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Effect of soil management on carbon stock and soil aggregation in an area of natural regeneration and surrounding systems in the Atlantic Forest biome

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ABSTRACT

This study aimed to quantify total organic carbon (TOC), carbon of humic substances (HS), and their stocks and evaluate the soil structural stability of areas with different uses under sandy loam soil texture. Soil samples were collected from managed areas and a reference area: Permanent Pasture (PP), No-Till (NT), Private Natural Heritage Reserve in the process of natural regeneration (PNHR) and Native Forest (FN). Dry mass analysis, carbon stock quantification, chemical fractionation of soil organic matter and soil aggregation were carried out. The NF area had the highest deposition of litter mass (ML). The PP and NT areas had the highest bulk density (Bd). TOC and Stock-C contents were higher in PNHR, followed by NF, and stratification index (STRATI) was also higher in the regeneration area. The NT, PNHR, and NF areas had a higher proportion of carbon fulvic acid fraction (C-FA) than carbon humic acid fraction (C-HA), but the fraction with the highest representation in all areas was carbon humin fraction (C-HUM). The PP, PNHR, and NF areas obtained the best aggregate stability indicators, as well as a higher proportion of macroaggregates, with the NT area having low aggregate stability. Recovery of C contents was observed in recent years in the area of PNHR, leading to a greater storage of C, which shows a quantitative recovery of C in the soil in this area after four years of natural regeneration. Furthermore, the PP and NT areas present a lower capacity for C sequestration, mainly due to the management conditions.

Keywords: aggregate stability, climate change, crop production, soil quality.

Efeito da gestão do solo no estoque de carbono e na agregação do solo em área de regeneração natural e em sistemas ao entorno no bioma Mata Atlântica

RESUMO

O objetivo deste trabalho foi quantificar os teores de carbono orgânico total (COT), carbono das substâncias húmicas (SH), e seus estoques, assim como avaliar a estabilidade estrutural do solo de áreas com diferentes formas de uso sob solo de textura franco arenosa. Amostras de solo foram coletadas em três áreas e uma área de referência: Pastagem permanente (PP), plantio direto (PD), Reserva Particular de Patrimônio Natural em processo de regeneração natural (RPPN) e área de Mata nativa (MN). Foram realizadas análises de massa da serapilheira (MS), densidade do solo (Ds), teores de COT, e estoque de C (EstC), variação do EstC (ΔEstC) e índice de estratificação (IE), fracionamento químico da matéria orgânica do solo (MOS) e determinações dos teores de C dos ácido fúlvico (C-AF), ácido húmico (C-AH) e humina (C-HUM), e seus respectivos estoques (EstC-AF, EstC-AH e EstC-HUM), ΔEstC de cada fração, extrato alcalino (EA=AF+AH), relação C-AF/C-AH e relação EA/C-HUM, além da análise de agregação, sendo determinado o diâmetro médio ponderado (DMP), diâmetro médio geométrico (DMG), índice de sensibilidade (IS) e nível de ordem (NOrd). A área de MN apresentou maior deposição de MS. As áreas de PP e PD tiveram maior Ds. Teores de COT e EstC foram superiores em RPPN, seguido de MN, sendo o IE também foi superior na área em regeneração, e a ΔEstC positiva apenas nesta área. Áreas de PD, RPPN e MN obtiveram maior proporção de C-AF comparado ao C-AH, porém a fração com maior representatividade em todas as áreas foi o C-HUM. As áreas de PP, RPPN e MN obtiveram os melhores indicadores de estabilidade de agregados, como DMP, DMG, IS e NOrd, assim como maior proporção de macroagregados, sendo a área de PD com baixa estabilidade de agregados. De maneira geral foi observada recuperação nos teores de C nos últimos anos na área de RPPN, acarretando maior estocagem de C, o que demonstra recuperação quantitativa de C no solo nesta área após quatro anos de regeneração natural. Ademais, as áreas de PP e PD apresentam menor capacidade de sequestro de C, devido principalmente as condições de manejo impostas nas áreas.

Palavras-chave: estabilidade de agregados, mudanças climáticas, produção agrícola, qualidade do solo.

1. INTRODUCTION

There has been a growing global concern about climate change and its consequences in recent decades. It is estimated that by 2100, the global average temperature will increase between 1.8ºC and 4.0ºC, leading to melting glaciers and polar ice caps, rising sea levels, and an increase in tropical storms and hurricanes (Blank, 2015). In Brazil, this increase can be up to 4ºC in the interior of the country and up to 3ºC on the coast, accompanied by a decrease in rainfall (Blank, 2015), generating impacts on the process of agricultural and livestock production (Cenci and Lorenzo, 2020).

Climate change and global warming are mainly due to greenhouse gas (GHG) emissions, mainly emitted by combustion processes for energy generation (Dintwe and Okin 2018; Campos *et al.,* 2020). It is urgent to promote changes and adaptations that can control or decrease their emission rates (Cenci and Lorenzo, 2020), especially of carbon dioxide (CO_2), the main pollutant gas emitted into the atmosphere, with a lifetime of more than 100 years (Sun and Zhong, 2023).

Studies show that some agricultural systems or environmental management techniques can potentiate the mitigation of CO_2 emissions to the atmosphere, promoting the maximization of carbon sequestration and stock (C) in the soil (Carvalho *et al.,* 2010). These are the systems or techniques considered by conservationists for promoting practices that contribute to the increase of soil organic matter (SOM), such as not disturbing the soil and no-tillage, used in crop succession and no-till systems (NTS) (Risal and Parajuli, 2022; Santos *et al.*, 2023).

The premises of NTS are: minimum soil disturbance, continuous vegetation cover, and crop rotation (Silva *et al.*, 2020). Crop rotation is the orderly alternation of different crops in a given cycle in the same area and season (Franchini *et al.*, 2011). The use of crop rotation and the use of suitable species provides a considerable amount of mulching and nutrient cycling, increasing the storage of C and nitrogen (N) in the soil (Silva *et al.*, 2020), enhancing C sequestration (Boddey *et al.*, 2010). Crop succession, on the other hand, is the arrangement of two crops in the same agricultural area for an indefinite period, each grown in a season (Franchini *et al.*, 2011).

The main objective of evaluating the quality of edaphic attributes according to the management system is to preserve the soil and maintain or increase its productive capacity. However, when the area is in a state of degradation, it is necessary to use management techniques for recovery. Human interference in the environment, especially if done inappropriately, can affect the quality and productive capacity of an area's environmental resources, triggering degradation processes (Alves *et al.*, 2023; Batista *et al.*, 2023).

Among the human interference practices with high soil degradation potential, clay mining is an example of extractivism that causes a high level of degradation, altering the soil, relief, and landscape of the area (Rouhani *et al.*, 2023; Madhav *et al.*, 2024). Clay extraction is of great importance in the civil construction segment and generates employment; however, it is a major cause of environmental damage, with the soil being the main affected medium. This extractivism causes high levels of erosion, the opening of pits that are often abandoned with the end of extraction, and the formation of artificial lakes from these pits are common (Geller *et al.*, 2012).

