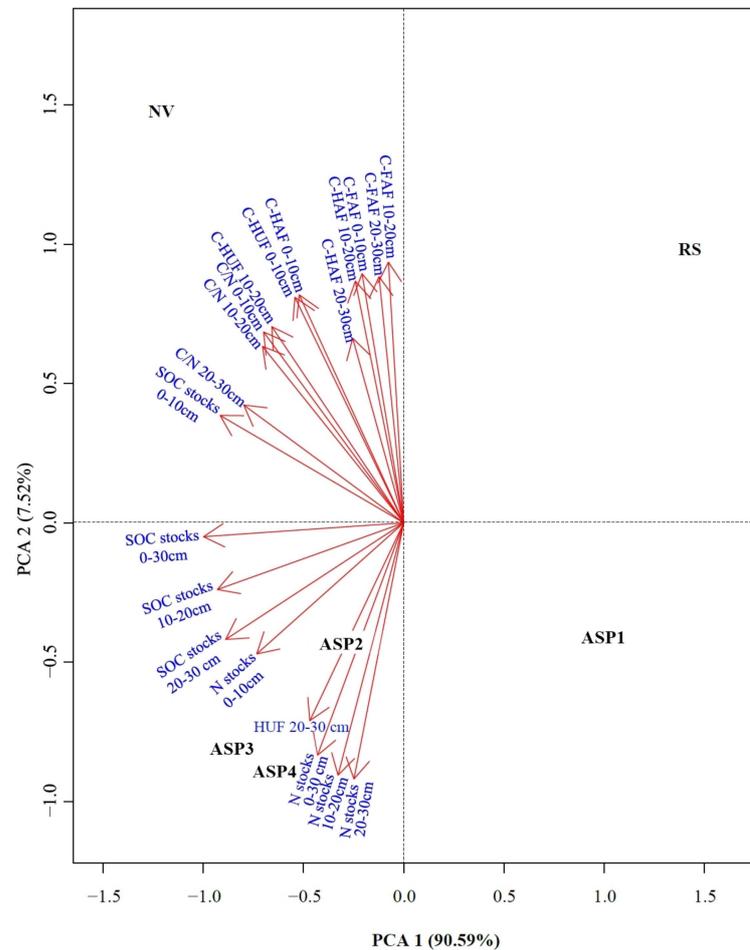
The cluster analysis identified two distinct groups based on the similarity between the evaluated systems (Figure 4). Group 1 (G1) was composed of the RS and ASP1, while Group 2 (G2) was composed of the ASP2, ASP3, ASP4, and NV systems. Our results indicated that even the short time of the ASP systems implementation, improvements in soil quality were observed since they were grouped with NV.



**Figure 4.** Dendrogram from hierarchical clusters analysis showing the formation groups for land use and management systems according to the soil attributes at 0–30 cm soil depth layer. G1: Group 1; and G2: Group 2. Systems: *E. camaldulensis* × *E. tereticornis* hybrid intercropped with buffel grass using the arrangements 2 × 3 + 10 m (ASP1) and 2 × 3 + 15 m (ASP2); *E. urophylla* × *E. grandis* hybrid intercropped with buffel grass using the arrangements 2 × 3 + 10 m (ASP3) and 2 × 3 + 15 m (ASP4).

Additionally, the principal components analysis (PCA 1 and PCA 2) contributed to support our findings, explaining 90.59% and 7.52% of the variance, respectively, for all the

evaluated soil depths (Figure 5). Higher C-FAF and C-HAF values were found at all depths assessed in the reference system (NV). For the NV, we also found high values of SOC stocks at 0–10 cm soil depth and C content in the HUF at 0–10 and 10–20 cm soil depths. The ASP2, ASP3, and ASP4 systems showed the higher N stocks for all the evaluated soil depths and SOC stocks for 10–20, 20–30, and 0–30 cm depths.



**Figure 5.** Relationship between principal components (PCA 1 and PCA 2) discriminating the land use and management systems according to the soil attributes at 0–10, 10–20, and 20–30 cm depth layers. Systems: *E. camaldulensis* × *E. tereticornis* hybrid intercropped with buffel grass using the arrangements 2 × 3 + 10 m (ASP1) and 2 × 3 + 15 m (ASP2); *E. urophylla* × *E. grandis* hybrid intercropped with buffel grass using the arrangements 2 × 3 + 10 m (ASP3) and 2 × 3 + 15 m (ASP4).

#### 4. Discussion

Our results showed a reduction in SOC levels with soil depth and, in general, higher SOC values in native vegetation followed by the ASP systems (Table 1). Increases in soil SOC contents are due to greater litter deposition and the presence of abundant and extensive root systems, and the production of exudates by plants [8,11]. Thus, higher levels of soil organic matter are generally found in the topsoil [39].

Increases in SOC levels were observed in the ASP2, ASP3, and ASP4 systems (Table 1). Similar results were found by Tonucci [40] after the introduction of ASP systems in the Cerrado biome, and these results were favored by the presence of different components that favor the production of aboveground biomass. In contrast, the greater exposure of the soil and the consequent reduction in the production of plant biomass in the ASP1 (*E. camaldulensis* × *E. tereticornis* hybrid intercropped with buffel grass) and RS systems resulted in a reduction of COS contents. Fikreyesus [41] and Iqbal [42] pointed out an inhibitory effect from leaves, roots, barks, fruits, and soil samples of *E. camaldulensis* on the

plant growth and production, suggesting the implementation of corrective practices, such as the removal of excess leaves, before intercropping plants between the eucalyptus lines in agroforestry arrangements with this species. We found that the higher spacing in the ASP2 system using the same *E. camaldulensis* × *E. tereticornis* hybrid may have reduced this inhibitory effect on the production of plant biomass between the lines of the trees, contributing to the growth of annual and forage crops. This arrangement in ASP2 favored the inputs of plant residues and roots increasing the SOC levels compared to the ASP1 system.

