

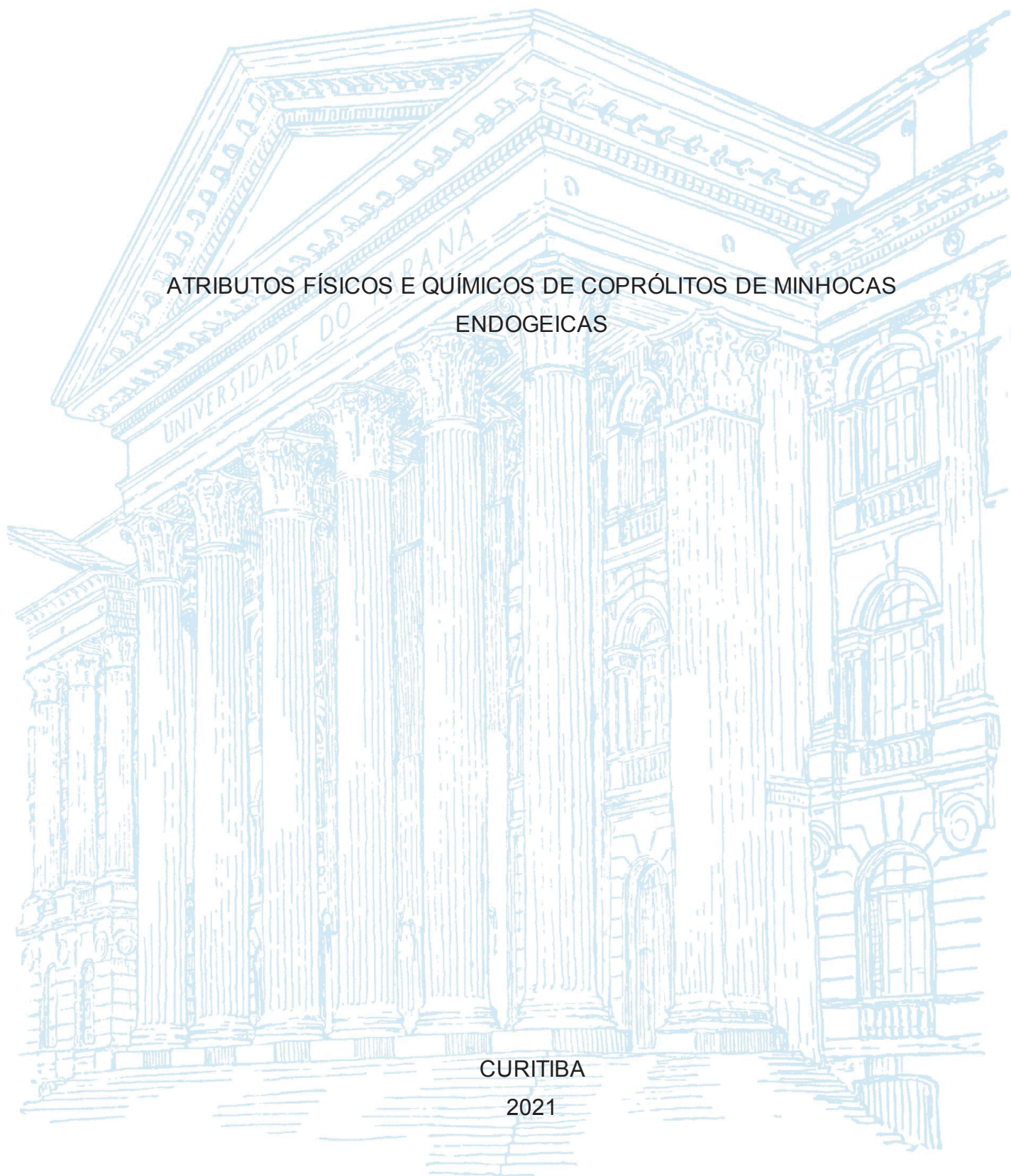
UNIVERSIDADE FEDERAL DO PARANÁ

ALESSANDRA SANTOS

ATRIBUTOS FÍSICOS E QUÍMICOS DE COPRÓLITOS DE MINHOCAS
ENDOGEICAS

CURITIBA

2021



ALESSANDRA SANTOS

ATRIBUTOS FÍSICOS E QUÍMICOS DE COPRÓLITOS DE MINHOCAS
ENDOGEICAS

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Coorientadores: Prof. Dr. Antônio Vargas Motta
Miguel Cooper
Carlos Manoel Pedro Vaz

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Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em CIÊNCIA DO SOLO da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **ALESSANDRA SANTOS** intitulada: **ATRIBUTOS FÍSICOS E QUÍMICOS DE COPRÓLITOS DE MINHOCAS ENDOGÊICAS**, sob orientação do Prof. Dr. GEORGE GARDNER BROWN, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua **APROVAÇÃO** no rito de defesa.

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“A tarefa não é tanto ver aquilo que ninguém viu, mas pensar o que ninguém
ainda pensou sobre aquilo que todo mundo vê.”
(Arthur Schopenhauer)

RESUMO

Animais do solo são importantes para a produtividade primária, decomposição e mineralização de resíduos orgânicos, aumentando a ciclagem de nutrientes e a disponibilidade para as plantas. Fauna edáfica de maior tamanho, como os engenheiros de ecossistema, particularmente minhocas, podem impactar a gênese do solo, as propriedades físicas do solo e a disponibilidade de nutrientes e recursos para outros organismos, mas os impactos são altamente dependentes das espécies de minhoca em questão. Além disso, pouco se sabe sobre a estrutura física dos coprólitos de minhocas, principalmente sua porosidade interna e o papel de diferentes espécies em conferir diferentes características físicas em coprólitos produzidos no mesmo solo ou em diferentes solos. Assim, um experimento foi realizado para analisar atributos químicos e físicos de coprólitos produzidos por quatro espécies de minhocas endogeicas tropicais (*Rhinodrilus alatus*, *Glossoscolex paulistus*, *Glossoscolex* n.sp. e *Pontoscolex corethrurus*) em dois solos de textura contrastante (argilo siltoso e argilo arenoso). Coprólitos de diferentes espécies apresentaram tamanhos, formas, tipos e números de poros diferentes. Poros arredondados ocorreram em todas as classes de tamanhos de poros e foram evidentes em todas as espécies estudadas, independentemente do tipo de solo, indicando que as minhocas são essenciais no rearranjo das partículas. Apesar de sua baixa conectividade, esses poros de transmissão arredondados podem ser muito importantes para o crescimento e penetração da raiz. Avaliações com porosimetria de mercúrio mostraram que os coprólitos tinham uma distribuição de tamanho de poro diferente que dependia mais da textura do solo do que as espécies de minhocas. Os atributos químicos dos coprólitos variaram de acordo com as espécies de minhocas e a textura do solo, mas os coprólitos geralmente apresentaram níveis mais elevados de carbono orgânico, fósforo inorgânico, Ca, Mg, K, Na e N total em comparação com o solo testemunha. Coprólitos de todas as espécies também tinham partículas mais finas (especialmente argila) em comparação com o solo testemunha. No entanto, em geral, os efeitos da textura do solo nos atributos físicos e químicos dos coprólitos das minhocas parecem ser maiores do que os impactos das próprias espécies.

Palavras-chave: Oligochaeta. Nutrientes do solo. Distribuição de tamanho de poros. Textura. Porosimetria de mercúrio. Microtomografia.

ABSTRACT

Soil animals are important for primary productivity, decomposition and mineralization of organic residues, increasing nutrient cycling and availability to plants. Larger soil fauna like ecosystem engineers, particularly earthworms, can impact soil genesis, soil physical properties and the availability of nutrients and resources to other organisms, but these impacts are highly dependent on the earthworm species in question. Furthermore, little is known regarding the physical structure of earthworm casts, particularly its internal porosity and the role of different species in conferring different physical characteristics in casts produced in the same or different soils. Hence, an experiment was carried out to analyze chemical and physical properties of casts produced by four tropical endogeic earthworm species (*Rhinodrilus alatus*, *Glossoscolex paulistus*, *Glossoscolex* n.sp., and *Pontoscolex corethrurus*) in two soils of contrasting texture (sandy loam and silty clay). Casts of the different species had different sizes, shapes, types and numbers of pores. Rounded pores occurred in all classes of pore sizes and were evident in all species studied regardless of the soil type, indicating that earthworms are essential in particle rearrangement. Despite their low connectivity, these rounded transmission pores, might be very important for root growth and penetration. Mercury porosimetry evaluations showed that castings had a different pore size distribution that depended more on soil texture than the earthworm species. The chemical properties of casts varied depending on the earthworm species and the soil texture, but casts generally had higher organic carbon levels, inorganic phosphorus, Ca, Mg, K, Na, and total N compared to the control soil. Casts of all species also had more finer particles (specifically clay) compared to the control soil. Nonetheless, overall, soil texture effects on both the physical and chemical properties of the earthworm casts appeared to be greater than the impacts of the species themselves.

Keywords: Oligochaeta. Soil nutrients. Pore size distribution. Texture. Mercury porosimetry. Microtomography.

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1 GENERAL INTRODUCTION

The populations and activity of soil fauna can be related to important environmental characteristics, like soil type, climate, and predominant vegetation (Lavelle & Pashanasi, 1989). Furthermore, the soil fauna is sensitive to changes in the system's biological, physical, and chemical properties, often resulting from soil management and cultivation practices (Baretta *et al.*, 2011). On the other hand, soil fauna can have important impacts on plant productivity, decomposition and nutrient cycling, as well as on soil physical properties (Lal, 1988; Lavelle *et al.*, 1992; Lavelle *et al.*, 1997; Velasquez *et al.*, 2019).

Earthworms are among the organisms most widely recognized in soils. They play an essential role in soils, acting as ecosystem engineers, altering the soil's physical properties to such a degree that it affects the availability of resources and nutrients to other organisms, including plants (Lavelle *et al.*, 1997). Furthermore, earthworm burrowing and casting has important impacts on the distribution of water and air in the soil profile (Blanchart *et al.*, 2004), and can have major impacts on plant growth and productivity.

Earthworms can vary in size from a few millimeters to over 2 m long, and from a few milligrams in size (particularly as juveniles or cocoons), up to over 500 g fresh biomass. Some tropical countries like Brazil have a very large number of giant earthworm species, locally called minhocuçus (Brown & James, 2007). However, most species range from 5 to 15 cm length (Lavelle & Spain, 2001), weighing from 200 mg up to 3 g fresh mass as adults. Earthworms can be distinguished according to the ecological category, being classified as epigeic species (inhabit the litter and upper layers of the soil and normally eat decomposing plant litter); anecic species (move vertically in the soil from the surface to the deeper layers, building vertical galleries and transporting material from the surface to the interior of the galleries); and endogeic species (do not create permanent galleries and are responsible for major soil aggregation and stabilization of organic matter, living in the soil profile, feeding basically on organic matter and varying widely in size).

Endogeic species ingest from around 1 up to more than 30 times their individual body weight (fresh mass) per day (Lavelle, 1988). A large part of the soil in the arable layer passes through the intestinal tract of earthworms several times (Darwin, 1881), but it is known that their stability and duration inside the soil varies

according to the type of soil and the worm species present in it (Hedde *et al.*, 2013). Cast production in an area varies depending on the population and species of earthworms present and the land use system. In pastures with a high population of endogeic species like *Pontoscolex corethrurus* (Müller, 1857), cast production can reach more than 300 t ha⁻¹ year⁻¹ (Lavelle, 1988).

As the soil passes through the intestine, major physical, chemical and biological changes take place (Brown *et al.*, 2001). Nitrogenous compounds from earthworm metabolism (urine excretion) are added (Lee, 1985), and microbial activity (especially fermenting species) is activated via mucus secretion by the earthworms. Hence, the castings are hotspots for root propagation (Nijhawan & Kanwar, 1952; De Vleeschauwer & Lal, 1981; Molungoy & Bedoret, 1989; Römbke *et al.*, 2005), and microbial activity (Wüst *et al.*, 2011), stimulating the mineralization of nutrients (Lubbers *et al.*, 2011, 2013; Majeed *et al.*, 2013) and release of plant growth promoting substances (Krishnamoorthy & Vajranabhaiah, 1986). Furthermore, earthworm casts generally have higher levels of organic carbon, inorganic P, Ca, Mg, K, Na, and total N compared to uningested soil (Fiuza *et al.*, 2011; Quadros *et al.*, 2002; Santos *et al.*, 1996; Hulugalle & Ezumah, 1991), depending on the species studied.

Many tropical earthworm species, particularly in Latin America also have calciferous glands (Righi, 1997) that secrete calcium carbonate into the esophagus. Furthermore, most endogeic species also have a strong muscular gizzard that can affect the dispersion of soil particles and modify clay platelet orientation (Barois *et al.*, 1993), affecting soil minerals. Hence, many earthworm species can influence the availability of heavy metals in soils (Zorn *et al.*, 2005; Lukkari *et al.*, 2006; Sizmur *et al.*, 2011; Duarte *et al.*, 2012), although the effects vary depending on the metal and the species of worm in question (Kizilkaya, 2004; Udovic *et al.*, 2007; Udovic & Lestan, 2007).

Earthworm casts often have higher fine sand, silt, and clay levels than the adjacent soil (Shipitalo & Protz, 1988; Trigo & Díaz Cosín, 1992). These differences in nutrient availability and cast texture occur due to the ability of earthworms to select soil that is richer in organic matter and smaller particles of the mineral fraction (Harris *et al.*, 1966), but perhaps also because these particles are at the size limit that they can ingest (Jouquet *et al.*, 2008; Jager *et al.*, 2003).