With the end of the extractivist process in the area, it is essential to search for strategies that allow the area to return to conditions close to what it was originally; one of these strategies is natural regeneration (Fonseca *et al.*, 2017). Although the importance of restoring and preserving degraded areas is well known, studies that guide and evaluate the restoration of these areas are still insufficient due to the complexity of the ecological processes involved (Novak *et al.*, 2019).

When protecting the natural heritage, the Conservation Units (CUs) represent one of the best strategies. Each group of Conservation Units, either Integral Protection Units or Sustainable Use Units, and their respective categories present different objectives and established concepts, focusing on conservation, restoration, or the environmental recovery of degraded areas (Sessegolo, 2006). In areas with clay extraction, the CUs, especially the Private Natural Heritage Reserve (PNHR), an Integral Protection Unit, are a promising strategy in restoring the degraded ecosystem (Abrão and Marra, 2022).

Accurately quantifying the changes in C stocks according to land use change is of paramount importance in the search for climate change mitigation strategies, as well as in the decision-making process of the best type of management to be adopted (Powers *et al.*, 2011; Ozório *et al.*, 2019; Magalhães *et al.*, 2016). For this, it is necessary to measure and understand soil quality (SQ), specifically the processes involved in the flow and storage of C in the soil (Medeiros *et al.*, 2023).

In areas subject to scientific study, the SQ is measured through indicators, whether physical, chemical, or biological (Aratani *et al.*, 2009), sensitive to management changes (Vezzani and Mielniczuk, 2009; Maia and Parron, 2015). SOM meets the requirements of a good indicator. Its contents can be altered with greater or lesser intensity, depending on the management performed, with influence on the physical, chemical, and biological attributes of the soil, reflecting on the stability of the edaphic system in sustaining productivity, aligned with environmental sustainability (Babu *et al.*, 2023; Tonucci *et al.*, 2023).

The main constituent of SOM is C, which is why the main technique for measuring the SOM of an area is through soil organic carbon levels and stocks. The volume of C input to the soil is determined by the rates of deposition, decomposition, and renewal of SOM residues, while the volume of output is determined by the rate of mineralization and loss of C to the atmosphere (Chacon *et al.*, 2023; Reichenbach *et al.*, 2023).

Another effective technique to demonstrate the influence of the management system under the SQ is the chemical fractionation of the SOM (Rosset *et al.*, 2016; Silva *et al.*, 2020). With the evaluation of the C content of humic substances (HS), it is possible to identify the degree of stability of the SOM (Borges *et al.*, 2015; Knox *et al.*, 2015). The contents and proportions of C in each HS vary significantly depending on soil management and depth (Pfleger *et al.*, 2017), bringing detailed and conclusive results on the dynamics of SOM over time (Bernoux *et al.*, 1999; Pinheiro *et al.*, 2004).

Aggregate stability is also considered a sensitive physical indicator for assessing SQ, playing an important role in C sequestration (Tisdall and Oades, 1982; Tadini *et al.*, 2022). The quantitative analysis and interpretation of attributes related to aggregate stability are important in evaluating soil conservation status, enabling better management of the edaphic environment (Stefanoski *et al.*, 2013).

Studies that guide and evaluate the edaphic quality in cultivated soils and areas in the recovery process of sandy soils in the state of Mato Grosso do Sul are still incipient. Thus, the study hypothesized that different forms of land use and management can promote different changes in soil attributes. The process of natural regeneration can show significant recoveries in MOS contents and stocks and in the state of soil aggregation in the PNHR area in the short term. The present work aimed to quantify the contents of total organic carbon, carbon of humic substances, as well as evaluate the stability of aggregates of areas with traditional management systems, area in the process of natural regeneration and native area under sandy loam soil texture in the Brazil tropical region.

2. MATERIAL AND METHODS

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

The study was conducted with soil samples collected in four areas with different management systems and known history, located in the district of Porto Morumbi, municipality of Eldorado, Cone-sul planning region of Mato Grosso do Sul, Brazil (Figure 1).

The study areas are within the Environmental Preservation Area (EPA) of the Paraná River Islands and Floodplains (ICMBio, 2019), at 23°48' S and 54º06' W, with an average altitude of 272 meters. According to the Köppen classification (Peel *et al.*, 2007), the climate of the region is subtropical, Cfa-type, with an average temperature of the coldest month between 14 and 15ºC and precipitation ranging from 1,400 to 1,700 mm per year (Mato Grosso do Sul, 2015). The soils in the study area were classified as Argissolo Vermelho-Amarelo Distrófico, which have a sandy texture at the superficial horizons (Santos *et al.*, 2018). The classification corresponded with Paleudalfs in the USA Soil Taxonomy (USDA, 2014) or the Acrisols in the FAO classification system (FAO, 2015). Sandy texture, with 637, 251, and 112 g kg^{-1} of sand, silt, and clay, respectively (Santos *et al.*, 2018).

Three managed areas (two productive and one in the natural regeneration process) and an adjacent reference area of Native Forest (NF) without anthropic action, with Atlantic Forest vegetation and a Semideciduous Seasonal Forest physiognomy, were evaluated. The three managed areas comprise: permanent pasture with *Brachiaria brizantha* (PP), no-tillage in a succession of soybean (summer – first crop) and corn (second crop) (NT), and a Private Natural Heritage Reserve area in the process of natural regeneration with secondary vegetation (PNHR) (Figure 1). The respective use and management histories of the study areas are presented in Table 1, shown in images in Figure 1, and described according to the chronology of use in

Figure 2.

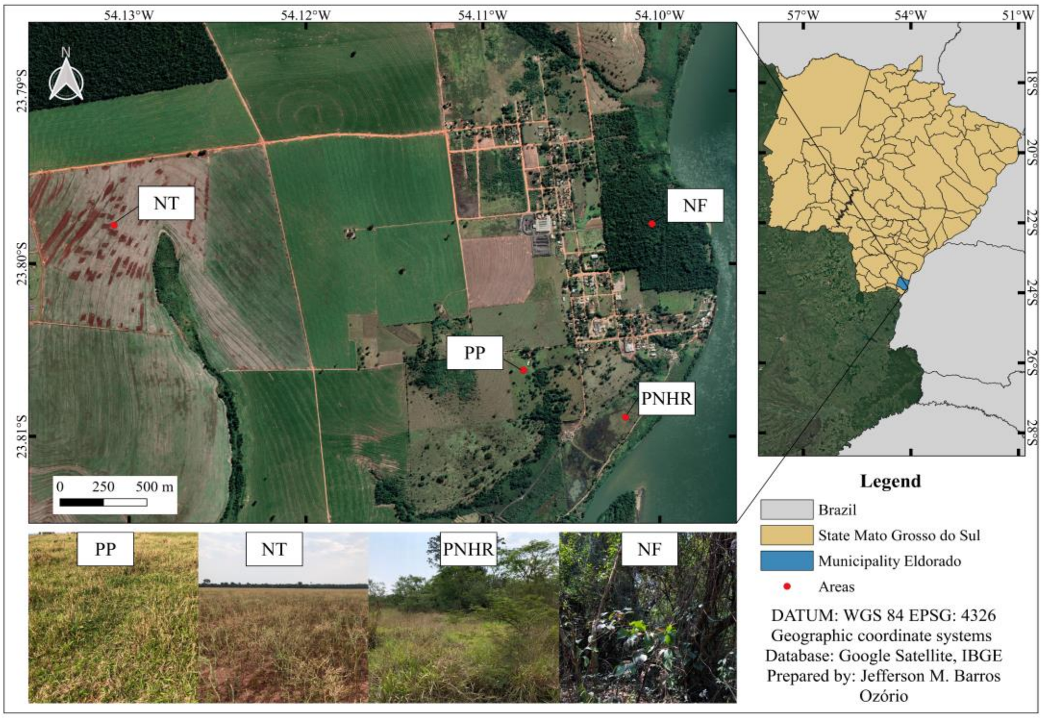

Figure 1. Geographic location of the study area, Eldorado, Mato Grosso do Sul, Brazil. Qgis 3.28.0 "Firenze". PP: Permanent pasture, NT: no-tillage, PNHR: Private Natural Heritage Reserve, NF: Native Forest.

