


Biogenic and physicogenic aggregates: formation pathways, assessment techniques, and influence on soil properties

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ABSTRACT: The soil particles can be gathered through physical and/or chemical processes in association with the biological activity, leading to the formation of aggregates. Soil aggregates has several functions in the soil, increasing macroporosity and water circulation - consequently reducing soil erosion and mechanical resistance to root growth, contributing to greater fixation of plants to the soil and absorption of water and nutrients, and protection of intra-aggregate organic matter. The aggregates were initially classified morphologically and in terms of their stability. In recent years, another way of evaluating aggregates, regarding their formation or origin pathway, has gained prominence in the studies conducted in Brazil. As for their origin, the aggregates can be classified morphologically as physicogenic, biogenic, or intermediate. This manuscript presents the techniques used to sample aggregates, the morphological patterns for their distinction observed in different soil classes and management types, and the chemical and physical properties. Additionally, we present analyses that are not commonly used to evaluate aggregates but which have the potential to be used as tools for a better understanding of their origin and to evaluate their modifications when subject to different types of management. In practical terms, identifying the aggregate origin and determining the related attributes allows recognizing the effect of vegetation/soil/management on soil aggregate forming agents, mainly roots and soil fauna, which reflects soil quality. For future studies, and especially to determine the importance of biogenic aggregation in improving edaphic properties, we suggest the use of micromorphology, near-infrared spectroscopy, X-ray computed tomography, clay dispersion analyses in addition with chemical, physical, and biological analysis. This approach can contribute to the identification of other patterns related to pedogenesis and the pathways of aggregate formation.

Keywords: aggregate origin, organic matter, soil structure, soil fauna, soil-environmental, indicators.

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CONTEXTUALIZATION OF THE STATE OF THE ART

The link between soil biological activity, soil organic matter (SOM) decomposition and stabilization, and soil aggregation dynamics has been recognized and intensively studied since the 1900s (Six et al., 2002, 2004). Numerous studies on soil aggregation emphasize the evaluation of aggregate stability through various aggregation indexes, such as aggregate stability index, average and weighted aggregate diameter, percentage of macroaggregates, among others. However, studies that evaluate the pathways of formation of soil aggregates are still incipient.

In the last ten years, another form of aggregate evaluation has been standing out in Brazil: the assessment of the pathways of aggregate formation or genesis. As for their genesis, aggregates can be morphologically classified as physiogenic, biogenic, or intermediate (Batista et al., 2013; Loss et al., 2014; Silva Neto et al., 2016; 2021; Fernandes et al., 2017; Melo et al., 2019a; Ferreira et al., 2020; Pinto et al., 2021). And since there is little information available on this topic, this review article presents the techniques employed for sampling aggregates, the morphological patterns for their distinction observed in different soil classes and management types, and the chemical and physical properties associated with these aggregates. Additionally, analyses that are not commonly used to evaluate aggregates are also presented but can be used as tools for a better understanding of their genesis and to evaluate their modifications when submitted to different types of management.

Soil aggregation: origin, classification, and criteria for identification

Unit particles (sand, silt and clay) in the soil can organize themselves into composite units formed from interactions between physical, chemical, and biological processes, and this process is defined as aggregation, and the end product is soil aggregates (Lepsch, 2011). Morphologically the aggregates that compose the soil structure can be classified according to their shape, and the most common are: granular, angular, or subangular blocks, prismatic, columnar, laminar (Santos et al., 2015); these being the patterns observed most frequently in Brazilian soils. From a physical point of view, aggregates can be evaluated for their stability by determining the mean weight diameter (MWD) of the aggregates evaluated by wet sieving (MWD_w) or dry sieving (MWD_d) (Teixeira et al., 2017). The aggregate stability index can be estimated from the relationship between MWD_w and MWD_d (Salton et al., 2017).

Aggregates, in general, are formed in two stages that can occur simultaneously in the soil. The first is the step of approximation of the individualized particles. This approximation can occur by mechanical or physical forces, such as the wetting and drying cycles that soil is subjected to; root growth in the soil; and the movement of edaphic organisms. The approximation between particles can also be carried out through cationic bridges or electrostatic attractions. After the approximation of the unit particles, the second stage consists in the stabilization of this first approximation, called cementation, and can have as cementing agents: organic matter, more effective due to its larger specific surface and the presence of surface charges; silicate clays, which can also act as a raw material; iron and aluminum oxides, and carbonates; and others (Oades and Waters, 1991; Six et al., 2002).

Soil aggregates are still largely identified, classified, and evaluated by the method used to visualize or isolate these structural units (Lavelle et al., 2020). Studies that identify, classify, and evaluate aggregates based on morphological characteristics (Bullock et al., 1985) have gained prominence in the last two decades, especially those related to the origin or formation pathways of aggregates (Pulleman et al., 2005; Velasquez et al., 2007; Batista et al., 2013; Loss et al., 2014, 2017; Mergen Junior et al., 2019a,b; Melo et al., 2019a; Ferreira et al., 2020; Pinto et al., 2021). The separation of aggregates according to their morphology is a simple and low-cost method based on visual characteristics

as it does not require sophisticated equipment, and can be applied by non-scientific operators, academics, technicians, or farmers (Lavelle et al., 2020), as well as scholars and connoisseurs of soil science.

Biogenic, physcogenic and intermediate aggregates

According to their origin or formation pathway, soil aggregates can be classified as physcogenic or of physcogenic origin (resulting from physical and chemical processes in the soil); biogenic, or of biogenic origin (formed by the action of biological agents) (Pulleman et al., 2005; Velasquez et al., 2007) (Figure 1); or intermediates, without evidence of a specific origin (Pulleman et al., 2005). They are differentiated based on the visualization of morphological features such as shape, size, presence of roots, porosity (Bullock et al., 1985; Pulleman et al., 2005), subunit arrangements and junctions.

Physcogenic aggregates are identified by their angular or prismatic shape and are directly related to the approximation of the soil unit particles as a function of the wetting and drying cycles and by the action of soil cementing agents (organic matter, iron, and aluminum oxides and hydroxides, and microbial activity) (Bullock et al., 1985; Pulleman et al., 2005). Biogenic aggregates have rounded shapes and are related to the action of the soil fauna (ecosystem engineers). An example is the passage of soil material through the gut system of the edaphic macrofauna, especially the Oligochaeta (earthworms), added to the physical and cementing action of the root system in association with the action of microorganisms (fungi and bacteria). The most studied ecosystem engineers are ants, earthworms, and termites, and they form, in addition to stable biogenic aggregates, biostructures such as galleries, channels and chambers (Lavelle et al., 2020). Intermediate aggregates do not have a defined morphology and may be biogenic aggregates that have lost their rounded shape due to aging or physcogenic aggregates linked with a small coprolite. Although intermediate aggregates are not associated with a formation pathway, their quantification is useful to minimize errors in identifying biogenic and physcogenic aggregates.

Other edaphic organisms can also modify soil structure but to a lesser extent and intensity, such as Coleoptera and Enchytraeidae. Batista (2011) observed the presence of Enchytraeidae coprolites in intermediate aggregates (Figures 2a and 2b), showing that these small earthworms also act in modifying soil structure, but at smaller scales. At the rhizosphere scale, biological activity acts in the formation of aggregates through the activity of bacteria and fungi. These microorganisms produce their own muscilages that are important “glue” binding substances of the soil, thus promoting increased soil stability (Oades, 1984). Batista (2011) observed spores of arbuscular mycorrhizal fungi in biogenic aggregates (Figure 2c). These fungi make a symbiotic association

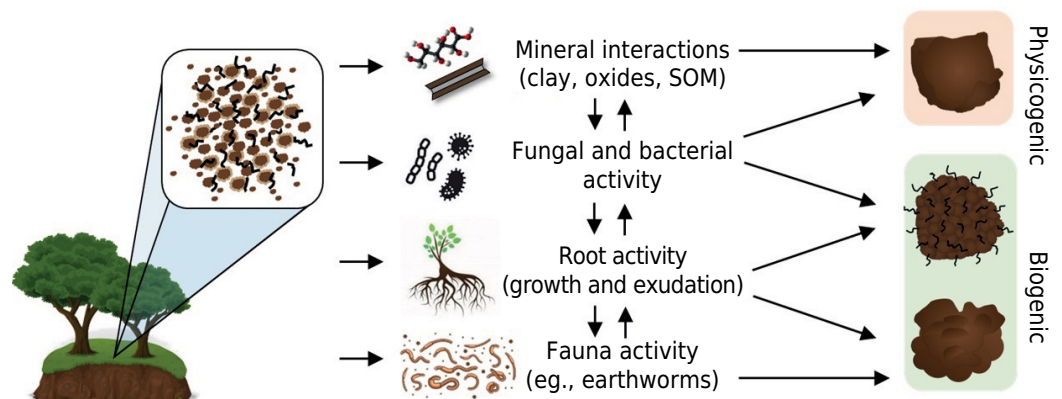


Figure 1. Theoretical model for the study of soil aggregate formation pathways. Silva Neto et al. (2021).

with roots and produce glomalin, a glycoprotein that contains high content of metal ions (Rillig, 2004; Wang et al., 2017), and when in contact with soil, has a cementing effect (Santos et al., 2020), favoring the stabilization of aggregates.