Hedde *et al.* (2013) showed how earthworm effects on aggregate stability depend on the soil and species characteristics. Endogeic earthworms, particularly

compacting species (Blanchart, 1997) like *P. corethrurus* create compact castings that are highly resistant to breakdown. Cast stabilization is a complex, rather poorly understood phenomenon, that involves several simultaneous processes, and the ultimate result depends on a variety of factors including soil texture, clay mineralogy, water and organic matter content, earthworm ecological category, microbial activity, cast age, where it is deposited and environmental forces like rainfall and temperature (Zhang & Schrader, 1997; Shipitalo & Le Bayon, 2004; Bottinelli *et al.*, 2010; Bottinelli *et al.*, 2014).

Although much is known of the chemical and physical characteristics of earthworm casts, their internal structure, porosity and relationship with soil physical attributes such as texture, and the differences between casts produced by native tropical species are still poorly known, particularly for larger species like the minhocuçu.

In order to elucidate the issues raised above, two large endogeic earthworm species (minhocuçu) were chosen for this study: *Rhinodrilus alatus* Righi, 1971, *Glossoscolex paulistus* Michaelsen, 1926. Furthermore, two other native endogeic earthworm species were chosen: *Glossoscolex* n.sp. (a new species, not yet described), and *P. corethrurus*.

R. alatus belongs to the Rhinodrilidae family, and is one of the best-known Brazilian earthworms, as it has been intensively collected and commercialized nationwide for over 80 years (Drummond *et al.*, 2008). The adults of this species measure around 60 cm in length (but can reach 1.2 m) and inhabit an area of about 20,000 km² in the Cerrado region of central Minas Gerais (Drummond *et al.*, 2013), where seasonal rains drive activity and inactivity (diapause) for about half of the year, respectively (Drummond *et al.*, 2015). Although its life-history has been relatively well studied (Drummond *et al.* 2008; 2015), little is known regarding the impacts of this species on soil properties and processes.

G. paulistus is an endemic Glossoscolecoid earthworm from the south-central region of the State of São Paulo, easily found around the cities of Rio Claro and Piracicaba (Righi, 1971). Members of the Glossoscolecidae family are found from Panama to Argentina, and species native to Brazil are generally endemic to specific regions. *G. paulistus* stands out for its relatively large size, with length from 28 to 35 cm and diameter of 1 cm. It is a geophagous species, feeding on soil organic matter without recognizable structure.

The new *Glossoscolex* species is a Glossoscolecid earthworm also endemic to São Paulo, found mainly in periodically flooded areas in the municipality of Rio Claro. The length of this species is approximately 23 cm (observed data).

Pontocolex corethrurus is a peregrine species of the Rhinodrilidae family, native to the neotropical region, but without a definitively known origin (Ortiz-Ceballos et al., 2019). It is the most widely distributed species in Brazil (Brown *et al.*, 2006), showing tolerance to a wide range of soil physicochemical properties, including temperature and humidity. It can be found in the most varied types of soils, usually in the upper layers, down to 30 cm deep, inhabiting from sandy to clayey soils of red or black earth, as well as in very humid places such as swamps, and dry soils on the tops of hills (Vannucci, 1953). The species is endogeic, geophagous, and can easily assimilate soil organic matter, even in very poor soils, having great ability to colonize different habitats (Hamoui, 1991).

Within this context, the present study evaluated casts of several earthworm species produced in two soils with different texture, characterizing their chemical and physical properties, internal structure and porosity and comparing them with uningested control soil.

Casts were produced in the laboratory, using the four earthworm species, and microtomographic analyzes were carried out on the casts in the two different soils to assess internal structure and pore types (Chapter 1), mercury porosimetry, and pore distribution using 3D microtomography (Chapter 2) and chemical and physical analysis (Chapter 3), to determine differences among species and control soils.

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2 CHAPTER I: MICROTOMOGRAPHIC ANALYSIS SHOW POROSITY OF ENDOGEIC EARTHWORM CASTS

2.1 RESUMO

As minhocas estão entre os animais mais importantes do solo, com atividades de engenharia de ecossistemas que afetam a agregação e a porosidade do solo. As espécies endogeicas tropicais podem produzir grandes quantidades de coprólitos, mas pouco se sabe sobre sua estrutura física, particularmente sua porosidade interna, e as diferenças nos coprólitos produzidos por espécies de diferentes tamanhos em diferentes solos. Portanto, no presente estudo, a microtomografia computadorizada foi usada para analisar coprólitos de minhocas em dois solos de textura variável. Quatro espécies de tamanho variável, variando de 0,5 a 50 g de peso fresco (*Rhinodrilus alatus*, *Glossoscolex paulistus*, *Glossoscolex* n.sp. e *Pontoscolex corethrurus*) foram incubadas em laboratório em um solo franco-arenoso ou um solo argiloso para a produção de coprólitos frescos, que foram então secos ao ar e analisados para determinar a porosidade total, distribuição e número de poros. Todas as minhocas mostraram ingestão seletiva de tamanho de partícula e produziram coprólitos globulares compactos. Todos os coprólitos também apresentaram diferenças no formato e no número de poros. Poros arredondados ocorreram em todas as classes de tamanhos de poros e em todas os coprólitos estudados, independentemente da textura do solo, indicando que as minhocas desempenham um papel importante no rearranjo das partículas e dos poros do solo. Embora de baixa conectividade, esses poros arredondados são poros de transmissão importantes para o crescimento e penetração da raiz. As diferentes espécies de minhocas tiveram um impacto importante na porosidade geral do coprólito, mas foi principalmente a textura do solo que controlou a porosidade total, o número de poros e a morfologia dos poros nos coprólitos.

Palavras-chave: Morfologia de poros. Número de poros. Porosidade de coprólitos. Oligochaeta.

2.2 ABSTRACT

Earthworms are among the most important animals in soils, with ecosystem engineering activities that impact soil aggregation and porosity. Tropical endogeic species can produce huge amounts of casts, but little is known of their physical structure, particularly their internal porosity, and the differences in castings produced by different sized species in different soils. Hence, in the present study computerized microtomography was used to analyze earthworm casts in two soils of varying texture. Four species of varying size, ranging from 0.5 to 50 g fresh weight (*Rhinodrilus alatus*, *Glossoscolex paulistus*, *Glossoscolex* n.sp. and *Pontoscolex corethrurus*) were incubated in the laboratory in a sandy loam or a silty clay soil to produce fresh castings, which were then air dried and analyzed to determine total porosity, distribution, and number of pores. All earthworms showed selective particle size ingestion, and produced compact globular castings. All the casts also showed differences in the shapes and number of pores. Rounded pores occurred in all classes of pore sizes and

in all castings studied regardless of soil texture, indicating that earthworms play an important role in soil particle and pore rearrangement. Although of low connectivity, these rounded pores are transmission pores important for root growth and penetration. The different earthworm species had an important impact on the overall cast porosity, but it was mainly the soil texture that controlled total porosity, number of pores, and pore morphology in the casts.

Keywords: Pore morphology. Number of pores. Porosity of casts. Oligochaeta.

2.3 INTRODUCTION

Soil structure influences both water flows and gas diffusion as well as energy and nutrient fluxes in soils (Hassink et al., 1993; Görres et al., 2001). Soil pore sizes and their distribution are dynamic properties that can be altered by crop management, machinery traffic, root growth, and bioturbation. Changes in soil pore space play important roles in the regulation of, and interaction between soil microbial and faunal communities (Elliott et al., 1980; Hassink et al., 1993). They also have significant effects on soil fertility, affecting relationships between water drainage, nutrient absorption, aeration, and temperature, ultimately influencing crop development and productivity (Letey, 1985).

Among the soil animals, earthworms are well recognized as important bioturbators, influencing soil properties and processes through ecosystem engineering (Lavelle et al., 2016). However, their effects vary depending on the earthworm's ecological category (anecic, endogeic, epigeic) and species (Lee & Foster, 1991; Barois et al., 1999; Blanchart et al., 1999). Anecic earthworms for example, bury surface litter, forming "middens" and vertical burrows. Qualitative micromorphological analyses of anecic earthworm structures suggest that the drilosphere (soil influenced by earthworms) has smaller pores and less total porosity than the adjacent soil (Binet & Curmi, 1992) due to the selective ingestion of smaller soil particles by anecic earthworms, and that their middens built from casts are more stable, with both chemical and physical differences when compared to the adjacent soil (Shipitalo & Protz, 1988; Ziegler & Zech, 1992; Zhang & Schrader, 1993).

Endogeic compacting earthworm species like the pantropical *Pontoscolex corethrurus* (Blanchart et al., 1999) can also reduce soil porosity and increase bulk density in sieved (disaggregated) soil macrocosms (Alegre et al., 1996; Hallaire et al.,

2000), and in degraded Central Amazonian pastures (Chauvel et al., 1999; Barros et al., 2004). However, when additional organic matter sources are provided, and in compacted soils, their activity can improve soil porosity (Zund et al., 1997; Hallaire et al., 2000). On the other hand, the temperate widespread endogeic species *Allolobophora chlorotica* and *Aporrectodea rosea* can increase soil organic matter translocation, N and C mineralization, and soil porosity, improving infiltration and water retention in the profile (Amossé et al., 2015).

Hence, the soil structure created by earthworm species depends highly on the earthworm size, its feeding and burrowing habit, the type of casts it produces, and the soil's texture (Blanchart et al., 1999). Casts can have different sizes, stability, and duration in the soil (Shipitalo & Le Bayon, 2004), particularly depending on their position (surface vs. within-soil) (Decaëns, 2000), and their effects on organic matter dynamics and microbial activity can also be very different (Brown et al., 2000). Globular casts are large and produced by "compacting" earthworm species and therefore tend to be more stable and durable than granular casts, produced by "decompacting" species (Blanchart et al., 1997). Furthermore, there can be interactions between these aggregates, since decompacting species can ingest casts of compacting species and vice versa (Blanchart et al., 1997; 1999; Jiménez et al., 1998), with granular casts opening the way for the mineralization of physically protected carbon, and globular casts "protecting" potentially mineralizable carbon (Martin, 1991). These physical differences can influence ecological processes not only at the local scale but also at the landscape scale, as well documented in Darwin (1881).

Cast properties are also related to soil moisture and water retention, where the discontinuity created by the differences in pore size between the casts and the surrounding soil can prevent the movement of water and nutrients from the casts to the soil (West et al., 1991; Binet & Curmi, 1992). However, little is known about the physical structure of casts produced by most earthworm species, particularly tropical endogeic earthworms, especially their internal porosity, and relationship with earthworm size and ingested soil texture.

Hence, in the present study, we used two species of native Brazilian giant earthworms, *Rhinodrilus alatus* Righi, 1971 and *Glossoscolex paulistus* Michaelsen, 1926, as well as two other species of endogeic earthworms, *Glossoscolex* n.sp. (new species), and *Pontoscolex corethrurus* Müller, 1857.

The species chosen, in addition to being endogeic, have important characteristics for the study: the first two species of giant earthworms have been poorly studied, and their ecology, cast formation and the soil processes to which they are closely related, are practically unknown; the giant earthworm species are endemic to different areas (geographically, chemically and physically); the species *Glossoscolex* n. sp. is a still undescribed species and its ecology is unknown; *P. corethrurus* is a pantropical species, widely studied, and present at the sites where the other earthworms are found.

Thus, this chapter aims to evaluate the porosity of casts of four Brazilian endogeic earthworm species, produced in two soils with contrasting texture.

2.4 MATERIAL AND METHODS

2.4.1 SOILS AND SPECIES USED

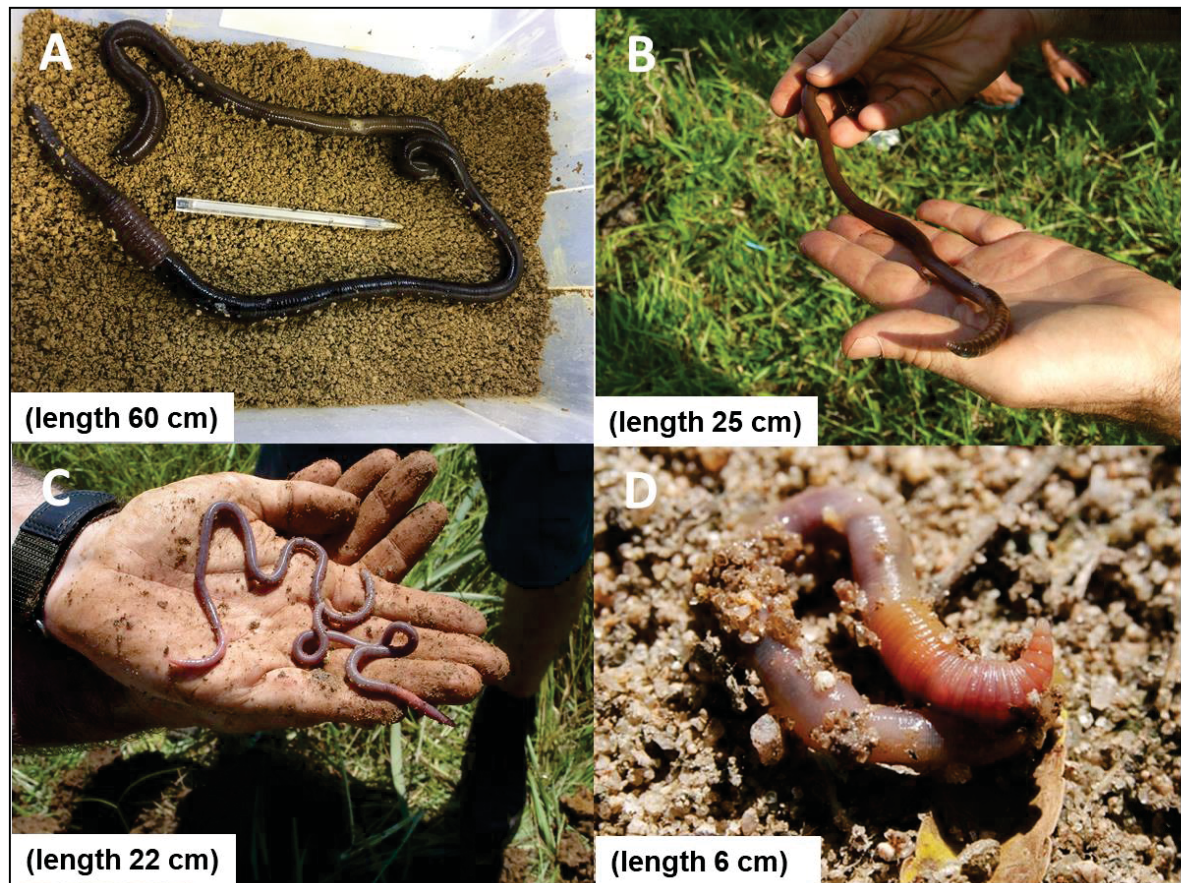
Two soils with contrasting texture were collected: the first was a silty clay Cambisol (4% sand, 44% clay, and 52% silt) from native Cerrado vegetation at the National Forest of Paraopeba (19°15'17.2"S, 44°24'04.9"W, altitude 725 m) and the second was a sandy loam Acrisol (74% sand, 13% clay and 13% silt) from a cattle pasture at the Fazenda Santa Rosa, Assistência district (22°30'35.7"S, 47°36'42.1"W, altitude 515 m), Rio Claro – SP. The choice of sampling sites was due to the endemism of the native species and the presence of a common peregrine species at both sites (*P. corethrurus*). Soils were collected at a depth of 0 - 30 cm.

