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Stability, labile organic carbon, and glomalin of biogenic aggregates in sandy soils under management systems in the subtropical region of Brazil

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ABSTRACT: Soil aggregates and their stability affect a wide range of soil properties. This study aimed to (a) verify whether biogenic aggregation provides higher macro- and microaggregate stabilization, and (b) evaluate whether biogenic aggregates are associated with higher labile organic carbon and glomalin contents. Three management systems were evaluated (permanent pasture, PP; no-tillage system, NT; and no-tillage + Brachiaria system, NT+B) as well as a reference area (Atlantic Forest biome vegetation, NF). According to their origin or formation pathway, the aggregates were separated, identified, and classified as biogenic (formed by biological processes) and physicogenic (resulting from chemical and physical actions). The PP system provided the greatest stabilization of the macroaggregates, regardless of the formation pathway, as reflected by a greater mean weight diameter (MWD). The PP system also influenced the degree of microaggregate stability by increasing the bond strength and reducing the dispersion of the clay fraction. Finally, the PP system elevated the contents of labile organic carbon (POXC), easily extractable glomalin (GRSP-EE), and total glomalin (GRSP-T) under both formation pathways. The NT+B system favored the stabilization of macroaggregates, especially in the subsurface soil layer, compared with the NT area. In the aggregates of the NT and NT+B areas, the highest values observed were for water-dispersible clay (WDC) and the lowest values observed were for non-dispersible clay (NDC), a pattern opposite to that observed in the aggregates of the PP and NF areas. In the biogenic aggregates of all areas, a high POXC content was quantified, and biogenic aggregation proportionally increased the values of MWD, GRSP-EE, and GRSP-T relative to physicogenic aggregation. The results showed that grain production systems, pasture systems, and non-anthropized environments differentially influenced aggregation and the concentrations of organic fractions associated with aggregate stability. This study highlights the need for future studies using these indicators to monitor the quality of soils, especially those with sandy texture, which are considered more fragile.

Keywords: soil aggregate formation pathway, physicogenic aggregates, no-tillage system.

INTRODUCTION

Over the past 20 years, soil protection research has focused on frequently discussed issues, such as the intensification of erosive processes, structural degradation, potentially toxic elements, and reduced soil biodiversity and soil organic matter (SOM) content. These adverse factors lead directly to problems such as soil degradation and reduced natural fertility, culminating in a decrease in the soil productive capacity (Holátko et al., 2021). Soil quality is defined as the soil ability to maintain or improve water and air quality, as well as to promote the health of plants and animals (Doran, 2002). Soil aggregation and SOM content are the main indicators of soil quality and the environmental sustainability of agricultural practices.

Aggregates and their stability influence a wide range of soil properties, including carbon stabilization, porosity, water infiltration and aeration, compaction and densification, water retention, hydraulic conductivity, and the ability to resist erosion (Cheng et al., 2015). Many researchers (Six et al., 2000, 2004; Bronick and Lal, 2005; Briedis et al., 2012; Tivet et al., 2013; Melo et al., 2019a, 2021; Lavelle et al., 2020; Pereira et al., 2021) consider aggregate stability as a reflection of soil structure and, consequently, of edaphic quality in general because it depends on an integrated balance between chemical, physical, and biological factors. A better understanding of the balance and interactions between these factors can improve our understanding of soil restoration and/or recovery, and thus the ability of soils to perform services ranging from food production to services of an ecosystemic nature (Lavelle et al., 2020).

Conceptually, soil aggregation processes can be explained via the hierarchical organization of different aggregation stages (Tisdall and Oades, 1982; Oades, 1984) in which aggregates are sequentially formed. First, microaggregates are formed, which subsequently act as the raw materials for macroaggregate formation (Six et al., 2004). When considering the origin or formation pathway of soil aggregates, they can be classified either as aggregates that are biogenic or of biogenic origin (i.e., formed by biological processes, with emphasis on the activity of roots and meso- and macrofauna), or as aggregates that are physicogenic or of physicogenic origin (i.e., resulting from chemical and physical processes, such as the formation of organo-mineral complexes, flocculation, and drying and wetting cycles) (Pulleman et al., 2005; Velasquez et al., 2007; Batista et al., 2013; Loss et al., 2014).

Some studies have evaluated the existence of a third class of aggregates (the intermediates) and their influence on soil properties (Pulleman et al., 2005; Batista et al., 2013; Ferreira et al., 2020). However, a specific origin or formation pathway has not yet been identified for this aggregate class (Pereira et al., 2021) because it commonly encompasses aggregates that cannot be classified as biogenic or physicogenic. For the more established aggregate classes, physical, chemical, and biological analyses are often performed to understand the contribution of the aggregation pathways to soil properties. In addition to compiling studies that have reported improvements in soil attributes due to biogenic aggregation, Pereira et al. (2021) also suggested the use of other methods or indicators that can be applied to evaluate soil structure and/or aggregates.

Macroaggregates stability is commonly determined by wet sieving based on the early study by Yoder (1936), Kemper and Koch (1966), and Kemper and Rosenau (1986), and it is represented by the mean weight diameter (MWD). Microaggregate stability is usually evaluated using traditional methods, such as the mechanical analysis of water-dispersible clay (Rengasamy, 2002). Recently, Melo et al. (2019a) proposed a method that quantifies three clay classes in soil, which are classified according to their structural behavior. According to their analysis, they obtained the following classes: water-dispersible clay (WDC), which is released from the aggregates and remains dispersed after a certain period (period that can vary depending on the behavior of the released clay); water-re-flocculable clay (WRC), which is released from the soil aggregates, but due to the characteristics of the particles (e.g., high zero charge point) or the conditions of the medium (e.g., high electrolyte concentration), it tends to flocculate; and non-dispersible clay (NDC), which is not released from soil aggregates after the application of disruptive forces. This method has been shown to improve the accuracy of evaluating microaggregate stability and the understanding of clay flocculation phenomena (Melo et al., 2019a; 2021).

The functionality of SOM depends on the contributions (mainly in terms of quality and quantity) of different organic materials and fractions to the soil. For example, the concentration of labile organic carbon (extracted with permanganate; POXC) in soil generally varies more than the concentration of total organic carbon (Culman et al., 2012a), and it is more closely associated with mineralization, nutrient availability, and aggregate stability. Extracted with permanganate is used for studies on such characteristics because it is a sensitive indicator of stabilization dynamics (e.g., long-term carbon sequestration) (Hurisso et al., 2016) and SOM mineralization (e.g., short-term nutrient availability) (Culman et al., 2013). Another important organic fraction usually applied as an indicator of SOM dynamics is glomalin, which represents a stable form of carbon storage in the soil (Rillig, 2004). Glomalin plays a crucial role in stabilizing soil aggregates and soil organic carbon (Wilson et al., 2009; Holátko et al., 2021).

Glomalin is a hydrophobic glycoprotein produced by the hyphae of arbuscular mycorrhizal fungi, specifically on the intra-radicular hyphae on roots and on the surface of extra-radicular hyphae in the rhizosphere. To a certain extent, glomalin can also be released from the mycelial surface into the soil (the external mycelium is also responsible for the exudation of glomalin, or incorporation into the cell walls and spores) (Wright et al., 1996; Gao et al., 2019; Holátko et al., 2021). Glomalin is operationally defined and extracted from soil as “glomalin-related soil protein” (GRSP) (Rillig, 2004). GRSP is considered as a microbial “glue” that contributes to the formation and stabilization of soil aggregates (Wright et al., 2007). Numerous studies have investigated the influence and action of GRSP on aggregates of different sizes (Wright et al., 2007; Wilson et al., 2009; Gao et al., 2019; Santos et al., 2020; Holátko et al., 2021). However, there have been no studies evaluating the effects of this binding agent on soil aggregate formation pathways, nor on its efficiency in validating the separation of aggregate classes.

