

# Organic matter fractions of soil aggregates under agroecological production systems in the southeast of Brazil<sup>1</sup>

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**ABSTRACT** - Agroecological management is considered a model of sustainable agriculture that offers social, environmental and economic benefits. The hypotheses of this study were that agroecological production systems can promote changes in the physical fractions of soil organic matter (SOM) associated with aggregates of different origins, and that the greater diversity of plant species in the production system may favour an increase in the carbon concentration of these aggregates. The aim of this study was to a) determine the mass of the free light fraction (FLF), intra-aggregate light fraction (ILF) and light organic matter fraction (LOM) of biogenic and physiogenic soil aggregates; and b) quantify the organic carbon content of the three light SOM fractions and the residual particulate SOM fraction. Five production systems were evaluated: AgF – Agroforestry system; CSun – Coffee grown in full sun; CSha – Coffee grown in shade; FLE – Flemingia grown in alleys; and NT – No-tillage. The aggregates were separated, identified and classified according to their origin or formation pathway into biogenic (formed by biological processes) or physiogenic aggregates (resulting from chemical and physical actions). From these, the mass and carbon content of the FLF, ILF, LOM and residual particulate fractions were quantified. The greater diversity of plant species found in the AgF system has not yet favoured an increase in the carbon content of the aggregates. The longer set-up time and the management practices of the CSun system has led to an increase in the carbon content of the organic fractions of the aggregates (3.44–1.63 g kg<sup>-1</sup> for C-LOM; 1.93–1.13 g kg<sup>-1</sup> for C-FLF). The lowest overall mean values for LOM and ILF were found mainly in the aggregates of the NT system (1.22–1.67 and 1.55–2.20 g kg<sup>-1</sup>, respectively), being associated with the shorter time and greater mobilisation of the arable layer. Biogenic aggregation afforded the highest overall mean values for LOM, and increased the mass of the light fractions (15.5%–27.8% for FLF; 23.4%–8.0% for ILF) and the carbon in the C-LOM, C-FLF and residual particulate fractions (42.3%, 12.9%, and 35.0%, respectively) in the surface layer, suggesting an improvement in soil quality.

**Key words:** Biogenic aggregates. Carbon protection. Density fractions.

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## INTRODUCTION

In the agricultural production process, there are adverse changes to soil properties when the management of agrifood systems does not adopt sustainable techniques. This can affect and compromise the quality of the soil, promoting unfavourable changes in the soil system, e.g. increased environmental degradation, a reduction in natural fertility, and a decrease in crop productivity. In this respect, agroecological management is seen as a model of sustainable agriculture. The model proposes to rescue the dignity of farmers, who throughout history have domesticated plants and animals, and maintained much of the genetic diversity used by man (NODARI; GUERRA, 2015).

Changes in the transition process of the use and management of arable land can be measured and evaluated using soil quality indicators. Soil aggregation and the soil organic matter (SOM) are efficient indicators for this evaluation, especially when analysed together (organic matter associated with soil aggregation), due to the strong interaction between the two (BRONICK; LAL, 2005). Currently, compilations of Brazilian (PEREIRA *et al.*, 2021) and international (GUHRA; STOLZE; TOTSCHKE, 2022; LAVELLE *et al.*, 2020) studies involving the formation of aggregate pathways (or the origins of aggregates) and compartmentalisation of the associated SOM have shown considerable satisfactory results in employing these attributes for monitoring soil quality, with emphasis on studies carried out in different soil use and management systems.

Additionally, Batista *et al.* (2013), Ferreira *et al.* (2020), Loss *et al.* (2014), Pinto *et al.* (2021), Pulleman *et al.* (2005), Rossi *et al.* (2016) and Schultz *et al.* (2019) found that monitoring agricultural areas in production systems is more accurate when the SOM is evaluated using physical fractionation methods, as these are of low cost, and are highly practical and efficient in the separation process due to the action of density and ultrasonic energy, the activity of dispersing agents and to sieving (CAMBARDELLA; ELLIOTT, 1993; CONCEIÇÃO *et al.*, 2008; GAVINELLI *et al.*, 1995; SOHI *et al.*, 2001).

Based on the above, the hypotheses of this study were that **H1**) agroecological production systems can promote changes in the physical fractions of the soil organic matter associated with aggregates of different origins; and **H2**) a greater diversity of plant species in the production system may favour an increase in the carbon concentrations of these aggregates. The aims of the study were **a**) to determine the mass of the free light fraction (FLF), intra-aggregate light fraction (both in sodium iodide), and the light organic matter fraction (in water) of biogenic and physiogenic soil aggregates; and **b**) quantify the organic carbon content of the three light fractions and the residual particulate fraction.

## MATERIAL AND METHODS

### Location, climate and soil of the study area

The study was carried out in an area of the Integrated Agroecological Production System (SIPA) known as the 'Fazendinha Agroecológica do km 47' (ALMEIDA; RIBEIRO; GUERRA, 1999), located at Embrapa Agrobiologia, in Seropédica in the state of Rio de Janeiro, in the southeast of Brazil (22°45' S and 43°41' W; at an altitude of 33 metres). The climate in the region is classified as type Aw (NEVES *et al.*, 2005). According to Santos *et al.* (2018), the soil was classified as a Red-Yellow Argisol, presenting a sandy texture in the surface layer and located in an area of gently undulating relief.

### History of the systems under evaluation

Five production systems under agroecological management were evaluated: AgF – Agroforestry system with 10 years of growth, cultivated with banana (*Musa sapientum*), jussara palm (*Euterpe edulis*), cocoa (*Theobroma cacao*), papaya (*Carica papaya*), Brazilian fern (*Schizolobium parahyba*), annatto (*Bixa orellana*) and açaí (*Euterpe oleracea*); CSun – Coffee (*Coffea canephora*) cultivated in full sun, with 15 years of growth; CSha – Coffee (*Coffea canephora*) shaded by Gliricidia (*Gliricidia sepium*), with 15 years of growth; FLE – Flemingia (*Flemingia macrophylla*) and the common bean (*Phaseolus vulgaris*) grown in alleys, with 10 years of growth; and NT – No-till planted with maize (*Zea mays*) and eggplant (*Solanum melongena*), with 6 years of growth. In the CSun and CSha systems, 'bokashi' fertiliser was applied annually (an organic fertiliser of vegetable and/or animal origin, submitted to a controlled fermentation process).

Barnyard manure, in doses necessary for an input of 50 to 100 kg ha<sup>-1</sup> N, was applied to the NT system in the sowing furrow when planting, with a cover dressing of poultry litter applied in doses of between 100 and 200 kg ha<sup>-1</sup> N. No variations in topography, climate or soil texture were found between the production systems.

### Collecting the samples and separating the aggregates

The samples were collected in May 2014. Four plots were marked out in each system, from which single undisturbed samples (clods) were randomly collected to form one composite sample per plot, giving four composite samples per sampled area. Each composite sample corresponds to one pseudo-repetition, collected in the 0–0.05 and 0.05–0.10 m layers, making up a set of 40 sample units (five evaluated areas x four pseudo-repetitions x two layers).

After collection, the samples were air-dried and sieved through a 9.7-mm and 8.0-mm mesh, with any aggregates retained in this interval selected for study. The aggregates were examined under a binocular magnifier and, using a method adapted by Pulleman *et al.* (2005), manually separated by origin or formation pathway based on the morphological patterns established by Bullock *et al.* (1985); the aggregates were then validated using studies included in Pereira *et al.* (2021). The two types of aggregate were separated by a visual verification of their morphological characteristics, such as shape, size, presence of roots, porosity (BATISTA *et al.*, 2013; BULLOCK *et al.*, 1985; MELO *et al.*, 2019; PEREIRA *et al.*, 2021; PINTO *et al.*, 2021; PULLEMAN *et al.*, 2005), and the arrangement and junctions of subunits (PEREIRA *et al.*, 2021).