Four endogeic earthworm species were used: *R. alatus*, *Glossoscolex* n.sp. (new species not yet described), *G. paulistus* and *P. corethrurus* (Figure 1). Two of them (*R. alatus*, *G. paulistus*) are fairly large earthworms (locally called minhoca), widely commercialized as fish bait: *R. alatus* grows up to 1.2 m long but is normally around 50-60 cm long and adults tend to weigh around 50 g, while *G. paulistus* is normally around 25 cm long and weighs ~15 g. The new *Glossoscolex* sp. is thin (8 mm) and long (22 cm), with adults weighing around 3 g, while *P. corethrurus* are generally small (around 6 cm long) and tend to weigh around 0.5 g when adult.

The experiment was carried out in the dark in an incubator of 22 ± 1 °C (Full Gauge Imperial III) located in an air-conditioned laboratory at Embrapa Forestry in Colombo, Paraná - Brazil. Five individuals of *R. alatus* and *G. paulistus* were placed individually in pots measuring 24 x 17 x 10 cm, while three individuals of *Glossoscolex*

n.sp. and *P. corethrurus*, were placed in each of five pots. Due to their different sizes, 500 g soil were used to incubate *R. alatus* and *G. paulistus*, while 100 g were used for *P. corethrurus*, and 250 g for *Glossoscolex* n.sp., all maintained at 70 % container capacity. The casts produced within a 24 h period by each earthworm species were collected and dried in an oven at 40 °C until they presented constant weight (four to five days), and the incubations were performed separately for each soil type. Casts of the smaller species (*Glossoscolex* n.sp. and *P. corethrurus*) were accumulated until enough volume and weight were available for the analyses.

FIGURE 1. EARTHWORM SPECIES STUDIED: A - *Rhinodrilus alatus*, B - *Glossoscolex paulistus*, C - *Glossoscolex* n.sp., D - *Pontoscolex corethrurus*.



SOURCE: (A) Alessandra Santos; (B) George Brown; (C) Samuel James; (D) Marie Bartz (2021).

2.4.2 MICRO-CT ANALYSIS

Micro-CT images were acquired with a SkyScan micro-CT X-ray scanner, model 1172 (Bruker, Belgium), at Embrapa Instrumentation in São Carlos – SP. The X-ray tube operates at 100 kV and 100 mA. It uses an aluminum/copper filter (0.5 / 0.04 mm

thick) positioned between the sample and the detector to block out soft X-rays and reduce the beam hardening and artifacts, improving image quality. The samples were fixed to the micro-CT scanner base with double-sided tape to prevent movement of the samples during rotation. A modified Feldkamp cone-beam with a modified algorithm was used to reconstruct the two-dimensional cross-sectional images, with post-alignment, smoothing, beam hardening, and ring artifact correction (Vaz *et al.*, 2011). Micro-CT images were binarized using the global three-dimensional segmentation method (Kravchenko *et al.*, 2009; Papadopoulos *et al.*, 2009; Vaz *et al.*, 2011).

The quantification of the total pore area, which assesses the amount of macro and mesopores, was performed according to the classification presented by Bullock *et al.* (1985) from 2D images. The “pore shape” parameter was obtained by the method of Cooper *et al.* (2016), where the SPIA software (Soil Pore Image Analysis) was used to classify soil porosity according to size and shape from data obtained by image analysis processes using Noesis Visilog 5.4. The number of images of each casting, species and soil type used for the analyses are shown in supplementary Table 1.

Micro-CT analysis using SPIA software mainly analyzes mesopores and macropores, as the resolution of the scanning is set at 3 μm . The micropore information is lost during the analysis and the choice of parameters that allow better visualization in the software.

The SPIA software classifies porous space based on a combination of shape and size. The size is defined based on the pore area and the equivalent diameter subdivided into classes of diameter. The shapes are defined according to Cooper *et al.* (2016). The pores were classified into three groups: rounded (isolated cavities), elongated (channels and fissures), or complex (chambers and stacking pores). Two indices were used to establish the shape of the pores. The first index (I_1) distinguished rounded pores from elongated and complex pores (Equation 1). A value of 1 for I_1 (Equation 1) defines a perfectly circular pore, and values greater than one indicate that the shape of the pore deviates from circular (Bouma *et al.*, 1977).

$$I_1 = P/4\pi A \quad (1)$$

where P represents the pore area perimeter (μm) and A represents pore area (μm^2). The second index (I_2 - Equation 2), used in conjunction with I_1 , allowed greater precision in separating elongated and complex pores.

$$I_2 = \frac{\frac{1}{m} \sum_i (NI)_i}{\frac{1}{n} \sum_j (DF)_j} \quad (2)$$

where NI represents the number of intercepts of an object in the i direction ($i = 0^\circ, 45^\circ, 90^\circ, 135^\circ$), DF represents Feret's diameter of an object in the j direction ($j = 0^\circ$ and 90°), m represents the number of directions i , and n represents the number of directions j .

Further details on the shape classification method used are provided in Cooper *et al.* (2016). The total pore area (TAP) was calculated as the relationship between the area of all pores, and the image total area (Hallaire & Cointepas, 1993). The TAP value is a function of the image resolution and the area corresponding to a single pixel (Cooper *et al.*, 2016). Values for all soil physical parameters measured (TAP, total number of pores, and number and area of pores of different sizes/shapes) were obtained from the images, and mean values calculated for all images processed per casting, per species and per soil type.

2.4.3 SOIL PARTICLE SIZE ANALYSIS

Particle size analysis was performed on the castings of all species as well as the uningested control soils, with five replicates, following the standard Brazilian method (pipette) described in Embrapa (2017), using sodium hydroxide as a chemical dispersant and slowly stirring for 16 hours.

2.4.4 STATISTICAL ANALYSIS

The experiment was carried out in a completely randomized design in a 2x4 factorial scheme, with the factors tested (2 different soils, Cambisol and Acrisol) and four earthworm species (*R. alatus*, *G. paulistus*, *Glossoscolex* n.sp. and *P. corethrurus*). The experiment was conducted with three replicates, each consisting of a pot with soil with one individual for the larger species (*R. alatus* and *G. paulistus*) and three individuals for the smaller ones (*Glossoscolex* n.sp. and *P. corethrurus*).

To investigate the differences between the casts of each species in the two soils, an analysis of the comparison of means was performed. The data were submitted to the Shapiro-Wilk normality test and ANOVAs were performed considering a factorial

design (4x2) where the effects of ingestion by four earthworm species was analyzed for the two soils with varying texture. Variables with significant differences between casts and control soil ($P < 0.05$) were tested using the post-hoc Tukey test at 5% probability.

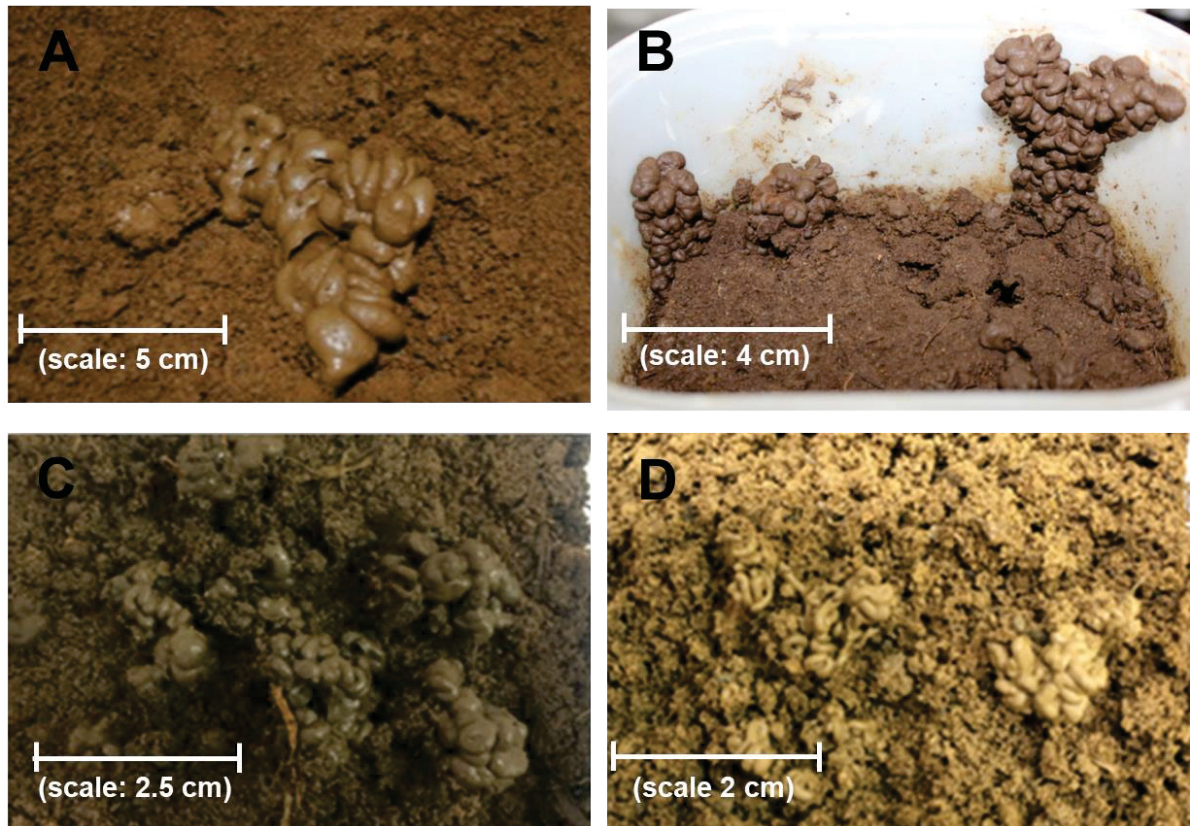
2.5 RESULTS

As previously mentioned, the analyses presented below from the micro-CT images are of mesopores and macropores.

The castings of most earthworm species had different particle size distributions than the original soil (in both soil types) although textural class of the casts of all species were similar, except for those of *Glossoscolex* n.sp. in the silty clay, classified as silty clay loam (Table 1). Casts of this species had much higher silt and much lower clay contents than the control soil, while those of the other three species had slightly higher clay contents, revealing that all species displayed differential ingestion of smaller soil particle sizes in this silty clay. In the sandy loam soil, preferential (higher) ingestion of smaller (clay) particles and lower ingestion of sand was detected in the same three species, but not in *Glossoscolex* n.sp. Higher ingestion of silt particles was also detected for *P. corethrurus* and *R. alatus*. According to Blanchart et al. (1999) all four species could be categorized as compacting earthworm species, due to the formation of globular casts (Figure 2).

