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Edaphic properties in a eucalyptus-forest ecotone in the Nova Baden State Park, Southeastern Brazil

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ABSTRACT: State parks are integral protection units created to protect ecosystems. However, anthropic activities may have been previously performed before their creation, forming transitional areas. Studies that evaluate the modifications of edaphic properties in these environments are scarce. The aim of this study was to evaluate the changes in soil properties and litter stock in areas of eucalyptus-forest ecotones. Litter stock ($Stock_{litter}$) and total soil organic carbon content and stock (TOC_{soil}), weighted mean diameter (MWD), origin of aggregates (biogenic or physicogenic) and respective content of total organic carbon (TOC_{Bio} and TOC_{Phy}), total organic carbon (TOC_{AG}), soil organic matter chemical fractions [fulvic acid (FAC), humic (HAC), and humin (HUMC)], and particulate, mineral-associated, free light and intra-aggregate light (POC, MAOC, FLFC, and ILFC) soil organic matter, fertility, and glomalin-related soil protein (GRSP) fractions were evaluated in aggregates (layer 0.00-0.10 m) in areas of eucalyptus-forest ecotone (Eco), preserved forest fragment (For), and eucalyptus plantation (Euc), in Nova Baden State Park, Lambari–MG. In the Euc and Eco areas, the highest $Stock_{litter}$ content was found. Higher values of TOC_{soil}, TOC_{AG}, GRSP, and MWD were observed in the Euc area. In the biogenic aggregates, the highest content of TOC_{Bio}, HAC, HUMC, POC, MAOC, FLFC, and ILFC was determined in detriment of the physicogenic ones. The dynamics of edaphic properties in the Eco area showed greater similarity with the For area than in the Euc area. In general, all vegetation covers contribute to the maintenance of soil quality.

Keywords: accumulated litter, biogenic and physicogenic aggregates, glomalin, total organic carbon.

INTRODUCTION

In Brazil, Law No. 9.985, instituted on July 18, 2000, established criteria and norms for creating, implementing, and managing Conservation Units (UCs). Since then, the conservation of areas with relevant natural resources and its management based on the preservation, maintenance, sustainable use, restoration, and recovery, within the UCs, was legitimized, ensuring the maintenance of their potential for future generations (Brasil, 2000). The UCs must be managed by a forest management plan, which establishes the zoning and rules for using the area and managing its natural resources (Brasil, 2000). The creation of this plan involves surveying the climatic, topographic, geological, pedological, and vegetation conditions of the protected area (Souza and Soares, 2013).

However, in some cases, UCs are established in areas where anthropic activities have been previously performed, such as the installation of monospecific plantations of exotic forest species, including eucalyptus. This is the case of the Nova Baden State Park (PENB), located in southeastern Brazil. It belongs to the category of national parks, which was established within the sphere of the state public power. This UC category belongs to the Full Protection Unit (UPI) group, which encompasses the UCs with more restrictive use. Only the indirect use of natural resources is admitted, which includes scientific research, environmental education activities, ecological tourism, and recreation in contact with nature (Brasil, 2000).

However, the information generated in UCs is still scarce, including the influence of eucalyptus plantations and transition areas between these plantations and native vegetation (ecotones) on soil quality. In this regard, the properties related to soil quality, nutrient cycling, and biodiversity in UPIs are included. Among these properties, we highlight those that reflect i) the relative proportion of the fraction leaves of eucalyptus, leaves of native species and/or agricultural crops (litter stock); ii) the compartmentalization of soil organic matter (SOM) and the genesis of soil aggregates (Pulleman et al., 2005; Vidotto et al., 2007; Denardin et al., 2014; Silva Neto et al., 2016; Fernandes et al., 2017; Schultz et al., 2019; Suárez et al., 2019; Yao et al., 2019); and/or iii) the soil chemical properties (Pinheiro et al., 2009).

These properties present wide variation, resulting from the interrelation between formation factors, vegetational cover, and land-use (Silva Neto et al., 2011). We can add other aspects related to those previously mentioned, such as glomalin, configured as a glycoprotein produced by arbuscular mycorrhizal fungi (Bedini et al., 2007; Lima et al., 2013). It influences the formation and stabilization of aggregates (Hickmann et al., 2011) that can be classified in terms of their genesis, origin or formation pathways as biogenic or physicogenic.

Biogenic aggregates are formed by the activity of microorganisms, roots, and soil fauna organisms (called ecosystem engineers), whereas physicogenic aggregates arise owing to chemical factors, organic matter input to the soil, and soil wetting and drying cycles (Jouquet et al., 2006; Silva Neto et al., 2010; Loss et al., 2014; Silva Neto et al., 2016; Fernandes et al., 2017; Suárez et al., 2019; Pereira et al., 2021). In both aggregates, total organic carbon values can still be evaluated (Nichols and Wright, 2005; Pulleman et al., 2005; Loss et al., 2014; Schultz et al., 2019; Ferreira et al., 2020; Pinto et al., 2021; Pereira et al., 2021; Rossi et al., 2023; Silva et al., 2023).

In the Atlantic Forest biome, Pinto et al. (2022) observed that biogenic aggregation provided higher levels of labile organic carbon; and contributed to increased macroaggregate stability and glomalin-related soil protein levels. This suggests an improvement in soil properties and, consequently, in soil quality. Biogenic aggregation can be considered a more sensitive, efficient, economical and reliable indicator for assessing soil quality in different soil and environmental conditions (Pereira et al., 2021).

In this context, the study formulated the general hypothesis that the assessment of soil quality through the analysis of its biological, chemical and physical properties can contribute to underpin the management plan of protected areas (UCs), and help in decision-making for the conservation and recovery of native biodiversity, which could indicate —or not— the removal of eucalyptus plantations. To test the general hypothesis, the study aimed to evaluate: i) the litter stock; ii) total soil organic carbon; iii) stability, classification, total organic carbon, organic matter fractions, fertility, and glomalin in aggregates in areas of eucalyptus-forest ecotones, preserved forest fragments, and eucalyptus plantations in PENB, which is located in the municipality of Lambari, Minas Gerais, Brazil.

MATERIALS AND METHODS

Geographical characterization of the study area

The study was conducted at PENB, which has a total area of approximately 215 ha, located in the municipality of Lambari, state of Minas Gerais, between meridians 45° and 46° W and parallels 21° 30′ and 22° S. The PENB is part of a large remaining fragment of Atlantic Forest, limited by the Serra das Águas de Lambari and the Biological Reserves of Santa Clara and Engenho Velho, located in the municipalities of Cambuquira and Campanha, respectively, in Minas Gerais (Figure 1).