The present study included **biogenic aggregates**: those in which rounded shapes can be seen that originate in the intestinal tract of the soil macrofauna, especially Oligochaeta (earthworms), or those showing the presence and activity of roots; and **physiogenic aggregates** (also known as **physicogenic**) – defined as those that present an angular shape resulting from the interaction between carbon, clay, cations, and the wetting and drying cycles of the soil. These were crushed and passed through a 2.0-mm mesh sieve to obtain the air-dried fine earth

fraction (ADFE) (TEIXEIRA *et al.*, 2017). The chemical attributes associated with the fertility of the biogenic and physiogenic aggregates of each production system in the 0–0.05 and 0.05–0.10 m layers were characterised and are shown in Table 1.

#### SOM analysis of the aggregates

The light fractions of the soil organic matter (free light fraction [FLF] and intra-aggregate light fraction [ILF]) were determined in sodium iodide solution (NaI) with a density of 1.80 Mg m<sup>-3</sup> ( $\pm 0.02$ ), using the procedure proposed by Sohi *et al.* (2001) and adapted by Machado (2002). After obtaining the ILF, 35 mL of sodium hexametaphosphate solution (NaPO<sub>3</sub>)<sub>n</sub>, 5.0 g L<sup>-1</sup> was added to the pellet remaining in the tube, which corresponds to the heavy fraction, and the mixture was stirred for approximately 14 h at 250 rpm. The dispersed material was passed through a 0.053-mm mesh sieve with the aid of a water jet. The retained material, known as residual particulate organic matter (POMred), was dried in an oven at 60 °C (GAVINELLI *et al.*, 1995). The light organic matter fraction (LOM) in water was separated by flotation in an aqueous medium (ANDERSON; INGRAM, 1989).

The organic carbon of the FLF, ILF, POMred and LOM fractions of the SOM was determined as per Yeomans and Bremner (1988), to obtain the C-FLF, CILF, POCres and C-LOM, respectively.

**Table 1** - Chemical characterisation of biogenic and physiogenic aggregates in the 0–0.05 and 0.05–0.10 m layers of the agroecological production systems in the southeast of Brazil

System	pH	COT	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Al <sup>3+</sup>	H+Al	S	T	K <sup>+</sup>	P
	H <sub>2</sub> O	g kg <sup>-1</sup>	cmol <sub>c</sub> dm <sup>-3</sup>				mg dm <sup>-3</sup>			
0–0.05 m Biogenic										
AgF	5.9	24.6	3.0	2.0	0.0	2.4	5.3	7.7	125	42
CSun	6.0	30.7	3.1	3.6	0.0	2.1	7.4	9.5	285	35
CSha	6.0	33.7	2.8	2.7	0.0	1.7	5.6	7.3	64	25
FLE	5.3	22.0	1.6	1.2	0.0	1.5	2.9	4.4	44	35
NT	6.4	15.7	1.6	1.0	0.0	0.5	2.8	3.2	56	44
Physiogenic										
AgF	5.9	24.5	2.6	2.8	0.0	2.4	4.7	7.0	106	36
CSun	6.2	22.7	3.5	2.3	0.0	1.8	6.4	8.1	234	46
CSha	6.0	29.7	2.5	2.2	0.0	1.3	4.8	6.1	54	27
FLE	5.3	17.8	1.5	0.9	0.0	1.5	2.5	4.0	33	36
NT	6.4	13.9	1.5	1.1	0.0	0.4	2.7	3.2	54	39
0.05–0.10 m Biogenic										
AgF	6.0	21.4	2.4	1.6	0.1	2.4	5.3	7.6	481	12
CSun	6.3	29.5	2.2	2.4	0.1	2.0	5.6	7.6	360	08
CSha	6.1	21.5	2.1	2.2	0.1	1.7	4.7	6.4	143	04

Continuation Table 1

FLE	5.6	15.6	1.5	1.0	0.1	2.1	2.7	4.8	75	09
NT	6.5	14.7	1.4	1.2	0.1	1.0	3.2	4.2	190	03
Physiogenic										
AgF	5.9	17.9	2.1	1.8	0.1	1.8	3.9	7.4	653	11
CSun	6.3	24.8	2.2	2.4	0.1	1.8	3.1	7.2	310	06
CSha	5.9	16.7	2.0	2.3	0.1	1.7	2.5	6.7	137	03
FLE	5.4	14.2	1.5	0.9	0.1	1.5	1.7	4.2	73	09
NT	6.5	12.0	1.2	1.4	0.1	0.8	1.8	4.1	162	03

pH – Active acidity; TOC – Total organic carbon; Ca<sup>2+</sup> – Exchangeable calcium; Mg<sup>2+</sup> – Exchangeable magnesium; Al<sup>3+</sup> – Exchangeable aluminium; H+Al – Potential acidity; S – Sum of bases; T – Cation exchange capacity at pH 7.0; K<sup>+</sup> – Exchangeable potassium; P – Available phosphorus; AgF – Agroforestry System; CSun – Coffee grown in full sun; CSha – Coffee shaded by *Gliricidia*; FLE – Alleys of *Flemingia* and beans; and NT – No-till with maize and eggplant. Source: Rossi *et al.* (2016)

### Statistical analysis

The study considered a completely randomised design in a 5 x 2 factorial scheme (five production systems x two aggregate formation pathways). The data were tested for normality of the residuals using the Shapiro-Wilk test, and for homoscedasticity of the variances by the Bartlett test. Variables that did not show a normal distribution or homoscedasticity were transformed based on the Box-Cox test and retested.

The data were evaluated by analysis of variance (ANOVA) followed by Tukey's test whenever the assumptions of normality and homoscedasticity were met (both transformed and untransformed variables). In cases where the data transformation was inefficient, the Kruskal-Wallis test followed by Fisher's least significant difference criterion were used to evaluate the production systems for each type of aggregate. The Wilcoxon test was used to compare variables between the types of aggregate in each production system. All the tests were carried out at 5% significance using the 'Openxlsx' and 'ExpDes.pt' packages of the R Software (R CORE TEAM, 2020).

Multivariate principal component analysis (PCA) and dendrogram analysis were also carried out. The dendrogram is a tree diagram that displays the groups formed by the hierarchical clustering of the observations at each step and their respective levels of dissimilarity. Both were built using the R Software (R CORE TEAM, 2020) with the 'Openxlsx', 'FactoMineR', 'Factoextra', 'Stats', 'Dendextend', 'Igraph' and 'Ggplot2' packages.

## RESULTS AND DISCUSSION

The organic fraction content was influenced by the production systems and the formation

pathways, with emphasis on the light organic matter in water (LOM) in the 0.05–0.10 m layer and light intra-aggregate organic carbon (CILF) in the 0–0.10 m layer (Tables 2 and 3). From the results of these two attributes, significant interactions were found between the factors under evaluation, showing these fractions to be more sensitive. There was also a wide variation in the results of the attributes under study (Tables 2 and 3; and Figure 1). In the multivariate principal component analysis (PCA), an accumulated variance was seen for principal components (PC) 1 and 2 of 81.1% and 73.2% (Figure 2, 0–0.05 layer; and Figure 3, 0.05–0.10 m layer, respectively). The dendrograms obtained from the cluster analysis indicated the formation of two main groups separating the systems, and four secondary groups separating the aggregate classes (Figure 4A; 0–0.05 layer).

The CSun system increased the mass and carbon content of the organic fractions in the aggregates for both formation pathways, and also in the overall mean values of the system (Table 2), for which the highest levels of LOM, FLF and ILF were quantified (Table 2), with their respective levels of organic carbon (C-LOM, C-FLF and CILF) (Table 3) and residual particulate matter (POCres) (Figure 1A,C). Analysing the PCA in Figure 2A shows the separation of CSun from the other systems in the 0–0.05 m layer. The attributes under evaluation helped position the system in the upper right quadrant (eigenvectors with positive values) of principal component 1 (PC1; main axis), with 65.7% variability in the data, showing a significant and positive correlation coefficient ( $\geq 0.70$ ) with the LOM attribute (0.70) and a higher correlation coefficient ( $\geq 0.80$ ) with the variables PorcPOCres (0.83), PorcC-FLF (0.83), FLF (0.89), ILF (0.87), CILF (0.88), POCres (0.88) and C-FLF (0.93).