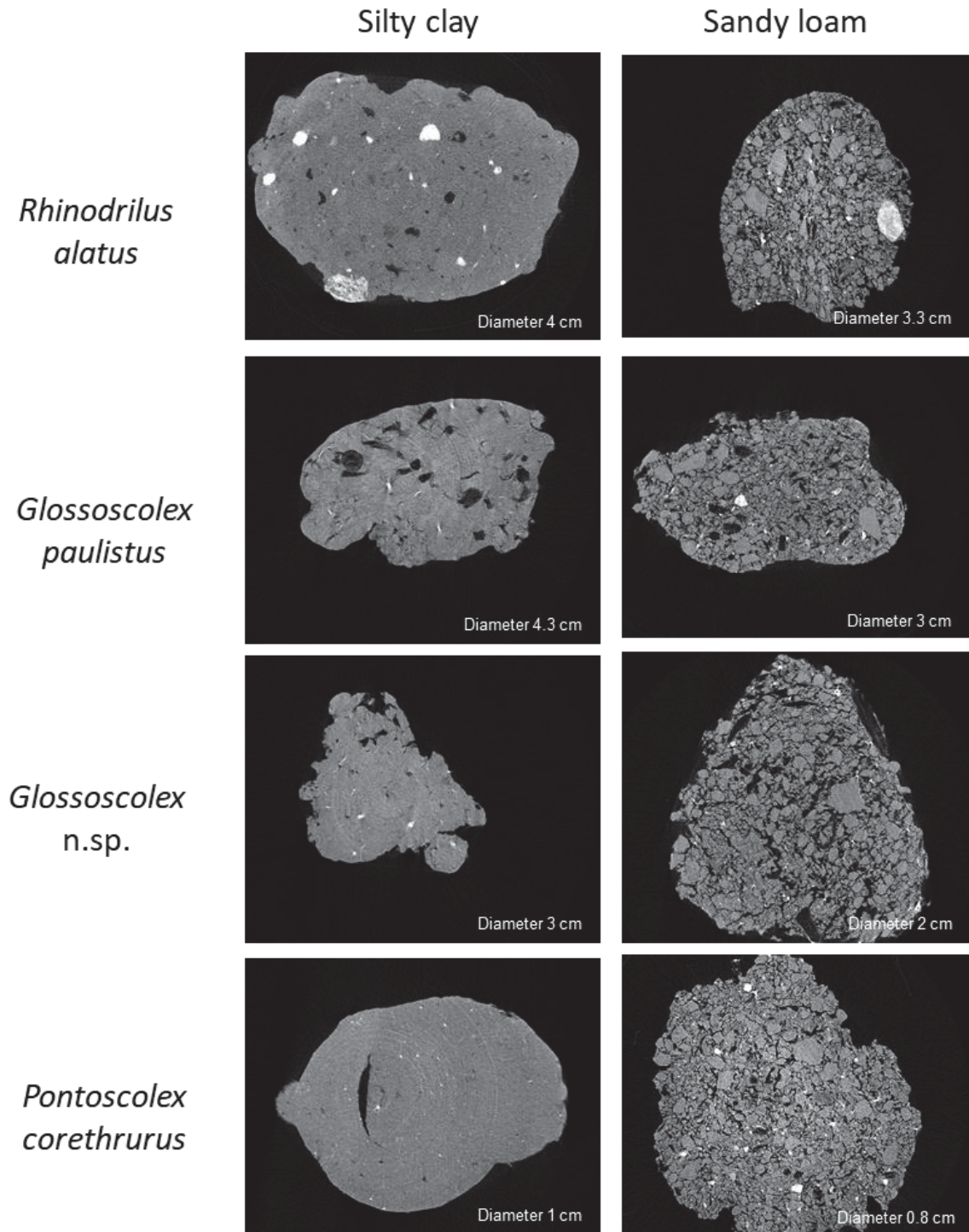
Binarized Micro-CT images of the casts of the four species studied in the two soils are shown in Figure 3. There was a remarkable variability in size of the casts depending on the species (Figure 2) and in their internal structure depending on the initial soil texture (Figure 3). Casts of the larger earthworm species (*R. alatus* and *G. paulistus*) were larger in size, while those of the other two species were smaller.

FIGURE 2. CASTS OF EARTHWORMS SPECIES STUDIED: A - *Rhinodrilus alatus*, B - *Glossoscolex paulistus*, C - *Glossoscolex* n.sp., D - *Pontoscolex corethrurus*.



SOURCE: (A, C, D) Alessandra Santos; (B) Guilherme X. Cardoso (2021).

FIGURE 3. BINARY IMAGES OF A CROSS-SECTION OF THE CASTINGS OF FOUR TROPICAL ENDOGEIC EARTHWORM SPECIES (*R. alatus*, *G. paulistus*, *Glossoscolex* n.sp., *P. corethrurus*) IN TWO SOILS OF CONTRASTING TEXTURE (SILTY CLAY AND SANDY LOAM) OBTAINED BY MICROCOMPUTERIZED TOMOGRAPHY. WHITE = CONSOLIDATED MATERIAL (QUARTZ); BLACK = PORES; GRAY = SOLID MATERIAL. NOTE DIFFERENCES IN THE SIZE OF THE CASTS (DIAMETER OF THE CASTS SHOWN IN BOTTOM LEFT OF EACH FIGURE).



SOURCE: The author (2021).

Soil texture had little effect on the size of the casts of each species, but the casts of all species in the silty clay visibly showed less porosity, while casts formed in the sandy loam displayed many more pores (black spaces) in the cast's matrix (grey color in Figure 3). This resulted in casts with much higher total porosity in the sandy loam (from min. of 8.2 % for *G. paulistus* up to 10.7 % for *Glossoscolex* n.sp.), than the silty clay soil (from min. of 1.2 % for *Glossoscolex* n.sp. up to 5.2 % for *P. corethrurus*), which had much higher clay and lower sand contents (Tables 1 and 2).

TABLE 1. SOIL PARTICLE SIZE DISTRIBUTION IN THE CONTROL SOILS FROM. PARAOPÉBA-MG AND RIO CLARO-SP AND CASTINGS OF FOUR TROPICAL ENDOGEIC EARTHWORM SPECIES CULTIVATED IN BOTH SOILS. SIGNIFICANT DIFFERENCES BETWEEN CAST AND CONTROL PARTICLE SIZES (CLAY, SILT, FINE AND COARSE SAND) ARE HIGHLIGHTED IN BOLD.

Soil	Clay	Silt	Fine sand	Coarse Sand	Textural class (USDA)
%					
Silty clay					
Control	46.2A	50.5B	1.3B	1.9AB	silty clay
<i>R. alatus</i>	45.6A	50.8B	1.4B	2.2B	silty clay
<i>G. paulistus</i>	46.1A	49.3B	1.8A	2.8A	silty clay
<i>Glossoscolex</i> n.sp.	31.8B	65.0A	1.3B	1.8AB	silty clay loam
<i>P. corethrurus</i>	46.7A	50.1B	1.3B	1.9AB	silty clay
Sandy loam					
Control	12.9C	13.3D	36.1A	37.6A	sandy loam
<i>R. alatus</i>	18.7A	20.7B	29.7C	30.9C	sandy loam
<i>G. paulistus</i>	15.4B	22.9A	30.2BC	31.5BC	sandy loam
<i>Glossoscolex</i> n.sp.	13.5C	14.7D	35.2A	36.6A	sandy loam
<i>P. corethrurus</i>	18.8A	18.2C	30.9B	32.2B	sandy loam

SOURCE: The author (2021).

LEGEND: different capital letters represent statistically significant differences between casts and/or control within the same texture.

The total number of pores (Table 2) and their distribution in the three distinct pore classes (rounded, elongated, complex), showed important differences between species and especially soil types (Figure 4). Small pores classified morphologically as rounded and that connect the larger pores (elongated and/or complex) were visible in all casts, but were more prevalent in the sandy loam.

In the silty clay soil, highest rounded porosity was found in *Glossoscolex* n.sp. casts (89% of TAP – total area of pores), followed by *G. paulistus* (79% of TAP).

Elongated pores represented about 89% of TAP in *P. corethrurus* casts, but for the remaining species, only about 10% of TAP. Complex pores were rare, representing only about 3% of TAP, except for *R. alatus* casts where they represented 16% of TAP.

TABLE 2. TOTAL PORE AREA AND TOTAL NUMBER OF PORES (MEAN \pm STANDARD DEVIATION) IN CASTINGS PRODUCED BY FOUR TROPICAL ENDOGEIC EARTHWORM SPECIES IN TWO SOILS OF CONTRASTING TEXTURE (SILTY CLAY AND SANDY LOAM).

Total area of pores TAP (%)							Total number of pores					
Silty clay												
	Mean	sd		Max	Min	CV (%)	Mean	sd		Max	Min	CV (%)
<i>R. alatus</i>	1.81	± 0.9	Bab	2.88	1.13	52	118	± 30.0	Bab	151	92	25
<i>G. paulistus</i>	2.94	± 2.8	Bab	6.14	0.92	95	138 ± 67.0	Ba	211	79	48	
<i>Glossoscolex</i> n.sp.	1.15 ± 0.4	Bb	1.60	0.91	33	46 ± 7.20	Bab	54	40	15		
<i>P. corethrurus</i>	5.16 ± 0.2	Ba	5.30	4.90	4	23 ± 5.10	Bb	28	18	22		
Sandy loam												
	Mean	sd		Max	Min	CV (%)	Mean	sd		Max	Min	CV (%)
<i>R. alatus</i>	8.34	± 1.7	Ans	9.59	6.37	20	1101	± 145.0	Ans	1261	978	13
<i>G. paulistus</i>	8.18	± 2.6	Ans	10.98	5.85	31	983	± 240.8	Ans	1242	766	24
<i>Glossoscolex</i> n.sp.	10.69	± 1.8	Ans	12.70	9.26	16	758	± 356.9	Ans	1164	616	47
<i>P. corethrurus</i>	10.20	± 1.9	Ans	11.89	8.16	18	1442	± 388.6	Ans	1877	1320	26

SOURCE: The author (2021).

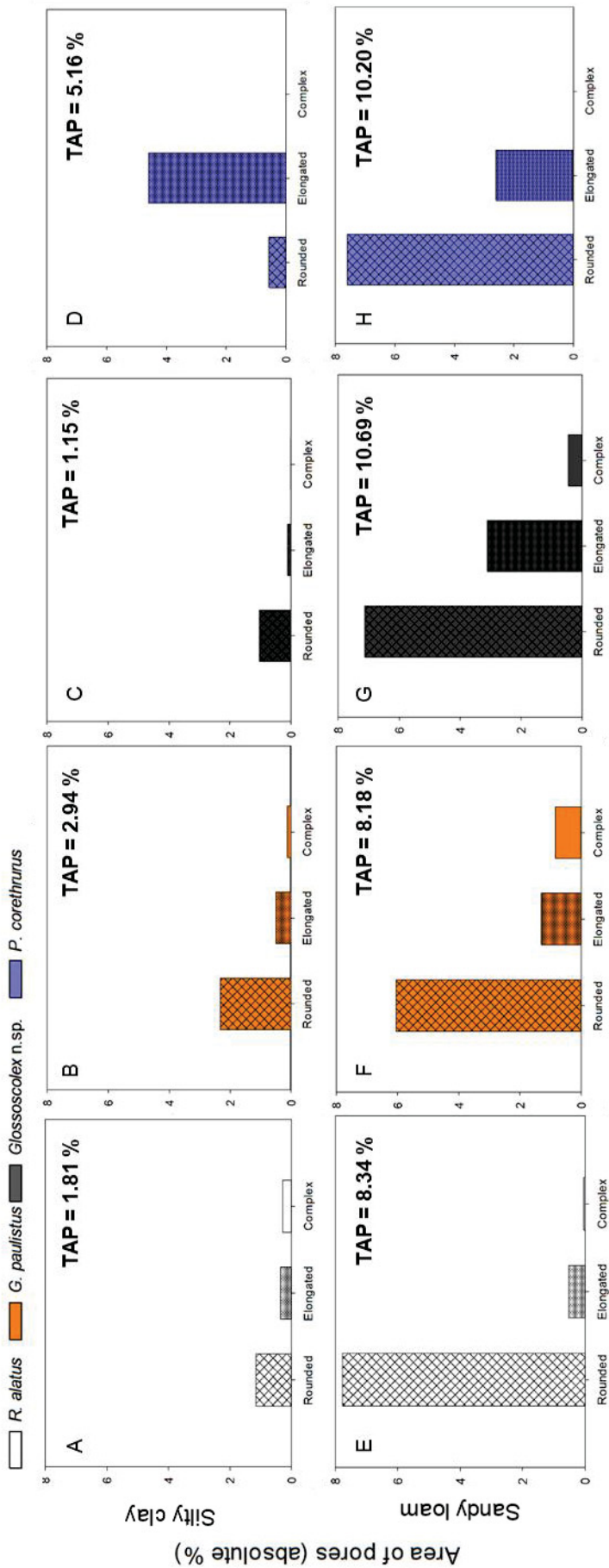
LEGEND: Different capital letters represent significant differences for the same species between the two soil types (texture); **different lower-case letters mean significant differences between the species studied within the same texture.

In the sandy loam soil, rounded porosity was prevalent in the casts of all species, and represented up to 93% of TAP in *R. alatus* casts. Elongated pores represented from 6 (*R. alatus*) to 30% of TAP (*P. corethrurus*), while complex pores were again poorly represented overall and even nonexistent in *P. corethrurus* castings (Figure 4).

For pore diameter, there were clearly visible differences in the distribution of the pore size classes between the two soil types and some differences between species. In the silty clay, *P. corethrurus* casts had pore diameters predominantly in the 100-200 μm class, while *G. paulistus* had casts with predominant pore diameters of 100-200 and 300-500 μm (Figure 5A). For the other two species, proportion of different sized pores was relatively constant, except for the largest pores (>1000 μm), which represented the smallest proportion of total porosity. In the sandy loam, the pore size distribution followed log-normal trend with highest proportion of pores with diameters

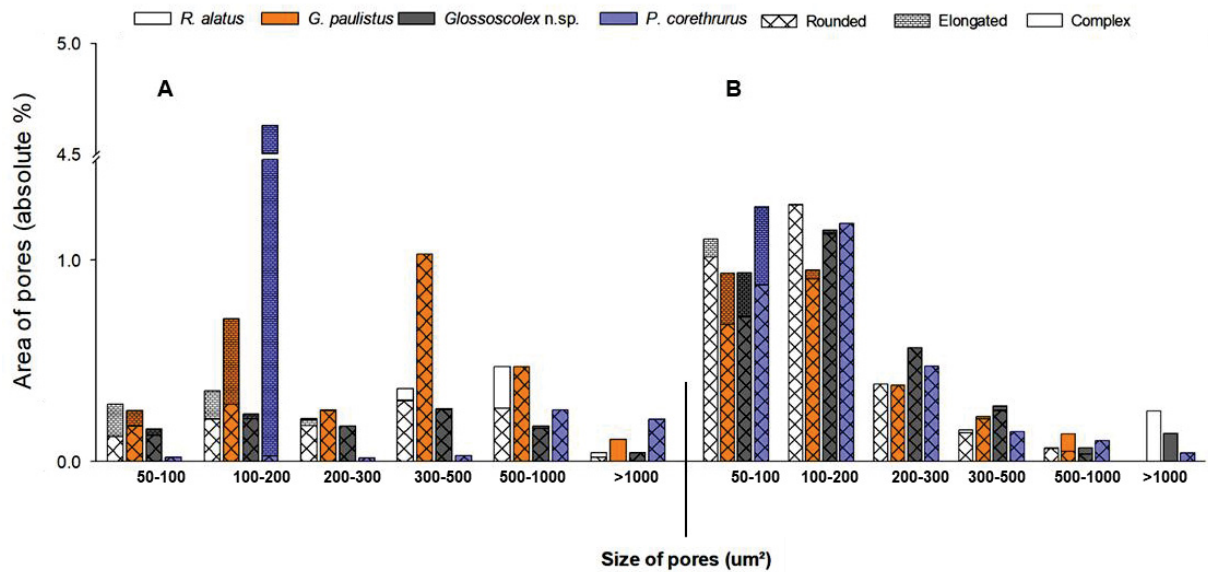
of 50-100 and 100-200 μm , followed by a marked decrease in the area of pores of larger sizes for casts of all species (Figure 5B).