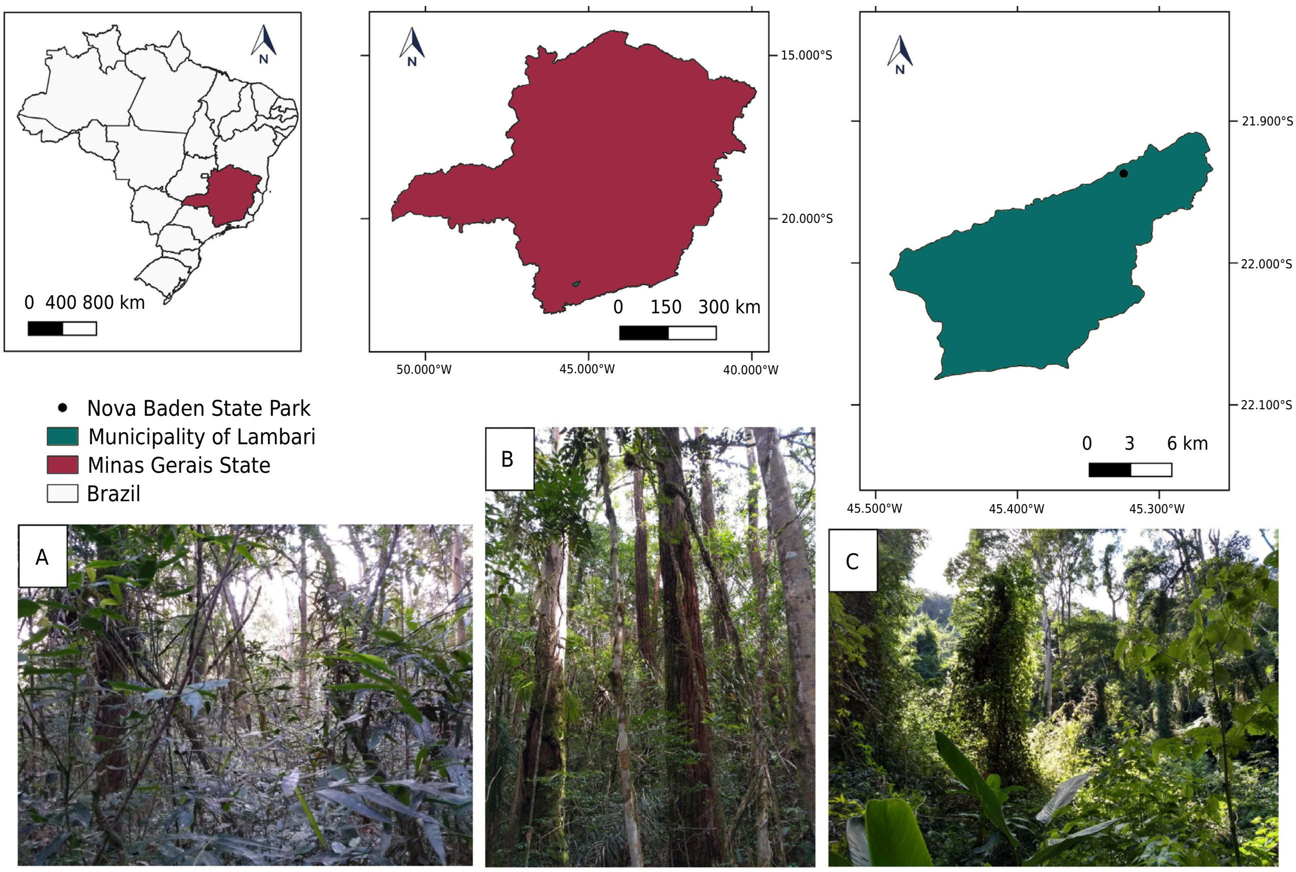

Figure 1. Geographic location of the study area - Nova Baden State Park, Lambari (Minas Gerais). Forest-eucalyptus ecotone area (a), eucalyptus area (b), and native forest fragment area (c), Southeastern Brazil.

According to Köppen classification system, the region's climate is Cwa, i.e., humid subtropical with dry winters and hot summers (Alvares et al., 2013). The relative humidity is approximately 75 %, occasional frosts occur, and average annual rainfall is between 1,500 and 2,000 mm. The terrain varies from mountainous to rugged, with altitudes from 1,300 to above 1,900 m. The vegetation is distributed in the phytophysiognomies Montane Semi deciduous Forest, Alluvial Semi deciduous Seasonal Forest with *Euterpe edulis*, High Montane Semi deciduous Seasonal Forest, and Marshy Field with *Hedychium coronarium.* The predominant soil classes in the area range from *Latossolo Vermelho-Amarelo* (Ferralsol) in the middle and upper thirds of the elevations; *Latossolo* with humic A horizon in the lower portion; *Gleissolo* (Gleisol) in the lowlands and areas that form the plain; and *Cambissolo Háplico* (Cambisol) in the areas of mountainous terrain (IUSS Working Group WRB, 2015; Santos et al., 2018).

Areas selected for the study

Three areas were selected: a well-preserved fragment of montane mixed ombrophilous forest (Forest or For); a eucalyptus plantation (Eucalyptus or Euc); and an ecotone of forest-eucalyptus plantation (Ecotone or Eco). In the Forest area, the canopy was 15–25-m high, with emergent individuals of *Araucaria angustifolia* (Bertol.) Kuntze and *Cariniana legalis* (Mart.) Kuntze reaching 30 m. In the intermediate stratum, we could observe individuals of *Astronium graveolens* Jacq., *Cabralea canjerana* (Vell.) Mart., *Machaerium nyctitans* (Vell.) Benth., *Cecropia hololeuca* Miq., *Piptadenia gonoacantha* (Mart.) J. F. Macbr., and *Erythrina* sp. L. among other species. In the understory, there were arboreal-shrubby individuals of 5 to 12 m and an herbaceous stratum with subshrubs of *Piper* spp. *Sanchezia* sp., *Cyathea* sp., and herbs and lianas.

In the Eucalyptus area, individuals of *Eucalyptus robusta* Sm. and *Corymbia citriodora* (Hook.) K.D. Hill and L.A.S. Johnson are predominant. With the abandonment of the area approximately 100 years ago, *Cedrela fissilis* Vell. and *Araucaria angustifolia* dominate the upper stratum, but generally with smaller sizes compared to those of the Forest area. In the intermediate stratum, we found individuals from 3 to 15 m of *Euterpe edulis* Mart., *Alchornea triplinervia* (Spreng.) Müll. Arg., *Croton floribundus* Spreng., *Allophylus edulis* (A. St.-Hil. et al.) Hieron. ex Niederl, *Cupania oblongifolia* Mart., *Psychotria vellosiana* Benth., *Zanthoxylum rhoifolium* Lam., and *Xylopia brasiliensis* Spreng. Subshrub individuals of *Piper* spp. and *Cyathea* sp. of 0.5 to 1.5 m and herbaceous *Goeppertia* spp. and lianas comprised the lower stratum.

In the Forest and Eucalyptus areas, a high density of epiphytes was recorded, mainly Bromeliaceae, and several species of bryophytes, lichens, and fungi on the tree trunks. In the Ecotone area, the structure and composition of native species was similar to the Eucalyptus area; however, exclusive native tree species were also present, such as *Prunus sellowii* Koehne, *Tibouchina granulosa* (Desr.) Cogn., and *Coffea arabica* L. and a lower density of *E. robusta* and *C. citriodora*. Table 1 shows the chemical and physical soil properties of the evaluated areas.

Collecting litter and soil

Sampling was carried out in May 2019, a period in which there is a reduced in pluviometric precipitation. In each area, five sampling points (replicates or pseudo repetition) were established; these were spaced by 10 m in a transect (50 m), where the circumscribed a) litter was collected using a metal collector (25 × 25 cm), and b) composite deformed and undeformed soil samples in the surface layer. The litter was dried in a forced air circulation oven (65 °C, 72 h) to obtain the average value (Stock$_{litter}$, kg ha^{-1}). In the Eucalyptus and Ecotone areas, the relative proportion (%) of eucalyptus leaves in the litter stock was evaluated.