Table 1. History and description of the change in management of the different study areas.

Area	Management history
PP	Area of 5 hectares, cultivated with *Urochloa brizantha* Hochst Stapf cv. for 12 years. The area is used for grazing beef cattle at a stocking rate of 1.2 animal units (AU) ha^{-1} with visible signs of degradation.
NT	Area of 50 hectares, cultivated with no-tillage in a succession of soybean (first-crop season - summer) and corn (second-crop season) cultures for the last 12 years.
PNHR	Area of 15 hectares. Private Natural Heritage Reserve - Forest remnant of the Atlantic Forest biome; area degraded due to clay extraction activity and in the process of natural regeneration for four years.
NF	Area of 20 hectares. The native vegetation of the Atlantic Forest - Semideciduous Seasonal Forest. It is considered in this study as the original soil condition without anthropic action.

PP: Permanent Pasture, NT: No-tillage, PNHR: Private Natural Heritage Reserve, NF: Native Forest.

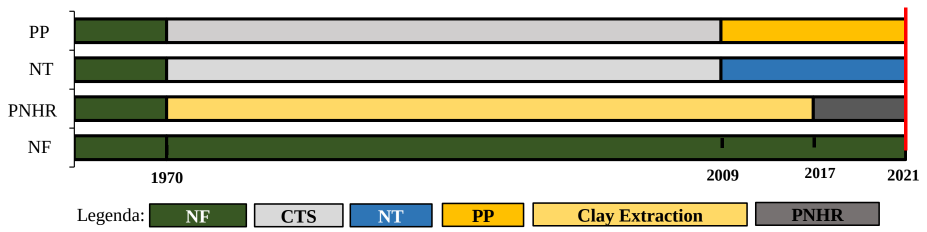

Figure 2. History of uses and changes in the use of the areas, with the respective implementation dates of each management system: NF: Native Forest; NT: no-tillage; PP: Permanent Pasture; PNHR: Private Natural Heritage Reserve, CTS: conventional tillage system.

2.2. Soil sample collection

For the collection of soil samples carried out in October 2021, five 400 m² plots were first demarcated in each area, each representing one pseudo repetition. Composite soil samples were collected in the layers of 0.00-0.05, 0.05-0.10, 0.10-0.20, and 0.20-0.40 m, with each composite sample represented by 10 single samples. After collection, the samples were air dried, crushed, and passed through a 2 mm sieve, thus obtaining fine air-dried soil (FADS). Unformed soil samples were also collected with a 100 cm³ volumetric ring for later bulk density (Bd) analysis in all the areas and layers mentioned above.

In addition, samples of plant litter deposited on the ground in the four study areas were collected with a collection frame with dimensions of 0.25 m^2 in five repetitions. Furthermore, soil monoliths were collected in the 0.00-0.05 and 0.05-0.10 m layers in five repetitions, with dimensions of 0.2 x 0.2 x 0.05 m, preserving their structure, for further analysis of aggregate stability. Notably, in the PNHR area, samples were collected around the lakes and in the furrows left by clay extraction.

2.3. Analyses performed

For the analysis of dry mass (DM), samples of plant litter were dried in an oven at 65ºC until a constant mass was obtained and then weighed on an analytical balance. For the Bd analysis, the undisturbed samples were dried in an oven at 105ºC for 24 hours and then weighed, with Bd calculation performed using the mass/volume ratio of the volumetric ring (Almeida *et al.,* 2017).

Total organic carbon (TOC) analysis was performed using the wet oxidation method of SOM by potassium dichromate in a sulfuric medium under constant heating, titrated with ferrous ammoniacal sulfate solution (Yeomans and Bremner, 1988). With the TOC and Bd contents, carbon stocks (Stock-C) were calculated using the equivalent mass method (Reis *et al.,* 2018; Signor *et al.,* 2014). Subsequently, the carbon stock variation (ΔStock-C) was calculated to verify trends in C accumulation or loss, obtained by the difference of the Stock-C of the system compared to the reference system (NF) and divided by the layer thickness. The stratification index (STRATI) was also calculated through the relationship between the TOC contents of the 0.00-0.05 and 0.20-0.40 m layers (Franzluebbers, 2002).

The chemical fractioning of the SOM was performed according to the differential solubility technique (Swift, 1996), as adopted by Benites *et al.* (2003), separating the fulvic acid (FA), humic acid (HA), and humin (HUM) fractions of each sample, and then determining the carbon content (C) of each fraction. From the C contents of HA and FA, alkaline extract (AE) (AE = HA+FA), and HUM, the HA/FA and AE/HUM ratios were calculated to verify the humification processes of the SOM. Stock-C calculations of each humic substance (HS) fraction were performed (Ellert and Bettany, 1995; Sisti *et al.*, 2004) and ΔStock-C to check the trends of C accumulation or loss of the fractions compared to NF.

To analyze aggregate stability, the collected monoliths were air dried, then manually disaggregated at the point of weakness. Sieving in a set of 8.00 mm and 4.00 mm sieves was performed, and 50 g of aggregates were removed from the fraction retained on the 4.00 mm sieve. They were saturated by capillarity for 5 minutes and sieved in water through a set of sieves with mesh sizes of 2.00, 1.00, 0.50, 0.25, and 0.125 mm, using the method described by Kemper and Chepil (1965) in a Yoder-type mechanical shaker (Yoder, 1936). Information such as weighted mean diameter (WMD), geometric mean diameter (GMD), sensitivity index (SI), and order level (OLev) were calculated according to the percentage of aggregates retained in each sieve class. With this data, the percentage of aggregate classes was also obtained, being divided into macro (>2.00 mm), meso (1.00+0.50+0.125 mm), and microaggregates (<0.125 mm) (Ozório *et al.*, 2024).

The results were analyzed for normality and homogeneity of variance using the Shapiro-

Wilk and Bartlett tests, respectively. Subsequently, the results were submitted to analysis of variance with application of the F test, and the means were compared to each other (one by one) using the 5% student t-test ($p < 0.05$) with the help of the R program (R Core Team, 2019).