**Table 2** – Values for the mass of light organic matter in water (LOM), free light fraction (FLF) and intra-aggregate light fraction (ILF) of biogenic (Biog) and physiogenic (Physic) aggregates in the in 0–0.05 and 0.05–0.10 m layers of the agroecological production systems in the southeast of Brazil

System	0–0.05 m								
	LOM (g kg <sup>-1</sup> )			FLF (g kg <sup>-1</sup> )			ILF (g kg <sup>-1</sup> )		
	Biog	Physic	$\bar{X}_1$	Biog	Physic	$\bar{X}_1$	Biog	Physic	$\bar{X}_1$
AgF	2.61	1.69	2.15 cd	4.04	3.69	3.86 b	1.43	1.21	1.31 c
CSun	24.15	14.68	19.41 a	10.44	7.64	9.03 a	4.55	3.30	3.93 a
CSha	11.04	6.21	8.62 b	3.06	3.78	3.42 b	1.60	1.24	1.42 bc
FLE	2.84	2.62	2.73 c	5.28	4.57	4.92 b	2.43	2.18	2.30 b
NT	1.44	1.00	1.22 d	4.00	3.50	3.75 b	1.62	1.49	1.55 bc
$\bar{X}_2$	8.42 A	5.24 B		5.36 A	4.64 A		2.32 A	1.88 B	
CV (%)		91.67			36.89			29.94	
System	0.05–0.10 m								
	LOM (g kg <sup>-1</sup> )			FLF (g kg <sup>-1</sup> )			ILF (g kg <sup>-1</sup> )		
	Biog	Physic	$\bar{X}_1$	Biog	Physic	$\bar{X}_1$	Biog	Physic	$\bar{X}_1$
AgF	1.52 bA	2.19 bA	1.85	2.85	3.51	3.18 ab	1.97	1.31	1.64 b
CSun	20.91 aA	10.30 aB	15.61	6.20	4.90	5.55 a	2.54	2.60	2.57 a
CSha	4.07 bA	4.58 abA	4.32	6.10	3.00	4.55 ab	1.88	1.36	1.62 b
FLE	2.27 bA	2.98 bA	2.62	3.97	3.43	3.70 ab	2.30	2.37	2.33 a
NT	1.92 bA	1.43 bA	1.67	3.18	2.59	2.89 b	2.09	2.30	2.20 ab
$\bar{X}_2$	6.14	4.30		4.46 A	3.49 A		2.16 A	2.00 A	
CV (%)		46.15			44.46			38.17	

For mean values followed by the same lowercase letter in a column, the systems do not differ for the same type of aggregate. For the same uppercase letter on a line, the types of aggregate do not differ for the same evaluated system. AgF – Agroforestry System; CSun – Coffee grown in full sun; CSha – Coffee shaded by Gliricidia; FLE – Alleys of Flemingia and beans; and NT – No-till with maize and eggplant.  $\bar{X}_1$  – Overall mean value of the evaluated system; and  $\bar{X}_2$  – Overall mean value of the type of aggregate. (ANOVA + Tukey’s test with no data transformations; ANOVA + Tukey’s test with data transformations; and the Kruskal-Wallis test + Fisher’s least significant difference)

**Table 3** - Carbon content of the light organic matter in water (C-LOM), free light fraction (C-FLF) and intra-aggregate light fraction (CILF) of biogenic (Biog) and physiogenic (Physic) aggregates in the 0–0.05 and 0.05–0.10 m layers of the agroecological production systems in the southeast of Brazil

System	0–0.05 m								
	CLOM (g kg <sup>-1</sup> )			CFLF (g kg <sup>-1</sup> )			CILF (g kg <sup>-1</sup> )		
	Biog	Physic	$\bar{X}_1$	Biog	Physic	$\bar{X}_1$	Biog	Physic	$\bar{X}_1$
AgF	0.74	0.29	0.51 b	0.68	0.59	0.63 b	0.33 bcA	0.35 bA	0.34
CSun	3.68	3.21	3.44 a	2.08	1.77	1.93 a	0.88 aA	0.60 aB	0.74
CSha	1.55	0.93	1.24 b	0.58	0.76	0.67 b	0.31 cA	0.39 bA	0.35
FLE	0.66	0.25	0.45 b	0.83	0.62	0.73 b	0.52 bA	0.38 bB	0.45
NT	0.27	0.17	0.22 b	0.61	0.51	0.56 b	0.33 bcA	0.29 bA	0.31
$\bar{X}_2$	1.38 A	0.97 A		0.96 A	0.85 A		0.47	0.40	
CV (%)		100.20			42.93			23.39	
System	0.05–0.10 m								
	CLOM (g kg <sup>-1</sup> )			CFLF (g kg <sup>-1</sup> )			CILF (g kg <sup>-1</sup> )		
	Biog	Physic	$\bar{X}_1$	Biog	Physic	$\bar{X}_1$	Biog	Physic	$\bar{X}_1$

Continuation Table 3

	Biog	Physic	$\bar{X}_1$	Biog	Physic	$\bar{X}_1$	Biog	Physic	$\bar{X}_1$
AgF	0.20 bA	0.28 bA	0.24	0.49	0.47	0.48 b	0.32 bA	0.29 aA	0.30
CSun	2.18 aA	1.09 aB	1.63	1.43	0.82	1.13 a	0.50 bA	0.43 aA	0.46
CSha	0.62 bA	0.50 bA	0.56	1.06	0.57	0.81 ab	1.34 aA	0.42 aB	0.88
FLE	0.31 bA	0.35 bA	0.33	0.56	0.56	0.56 b	0.36 bA	0.35 aA	0.35
NT	0.22 bA	0.19 bA	0.21	0.48	0.43	0.46 b	0.39 bA	0.38 aA	0.38
$\bar{X}_2$	0.71	0.48		0.80 A	0.57 A		0.58	0.37	
CV (%)		41.94			55.04			74.30	

For mean values followed by the same lowercase letter in a column, the systems do not differ for the same type of aggregate. For the same uppercase letter on a line, the types of aggregate do not differ for the same evaluated system. AgF – Agroforestry System; CSun – Coffee grown in full sun; CSha – Coffee shaded by *Gliricidia*; FLE – Alleys of *Flemingia* and beans; and NT – No-till with maize and eggplant.  $\bar{X}_1$  – Overall mean value of the evaluated system; and  $\bar{X}_2$  – Overall mean value of the type of aggregate. (ANOVA + Tukey's test with no data transformations; ANOVA + Tukey's test with data transformations; and the Kruskal-Wallis test + Fisher's least significant difference)

Other studies carried out in the same agricultural production systems found a pattern of superior soil attributes in the aggregates of the coffee systems (especially in full sun), e.g., Moura *et al.* (2019), for organic P fractions with different degrees of lability, and Rossi *et al.* (2016), for chemical attributes related to fertility and total organic carbon, both particulate and associated with minerals. The longer time of the CSun system (15 years), and maintaining the ground cover between crop rows using species of forage grasses may have favoured greater levels of carbon in the aggregates. The plant material from the various species (coffee and grasses), in addition to helping maintain the soil moisture, help to increase the supply of SOM (MOURA *et al.*, 2019; ROSSI *et al.*, 2016), and explain the results for the organic fractions in the aggregates of the system.