FIGURE 4. TOTAL PORE AREA (TAP, ABSOLUTE %) AND AREA OF PORES OF DIFFERENT SHAPES IN CASTINGS PRODUCED BY FOUR TROPICAL ENDOGEIC SPECIES IN TWO SOILS OF CONTRASTING TEXTURE. A, B, C, D - SILTY CLAY;; E, F, G, H - SANDY LOAM;.: A AND E - *R. alatus*; B AND F - *G. paulistus*; C AND G - *Glossoscolex* n.sp.; D AND H - *P. corethrurus*.



SOURCE: The author (2021).

FIGURE 5. PORE SIZE DISTRIBUTION IN DIFFERENT CLASSES OF SIZE (FROM 50 TO >1000 μm) AND SHAPE (ROUNDED, ELONGATED, AND COMPLEX) IN CASTINGS OF FOUR TROPICAL ENDOGEIC EARTHWORM SPECIES PRODUCED IN TWO SOILS OF CONTRASTING TEXTURE (A = SILTY CLAY AND B = SANDY LOAM).

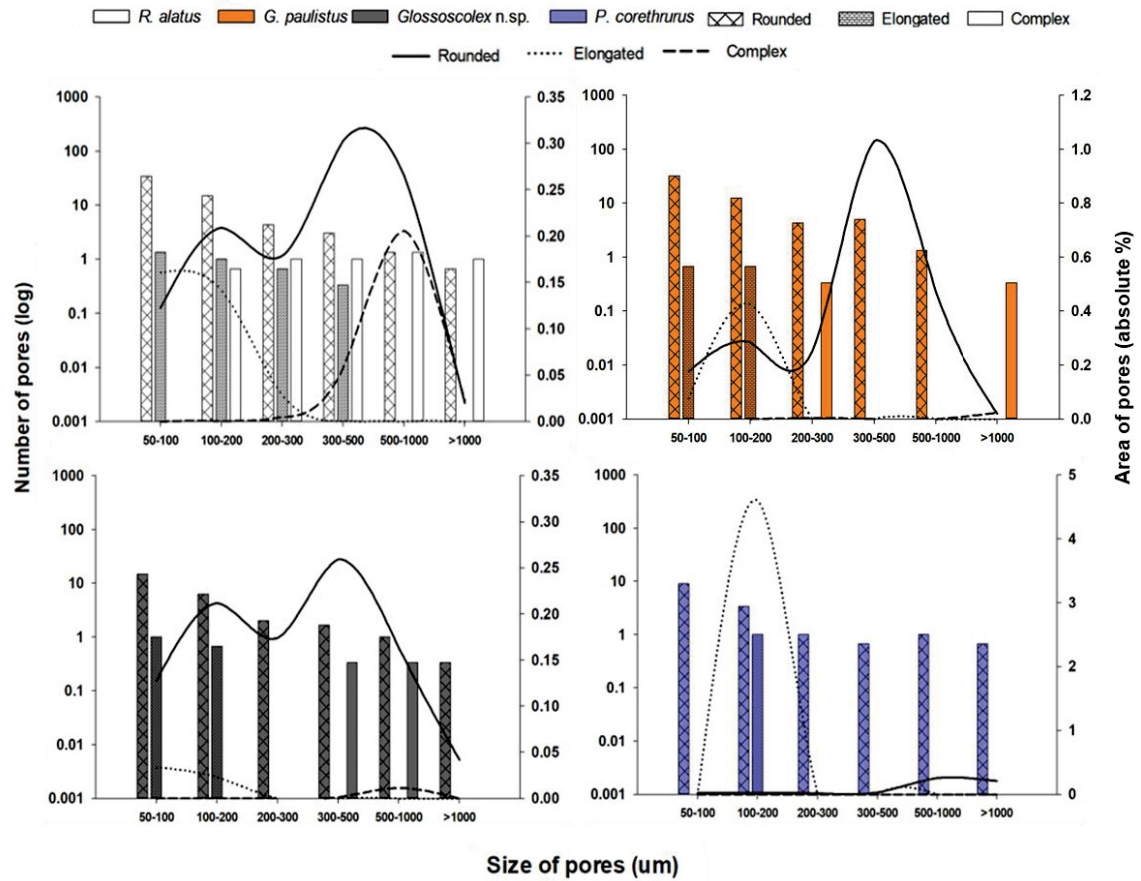


SOURCE: The author (2021).

When the area and number of pores was plotted together for the different size classes (Figure 6 and 7), clear differences between the species became evident. In the silty clay, the species with the highest average number of pores (NP) was *G. paulistus* (NP = 138; Table 2) mainly of the rounded type up to diameters of 1000 μm (Figure 6). For *P. corethrurus*, rounded pores also dominated in all diameter classes, but the presence of a single large elongated pore in the 100-200 μm class resulted in the largest area (Figure 6). In the sandy loam, rounded pores were also dominant in terms of total number, with the highest number of pores for *P. corethrurus* casts (NP = 1442; Table 2). Pores of the other shapes were mostly elongated pores in the smaller diameter classes (< 200 μm) for all species, and complex pores with diameter > 200 μm for all species except *P. corethrurus* (Figure 7). The smaller, rounded pores were the ones most contributing to the TAP.

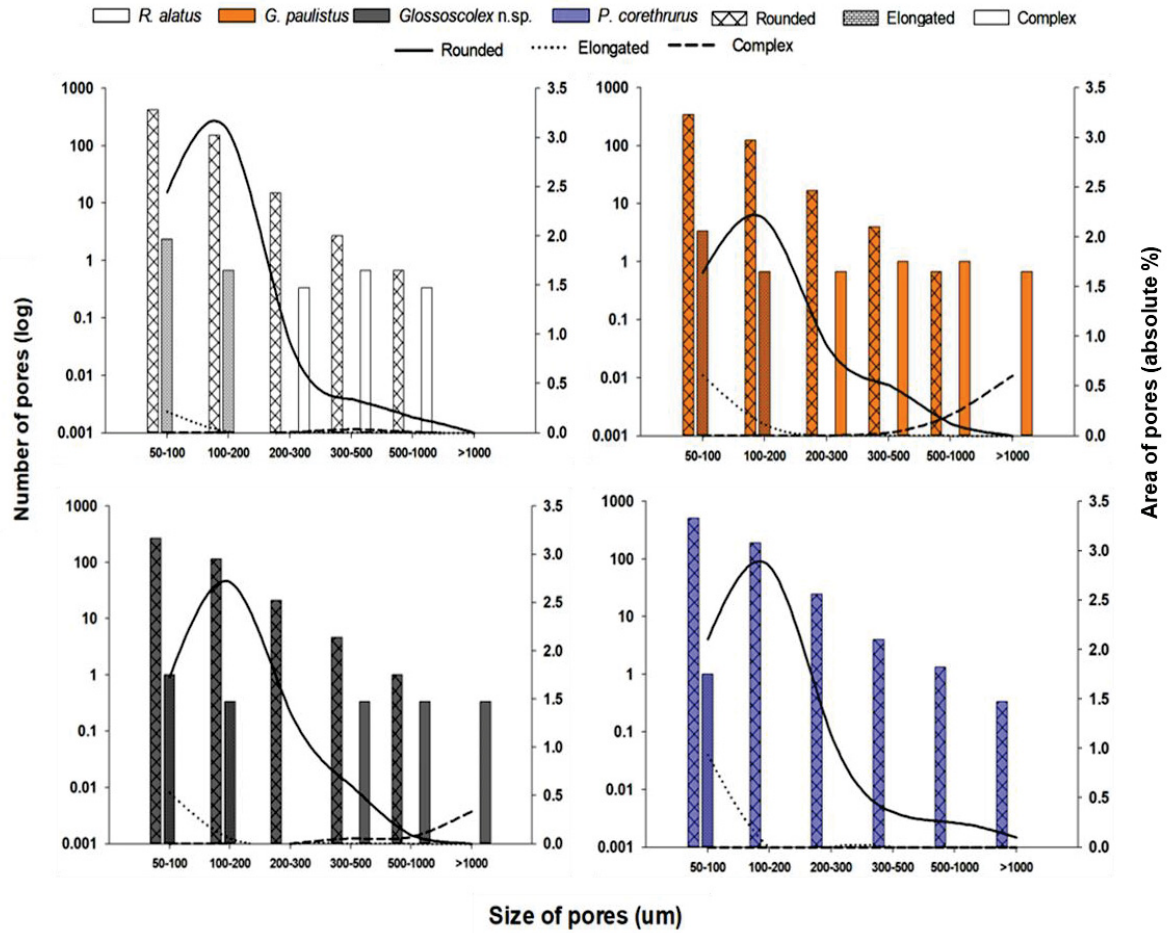
Although *Glossoscolex n.sp.* casts had the highest TAP, the number of pores found was the lowest, probably due to the presence more elongated (50-200 μm) and larger (300>1000 μm) complex pores that increased TAP, or due to a higher connectivity, since higher connectivity between pores increases TAP.

FIGURE 6. NUMBER AND AREA OF PORES IN DIFFERENT CLASSES OF SIZE (from 50 to >1000 μm) AND SHAPE (ROUNDED, ELONGATED, AND COMPLEX) IN CASTS OF FOUR TROPICAL ENDOGEIC EARTHWORM SPECIES FROM THE SILTY CLAY SOIL. * LINES CORRESPOND TO THE PORE AREA AXIS ACCORDING TO EACH SHAPE CLASS.



SOURCE: The author (2021).

FIGURE 7. NUMBER AND AREA OF PORES IN DIFFERENT CLASSES OF SIZE (from 50 to >1000 μm) AND SHAPE (ROUNDED, ELONGATED, AND COMPLEX) IN CASTS OF FOUR TROPICAL ENDOGEIC EARTHWORM SPECIES FROM THE SANDY LOAM SOIL. * LINES CORRESPOND TO THE PORE AREA AXIS ACCORDING TO EACH SHAPE CLASS.



SOURCE: The author (2021).

2.6 DISCUSSION

The internal structure of earthworm casts remains largely undescribed, except for a couple species from temperate (*Dendrodrilus rubidus*, *Aporrectodea chlorotica*) and tropical regions (*P. corethrurus*, *Millsonia anomala*) whose casts have been evaluated using thin slices and electron microscopy (Bal, 1982; Blanchart *et al.*, 1993, 1997, 1999; Decaëns, 2000). Castings of the tropical endogeic species had a thin, external hydrophobic cortex (Blanchart *et al.*, 1999; Decaëns, 2000), which helps protect them from degradation and desiccation. However, as casts age and dry out, the cortex is broken and the internal parts of the cast are exposed to atmospheric conditions and internal porosity tends to increase, particularly the proportion of larger pores (Duboisset, 1995). In the present experiment, the casts used had been dried for 48 h at 40 °C.

Computerized tomography is a highly useful non-destructive technique to view soil structure and porosity (sizes, shapes, distribution), and has been mostly used for intact soil columns (e.g., Jégou *et al.*, 1999; Jassogne *et al.*, 2007; Cnudde & Boone, 2013). Under these conditions, earthworm burrowing (mainly) and casting activity have frequently been shown to affect soil structure, particularly through macropore and gallery formation (e.g., Jégou *et al.*, 2002). However, only recently has micro-CT been used to describe soil aggregates like earthworm casts (Lucas *et al.*, 2020; Le Bayon *et al.*, 2020). In the latter study the authors used this technique mainly to differentiate casts of different species and from aggregates in soil columns without earthworms, primarily based on the aggregate volume, the volume of aggregate grains and the volumetric mass of aggregates. Nonetheless, the type of pores within the casts and their sizes were not evaluated, and this technique has not yet been used for this purpose in earthworm castings.

In the present study micro-CT showed differences in porosity between the casts of four earthworm species produced in two soils of contrasting texture. Casts produced in the silty clay had higher density than those produced in the sandy loam, and therefore, much lower total porosity. Many earthworms selectively feed on particles of different sizes in soils (Barois *et al.*, 1999), and in the present case all four species showed selective ingestion of fine particles. However, *R. alatus*, *G. paulistus* and *P. corethrurus* ingested more clay in both soils, while *Glossoscolex* n.sp. ingested less clay and more silt only in the silty clay soil, resulting in casts with a distinct textural

class compared to the control soil. Larger particles reinforce the gizzard grinding function, while smaller particles tend to have higher C-contents, both of which facilitate the assimilation of nutrients from the ingested soil (Marhan & Scheu, 2005). However, the ingestion of smaller particles, together with the egestion of soil from muscular earthworm species may end up creating compacting casts with lower overall porosity than non-biogenic aggregates (Blanchart *et al.*, 1997).