The role of WDC, WRC, NDC, and GRSP indicators in stabilizing aggregates of different origins in agricultural areas is still poorly understood. This study thus uses these indicators in conjunction with those for macroaggregation (i.e., MWD) and SOM (i.e., POXC) to obtain a better understanding of the genesis of aggregates and to evaluate the changes in the structural units (i.e., biogenic and physicogenic aggregates) when subjected to different types of management. Based on the above information and on the hypothesis that soil management systems (e.g., pasture and grain production systems) promote changes in the state of aggregation and the concentrations of organic fractions associated with biogenic and physicogenic aggregate stability in sandy-textured soils, this study aimed to (a) verify whether biogenic aggregation provides better macro- and microaggregate stabilization; and (b) evaluate whether biogenic aggregates are associated with higher labile organic carbon and glomalin contents.

MATERIALS AND METHODS

Location, climate, and soil of the study area

The study was conducted in the municipality of Terra Roxa, located in the west of the state of Paraná (southern Brazil) at coordinates 24° 11’ 34” S and 54° 06’ 62” W, and an average altitude of 319 m. The climate of the region is humid subtropical with hot summers (Cfa) according to Köppen’s classification system (Alvares et al., 2013). The soil in the study area is classified as *Argissolo Vermelho-Amarelo Distrófico*, with a sandy texture in the superficial horizons (Santos et al., 2018). This type of soil corresponds to Paleudalfs in the USA Soil Taxonomy (Soil Survey Staff, 2014) or Acrisols in the FAO classification system (IUSS Working Group WRB, 2015).

History of the evaluated areas

Three areas under different management systems and a reference area were evaluated: i) permanent pasture (PP); ii) no-tillage system (soybean/corn succession) (NT); iii) no-tillage + Brachiaria system (corn and Brachiaria ruziziensis intercropping, succession with soybean) (NT+B); and iv) Atlantic Forest biome vegetation (FN). The history, descriptions, and locations of the sample areas are presented in table 1, and the characterization of the physical and chemical properties down to a depth of 0.10 m are shown in table 2. Sampling was conducted in August 2020. In the NT and NT+B areas, corn (*Zea mays* L.) was the annual crop that preceded the collection period, and soybeans (*Glycine max* L.) were sown into these systems in October 2020. In the NT+B area, the collection was performed 35 days after Brachiaria desiccation. All sample areas belonged to the same farm and were under the same conditions of relief, climate, and soil class.

Collecting the samples and separating the aggregates

In each sample area, five 400 m^2 plots were demarcated and undeformed samples (clods) were collected. Five pseudorepetitions were then collected from the 0.00-0.05 and 0.05-0.10 m layers in each of the four sample areas, creating a set of 40 sample units. After collection, the sample units were air-dried and then subjected to sieving

Table 1. History, description and location of the sample areas

Areas	Description and location
Permanent pasture	±45 years of permanent pasture with coast-cross (*Cynodon dactylon*) and continuous stocking with dairy cattle at 2.0 AU ha^{-1}. The area has been under this management for 45 years, being reformed every 15 years with lime application of approximately 2.0 t ha^{-1}. Coordinates: 24° 11’ 34.86” S and 54° 06’ 49.06” W. Altitude: 312 m.
No-tillage system	±28 ha; being 20 years of conventional cultivation system (CT) + 25 years of NT in a succession of soybean (summer) and corn (winter). Soil correction is made through periodic recommendations based on soil analysis, with the application of calcareous or dolomitic limestone and agricultural gypsum every 3 or 4 years, in the amount of 1.0 and 2.0 t ha^{-1}, respectively. The area receives base fertilization with approximately 200 kg ha^{-1} at soybean sowing with NPK formulation in the proportion of 04–30–10, and with 10–15–15 formulation at second-crop corn sowing, following recommendations in the function of soil analyses. Coordinates: 24° 11’ 31.34” S and 54° 06’ 52.04” W. Altitude: 322 m.
No-tillage + Brachiaria system	±28 ha; being 20 years of CT, later 19 years of NT in succession of soybean (summer) and corn (winter) and in the last six years corn and *Brachiaria ruziziensis* intercropping in winter crops (25 years of NT+ Brachiaria in total). The area receives limestone application for correction every four years, with the application of approximately 2.0 t ha^{-1}. For soybean sowing, the formulated 15–15–15, 200 kg ha^{-1} of NPK plus potassium chloride is applied in coverage. For second-crop corn sowing, the area receives NPK application in the proportion of 15–15–15 240 kg ha^{-1} and ammonium sulfate in coverage. Coordinates: 24° 11’ 28.30” S and 54° 06’ 50.60” W. Altitude: 322 m.
Atlantic Forest biome vegetation	±28 ha; area of vegetation of the Atlantic Forest biome - Semideciduous Seasonal Forest, without signs of anthropic action. Coordinates: 24° 11’ 15.22” S and 54° 06’ 47.46” W. Altitude: 310 m.

using 9.7-, 8.0-, and 4.0-mm mesh sieves. The aggregates retained in the two intervals of 9.7> Ø ≥8.0 and 8.0> Ø ≥4.0 were selected for the study. The aggregates obtained in each interval were examined under binocular magnification and manually separated according to their origin or route of formation, according to the morphological patterns established by Bullock et al. (1985) using the protocol adapted by Pulleman et al. (2005) and validated by the studies gathered in Pereira et al. (2021).

Differentiation between the aggregates in the two intervals was performed based on the visualization of morphological features, such as shape, size, presence of roots, porosity (Bullock et al., 1985; Pulleman et al., 2005; Batista et al., 2013; Melo et al., 2019b; Pinto et al., 2021; Pereira et al., 2021), and subunit arrangements and junctions (Pereira et al., 2021). Thus, the aggregates were classified as either biogenic - those in which it is possible to see rounded shapes formed by the intestinal tracts of soil macrofauna, mainly Oligochaeta (earthworms), or those in which it is possible to see the presence and activity of roots - and physicogenic - those that presented angular shapes resulting from the interaction between carbon, clay, cations, and soil wetting and drying cycles (Figure 1).

Stability analysis of macro- and microaggregates

After identification, 25 g of biogenic and physicogenic aggregates in the interval of 8.0> Ø ≥4.0 mm (i.e., macroaggregates) were weighed and subjected to wet stability

Table 2. Characterization of physical and chemical soil attributes in the 0.00-0.10 m layer in the study areas in the subtropical region of Brazil

Areas	Sand	Silt	Clay	TOC	$pH(H_2O)$	Ca^{2+}	Mg^{2+}	H+Al	S	T	V	K	P
	g kg^{-1}					$cmol_c$ dm^{-3}					%	mg dm^{-3}	
PP	610	190	200	16.10	5.80	4.30	2.20	2.30	7.00	9.30	75	203	51
NT	750	130	120	5.90	6.80	3.20	1.10	0.20	4.70	4.90	96	100	42
NT+B	770	120	110	8.30	6.60	3.10	1.30	0.50	5.00	5.40	91	171	41
FN	670	210	120	7.00	5.50	1.30	0.70	1.80	2.20	3.90	55	50	23

PP: Permanent pasture; NT: No-tillage system; NT+B: No-tillage + Brachiaria system; NF: Atlantic Forest biome vegetation; S: Exchangeable sum of bases; T: T-value, corresponds to the cation exchange capacity at pH 7.0; V: Ssaturation by bases; and TOC: total organic carbon.