Table 1. Chemical and physical properties of the soil in the 0.00–0.10 m layer in the study areas, Southeastern Brazil

Areas	pH(H_2O)	Ca^{2+}	Mg^{2+}	H+Al	SB	CEC	K	P	BS
		cmol$_c$ dm^{-3}					mg dm^{-3}		%
Euc	5.3	2.4	1.9	16.5	4.7	21.2	55	04	22
Eco	4.9	2.1	1.1	12.5	3.6	16.0	47	02	23
For	5.4	4.7	0.8	12.0	7.3	19.3	78	03	38
	Sand	**Silt**	**Clay**	**Texture class**				**Bd**	
	g kg^{-1}							Mg m^{-3}	
Euc	366	392	242	Loam				0.87	
Eco	298	263	399	Clay loam				0.94	
For	180	455	364	Silty clay loam				1.03	

pH(H_2O): active acidity; Ca^{2+}: exchangeable calcium; Mg^{2+}: exchangeable magnesium; H+Al: potential acidity; SB: sum of exchangeable bases; CEC: cation exchange capacity at pH 7.0; K: Exchangeable potassium; P: available phosphorus; BS: bases saturation; Bd: bulk density; Euc: Eucalyptus plantation; Eco: Forest-eucalyptus ecotone; and For: Forest fragment.

At each sampling point, one composite deformed soil sample was taken, made up of five simple soil samples. Totaling five composite deformed soil samples per sampling area. Each composite deformed soil sample corresponds to a replicate, collected in the 0.00–0.10 m layer, configuring 15 sampling units (three areas × five replicate). In the laboratory, they were air dried, crumbled, and passed through a 2.0-mm-mesh sieve (Teixeira et al., 2017), for determination of total soil organic carbon (TOC_{soil}, g kg^{-1}) via wet oxidation of soil organic matter with potassium dichromate in acid medium (Yeomans and Bremner, 1988). Bulk density (Bd, Mg m^{-3}) was determined in unformed samples collected using a Kopeck ring to calculate the carbon stock ($Stock_{TOCsoil}$, Mg ha^{-1}), by the equation 1 (Veldkamp,1994), found in Oliveira (2014):

$$Stock_{TOC_{soil}} = \left[\frac{TOC_{soil} \times Bd \times e}{10}\right], \quad \text{Eq. 1}$$

in which: *e* is the layer thickness (cm).

Within each point, five undeformed soil samples (clods) were randomly collected to form one composite soil sample, totaling five composite soil samples per area sampled. Each composite soil sample corresponds to a replicate, collected in the 0.00-0.10 m layer, totaling 15 sampling units (three areas × five replicate). After collection, the sample units were air-dried and then subjected to sieving using 9.7-, 8.0-, and 4.0-mm mesh sieves. Aggregates retained in the two intervals of 9.7> Ø ≥8.0 and 8.0> Ø ≥4.0 were selected for the study. In the 8.0> Ø ≥4.0 mm aggregates, sub-samples were taken for evaluation of chemical properties, glomalin, and stability of aggregates.

The pH was determined in water at 1:2.5 (m:v) ratio; exchangeable Ca^{2+}, Mg^{2+}, and Al^{3+} were extracted with KCl 1 mol L^{-1}, and determined by titrimetry; P, K, and Na^+ were extracted with Mehlich^{-1} solution, P was determined by colorimetry, and K^+ and Na^+ by flame photometry; and H+Al (potential acidity) was extracted with 0.5 mol L^{-1} calcium acetate solution (pH 7.1–7.2) and determined by titrimetry (Teixeira et al., 2017). Total organic carbon of aggregates (TOC_{AG}) was also determined (Yeomans and Bremner, 1988).

Glomalin was quantified as glomalin-related soil protein (GRSP). The readily extractable GRSP fraction (GRSP-EE) was obtained from autoclave extraction using 20 mmol L^{-1} sodium citrate solution (pH 7.0) (121 °C, 30 min). The total GRSP fraction (GRSP-T) was obtained from autoclave extraction using 50 mmol L^{-1} sodium citrate solution (pH 8.0) (121 °C, 60 min). The GRSP fractions were quantified by the Bradford method with modifications proposed by Wright and Upadhyaya (1996), using bovine serum albumin as the standard.

Stability of the aggregates was calculated using the weighted mean diameter (MWD) of the aggregates by the wet (WW) and dry (DW) determination methods. In both methods, 25 g of the 8.0–4.0 mm aggregates were placed on top of a set of sieves of 2.0, 1.0, 0.5, 0.25, and 0.105 mm for 15 min in the Yooder apparatus (WW) and in the ROTAP apparatus with controlled speed and rotary movements with vibration (DW). Material retained on each sieve was transferred to Petri dishes and dried in an oven (105 °C, 72 h). With the mass of aggregates, we calculated the weighted mean diameter (MWD_{WW} and MWD_{DW}) (Teixeira et al., 2017).

Aggregates of 9.7> Ø ≥8.0 mm were then taken to the laboratory, examined under a binocular stereomicroscope, and classified according to their origin or formation pathways. For this study, two classes (biogenic and physicogenic) were identified and evaluated from the morphological patterns established by Bullock et al. (1985). This was done using a protocol adapted by Pulleman et al. (2005) and validated by the studies gathered by Pereira et al. (2021). Differentiation between the aggregates 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; Melo et al., 2019; Pereira et al., 2021), and subunit arrangements and junctions (Pereira et al., 2021).

Biogenic classes were those that was possible to visualize rounded shapes. These were produced in the intestinal tract of soil macrofauna, mainly Oligochaeta (earthworms). Biogenic classes also include samples where it was possible to visualize the presence and activity of roots. Physicogenic class was defined as those that had angular shapes resulting from the interaction between carbon, clay, cations, and soil wetting and drying cycles. After identification, the relative contribution (% by mass) of each aggregate class from each sample area was determined. To determine relative mass contribution, 100 g of aggregate was weighted for each replicate and area (Pereira et al., 2021).

Aggregates were crushed and passed through a 2.0-mm-mesh sieve (Teixeira et al., 2017) to analyze organic matter fractions. Humic substances (HS) were obtained by extraction and separation by differential solubility of organic matter in basic or acid medium (fulvic and humic acids, respectively FAC and HAC) and the residue (humin, HUMC) (Benites et al., 2003). Total organic carbon of biogenic, physicogenic aggregates (TOC_{Bio} and TOC_{Phy}), and humic fractions was determined (Yeomans and Bremner 1988).

Physical grain size fractionation of the soil organic matter (SOM) fractions into particulate fractions (related to the soil sand fraction) and mineral-associated fractions (related to clay and silt fractions) was performed by the Cambardella and Elliot (1993) method. Physical densimetric fractionation of SOM in the light-free fraction (FLF) and light intra-aggregate fraction (ILF) (Sohi et al., 2001; Machado, 2002) was performed by separating the little altered organic residues from the more humified organic material, through density. We determined the organic carbon of the particulate fraction (POC), free light fraction (FLFC), and intra-aggregate light fraction (ILFC) of the SOM (Yeomans and Bremner, 1988) and the organic carbon of the mineral-associated fraction (MAOC) of the SOM by the difference between TOC and POC.

Statistical analyses

The study was statistically analyzed in a unifactorial scheme in a completely randomized design for the variables related to litter stock, chemical properties, glomalin and aggregate stability. Statistical study was carried out in a 3 × 2 factorial scheme (three areas evaluated × two aggregate formation pathways) in a completely randomized design for the variables related to aggregate formation pathways. All the data was analyzed for homoscedasticity of variance using Levene’s test. Variables that were not considered homogeneous were transformed and tested again.

