



## **Influence of land use and occupation on the chemical and physical fractions of organic matter in cultivated and native areas in the Atlantic Forest biome**

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### **ABSTRACT**

This study quantified the C content of the chemical and physical fractions of SOM in different management systems in an Argis soil of sandy texture. The study was carried out in a reference area of Native Forest (NF), and in three managed areas: Permanent Pasture (PP), No-Tillage System (NTS) and an area of Private Natural Heritage Reserve (PNHR) in the process of natural regeneration. Soil samples were collected in the layers 0.0-0.05, 0.05-0.10 and 0.10-0.20 m. We assessed the soil density (Sd), total organic carbon (TOC) content, chemical fractionation of SOM with determination of the C contents of the fulvic acids (FA), humic acids (HA) and humin (HUM), with subsequent calculations of the HA/FA ratios, AE/HUM, stock (StockC), physical granulometric fractionation and determination of C contents of particulate organic matter (C-POM) and carbon management index (CMI). Higher TOC contents were observed for the NF area. The C-HA and C-HUM contents were higher in the NF and NTS. NF showed higher C-POM levels in all layers evaluated. For the C-MOM, the NTS area was superior to the other managed areas. The managed areas had lower StockPOM values than the NF. The managed areas had lower CMI values in relation to NF. The NTS area showed that, even in crop succession, it contributes to the improvement of the soil organic fraction over the adoption time. On the other hand, the areas of PP and PNHR showed that inadequate management favors the reduction of edaphic quality.

**Keywords:** carbon in soil, carbon management index, chemical fractionation, physical fractionation.



# Influência do uso e ocupação do solo sob as frações químicas e físicas da matéria orgânica em áreas cultivadas e nativa no bioma Mata Atlântica

## RESUMO

O objetivo deste trabalho foi quantificar os teores de C das frações químicas e físicas da MOS em diferentes sistemas de manejo em um Argissolo textura arenosa. O estudo foi realizado em uma área de referência de Mata Nativa (MN) e três áreas manejadas: pastagem permanente (PP), sistema plantio direto (SPD) e área de Reserva Particular de Patrimônio Natural em processo de regeneração natural (RPPN). Foram coletadas amostras de solos das camadas de 0-0,05, 0,05-0,10 e 0,10-0,20 m. Foi determinado a densidade do solo (Ds) os teores de carbono orgânico total (COT) o fracionamento químico da MOS com determinação dos teores de C dos ácidos fúlvicos (AF), ácidos húmicos (AH) e humina (HUM), com posteriores cálculos das relações AH/AF, EA/HUM, estoque (EstC), fracionamento físico-granulométrico e determinação dos teores de C da matéria orgânica particulada (C-MOP) e índice de manejo de carbono (IMC). Observou-se maiores teores de COT para a área de MN, especialmente nas camadas de 0-0,05 e 0,05-0,10 m. Os teores de C-HUM predominam em relação aos teores de C-AH e C-AF. Os teores de C-AH e de C-HUM foram superiores em MN e SPD. A MN apresentou maiores teores de C-MOP em todas as camadas avaliadas. Para o C-MOM a área de SPD foi superior as demais áreas manejadas. As áreas manejadas apresentaram valores de EstMOP inferiores à MN. Nas duas primeiras camadas, a área de MN apresentou maiores EstMOM. As áreas manejadas apresentaram valores inferiores de IMC em relação a MN. A área de SPD demonstrou que, mesmo em sucessão, contribui para melhoria da fração orgânica do solo ao longo do tempo de adoção, se aproximando às condições da MN. Já as áreas de PP e RPPN evidenciaram que o manejo inadequado favorece na diminuição da qualidade edáfica.

**Palavras-chave:** carbono do solo, fracionamento físico, fracionamento químico, índice de manejo de carbono.

## 1. INTRODUCTION

Soil quality (SQ) is complex and is based on its ability to support ecosystem services, balancing physical, chemical and biological quality. It is totally dependent on the management system adopted and on the relationship between the ecosystem and the environment (Doran and Parkin, 1994). Studies on SQ have been improved by several authors, who have developed methods and quality indices which allow different applications for different types of soils and regions. The indicators applied must be sensitive to the management and use of the edaphic environment, being efficient and accurate in identifying changes in soil attributes also in a short evaluation period (Aziz *et al.*, 2013; Marques *et al.*, 2015; Magalhães *et al.*, 2016; Lal, 2018).

Soil organic matter (MOS), by determining the organic carbon content (C), is one of the most sensitive indicators to assess changes in the quality of the edaphic environment (Borges *et al.*, 2015). In addition, in natural environments the stock of C is in balance between the rates of entry and exit, and when they present some type of disturbance that influences litter deposition (Barros and Fearnside, 2016), it ends up modifying the dynamics of C stock in these areas (Rosset *et al.*, 2014; Loss *et al.*, 2015; Koven *et al.*, 2017). However, in many cases, only the quantification of C is not enough to identify possible changes in the quality of the edaphic environment (Diniz *et al.*, 2020).

SOM fractionation techniques are important for evaluation because they are able to express changes in the quality of the soil organic fraction (Rosset *et al.*, 2014; 2016), even in a short period of time (Loss *et al.*, 2015). This happens because most fractions of SOM are located in

different compartments and differ in cycling time, rates of microbial and biochemical degradation, accessibility of microorganisms and interactions with the mineral part of the soil (Kunde *et al.*, 2016).

Chemically, SOM is divided into two parts, one composed of the unhumified fraction, composed of little decayed plant and animal remains and organic compounds with biochemical categories of proteins, sugars, waxes, greases and resins; and the other of humic substances (HS). HS are separated into three fractions: fulvic acids (FA), humic acids (HA) and humin (HUM); and are differentiated according to their molecular weight, increasingly FA>HA>HUM; they are soluble in different pH ranges, among other characteristics. FA are soluble in alkaline or acid pH, HA are soluble in alkaline pH and HUM is insoluble in any pH range (Benites *et al.*, 2003; Gazolla *et al.*, 2015; Olk *et al.*, 2019).

The HUM fraction is responsible for the aggregation of mineral particles and, in most tropical soils, represents much of the humified C. The HA represent the intermediate fraction, between the organic compounds of higher chemical stability (HUM) and the occurrence of free oxidized organic acids in the soil solution (FA). The FA have higher solubility, being mainly responsible for cation transport mechanisms in the soil, and being the most unstable fraction of the humification process (Baldotto and Baldotto, 2014; Lehmann and Kleber, 2015).

The physical-granulometric fractionation divides the SOM into two organic fractions: particulate organic matter (POM), with fractions of more than 53  $\mu\text{m}$  in size and mineral organic matter (MOM), with fractions of less than 53  $\mu\text{m}$  in size (Cambardella and Elliott, 1992), with subsequent determinations and calculations of their respective C contents (C-POM and C-MOM). C-POM is sensitive in identifying changes in land use, even within a short period of time (Loss *et al.*, 2015; Rosset *et al.*, 2019), whereas C-MOM is less altered by changes in land use due to longer cycling time (Bayer *et al.*, 2004).