TECHNIQUES FOR SEPARATING AND EVALUATING AGGREGATES

The study of aggregation based on the analysis of separation and morphological classification of aggregates is a practical, accessible, replicable, and easily understood and interpreted indicator that can be used to determine whether soil conditions are improving, remaining the same, or worsening (Ferreira et al., 2020) as a function of the use or management adopted. The method consists of five fundamental steps: collection; separation; source identification and classification; quantification of the relative percentage; and storage for characterization of the physical, chemical, and biological properties of the aggregates (Figure 3).

Sample collection

Undeformed soil samples are collected to separate and subsequently identify the formation pathways of aggregates as well as analyze their structural stability. For the analysis,

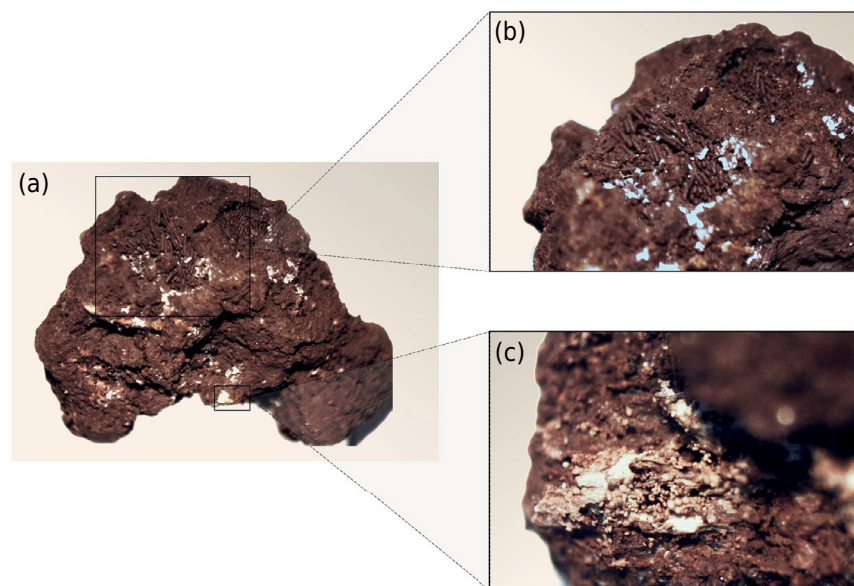


Figure 2. (a) Intermediate aggregates with small-welded coprolites. In detail, the increase (b) possibly *Enchytraeidae* feces and (c) mycorrhizal fungi spores from the corn/cotton area in the dry season of evaluation.

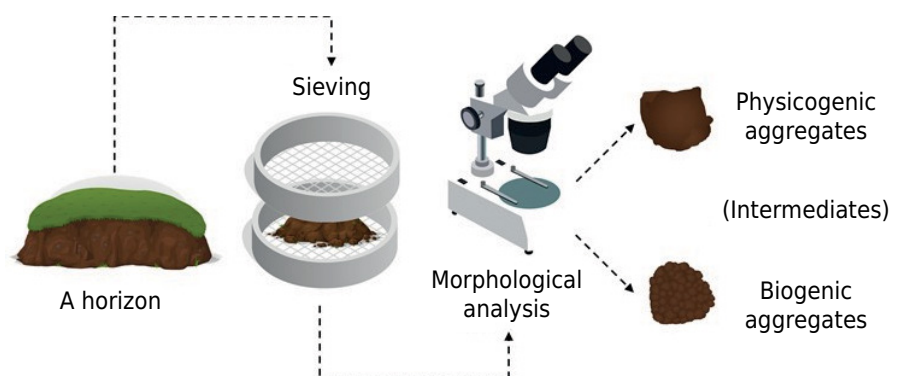


Figure 3. Summary of steps for morphological analysis of soil aggregates - process of separation and visual evaluation of aggregates. Source: Silva Neto et al. (2021).

a trench of at least 0.25 × 0.20 × 0.10 m (width × length × height) is opened, and, with the help of a metal probe or a square spade, blocks or clods of soil are collected. For example, in soil use systems with row crops, such as no-till, conventional tillage, and crop-livestock integration, collections are made between the rows of the main crops. Samples are collected from the most superficial soil layers, usually up to the first 0.10 m depth, stratified (0.00-0.05 and 0.05-0.10 m) or at a single depth (0.00-0.10 m). This depth is due to the greater biological activity of the soil macrofauna and the greater presence and activity of the root system. After collection, the samples are bagged, labeled, and transported to the laboratory to preserve their structural integrity, where they will be dried in the shade and carefully detached in the lines of weakness to separate the aggregates for subsequent sieving. The undeformed samples must be friable to carry out aggregate separation (Batista et al., 2013; Batista, 2015; Loss et al., 2014, 2017; Salton et al., 2017).

Separation of aggregates

According to the diameter of the sieves used for this purpose, the aggregates are morphologically identified by size. In the literature, there are some variations in relation to the size of the meshes used. One of the pioneering studies on this theme was that by Pulleman et al. (2005), in which the authors used aggregates of size between 12.5-4.0 mm in diameter. In this study, Pulleman et al. (2005), based on descriptions by Bullock et al. (1985), described, methodologically, what biogenic and physicogenic aggregates are and highlighted for the first time the class of intermediate aggregates. In Brazil, some studies used aggregates ranging from 19 to 8 mm (Melo et al., 2019a); 9.7 to 4.0 mm (Batista, 2015; Ferreira et al., 2020); 9.5 to 4.0 mm (Velasquez et al., 2007; Loss et al., 2017; Ventura et al., 2018; Mergen Junior et al., 2019a,b); 9.7 to 8.0 mm (Batista et al., 2013; Loss et al., 2014; Rossi et al., 2016; Fernandes et al., 2017; Lima et al., 2020; Pinto et al., 2021). Pulleman et al. (2005) point out that small aggregates (1 to 2 mm) are very fragile, which makes separation hard; however, larger aggregates (19 to 8 mm) makes separation easier, which decreases the error associated with aggregation pathway classification (Jouquet et al., 2009; Melo et al., 2019a). Lavelle et al. (2020) used aggregates >2.0 mm in diameter (large macroaggregates) in their study, claiming easy separation and identification.

Identification of the aggregate formation pathway

After separating the aggregates by size, they are observed under a binocular microscope and classified manually, according to morphological patterns, into physicogenic, biogenic and intermediate (Figures 4, 5, 6, 7 and 8).

The relative contribution of the aggregates can be determined after classification, and the results obtained are expressed in mass. For this purpose, all the aggregates (biogenic, intermediate and physicogenic) that have been identified can be weighed and thus quantify the fraction of aggregates according to the formation pathway in relation to the initial mass (Loss et al., 2017; Mergen Junior et al., 2019a,b; Pinto et al., 2021). Another possibility to assess the percentage of each fraction can be done by weighing a certain mass of the total aggregates before morphological separation, for example, 100 g (Batista et al., 2013; Batista, 2015; Ferreira et al. 2020); 200 g (Melo et al., 2019a) or 500 g (Pulleman et al., 2005) and, after identification, the percentage of each type of aggregate in relation to the initial mass is calculated. It is important to highlight that the larger the classified aggregates, the larger the total soil mass evaluated must be to minimize sampling errors since the number of evaluated aggregates is inversely proportional to their diameter.

Examples of aggregates under different soil-environmental conditions in Brazil

In the literature, studies that attempt to explain the qualitative and quantitative aspects related to the origin of aggregates as a function of edaphic and environmental

conditions (e.g., vegetation, texture, climate, relief, biological activity, and management practices) in tropical and subtropical regions are still incipient. This is probably due to the complexity and difficulty of references that allow more accurate identification of training processes (Loss et al., 2014). The simple visual separation of aggregates into biogenic and physicogenic (and intermediate, depending on the study), for example, can provide important information about aggregation, reflecting the state of soil quality, as in the case of the study by Lavelle et al. (2020). Besides having distinct morphological characteristics, physicogenic, intermediate, and biogenic aggregates can also differ in chemical characteristics. In the studies by Batista et al. (2013) and Batista (2015), physicogenic aggregates were closer to intermediate aggregates from a chemical point of view, which distanced themselves from biogenic ones.