All the data was then submitted to analysis of variance using the *F*-test (ANOVA) when the assumption of homogeneity was met (variables transformed or not) and the means were compared using Bonferroni's parametric *t*-test. The statistical tests applied were carried out at a 5 % significance level using the software Sisvar, version 5.6 (Ferreira, 2011).

In addition, multivariate principal component analysis (PCA) and hierarchical grouping (cluster dendrogram) were carried out based on the properties evaluated, to help distinguish between the areas. Multivariate analyses were also carried out at a 5 % significance level using the R Software (R Development Core Team, 2020) using the "Ggplot2" package.

RESULTS

Litter stock, soil total organic carbon content, and stock

The highest values of litter stock ($Stock_{litter}$) were quantified in the Eucalyptus and Ecotone areas. The highest values of organic carbon content were quantified in the Eucalyptus area, which did not differ from the Forest and Ecotone area. As for the carbon stock, the highest values were observed in the Forest and Eucalyptus areas (Table 2).

Chemical, biological and physical properties of 8.0–4.0 mm aggregates

Values of pH, Ca^{2+}, Mg^{2+}, Al^{3+}, and H+Al quantified in the aggregates did not differ among areas (Table 3). The highest content of P and Na^{+} was observed in the Eucalyptus area when compared with the other areas, which did not differ among themselves. The K^{+} was higher in the Eucalyptus and Forest areas than in the Ecotone area, but it did not differ among themselves.

In the Eucalyptus area, we observed higher values of GRSP-T and MWD_{DW} compared with the other areas. It also presented higher values of TOC_{AG} compared with the Ecotone area. Forest area presented intermediate values of TOC_{AG}, which did not differ from the other areas (Table 4). There were no differences between the areas for GRSP-EE or MWD_{WW}.

Hierarchical grouping analysis (Figure 2a), integrating the chemical properties (pH, Ca^{2+}, Mg^{2+}, P, Na^{+}, Al^{3+} and H+Al), TOC_{AG}, GRSP-T, GRSP-EE, MWD_{DW} and MWD_{WW} of the aggregates, separated the areas in two groups. The first group was formed only by the Eucalyptus area, which presented dissimilarity of approximately 60 % compared to the second group, formed by the Forest and Ecotone areas. The dissimilarity between these two areas was lower than that of the Eucalyptus area, around 40 %, which may be associated with the spatial proximity between them, as well as the same position in the landscape.

Table 2. $Stock_{litter}$, total soil organic carbon content, and stock in the 0.00-0.10 m layer (TOC_{soil} and $Stock_{TOC_{soil}}$, respectively) in areas under eucalyptus plantation (Euc), forest-eucalyptus ecotone (Eco), and forest fragment (For), Southeastern Brazil

Areas	$Stock_{litter}$	TOC_{soil}	$Stock_{TOC_{soil}}$
	kg ha^{-1}	g kg^{-1}	Mg ha^{-1}
Euc	21.67 a	44.67 a	38.87 a
Eco	24.89 a	34.25 b	32.11 b
For	17.33 b	38.06 b	39.07 a
CV%	26.3	5.7	7.5

Averages followed by the same letters do not differ significantly by Bonferroni's *t*-test at 5 %. CV: coefficient of variation. $Stock_{litter}$: stock of litter; TOC_{soil}: soil total organic carbon; and $Stock_{TOCsoil}$: stock of soil total organic carbon.

Table 3. Chemical properties of the aggregates in areas under eucalyptus plantation (Euc), forest-eucalyptus ecotone (Eco), and forest fragment (For) in the 0.00-0.10 m layer, Southeastern Brazil

Areas	pH(H_2O)	Ca^{2+}	Mg^{2+}	Al^{3+}	H+Al	K^+	Na^+	P
		cmol$_c$ dm^{-3}						mg dm^{-3}
Euc	4.42 a	1.51 a	1.97 a	2.41 a	17.13 a	0.01 a	0.006 a	1.83 a
Eco	4.36 a	0.39 a	2.86 a	1.59 a	9.96 a	0.005 b	0.001 b	1.61 b
For	4.80 a	2.63 a	1.87 a	1.30 a	10.77 a	0.01 a	0.002 b	1.48 b
CV%	9.6	34.3	27.0	20.0	29.9	39.2	27.4	24.3

Averages followed by the same letters do not differ significantly by the Bonferroni's t-test at 5 %. CV: coefficient of variation. pH(H_2O): active acidity; Ca^{2+}: exchangeable calcium; Mg^{2+}: exchangeable magnesium; Al^{3+}: exchangeable acidity; K^+: exchangeable potassium; Na^+: exchangeable sodium; and P: available phosphorus.

Table 4. Total organic carbon of aggregates (TOCAG), soil protein related to total glomalin (GRSP-T) and easily extractable (GRSP-EE), and weighted mean diameter dry (MWDDW) and wet (MWDWW) in areas under eucalyptus plantation (Euc), forest-eucalyptus ecotone (Eco), and forest fragment (For) in the 0–0.10 m layer, Southeastern Brazil

Areas	TOC_{AG}	GRSP-T	GRSP-EE	MWD_{DW}	MWD_{WW}
	g kg^{-1}	mg g^{-1}		mm	
Euc	39.00 a	11.53 a	3.31 a	4.83 a	4.35 a
Eco	32.06 b	4.25 c	2.81 a	4.62 b	4.38 a
For	34.17 ab	8.30 b	3.02 a	4.58 b	4.08 a
CV%	8.8	6.5	11.9	2.2	5.8

Averages followed by the same letters do not differ significantly by the Bonferroni's *t*-test at 5 %. CV: coefficient of variation.

In the principal components analysis, it was considered the first two principal components (PC1 and PC2), which explained 66.78 % of the total variability of the data (Figure 2b). This analysis indicated an association of the Eucalyptus area with K^+, Na^+, P, H+Al, TOC_{AG}, GRSP-T, GRSP-EE, and MWD_{DW}, and an association of the Forest and Ecotone areas with Mg^{2+} and MWD_{WW}, respectively. The properties that contributed the most to separate the areas, that is, that presented significant influence (correlation coefficient >0.70) on the principal component 1, which explained the greatest variability of the results (41.04 %), were GRSP-T (0.91), Na^+, (0.88), TOC_{AG} (0.82), MWD_{DW} (0.80), K^+ (0.79) and P (0.73).

Relative proportion and organic matter fractions of biogenic and physicogenic aggregates

Regarding the morphological classification of aggregates, the biogenic morphological class predominated in all areas (88, 89, and 93 % in Eucalyptus, Ecotone, and Forest, respectively), compared to physicogenic (7, 11, and 12 %, respectively) (Figure 3a). However, the relative proportions of these two aggregate formation pathways did not differ among the areas, probably owing to the low degree of anthropogenic disturbance in the study areas.

Higher values of TOC_{Bio} and TOC_{Phy} (47.70 and 39.81 g kg^{-1}) were quantified in the Eucalyptus area, compared to the Ecotone (32.44 and 24.21 g kg^{-1}) and Forest (27.72 and 24.70 g kg^{-1}) areas (Figure 3b). The results for these properties corroborate the results verified for $Stock_{litter}$, TOC_{soil} and $Stock_{TOCsoil}$ (Table 2), TOC_{AG}, GRSP-T and MWD_{DW} (Table 4). The TOC_{Bio} values were higher than TOC_{Phy} values in the Eucalyptus and Ecotone areas (Figure 3b). There were no differences between the TOC_{Bio} and TOC_{Phy} values in the Forest area.