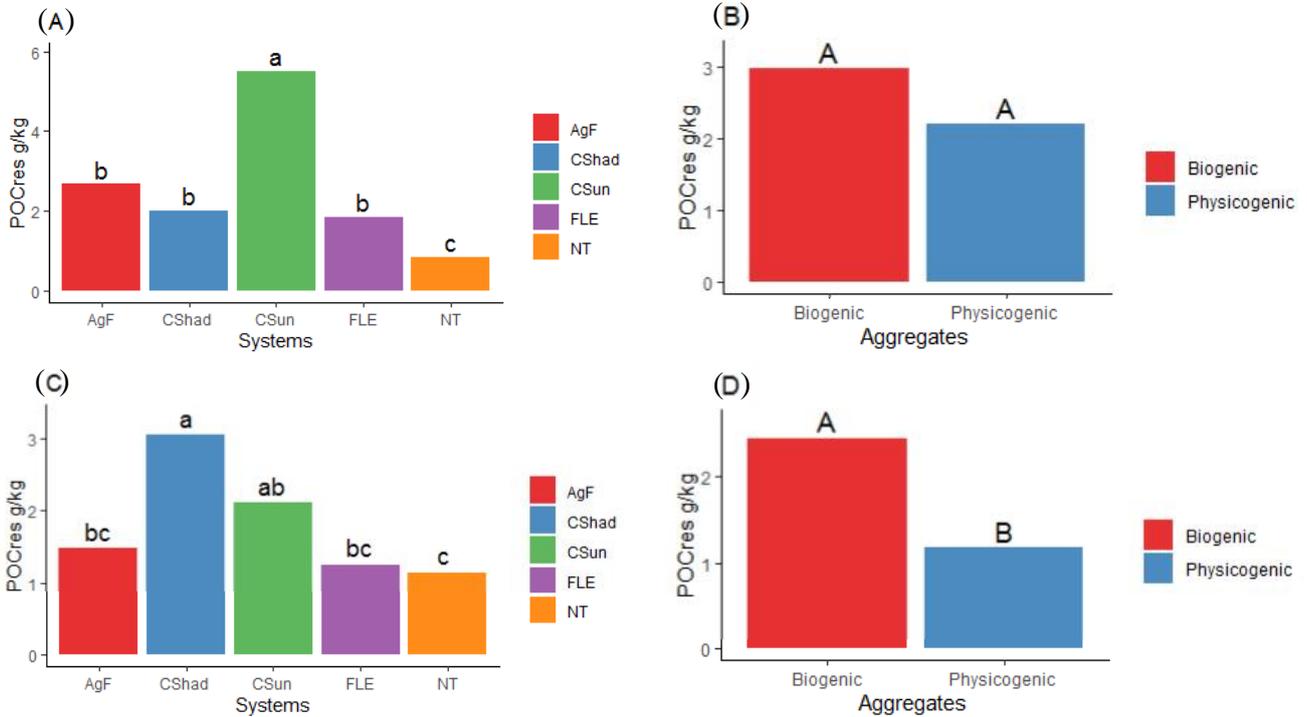
For the CSun system in the surface layer, the data from the clustering analysis are corroborated by the pattern found in the PCA (Figure 2A) and in the applied statistical tests (Tables 2 and 3; Figure 1A, C). An evaluation of the dendrogram in Figure 4 shows the existing dissimilarities between the production systems, especially in the 0–0.05 m layer. The dendrogram showed the formation of two main groups, with the CSun system (the more heterogeneous group) separated from CSha, AgF, FLE and NT systems (the more homogeneous group) with approximately 40.0% dissimilarity (Figure 4A), a pattern not seen in the 0.05–0.10 m layer (Figure 4B). These results suggest that the factors associated with the coffee system grown in full sun play a more intense role in the physical compartmentalisation of the SOM in the aggregates of the surface layer, promoting a more marked separation of the CSun system from the other systems.

The PCA in Figure 3A showed the approximation of the CSun system (upper right quadrant) and the CSha system (lower right quadrant) in the 0.05–0.10 m layer. Both separated from the other systems along PC2 (least-relevant axis) with 24.6% of the variation in the results. The variables PorcPOCres (0.77), PorcC-FLF (0.78), CILF (0.83), FLF (0.84), POCres (0.84) and C-FLF (0.89) showed the greatest correlation ( $\geq 0.70$ ) with PC1 (most-relevant axis, 48.6%) in Figure 3A. From the point of view of lability and physical protection, FLF (or C-FLF) is considered more labile and less protected than ILF (or ILFC) and POCres. According to Conceição *et al.* (2008), the light SOM fractions have a variable composition, ranging from plant materials with a high degree of lability (FLF) to organic compounds in a more advanced stage of decomposition (ILF), with the following stabilisation mechanisms: the time deposited in the soil, the intrinsic recalcitrance of the organic molecule, and the physical protection from occlusion in aggregates.

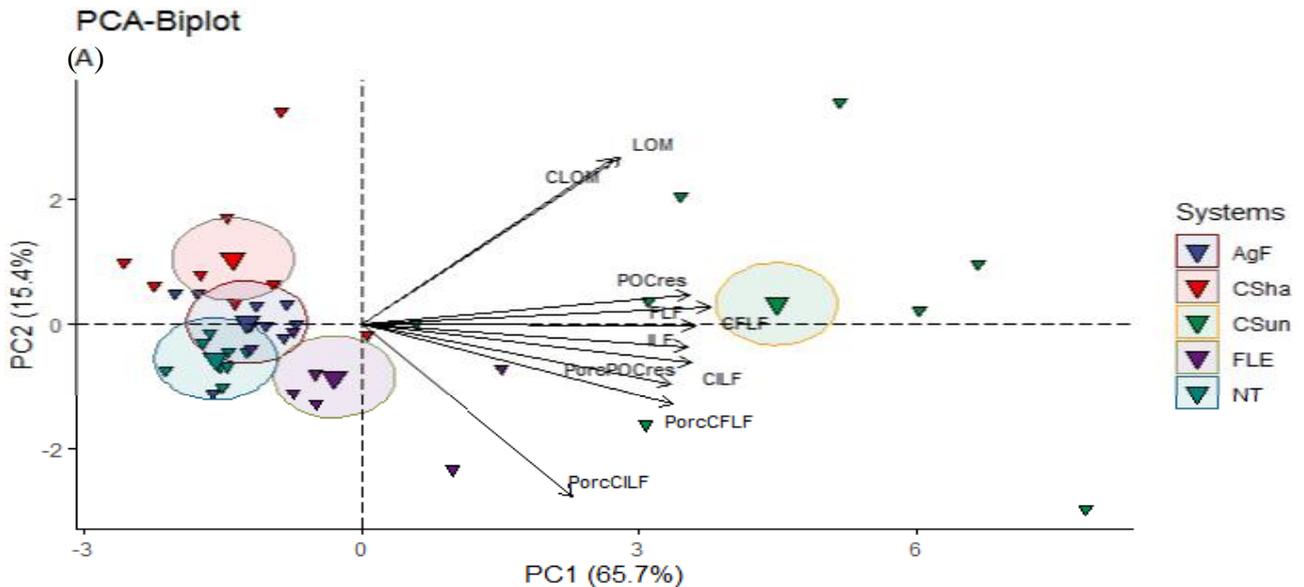
POCres is considered to belong to the heavy SOM fraction, corresponding to the carbon of the particulate organic matter (diameter of the sand fraction:  $2.0 > \varnothing \geq 0.053$  mm) that was not recovered in the densimetric fractionation, and whose carbon content is generally related to the efficiency of the chemical agent used in separating the light SOM fractions (CONCEIÇÃO *et al.*, 2007). The attributes FLF, C-FLF, CILF and POCres had a larger influence on the PCA data due to the greater occurrence of high values for the correlation coefficient with the main axis, suggesting the feasibility of using densimetric physical fractionation of the SOM as an indicator of soil quality.