Considering the physical fractionation of SOM, the carbon management index (CMI) is calculated, which is a relative measure of the impacts of soil management, and combines quantitative and qualitative characteristics to analyze the quality of the areas (Blair *et al.*, 1995). This method makes it possible to infer whether current management practices are harmful to the maintenance of SOM and, consequently, of soil quality over the years of cultivation (Conceição *et al.*, 2014; Nanzer *et al.*, 2019).

Therefore, in addition to the quantification of C, it is important to characterize the quality of C stored underground as a consequence of the adoption of different management systems under different soil conditions and regional climate. This study therefore evaluated soil quality by chemical and physical fractionation of soil organic matter in areas with sandy soil and different management systems.

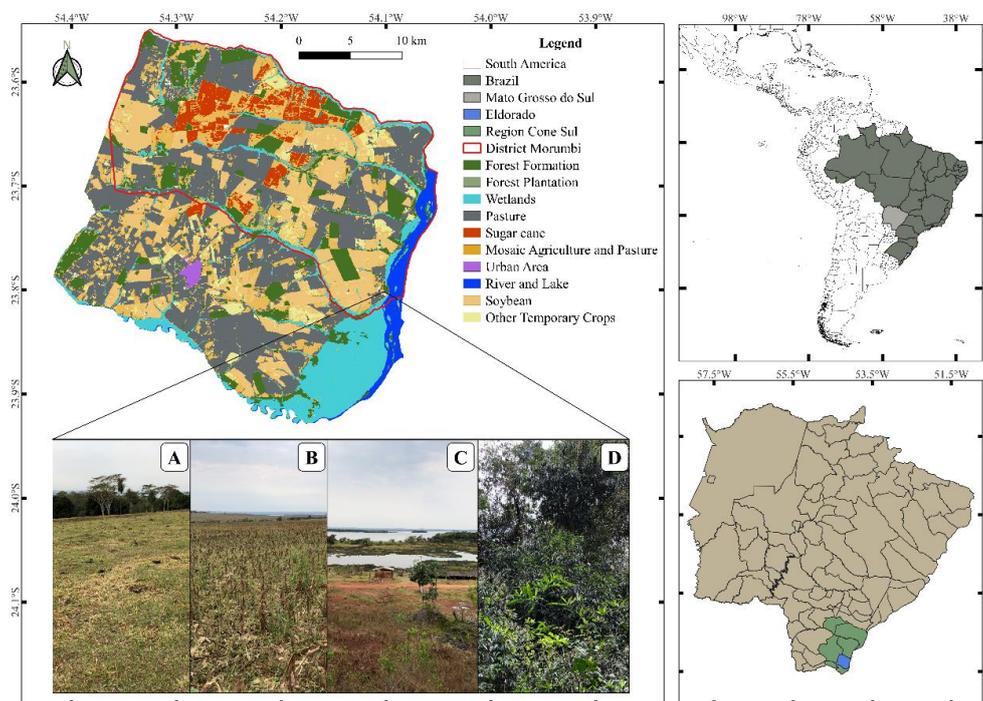
## 2. MATERIAL AND METHODS

### 2.1. Location, Climate, Soil and History of Study Areas

Soil samples were collected in different management systems with known history, located in the district of Porto Morumbi, municipality of Eldorado, Cone-sul region of Mato Grosso do Sul, Brazil (Figure 1). The study areas are located at coordinates 23°48' latitude S and 54°06' longitude W, with an average altitude of 272 meters, and located within the Environmental Preservation Area (APA) of the Islands and Floodplains of the Paraná River (Ilhas e Várzeas do Rio Paraná) (ICMBio, 2019). The climate of the region is subtropical – Cfa, according to Koppen classification (Peel *et al.*, 2007) with average temperature of the coldest month between 14 and 15°C and rainfall ranging from 1,400 to 1,700 mm per year (Mato Grosso do Sul, 2015).

Three managed areas and an adjacent reference area (Native Forest - NF - Atlantic Forest Vegetation with phyto physiognomy of Semidecidual Seasonal Forest) without anthropic action were evaluated. The three managed areas are: permanent pasture with the species *Brachiaria brizantha* (PP), no-tillage system in succession of soybean (summer) and corn crops (second

harvest) (NTS), as well as a Private Natural Heritage Reserve in process of natural regeneration with secondary vegetation (PNHR) (Table 1).



**Figure 1.** Experimental location map, with land use and occupation data for the city of Eldorado - MS, Brazil. Data

**Source:** MapBiomass project (2021). Qgis Development Team (2021).

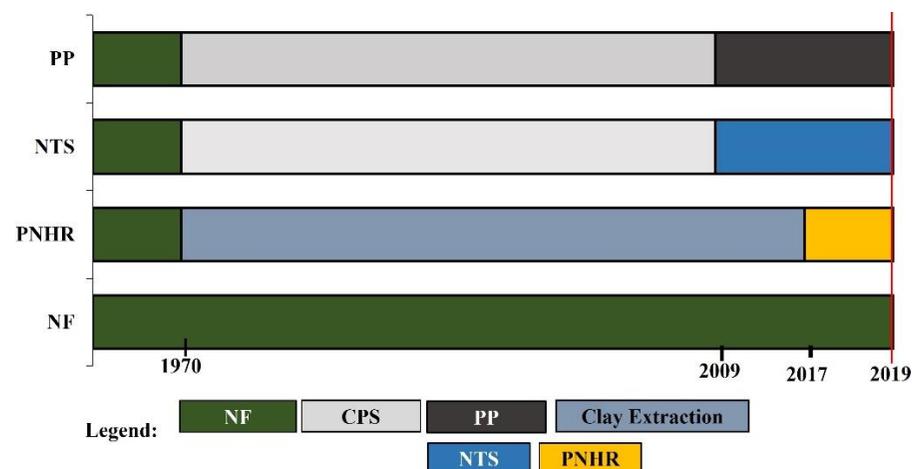
**Table 1.** History and description of the changes in management systems of the different study áreas.

Area	Management history
PP	Area of 5 hectares, cultivated with the species <i>Brachiaria brizantha</i> Hochst Stapf cv. MG4 permanently for 10 years. Area used for grazing cattle with a capacity of 1.2 animal unit (AU) ha <sup>-1</sup> with visible signs of degradation.
NTS	Area of 50 hectares, cultivated under a no-tillage system in succession of soybean (summer) and corn (second harvest) crops, and this type of system has been employed in the area for the last 10 years.
PNHR	Area of 15 hectares. Private Natural Heritage Reserve - Forest remnant of the Atlantic Forest biome, degraded area and in process of natural regeneration for 2 years.
NF	Area of 20 hectares. Native vegetation of Atlantic Forest - Semideciduous Seasonal Forest. Represented in the study as the original condition of the soil, without anthropic action.