3. RESULTS

The PP, NT, and PNHR areas had similar values of dry mass litter deposited on the soil, around 1300.00 kg ha^{-1}, representing 58% of the material found in the NF area, which had 2218.00 kg ha^{-1} (Figure 3A).

The PP and NT areas presented similar and higher Bd values throughout all layers evaluated, around 1.40 Mg m^{-3} at the surface, reaching 1.61 Mg m^{-3} at 0.20-0.40 m. Lower Bd values were found in the PNHR and NF areas, in the surface layer, with values of 1.19 and 1.25 Mg m^{-3}, respectively. In the layers, 0.05-0.10 and 0.10-0.20 m, both areas obtained values close to 1.40 Mg m^{-3}, and in the last layer evaluated, the PNHR area obtained 1.54 Mg m^{-3}, while NF, 1.28 Mg m^{-3}, being different from each other. There was a tendency for higher Bd values to occur as the depth increased, while the NF area showed lower Bd values in the 0.20-0.40 m layer (Figure 3B).

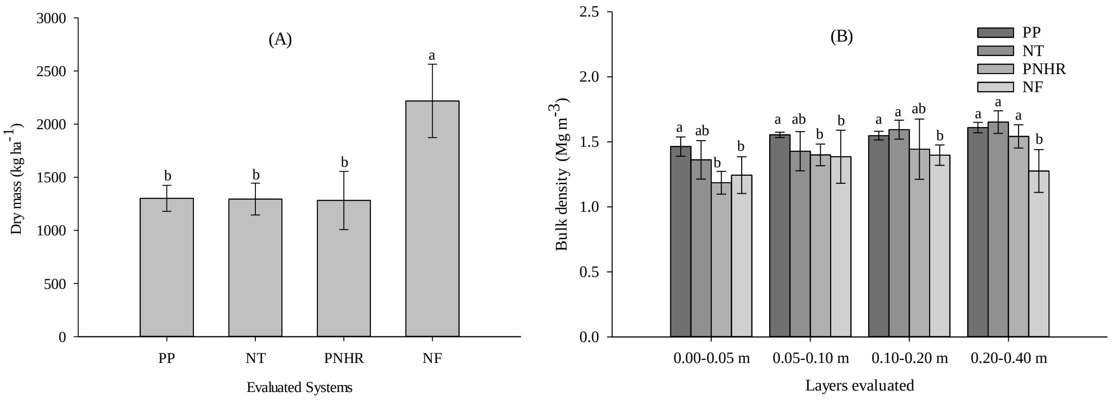

Figure 3. Dry mass of plant litter (A) and bulk density (Bd) of the different land use systems. PP: Permanent pasture, NT: no-tillage, PNHR: Private Natural Heritage Reserve, NF: Native Forest. Means followed by equal letters in each layer did not differ by the t student test ($p<0.05$).

In the superficial layer, the PNHR area showed the highest TOC content, 19.94 g kg^{-1}, followed by NF, 12.65 g kg^{-1} (Figure 4A). The contents were significantly lower in PP and NT in the same layer, around 6.60 g kg^{-1}. In the 0.05-0.10 m layer, the area of PNHR still obtained the highest TOC content. The NF area had a content of 6.79 g kg^{-1}, similar to the NT area. The PP area showed the lowest content in this layer, 3.80 g kg^{-1}, with a significant drop in TOC with increasing depth (Figure 4A).

In the 0.10-0.20 m layer, NT and NF maintained the contents near 6.00 g kg^{-1}, and PP and PNHR near 3.00 g kg^{-1}. In the 0.20-0.40 m layer, NT, PNHR, and NF had similar TOC contents. In all systems, TOC contents decreased with depth (Figure 4A).

The Stock-C (Figure 4B) generally followed the same patterns as the results of the TOC contents (Figure 4A). In the superficial layer, the area of PNHR showed the highest Stock-C, 24.84 Mg ha^{-1}, while NF had 15.76 Mg ha^{-1}. The PP and NT areas in the same layers obtained low Stock-C, with the NT area stocking around 8.00 Mg ha^{-1} in both layers and the PP area, 8.31 and 5.27 Mg ha^{-1} in the 0.00-0.05 and 0.05-0.10 m layers, respectively (Figure 4B).

In the 0.05-0.10 m layer, the NT, PNHR, and NF areas did not exhibit significant differences for Stock-C, while the PP area had the lowest Stock-C. In the 0.10-0.20 m layer, the PNHR area was similar to the PP area, with lower Stock-C, and in the 0.20-0.40 m layer, NT obtained lower Stock-C compared to NF (Figure 4B).

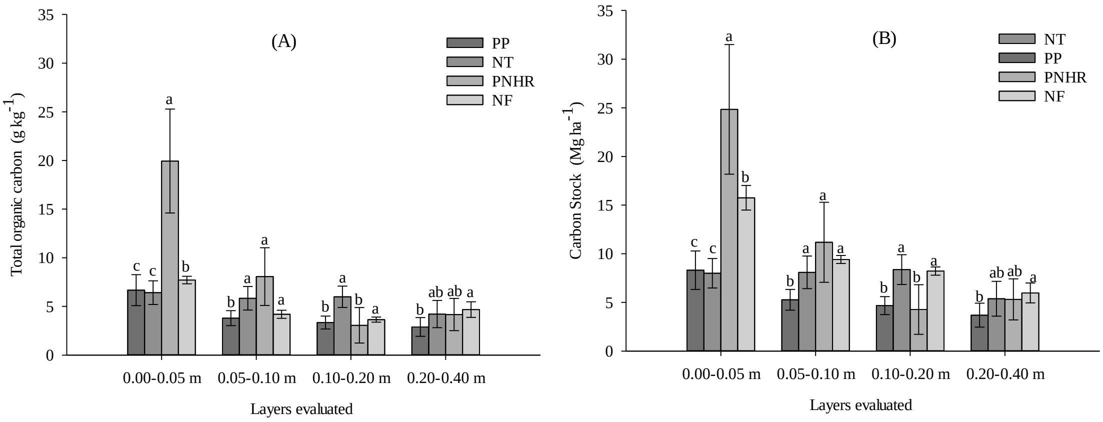

Figure 4. Total organic carbon content (A) and carbon stock (B) of the different land use systems. PP: Permanent pasture, NT: no-tillage, PNHR: Private Natural Heritage Reserve, NF: Native Forest. Means followed by equal letters in each layer do not differ by the t student test ($p<0.05$).

The highest STRATI observed was in the PNHR area, with a value of 5.28, while the lowest was in NT, 1.65, with the PP and NF areas having intermediate and similar values, 2.89 and 2.78, respectively (Figure 5A). For ΔStock-C, the PP and NT areas showed negative variation in almost all layers and the 0-0.40 m profile. The PNHR area showed positive variation in the 0.05-0.10 and 0.10-0.20 m layers and the 0-0.40 m profile (Figure 5B).