Humic acid (HAC), fulvic acid (FAC) and humin (HUMC) fractions showed a similar pattern to that observed for TOC_{Bio} and TOC_{Phy} (Figure 2b), with higher levels observed in the physicogenic and biogenic aggregates in the Eucalyptus area compared to the Forest and Ecotone areas (Table 5). These results are also similar to those found for the properties

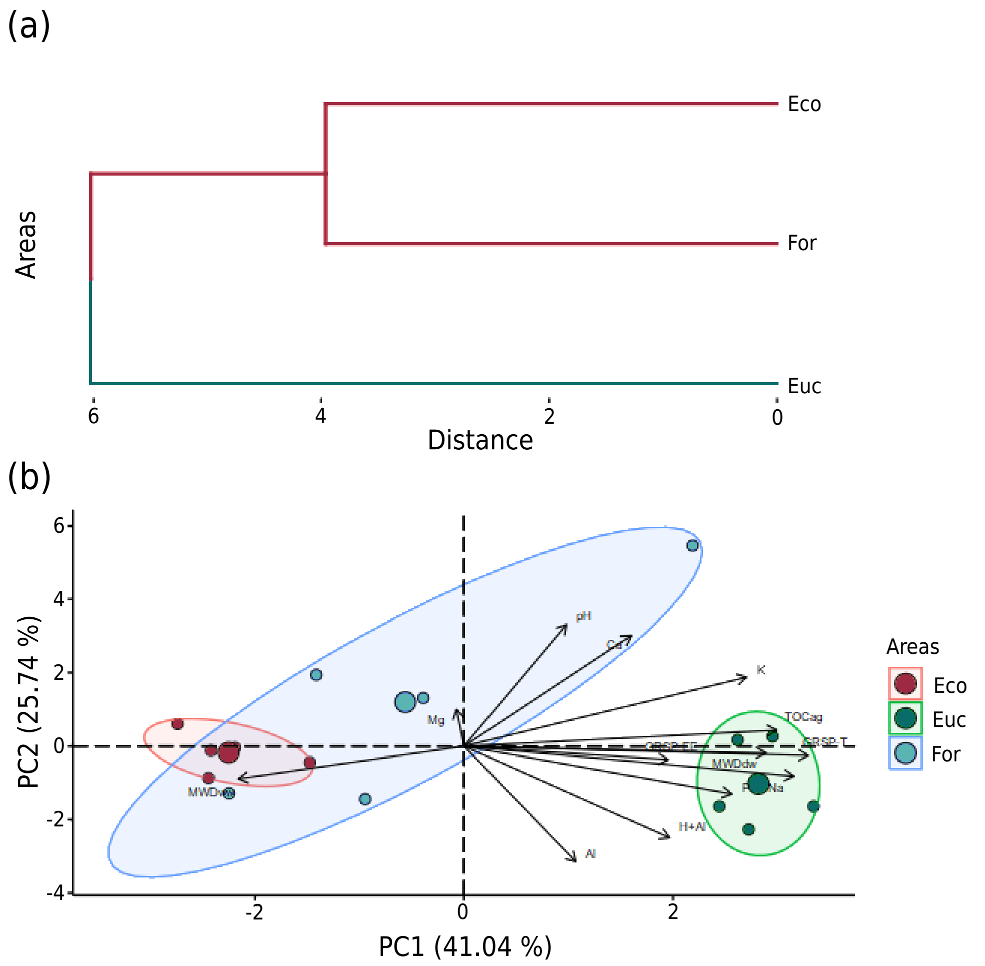

Figure 2. Hierarchical clustering dendrogram (a) and principal component analysis (b) integrating chemical properties, total organic carbon, glomalin, and stability of aggregates in areas under eucalyptus plantation (Euc), forest-eucalyptus ecotone (Eco), and forest fragment (For) in the 0.00-0.10 m layer, Southeastern Brazil. pH: active acidity; Ca^{2+}: exchangeable calcium; Mg^{2+}: exchangeable magnesium; Al^{3+}: exchangeable acidity; K: exchangeable potassium; Na^{+}: exchangeable sodium; P: available phosphorus; GRSP-T: total glomalin-related soil protein; GRSP-EE: easily extractable glomalin-related soil protein; MWDdw: dry weighted mean diameter; MWDww: wet weighted mean diameter; and TOC_{AG}: Total organic carbon of aggregates.

Stocklitter, TOC_{soil} and $Stock_{TOCsoil}$ (Table 2), TOC_{AG}, GRSP-T and MWD_{DW} (Table 4). No differences were found between the Forest and Ecotone areas for these fractions (Table 5).

Higher content of HAC and HUMC was observed in biogenic aggregates than in physicogenic ones only in the Eucalyptus area (Table 5). In relation to the carbon distribution in these fractions, high carbon concentrations in %HUMC were found for both formation pathways in all areas. Results of %HAC and %HUMC were, in general, higher in the Eucalyptus area than in other areas. %HS predominated in relation to non-humified carbon, both in biogenic and physicogenic aggregates, in the three areas. Proportion of %HS in relation to percentage of non-humified carbon (%HNC) in biogenic and physicogenic aggregates was higher in the Eucalyptus area when compared to the Forest and Ecotone areas (Table 5).

For the particle size physical fractions (particulate organic carbon and associated to minerals, POC and MAOC) and densimetric fractions (organic carbon of the light free and light intra-aggregate fractions, FLFC and ILFC), the highest content was also observed in the Eucalyptus area in both aggregates formation pathways (Table 6). Higher carbon content was observed only in the MAOC fraction in biogenic aggregates in the Ecotone area compared to the Forest area (Table 5).

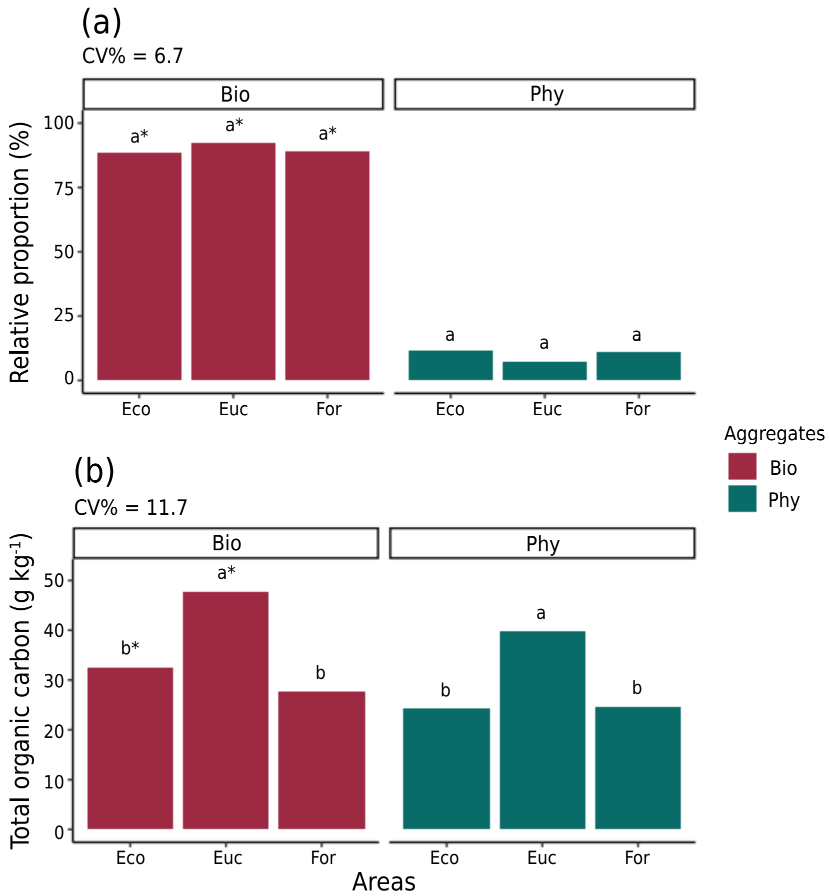

Figure 3. Relative proportion and total organic carbon (TOC) of biogenic (Bio) and physicogenic (Phy) aggregates of biogenic and physicogenic aggregates in areas under eucalyptus plantation (Euc), forest-eucalyptus ecotone (Eco), and forest fragment (For) in the 0.00-0.10 m layer, Southeastern Brazil. Averages followed by the same letters do not differ significantly by the Bonferroni's *t*-test at 5 %. (*) Indicates a significant difference between biogenic and physicogenic aggregates by Bonferroni's *t*-test at 5 %.