In the present case, level of compaction and porosity of the casts of all earthworm species appeared to be more dependent on the texture of the original soil than on the ability of the earthworm to create compact casts. Interestingly, *P. corethrurus* is generally considered a highly compacting species, producing casts that are harder than the compact aggregates produced by bulldozing an Amazonian soil (Chauvel *et al.*, 1999). The casts of this species did not show significant differences from those produced by the larger species in both soils used here. Hence, all of the species used here are compacting, though the level of compaction and the ultimate porosity, as well as the internal configuration (type of pores) generated in cast production was highly dependent on the soil texture.

As soil passes through the earthworm gizzard and the intestine, micro-aggregates are broken down and clay platelets are often re-oriented due to intense mixing activities (Barois *et al.*, 1993; Shipitalo & Protz, 1988). In both soils, but particularly in the silty clay, the coalescence of these dispersed soil particles formed casts with low porosity and a predominance of rounded pores. The high cohesion of the soil particles in the casts is due to attraction forces, microbial and earthworm-secreted muco-polysaccharides, and muscular activities of the earthworm upon egesting the soil (Barois & Lavelle, 1986, Lavelle & Spain, 2001; Shipitalo & Le Bayon, 2004).

Rounded pores are considered to have low connectivity, resulting in low water conduction (Souza *et al.*, 2006), being considered transmission pores that are more important for the root growth and penetration in the soil (Pires *et al.*, 2008). The elongated pores found in low quantity in the casts of *R. alatus*, *G. paulistus*, and *Glossoscolex* n.sp. in both soils, are more important when related to the total pore area. Particularly in the casts of *P. corethrurus*, this form represented a higher proportion of total pores and pore area (89%), and this elongated porosity may be due to a morphological characteristic of the casts of these earthworms. The earthworm appears to create a small fold in the casts (an empty space in the formation) when

egesting the cast on the soil surface, which acquires an elongated shape after drying (Figure 2). Although this class is considered important for water movement, the elongated shape may also affect cast stability: the more elongated the pore, the more easily the casts can be broken down.

The complex pores appeared in low quantity in both soils, and were not present in casts of *P. corethrurus*. This form of pores is known to promote greater water movement through the soil profile (Souza *et al.*, 2006; Castilho *et al.*, 2015). In casts, the presence of a cortex with more dense material at the edges may contribute to complex porosity, affecting air and water movement from the soil into and out of the casts, and hence its moisture and O₂ contents. High moisture and low O₂ contents, together with the high inorganic N and microbial activity in earthworm casts has been known to increase greenhouse gas emissions, particularly of N₂O but also CH₄ (Blanchart *et al.*, 1999; Drake & Horn 2007; Santos *et al.*, 2021). For earthworm casts lacking complex porosity, such as those of *P. corethrurus*, O₂ levels may be lower, and drying slower. These phenomena are important determinants of microbial activity as well as physico-chemical reactions in the casts that determine their overall functional roles in soils, and are in dire need of further investigation.

2.7 CONCLUSIONS

The four species of earthworms studied showed differences in the porosity (number and area of pores) and pore structure in the casts. However, soil texture was a more important factor in determining the total pore area, number, and pore morphology of these soils. When compared to the other species studied, *P. corethrurus* casts showed the greatest differences in the number of pores concerning the two soils and also in the classes in which these pores are found, with the silty clay having mostly elongated pores and the sandy loam both rounded and elongated pores, but without any complex pores.

Micro-CT was an efficient means of describing the porosity of biogenic aggregates (earthworm casts) and has high potential to help further reveal the complex and important roles of both texture and earthworm species in determining overall functional impacts, particularly physicochemical reactions, but also microbiological activity of earthworm castings in soils.

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3 CHAPTER II: SOIL TEXTURE AS A DETERMINING FACTOR IN THE PHYSICAL PROPERTIES OF EARTHWORMS CASTS

3.1 RESUMO

A alimentação seletiva das minhocas e a transformação de nutrientes em seus intestinos podem levar a mudanças importantes nas propriedades químicas, físicas e microbiológicas do solo, bem como nos processos do solo em seus coprólitos. No entanto, a estrutura e a porosidade dos coprólitos da maioria das espécies de minhocas, particularmente nos trópicos, são pouco conhecidas. Assim, no presente estudo quantificou-se as mudanças na porosidade total, bem como na distribuição dos poros nos coprólitos de várias espécies de minhocas. Quatro minhocas endogeicas brasileiras (*Rhinodrilus alatus*, *Glossoscolex paulistus*, *Glossoscolex* n.sp. e *Pontoscolex corethrurus*) foram mantidas em dois solos de textura contrastante (argilo siltoso e franco arenoso) e seus coprólitos coletados e analisados por porosimetria de mercúrio e microtomografia computadorizada 3D. A distribuição dos poros nos coprólitos foi muito diferente dependendo da textura original do solo. Os coprólitos no solo argilo siltoso tinham uma distribuição de tamanho de poro mais fina (entre 0,01 e 0,1 μm - microporos), formada principalmente pelo rearranjo de partículas finas. No solo franco arenoso, os coprólitos apresentaram maior variação no tamanho dos poros, dependendo do volume de intrusão de Hg. No entanto, todas as espécies produziram a mesma faixa de tamanho de poros, concentrada em tamanhos de 10 a 100 μm (mesoporos). Portanto, embora diferentes espécies de minhocas produzam coprólitos de tamanhos diferentes, é principalmente a textura do solo que afeta os tamanhos e distribuição dos poros nesses macroagregados biológicos (coprólitos).

Palavras-chave: Minhocuçus. Porosidade. Oligochaeta.

3.2 ABSTRACT

The selective feeding of earthworms and the transformation of nutrients in their intestines can lead to important changes in soil chemical, physical and microbiological properties, as well as soil processes in earthworm casts. However, the structure and porosity of the casts of most earthworm species, particularly in the tropics are poorly known. Hence, the present study quantified the changes in total porosity and pore distribution in casts of several earthworm species. Four Brazilian endogeic earthworms (*Rhinodrilus alatus*, *Glossoscolex paulistus*, *Glossoscolex* n.sp. and *Pontoscolex corethrurus*) were kept in two soils of contrasting texture (A - silty clay and B - sandy loam) and their casts collected and analysed using mercury porosimetry and 3D computed microtomography. The distribution of pores in the casts was very different depending on the original soil texture. Casts in the silty clay had a finer pore size distribution (between 0.01 and 0.1 μm - micropores) formed mainly by the rearrangement of fine particles. In the sandy loam, casts showed a wider variation in pore size, depending on Hg intrusion volume. However, all species produced the same pore size range, concentrated in sizes from 10 to 100 μm (mesopores). Therefore, although different species of earthworms produce casts of different sizes, it is mainly soil texture that affects pore size and pore distribution in these biological macroaggregates (casts).

Keywords: Earthworms. Porosity. Oligochaeta.

3.3 INTRODUCTION

Earthworms significantly influence soil structure, its development and maintenance (Jouquet *et al.*, 2008; Lavelle, 2011) and are generally recognized as good indicators of soil quality (Birkás *et al.*, 2004; McKenzie *et al.*, 2011). For this reason, they are commonly identified as the most important engineers in the soil ecosystem (Lavelle, 2011).

The effect of earthworms on soil structure is mainly due to the ingestion and mixing of particles and organic matter, which are then egested in the form of casts. The daily cast production by an earthworm can be dozens of times greater than its individual body weight (Shipitalo & Le Bayon, 2004; Bottinelli *et al.*, 2010). Juveniles and smaller individuals tend to ingest more soil and produce more casts, while adults and larger species tend to cast proportionately less per unit biomass (Barois *et al.*, 1999). However, proportions are highly variable, ranging from less than 1 times up to >30 times their body weight per day, depending on the soil nutrient (especially organic matter) content, bulk density, temperature and moisture, as well as earthworm species and age (Barois *et al.*, 1999; Perreault & Whalen, 2006; Blanchart *et al.*, 2009). Nonetheless, with these high levels of cast production, earthworms can deeply affect soil aggregation and structure (Blanchart *et al.*, 2004, 2009), particularly when they are abundant and active.

Soil porosity is a complex interconnected network of open spaces in a dense, solid matrix, responsible for the conduction of water, air and solutes, and the maintenance of roots and microbiota. Under unsaturated soil conditions, most water and nutrient flows occur in the intra-aggregate pores (Ben-Hur *et al.*, 2009; Peth *et al.*, 2010). The structure of intra-aggregate pores is highly correlated with the soil's hydraulic properties (Eynard *et al.*, 2006), which influences both solute movement and aggregate stability (Horn, 2004; Goebel *et al.*, 2011). However, aggregate stability can also be influenced by the degree of water repellency associated with organic matter and water contents and pore size distribution (Papadopoulos *et al.*, 2009; Lipiec *et al.*, 2009; Vogelmann *et al.*, 2013; Stoffregen & Wessolek, 2014).

The soil structure is defined as how the primary soil particles (sand, silt, and clay) are combined and arranged with other solid soil components to form agglomerates or

aggregates. Aggregates are formed by fixing mineral particles with organic and inorganic substances, and the dynamics of this complex process is the result of many interacting factors, including the environment, soil management, plant activity, soil formation, and soil properties such as mineral composition, texture, organic carbon concentration, microbial and fauna activities (especially bioturbation), chemistry, nutrient reserves and moisture.

The biological aggregation on the soil surface, consists mainly of biogenic aggregates that greatly influence water movement and gas diffusion into the soil (Capowiez *et al.*, 2006; Gerke, 2006; Alaoui *et al.*, 2011). Hence, the study of the biological formation processes as well as the physical characteristics of these biological aggregates is important in order to understand their contribution to soil functioning, particularly water and gas exchanges, but also microbial processes.

In this regard, imaging techniques such as X-ray computed tomography (CT) or photographs of soil sections impregnated with resin (micromorphology) provide a means of examining soil structure and porosity, as well as quantification of pore area, volume, size, shape characteristics and location in the soil matrix (Vogel & Roth, 1998; Cnudde & Boone, 2013). This is particularly useful for measuring pore area and size, and for assessing changes induced by natural (pedogenetic), anthropic (Jassogne *et al.*, 2007; Cássaro *et al.*, 2017) or biological agents (Jégou *et al.*, 1999, 2002; Blanchart *et al.*, 1993, 1997). Mercury porosimetry is another useful technique for describing the pore sizes in an aggregate or a soil clod (Auvinet & Bouvard, 1989), but has been little used so far on biogenic aggregates such as earthworm castings (Blanchart *et al.*, 1993; Chauvel *et al.*, 1999; Barros *et al.*, 2004).

Considering the fact that earthworm activities are highly variable, depending on environmental conditions (Eijsackers, 2011; Caro *et al.*, 2012) and species, the present study was undertaken to quantify the changes in porosity and pore distribution and area in earthworm casts of different species.

3.4 MATERIAL AND METHODS

3.4.1 SOILS AND SPECIES USED

Two soils with contrasting texture were collected: the first was a silty clay Cambisol (4% sand, 44% clay, and 52% silt) from native Cerrado vegetation at the

National Forest of Paraopeba (19°15'17.2"S, 44°24'04.9"W, altitude 725 m) and the second was a sandy loam Acrisol (74% sand, 13% clay and 13% silt) from a cattle pasture at the Fazenda Santa Rosa, in Assistência district (22°30'35.7"S, 47°36'42.1"W, altitude 515 m), near Rio Claro – SP.

Four Brazilian endogeic earthworm species were used: *Rhinodrilus alatus* Righi 1971, *Glossoscolex* n.sp. (new species still undescribed), *Glossoscolex paulistus* Michaelsen 1926 and *Pontoscolex corethrurus* (Müller, 1857).

The experiment was carried out in the dark, in an incubator in an air-conditioned (22 ±1°C) laboratory (Full Gauge Imperial III Incubator), at Embrapa Forestry in Colombo-PR, Brazil. Five *R. alatus* or *G. paulistus* were placed individually into five 24 x 17 x 10 cm containers (plastic boxes with a lid). Due to their smaller size, three individuals of *Glossoscolex* n.sp. and *P. corethrurus* were added to each box (n=5 replicates also). Due to the different size of the species used, 500 g of soil were used to incubate *R. alatus* and *G. paulistus*, 100 g for *P. corethrurus*, and 250 g for *Glossoscolex* n.sp., corrected to 70% container capacity. The casts produced within 24 hours by each earthworm species were collected and dried in an oven at 40 °C, and the experiment conducted separately for each of the two soil types.

Particle size analysis of the casts (see Chapter I), revealed that the castings of all earthworm species had a higher percentage of finer particles, particularly of clay in casts of *R. alatus*, *G. paulistus* and *P. corethrurus*, and of silt in the casts of *Glossoscolex* n.sp. (see Table 1; Chapter I).

3.4.2 MICRO-CT ANALYSIS

For each earthworm species and soil type, three individual casts were analyzed by X-ray micro-CT, for a total of 24 samples. Each cast was air-dried and placed at the end of a small carbon rod before being digitized. Micro-CT images were acquired with a SkyScan micro-CT X-ray scanner, model 1172. The scanning protocol and scanner parameters were identical for all samples, with the X-ray tube operating at 100 kV and 100 mA, and using an aluminum/copper filter (0.5/0.04 mm thickness) positioned between the sample and the detector to block smooth X-rays and reduce the hardening of the beam and artifacts, improving image quality. A modified Feldkamp conical beam algorithm was used to reconstruct the two-dimensional transversal images, with processes of post-alignment, smoothing, beam hardening and correction of ring

artifacts (Vaz *et al.*, 2011). A total of 383-800 images were used for *R. alatus* casts, 299-941 images for *G. paulistus* casts, 371-604 images for *Glossoscolex* n.sp. casts, and 175-409 images for *P. corethrurus* casts (Table 3). The images were binarized using the global three-dimensional segmentation method (Kravchenko *et al.*, 2009; Papadopoulos *et al.*, 2009; Vaz *et al.*, 2011).