For CSha, AgF, FLE and NT, the system of coffee shaded with *Gliricidia* favoured the formation of aggregates with higher overall mean values for LOM in the 0–0.10 m layer (Table 2), and C-FLF, CILF and

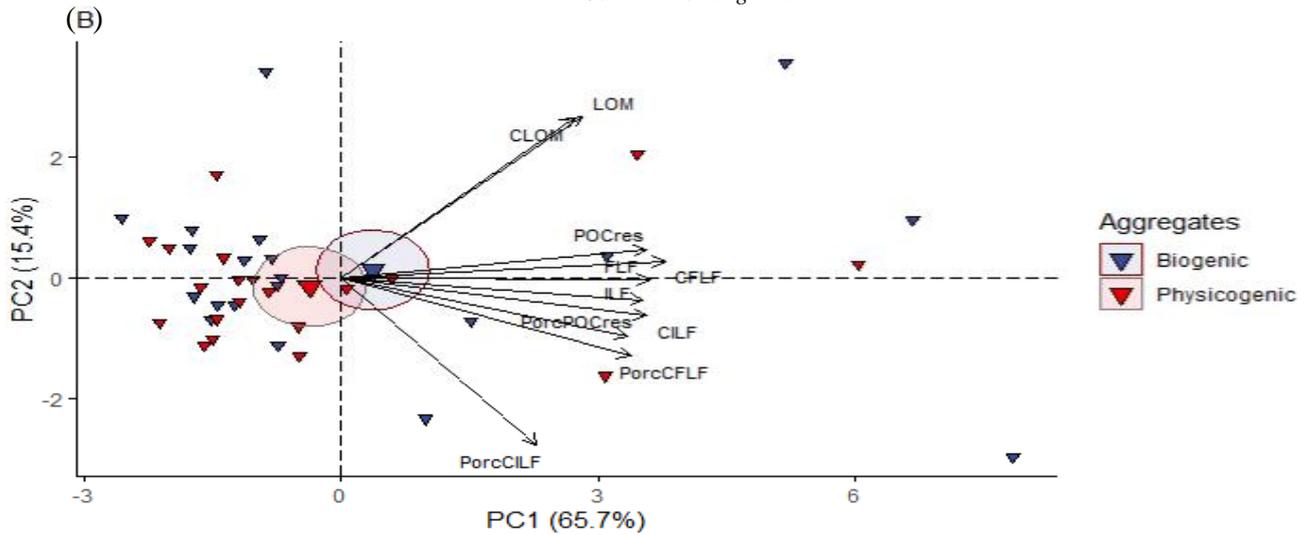
**Figure 1** - Residual particulate organic carbon (POCres) content of biogenic and physiogenic aggregates in agroecological production systems in the southeast of Brazil. (A and B) 0–0.05 m layer; and (C and D) 0.05–0.10 m layer. For mean values followed by the same lowercase letter, the systems do not differ for the same type of aggregate. For the same uppercase letter, the types of aggregates do not differ for the same evaluated system. AgF – Agroforestry System; CSun – Coffee grown in full sun; CShad – Coffee shaded by Gliricidia; FLE – Alleys of Flemingia and beans; and NT – No-till with maize and eggplant. (ANOVA + Tukey’s test with no data transformations; ANOVA + Tukey’s test with data transformations; and the Kruskal-Wallis test + Fisher’s least significant difference)



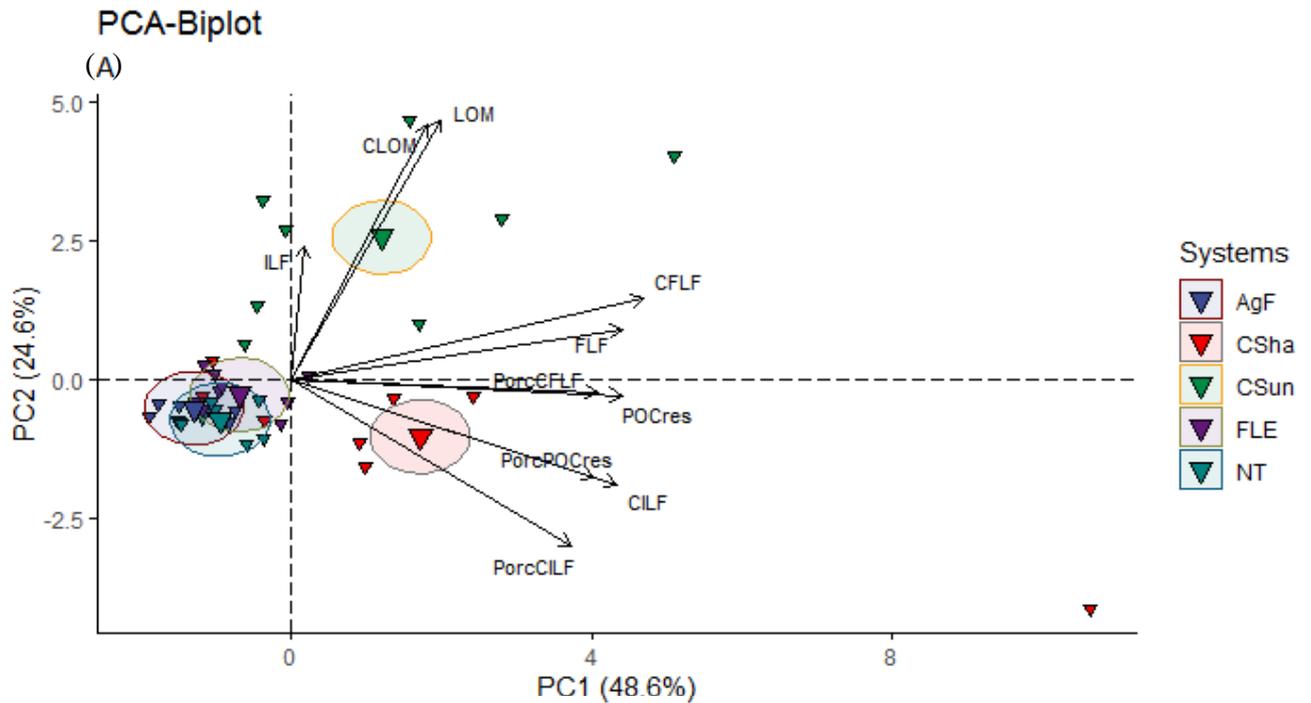
**Figure 2** - Principal component analysis considering the SOM fractions of biogenic and physiogenic aggregates in the 0–0.05 m layer of the agroecological production systems. (A) Mean values of the evaluated systems; and (B) Mean values of the types of aggregate. AgF – Agroforestry System; CSun – Coffee grown in full sun; CSha – Coffee shaded by Gliricidia; FLE – Alleys of Flemingia and beans; and NT – No-till with maize and eggplant; LOM – Light organic matter in water; FLF – Free light fraction; ILF – Intra-aggregate light fraction; C-LOM – Light organic matter carbon in water; C-FLF – Free light fraction carbon; CILF – Intra-aggregate light fraction carbon; POCres – Residual particulate organic carbon; PorcC-FLF – Proportion free light fraction carbon; PorcCILF – Proportion of intra-aggregate light fraction carbon; and PorcPOCres - Proportion of residual particulate organic carbon