PP: Permanent Pasture, NTS: No-Tillage System, PNHR: Private Natural Heritage Reserve, NF: Native Forest.

All four areas studied are on soil classified as Typical Dystrophic Red Argiole (Santos *et al.*, 2018), equivalent Acrisols (IUSS Working Group Wrb, 2015) and Ultisols (NRCS, 2014) of sandy texture (Santos *et al.*, 2018), making up four different systems, analyzed in a completely randomized design. The use and management of the present study areas are displayed in Table 1, and are described according to the chronology of use in Figure 2.

In each of the four study areas, disturbed soil samples from the 0-0.2 m layer were collected for soil physical and chemical characterization analyses (Table 2).



**Figure 2.** History of uses and changes in use of areas, with the respective implementation dates of each management system: NF: Native Forest; CPS: Conventional Preparation System; NTS: No-tillage System; PP: Permanent Pasture; PNHR: Private Natural Heritage Reserve.

**Table 2.** Physical and chemical attributes of the soil in the 0-0.2 m layer of the four areas studied in the district of Porto Morumbi, Eldorado, MS.

MS	Sand	Silt	Argila	pH	OM	P	K	Ca	Mg	Al	H+Al	SB	CEC	V
	g kg <sup>-1</sup>			CaCl <sub>2</sub>	g dm <sup>-3</sup>	mg dm <sup>-3</sup>		cmol <sub>c</sub> dm <sup>-3</sup>					%	
PP	860	43	97	4.59	14.76	6.79	0.04	0.80	0.60	0.13	1.40	1.44	2.84	50.70
NTS	794	59	147	4.07	20.77	13.88	0.17	1.10	0.80	0.39	2.80	2.07	4.87	42.50
PNHR	894	26	80	4.13	13.39	10.44	0.05	0.60	0.30	0.30	1.80	0.95	2.75	34.50
NF	832	44	124	4.69	26.78	12.01	0.15	3.00	1.10	0.10	2.40	4.25	6.65	63.90

MS: Management system; PP: permanent pasture; NTS: no-tillage system; PNHR: Private Natural Heritage Reserve; NF: native forest. Physical characterization – Granulometry: pipette method. Chemical characterization – Mehlich (P and K); KCl 1N (Ca, Mg and Al); Calcium Chloride (pH); Calcium Acetate pH 7 (H + Al); CEC: Cationic exchange capacity; OM: Organic matter; V: Base Saturation; SB: Sum of bases.

For each of the four study areas, composite disturbed soil samples were collected in five replicates in the layers of 0.00-0.05, 0.05-0.10 and 0.10-0.20 m, each composite sample being represented by five simple samples. In all areas and layers, undisturbed samples were also collected with the aid of a volumetric ring with five replicates.

After collection, a procedure was performed to obtain air-dried fine earth (TADS). The Sd was determined by the methodology described by Claessen (1997). The total organic carbon (TOC) was obtained through the method of (Yeomans and Bremner, 1988).

The chemical fractionation of soil organic matter (SOM) was determined following the differential solubility method established by the International Society of Humic Substances (Swift, 1996), according to the adaptation of Benites *et al.* (2003), based on the characteristics of differential solubility by differentiating the fractions of fulvic acid (FA), humic acid (HA) and humin (HUM), with subsequent determinations of the C-FA, C-HA and C-HUM contents.

From the analyses of C of FA, HA and HUM, the values of alkaline extract (AE) ( $AE = HA + FA$ ) and the ratios of HA/FA and AE/HUM were calculated to verify the humification processes of the SOM. In addition, the C stocks of the humic fractions were calculated according to the equivalent mass method (Reis *et al.*, 2018; Ozório *et al.*, 2020). The physical-granulometric fractions of the MOS were determined according to the method of Cambardella and Elliott (1992), obtaining the particulate organic matter (POM) and mineral organic matter (C-MOM). Subsequently, C stocks of particulate organic matter (StockC-POM) and mineral organic matter (StockC-MOM) were calculated following the equivalent mass method (Reis *et al.*, 2018; Ozório *et al.*, 2020). Then, the following indices were calculated to evaluate the quality of the soil organic fraction: carbon stock index (CSI), lability of SOM (L), lability index (LI) and carbon management index (CMI) according to Blair *et al.* (1995).

After the laboratory analyses were performed, the results were assessed in a completely randomized design, submitted to variance analysis employing the F-test, and the mean values were compared by the Tukey test at 5% probability with the aid of the R Core Team program (2021). All tests were performed using ExpDes.pt (Ferreira *et al.*, 2018). A complementary analysis was also performed using the multivariate technique of principal component analysis – PCA, to assess the interrelationships involving all variables and explain these variables in terms of their inherent dimensions (Silva *et al.*, 2020). In order to identify the correlation between the variables, a correlation matrix was performed using Pearson's correlation method (Bravo *et al.*, 2020).

### 3. RESULTS AND DISCUSSION

#### 3.1. Chemical fractions of soil organic matter

The areas of PP, NTS and PNHR had similar soil density values (Sd) ( $p < 0.05$ ) in all layers evaluated. In the 0.00-0.05 m layer, the values ranged from  $1.37 \text{ Mg m}^{-3}$  to  $1.52 \text{ Mg m}^{-3}$ . In the layer 0.05-0.10 m there was variation from  $1.39 \text{ Mg m}^{-3}$  to  $1.44 \text{ Mg m}^{-3}$ . The NTS area showed ( $p < 0.05$ ) higher Sd in relation to NF in the layers of 0.00-0.05 and 0.05-0.10 m. In the 0.10-0.20 m layer the Sd values were similar in all the areas evaluated (Table 3).

The higher Sd in the NTS area may be a consequence of the short management time without soil revolving and the succession of crops, requiring more time for positive changes in the soil's physical attributes (Anghinoni, 2007), as well as the frequent traffic of machinery that favor this result. The results corroborated the studies of Rosset *et al.* (2014), Corrêa *et al.* (2016) and Falcão *et al.* (2020) also in areas of soybean/corn succession.

**Table 3.** Carbon contents of fulvic acid (C-FA), humic acid (C-HA) and humin (C-HUM), HA/FA ratio, alkaline extract (EA)/HUM and carbon stock of fulvic acid fractions (Stock- FA), humic acid (Stock-HA) and humin (Stock-HUM) in different areas evaluated.