Figure 4 shows examples of physicogenic, intermediate, and biogenic aggregates in no-till areas under a crop rotation plan (grass/legume) in an Atlantic Forest environment (São Paulo, Brazil). The origin of aggregates is directly related to the adopted management system because the management normally causes modifications in the aggregation, resulting in changes in the aggregate formation pathways (Pulleman et al., 2005; Loss et al., 2014).

Some examples of aggregates formed by the biogenic pathway in different soil classes in the Guapi-Macacu basin (RJ, Brazil) in planted pasture areas are presented in figure 5. Forage grasses, especially brachiaria, can promote greater organic carbon addition to the soil via rhizodeposition, providing better conditions for the edaphic fauna and the formation of aggregates of biogenic origin (Pulleman et al., 2005; Loss et al., 2014; Mergen Junior et al., 2019a,b).



Figure 4. Soil aggregates in areas of no-tillage system with crop rotation in the Atlantic Forest biome: (a) physicogenic; (b) intermediates; and (c) biogenic. Source: Batista (2015).

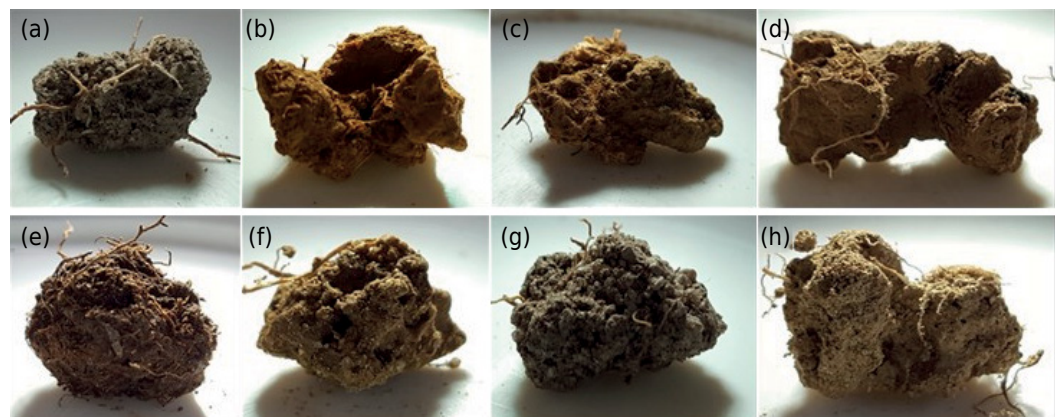


Figure 5. Biogenic soil aggregates in different soil taxonomic classes. (a) *Planossolo Háplico* (Alfisol/Planosol); (b) *Nitossolo Bruno* (Alfisol/Nitisol); (c) *Argissolo Vermelho* (Ultisol/Acrisol); (d) *Latossolo Vermelho-Amarelo* (Oxisol/Ferralsol); (e) *Gleissolo Melânico* (Entisol/Gleysol); (f) *Cambissolo Flúvico* (Inceptisol/Cambisol); (g) *Argissolo Acinzentado* (Ultisol/Acrisol); (h) *Gleissolo Háplico* (Entisol/Gleysol). Source: Pinto et al. (2019).

Application of organic residues from swine and poultry farming can also influence the origin of aggregates. Figure 6 shows examples of these units, identified and classified in areas subjected to constant application of these types of waste, highlighting the swine manure on the *Argissolo Vermelho-Amarelo* (Ultisol; Acrisol) in Braço do Norte (Santa Catarina, Brazil) and poultry and swine manure on the typical *Latossolo Vermelho Eutroférico* (Oxisol; Ferralsol) in Londrina (Paraná, Brazil). Some practices such as the maintenance of plant residues on the soil surface and the absence of disturbance of this layer in association with the addition of organic waste can provide favorable physical and chemical conditions for soil fauna, which are primarily responsible for the formation of biogenic aggregates (Batista et al., 2013; Mergen Junior et al., 2019b; Pinto et al., 2021).

Some examples of biogenic and physicogenic aggregates in no-till areas in the Cerrado of Minas Gerais under *Latossolo Vermelho Distrófico típico* (Oxisol; Ferralsol) are presented in figure 7. The presence and contribution of roots in the formation of biogenic aggregates can be clearly observed (Figures 7a, 7b, 7c and 7d) compared to physicogenic aggregates, which have angular and prismatic shapes typical (Figures 7e, 7f, 7g and 7h). It is worth noting that soil and water conservation management systems provide a more balanced distribution between the percentage amounts of physicogenic and biogenic aggregates in the soil surface layer (Melo et al., 2019a; Pinto et al., 2021).

Figure 8 presents examples of aggregates of different morphological types in no-till areas with different installation times under *Latossolo Vermelho Eutroférico* (Oxisol; Ferralsol) in Guaíra (Paraná, Brazil). The high clay contents on the soil surface can favor the formation of physicogenic aggregates (Ferreira et al., 2020). Soil properties such as texture, moisture, polyvalent cation content, and organo-mineral interactions play important roles in the origin of these aggregates (Pulleman et al., 2005; Batista et al., 2013; Loss et al., 2014; Ferreira et al., 2020).

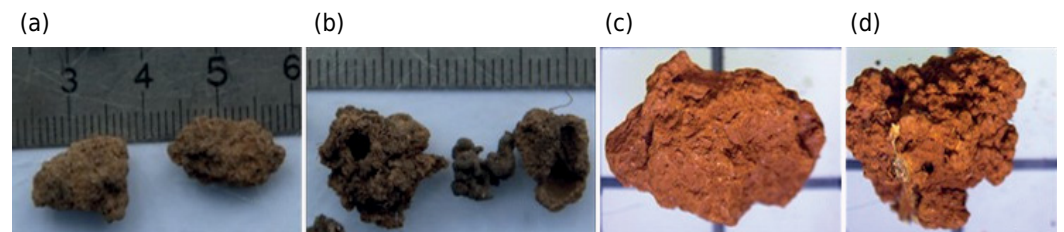


Figure 6. Soil aggregates in *Argissolo Vermelho-Amarelo* (Ultisol/Acrisol) (a-b) and *Latossolo Vermelho* (Oxisol/Ferralsol) (c-d) in southern Brazil. Physicogenic soil aggregates (a; c) and biogenic soil aggregates (b; d). Source: Loss et al. (2017) and Melo et al. (2019a).

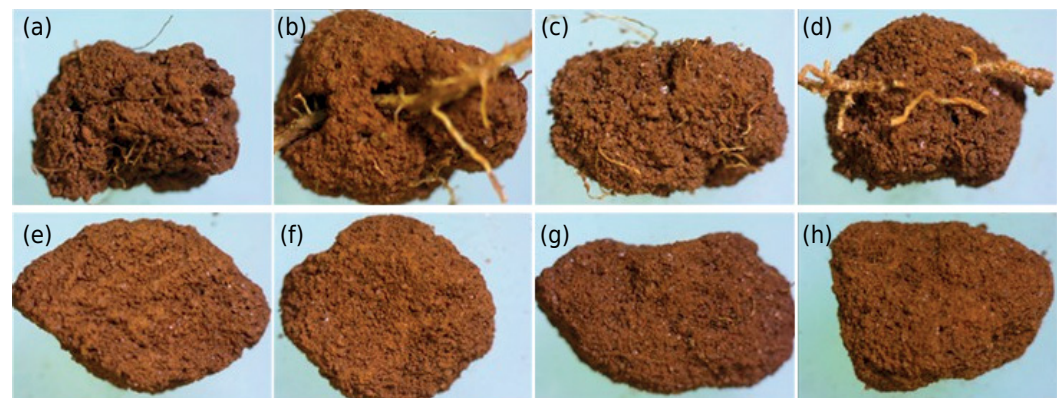


Figure 7. Soil aggregates in areas of no-tillage system in the Cerrado biome. Biogenic soil aggregates (a, b, c and d) and physiogenic soil aggregates (e, f, g and h). Source: Pinto et al. (2021).