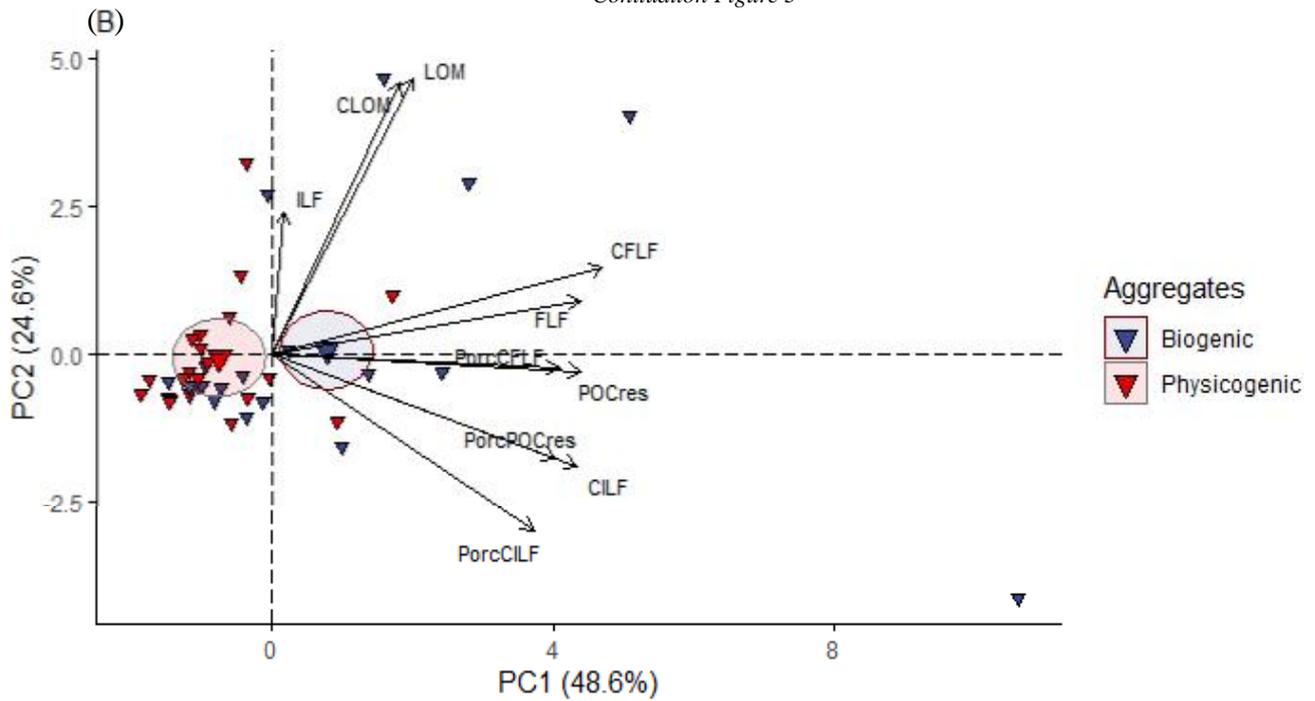
Continuation Figure 2



**Figure 3** - Principal component analysis considering the SOM fractions of biogenic and physiogenic aggregates in the 0.05–0.10 m layer of the agroecological production systems. (A) Mean values of the evaluated systems; and (B) Mean values of the types of aggregate. AgF – Agroforestry System; CSun – Coffee grown in full sun; CSha – Coffee shaded by Gliricidia; FLE – Alleys of Flemingia and beans; and NT – No-till with maize and eggplant; LOM – Light organic matter in water; FLF – Free light fraction; ILF – Intra-aggregate light fraction; C-LOM – Light organic matter carbon in water; C-FLF – Free light fraction carbon; CILF – Intra-aggregate light fraction carbon; POCres – Residual particulate organic carbon; PorcC-FLF – Proportion free light fraction carbon; PorcCILF – Proportion of intra-aggregate light fraction carbon; and PorcPOCres - Proportion of residual particulate organic carbon



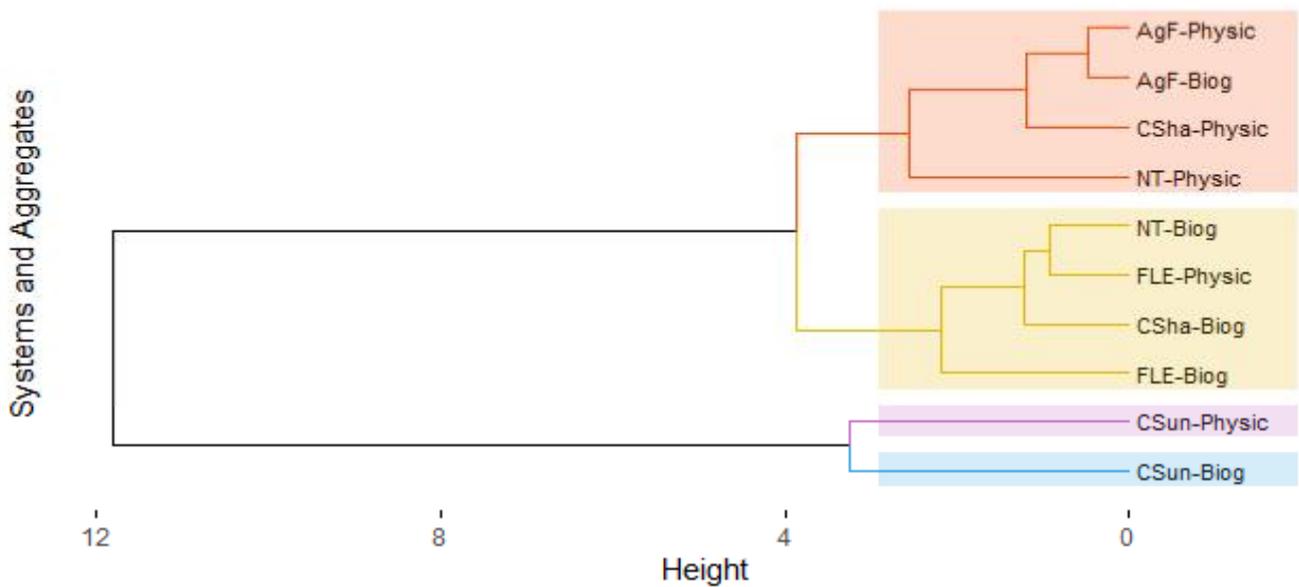
Continuation Figure 3



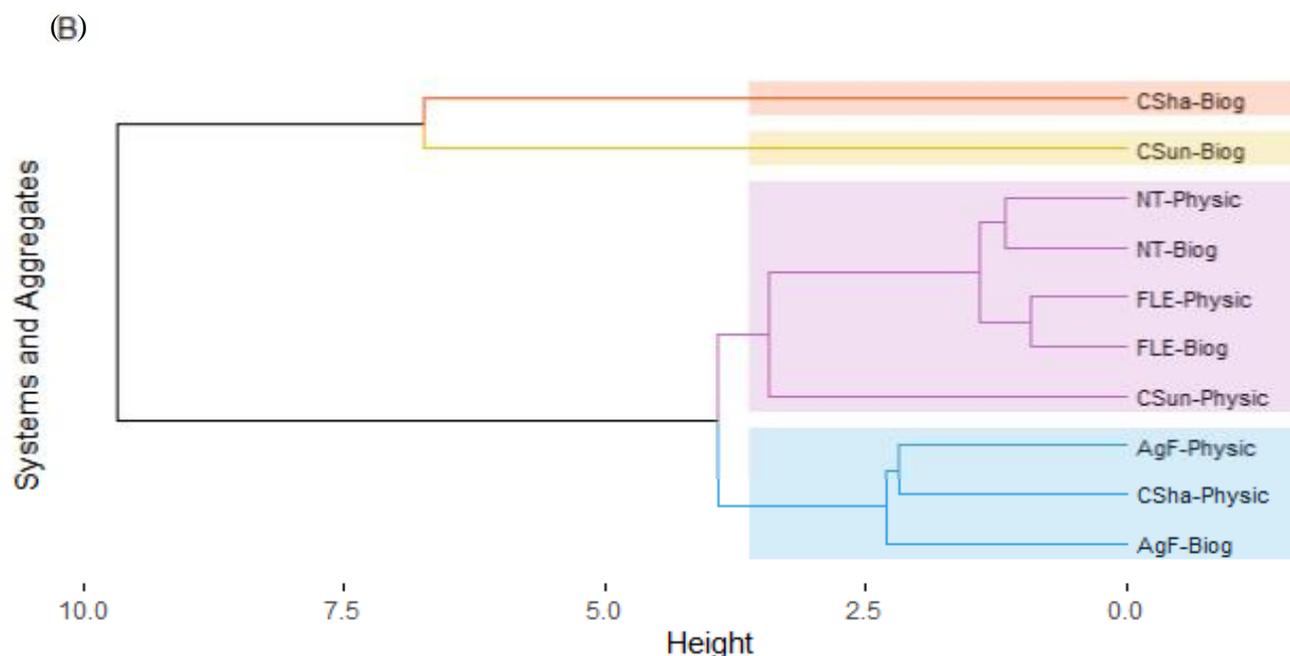
**Figure 4** - Hierarchical cluster analysis considering the SOM fractions of biogenic (Biog) and physiogenic (Physic) aggregates in agroecological production systems in the southeast of Brazil. (A) 0–0.05 m layer; and (B) 0.05–0.10 m layer. AgF – Agroforestry System; CSun – Coffee grown in full sun; CSha – Coffee shaded by Gliricidia; FLE – Alleys of Flemingia and beans; and NT – No-till with maize and eggplant

### Cluster Dendrogram

(A)



Continuation Figure 4



POCres in the 0.05–0.10 m layer (Table 3 and Figure 1C). A study of the PCA shown in Figure 2A, shows the same systems positioned in the upper and lower left quadrants (eigenvectors with negative values) in the 0–0.05 m layer. To corroborate the observed patterns, an analysis of Figure 3A shows that the separation between CSun and CSha occurred as a function of PC1, which explained 48.6% of the variability in the results. Additionally, the discriminating variables (correlation coefficient  $\geq 0.70$ ) for PC2 in Figure 3A were LOM (0.89) and C-LOM (0.87) in the 0.05–0.10 m layer. The CSha system has also been installed for a longer period (15 years) compared to the AgF, FLE and NT systems, with plant residue that is homogenised together with that of the *Gliricidia* (leaves, branches and reproductive structures) to make up a larger layer of litter. The action of the decomposition process on the litter influenced the LOM concentrations.