MS	Sd	TOC	C-FA	C-HA	C-HUM	HA/FA	AE/HUM	Stock-FA	Stock-HA	Stock-HUM
		g kg <sup>-1</sup>				-	-		Mg ha <sup>-1</sup>	
0-00.05 m										
PP	1.44ab	7.28c	2.22a	1.03c	3.72c	0.48b	0.88b	2.83a	1.31c	4.74c
NTS	1.52a	11.46b	2.48a	1.83b	5.46b	0.76b	0.81b	3.16a	2.34b	6.96b
PNHR	1.37ab	7.61c	2.41a	1.31c	2.82c	0.55b	1.33a	3.07a	1.67c	3.59c
NF	1.27b	16.42a	2.30a	2.95a	7.25a	1.31a	0.74b	2.93a	3.76a	9.23a
CV (%)	12.43	15.17	19.61	14.12	18.21	21.65	20.15	19.61	14.12	18.21
0.05-0.10 m										
PP	1.39ab	6.79b	1.92a	0.75c	3.39b	0.41c	0.79b	2.39a	0.94c	4.21b
NTS	1.54a	10.19b	2.01a	1.80b	5.69a	0.92ab	0.68b	2.50a	2.25b	7.08a
PNHR	1.44ab	5.75d	2.11a	1.43b	2.36b	0.68bc	1.52a	2.63a	1.79b	2.94b
NF	1.25b	10.21a	2.22a	2.30a	6.39a	1.18a	0.76b	2.77a	3.23a	7.95a
CV (%)	17.15	13.75	16.37	15.20	13.14	23.49	20.09	16.37	15.2	13.14
0.10-0.20 m										
PP	1.45a	7.04c	2.22a	1.23c	2.94b	0.55b	1.17b	2.83a	1.58c	3.75b
NTS	1.45a	10.67b	2.20a	2.17ab	4.88a	1.07b	0.93b	2.81a	2.77ab	6.22a
PNHR	1.37a	6.56c	1.94ab	1.80bc	1.92b	0.93b	2.05a	2.48ab	2.30bc	2.46b
NF	1.29a	13.41a	1.39b	2.57a	5.30a	1.92a	0.74b	1.77b	3.28a	6.77a
CV (%)	16.43	17.22	20.71	17.61	16.90	28.71	22.45	20.71	17.61	16.90

Means followed by the same lowercase letter in the column for each system and layer do not differ statistically by the Tukey test (5%). MS: Management system; PP: permanent pasture; NTS: no-tillage system; PNHR: Private Natural Heritage Reserve; NF: native forest. CV(%): coefficient of variation.

The NF area had the highest TOC contents, especially in the 0.00-0.05 and 0.05-0.10 m layers, with contents of 16.41 and 13.59 g kg<sup>-1</sup>, respectively. In the 0.10-0.20 m layer, the areas of NTS and NF were similar ( $p < 0.05$ ) (Table 3). The highest content of TOC in the area of NF may be related to the continuous deposition of litter along with the absence of anthropic actions, especially soil revolving, favoring the increase of TOC contents (Loss *et al.*, 2015; Nanzer *et al.*, 2019). Also comparing NF areas of the Atlantic Forest biome, Rosset *et al.* (2016; 2019), Martins *et al.* (2020), Troian *et al.* (2020) and Ozório *et al.* (2019) found higher contents of TOC in NF in relation to areas of PP and NTS.

The different TOC contents are the result of alteration, production and decomposition of organic residues and depend directly on natural factors associated with pedogenetic processes, but are mainly altered by anthropic actions in soil management (Lal, 2018; Falcão *et al.*, 2020; Santos *et al.*, 2021). The highest TOC contents in the NTS area in relation to the areas of PP and PNHR may be associated with the absence of soil revolving due to the cultivation of corn/soybean in succession in the area. In addition, the low levels of TOC in the areas of PP and PNHR are due to their advanced stage of degradation, with animal overcrowding in PP and the history of soil exploration/extraction of raw material destined to the region's potteries for several decades in the PNHR area.

In all areas studied, C-HUM contents predominated in relation to C-HA and C-FA contents (Table 3). This fact is related to the greater recalcitrance of this fraction compared to the FA and HA fractions (Han *et al.*, 2016). Similar results were found by Rosset *et al.* (2016) and Rosa *et al.* (2017) under different soil conditions, climate and management systems.

The C-FA contents ranged from 1.39 to 2.48 g kg<sup>-1</sup>, but there were no differences ( $p < 0.05$ ) between the studied areas, except for the 0.10-0.20 m layer, with lower content in the NF and higher content in the areas of PP and NTS (Table 3). The FA have a lower nitrogen carbon (C/N) ratio compared to the other fractions, facilitating their decomposition by soil microorganisms (Dobbss *et al.*, 2009). Moreover, this fraction is responsible for the process of transporting cations in the soil, being also fundamental for the cycling of C and nutrients. However, this fraction is highly sensitive to changes in management and can be easily lost due to inadequate management in certain areas (Baldotto and Baldotto, 2014).

The C-HA contents ranged from 0.75 to 2.95 g kg<sup>-1</sup>, with higher levels observed in the NF area in all evaluated layers, similar ( $p < 0.05$ ) to the NTS area in the 0.10-0.20 m layer. In general, the areas of PP and PNHR had lower levels of C-HA ( $p < 0.05$ ) in relation to the other areas (Table 3). This may be related to soil management used in these areas over the last few years, which do not advance the SOM humification process, with consequent lower C levels of the chemically more stable fractions of C (Guimarães *et al.*, 2013).

The C-HUM contents ranged from 1.92 to 7.25 g kg<sup>-1</sup>, as did those of C-HA; the C-HUM contents were higher in the NF area in all layers evaluated, being similar ( $p < 0.05$ ) only in relation to the NTS area in the 0.10-0.20 m layer. These results of C-HUM contents are related to TOC contents (Table 3), mainly because the HUM fraction represents the majority of the soil TOC. It is noteworthy that the C-HUM contents in the NTS area were higher ( $p < 0.05$ ) than the other two anthropized areas in all evaluated layers (Table 1). Due to the lower soil disturbance due to non-revolving, over the years of cultivation the NTS provides greater stability of C (Guimarães *et al.*, 2013) with predominance of the HUM fraction (Rosset *et al.*, 2016). On the other hand, the lowest levels of C-HUM in the areas of PP and PNHR (Table 3) are associated with non-conservationist management of these areas over the last years, as also evidenced in the lower TOC contents of these areas.

With the exception of the NF area in all layers, and the NTS area in the 0.10-0.20 m layer, the values of the HA/FA ratio were below 1.00 (Table 3). The HA/FA ratio is useful, mainly to reflect the quality of humus, in which the higher the ratio, the higher the condition of SOM humification and the better the quality and stability of the soil organic fraction (Pfleger *et al.*,

2017, Diniz *et al.*, 2020).

Considering the results, it can be affirmed that the areas of PP and PNHR have lower stabilization of the SOM, with consequent lower quality of the soil organic fraction, with a higher proportion of FA in relation to HA, with damage to other edaphic attributes, such as soil structural stability.