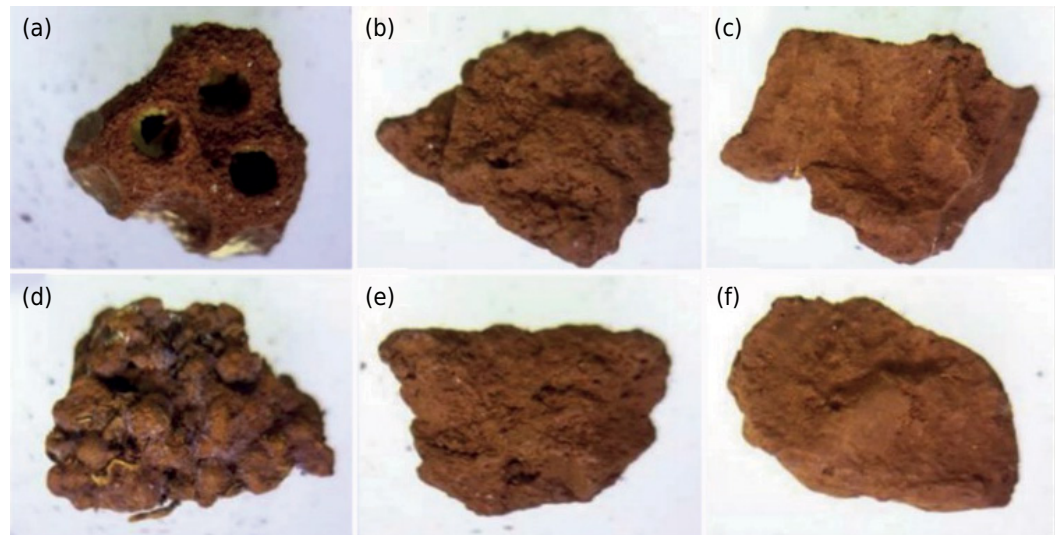


Figure 8. Soil aggregates in no-tillage system. Biogenic soil aggregates (a and d), intermediates (b and e), and physcogenic soil aggregates (c and f). Source: Ferreira et al. (2020).

Aggregate analysis

After the morphological separation of the different aggregates, physical and chemical analyses can be performed on each type of aggregate, in order to understand the contribution of aggregation pathways on soil properties, such as particle size composition (Mergen Junior et al., 2019b); degree of dispersion of clays (Melo et al., 2019a); aggregate stability by calculating the mean-weight diameter (MWD) and geometric (MGD) indices of the aggregates (Batista et al., 2013; Batista, 2015; Silva Neto et al., 2016; Rossi et al., 2016; Loss et al., 2017; Fernandes et al., 2017; Lima et al., 2020), mass distribution of aggregates in the mean diameter classes:¹ $X > \emptyset \geq 2.0$ mm (macroaggregates); $2.0 > \emptyset \geq 0.25$ mm (mesoaggregates) and $\emptyset < 0.25$ mm (microaggregates) (Silva Neto et al., 2016; Mergen Junior et al., 2019b; Loss et al., 2020); characterization of the sorting complex of aggregates - pH, Ca^{2+} , Mg^{2+} , Al^{3+} , K^+ , Na^+ , H + Al and P (Batista et al., 2013; Loss et al., 2014; Batista, 2015; Rossi et al., 2016; Mergen Junior et al., 2019a; Melo et al., 2019a); quantification of total organic carbon and total nitrogen contents (Batista et al., 2013; Batista, 2015; Rossi et al., 2016; Loss et al., 2017; Mergen Junior et al., 2019a; Ferreira et al., 2020; Pinto et al., 2021); and potentially mineralizable carbon (Pinto et al., 2018); determination of carbon contents in macro, meso and microaggregates (Loss et al., 2020); particle size physical fractionation (Pulleman et al., 2005; Loss et al., 2014; Batista, 2015; Ventura et al., 2018; Ferreira et al., 2020; Pinto et al., 2021); physical densimetric fractionation (Pinto et al., 2021); organic and inorganic P content (Moura et al., 2019) and chemical fractionation of the organic matter contained in the aggregates (Fernandes et al., 2017; Ventura et al., 2018; Melo et al., 2019a; Ferreira et al., 2020; Pinto et al., 2021).

Regarding the stability of aggregates, which can be done wet or dry (Salton et al., 2017), attention should be paid to the mesh diameter of the sieves used to separate the aggregates, as the larger diameter sieve, which can be 19, 12.5, 9.7, 9.5 mm, for example, will be the upper limit of the class of larger aggregates to compose the class center, in mm, attribute that will be used for the calculation of MWDw and MWDd. The aggregate stability index will be estimated from the relationship between MWDw and MWDd (Teixeira et al., 2017), which seems to be more appropriate than calculating MWD or MGD alone. Some authors used sieves of 9.7 to 4.0 mm to obtain aggregates and subsequent morphological identification; to analyze the stability of aggregates, these

¹ The value of the largest diameter will depend on the opening mesh of the first sieve used to separate the aggregates.

are passed through an 8.00 mm mesh sieve first to then compose the set of sieves used for the evaluation of structural stability (Batista et al., 2013; Loss et al., 2014). Other techniques used to evaluate soil structure and/or aggregates are micromorphology, near-infrared spectroscopy (NIRS), and computed tomography.

Micromorphology

Micromorphology is an important tool for the study of soil aggregates. Through micromorphological analysis, it is possible to describe, measure, and interpret soil constituents and their spatial arrangements and pedological features through microscopic analysis of thin slides of undeformed soil samples (Bullock et al., 1985; Stoops, 2003). In the study of soil aggregates, micromorphological techniques allow (i) to analyze the size, shape, and arrangement of aggregates and inter-and intra-aggregate pores; (ii) to evaluate pedality - the degree of structure development at the microscopic scale; (iii) assist in identifying the pedogenetic processes involved in the formation of aggregates and, consequently, soils; (iv) identify characteristics originating from different groups of organisms and their modifications; and (v) quantify the effect of different groups of edaphic fauna on the development of soil (micro)structure and organic matter dynamics. In summary, micromorphology is a method of studying soil microstructure, which aims to contribute to understanding soils, their identification, origin, and relative chronology (Stoops et al., 2018).

In micromorphology, the term structure has received several definitions over time. The most widespread and expressed concept in Bullock et al. (1985) considers structure as the physical constitution of a soil, expressed by the size, shape, and arrangement of solid particles and pores, forming aggregates or not. As for aggregates, they are defined as structural soil units whose arrangement and distribution produce geometric shapes and varied dimensions (Castro and Cooper, 2019). The basic unit in the micromorphological analysis is the elementary or primary aggregate, internationally called *ped* ($\emptyset < 0.1$ mm), identified only by optical microscopy. As for origin, Stoops and Buol (1985) distinguish five types of microaggregates produced by different processes: structural or weft, zoogenetic, relics, geochemical and complex.

Regarding biogenic aggregates, the effects of soil fauna on aggregates can be multiple and complex, with more than 50 different types of characteristic features being identified in the micromorphological analysis (Kooistra and Pulleman, 2018). Micromorphological studies of soil fauna characteristics can offer a valuable contribution as part of an integrated approach that aims to elucidate the role of soil fauna diversity and its functions in soils. An example of this integrated approach is the study by Pulleman et al. (2005), using micromorphology and physical and chemical soil analysis to determine the effect of earthworms on macro and microaggregate formation and carbon sequestration.

In a study developed under laboratory conditions, to evaluate the influence of the biological component (earthworms and brachiaria) on aggregate formation, an experiment was conducted for three months using fine air-dried soil ($\emptyset < 2.00$ mm), packed in (polyvinyl chloride) PVC columns (Silva Neto et al., 2021). Undisturbed samples were collected at the end of the study to assess the formation of aggregates using micromorphology techniques. The study verified the influence of earthworms (*Lumbricus rubellus*), brachiaria (*Brachiaria Decumbens*), and the combination of these two components in the formation of aggregates (Figure 9). When comparing the samples of treatments with earthworm and brachiaria, the importance of earthworms in aggregation is identified. In the brachiaria + earthworm treatment slides, plasma formation is observed through the coalescence of the microaggregates, originating zones of polyhedral blocks and loose aggregates. Porosity occurs predominantly by composite packing. The contribution of roots to the formation of aggregates was observed, forming a looser plasma (with microaggregates). Quartz grains have a plasma coating and some blackened spots (organic matter). Blackened spots of organic material along the blade were also observed.