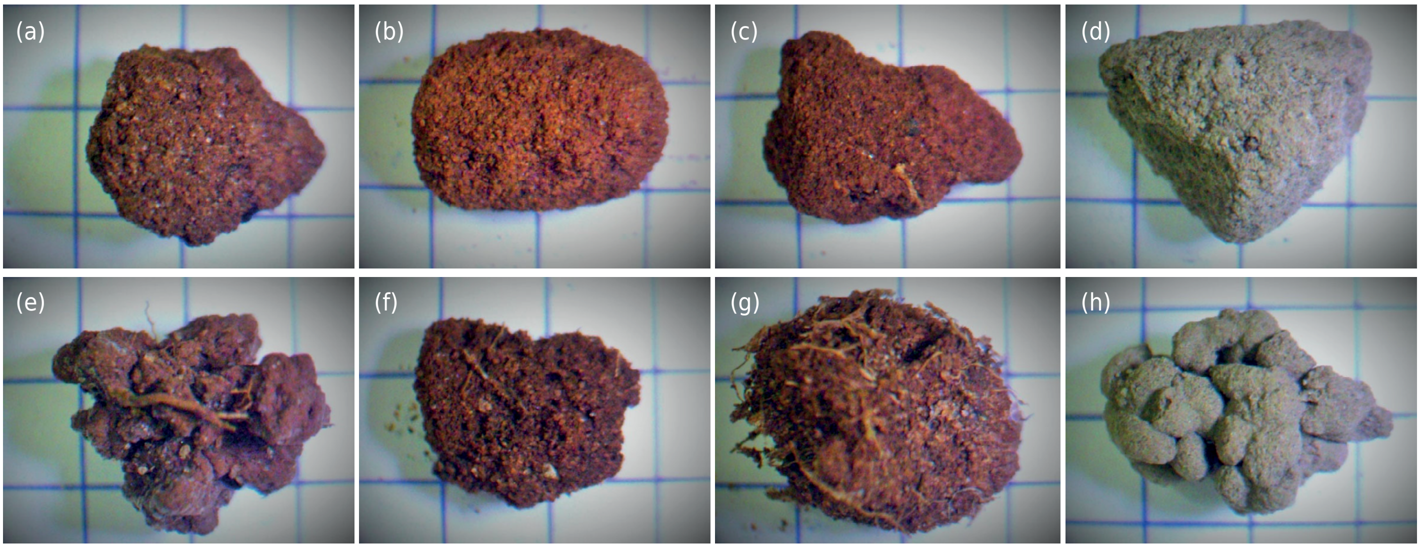

Figure 1. Examples of the morphological differentiation of biogenic (Bio) and physicogenic (Phy) aggregates in areas under different management systems and native forest in southern Brazil. (a) Phy from permanent pasture; (b) Phy from no-tillage system; (c) Phy from no-tillage + Brachiaria system; (d) Phy from Atlantic Forest biome vegetation; (e) Bio from permanent pasture; (f) Bio from no-tillage system; (g) Bio from no-tillage + Brachiaria system; and (h) Bio from Atlantic Forest biome vegetation.

analysis using vertical sieving with a set of sieves of decreasing mesh size, namely 2.0, 1.0, 0.50, 0.25, and 0.105 mm, for 15 min in the Yoder apparatus (Yoder, 1936). The material retained on each sieve was transferred to Petri dishes and dried in an oven at 105 °C until a constant mass was obtained (Teixeira et al., 2017). Based on the aggregate mass data, the MWD was calculated using equation 1.

$$MWD = \sum (xi\ yi) \qquad \text{Eq. 1}$$

in which: MWD is the mean weight diameter; *xi* is the mean diameter of the *i*-th aggregate size class (mm); *yi* is the proportion of the *i*-th aggregate size class with respect to the total, corrected for moisture; and *i* represents one of the aggregate size classes, which were 8.0≥ Ø >2.0, 2.0≥ Ø >1.0, 1.0≥ Ø >0.50, 0.50≥ Ø >0.25, and 0.25≥ Ø >0.105 mm.

Three clay classes were quantified to evaluate microaggregate stability according to the method proposed by Melo et al. (2019a), with adaptations from Melo et al. (2021): i) water-dispersible clay (WDC), or clay that is mechanically disaggregable in water and does not flocculate in suspension; ii) water-re-flocculable clay (WRC), or clay that is mechanically disaggregable and flocculates in suspension; and iii) non-dispersible clay (NDC), or clay that is not mechanically disaggregable. Subsequently, the WDC, WRC, and NDC data were normalized according to the total clay content.

Analysis of labile organic carbon and glomalin

Labile organic carbon was quantified via oxidation with $KMnO_4$ 0.02 mol L^{-1} used to extract POXC (Weil et al., 2003; Culman et al., 2012b). First, 2.50 g of aggregates were weighed and transferred to a 50 mL polypropylene centrifuge tube, to which 20 mL of 0.02 mol L^{-1} $KMnO_4$ was added. Then, the tubes were agitated for 2 min in a horizontal agitator at 240 oscillations min^{-1}. After shaking, the tubes were allowed to stand upright and stabilize for 10 min. After stabilization, 0.50 mL of the supernatant was pipetted into another 50 mL centrifuge tube containing 49.50 mL distilled water. The absorbance of each sample was measured via colorimetry at 550 nm using a spectrophotometer. The POXC (g kg^{-1}) was calculated using equation 2.

$$\text{POXC} = [0.02\ mol\ L^{-1} - (a + b\ Abs)] \times (9000\ mg\ C\ mol^{-1}) \times (0.02\ L\ Wt^{-1}) \qquad \text{Eq. 2}$$

in which: POXC is the labile organic carbon extracted with $KMnO_4$; 0.02 mol L^{-1} is the initial concentration of the $KMnO_4$ solution; a is the intercept of the standard curve; b is the slope of the standard curve; Abs is the sample absorbance reading at 550 nm; 9000 mg is the amount of C oxidized by one mole of MnO_4, with Mn^{7+} being reduced to Mn^{4+}; 0.02 L is the volume of $KMnO_4$ solution that reacted with the soil; and Wt is the mass of the sample (kg) used in the reaction.

Glomalin was quantified as "glomalin-related soil protein" (GRSP). The substance can be classified into two fractions: easily extractable GRSP (GRSP-EE, representing the newly produced fraction) and total GRSP (GRSP-T, the sum of recent and old fungal protein production) (Wright and Upadhyaya, 1996, 1998; Rillig, 2004). The GRSP-EE fraction was obtained by autoclave extraction using a 20 mM sodium citrate solution (pH 7.0) (121 °C, 30 min). The same procedure was applied for the GRSP-T fraction using a 50 mM sodium citrate solution (pH 8.0) (121 °C, 60 min). The GRSP fractions were quantified using the Bradford method, with modifications proposed by Wright and Upadhyaya (1996) using standard bovine serum albumin.

Statistical analysis

The data were tested for normality of errors based on an analysis of the residuals according to the Shapiro-Wilk test, and for homoscedasticity by the Bartlett test. Variables that did not show a normal distribution or homoscedasticity were transformed according to the Box-Cox test and were retested. Data were evaluated using analysis of variance followed by Tukey's test when the assumptions of normality of errors and homoscedasticity were met (transformed or untransformed variables). In cases where data transformation was insufficient, the Kruskal-Wallis test followed by Fisher's least significant difference criterion was used to evaluate land use and management systems for each aggregate type, and the Wilcoxon test was used to compare variables among aggregates under each land use and management system. Principal component analysis (PCA) based on Pearson's correlation matrix was also performed for the evaluated properties. All tests were performed at a 5.0 % significance level using R (R Development Core Team, 2020).

RESULTS

Effects of management systems and formation pathways on aggregation status

Soil aggregation status, which was evaluated according to the stability of macro- (via MWD) and microaggregates (via WDC, WRC, and NDC), made it possible to identify differences among the management systems (Figure 2 and Table 3).

Pasture system provided the greatest macroaggregate stabilization regardless of the formation pathway, as reflected in the higher MWD values, which ranged from 4.46 to 4.83 mm for biogenic aggregates and from 4.42 to 4.59 mm for physicogenic aggregates (Figure 2; 0.00-0.10 m layer). The pasture system also influenced the degree of microaggregate stability; in this system, the lowest WDC values and the highest NDC values were verified for both classes of aggregates (Table 3; 0.00-0.10 m layer). It is worth mentioning that for the WDC and NDC results of the aggregates from the PP area, a similar pattern was verified to the ones observed for the aggregates from the NF area (Table 3; 0.0–0.10 m layer). Furthermore, in the NF area, the highest WRC values for both aggregate classes were also quantified in the subsurface layer (Table 3; 0.05-0.10 m layer).