However, it is important to highlight that in soils under tropical climate conditions, along with the presence of soils with more sandy texture, the HA/FA ratio is usually lower due to the high rate of decomposition of plant residues under the soil. For the AE/HUM ratio, the PNHR area had higher values in all evaluated layers, ranging from 1.33 to 2.05, differing ( $p < 0.05$ ) from all other areas (Table 3). Higher values of this relationship indicate greater presence of less stable fractions of C (FA and HA), in relation to the fraction of greater chemical stability (HUM).

The StockC-FA ranged from 1.77 to 3.16 Mg ha<sup>-1</sup>, but did not differ ( $p < 0.05$ ) between the management systems evaluated in the layers 0.00-0.05 m and 0.05-0.10 m. In the 0.10-0.20 m layer, the NF area had lower StockC-FA in relation to the PP and NTS areas (Table 3). Among the soil humic substances, the FA fraction is the first to undergo quantitative changes, as it reflects the first stage for the stabilization of the SOM (Rosa *et al.*, 2017).

The NF area had the highest StockC-HA in the first two layers, 3.76 Mg ha<sup>-1</sup> and 3.23 Mg ha<sup>-1</sup>, respectively, similar ( $p < 0.05$ ) to the NTS area in the 0.10-0.20 m layer. The PP area stood out negatively, with lower values of StockC-HA in all layers, similar to PNHR in the layers of 0-0.05 and 0.10-0.20 m, and with lower stock (0.94 Mg ha<sup>-1</sup>) in the 0.05-0.10 m layer (Table 3).

The highest StockC-HUM in the 0.00-0.05 m layer was observed in NF with a value of 9.23 Mg ha<sup>-1</sup>, differing ( $p < 0.05$ ) from the other areas. In the layers 0.05-0.10 m and 0.10-0.20 m, the StockC-HUM was similar ( $p < 0.05$ ) between the NTS and NF areas, different from that observed in the PNHR and PP areas, which had the lowest StockC-HUM (Table 3). These results corroborate the C contents of the SOM fractions and also the TOC contents, mainly because the HUM fraction represents the majority of the soil TOC.

It is important to highlight that, among the managed areas, the NTS, even in soybean/corn succession implemented since 2009, had higher levels and C stock of the most stable fractions of the SOM, being similar to NF in the most superficial layer (Table 3). Rosset *et al.* (2016) report that the accumulation of C in the most recalcitrant fractions of SOM tends to increase as a function of the time of adoption of the NTS.

Through the quantitative results of C contents, with qualitative inferences in relation to the chemical fractions of the SOM (Table 3), it is possible to observe that the PNHR area, due to the anthropic actions of soil exploration for clay extraction, had low C stocks of the most stable fractions of the SOM (HA and HUM) in addition to the lowest HA/FA ratio and highest AE/HUM ratio (Table 3). The same thing happened in the PP area, because this area is in an advanced stage of degradation, impairing the processes of humification of the SOM, with consequent lower chemical stabilization.

### 3.2. Physical Fractions of Soil Organic Matter

In all layers evaluated, the NF area had higher ( $p < 0.05$ ) carbon content of particulate organic matter (C-POM) when compared to the three managed systems, reaching 4.04 g kg<sup>-1</sup> in the 0.00-0.05 m layer (Table 4). These higher levels of C-POM in the surface layer coincide with the pattern of the highest TOC contents observed in this area (Table 3). Similar data were found by Kunde *et al.* (2016); Rosset *et al.* (2019); Bieluczyk *et al.* (2020); Ferreira *et al.* (2020) and Santos *et al.* (2021), comparing different types of native vegetation with managed areas.

Comparing only the managed areas, it was observed that the NTS area showed higher C-POM ( $p < 0.05$ ) than PP in the 0.00-0.05 m layer and PNHR in all layers, with values of 2.19 g kg<sup>-1</sup>, 1.85 g kg<sup>-1</sup> and 1.68 g kg<sup>-1</sup>, respectively, for the layers 0.00-0.05, 0.05-0.10 and 0.10-0.20

m (Table 4). These higher levels observed in NTS are due to the minimal soil disturbance of this area, added to the accumulation of plant residues over the years of cultivation, as also observed by Melo *et al.* (2016) and Rosset *et al.* (2019) in NTS areas in succession of soybean/corn crops. In general, the highest levels of C-POM observed in the soil surface layer occur due to the higher intake of plant residues in this layer, together with the absence of anthropic actions that impair the accumulation of particulate C (Rosset *et al.*, 2014; Kunde *et al.*, 2016; Nanzer *et al.*, 2019).

**Table 4.** Carbon contents of particulate organic matter (C-POM), mineral organic matter (C-MOM), POM carbon stock (Stock POM) and MOM (Stock MOM), carbon stock index (CSI), lability (L), lability index (LI) and carbon management index (CMI) of the different areas evaluated in the district of Porto Morumbi, municipality of Eldorado, Mato Grosso do Sul.

MS	C-POM	C-MOM	Stock MOP	Stock MOM	CSI	L	LI	CMI
	g kg <sup>-1</sup>		Mg ha <sup>-1</sup>					
0.00-0.05 m								
PP	1.70c	5.56c	2.17c	7.08c	0.44c	0.31a	0.95a	42.13b
NTS	2.19b	9.26b	2.80b	11.79b	0.69b	0.23b	0.73b	50.95b
PNHR	1.74c	5.85c	2.22c	7.45c	0.46c	0.30ab	0.92ab	42.61b
MN	4.04a	12.37a	5.14a	15.75a	1.00a	0.32a	1.00a	100.00a
CV (%)	8.06	5.89	8.06	5.89	4.70	12.34	12.85	9.55
0.05-0.10 m								
PP	1.78b	5.25c	2.21b	6.54c	0.51c	0.34a	1.27a	65.51b
NTS	1.85b	8.51b	2.31b	10.58b	0.76b	0.21b	0.80b	61.67b
PNHR	1.35c	4.95c	1.68c	6.16c	0.46d	0.27b	1.01ab	47.07c
MN	2.89a	10.70a	3.60a	13.31a	1.00a	0.27b	1.00a	100.00a
CV (%)	7.54	4.53	7.54	4.53	3.22	13.01	15.14	10.27
0.10-0.20 m								
PP	1.45b	5.33b	1.85b	6.80b	0.66b	0.27ab	0.89a	59.04bc
NTS	1.68b	8.49a	2.15b	10.84a	1.00a	0.19b	0.64b	64.21b
PNHR	1.11c	4.63b	1.42c	5.91b	0.56c	0.24ab	0.77ab	44.46c
MN	2.41a	7.79a	3.08a	9.95a	1.00a	0.31a	1.00a	100.00a
CV (%)	10.24	7.00	10.24	7.00	6.27	16.91	15.63	13.49

Means followed by equal letters in the column, in each layer, do not differ from each other by Tukey's test (5%). MS: management system; PP: permanent pasture; NTS: no-tillage system; PNHR: Private Natural Heritage Reserve and NF: native forest; CV= Coefficient of variation.