The significant increases in soil N contents observed in the ASP systems were favored by the arrangements of the tree component [11] combined with annual and forage species. In addition, the implementation of ILPF systems and management of intercropped crops and forages over time were carried out with nitrogen fertilization, contributing to the increase in soil N contents (Table 1). Additionally, C/N ratios were lower in fertilized treatments (ASP systems), corroborating with results obtained by De Mastro [39]. The higher C/N ratio was verified in the NV system (Table 1).

The C contents in the humic fractions of SOM were higher in the native vegetation (Table 2). Kotzé [1] also observed significant reductions in different humic fractions of SOM in semi-arid agroecosystems in South Africa. In soils under native ecosystem there is an accumulation of stable fractions of soil organic matter due to higher litter deposition and absence of anthropic intervention. Thus, after the conversion of land use, the residence time of these humic substances is determined according to the soil management practices adopted [1,43]. Additionally, the absence of soil disturbance favors the polymerization and increase in the levels of humic compounds [44,45].

As observed in this study, Beutler [14] also found a high correlation between SOC and C-HUF levels. Silva [46] attributed the increase in recalcitrant fractions in the soil to increases in the production of plant residues with a higher C/N ratio and lignin, decreasing the decomposition process. In addition, FAF is more easily used as an energy source by microorganisms [15,16]. As for the C-HAF/C-FAF ratio, the values were similar between the systems evaluated (Table 2), corroborating to the studies by Cardozo Jr [47] and Baldotto [2]. The values of (C-FAF + C-HAF)/C-HUF ratio in the ASP systems can be attributed to the addition of N fertilizer, which decreased the C/N ratio and favored the microbial decomposition [48,49]. Cardozo Jr [47] also observed a reduction in the ratio (C-FAF + C-HAF)/C-HUF in an agroforestry system when compared to the area of native vegetation.

Several studies have found results similar to the present study [6,10,11,13,50,51], showing that integration of the trees with annual crops and forages allows increases in the SOC and N contents and stocks (Table 1 and Figure 3). Improvements in soil quality were also seen with the increase in the levels of SOM. Chen [8] observed an increase in soil aggregation and a consequent decrease in soil erosion processes, while Baldotto [2] observed a positive correlation between increases in SOC levels and increase in soil fertility. Li [52] reported that SOC and N contents were responsible for maintaining soil microbial biomass. The constant deposition of organic matter, mainly leaves, provided better conditions for the establishment and maintenance of microbial populations [14,53].

The SOC and N stocks were also higher in the ASP and NV systems (Figure 3). Similar results were observed by Sarto [54], evaluating eight years old silvopastoral system composed by eucalyptus and pastures, showing SOC and N stocks similar to the area under native Cerrado. The higher SOC contents in native systems result from the constant input of organic matter through the process of recycling tree leaves [15]. Litter production of approximately 4,000 kg ha<sup>-1</sup> year<sup>-1</sup> was observed in a transition region between the Cerrado and Caatinga biomes, 64% of which was composed of leaves, with a decomposition rate of 3 mg day<sup>-1</sup> [55]. In this context, in a multiple cropping system, such as an agroforestry system, the presence of tree species with less removal of plant residues, can significantly decrease SOC losses and reduce the greenhouse gas emissions to the atmosphere [47].

The highest HI were observed in the RS system (Figure 3C), indicating a higher proportion of humic fractions of SOM in relation to the SOC content. This result is due to a low proportion of labile C present in this system, resulting from the small input of organic matter. While the lowest HI found in the ASP and reference (NV) systems are the result of constant inputs of organic matter over time [56,57]. According to Dias [15], the presence of organic matter with high lability favors microbial activity and increase the nutrient cycling process.

In short, we observed an increase in the SOM quality in the ASP2, ASP3, ASP4, and NV systems, compared to the ASP1 and RS systems. Previous studies carried out by Dias [15] and Silva [45] reported a decrease in the soil quality and SOM compartments after conversion of the native Cerrado into different agricultural systems due to greater soil disturbance and small input of organic matter. However, our results indicate that the use of integrated production systems makes it possible to recover the quality of SOM, as reported by Baldotto [2], with significant increases in SOC and N stocks. Lal [58] also indicated the use of integrated production systems as a strategy for restoring SOM due to the higher inputs of plant biomass above and belowground, increasing SOC and decreasing losses by erosion, mineralization, and leaching.

## 5. Conclusions

Our results showed that ASP systems increase C and N stocks over time and, consequently, contribute to improve soil quality in the studied soil and have important implications for climatic aspects. Thus, this study supports the hypothesis that ASP systems can lead to a significant amount of accumulation and high soil C and N stocks in a transition region between the Cerrado and Caatinga biomes in Brazil, demonstrating that these systems can be adopted as alternative for sustainable production and as an important technique for the recovery of degraded pastures. In addition, ASP systems have proved to be important production models that can contribute to mitigating climate change, as they make it possible to obtain high annual rates of SOC and N accumulation.

Higher stocks and annual soil C accumulation rates were obtained in ASP systems when cultivated with the *E. camaldulensis* × *E. tereticornis* hybrid with 15 m between tree planting lines and in ASP systems cultivated with the *E. urophylla* × *E. grandis* hybrid in the 10 and 15 m arrangements between tree planting lines, showing that the choice of the ASP system has an important influence on the storage of C in the soil.

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