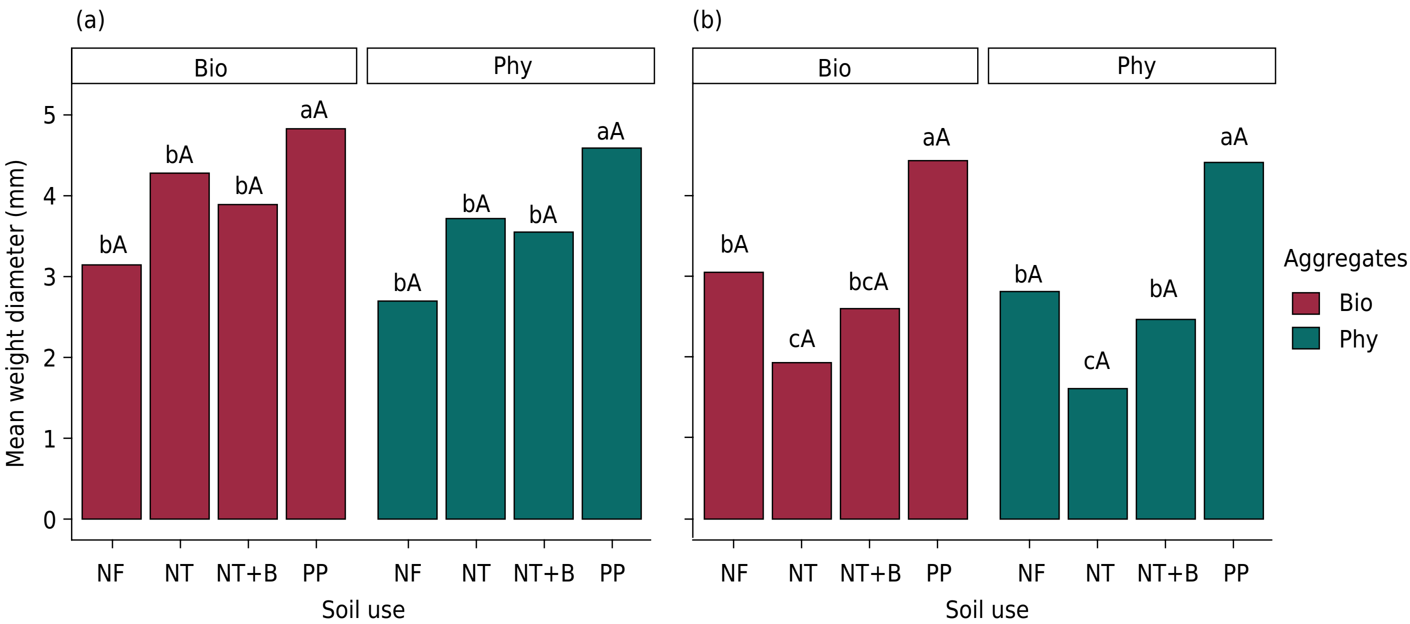

Figure 2. Mean weight diameter (MWD) of biogenic (Bio) and physicogenic (Phy) aggregates from areas under management systems in the subtropical region of Brazil. Means followed by the same lower-case letter do not differ between management systems for the same type of aggregate. Means followed by the same capital letter in the row do not differ among the types of aggregates for the same evaluated system (ANOVA + Tukey's test without data transformations; ANOVA + Tukey's test with data transformations; and Kruskal-Wallis test + Fisher's minimum significant difference). (a) 0.00-0.05 m soil layer; and (b) 0.05-0.10 m soil layer. PP: Permanent pasture; NT: No-tillage system; NT+B: No-tillage + Brachiaria system; and NF: Atlantic Forest biome vegetation.

Table 3. Water-dispersible clay (WDC), water-re-flocculable clay (WRC) and non-dispersible clay (NDC) of biogenic (Bio) and physicogenic (Phy) aggregates from areas under management systems in the subtropical region of Brazil

Areas	WDC		WRC		NDC	
	Bio	Phy	Bio	Phy	Bio	Phy
	%					
0.00-0.05 m soil layer						
PP	62.73 bcA	54.66 bcB	16.49 aA	11.36 aA	20.79 abA	33.98 abA
NT	74.28 abA	59.87 abB	14.63 aA	14.57 aA	11.09 bA	26.36 bA
NT+B	74.02 aA	72.39 aB	8.11 aA	11.09 aA	17.83 bA	16.51 bA
NF	57.62 cA	48.94 cB	6.81 aA	15.85 aA	35.57 aA	35.21 aA
0.05-0.10 m soil layer						
PP	53.54 bA	49.20 bA	0.03 abA	0.00 bA	46.43 aA	50.80 aA
NT	83.44 aA	70.41 aA	0.00 bA	0.21 bA	16.56 bA	29.38 bA
NT+B	66.94 aA	70.10 aA	3.38 abA	0.00 bA	29.69 bA	29.90 bA
NF	47.86 bA	50.98 bA	7.27 aA	3.78 aA	44.87 aA	45.24 aA

Means followed by the same lower-case letter in the column do not differ between management systems for the same type of aggregate. Means followed by the same capital letter in the row do not differ among the types of aggregates for the same evaluated system (ANOVA + Tukey's test without data transformations; ANOVA + Tukey's test with data transformations; and Kruskal-Wallis test + Fisher's minimal significant difference). PP: Permanent pasture; NT: No-tillage system; NT+B: No-tillage + Brachiaria system; and NF: Atlantic Forest biome vegetation.

Among the NT, NT+B, and NF areas, it was observed that the NT+B system favored the stabilization of macroaggregates the most, especially in the subsurface layer. The MWD values (2.63 and 2.48 mm in the biogenic and physicogenic aggregates, respectively) observed for this system were close to those verified in the reference area (3.07 and 2.83 mm in the biogenic and physicogenic aggregates, respectively). In the subsurface layer of the NT area, MWD values below 2.00 mm were observed in the biogenic (1.94 mm) and physicogenic (1.61 mm) aggregates (Figure 2; 0.05-0.10 m layer). Aggregates from the grain production systems (NT and NT+B) had the highest WDC values and lowest NDC values, a pattern opposite to that observed for aggregates from the PP and NF areas (Table 3; 0.00-0.10 m layer). Among the formation pathways, the quantified MWD values for aggregates from the biogenic pathway were higher than those from the physicogenic pathway by 5.0 (PP), 10.0 (NT+B), 16.0 (NT), and 17.0 % (NF) in the 0.00-0.05 m layer. In the 0.05-0.10 m layer, the increase was 6.0 (NT+B), 8.0 (NF), and 20.0 % (NT). In the biogenic aggregates, the highest WDC values were observed at the surface (Table 3; 0.00-0.05 m layer).

Influence of management systems and formation pathways on organic fractions

Organic fractions associated with aggregate stability were influenced both by the management system and formation pathway, most notably by POXC at the surface (Table 4; 0.00-0.05 m layer).

Only the pasture system increased the contents of POXC, GRSP-EE, and GRSP-T under the two formation pathways; the highest contents of these properties were quantified in these aggregates (Table 4; 0.00-0.10 m layer). The NT+B system had an increased GRSP-T content in the subsurface biogenic aggregates compared to that in the NT and NF areas (Table 4; 0.05-0.10 m layer). In the physicogenic aggregates of the grain production systems (NT and NT+B), higher GRSP-T content was observed compared to that of the physicogenic aggregates in the control area (NF) (Table 4; 0.00-0.10 m layer).