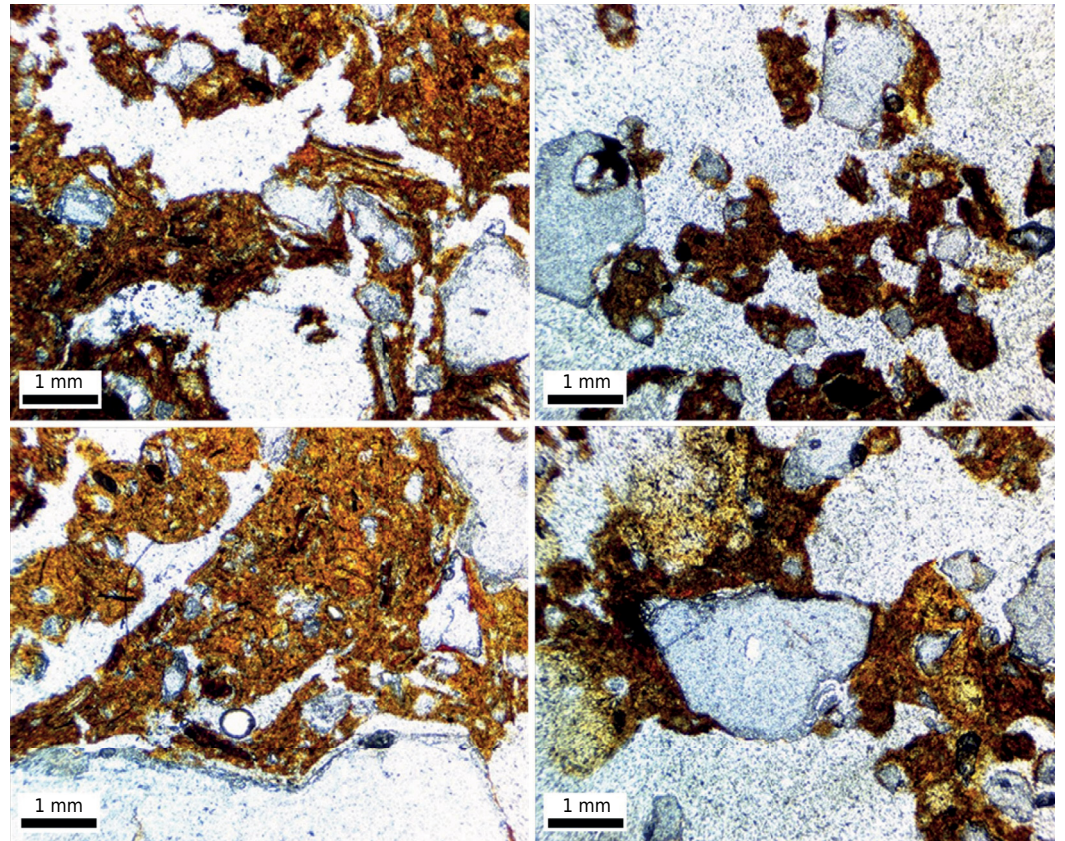


Figure 9. Photomicrographs of soil micromorphology slides in cylinder with brachiaria + earthworms.

Combining micromorphological methods with other techniques, such as morphological (macro) identification of aggregates, provides a promising approach for future studies on the role of soil fauna (Kooistra and Pulleman, 2018).

NIRS (Near Infrared Spectroscopy)

Studies on the use of near infrared spectroscopy (NIRS) in soil science have increased rapidly in recent decades. This technique is based on measuring the intensity of absorption of electromagnetic radiation in the near infrared region (700-1100 nm) - the region of the infrared electromagnetic spectrum that is closest to the visible region in terms of wavelength. The spectral curves or "signatures" are formed from the irradiance of energy that interacts with the ground and reflects at different wavelengths, and sensors capture this information. The use of reflectance spectroscopy in determining soil attributes is possible because the spectrum is a direct result of the soil's organic constituents, minerals, and physical properties, such as texture, mineralogy and soil moisture content (Stenberg et al., 2010).

The NIRS has been proposed as an alternative technique to assist conventional methods for determining soil properties, which generally involve laboratory analysis using chemical reagents that can generate environmental impacts (Nanni and Demattê, 2006). This technique brings several advantages, such as easier sample preparation, speed and ease of execution of the analysis, no chemical reagents (no harm to the environment), and a small amount of soil to take readings. However, to use this technique - relating the NIRS spectral signature of the sample to soil properties - requires establishing a database of soil samples in which the evaluated properties have been measured. In Brazil, the Brazilian Soil Spectral Library (BESB) is an online collection built collaboratively among 41 institutions from 26 Brazilian states, which follows a well-defined flow of receiving soil samples and reading the spectra (ten Caten et al., 2021). It contains data

and information on the spectral behavior of 39,284 soil samples from different country states (Demattê et al., 2019).

Regarding the study of aggregates, recent research suggests that NIRS may allow the characterization of macroaggregates by a specific spectral signature reflecting the quantity and nature of organic matter and mineral particles associated in specific ways in each biogenic or physicogenic structure (Hedde et al., 2005; Velasquez et al., 2007; Zhang et al., 2010; Zangerlé et al., 2011, 2014; Domínguez-Haydar et al., 2020). In addition, the NIRS can show how earthworm diversity is linked to the quality of soil organic matter (Huerta et al., 2013). An example of this technique is the study by Zangerlé et al. (2016), combining visual morphological analysis and NIRS to identify the origin of soil macroaggregates and quantify the respective contributions of locally found species, with a focus on earthworms. Therefore, this method also represents an important tool for studying soil aggregates regarding their origin and physical and chemical properties.

Computed Tomography

Regarding studies involving soil science, the first works used X-ray computed tomography (CT) date from the 1980's (Petrovic et al., 1982; Crestana et al., 1985). These authors used CT to determine soil density, the spatial distribution of water content, retention, and water movement in the soil. The CT has been widely used in studies involving undeformed soil samples, as it is a non-destructive (2D or 3D) imaging technique for the characterization and quantification of soil physical properties at high resolution (mm to μm scale) (Gantzer and Anderson, 2002; Yang et al., 2018). Assessments of soil pore distribution and properties (Hu et al., 2015; Yang et al., 2018) have been successfully performed using CT.

Using the X-ray computed microtomography technique (X-ray μCT), Dal Ferro et al. (2013) evaluated the distribution of pore size and aggregate morphology (sieved at 5-6 mm) in a long-term experiment using organic and mineral fertilizer for 12 years. The results highlighted the central role of organic matter in pore space distribution, as there was a shift from small (80-320 μm) to large (>560 μm) pores in the organic fertilization treatment. In another study on aggregate porosity, X-ray μCT was used to elucidate better the relationship between organic matter added to the soil and the resulting aggregate structure properties (Nakano et al., 2015). The authors investigated the internal structures of soil aggregates cultivated with rice and wheat with the addition of two types of organic fertilizer (from rice straw and cattle manure) and two types of plant residues (rice straw and wheat straw) beyond control (no fertilization). The X-ray μCT images allowed observing organic substances such as manure, roots, and seeds in the aggregates. And it was possible to distinguish the porous and solid phases of the aggregates. The authors concluded that, through the use of images produced by X-ray μCT , the application of manure to the soils effectively increased the intra-aggregate porosity. The addition of straw to the soil alone favored the presence of pores running from the core of the aggregate to its surfaces, and spongy pores were identified in the aggregates in the treatments with manure addition. This pattern suggests that differences in the organic fertilizers used may affect the pore network within the intra-aggregate space.

Effects of biological activity on aggregate formation were assessed in an experiment that consisted of a 12-week laboratory incubation with soil macrofauna (*Oligochaeta*) and grassy vegetation (*Brachiaria Decumbens* cv); the authors observed that in the evaluation of porosity by X-ray μCT , the biogenic aggregates presented a higher proportion of larger pores compared to the physicogenic ones (Figure 10) (Silva Neto et al., 2021).