Table 5. Chemical fractionation of organic matter (g kg^{-1} and %) of biogenic (bio) and physicogenic (phy) aggregates in areas under eucalyptus plantation (Euc), forest-eucalyptus ecotone (Eco), and forest fragment (For) in the 0.00-0.10 m layer, Southeastern Brazil

Areas	HAC		FAC		HUMC		HAC	
	bio	phy	bio	phy	bio	phy	bio	phy
	g kg^{-1}						%**	
Euc	10.11 a*	8.24 a	8.23 a	7.66 a	31.38 a*	27.05 a	21.19 ab	20.66 a
Eco	5.28 b	5,06 b	5.91 b	4.90 b	13.75 b	11.75 b	16.36 b	19.72 a
For	5.83 b	4.78 b	4.46 c	4.75 b	13.00 b	12.67 b	23.75 a	19.43 a
CV%	20.2		13.7		14.0		16.7	
	%FAC		%HUMC		%HS		%HNC	
	bio	phy	bio	phy	bio	phy	bio	phy
	%**							
Euc	16.36 a	18.31 a	63,17 a	65.80 a	97.88 a	97.67 a	2.12 c	2.33 b
Eco	18.23 a	20.22 a*	39.56 b	43.53 b	74.15 c	83.47 b*	25.85 a*	16.53 a
For	16.03 a	19.38 a*	49.35 b	49.05 b	85.16 b	87.86 b	14.84 b	12.14 a
CV%	9.1		11.5		4.7		18.8	

Means followed by the same letter in the column do not differ by the Bonferroni's t-test at 5 %. (*) Significant difference between biogenic and physicogenic aggregates by the Bonferroni's t-test at 5 %. (**) Percentage of the fraction evaluated in relation to TOC. HAC: Humic carbon-acids; FAC: Fulvic carbon-acids; HUMC: Humic carbon; %HS: percentage of humic substances; and %HNC: percentage of non-humified carbon. CV: Coefficient of Variation.

Table 6. Particle size fractionation of organic matter of biogenic (bio) and physicogenic (phy) aggregates in areas under eucalyptus plantation (Euc), forest-eucalyptus ecotone (Eco), and forest fragment (For) in the 0.00-0.10 m layer, Southeastern Brazil

Areas	POC		MAOC		FLFC		ILFC	
	bio	phy	bio	phy	bio	phy	bio	phy
	g kg^{-1}							
Euc	10.80 a*	9.15 a	36.90 a*	29.30 a	2.68 a*	1.71 a	1.88 a*	1.32 a
Eco	5.04 b*	3.30 b	27.39 b*	21.00 b	1.79 b	1.19 a	0.73 b	0.62 b
For	6.69 b*	4.04 b	21.74 c	19.47 b	1.37 b	1.18 a	0.52 b	0.58 b
CV%	16.0		12.3		28.1		14.9	
	%POC		%MAOC		%FLFC		%ILFC	
	bio	phy	bio	phy	bio	phy	bio	phy
	%**							
Euc	22.90 a	23.14 a	77.10 b	76.86 b	5.64 a*	4.31 a	3.98 a*	3.34 a
Eco	15.43 b	13.24 b	84.57 a	86.76 a	5.60 a	4.93 a	2.26 b	2.57 ab
For	24.38 a*	16.59 b	75.72 b	83.39 a*	5.60 a	4.75 a	1.93 b	2.38 b
CV%	18.3		4.3		16.7		17.0	

Means followed by the same letter in the column do not differ by the Bonferroni's *t*-test at 5 %. (*) Significant difference between biogenic and physicogenic aggregates by the Bonferroni's *t*-test at 5 %. (**) Percentage of the fraction in relation to TOC. POC: particulate organic carbon; MAOC: mineral-associated organic carbon; FLFC: free light fraction carbon; and ILFC: carbon: intra-aggregate light fraction. CV: coefficient of variation.

In biogenic aggregates, high content of POC, MAOC, FLFC, ILFC, %POC, %FLFC, and %ILFC was quantified in most areas (Table 5). Results corroborate those verified for TOC_{Bio} (Figure 2b), HAC and HUMC attributes (Table 5). Proportions of %MAOC in relation to %POC predominated in the biogenic and physicogenic aggregates in all areas (Table 6).

Hierarchical grouping analysis for organic matter properties in biogenic and physicogenic aggregates separated the areas and the respective aggregate formation pathways into two large groups (Figure 4a). One group was formed by the Eucalyptus area, where we observed dissimilarity around 80 %, compared to the Ecotone and Forest areas, which were associated by a dissimilarity of approximately 25 % (Figure 4a).

The PCA revealed that the organic matter properties of the different aggregate formation pathways contributed expressively to the differentiation between the areas. Principal components 1 and 2 explained 88.43 % of the total variability of the data (Figure 4b). Principal component 1 explained 82.43 % of the variability of the data and presented high positive correlation coefficients (>0.83) with the properties TOC, HAC, FAC, HUMC, POC, MAOC, FLFC, and ILFC. Principal component 2 explained only 6.0 % of the data variability and showed inexpressive correlations with the attributes (< 0.70) (Figure 4b).

DISCUSSION

Litter stock and soil total organic carbon storage

Relative proportion of eucalyptus leaves in the Eucalyptus area was 14 %, almost three times higher than that observed in the Ecotone area (5 %). The slow decomposition of this material due to the efficient internal retranslocation of N and P (Cunha et al., 2019) may have contributed to the high $Stock_{litter}$ values in such areas (Table 2).

The pattern of higher TOC_{soil} values in the Eucalyptus area compared to the Forest (Table 2) was the opposite of that observed in a study conducted in the municipality of Chapecó (SC), in which higher values of this property were seen in the Mixed Ombrophilous Forest area than in a plantation of *Eucalyptus saligna* Sm (Denardin et al., 2014). This divergence was probably due to the difference in age of the plantations, which was eight years in that study, and 100 years in the present study.