TABLE 3. PARAMETERS USED FOR THE BINARIZATION OF THE MICRO-CT IMAGES USING THE CTANALYSER SOFTWARE.

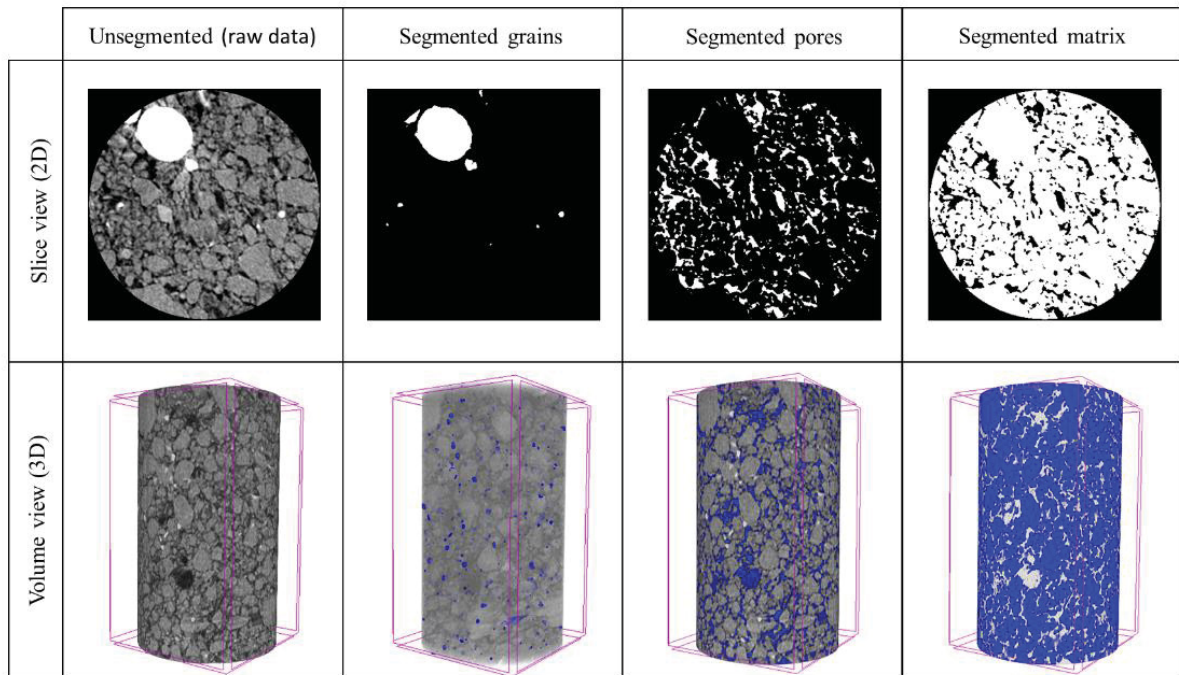
Soil type	Species	Casts	Number of slices	Lower grey threshold	Upper grey threshold	Lower vertical position	Upper vertical position	Pixel size
			n°	µm				
Silty clay	<i>Rhinodrilus alatus</i>	1	643	66	255	1181	6237	7.87
		2	422	69	255	1206	4594	8.04
		3	800	66	255	1201	8315	8.90
	<i>Glossoscolex paulistus</i>	1	369	85	255	7168	11383	11.45
		2	941	70	255	424	7814	7.86
		3	732	74	255	403	5399	6.83
	<i>Glossoscolex n. sp.</i>	1	327	90	255	2064	4292	6.83
		2	498	81	255	500	2963	4.95
		3	405	88	255	1104	3106	4.95
	<i>Pontoscolex corethrurus</i>	1	371	75	255	1709	3542	4.95
		2	175	89	255	961	1823	4.95
		3	425	76	255	218	2319	4.95
Sandy loam	<i>Rhinodrilus alatus</i>	1	509	60	255	444	3916	6.83
		2	485	50	255	524	4825	8.88
		3	383	66	255	542	3937	8.88
	<i>Glossoscolex paulistus</i>	1	670	51	255	376	4956	6.84
		2	348	61	255	2284	5016	7.87
		3	938	67	255	376	6791	6.84
	<i>Glossoscolex n. sp.</i>	1	299	54	255	1157	3197	6.84
		2	604	57	255	1177	4377	5.30
		3	416	36	255	862	2425	3.76
	<i>Pontoscolex corethrurus</i>	1	409	63	255	205	1878	4.10
		2	198	54	255	631	1574	4.78
		3	302	57	255	258	1698	4.78

SOURCE: The author (2021).

The casts were segmented by limit intensity to differentiate the components that were visually identified in the images: (a) the elements of high attenuation were interpreted as mineral grains of high density, (b) elements of low attenuation were interpreted as pores and (c) intermediate attenuation elements were interpreted as an

undifferentiated matrix of the aggregates. An example of this segmentation is shown in Figure 8 for an *R. alatus* cast in the sandy loam soil.

FIGURE 8. EXAMPLE OF SEGMENTATION INTO THREE COMPONENTS: GRAINS, PORES AND UNDIFFERENTIATED MATRIX FOR AN EARTHWORM CAST OF *Rhinodrilus alatus* IN THE SANDY LOAM SOIL. ABOVE IN 2D (SELECTED SLICE 690 OF ROI 1), THE SEGMENTED SURFACES IN BLUE REPRESENT 15% FOR GRAINS, 30% FOR PORES AND 55% FOR UNDIFFERENTIATED MATRIX, FOR THE ATTENUATION COEFFICIENT.



SOURCE: The author (2021).

From the creation of the 3D model using the CTAnalyser software, we aimed for the best relationships between parameters for each micro-CT section in the image, using the values detailed in Table 3.

Total porosity and the amount of macro and mesopores were quantified following the classification of Bullock *et al.* (1985). Pore shape categories followed the classification of Cooper *et al.* (2010), which classifies pores as rounded (isolated channels and holes), elongated (fissures), or complex (empty). The porosity of the sample was calculated through digital image analysis (Cooper *et al.*, 2005; Juhász *et al.*, 2007) using Noesis Visilog 5.4 and the SPIA software (Soil Pore Image Analysis) to classify pores according to size and shape. Pore volume and percentage of total porosity were calculated using the average of the three replicates from the 3D reconstructions based on micro-CT, using the software CTAnalyzer, CTvol 3D Visualization and CTvox 3.0.

3.4.3 POROSIMETRY BY MERCURY INTRUSION

Mercury is a non-wetting liquid with a high surface tension value and contact angle and needs to be pressurized to be introduced into soils (van Brakel *et al.*, 1981). According to the capillary or Washburn Equation (Eq.1), the pressure P necessary for mercury to invade a cylindrical capillary of radius r is:

$$P = (2\sigma_{Hg} \cos\beta_{Hg})/r \quad (1)$$

where σ_{Hg} is the surface tension of mercury and β_{Hg} is the contact angle between mercury and the capillary wall.

The determination of porosimetry by mercury intrusion was performed using an AutoPore IV-Micromeritics equipment, according to the procedure described by the ISO15901-1/2005 standard (ISO, 2005). The measurements were made with a gradual reading for each pressure applied (stepwise mode), using the following parameters: Hg density = 13.53 g dm⁻³, Hg surface tension (γ) = 485.0 dyn cm⁻¹, Hg contact angle (ϕ) = 130.0 deg, fill pressure = 0.51 psi, maximum intrusion pressure = 40.000 psi, and balance time = 10 s.

3.4.4 STATISTICAL ANALYSIS

The experiment was carried out in a completely randomized design in a 2 x 4 factorial scheme, with the factors tested (2 different soils: Cambisol and Acrisol) and four earthworm species (*R. alatus*, *G. paulistus*, *Glossoscolex* n.sp. and *P. corethrurus*), conducted using three replicates, each consisting of a pot with soil with one individual for the larger species (*R. alatus* and *G. paulistus*) and three individuals for the smaller ones (*Glossoscolex* n.sp. and *P. corethrurus*).

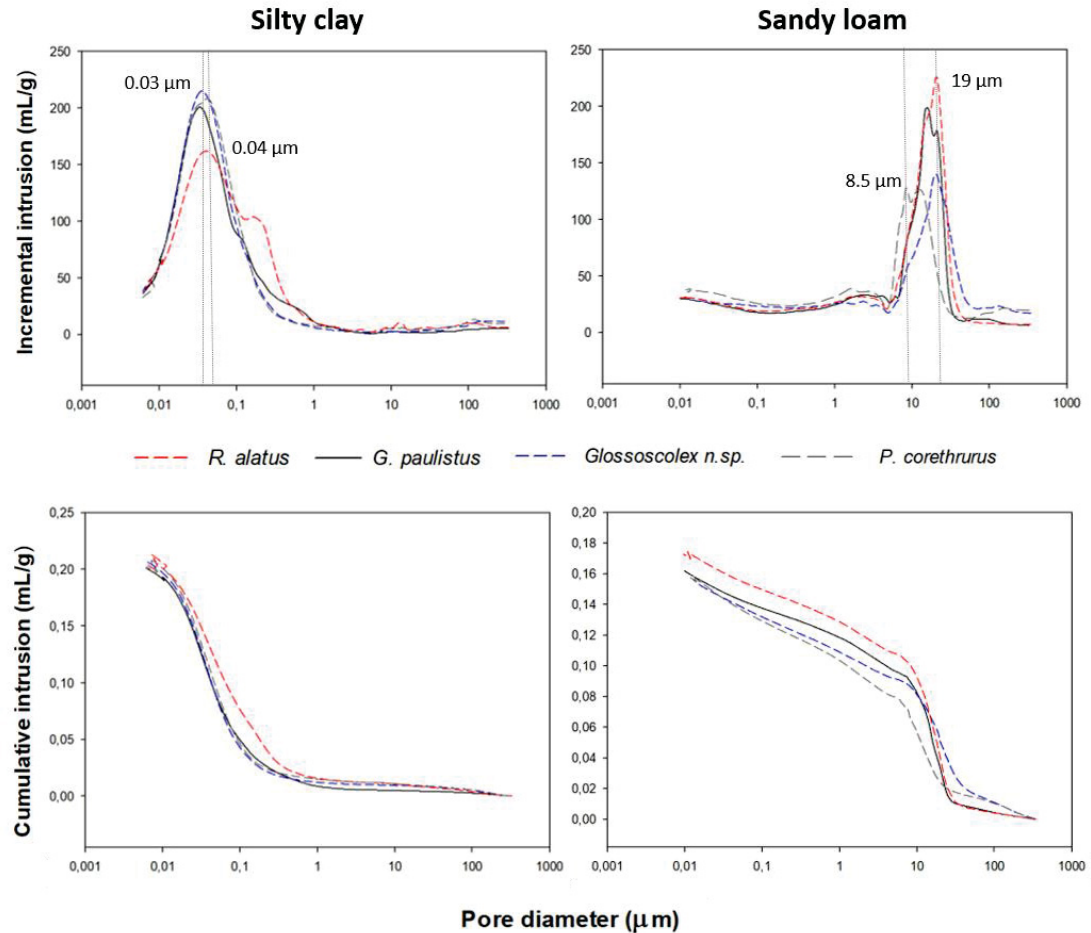
The data were submitted to the Shapiro-Wilk normality test and ANOVAs were performed considering a factorial design (4x2) where the effect of the four earthworm species was analyzed on the two soils with different texture. Factors with a significant P value (<0.05) were tested using the post-hoc Tukey test at 5% probability. For the porosimetry measurements, derived and accumulated values of mercury intrusion were calculated.

A Principal Component Analysis (PCA) was performed using the pore volume values (mm^3) (total, open, and closed) and the significance of the PCA model was assessed using Monte Carlo permutations ($P < 0.05$), with the ADE-4 package for R (Dray & Dufour, 2007).

3.5 RESULTS

The pore size showed a different distribution between the two soils with different texture (Figure 9). Although both displayed a unimodal pore size distribution, in the silty clay most of the intrusion volume (around 150 to 212 mL g^{-1}) was into very fine pores ranging in size from 0.01 to 0.1 μm (micropores), formed mainly by the arrangement of fine particles. Pore sizes of the species tended to be similar (one main peak in the incremental intrusion – the highest value for *G. paulistus* was 0.03 μm), but for *R. alatus* there was also a small peak (about 100 mL g^{-1}) representing a pore volume varying in size from 0.1 to 1 μm (with the peak being at 0.04 μm), which may correspond to small crack cavities in the cast. On the other hand, in the sandy loam, around half of the Hg intrusion occurred into larger pores with size from around 10-100 μm diameter (with peaks at 8.5 μm for *P. corethrurus* and 19 μm for *R. alatus*), and the incremental volume was more variable (125-224 mL g^{-1}), indicating higher variability in pore size of the casts of different species. Pore sizes of the casts of the smaller species (*P. corethrurus* and *Glossoscolex n.sp.*), tended to be smaller than those of the larger *R. alatus* and *G. paulistus*.

FIGURE 9. INCREMENTAL AND CUMULATIVE MERCURY INTRUSION POROSIMETRY IN CASTS OF FOUR TROPICAL ENDOGEIC SPECIES (*Rhinodrilus alatus*, *Glossoscolex paulistus*, *Glossoscolex n.sp.*, *Pontoscolex corethrurus*) IN TWO SOILS OF CONTRASTING TEXTURE (OVEN-DRIED SAMPLES PRE-CONSOLIDATED AT 175 KPA).