Melo et al. (2019a) used the X-ray μCT technique to evaluate the influence of the application of poultry and swine manure and biogenic aggregation on the porosity of a *Latossolo Vermelho Eutroférico* (Oxisol; Ferralsol) (Figure 11). The authors noted that biogenic

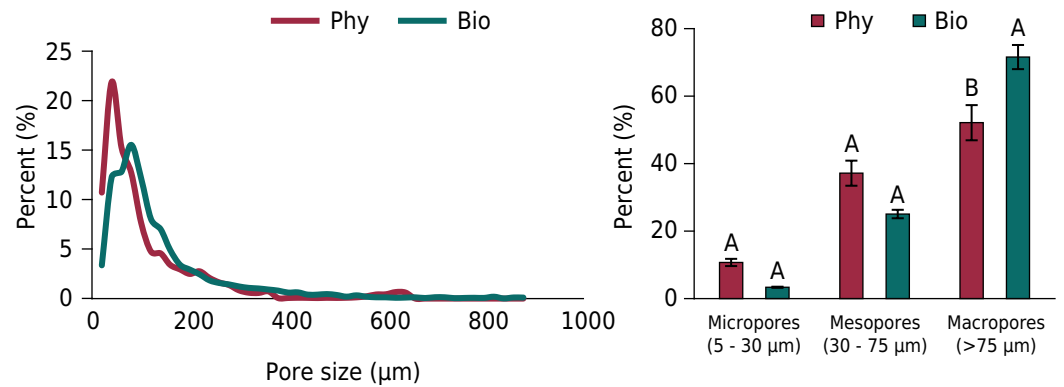


Figure 10. Distribution and mean values of soil aggregates pore size. Phy: physcogenic; Bio: biogenic. Source: Silva Neto et al. (2021).

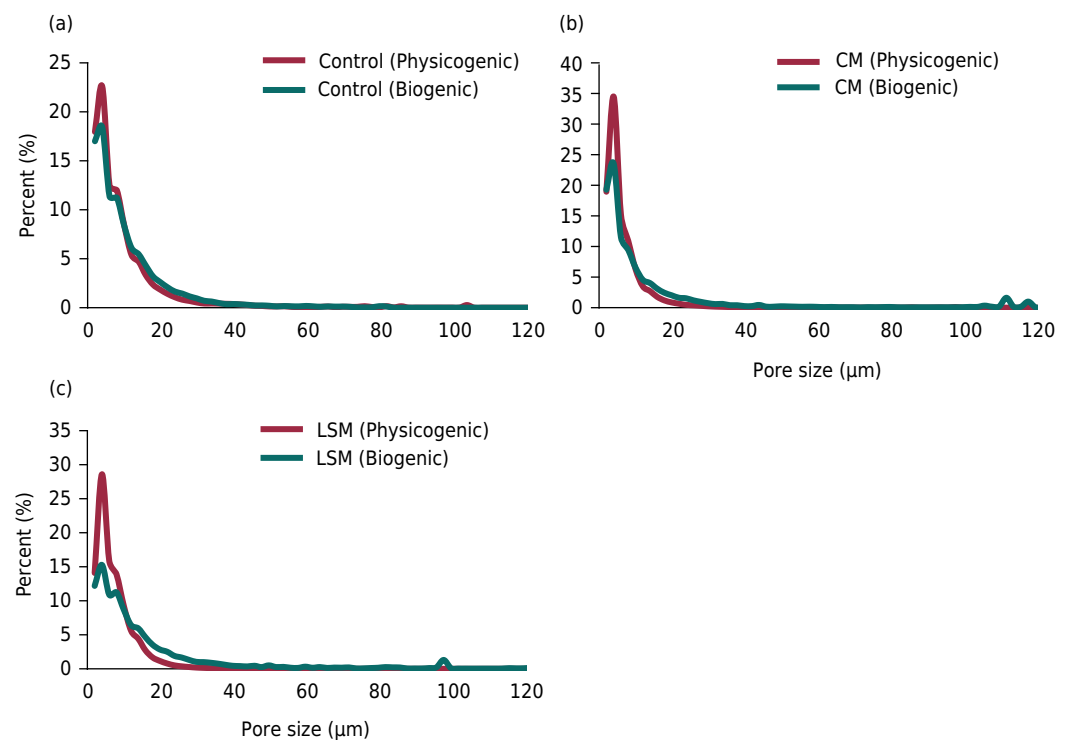


Figure 11. Manure application effects on the distribution of soil aggregates pore size in a *Latosolo* (Oxisol/Rhodic Ferralsol). Control: without manure application (a); CM: chicken manure (b); and LSM: liquid swine manure (c). Source: Melo et al. (2019a).

aggregates always showed a larger volume of higher pore diameter than fisiogenic aggregates, especially above 10 μ m. These authors also attributed the existence of pores between 100 and 120 μ m, present in biogenic aggregates under the influence of manure, to the activity of “ecosystem engineers”.

X-ray μ CT is a promising tool to study soil structure, especially the visualization and quantification of different components at the aggregate scale (Le Bayon et al., 2021). It can thus provide additional information about soil aggregates’ origin and formation processes, whether the edaphic fauna forms them or not. Through X-ray μ CT, it is possible to evaluate and identify differences in the physical and chemical characteristics of biogenic x physcogenic origin aggregates. Le Bayon et al. (2021) used X-ray μ CT to try to differentiate aggregates formed by distinct species of earthworms (biogenic aggregates) from aggregates not formed by earthworms (e.g., physcogenic aggregates); and also evaluate whether X-ray μ CT can serve as a “tomographic signature” specific

to the earthworm species studied. The results showed that the X-ray μ CT analysis was able to differentiate aggregates formed by earthworms from aggregates not formed by earthworms, using the relative volume of the components inside the aggregates, the volumetric mass of the aggregates, and their global volume. The authors found that the proportion of the volume of mineral grains within the aggregates is significantly different according to the studied earthworm species. Le Bayon et al. (2021) concluded that the X-ray μ CT technique is a powerful and promising tool for studying biogenic aggregates' composition and formation. However, the authors emphasize that future research is needed to elucidate aggregate components' forms and spatial distribution, particularly the different states of organic matter decomposition.

Clay dispersion

The quantification of clay dispersion is ultimately a measure of the stability of soil microaggregates (Melo et al., 2019b). According to the theory proposed by aggregation hierarchy and Tisdall and Oades (1982), the unitary particles aggregate into small microaggregates of up to 20 μ m, which subsequently aggregate in higher hierarchical levels. Consequently, the existence of clay particles dispersed in water implies the rupture or non-existence of these microaggregates in the soil.

There are two major groups of methods for assessing clay dispersion: those that assess its spontaneous dispersion in water and those that measure its dispersion in water after applying a disruptive force (Melo et al., 2019b). For highly weathered soils, methods with disruptive forces are notoriously more widely used and will focus on this topic. Here, clay quantified from these methods will be referred to as mechanical water-dispersible clay (MWDC). When MWDC is measured, two processes are simultaneously measured: the ability of the microaggregates to remain stable after the application of the disruptive force and the clay ability to reflocculate after being detached from the microaggregates. MWDC, therefore, is composed of the clay fraction that did not resist disruptive forces and, after dispersing, did not reflocculate, remaining in suspension.

Studies evaluating the dispersion of the clay fraction as a function of the aggregate formation pathway are scarce. From the present review, only the study by Melo et al. (2019a) encompassed measurements of MWDC. These authors evaluated the effect of two doses of chicken litter and liquid swine manure on biogenic aggregation and the chemical and physical properties of a very clayey *Latossolo Vermelho Eutroférico* (Oxisol; Ferralsol). The authors found no evidence that the higher organic matter content of the biogenic aggregates influenced by the addition of the manures increased the resistance of the clay fraction to disruptive forces. However, a strong negative association between MWDC and Al^{3+} content was observed. This pattern suggests that Al^{3+} neutralization, which was more dependent on the manure used than the aggregation pathway, inhibited particle reflocculation and intensifying dispersion. This result can be explained by the higher proportion of cations with lower charge sparsity, such as K^+ (Melo et al., 2020), when the saturation by Al^{3+} is low.

INFLUENCE OF THE AGGREGATE FORMATION PATHWAY ON SOIL PROPERTIES

Studies in Brazil have reported improvements in soil chemical and physical properties due to biogenic aggregation (Batista et al., 2013; Loss et al., 2014; Batista, 2015; Silva Neto et al., 2016; Loss et al., 2017; Melo et al., 2019a; Ferreira et al., 2020). To understand the influence of biogenic aggregation on these properties, data from these authors were compiled and presented in figures 12 to 16. The relative change of attributes caused by biogenic aggregation with physcogenic aggregation was calculated to combine studies with different soils and management. The relative contribution was calculated using equation 1.

$$\text{Relative change} = 100 \times \left(\frac{\text{Bio} - \text{Phys}}{\text{Phys}} \right) \quad \text{Eq. 1}$$

in which *Bio* is the observed value of a given property in biogenic aggregates, and *Fis* is the observed value for the same property in physcogenic aggregates.