LOM is a light SOM fraction, which is initially separated by granulometry ( $2.0 > \varnothing \geq 0.25$  mm) and later by density (flotation in an aqueous medium), i.e. it corresponds to all the organic matter (in different degrees of decomposition and transformation) with a size greater than 0.25 mm and density less than  $\pm 1.0$  g cm<sup>-3</sup> (H<sub>2</sub>O). Included in this set are the light fractions (FLF and ILF) and POCres (corresponding to the same LOM size interval). Once physically protected by being enclosed in macroaggregates, the labile fractions may eventually become substrates for the soil microfauna,

also acting as a nucleation site for the formation of microaggregates inside the larger aggregates, contributing to the process of hierarchical aggregation (BRIEDIS *et al.*, 2018; PINTO *et al.*, 2022; SIX; ELLIOTT; PAUSTIAN, 2000). Such fractions can be used as efficient markers in measuring the initial changes in soil quality associated with the different management systems, as they are more sensitive to short-term disturbances caused in the soil environment.

The FLE system stood out for influencing concentrations of the occluded fraction (ILF) of the SOM, with the highest overall mean values for ILF found in the aggregates in the 0–0.10 m layer, where the ILF content in the subsurface were similar to those found in the CSun system (Table 2). However, the same pattern was not seen in the results for CILF (Table 3). The results for ILF on the surface may be associated with the length of time of the system (10 years), which includes a shrub legume (*Flemingia*) and the common bean that form bands of plants grown in alleys (an alternative to green manure), and supply the soil with an important contribution of biomass that has high levels of nitrogen and carbon (MOURA *et al.*, 2019), albeit less than those supplied by the *Gliricidia* (tree legume) in the CSha system.

The lowest overall mean values for the organic fractions in the aggregate classes were found in the AgF system and particularly the NT system, with emphasis on

the surface LOM and ILF fractions (Table 2), where the C-LOM content found in the aggregates of these systems showed a similar pattern to the attributes of the LOM and ILF (Table 3). The results of the univariate statistical tests are similar to the PCA data in the 0–0.05 m layer (Figure 2A), where PC1 mainly separated the FLE, NT and AgF systems from the CSha system with 65.7%. A study of Figure 3A shows no difference in the positioning pattern of the AgF, NT or FLE systems, demonstrating an overlap of these systems in the 0.05–0.10 m layer. In the dendrogram corresponding to this layer, the CSun, CSha, AgF, NT and FLE systems were separated into two main groups with  $\pm 72.0\%$  dissimilarity; however there was no clear separation between the aggregates of systems evaluated in the subsurface, except for the biogenic aggregates in CSha and CSun (the more heterogeneous group).

Through the use of multivariate techniques, Rossi *et al.* (2016) observed a clear separation of the Flemingia-alley and no-till systems from the other systems. According to the authors, the systems with longer periods of conservation management and less use of cropping practices were similar and correlated for most of the soil attributes. Among the systems evaluated in the study, NT had been set up six years earlier (shortest time); in addition, its surface layer is less affected by soil mobilisation each year, affording less protection to the organic matter in the aggregates from microbial attack, and contributing with greater mineralisation of the most labile SOM fractions.

Of the two formation pathways, the biogenic pathway showed a greater capacity for maintaining and preserving the most labile SOM fractions. For the biogenic aggregates, the highest overall mean values for LOM were seen in the 0–0.05 m layer (Table 2), with an increase of  $\pm 103.0\%$  for this attribute in the biogenic aggregates in the 0.05–0.10 m layer of the CSun system (Table 2). For the light fractions, this increase was 15.5% and 27.8% for FLF and 23.4% and 8.0% for ILF (0–0.05 and 0.05–0.10 m layers, respectively). Biogenic aggregation can be defined as the complete set of processes exerted or mediated by the soil biota that trigger or alter the processes of chemical aggregation, such as the excretion of organic matter that acts as a coating or aggregation agent, and/or physical, such as the compaction resulting from mechanical stress due to root growth (GUHRA; STOLZE; TOTSCHKE, 2022).

In this study the biogenic pathway also increased the potential of the aggregates for accumulating organic carbon, suggesting improvements in soil quality from the agroecological management. The carbon content quantified in the biogenic aggregates compared to the physiogenic aggregates was proportionally higher by 42.3% (C-LOM), 12.9% (C-FLF) and 35.0% (POCres) in the 0–0.05 m layer; and by 47.9% (C-LOM), 40.6% (C-FLF) and 108.5% (POCres) in the 0.05–0.10 m layer.

It should be noted that, compared to the physiogenic aggregates, the greatest CILF content was seen in the biogenic aggregates in the 0–0.05 m layer of the CSun and FLE systems, and in the 0.05–0.10 m layer of CSha (Table 3).

Under ideal conditions of the soil microclimate, the main biological agents responsible for the biogenic pathway (macrofauna and roots) mainly enrich the aggregates with the most labile SOM fractions. This enrichment can come about in different ways: **a)** organic matter from fine and coarse dead roots, and root exudates from rhizosphere activity; **b)** organic root detritus occasionally ingested by invertebrates (Oligochaeta) and included in their coprolites; and **c)** organic matter derived from litter transformed by fragmentation and natural composting, and progressively incorporated into the soil by the processes of digestion and bioturbation associated with the feeding activities of macroinvertebrates, and the loss of dissolved organic matter (LAVELLE *et al.*, 2020).

Several studies have found similar results when evaluating physical SOM fractions (LOSS *et al.*, 2014; PINTO *et al.*, 2021; PULLEMAN *et al.*, 2005; ROSSI *et al.*, 2016). In the same region as this study, Schultz *et al.* (2019) found higher levels of particulate organic carbon in biogenic aggregates under tree vegetation (*Caesalpinia echinata*, *Pachira aquatica*, *Hevea brasiliensis* and *Matayba guianensis*). According to the authors, the data indicate that biogenic aggregates favour the protection and stabilisation of soil carbon, as well as being potential nutrient reservoirs. The results of this study corroborate those of Ferreira *et al.* (2020) and Melo *et al.* (2019). According to Ferreira *et al.* (2020), biogenic aggregates, in addition to indicating greater biological activity, also favour the maintenance and accumulation of carbon in the soil; for Melo *et al.* (2019), the greater the carbon input, the more intense the formation of biogenic aggregates.

Figure 2A shows the clear separation of the biogenic aggregates (upper right quadrant) from the physiogenic aggregates (lower left quadrant) along the two axes (PC1 65.7%; and PC2 15.4%), almost opposite each other; Figure 3A shows a separation along the less relevant axis only (PC2 24.6%). For both PCAs, the physical SOM fractions were more associated with biogenic aggregation. Additionally, the formation of four secondary groups with  $\pm 30.0\%$  dissimilarity was seen in the dendrogram of the 0–0.05 m layer, showing separation of the biogenic and physiogenic aggregates within the most heterogeneous group (CSun), and almost complete separation in the more homogeneous group (CSha, AgF, FLE and NT) (Figure 4A). The results of the multivariate analyses (PCA and clustering) highlight the greater levels of these attributes in the biogenic aggregates, and that the contribution of these structural units to the soil

properties may be more evident and substantial in the most superficial layer of the soil.