Regarding the formation pathways, the biogenic aggregates reaffirmed their ability to preserve and accumulate the most labile and soluble SOM fractions (e.g., POXC), as well as proportionally increase the contents of the other evaluated organic fractions

Table 4. Labile organic carbon (POXC), easily extractable glomalin (GRSP-EE) and total glomalin (GRSP-T) of biogenic (Bio) and physicogenic (Phy) aggregates from areas under management systems in the subtropical region of Brazil

Areas	POXC		GRSP-EE		GRSP-T	
	Bio	Phy	Bio	Phy	Bio	Phy
	g kg^{-1}					
	0.00-0.05 m soil layer					
PP	0.96 aA	0.84 aB	1.05 aA	0.85 aA	10.15 aA	7.12 aA
NT	0.85 bA	0.53 bB	0.64 abA	0.37 bB	4.28 bA	3.45 bA
NT+B	0.77 bA	0.60 bB	0.58 bA	0.46 bA	4.12 bA	3.72 bA
NF	0.64 bA	0.43 bB	0.66 abA	0.49 bA	3.17 bA	1.73 cA
	0.05-0.10 m soil layer					
PP	0.77 aA	0.61 aA	0.77 aA	0.73 aA	6.73 aA	4.89 aB
NT	0.30 bA	0.28 bA	0.35 bA	0.38 bA	2.20 cA	2.39 bA
NT+B	0.33 bA	0.35 bA	0.48 bA	0.37 bA	2.79 bA	2.70 bA
NF	0.37 bA	0.28 bA	0.49 bA	0.35 bA	1.23 cA	1.12 cA

Means followed by the same lower-case letter in the column do not differ between management systems for the same type of aggregate. Means followed by the same capital letter in the row do not differ among the types of aggregates for the same evaluated system (ANOVA + Tukey's test without data transformations; ANOVA + Tukey's test with data transformations; and Kruskal-Wallis test + Fisher's minimal significant difference). PP: Permanent pasture; NT: No-tillage system; NT+B: No-tillage + Brachiaria system; and NF: Atlantic Forest biome vegetation.

(GRSP-EE and GRSP-T), which are involved in aggregate formation and stabilization processes. In the biogenic aggregates at the surface of all study areas, higher POXC content was observed compared to the physicogenic aggregates (Table 4; 0.00-0.05 m layer). The GRSP-EE values quantified in the biogenic aggregates were higher relative to the physicogenic aggregates by 24.0 (PP), 26.0 (NT+B), 35.0 (NF), and 73.0 % (NT) in the 0.00-0.05 m layer; and 5.0 (PP), 30.0 (NT+B), and 40.0 % (NF) in the 0.05-0.10 m layer. For GRSP-T, the values observed in the aggregates of the biogenic pathway were 11.0 (NT+B), 24.0 (NT), 43.0 (PP), and 83.0 % (NF) higher compared to those of the physicogenic pathway in the 0.00-0.05 m layer; and 3.0 (NT+B), 10.0 (NF), and 38.0 % (PP) in the 0.05–0.10 m layer.

Dissimilarity among the evaluated soil use and management systems

In the PCA, we considered only the first two principal components (Comp. 1 and Comp. 2), which explained 70.97 and 77.11 % of the total variability of the data in the 0.00-0.05 and 0.05-0.10 m layers, respectively (Figures 3 and 4). As shown in figure 3, we observed the formation of three distinct groups: (1) the group formed by the aggregates (biogenic and physicogenic) of the pasture system; (2) the group formed by the biogenic aggregates of the grain production systems (NT and NT+B); and (3) the group formed by the physicogenic aggregates from the grain production systems (NT and NT+B). As shown in figure 4, the formation of the three groups was also verified; however, no separation of aggregate types were observed in the PP (1st group), NT and NT+B (2nd group), and NF (3rd group) areas.

The main axis (Comp. 1), which explained the greatest dissimilarity among management systems and formation pathways, separated the biogenic and physicogenic aggregates from the PP area (upper right quadrant) and biogenic aggregates from the NT and NT+B areas (lower right quadrant) from the other aggregates, representing 44.15 % of the data variability (Figure 3). The discriminant variables (correlation coefficient ≥0.45) were MWD (0.46), GRSP-EE (0.49), POXC (0.52), and GRSP-T (0.52). In figure 4, Comp. 1 also separated the aggregates of the PP area (upper and lower right quadrants) from

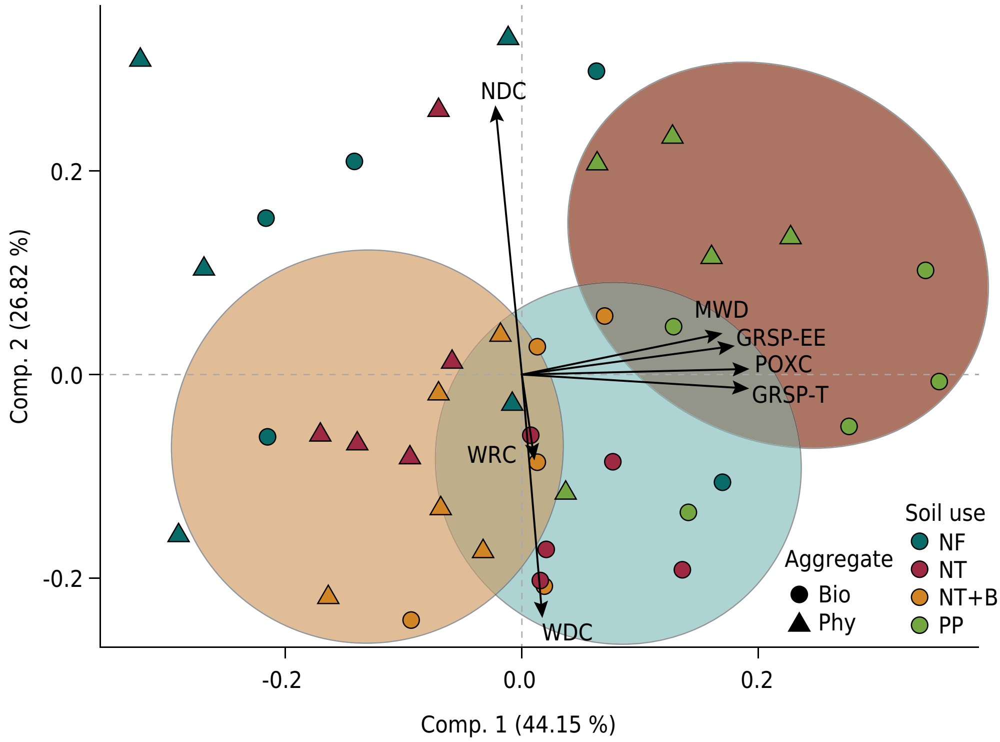

Figure 3. Principal component analysis integrating the aggregation state and the organic fractions associated with the stability of biogenic (Bio) and physicogenic (Phy) aggregates from areas under management systems in the 0.00-0.05 m layer, subtropical region of Brazil. PP: Permanent pasture; NT: No-tillage system; NT+B: No-tillage + Brachiaria system; NF: Atlantic Forest biome vegetation; MWD: mean weight diameter; WDC: water-dispersible clay; WRC: water-re-flocculable clay; NDC: non-dispersible clay; POXC: labile organic carbon; GRSP-EE: easily extractable glomalin; and GRSP-T: total glomalin.