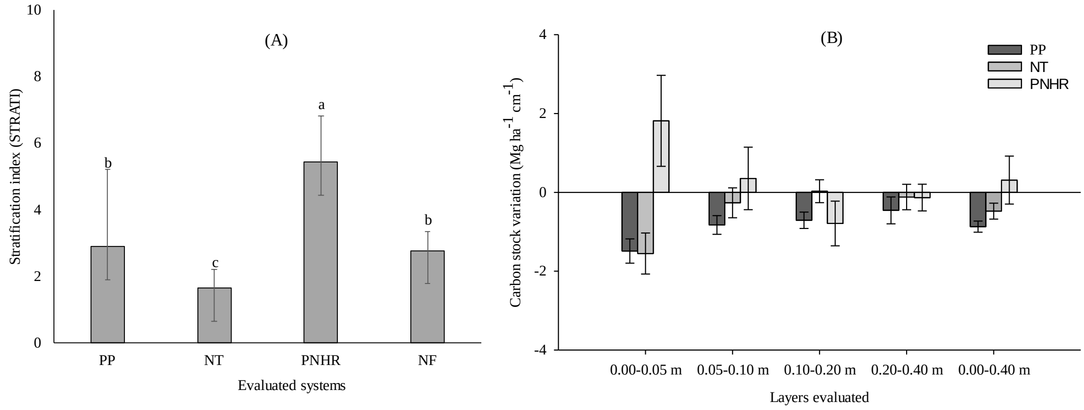

Figure 5. Stratification index of total organic carbon content (A) and carbon stock variation (B) of the different land use systems. PP: Permanent pasture, NT: no-tillage, PNHR: Private Natural Heritage Reserve, NF: Native Forest. Means followed by equal letters in each layer do not differ by the t student test ($p<0.05$).

Regarding the humic substances, in the 0.00-0.05 m layer, the highest levels of C-FA were found in the PNHR and NF areas, 2.80 and 2.54 g kg^{-1}, respectively. In the other layers evaluated, only the area of NF obtained higher contents, ranging from 2.42 to 1.91 g kg^{-1} (Table 2). The C-HA contents in the surface layer were highest in the PP, NT, and PNHR areas, near 2.65 g kg^{-1}, and the lowest in PP, 1.20 g kg^{-1}. In the following layers, PP and NF were the areas with the highest C-HA contents, and in all layers, there was a similarity in the C-HA contents between these two areas Table 2.

The most recalcitrant C fraction (C-HUM) was the one that predominated over the others in all layers. In the surface layer, the PNHR and NF areas had considerably higher levels of C-HUM, 18.12 and 11.36 g kg^{-1}, respectively, while PP and NT had levels close to 7.50 g kg^{-1}. Similar to the TOC and Stock-C results, the TOC-HUM contents of the PNHR and NF areas, with contents close to 6.0 g kg^{-1} in the 0.05-0.10 m layer, representing only 33% and 53% of the content found in the previous layer, respectively. The PP and NT areas obtained contents of 5.69 and 7.25 g kg^{-1} in the same layer, respectively. In the 0.10-0.20 and 0.20-0.40 m layers, the PP, NT, and NF areas were similar concerning the C-HUM contents (Table 2).

Table 2. Carbon content of the fulvic acid (C-FA), humic acid (HA), and humin (HUM) fractions, carbon stock of the fulvic acid (StockC-FA), humic acid (StockC-HA), and humin (StockC-HUM) fractions, C-HA/C-FA and AE/C-HUM ratios of the different land-use systems.

MS	C-FA	C-HA	C-HUM	StockC-FA	StockC-HA	StockC-HUM	C-HA/C-FA	AE/C-HUM
	g kg^{-1}			Mg ha^{-1}				
				0.00-0.05 m				
PP	1.36b	2.09a	7.56c	1.70b	2.61a	9.42c	1.54a	0.53a
NT	1.22b	1.20b	7.26c	1.52b	1.49b	9.04c	1.01b	0.35a
PNHR	2.80a	2.67a	18.12a	3.49a	3.33a	22.57a	0.94b	0.33a
NF	2.54a	2.39a	11.36b	3.16a	2.98a	14.15b	0.96b	0.45a
				0.05-0.10 m				
PP	1.07b	1.47ab	5.69a	1.49b	2.04b	7.88b	1.39a	0.54ab
NT	1.18b	1.11b	7.25a	1.64b	1.54b	10.05a	0.98a	0.34b
PNHR	1.32b	1.06b	6.04a	1.83b	1.46b	8.37ab	0.78a	0.44ab
NF	2.42a	1.84a	6.11a	3.35a	2.55a	8.47ab	0.78a	0.88a
				0.10-0.20 m				
PP	0.90b	1.43a	4.62a	1.26bc	2.00a	6.46b	1.68a	0.58ab
NT	1.14b	0.84b	6.77a	1.60b	1.17b	9.45a	0.75a	0.36b
PNHR	0.86b	0.70b	2.10b	1.20c	0.98b	2.94c	0.85a	0.87a
NF	1.98a	1.42a	5.16a	2.77a	1.98a	7.21b	0.72a	0.68a
				0.20-0.40 m				
PP	0.73c	1.06ab	5.25a	0.93c	1.35a	6.72ab	1.45a	0.37b
NT	1.03b	0.83b	6.51a	1.32b	1.05ab	8.27a	0.83a	0.36b
PNHR	0.77bc	0.72b	2.29b	0.99c	0.91b	2.89c	0.97a	0.70a
NF	1.91a	1.32a	4.49a	2.44a	1.68a	5.69b	0.69a	0.78a

Means followed by equal letters in the column in each layer do not differ by the t student test ($p \leq 0.05$). MS: Management system, PP: Permanent pasture, NT: no-tillage, PNHR: Private Natural Heritage Reserve, NF: Native forest.

The StockC-FA in the 0.00-0.05 m layer was higher in PNHR and NF, 3.49 and 3.16 Mg ha^{-1}, respectively, and lower in PP and NT. In the next layer, PP, NT, and PNHR areas were similar for StockC-FA, ranging from 1.49 to 1.83 Mg ha^{-1}, while the NF area had the highest StockC-FA, 3.35 Mg ha^{-1}. Similar behavior of the previous layer, at 0.10-0.20 m, but the managed areas showed values ranging from 1.20 to 1.60 Mg ha^{-1}, while NF had StockC-FA of 2.77 Mg ha^{-1}. In the 0.20-0.40 m layer, the PP and PNHR areas obtained the lowest values of StockC-FA, 0.93 Mg ha^{-1}, and 0.99 Mg ha^{-1}, respectively, differently from that observed in NF, 2.44 Mg ha^{-1} (Table 2).

The PP, PNHR, and NF areas obtained the highest StockC-HA in the surface layer, ranging from 2.61 to 3.33 Mg ha^{-1}, while NT had 1.69 Mg ha^{-1}. As with the results of StockC-FA, in the layer 0.05-0.10 m, the values of StockC-HA did not differ in PP, NT, and PNHR. In the 0.10-0.20 m layer, the PP and NF areas showed the highest StockC-HA, 2.00 and 1.98 Mg ha^{-1}, respectively, while NT and PNHR stored 1.17 and 0.98 Mg ha^{-1}, respectively. At 0.20-0.40 m, PP and NF had the highest StockC-HA, 1.68 and 1.35 Mg ha^{-1}, respectively, and the PNHR area had the lowest StockC-HA, 0.91 Mg ha^{-1} (Table 2).