It is also important to highlight the lowest levels of C-POM in the PNHR area, ranging from 1.11 to 1.35 g kg<sup>-1</sup>, demonstrating low potential for labile C accumulation in this area. The differences ( $p < 0.05$ ) in C-POM contents in the studied areas reinforce the potential of this fraction to be used as an indicator of soil quality due to the sensitivity to demonstrate changes in a short period of time, resulting from the use of the edaphic environment (Conceição *et al.*, 2013; Briedis *et al.*, 2018; Bongiorno *et al.*, 2019; Rosset *et al.*, 2019). Higher C-POM levels are related to the aggregation process, where these labile fractions are slowly occluded in soil aggregates, leading to physical protection of SOM (Tobiasová, 2011). However, through the change in land use and cultivation, the labile fractions are constantly exposed to microbial activity and subject to mineralization, hindering the occlusion process and, consequently, promoting the reduction of SOM levels (La Scala *et al.*, 2008) as reported by Gmach *et al.* (2018).

The NF area also had the highest levels of C-MOM in all layers, being similar ( $p < 0.05$ ) to

the NTS in the layer of 0.10-0.20 m, with contents ranging from 7.79 g kg<sup>-1</sup> to 12.37 g kg<sup>-1</sup> (Table 4). The NTS area had intermediate levels in the layers of 0.00-0.05 m and 0.05-0.10 m, with 9.26 g kg<sup>-1</sup> and 8.51 g kg<sup>-1</sup>, respectively. The highest levels of C-MOM (Table 4) are mainly related to the higher levels of TOC in these areas (Table 3), added to non-revolving, and to the contribution of POM stabilization over time, where the labile fraction of C becomes the most recalcitrant fraction, with consequent stabilization of the SOM over time (Ozório *et al.*, 2020).

Relating to the low TOC contents (Table 3), as well as low C-POM contents, the PP and PNHR areas had the lowest C-MOM levels in all three layers evaluated (Table 4). Since C-MOM has slow cycling, it is possible to infer that, because these areas have low vegetation cover and are considerably degraded, these low levels are justified. According to Mafra *et al.* (2015), the reduction of C-MOM in the managed areas in comparison with native vegetation area is associated with the breakdown of aggregates due to inadequate management over the years, exposing C to the action of microorganisms and external degradation agents, hindering the accumulation of TOC in the soil. However, this fraction is considered less sensitive to soil management in relation to POM, especially in the short term, due to being physically protected and considered more stable (Guimarães *et al.*, 2018).

The highest stocks of particulate organic matter (StockPOM) were found in the NF area in all layers evaluated, reaching 5.14 Mg ha<sup>-1</sup> in the 0.00-0.05 m layer, differing ( $p < 0.05$ ) from the other areas (Table 4). This fact results from the absence of anthropic activities and the greater deposition of residues newly incorporated into the soil, as can be observed in the highest levels of C-POM in this area. Similar results were also observed by Rosset *et al.* (2019) comparing different management systems in relation to native Atlantic forest vegetation.

The lowest StockPOM were observed in the areas of PP, NTS and PNHR, with 2.17, 2.80 and 2.22 Mg ha<sup>-1</sup> for the 0.00-0.05 m layer, 2.21, 2.31 and 1.68 Mg ha<sup>-1</sup> for the 0.05-0.10 m layer and 1.85, 2.15 and 1.42 Mg ha<sup>-1</sup> in the 0.10-0.20 m layer, respectively (Table 4). The values found in these managed areas in relation to the NF area corroborate the lowest levels of TOC (Table 3) and C-POM (Table 4), demonstrating that the forms of land use of these areas over the last few years have not been efficient in contributing to the increase in labile C stocks in the soil.

As for C-MOM contents, the NF and NTS areas had ( $p < 0.05$ ) the highest StockMOM in the most superficial layer, with 15.75 Mg ha<sup>-1</sup> and 11.79 Mg ha<sup>-1</sup>, respectively (Table 4). Bayer *et al.* (2004) reported that StockMOM is less altered by different forms of management. Carmo *et al.* (2012) observed that in deeper layers, this fraction is highly stable, undergoing little changes by the management system. If specific recovery practices are adopted in the PP area, such as reform of pasture with soil correction, in addition to forest density in the PNHR area, the entry of C into the soil can be reestablished and, consequently, promote the increase of stocks of labile fractions and subsequently recalcitrant, with consequent increases in the total StockC (Falcão *et al.*, 2020).

All managed areas presented CSI values lower than 1.00, except for the 0.10-0.20 m layer of the NTS area (Table 4). This fact indicates that these forms of management were not potentially efficient in stocking C in the soil. The CSI values observed in these areas followed the trend of the lowest TOC contents (Table 3). Among the managed areas, the NTS presented the highest ( $p < 0.05$ ) CSI (Table 4). Considering that prior to the implementation of the NTS, the area was managed under a conventional tillage system, the results of CSI infer that the land use in this area allowed the recovery of carbon stock, even if slowly. Similar results were obtained by Conceição *et al.* (2014), also in an area with alteration from CTS systems to NTS, and by Rosset *et al.* (2019) in NTS chronosequence, in areas previously cultivated with CTS. Zhang *et al.* (2020) observed that the adoption of management practices with a greater number of plant species can promote the accumulation of TOC in the soil more rapidly, resulting in a

greater amount of labile organic fractions in a short period after the adoption of this practice.

In general, the areas presented L values lower than 1.00, indicating the predominance of C in the fraction associated with minerals (MOM), which is desirable, because this fraction is more stable (Guimarães *et al.*, 2018). According to Santos *et al.* (2017), the system becomes more susceptible to C loss by the action of microorganisms when C-POM predominates, because in this fraction, C has lower stability and is exposed to the highest rate of decomposition.

The L values in the 0.00-0.05 m layer of the NF, PNHR and PP areas did not differ from each other ( $p < 0.05$ ), with values of 0.32, 0.30 and 0.31, respectively. It is also emphasized that in the 0.05-0.10 m layer the PP area had a value of 0.34, higher than the other areas ( $p < 0.05$ ) (Table 4). L is considered an excellent indicator of soil quality, being obtained through the ratio between POM and MOM fractions, and values closer to 1.00 suggest balance between these fractions (Benbi *et al.*, 2015). Similar results were obtained by Schiavo *et al.* (2011) in the state of Mato Grosso do Sul and Rosset *et al.* (2019) and Ozório *et al.* (2020) in the state of Paraná, both comparing managed and native areas. Except for the PP area, it was observed that for the other areas evaluated, the L of the SOM decreased according to the depth, especially in the area of NTS (Table 4). The same behavior was observed by Schiavo *et al.* (2011), Kunde *et al.* (2016) and Rosset *et al.* (2019).