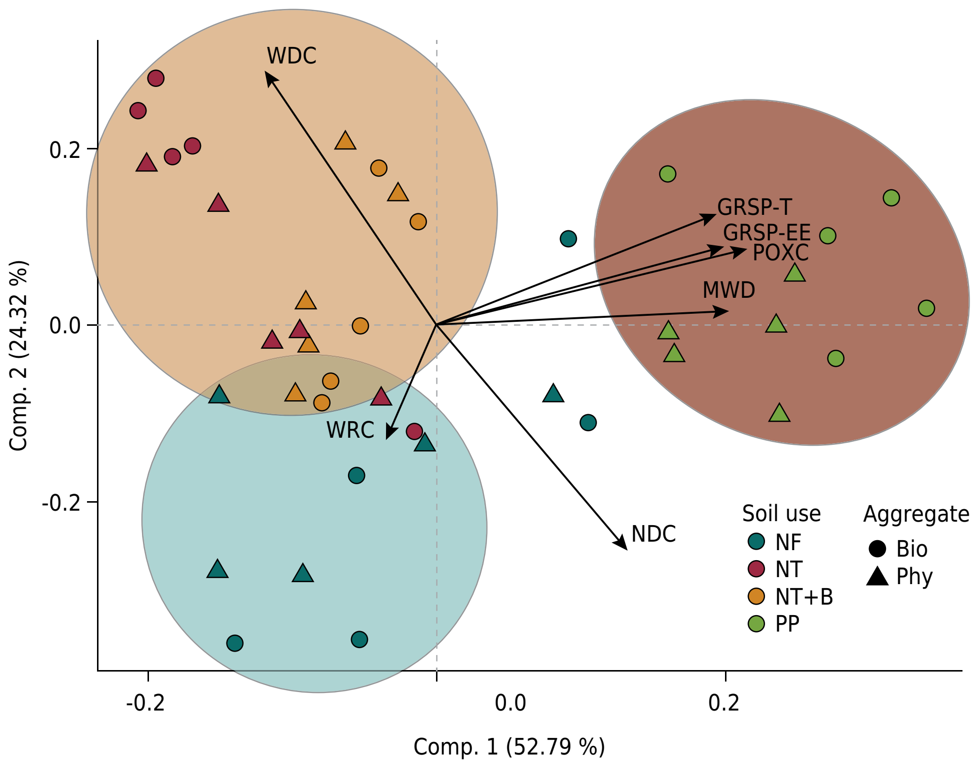

Figure 4. Principal component analysis integrating the aggregation state and the organic fractions associated with the stability of biogenic (Bio) and physicogenic (Phy) aggregates from areas under management systems in the 0.05-0.10 m layer, subtropical region of Brazil. PP: Permanent pasture; NT: No-tillage system; NT+B: No-tillage + Brachiaria system; NF: Atlantic Forest biome vegetation; MWD: mean weight diameter; WDC: water-dispersible clay; WRC: water-re-flocculable clay; NDC: non-dispersible clay; POXC: labile organic carbon; GRSP-EE: easily extractable glomalin; and GRSP-T: total glomalin.

the aggregates of the NT, NT+B, and NF areas (upper and lower left quadrants), representing 52.79 % of the data variability and showing a positive correlation coefficient (≥0.45) with the properties MWD (0.45), GRSP-EE (0.45), GRSP-T (0.45), and POXC (0.49).

In both PCAs, the properties MWD, POXC, GRSP-EE, and GRSP-T were most associated with the grassland system, corroborating the results of the statistical tests (Figure 2 and Table 4). For properties WDC, WRC, and NDC, a higher correlation with the axis of least relevance (Comp. 2) was observed in both PCAs (26.82 %, Figure 3; and 24.34 %, Figure 4). The WDC variable showed a medium negative correlation (-0.65; 0.00-0.05 m layer) and a medium positive correlation (0.65; 0.05-0.10 m layer) with Comp. 2, and it was most associated with the aggregates of the grain production systems (NT and NT+B).

DISCUSSION

Effects of management systems and formation pathways on aggregation status

The use of aggregate stability indicators, either for macro- (MWD) or microaggregates (WDC, WRC, and NDC), can be considered a valuable tool for measuring the impacts of different land use and management practices on agroecosystems. In this study, great effects of pasture system on macro- (MWD) and microaggregate (WDC and NDC) stabilization were found, regardless of their origin (Figure 2 and Table 3). The high MWD values of the aggregates in the PP area (Figure 2; 0.00-0.10 m layer) may be related to factors such as the amount of vegetal residues and manure; the presence of active roots and high biological activity; low topsoil disturbance; and soil texture with high clay content (Table 2). In pasture systems, the increased amount of plant residues and manure from cattle grazing, which improves the carbon content in the soil, can also improve the microhabitat in which microorganisms live, thus facilitating microbial growth and contributing to the effectiveness and increased density of fungal hyphae. Fungal hyphae directly improve aggregate stability by reorienting clay particles with extracellular polysaccharides (Ternan et al., 1996; Bronick and Lal, 2005) and indirectly improve it by providing carbon for other microorganisms that produce chemical binding agents.

The formation of large-diameter aggregates is generally attributed to grasses, especially forage crops and soil cover (perennial or annual), because of their abundant root systems and the constant renewal and decomposition of their tissues after harvest, crop management (Stumpf et al., 2016), or animal feeding. The roots, more specifically the fine roots, have an indirect effect on aggregate stability through the release of particulate organic matter, which stimulates microbial activity. However, roots penetrating soil layers can mechanically damage the existing aggregates (Six et al., 2004). This can occur by breaking up the aggregates, which then are reconstituted. In the same region as the present study, Stumpf et al. (2016) found that grass roots promoted the recovery of soil physical conditions in the topmost layer by forming new aggregates, leading to decreased soil density and increased macroporosity.

In this study, the presence of cattle in the pasture system (Table 1) resulted in the constant addition of manure, which increased SOM content and stimulated the production of root exudates. Additionally, in the PP area, there was a difference in the soil texture (sandy loam) compared to the NT and NT+B areas (Loamy sandy) (Table 2). The higher clay content and less soil disturbance in the PP area may contribute to a greater conservation of SOM, possibly through associations between transformed organic matter and mineral particles that form organo-mineral complexes (Six et al., 2004). The combination of all the aforementioned factors may favor the formation of more stable aggregates in this system, a pattern that can be considered for both biogenic and physicogenic aggregates

(Figure 2; 0.00-0.10 m layer). Corroborated by the POXC, GRSP-EE and GRSP-T results (Table 4; 0.00-0.10 m layer).

Dispersion of the clay fraction is a dynamic soil property (Melo et al., 2019a); therefore, aggregate stability is indicative of soil structural resistance in the face of disruptive agents, such as rain or mechanical revolving, under field conditions. The WDC class analysis assesses how soil clay responds to mechanical forces and hydration; hence, it is an assessment of microaggregate stability (Igwe and Obalum, 2013; Melo et al., 2019a). The low WDC values and high NDC values observed in the aggregates of the PP area (Table 3; 0.00-0.10 m layer) may be related to the low level of surface layer disturbance associated with the beneficial effect of the grass root system vegetation cover; these factors may increase the bond strength and reduce the dispersion of the clay fraction. This explanation could also justify the WDC results similarity between the pasture system's aggregates and the non-anthropized environment (NF) (Table 3; 0.00-0.10 m layer).

Carbon concentrations in the PP and NF areas in the 0.00–0.10 m layer were 16.10 and 7.0 g kg^{-1}, respectively (Table 2). However, NDC values were similar among the aggregates in these areas (Table 3; 0.00-0.10 m layer). In a study carried out in southern Brazil with Ferralsols (*Latossolos*) with a very clayey texture, Melo et al. (2021) observed that the initial increase in organic carbon content reduced the NDC value, whereas high increases in the contents of the same chemical properties led to increased NDC values. According to the authors, highly stable microaggregates (pseudo-silt and pseudo-sand) are favored under both high and low SOM concentrations. The NDC class indicates stronger aggregation, which occurs due to various organic and inorganic binding mechanisms when the dispersive net charge is assumed to be zero (Rengasamy et al., 2016; Melo et al., 2019a). According to Melo et al. (2019a), the WRC class is supposedly more transportable than the NDC class because the former remains in suspension, while mechanical disturbances occur in the soil solution (e.g., during rainfall). According to the authors, when mechanical disturbances are reduced, the WRC class has a greater aggregation potential than the WDC class. This could explain the higher WRC values quantified in the aggregates of the subsurface layer of the NF area (greater diversity in terms of vegetation cover) (Table 3; 0.05-0.10 m layer).

Between the grain production systems (NT and NT+B) and the reference area (NF), the beneficial effect of Brachiaria intercropped with corn on macroaggregate stability can be observed by analyzing two results: the first is the high MWD values of the biogenic and physicogenic aggregates in the subsurface layer; these values were similar to the ones verified in the same classes of aggregates in the NF area. The second is the low MWD values (<2.00 mm) identified in the biogenic and physicogenic aggregates of the NT area (without Brachiaria), also in the subsurface layer (Figure 2; 0.05-0.10 m layer). The use of grasses, especially Brachiaria, is a key factor for ensuring the viability of grain and fiber production systems, and for ensuring sustainable farming in sandy-textured soils (Donagemma et al., 2016). Furthermore, several studies (Briedis et al., 2012; Tivet et al., 2013) have observed that MWD values of less than 2.00 mm indicate that the soil is in an unfavorable physical condition with respect to its aeration, infiltration and redistribution of water, and root system establishment.