There was a high variation in the StockC-HUM according to the layer evaluated, especially in the PNHR area. In the 0.00-0.05 m layer, the PNHR obtained the highest StockC-HUM, with 22.57 Mg ha^{-1}, reaching 2.90 Mg ha^{-1} in the 0.20-0.40 m layer, equivalent to only 12.84% of the value observed in the surface layer (Table 2). The NF area had the second highest StockC-HUM in the surface layer, with 14.15 Mg ha^{-1}, while PP and NT obtained stocks of 9.04 and 9.42 Mg ha^{-1}, respectively.

For the HA/AF ratio, there was only a difference between the areas in the 0.00-0.05 m layer. PP showed the highest ratio, 1.54; PP was the only area with values higher than 1.00, with a predominance of the HA fraction concerning FA. Observing the AE/C-HUM ratio, the value in all areas was lower than 1.00, indicating a predominance of C-HUM concerning AE (Table 2).

For the 0-0.40 m section, the NT and PNHR areas showed positive variation in the StockC area for HUM. In the PP area, there was a negative variation in the Stock-C for all organic fractions (Figure 6).

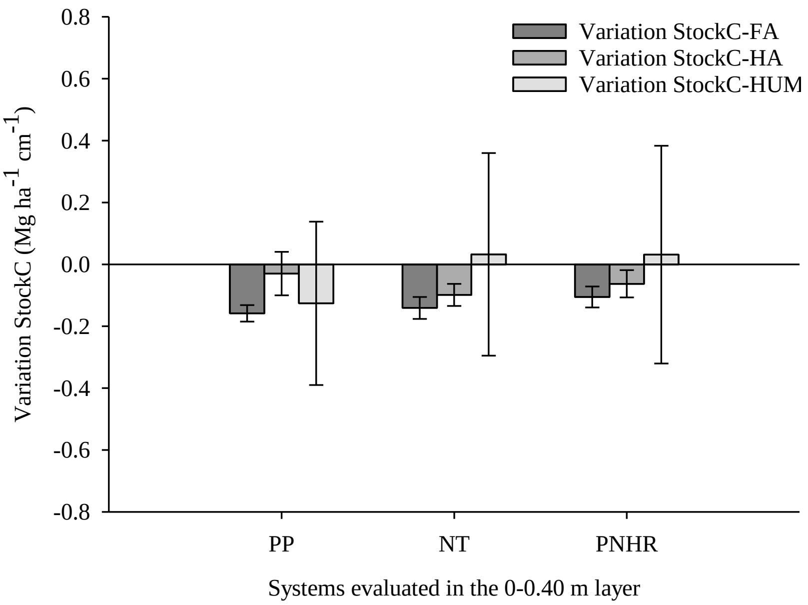

Figure 6. Variation of áreas no stock of humic substances of the different land-use systems. PP: Permanent pasture, NT: no-tillage, PNHR: Private Natural Heritage Reserve, according to the reference area.

Generally, aggregates were more stable in the PP, PNHR, and NF areas. In the 0.00-0.05 m layer, we observed WMD values close to 4 mm and GMD values close to 3 mm, while the same indexes in the NT area were close to 2 and 1 mm, respectively (Figure 7). In the 0.05-0.10 m layer, the PP and NT areas showed similar WMD and GMD values compared to the 0.00-0.05 m layer, while in PNHR and NF, there was a decrease in values, but still similar to PP (Figure 7B).

The PP and PNHR areas obtained SI close to 1.00 (NF reference value) in both layers, being similar, while NT obtained about half the value, 0.6 (Figures 8A and B). In both layers, the area with the highest OLev was PNHR, 200 and 100, respectively, followed by NF, 120 and 100. The other areas, PP and NT, had considerably lower OLev, 60 and 40, respectively, related to the low levels of Stock-C in these areas (Figures 8A and B).

The predominant aggregate class in the PP, PNHR, and NF areas were the macroaggregates (>2.0mm), around 80%. In NT, the predominant aggregates were mesoaggregates (0.25 to 2.00mm), around 40%, followed by macroaggregates. For the microaggregates (<0.25mm), there was a representation on the order of 20%, with NT having the highest percentage (Figures

9A and 9B).

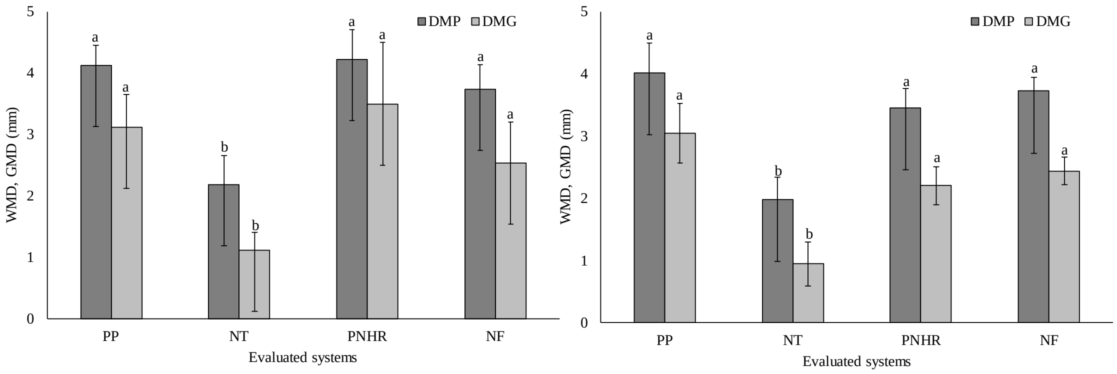

Figure 7. Weighted mean diameter and geometric mean diameter of aggregates in the 0.00-0.05 m (A) and 0.05-0.10 m (B) layers of the different land use systems. PP: Permanent pasture, NT: no-tillage, PNHR: Private Natural Heritage Reserve, NF: Native Forest. Means followed by equal letters in each layer do not differ by the t student test (p≤0.05).

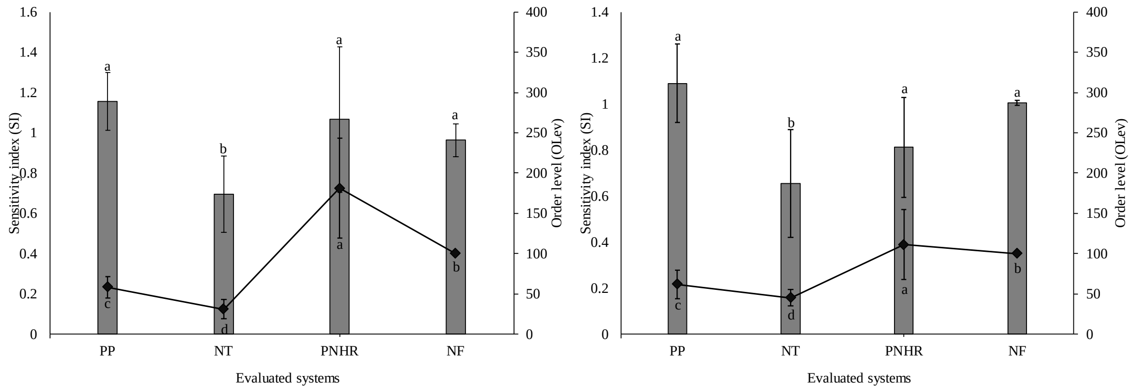

Figure 8. The sensitivity index (SI) and order level (OLev) of the 0.00-0.05 m (A) and 0.05-0.10 m (B) layers of the different land use systems. PP: Permanent pasture, NT: no-tillage, PNHR: Private Natural Heritage Reserve, NF: Native Forest. Means followed by equal letters in each layer do not differ by the t student test (p<0.05).