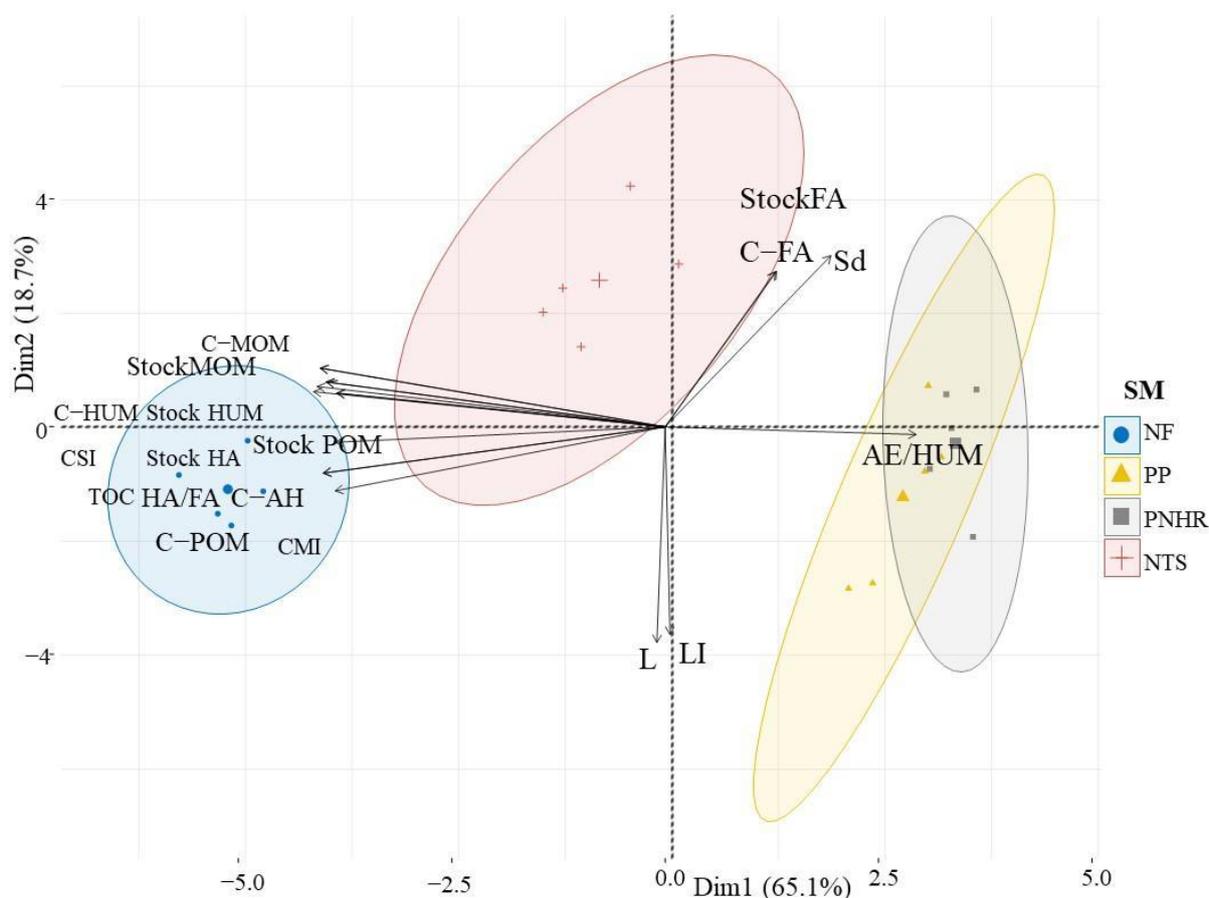
In the 0.00-0.05 m layer, for the managed areas, the values of the lability index (LI) were close to the NF, and the NTS area showed a difference ( $p < 0.05$ ) in relation to the PP and PNHR areas. For the other layers, only the NTS area presented values lower than the NF (Table 4).

In none of the management systems evaluated, CMI values similar or higher ( $p < 0.05$ ) to those of the NF area were observed in all layers evaluated. However, evaluating only the managed areas, the highest values were observed in the NTS areas, with 64.21 in the 0.10-0.20 m layer, and PP, 65.51 in the 0.05-0.10 m layer. These results are probably due to the non-revolving of the soil in both the NTS and PP, and even if the PP area is considerably degraded, the presence of grasses is fundamental, as it favors a certain stabilization of C in subsurface by the action of the root system (Nanzer *et al.*, 2019; Santos *et al.*, 2019).

In general, presenting behavior similar to the levels of TOC (Table 3), C-POM and C-MOM (Table 4), it is noteworthy that the PNHR area presented lower CMI values ( $p < 0.05$ ) than those found in NTS and PP in the 0.05-0.10 m layer and NTS in the 0.10-0.20 m layer, ranging from 42.61 to 47.07 (Table 4). These results reflect the state of degradation in which the area is after being overexploited for decades, demonstrating that, in addition to the isolation done in 2017, it requires other recovery practices so that there is an increase in the quantity and improvement of the quality of the SOM, with consequent improvement of other edaphic attributes. Satisfactory results of increase in the C contents and physical fractions of SOM and CMI in an isolated area, with subsequent practice of forest density through planting of native tree species were found by Santos *et al.* (2021) in the municipality of Mundo Novo, MS.