Higher MWD values in aggregates in the NT+B area (Figure 2; 0.05-0.10 m layer) may also be related to the collection time interval after desiccation of the annual forage. Generally, Brachiaria takes longer to dehydrate completely, around 20 days, thus requiring greater anticipation of desiccation in relation to sample collection and soybean sowing (Kluthcouski et al., 2004). This factor favors the increase of soil moisture and the reduction of temperature, benefiting the activity of the edaphic fauna and the root system. Shoot and root dry matter and, consequently, organic matter supplied by grasses crops in NT+B system improve microbial activity, which, along with root exudates, results in improved aggregate stability (Rosolem et al., 2016).

In the same region studied here, Rosset et al. (2019) found that the corn-Brachiaria (winter) consortium with four years of installation (2008–2012) was efficient at improving soil aggregation under Ferritic Ferralsols (*Latossolo Vermelho*). According to the authors, the soil structural quality increased due to the more abundant and aggressive root system of Brachiaria intercropped with corn compared with soybean/corn succession. The increased resistance of macroaggregates to disruptive forces can be attributed to the presence of polysaccharides that increase particle cohesion and the effects produced by the physical entanglement of fungal roots and hyphae. Arbuscular mycorrhizal fungi facilitate aggregation by shaping the plant community composition, influencing host plant root growth (via the symbiotic relationship between plants and arbuscular mycorrhizal fungi), and directly affecting mycelium growth (Rillig and Mummey, 2006).

The higher WDC values and lower NDC values observed for aggregates in the NT and NT+B areas (Table 3; 0.00-0.10 m layer) indicate that the minimal disturbance of the topsoil under the NT system mechanically affects the ability of the microaggregates to resist disruptive forces. It is worth noting that among the indicators of microaggregate stability studied via PCA, the WDC class was the most associated with the NT and NT+B areas (Figures 3 and 4). This reinforces the hypothesis that there is a direct relationship between the effects of grain production system management practices and the WDC indicator. In the same region as this study, Figueiredo et al. (2021) found that the dispersion degree of the clay fraction was affected by soil management systems without any effect from the cropping systems. In their study, the management system with the least soil disturbance (continuous no-tillage) exhibited the highest dispersion degree of the clay fraction compared to systems that promoted greater soil tillage, regardless of the agricultural implementation used or the intensity of soil disturbance. However, the negative mechanical effects of soil disturbance on clay fraction dispersion can be partially neutralized by proper soil chemical management and fertilization of the production system, as pointed out by Nunes et al. (2020).

Effects of the formation pathways on macroaggregate stability were evident according to the proportionally higher MWD values observed in biogenic aggregates compared to physicogenic ones (Figure 2). The pattern of the MWD results was similar to that reported by Silva Neto et al. (2021) in an incubation experiment with macrofaunal organisms (Oligochaeta) and grass vegetation (*Brachiaria decumbens*) in southeastern Brazil. According to the authors, different factors involving these organisms are responsible for binding the small soil subunits together, conferring greater stability to biogenic aggregates. In northeastern Brazil, Silva Neto et al. (2010) also observed that biogenic aggregates presented greater physical stability than physicogenic aggregates in soils covered by grass species (*Saccharum officinarum* and *Brachiaria decumbens*), demonstrating the effect of biological activity on the genesis of these aggregates.

Aggregation induced by biogenic pathways occurs at different scales and under the impacts and interactions of various organisms. These aggregates affect important functions in the soil, such as increased water repellency; increased capacity to hold and store water, nutrients, and contaminants; and increased habitat supply (Guhra et al., 2022). Numerous factors may explain the high stability of biogenic macroaggregates; for example, biogenic exudates from fungi, roots, earthworms, and bacteria influence water repellency, transport, and retention within preferential flow paths. That is, the hydro-repellent coating on some minerals or soil aggregate surfaces results from the slow accumulation of potentially hydrophobic organic substances produced by microbial byproducts and the root exudates and subsurface waxes from plant leaves (Morales et al., 2010).

Additionally, water stability increases when root exudates (i.e., mucilages) are incorporated into soil aggregates. Root exudation is involved in organo-mineral associations and the attachment of SOM to aggregates, which co-occur with the release of organic carbon from mineral surfaces mediated by organic binders (Guhra et al., 2022). Furthermore, earthworms significantly influence aggregate turnover and SOM dynamics during soil excavation and ingestion processes by destroying existing aggregates and forming biogenic macro- and microaggregates. These contain fine particles of organic matter that are physically protected against microbial degradation (Pulleman et al., 2005). Finally, fungi-derived organic matter (e.g., exudates and tissues) becomes stabilized through organo-mineral associations and aggregate formation, as the hyphae physically surround soil particles and fungal exudates serve as aggregation agents (Frey, 2019).

It is possible to evaluate the state of soil aggregation by studying soil morphology and aggregate formation pathways, which is associated with important soil properties such as structure, infiltration, water retention, and carbon storage. Soil structure affects plant growth by influencing the distribution of roots and, consequently, their ability to absorb water and nutrients (Velasquez and Lavelle, 2019). In addition, the activity of soil biota is an essential factor for pedogenesis, structure dynamics, nutrient turnover, and organic matter in soils. The beneficial role of soil biota in the formation and stabilization of aggregates as a function of the type of land use and management (or field treatment) can be effectively evaluated by quantifying the proportion of hydro-stable aggregates (Guhra et al., 2022).

In this study, the effect of formation pathways on microaggregate stability was verified based on the WDC results, with higher values occurring under the biogenic pathway (Table 3; 0.00-0.05 m layer). Despite the soil improvements provided by biogenic aggregation, Melo et al. (2019b) observed an increase in the potential dispersion of the clay fraction in the biogenic aggregates of NT areas with the application of organic waste (chicken litter and liquid swine manure) to Rhodic Hapludox soil (*Latossolo Vermelho Eutroférrico*) in the same region as the present study. Although the SOM content in the biogenic aggregates was higher owing to the addition of manure, the authors found no evidence that it was responsible for the increased resistance of the clay fraction to disruptive forces.

However, a strong negative association between water-dispersible clay and Al^{3+} content was found by Melo et al. (2019b). According to the authors, this pattern suggests that the neutralization of Al^{3+} inhibited the re-flocculation of the particles, intensifying dispersion. This result can be explained by the higher proportion of lower-valence cations (K^+, Ca^{2+}, and Mg^{2+}) when the Al^{3+} saturation is low (Melo et al., 2021; Pereira et al., 2021). This chemical phenomenon may have been favored in aggregates of biological origin because of their higher concentrations of nutrients. It is worth noting that this study and the work of Melo et al. (2019b) are the first to use the clay class technique in aggregates of different origins. Therefore, more studies should be conducted on aggregate genesis, other land use and management systems, and new methods for evaluating microaggregation.

Influence of management systems and formation pathways on organic fractions

Previous studies have verified that, in sandy-textured soils located in tropical climatic environments, the stabilization of aggregates is mainly controlled by the activities of specific fungi that act as aggregating agents in the edaphic system (Bossuyt et al., 2001). However, this stabilization also depends on the decomposability of the SOM (Sall et al., 2016). In this study, macro- (Figure 2) and microaggregation (Table 3) in the PP area were found to have the greatest influence on POXC, GRSP-EE, and GRSP-T concentrations in the aggregate formation pathways (Table 4; 0.00-0.10 m layer). The higher labile organic carbon content observed in aggregates in the PP area (Table 4;

0.00-0.10 m layer) confirmed that POXC is a sensitive indicator management-induced changes in SOM (Culman et al., 2012b; 2013) and a good predictor of SOM stabilization relative to other soil carbon fractions (Hurisso et al., 2016).

In the same region of the present study, Assunção et al. (2019) found contrasting results regarding POXC to those verified here; they found higher contents in a native forest area (alluvial semideciduous seasonal forest; 1130 mg kg^{-1} POXC), followed by the areas of NT systems (soybean/corn succession; 980 mg kg^{-1} POXC) and pasture (*Cynodon dactylon*; 900 mg kg^{-1} POXC). The authors justified these findings due to the greater contribution of SOM to the surface layer in the forest area. According to Hurisso et al. (2016), POXC content is most influenced by conservation-oriented management practices, the main objective of which is to promote SOM accumulation or stabilization (long-term carbon sequestration).