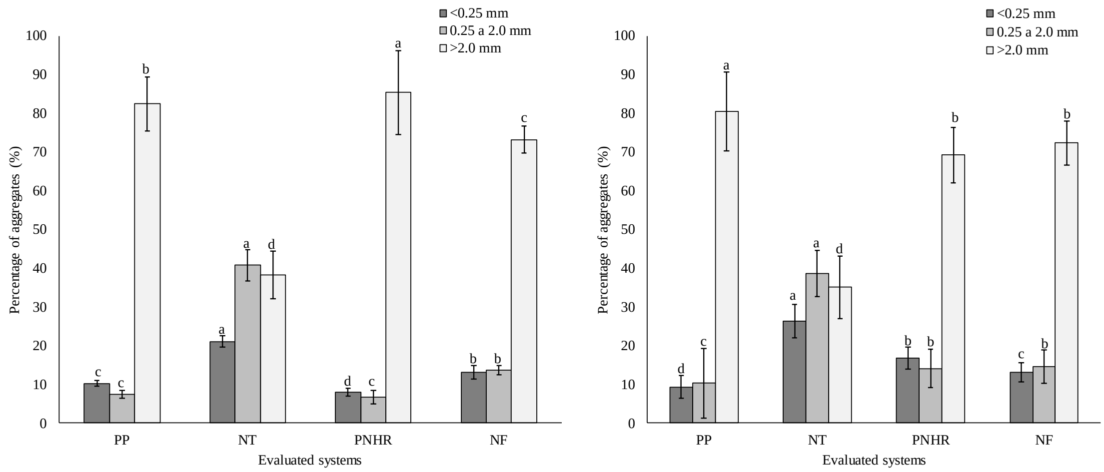

Figure 9. Percentage of aggregates retained in different size classes in the layers 0.00-0.05 m (A) and 0.05-0.10 m (B) of the different land-use systems. PP: Permanent pasture, NT: no-tillage, PNHR: Private Natural Heritage Reserve, NF: Native Forest. Means followed by equal letters in each layer did not differ by the t student test (p<0.05).

4. DISCUSSION

The area of NF showed a higher contribution of plant litter than the managed areas and the area under the natural regeneration process. This is mainly because it is an area with a low degree of permanent human interference over decades. This fact is considered to reflect the abundance of large vegetation with dense canopy, where there is a greater contribution of organic material deposited on the soil (Nunes and Pinto, 2007). Plant litter is an environmental indicator, making it possible to detect and compare the amounts of DM that areas with different vegetation contribute to the soil (Machado *et al.*, 2008). This variable can also indicate the evolution of areas in recovery through natural regeneration (Silva *et al.*, 2018).

None of the areas presented Bd with the capacity to restrict the root growth of the implanted species. The plants in sandy/medium texture soils present growth restriction in Bd above 1.75 Mg m^{-3} (Reinert *et al.*, 2008). The influence of management on soil structure can be observed in the PP and NT areas, as they present higher NT values in the reference area. Higher Bd in the PP area can be attributed to the fact that the pasture is degraded (low percentage of soil cover, evidence of preferential erosion gullies, and high presence of weeds), being vulnerable to the compaction process over the years of grazing, even with animal stocking of only 1.2 AU ha^{-1}.

In studies by Martins *et al.* (2020), the authors compared soil physical attributes of degraded pastures with recovered pastures and concluded that pasture degradation results in increased Bd, reducing total porosity and macroporosity. Another fact associated with this is the soil trampling by animals. While in the NT area, the frequent machinery traffic in sowing, spraying, and harvesting operations may have led to increased Bd (Rosset *et al.*, 2014; Lopes *et al.*, 2022b).

In unmanaged soils, Bd varies due to intrinsic soil characteristics and pedogenetic processes. Among the factors that influence soil structure, texture is the one that most influences physical behavior due to the distinct characteristics and behaviors that soil mineral particles present. Soils with high sand content tend to have higher Bd values because they have low microporosity (Pádua *et al.*, 2015; Marcolin and Klein, 2011).

In all areas, the highest TOC contents were found in the 0.00-0.05 m layer, decreasing with increasing depth. The same behavior was found in the study of Farias *et al.* (2022); Lopes *et al.* (2022a; 2022b), Rosset *et al.* (2022), and Pinto *et al.* (2023). The management defines the contribution of plant biomass that will be deposited in the soil, and the C in deeper layers comes from the decomposition of plant roots. Thus, it is favorable to use species that produce abundant straw and vigorous root systems to favor the contribution of C at depth (Salton and Tomazi, 2014).

Evaluating the systems against each other, it is evident that the different land-use types influence TOC and Stock-C contents, as seen in other studies (Rosset *et al.*, 2014; 2022; Azevedo *et al.*, 2018; Troian *et al.*, 2020; Lopes *et al.*, 2022a; 2022b). The areas of PNHR and NF, where there are no agricultural activities, showed the highest levels of C and Stock-C due to the absence of human interference, especially the lack of soil disturbance, which preserves the C compartment. Other studies showed similar results, i.e., unmanaged areas with higher C inputs than managed areas (Azevedo *et al.*, 2018; Troian *et al.*, 2020).

Furthermore, Pinheiro *et al.* (2021) results showed greater stabilization of organic material in forest areas when compared to pasture areas. There is a tendency to increase soil fertility, mainly due to increased C sequestration, via stabilized material. Protecting forested areas is an efficient way to ensure, in the long term, a positive gradient of C incorporation in these areas (Azevedo *et al.*, 2018).

The PNHR area showed higher contents and Stock-C in the superficial layers than the NF area. This shows that the natural regeneration of the area is advancing after four years, allowing more organic material to be deposited in the soil to be decomposed, which can also be proven by the area's higher STRATI. This is because the first years of regeneration are filled with primary vegetation with short cycles, which decomposes quickly (Ozório *et al.*, 2019). In a study carried out by Novak *et al.* (2019), the authors concluded that in areas of natural regeneration where any agricultural management was terminated, the vegetation and all its diversity gradually returned, occurring with greater deposition of plant residues of varied composition, incorporating MOS and, consequently, improving the attributes and SQ (Novak *et al.*, 2019). Using forest recomposition, Santos *et al.* (2021) observed an increase in Stock-C levels after one year of the recovery process of a degraded area in the municipality of Mundo Novo, in the Cone-Sul region of Mato Grosso do Sul.