## CONCLUSIONS

1. The agroecological production systems promoted distinct changes in the concentrations of the physical fractions of organic matter in the soil aggregates from the different origins. The greater diversity of plant species found in the ten-year agroforestry system has not yet resulted in any increase in the carbon content of the aggregates. The management practices and longer time of the system with coffee grown in full sun are related to increases in the mass and carbon content of the organic fractions of the aggregates. The lowest overall mean values for light organic matter and the intra-aggregate light fraction were mainly found in aggregates of the no-till system. These values are associated with the shorter time and the greater mobilisation of the arable layer;
2. Light organic matter in water was the most efficient fraction in separating the coffee cultivation systems (full sun and shaded) in the subsurface layer. The multivariate analyses showed a high correlation between the light fractions and residual particulate organic matter, demonstrating the effectiveness of densimetric fractionation;
3. Biogenic aggregation favoured an increase in the carbon content of the physical fractions of the soil organic matter, which suggests an improvement in soil quality, the differences being found mainly in the most superficial layer of the soil.

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## REFERENCES

- ALMEIDA, D. L.; RIBEIRO, R. L. D.; GUERRA, J. G. M. Sistema integrado de produção agroecológica ("Fazendinha Agroecológica Km 47"). *In: SIMPÓSIO DE AGRICULTURA ECOLÓGICA*, 2.; ENCONTRO DE AGRICULTURA ORGÂNICA, 1., 1999, Guaíba, RS. *Anais* [...]. Guaíba: Agropecuária, 1999. p. 152-159.
- ANDERSON, J. M.; INGRAM, J. S. I. **Tropical soil biology and fertility**: a handbook of methods. Wallingford: CAB International, 1989. 171 p.
- BATISTA I. *et al.* Caracterização dos agregados em solos sob cultivo no cerrado, MS. *Semina*, v. 33, p. 1-10, 2013.
- BRIEDIS, C. *et al.* How does no-till deliver carbon stabilization and saturation in highly weathered soils? *Catena*, v. 163, p. 13-23, 2018.
- BRONICK, C. J.; LAL, R. Soil structure and management: a review. *Geoderma*, v. 124, p. 3-22, 2005.
- BULLOCK, P. *et al.* **Handbook for soil thin section description**. Albrighton, England: Waine Research Publications, 1985. 152 p.
- CAMBARDELLA, C. A.; ELLIOTT, E. T. Methods for physical separation and characterization of soil organic matter fractions. *Geoderma*, v. 56, p. 449-457, 1993.
- CONCEIÇÃO, P. C. *et al.* Eficiência do politungstato de sódio no fracionamento densimétrico da matéria orgânica do solo. *Revista Brasileira de Ciência do Solo*, v. 31, n. 6, p. 1301-1310, 2007.
- CONCEIÇÃO, P. C. *et al.* Fracionamento densimétrico com politungstato de sódio no estudo da proteção física da matéria orgânica em solos. *Revista Brasileira de Ciência Do Solo*, v. 32, n. 2, p. 541-549, 2008.
- FERREIRA, C. R. *et al.* Dynamics of soil aggregation and organic carbon fractions over 23 years of no-till management. *Soil & Till Research*, v. 198, p. 104533, 2020.
- GAVINELLI, E. *et al.* A routine method to study soil organic matter by particle-size fractionation: examples for tropical soils. *Communications in Soil Science and Plant Analysis*, v. 26, p. 1749-1760, 1995.
- GUHRA, T.; STOLZE, K.; TOTSCHKE, K. U. Pathways of biogenically excreted organic matter into soil aggregates. *Soil Biology and Biochemistry*, v. 164, p. 108483, 2022.
- LAVELLE, P. *et al.* Soil aggregation, ecosystem engineers and the C cycle. *Acta Oecologica*, v. 105, p. 103561, 2020.
- LOSS, A. *et al.* Soil fertility, physical and chemical organic matter fractions, natural <sup>13</sup>C and <sup>15</sup>N abundance in biogenic and physicogenic aggregates in areas under different land use systems. *Soil Research*, v. 52, p. 685-97, 2014.
- MACHADO, P. L. O. A. **Fracionamento físico do solo por densidade e granulometria para a quantificação de compartimentos da matéria orgânica do solo**: um procedimento para a estimativa pormenorizada do seqüestro de carbono pelo solo. Rio de Janeiro: Embrapa Solos, 2002.
- MELO, T. R. *et al.* Biogenic aggregation intensifies soil improvement caused by manures. *Soil & Till Research*, v. 190, p. 186-93, 2019.
- MOURA, O. V. T. *et al.* Fósforo em agregados biogênicos e fisiogênicos sob diferentes sistemas de manejo agroecológico. *Revista Agrarian*, v. 12, p. 466-478, 2019.
- NEVES, M. C. P. *et al.* Sistema integrado de produção agroecológica ou Fazendinha Agroecológica do Km 47. *In: AQUINO, A.; ASSIS, R. L. de (ed.). Agroecologia: princípios e técnicas para uma agricultura orgânica sustentável*. Brasília: Embrapa Informação tecnológica; Seropédica, RJ: Embrapa Agrobiologia, 2005. p. 147-172.

- NODARI, R. O.; GUERRA, M. P. A agroecologia: estratégias de pesquisa e valores. **Estudos Avançados**, v. 29, p. 183-207, 2015.
- PEREIRA, M. G. *et al.* Biogenic and physicogenic aggregates: formation pathways, assessment techniques, and influence on soil properties. **Revista Brasileira de Ciência do Solo**, v. 45, p. 0210108, 2021.
- PINTO, L. A. S. R. *et al.* Aggregates physicogenic and biogenic under different management systems in the Cerrado region, Brazil. **Revista Brasileira de Ciência do Solo**, v. 45, p. 0200114, 2021.
- PINTO, L. A. S. R. *et al.* Soil quality indicators in conventional and conservation till systems in the Brazilian Cerrado. **Environmental Earth Sciences**, v. 81, p. 1-13, 2022.
- PULLEMAN, M. M. *et al.* Earthworms and management affect organic matter incorporation and microaggregate formation in agricultural soils. **Applied Soil Ecology Amsterdam**, v. 29, n. 1, p. 1-15, 2005.
- R CORE TEAM. **R**: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing, 2020.
- ROSSI, C. Q. *et al.* Vias de formação, estabilidade e características químicas de agregados em solos sob sistemas de manejo agroecológico. **Pesquisa Agropecuária Brasileira**, v. 51, n. 9, p. 1677-1685, 2016.
- SANTOS, H. G. *et al.* **Sistema brasileiro de classificação de solos**. 5. ed. rev. e ampl. Brasília, DF: Embrapa, 2018. 356 p.
- SCHULTZ, N. *et al.* Agregação do solo e atributos químicos em áreas com diferentes coberturas vegetais. In: TULLIO, L. (org.). **Características do solo e sua interação com as plantas 2**. 2. ed. [S. l.]: Atena Editora, 2019. v. 2, p. 1-12.
- SIX, J.; ELLIOTT, E.; PAUSTIAN, K. Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-till agriculture. **Soil Biology and Biochemistry**, v. 32, n. 14, p. 2099-2103, 2000.
- SOHI, S. P. *et al.* A procedure for isolating soil organic matter fractions suitable for modeling. **Soil Science Society. American Journal**, v. 65, n. 1, p. 1121-1128, 2001.
- TEIXEIRA, P. C. *et al.* **Manual de métodos de análise de solo**. 3. ed. rev. e ampl. Brasília, DF: Embrapa, 2017. 573 p.
- YEOMANS, J. C.; BREMNER, J. M. A rapid and precise method for routine determination of organic carbon in soil. **Communications Soil Science and Plant Analysis**, v. 19, p. 1467-1476, 1988.