The GRSP concentrations tend to be positively correlated with macroaggregate stability in water (Rillig, 2004), ranging from 2.0 to 15.0 g kg^{-1} in arable soils, grasslands, and natural ecosystems (Wright and Upadhyaya, 1996; Wright et al., 2000). The GRSP contents verified in this study were within the range indicated by previous authors (Table 4; 0.00-0.10 m layer). The GRSP-EE fraction consists of recently produced and relatively more labile fungal protein, whereas the GRSP-T fraction is the sum of recent and old fungal protein production and is chemically more recalcitrant. As previously mentioned, the high amounts of plant residues and manure continuously deposited into the soil and the presence of roots and fungal hyphae in the pasture system create nucleation sites for the growth of fungi and other microorganisms. This results in the mixture and extensive network of microbial and plant products (mucilages), which include GRSP (Santos et al., 2020). This knowledge justifies the high GRSP-EE and GRSP-T contents identified in the aggregates of the PP area (Table 4; 0.00-0.10 m layer). The GRSP has been suggested as an early warning indicator of soil quality when soil properties are often overlooked, such as in degraded soils (Rillig, 2004).

According to the PCA, POXC, GRSP-EE, GRSP-T, and MWD were mainly responsible for separating the aggregates of the pasture system from those of the other evaluated systems (Figures 3 and 4). These results show that the associations between binding agents and aggregate stability are more dependent on carbon concentrations and less dependent on the disturbance of the arable layer, and that they are more related to macroaggregates than to microaggregates. Wright et al. (2007) found that GRSP was related to increased aggregate stability and provided a quantitative measurement of specific SOM components.

As was observed for macroaggregation (Figure 2; 0.05-0.10 m layer), the beneficial effect of corn-Brachiaria intercropping on the GRSP-T content can be verified in two ways, the first via the higher quantification of GRSP-T values in the subsurface biogenic aggregates compared to the same class of aggregates in the NT and NF areas (Table 4; 0.05-0.10 m layer); and the second via the higher quantification of GRSP-T values in the physicogenic aggregates. These latter values were similar to those verified in the same class of aggregates in the NT area, and they were higher than those quantified in the aggregates of the NF area (Table 4; 0.00-0.10 m layer).

Plant species used in grain production systems, including both cash and cover crops, mainly provide surface carbon in the form of dead plant tissue, which is incorporated into the soil through both biotic and abiotic homogenization processes. However, the organic material released by root exudation and the decomposition of root organic residues is presumably more relevant for aggregating and stabilizing SOM (Guhra et al., 2022). Some studies have suggested that the NT system is better at increasing GRSP concentrations and colonization by arbuscular mycorrhizal fungi than the conventional non-conservationist tillage system, as this system causes a greater mechanical disturbance in the hyphal network (Holátko et al., 2021). Within the NT system, other parameters that can affect GRSP concentrations include crop rotation, succession, and intercropping practices, as verified by the results of the attributes among the NT, NT+B, and NF areas (Table 4).

Recently, Guhra et al. (2022) defined biogenic aggregation as the sum of all processes exerted or mediated by soil biota that trigger or alter physical (e.g., compaction by mechanical stress due to root growth) and chemical (e.g., excretion of organic matter that acts as a coating or aggregation agent) aggregation processes. Overall, the biogenic aggregates in the present study predominantly showed higher values of POXC, GRSP-EE, and GRSP-T compared to the physicogenic aggregates (Table 4; 0.00-0.10 m layer). The higher POXC content observed in the biogenic aggregates (Table 4; 0.00-0.05 m layer) showed that organic material with higher lability (i.e., higher bioavailability) is predominant in these aggregates and that the incorporation and maintenance of this material are favored in biogenic aggregates due to the soil fauna and plant root systems, especially under no-tillage management systems (Loss et al., 2014; Mergen Junior et al., 2019; Pinto et al., 2021). This pattern has also been verified by other authors that evaluated different SOM fractions; for example, Loss et al. (2014), Melo et al. (2019b), and Ferreira et al. (2020) studied carbon from the fulvic and humic acid fractions, and Silva Neto et al. (2021) studied microbial biomass carbon. This reinforces the hypothesis that biogenic aggregates lead to improved soil quality, especially regarding their greater ability to preserve and accumulate more soluble SOM fractions. The higher WDC content in the biogenic aggregates (Table 3; 0.00-0.10 m layer) can be explained by the higher POXC contents (Table 4; 0.00-0.05 m layer), as the relationship between the WDC and WRC classes depends on the surface charge balance of the minerals (Melo et al., 2021).

The proportionally higher GRSP-EE and GRSP-T contents quantified in the biogenic aggregates compared to the physicogenic aggregates (Table 4) are related to the biological agents responsible for the formation of these aggregates, particularly arbuscular mycorrhizal fungi. Several studies have shown that fungi play a more prominent role in aggregation than bacteria because they contribute to three different aggregation mechanisms: physical entanglement, production of hydrophobic substances, and production of extracellular polysaccharides. Sall et al. (2016) found that fungal activity plays an important role in macroaggregate formation in Senegal, mainly in sandy-textured soils. Another interesting factor of these results is the possibility of validating the method of separating the aggregate classes (Bullock et al., 1985; Pulleman et al., 2005; Pereira et al., 2021) via semi-quantitative biochemical analysis (i.e., the sodium citrate method; Wright and Upadhyaya, 1996, 1998; Rillig, 2004).

Arbuscular mycorrhizal fungi and their exudates (including GRSP) can decrease the permeability of the soil surface, increasing its hydrophobicity and stabilizing the aggregates (Holátko et al., 2021); together, these effects influence the MWD. The polysaccharides in GRSP are sticky and can hold small aggregates together, and the iron ions present in its composition (2.0–12.0 %) create bridges linking clay minerals and aliphatic amino acids. In these bridges, the complexes of organic substances (GRSP or humin) and minerals (clay) form a hydrophobic layer that protects the soil from damaging factors such as water and wind erosion (Nichols and Wright, 2004). The GRSP also contributes to increased organic carbon stocks and is significantly correlated with nitrogen in all soil types (Wilson et al., 2009).

Although the attributes MWD, POXC, GRSP-EE, and GRSP-T were most associated with the PP area according to the PCA (Figures 3 and 4), they also contributed to the separation of biogenic (lower right quadrant) and physicogenic (lower left quadrant) aggregates from the NT and NT+B areas on the soil surface (Figure 3), a pattern that was not observed in the subsurface layer (Figure 4). The results of the PCA showed that these attributes had higher values in biogenic aggregates and that the contribution of the biogenic pathway to soil properties may be more expressive in conservationist systems (e.g., no-tillage systems) and in the topsoil layer.

CONCLUSIONS

Grain production systems, pastures, and non-anthropic environments (Atlantic Forest biome vegetation) differently influence the aggregation and concentration of the organic fractions associated with aggregate stability. Compared to grain production systems, the edaphic and environmental conditions of the pasture system promoted greater stabilization of macro- and microaggregates, and favored the maintenance and storage of soluble organic carbon and glomalin fractions. Further, compared to soybean/corn succession, the intercropping of corn and *Brachiaria ruziziensis* as winter crops after six years was efficient at increasing the stability of macroaggregates and the concentration of the total glomalin fraction in the biogenic aggregates of the subsurface layers.

Biogenic aggregation results in a higher concentration of labile organic carbon and contributes to the elevation of macroaggregate stability and glomalin-related soil protein content. This suggests an improvement in soil properties and, consequently, in edaphic quality. Our study highlights the need for future studies using these indicators to monitor the quality of soils, especially those with a sandy texture, which are considered more fragile, and to infer the environmental sustainability of commonly adopted agricultural use and management practices.

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Formal analysis: Luiz Alberto da Silva Rodrigues Pinto (lead), Cristiane Figueira da Silva (supporting) and Thadeu Rodrigues de Melo (supporting).

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Resources: Marcos Gervasio Pereira (equal) and Jean Sérgio Rosset (equal).

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