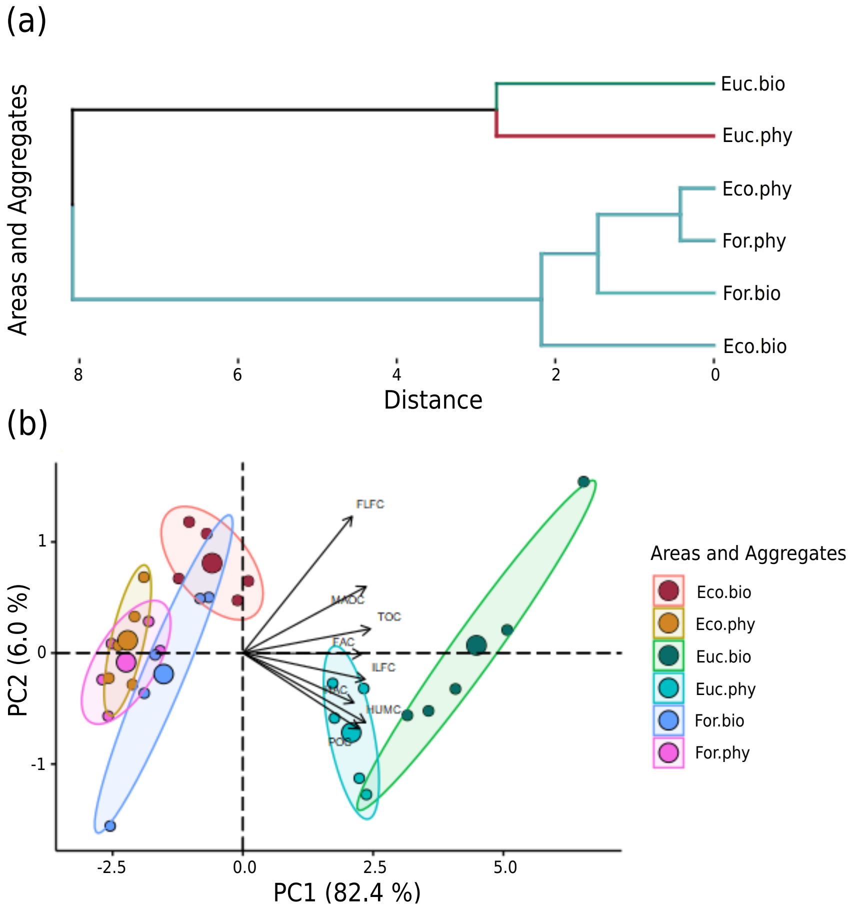

Figure 4. Hierarchical clustering dendrogram (a) and principal component analysis (b) integrating the chemical and physical fractions of organic matter of biogenic (bio) and physicogenic (phy) aggregates in areas under eucalyptus plantation (Euc), forest-eucalyptus ecotone (Eco), and forest fragment (For) in the 0.00-0.10 m layer, Southeastern Brazil. TOC: total organic carbon; POC: particulate organic carbon; MAOC: mineral-associated organic carbon; HAC: humic acids carbon; FAC: fulvic acids carbon; HUMC: humin carbon; FLFC: free light fraction carbon; and ILFC: intra-aggregate light carbon-fraction.

Besides age, the positioning of the Eucalyptus area at the lowest point of the landscape may also have contributed to the high TOC_{soil} and $Stock_{TOCsoil}$ values, which did not differ significantly from that quantified in the Forest area due to the disfavoring of the decomposition of soil organic matter (SOM) (Table 2). This pattern is based on the rainfall coming from the highest parts, where Forest and Ecotone areas are located, as it tends to accumulate in the lower part of the landscape. This pattern was verified in areas of forest and forest-field edge ecotone in the southern region of the state of Amazonas, where higher TOC_{soil} values were recorded in points of the landscape located in the depressions than in those in high areas (Vidotto et al., 2007). Therefore, the maintenance of SOM and soil carbon content and stocks can be affected by numerous factors, such as soil type and initial condition, climate, position in the relief, management practices, time to add the vegetation cover, among others (Denardin et al., 2014), which influence the relationship between carbon input and the decomposition/mineralization process of SOM.

It is worth noting that the Eucalyptus area was classified as having a loam texture in the first 0.10 m of depth, with lower clay contents (242 g kg^{-1}) compared to the Ecotono (clay loam) and Forest (silty clay loam) areas, with clay contents of around 399 and 364 g kg^{-1} (Table 1). This could compromise the accumulation of SOM in the Eucalyptus area. The TOC_{soil} and $Stock_{TOCsoil}$ results show that soil texture was not a limiting factor for the accumulation of SOM in the Eucalyptus area, since the medium-textured area is storing more carbon, especially when compared to the clay-textured Ecotone area (Table 2).

High value of $Stock_{TOCsoil}$ in the Forest area may be related to the stability of this ecosystem, reflected by the continuous addition of organic material via litter and subsurface due to the renewal of the root system (Table 2). Changes in this material occur in specific situations (droughts, frosts, and fires, among others), but whose effects, due to the periodicity and/or intensity, normally do not manifest themselves in the levels of carbon stored in the soil (Alves et al., 2008; Costa et al., 2008).

Fertility, glomalin and stability of aggregates

Variation between the areas regarding the composition of the plant community and, consequently, the chemical quality of the litter, probably influenced the difference between the areas with respect to the content of P and K^+ in the topsoil (0.00-0.10 m) (Table 3). In northern China, revegetation under agro-pastoral ecotone led to increased TOC levels in both deformed and aggregate samples, mainly owing to changes in macroaggregation (Yao et al., 2019). However, this increase was even greater when fabaceous species were used in the revegetation of the areas compared to other species after approximately 20 years.

High values of GRSP-T and MWD_{DW} (Table 4) may be associated with the high value of TOC_{soil} (Table 2), in the Eucalyptus area. Some authors found a significant positive correlation between glomalin fractions and TOC content in uncultivated and cultivated soils (Nichols and Wright, 2005; Lima et al., 2013), probably owing to the similarity between these properties regarding the dynamics of decomposition (Bedini et al., 2007). The high stability of aggregates is directly related to the carbon content in the soil (Hickmann et al., 2011).

According to Wright et al. (2007), GRSP is related to increased aggregate stability and can provide a quantitative measure of specific SOM components. Pinto et al. (2022) found relationships between oxidizable carbon, GRSP-EE, GRSP-T, and MWD in areas under different management systems, for example. According to the authors, these results show that the associations between binding agents and the stability of aggregates are more dependent on carbon concentrations and less disturbance of the arable layer, and are more related to macroaggregates than microaggregates. The GRSP levels can vary from 2.0 to 15.0 mg g^{-1} in arable soils, pastures and natural ecosystems (Wright and Upadhyaya 1996; Wright et al., 2000). The levels quantified in this study are within the range indicated by the authors.

It is worth noting that the MWD values in both methods of assessing the state of aggregation (wet and dry) were above 4.0 in all three areas (Table 4). Studies have observed that MWD values below 2.0 mm indicate soils in unfavorable physical conditions with regard to aeration; water infiltration and redistribution; and root system establishment (Briedis et al., 2012; Tivet et al., 2013). Multivariate analysis (Figure 2) was more efficient in separating the areas evaluated based on the analysis of chemical properties, glomalin and stability of 8.0–4.0 mm aggregates. The PCA (Figure 2a) showed that only the Mg^{2+} and MWD_{WW} properties were not influenced by the soil conditions related to the Eucalyptus area.

Aggregate formation pathways and associated organic matter

Relative proportions of these two aggregate formation pathways did not differ between the areas, probably due to the low degree of anthropogenic disturbance in the study areas (Figure 3a). As for relative proportion and SOM fractions of biogenic and physicogenic aggregates the pattern observed may indicate the similarity between the areas regarding the availability of favorable conditions for the activity of soil fauna, which is the main precursor of biogenic aggregates (Jouquet et al., 2006; Fernandes et al., 2017), such as microclimate and soil chemical and physical properties.