Values greater than 100 % indicate that the attribute was increased in biogenic aggregates in relation to physcogenic ones. In general, it is possible to observe that the biogenic aggregates presented predominantly higher values compared to the physcogenic aggregates. It is important to emphasize that for some of the properties surveyed (Na^+ , H+Al , and clay dispersed in water), the numerical increase does not mean an improvement in soil quality. However, its increase, at the levels observed by the authors, does not imply problems for soil fertility.

The lack of studies on the effect of biogenic aggregation on soil properties is notorious. Because of the greater presence of *Latossolos* (Oxisols; Ferralsols) in Brazil, the studies on these aggregates (five studies) are concentrated on this order, while only one study was found for the orders of *Argissolos* (Ultisols; Acrisols), *Cambissolos* (Inceptisols; Cambisols), and *Nitossolos* (Alfisols, Nitisols). However, this limitation of soil representation is mitigated in the present study since the relative changes were calculated due to the focus on biogenic aggregation on soil properties. Additionally, due to the small amount of information, there is a certain restriction to inferences for some of the variables presented in figures 12 to 16.

Regarding the chemical properties of the soil (Figures 12 and 13), available phosphorus was the only property that had its availability increased in biogenic aggregates in all cases. This fact may be related to the activity of “ecosystem engineer” organisms, for example, ants that, when building their nests and accumulating organic matter through excrement and construction material, can increase phosphorus availability in the soil (Frouz et al., 2005). For the other properties, the results depended on the evaluated conditions, whether soil, climate, or management. This suggests that, despite the tendency of biogenic aggregation to favor soil chemical properties, the context in which soil biota acts reflects its potential for soil improvement. Additionally, these factors also regulate the intensity of biological activity, affecting the proportion of biogenic aggregation in the soil since the soil fauna is, at the same time, a transforming agent and a reflection of the

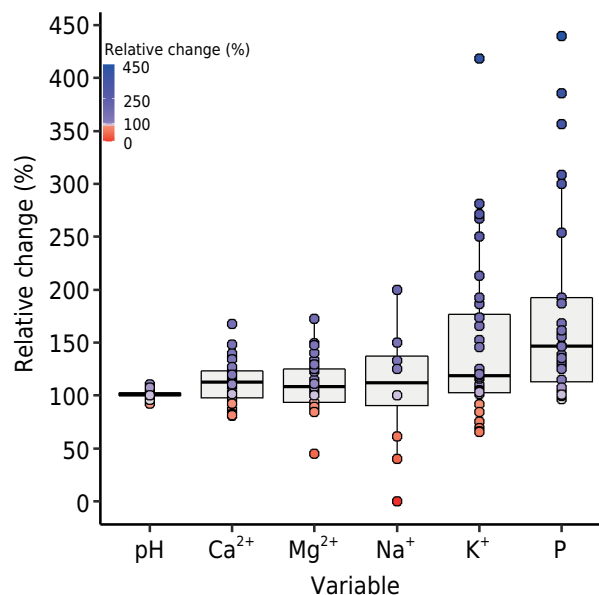


Figure 12. Changes in soil chemical properties (pH, Ca^{2+} , Mg^{2+} , K^+ , Na^+ and P) caused by biogenic aggregation in relation to physcogenic aggregation.

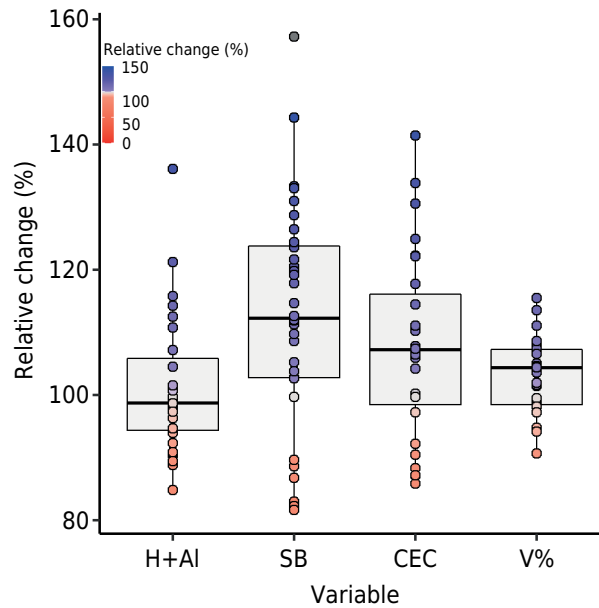


Figure 13. Changes in soil chemical properties (H+Al, SB (sum of bases), CEC (cation exchange capacity and V% (Value V) caused by biogenic aggregation in relation to physcogenic aggregation.

physical, chemical, and biological properties of soils (Blanchart et al., 2006). Despite the variability of responses, the potential of biogenic aggregates to increase cation exchange capacity, pH, and nutrient availability is noted, minimizing leaching and the demand for acidity correctives and fertilizers.

The increase in properties related to soil organic matter was more evident compared to soil chemical properties (Figures 14 and 15). Few were the attributes evaluated that presented a relevant proportion of values below 100 %. This consistency of results reinforces the importance of soil fauna and roots for the contribution of organic compounds to the soil and demonstrates that favoring biogenic aggregation brings benefits in terms of increasing organic matter content at different decomposition levels. It emphasizes the 600 % increase in carbon content and almost 300 % in nitrogen content associated with particulate organic matter, a potential nutrient supplier via mineralization due to its lower recalcitrance.

This enrichment of the soil in organic matter occurs not only because of its concentration in these aggregates but also because it promotes greater stability in the water of the aggregates, which favors the protection and accumulation of organic matter. The activity of “ecosystem engineers,” mainly Oligochaeta, can incorporate considerable amounts of particulate organic carbon (COp) into mineral particles, as well as initiate the formation of microaggregates (Pulleman et al., 2005). The ratio between the formation of macro and microaggregates, stabilization, and degradation is directly related to the dynamics of COp. Thus, biogenic aggregates can increase carbon retention in the soil (Six et al., 2000).

The results demonstrate raised consistent increase in the size of stable biogenic aggregates in water compared to physcogenic (Figure 16). This increase reached 140 % for the weighted average diameter and 200 % for the geometric average diameter. It is noteworthy that a study also found an increase in the contents of mechanically dispersible clay in water, suggesting that despite being more stable, these aggregates have greater potential for transporting fine particles when subjected to disruptive forces, such as the impact of raindrops. Therefore, it emphasizes the importance of soil cover to maintain the structural quality obtained by biogenic aggregation.

The study of the origin of aggregates allows us to identify the role of different agents involved in changing the soil structure. Evaluate the formation pathways in different configurations of management systems such as no-tillage system - NTS (Loss et al., 2014;

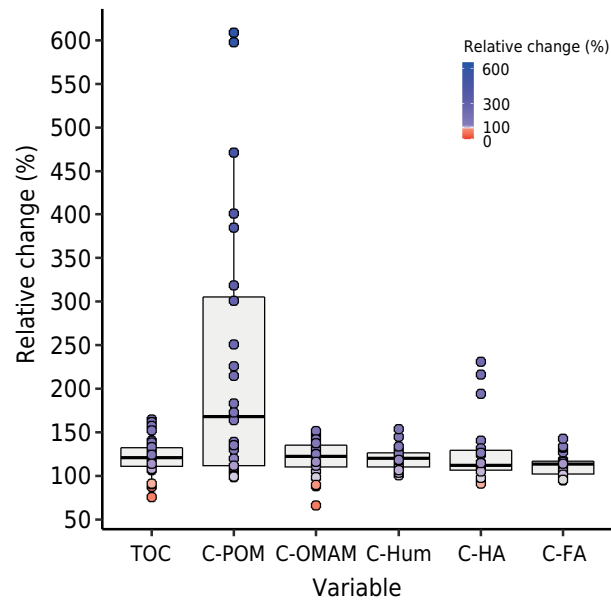


Figure 14. Changes on soil properties related to organic matter (Total organic carbon (TOC), C-Particulate organic matter (C-POM), C-organic matter associated to minerals (C-MAOM), C-humin (C-Hum), C-humic acids (C-HA), and C-fulvic acids (C-FA) caused by biogenic aggregation in relation to physcogenic aggregation.