Different results were seen in a study by Lopes *et al.* (2022b) in the same study area two years earlier, where the PNHR area obtained significantly lower C contents. This fact demonstrates that studies on the contribution of C to the soil according to the time are necessary to evaluate the effects of sequestration and fixation of C in the soil (Azevedo *et al.*, 2018).

It is important to highlight that the area in the recovery process showed higher Stock-C concerning the NF area, while PP and NT had lower Stock-C. Some studies prove the potential that pasture areas and no-tillage on straw present to increase soil Stock-C, even surpassing those of native areas when properly managed (Salton *et al.*, 2011), but with a slow, gradual increase (Rosset *et al.*, 2014). Such results suggest that both areas are not being managed in a way that provides the potential for C sequestration and incorporation into the soil, as was seen in the work of Troian *et al.* (2020) and Rosset *et al.* (2014).

By evaluating the data from this study over time with those from Lopes *et al.* (2022a), which were carried out in the same area, it was possible to prove in the NT area that crop succession did not increase C storage. The conversion of areas with less floristic heterogeneity for areas with crop rotation systems, such as no-till system (NTS), promotes a greater contribution of SOM to the soil and, consequently, an increase in TOC contents over the years of cultivation (Rosset *et al.*, 2016), a fact not observed in this NT area two years after the first evaluation.

In the NT area, there is only soybean (first-crop - summer) and corn (second-crop) in succession, not fitting into NTS, which has crop rotation as one of the premises, a system that promotes a greater contribution of organic material to the soil. In addition, between the implementation of a corn crop and the soybean crop, which occurs in mid-October, the NT area remains fallow for 60 days, which causes the decomposition of the already developing plant material of the corn crop straw, revealing portions of the area with exposed soil. According to Nunes *et al.* (2006), seeding on straw implies knowledge and correct definition of the cover species. The crop must have good biomass production and be persistent in physically protecting the soil and nutrients.

A good alternative to increase straw production would be to change the succession system to rotation or to introduce one more crop in the succession system, such as a forage crop alone or intercropped with corn. The cultivation of winter forage can contribute by increasing the plant remains kept on the soil surface, providing physical protection, increasing organic matter, maintaining soil moisture, and reducing weeds (Vernetti Junior *et al.*, 2009).

The PP area, due to the stage of soil degradation, did not show an increase in Stock-C two years after the first evaluation because pasture in this condition presents significant losses of C to the atmosphere (Lopes *et al.*, 2022b). However, properly managed pastures can effectively accumulate C in the soil, mainly due to the root system and the high contribution of plant material that pastures bring to the soil (Salton *et al.*, 2011), a fact observed when recovering pastures also in the Cone-sul region of Mato Grosso do Sul (Martins *et al.*, 2020; Lal *et al.*, 2007).

The area of PNHR showed higher STRATI than the area of NF, indicating a greater contribution of C at the surface. Greater C stratification indicates that there is a continuous entry of C in the area, increasing the TOC contents of the surface layer over time (Troian *et al.*, 2020), which can be proven by comparing with the study of Lopes *et al.* (2022b) in the same area in a previous period of two years. While the STRATI of the PP area remained similar to the reference area, PP and NF did not show similar C concentrations, indicating only the same level of C stratification in the different layers. The NT area obtained the lowest STRATI, indicating low SOM input due to the crops used in the succession system.

Several studies have proven that having a higher proportion of C in the HA fraction is more common than FA (Piccolo *et al.,* 2002; Rosset *et al.,* 2016). Areas of NT and NF obtained higher contents of C in the FA fraction compared to HA, i.e., C-HA/C-FA ratio lower than 1.00. These results indicate that the SOM of these areas has less stability because the FA fraction is the most soluble and mobile in the soil, being easily polymerized or mineralized, changing it quantitatively in the soil (Steverson, 1994; Lopes *et al.*, 2022a). The area with the highest SOM stability was the PP, indicating that the system favors the formation of the most stable fractions, probably because the organic material comes only from grasses (Fontana *et al.,* 2006).

In all areas and layers, HUM was the predominant fraction. The same was seen in other studies in the literature (Fontana *et al.,* 2006; Rosset *et al.,* 2016; Lopes *et al.,* 2022a). Moreover, the AE/HUM ratio lower than 1.00 indicates a greater proportion of the insoluble fraction in the soil compared to the soluble fraction (FA and HA). This fact indicates that the SOM tends to stabilize over time. The same was seen in the work of Rosset *et al.* (2016), concluding that not tilling the soil implies greater stability of C, with a predominance of the HUM fraction over the years of cultivation.

High WMD, GMD, and SI values in PP, PNHR, and NF indicate that these areas favor the soil ability to maintain structural stability. This fact can be associated with the high rate of water-stable macroaggregates and higher TOC contents (Loss *et al.*, 2015). In a study by Novak *et al.* (2019), with an evaluation of areas in natural recovery processes, the authors also observed better results of soil structure conservation. In a study by Cunha Neto *et al.* (2018), forest and pasture areas were the systems that presented the best aggregate stabilization. In the PP area, structural stability can be attributed to the influence of *Brachiaria* roots, even though the area is degraded, because the grass root system is the main particle aggregating agent in tropical soils, as well as the characteristics of the organic matter (Salton *et al.,* 2014), a fact proven in a study by Brandão and Silva (2012).

In the PNHR area, the factor attributed to soil aggregation is the increase in TOC content over the years of natural regeneration in the layer 0.00-0.10 m. The increment of C to the soil is fundamental for the formation of microaggregates, resulting in the formation of macroaggregates over the years (Tisdall and Oades 1982), add to this the natural regeneration and the interruption of clay extraction as occurred in years in previous decades.

The low aggregate stability in the NT area evidenced by the indicators WMD, GMD, SI, and OLev may be associated with the fact that there is no greater diversification of species implemented in the crop rotation system, with only the succession of soybean and corn crops, not fitting as an NTS, a system that provides an improvement in the state of soil aggregation, that at the same time increases the TOC content of the soil (Rosset *et al.* 2014; 2019). In addition, crop rotation provides various root systems in the area, improving the soil physical structure (Becker *et al.,* 2022). Thus, greater vegetation diversity favors physical and chemical processes related to soil aggregation (Loss *et al.,* 2015).

5. CONCLUSIONS

Despite the short time in the natural regeneration process, the Private Natural Heritage Reserve shows significant improvements in soil quality, especially in the surface layers, which are more sensitive to management changes, ensuring structural stability similar to that of the native forest. This shows that, after four years of natural regeneration, several important soil attributes begin to show qualitative improvements, with consequent benefits for the soil and environmental quality of the area, proving the efficiency of the natural regeneration process for recovering soil carbon stocks in areas previously used for clay extraction.

Due to land use and management practices, the areas with permanent pasture and direct sowing evaluated in this study showed slow carbon sequestration and storage potential compared to the reference and natural regeneration areas.

The area of permanent pasture contributes to the formation of stable aggregates, even with low carbon contents, indicating that the formation of aggregates is related to the characteristics of the carbon contributed.

The no-till area with only successive crops of soybeans and corn on sandy loam soil did not provide favorable conditions for improving the structural quality of the soil over the years of cultivation in the area evaluated.

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