Presence of trees and litter stock favor the activity of macrofauna, allowing them to act on the soil forming biogenic structures by homogenizing the mineral and organic fractions

(Suárez et al., 2019). In general, differences are observed between areas regarding this property when comparing regions of Atlantic Forest under different regeneration stages, as verified in southeastern Brazil (Silva Neto et al., 2016; Fernandes et al., 2017). In the Colombian Amazon, Suárez et al. (2019) classifying soil macroaggregates under forest area into biogenic aggregates from fauna (coprolites) and flora (root), physicogenic and non-macroaggregated fraction, quantified proportions of 16.0, 12.5, 18.7, and 52.3 %, respectively.

In southeastern Brazil, Schultz et al. (2019), evaluating the influence of tree vegetation and grasses on aggregate formation pathways in *Argissolo Vermelho-Amarelo* (*Udult*), verified a predominance of biogenic aggregates (70.5 and 63.0 %) compared to physicogenic (29.5 and 37.0 %) in an area of tree vegetation. In Paraná, Southern Brazil, under *Latossolo Vermelho Eutroférrico* (*Rhodic Ferralsol*), Ferreira et al. (2020) observed that the proportion of biogenic aggregates was higher (51.2 and 53.8 %) than that of intermediate (27.5 and 25.1 %) and physicogenic (21.3 and 21.1 %) aggregates in a native Atlantic Forest fragment in two sampling seasons. Presence of biogenic aggregates, invertebrates, and roots demonstrates high biological activity, which probably indicates a high quality in soil processes and optimal biological regulation in the functioning of the edaphic system (Velasquez and Lavelle, 2019).

The pattern of TOC results between the aggregate formation pathways (Figure 3b) resembles the pattern seen in other studies (Pulleman et al., 2005; Silva Neto et al., 2016; Fernandes et al., 2017; Schultz et al., 2019; Suárez et al., 2019). According to Pinto et al. (2022), the results verified in this study (Figure 3b) reinforce the hypothesis that biogenic aggregates lead to improved soil quality, especially with regard to their greater capacity to preserve and accumulate the more soluble fractions of SOM. In the Forest area, higher TOC content was recorded in biogenic aggregates compared to intermediate and physicogenic aggregates (Ferreira et al., 2020). Higher TOC levels were determined in biogenic aggregates compared to the physicogenic pathway in forest fragments under different relief conditions in southeastern Brazil (Pinto et al., 2021).

It is also noteworthy in this study that, in the biogenic aggregates of the Eucalyptus area, higher levels of organic carbon were quantified (numerically) compared to that of the sampled soil and the 8.0-4.0 mm aggregates (47.70, 44.67 and 39.00 g kg^{-1}, TOC_{Bio}, TOC_{soil}, and TOC_{AG}, respectively) (Figure 3b, Tables 2 and 3). In areas with the absence or low intensity of anthropic actions, it is assumed high activity of biological agents (macrofauna and flora), responsible for the addition and incorporation of carbon in aggregates of biogenic origin (Pinto et al., 2022). Thus, it can be inferred that the enrichment of organic carbon in biogenic aggregates was more evident in comparison with the deformed soil samples, and that these aggregates, besides indicating greater biological activity, also promote the accumulation of organic carbon in the soil (Ferreira et al., 2020; Pinto et al., 2021).

For organic matter fractions, higher levels of HAC and HUMC in biogenic aggregates (Table 5) were also verified by other authors, who suggest that the biogenic pathway can be adopted as a potential indicator in the evaluation of soil quality, owing to its greater sensitivity to different soil and environmental conditions (Silva Neto et al., 2010; Loss et al., 2014; Fernandes et al., 2017). Higher levels of FAC, HAC, HUMC, and %HS in biogenic aggregates compared to those in the physicogenic pathway (Table 5) indicate that the former aggregates have the ability to protect and stabilize humic substances, also favoring the maintenance and accumulation of carbon in these fractions (Pinto et al., 2021; Rossi et al., 2023).This pattern was also observed in other studies (Pulleman et al., 2005; Loss et al., 2014; Schultz et al., 2019).

In the Atlantic Forest biome, Rossi et al. (2023) found an increase in the organic carbon content of the humic and oxidizable fractions of SOM in biogenic aggregates, especially those formed under coffee systems grown in full sun, coffee shaded by gliricidia and agroforestry systems. Studying fragments of the Atlantic Forest at different stages of regeneration, Fernandes et al. (2017) quantified higher levels of HUMC in biogenic aggregates. For the authors, these results are related to the agents responsible for the formation of these aggregates, especially earthworms (edaphic macrofauna), since they have a direct action on biogeochemical cycling in the soil (Jouquet et al., 2006), positively affecting the ecology of the humification process (Fernandes et al., 2017; Rossi et al., 2023).

Results of the physical fractions of SOM (POC, FLFC, and ILFC) in biogenic aggregates indicate the predominance of organic material of high lability (great availability), whose incorporation dynamics, maintenance, and accumulation are benefited by edaphic biological activity (soil fauna and root system) (Loss et al., 2014; Pinto et al., 2022). Such aggregates provide a favorable environment for the physical protection of these fractions after encapsulation.

Results for the properties of $Stock_{litter}$, TOC_{soil}, $Stock_{TOCsoil}$ (Table 2), TOC_{AG}, GRSP-T, MWD_{DW} (Table 4), TOC_{Bio}, TOC_{Phy} (Figure 3 b), HAC, FAC, HUMC (Table 5), POC, MAOC, FLFC, and ILFC (Table 6) in the Eucalyptus area may be related to the characteristics of the area discussed above. As for multivariate analysis, in this study, the main axis separated the forest and ecotone areas, located in the upper and lower left quadrants, from the eucalyptus area, located in the upper and lower right quadrants, whose variables were more associated with the latter area (Figure 4b). This has corroborated the results of the statistical tests applied.

Evaluating the PCA results, Ferreira et al. (2020) found that the analysis showed a clear distinction between the environments evaluated, in which the attributes associated with SOM in the native forest area showed positive values and high correlation with PC1, unlike the managed areas, in which negative values were observed. Pinto et al. (2021) also verified in their study a clear separation between the evaluated areas and the pattern observed in the PCA in association with the statistical tests applied, evidencing the favoring of biogenic aggregation compared to the different fractions of SOM.

The variables TOC, HAC, FAC, HUMC, POC, MAOC, FLFC and ILFC were those that most contributed to the discrimination of areas in the study of aggregates formation pathways and SOM-associated (Figure 4b). This has reinforced the hypothesis of a possible direct relationship between these indicators in the Eucalyptus area, reflecting the edaphic conditions of the area, which favor the mechanisms of stabilization and accumulation of SOM, especially in biogenic aggregates.

CONCLUSIONS

Litter stock was higher in the Eucalyptus and Ecotone areas than in the Forest area, probably owing to the slow decomposition of the litter deposited by the eucalyptus.

Characterization of the chemical, biological, and physical properties of aggregates, as well as the compartmentalization of SOM-associated with aggregates of different origins (biogenic and physicogenic), allowed to discriminate the Forest and Ecotone areas from the Eucalyptus area.

In biogenic aggregates, higher levels of total organic carbon and its respective chemical and physical fractions were found than in the physicogenic ones, which highlighted the importance of studying the origin of aggregates in the evaluation of soil quality in different forest ecosystems.

Dynamics of edaphic properties in the Ecotone area is similar to the forest fragment, compared to the eucalyptus plantation. However, in general, all different vegetation covers contribute to the maintenance of soil quality in the Nova Baden State Park area.

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