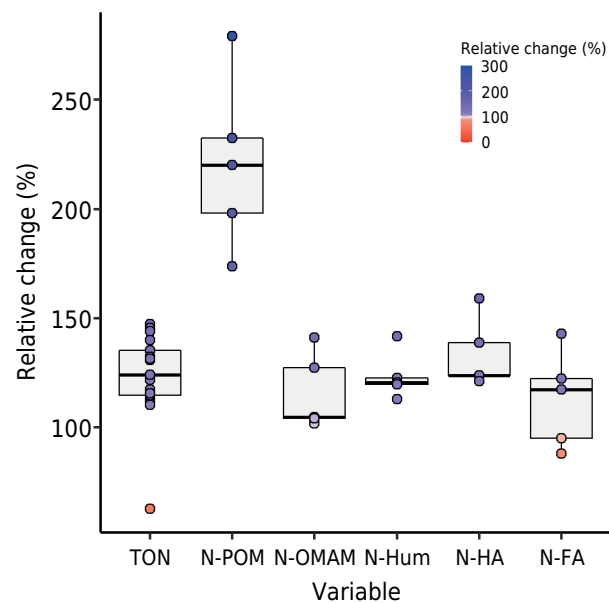


Figure 15. Changes on soil properties (Total organic nitrogen (TON), N-Particulate organic matter (N-POM), N-organic matter associated to minerals (N-MAOM), N-humin (N-hum), N-humic acids (N-HA) and N-fulvic acids (N-FA) related to organic matter caused by biogenic aggregation in relation to physcogenic aggregation.

Batista, 2015; Loss et al., 2017; Melo et al., 2019a; Ferreira et al., 2020; Pinto et al., 2021), crop-livestock integration - CLI (Batista et al., 2013), pasture (Loss et al., 2014; Silva Neto et al., 2016); application of manure in NTS (Loss et al., 2017; Melo et al., 2019a); conventional soil tillage system (Loss et al., 2014) and forest in the regeneration stage (Loss et al., 2014; Silva Neto et al., 2016) allowed to elucidate the influence of biological agents, roots and soil fauna, in improving the chemical, physical and biological properties of the soil as demonstrated above. These studies were developed in different soil classes and states of Brazil, being *Nitossolo Vermelho* (Alfisol; Nitisol) (Goiás, Brazil), *Argissolo Vermelho-Amarelo* (Ultisol; Acrisol) (Santa Catarina, Brazil), *Latossolo Vermelho* (Oxisol; Ferralsol) (Mato Grosso do Sul and São Paulo, Brazil), *Latossolo Vermelho-Amarelo* (Oxisol; Ferralsol) (Mato Grosso do Sul, Brazil), *Cambissolo Háplico* (Inceptisol; Cambisol) (Rio de

Janeiro, Brazil), and present distinct chemical and physical properties associated with the respective soil classes and formation factors.

In an attempt to verify the existence of a pattern for the formation of biogenic and physcogenic aggregates in the studies presented and, therefore, in different management systems, treatments, and soil classes, the results were analyzed together (Figure 17). Due to the heterogeneity of properties evaluated in the different studies, only those commonly quantified were selected. The results demonstrate that the effect of soil origin was more

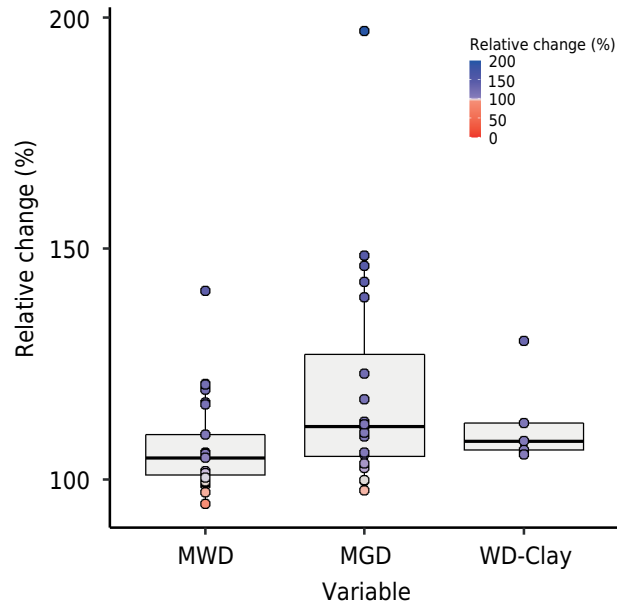


Figure 16. Changes in the Mean Weighed Dimater (MWD); Mean Geometric Dimeter (MGD); and Water-dispersible clay (WD-Clay) related to aggregation caused by biogenic aggregation in relation to physcogenic aggregation.

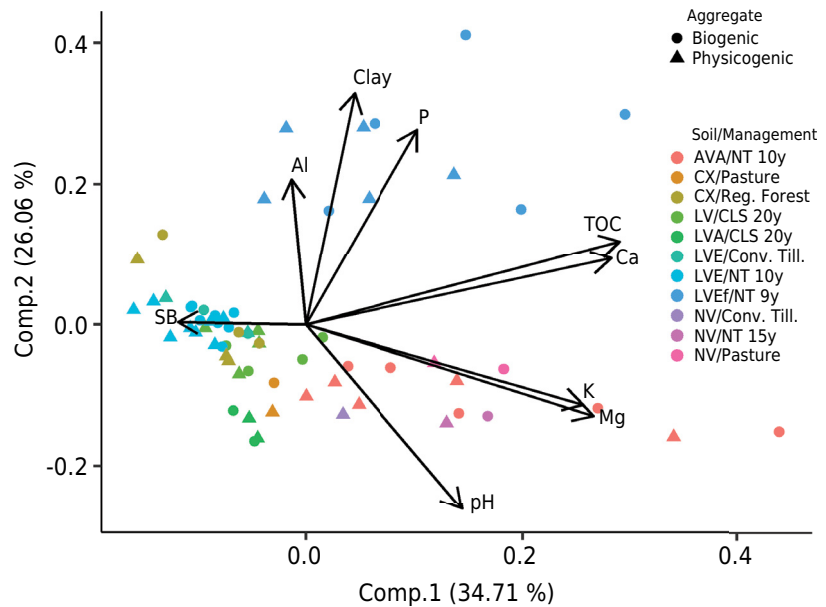


Figure 17. Principal Components Analysis (PCA) of physical and chemical attributes of biogenic and physcogenic aggregates. AVA: *Argissolo Vermelho Amarelo* (Ultisol/Acrisol); CX: *Cambissolo Háplico* (Inceptisol/Cambisol); LV: *Latossolo Vermelho* (Oxisol/Ferralsol); LVA: *Latossolo Vermelho Amarelo* (Oxisol/Ferralsol); LVE: *Latossolo Vermelho Eutrófico* (Oxisol/Ferralsol); LVEf: *Latossolo Vermelho distroférico* (Oxisol/Ferralsol); NV: *Nitossolo Vermelho* (Alfisol/Nitisol); CLS: crop-livestock systems; NT: no-tillage; CT: conventional tillage; Reg. Forest: forest under regeneration; 9y, 10y, 20y: years; SB: sum of bases; TOC: total organic carbon.

pronounced than the origin of aggregates. The different management systems and soil classes remained related regardless of the origin of the aggregates. This pattern evidences the intrinsic characteristics of pedogenesis compared to the origin of aggregates.

The lesser influence of management, in relation to pedogenesis, is evident in the comparison of areas under similar management (USM for ten years), which were grouped according to soil classes (RYA and LVE). Thus, it is possible to verify that there is no common pattern in the chemical and physical characteristics of the biogenic and physicogenic aggregates for the studied soil classes. The improvements in chemical, physical, and biological properties that biogenic aggregates promote are noticeable (Figures 12, 13, 14, 15 and 16), but the changes occur at different intensities (Figure 17).

FINAL CONSIDERATIONS







We highlighted some studies on the formation of aggregates for the different edaphic-environmental conditions in Brazil. To assist in the orderly expansion of knowledge about these processes, this study gathered the images and criteria used by studies carried out in the country for the morphological classification of the aggregates regarding the aggregation pathway. The results suggest that, for most studied variables, there is soil improvement by biogenic aggregation in relation to physical aggregation. Biogenic aggregates verified greater availability of nutrients, organic matter content in different degrees of decomposition, and stability in water. Such standards demonstrate the potential use of biogenic aggregates as indicators of the quality of the adopted management. However, its ability to promote improvement in soil properties seems to depend on soil and environmental conditions and the use and management of the soil, making it necessary to expand studies on the subject to clarify the potential contribution of biogenic aggregation for soil improvement under different conditions.







For future studies, and especially to assess the importance of biogenic aggregation in improving edaphic properties, it is suggested the use of micromorphology, near infrared spectroscopy, X-ray computed tomography, clay dispersion analyses, in conjunction with chemical, physical and biological analysis of aggregates, to contribute to the identification of other patterns related to pedogenesis and the pathways of formation of aggregates.



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