### 3.3. Analysis of principal components and correlation between variables

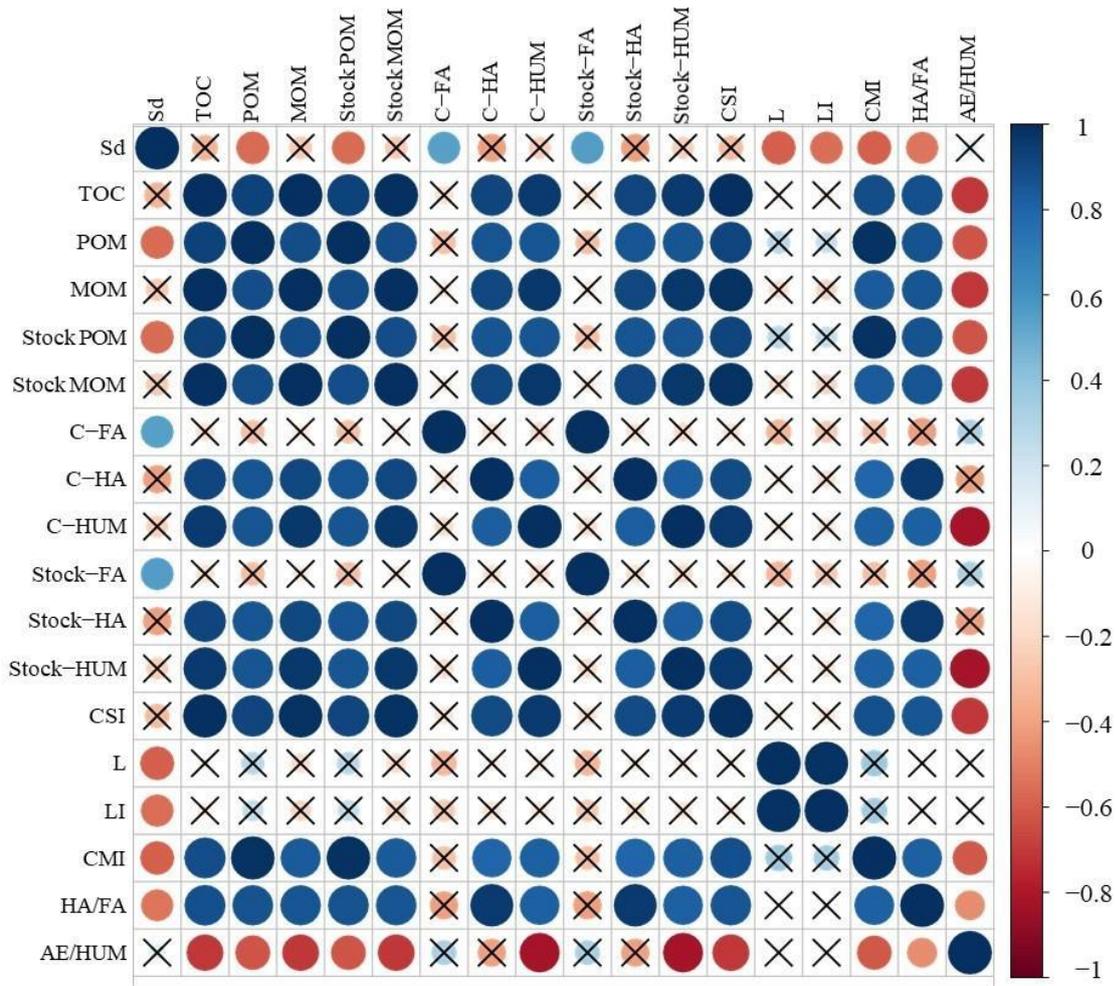
A multivariate analysis was performed using the data of the attributes Sd, TOC, C-FA, C-HA, C-HUM, HA/FA, AE/HUM, StockC-FA, StockC-HA, StockC-HUM, C-POM, C-MOM, StockC-POM, StockC-MOM, CSI, L, LI and CMI, in which the edaphic variables in the 0-0.2 m layer explain 83.8% of the data variation for the first two axes (Figure 3). The areas of NF and NTS were in different positions in relation to the areas of PP and PNHR, in which the latter were more related to the variable AE/HUM. The NF area was related to all other attributes, except for C-FA, StockC-FA and Sd. It is important to highlight that the NTS area is closer to NF, that is, it has the closest one similarity to all the attributes evaluated (Figure 3).



**Figure 3.** Principal Component Analysis - PCA for the different areas evaluated. PP: permanent pasture. NTS: no-tillage system. PNHR: Private Natural Heritage Reserve. NF: native forest. Carbon from fulvic acids (C-FA), humic acid (C-HA) and humin (C-HUM), HA/FA ratio, alkaline extract (AE)/HUM and carbon stock from fulvic acid fractions (StockFA), humic acid StockHA) and humin (StockHUM), carbon from particulate organic matter (C-POM) and mineral organic matter (C-MOM), carbon stock from POM (StockPOM) and MOM (StockMOM), carbon stock index (CSI), liability (L), liability index (LI) and carbon management index (CMI).

The areas of PP and PNHR, due to the arrangement of the groups, did not contribute effectively to the improvement of the edaphic quality within the parameters evaluated, and only the NTS area was closer to the NF. Rosset *et al.* (2014; 2016); Martins *et al.* (2020) and Troian *et al.* (2020) also observed the same behaviors found in this study, with the NF area of vegetation of the Atlantic Forest biome having better edaphic quality in relation to the other managed areas. Rosset *et al.* (2019) observed similarities between the areas of PP and NF. Unlike what was observed by Falcão *et al.* (2020), where the NF area of the Cerrado biome was similar to the areas of NTS and PP with six years of implementation.

In Pearson's correlation analysis presented in Figure 4, it is possible to highlight the positive and significant correlation ( $p < 0.05$ ) of the C-POM and C-MOM contents with the C-HA and C-HUM contents, and these four variables have a strong relationship with the TOC content. Such behavior is also observed in the stock values of these variables. These results show the importance of maintaining the TOC, to contribute to the different stages and composition of the SOM, and consequently in the improvement of soil quality, with greater structuring (Tisdal and Oades, 1982; Ferreira *et al.*, 2020), nutrient cycling (Santos *et al.*, 2019), mitigation of erosive processes that cause the loss of productive soil (Lal, 2018) and mainly reducing CO<sub>2</sub> emissions into the atmosphere.



**Figure 4.** Correction matrix between variables using Pearson's correlation method. Carbon from fulvic acids (C-FA), humic acid (C-HA) and humin (C-HUM), HA/FA ratio, alkaline extract (AE)/HUM and carbon stock from fulvic acid fractions (Stock-FA), humic acid (Stock-HA) and humin (Stock-HUM), carbon from particulate organic matter (C-POM) and mineral organic matter (C-MOM), carbon stock from POM (Stock POM) and MOM (Stock MOM), carbon stock index (CSI), lability (L), lability index (LI) and carbon management index (CMI). Correlations identified with "X" do not present significant correction ( $p < 0.05$ ).

Figure 4 also shows a significant correlation ( $p < 0.05$ ) between CMI values and C-HA and C-HUM contents. This shows that even though fractionation techniques are different once the HS is changed, it changes the C in quality and quantity (CMI). Thus, we evidenced the complexity of the SOM, and sensitivity that it presents in identifying changes in the use and occupation of soil (Lal, 2018).

#### 4. CONCLUSIONS

Based on our findings, the no-tillage system, even in the soybean/corn succession, contributes to carbon content and stocks, favoring the quantity and quality of organic matter. The no-tillage system also had characteristics closer to the reference area, compared to other available systems.

Among the managed areas, an area of direct planning system demonstrates the presence of fractions of greater stability and greater degree of humification of soil organic matter.

It is also concluded that degraded pastures and areas with intense exploitation accrue the presence of C in the soil, not offering environmental benefits in the mitigation of CO<sub>2</sub> emissions,

with significant losses of C in relation to areas of no-tillage system and native forest.

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