SOURCE: The author (2021).

Casts deposited in the silty clay had between 1.2% (*P. corethrurus*) and 5.5% (*G. paulistus*) total porosity, though the highest total pore volume was for *R. alatus* (6.90 mm³), closely followed by *G. paulistus* (6.87 mm³) (Table 4; Figure 10). Although differences were not significant, both smaller species (*Glossoscolex n.sp.* and *P. corethrurus*) had smaller total pore volumes (0.23 and 0.09 mm³, respectively), and smaller total cast porosity (1.2 and 1.6%), compared to the two larger species. The larger species had pore volumes that were around 75 and 30 times larger than those found in *P. corethrurus* and *Glossoscolex n.sp.* casts. Most of the pores were of the open type (more connected to each other) for *G. paulistus*, while most were of the closed type for *P. corethrurus*, while the other two species showed little difference in the proportion of these two types of pores. The only type of pore that displayed

significant differences between the species was the closed porosity, which was significantly higher in *R. alatus* casts than in the two smallest species (Table 4).

TABLE 4. VOLUME AND PERCENTAGE OF TOTAL POROSITY, CLOSED AND OPEN PORES, AND EULER NUMBER IN CASTS OF FOUR TROPICAL ENDOGEIC SPECIES (*Rhinodrilus alatus*, *Glossoscolex paulistus*, *Glossoscolex* n. sp., *Pontoscolex corethrurus*) IN TWO SOILS WITH CONTRASTING TEXTURE.

Soil	Species	Closed		Open		Total		Closed		Open		Total		Nº Euler
		-----mm ³ -----						-----%-----						
Silty Clay	<i>R. alatus</i>	3.31	Aa	3.58	ns	6.90	ns	1.72	ns	1.54	ns	3.23	ns	161424
	<i>G. paulistus</i>	2.35	ab	4.51	ns	6.87	ns	1.25	ns	4.32	ns	5.53	ns	149586
	<i>Glossoscolex</i> n. sp.	0.10	b	0.12	ns	0.23	ns	0.73	ns	0.91	ns	1.63	ns	21912
	<i>P. corethrurus</i>	0.07	b	0.02	B	0.09	B	0.85	ns	0.32	ns	1.16	ns	2702
Sandy Loam	<i>R. alatus</i>	0.50	B	7.52	ns	8.02	ns	0.89	ns	13.48	ns	14.28	ns	2693
	<i>G. paulistus</i>	0.54	ns	14.59	ns	15.13	ns	0.72	ns	14.42	ns	15.05	ns	24543
	<i>Glossoscolex</i> n. sp.	0.08	ns	7.68	ns	7.76	ns	0.28	ns	20.44	ns	20.67	ns	-31149
	<i>P. corethrurus</i>	0.03	ns	1.46	A	1.50	A	0.51	ns	16.93	ns	17.36	ns	4452

SOURCE: The author (2021).

LEGEND: Upper-case letters represent significant differences for the same species between soil types. Lower case letters represent significant differences between species within each soil type.

In the sandy loam, total cast porosity was much higher, ranging from around 14% for *R. alatus* casts to over 20% in *Glossoscolex* n.sp. casts (Table 4, Figure 10). In this soil, open porosity represented the vast majority of total porosity in the casts of all species. The largest total pore volume was seen in *G. paulistus* (15.13 mm³) casts, that had 10 times more pore volume than those of *P. corethrurus*, though differences between the species were not significant. In this soil, *P. corethrurus* casts had significantly ($p < 0.05$) higher total and open pore volume than in the silty clay soil. On the other hand, closed pore volume was significantly lower in the sandy loam than the silty clay soil in casts of *R. alatus* (Table 4). In fact, both soils have very different overall mean total porosity, being around 2.9 % in casts produced in silty clay and 16.8 % (almost 6 times higher) in casts produced in the sandy loam.

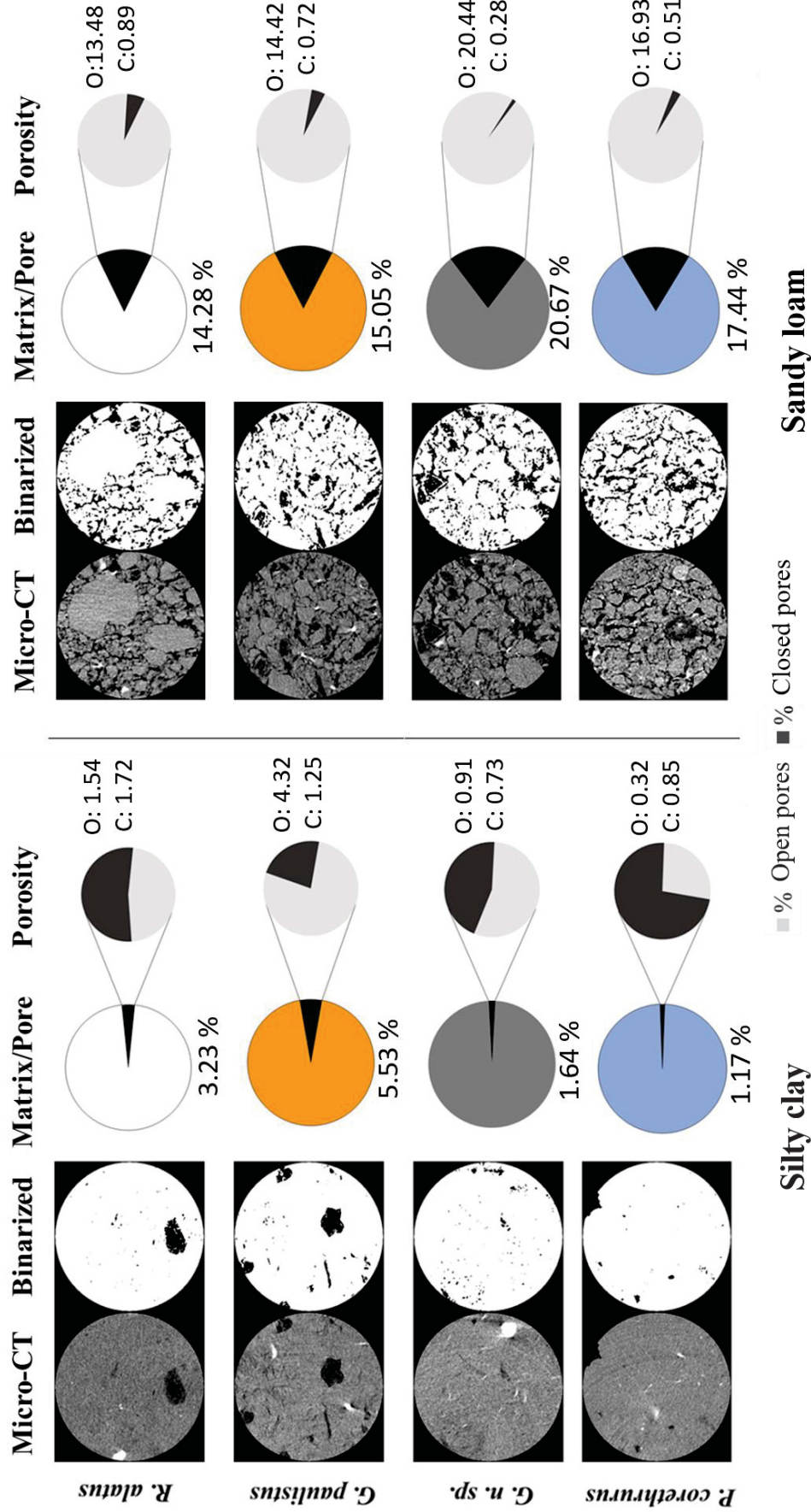
The connectivity function determined by the specific Euler number, provides information on pore connectivity within and between different classes of pores. When removing pore classes by size, from the smallest to the largest, connectivity gradually decreases (the Euler number increases) until a disconnected state is reached (the Euler number becomes positive). With this, the Euler number becomes a dimensionless indicator of how a pore is connected to other pores: smaller (or more

negative) Euler numbers mean greater pore connectivity (Brabant *et al.*, 2011; Wildenschild & Sheppard, 2013). A negative Euler number was observed only in *Glossocolex* n.sp. casts in the sandy loam soil (Table 4), indicating that these casts have a higher degree of connectivity between pores.

The 3-D reconstruction of the porous network within the casts of each earthworm species showed that the casts of *G. paulistus* in the silty clay had the densest network, followed by the casts of *R. alatus* (Figure 11). These images also reveal the differences between open porosity (more connected to each other) and closed porosity, with greater representation in the casts of *Glossoscolex* n.sp. and *P. corethrurus* that were smaller in size. In the sandy loam, the porous network of all species was relatively similar, and differences were more subtle and difficult to visualize.

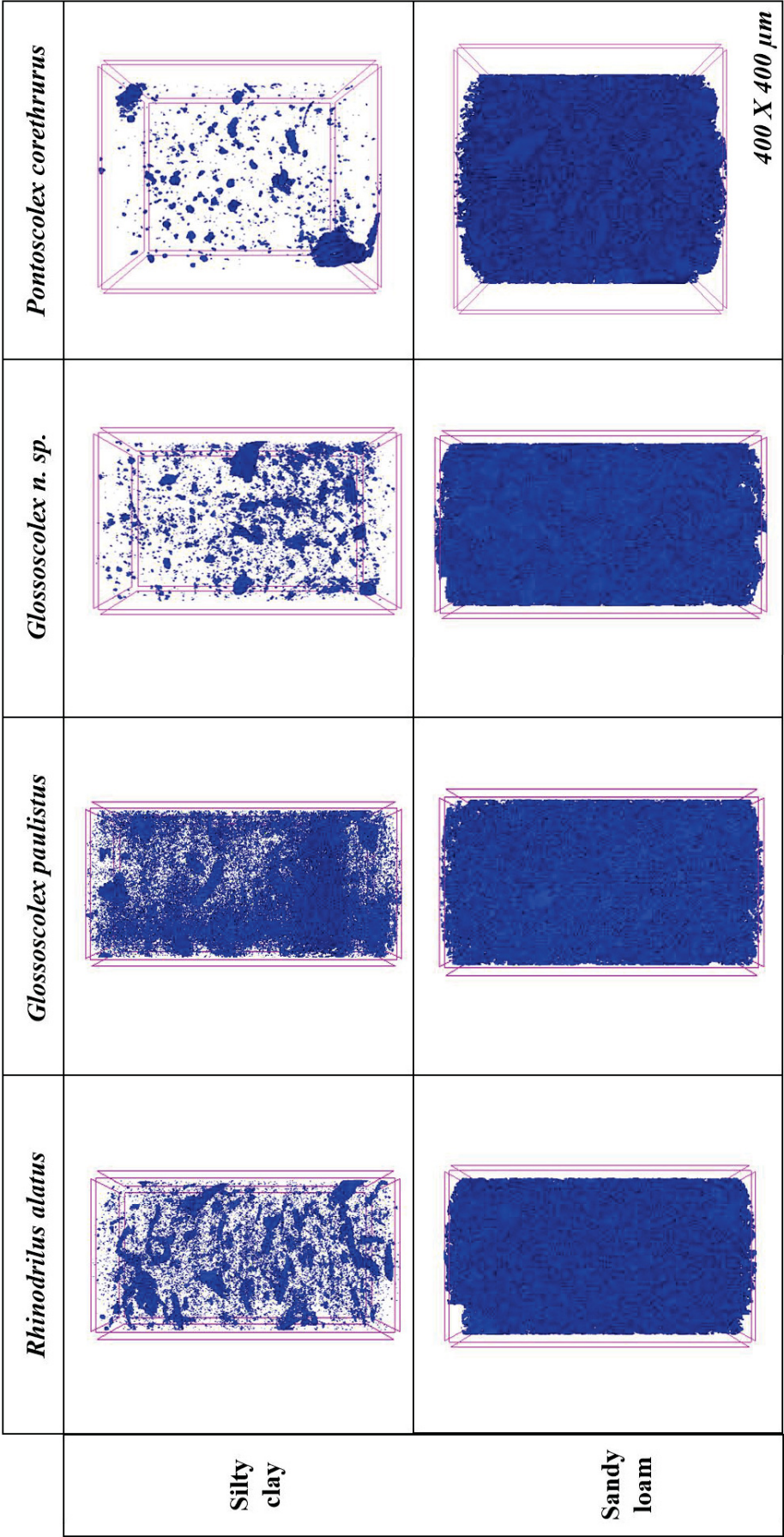
The PCA (Figure 12), highlighted the important role of both soil texture and earthworm species in influencing total and open porosity of the casts. The first axis (PC1) explained 67% of the data variance, and was closely related to TV (total volume) and OP (open porosity volume) of the casts, both of which were higher in the sandy loam soil. Nonetheless, the second axis (PC2) explained about 33% of the data and was closely related to cast CP (closed porosity volume) and the earthworm species that produced the casts.

FIGURE 10. PERCENTAGE TOTAL, OPEN AND CLOSED POROSITY IN CASTS OF FOUR TROPICAL ENDOGEIC SPECIES (*Rhinodrilus alatus*, *Glossoscolex paulistus*, *Glossoscolex n.sp.*, *Pontoscolex corethrurus*) IN TWO SOILS OF CONTRASTING TEXTURE, THROUGH 3D ANALYSIS OF MICRO-CT. MICRO-CT = CT IMAGE; BINARIZED = BINARIZED IMAGE; BLACK COLOR IN IMAGE = PORES; MATRIX/PORE = PERCENTAGE OF SOLIDS AND PORES (BLACK); POROSITY = PERCENTAGE OF OPEN (O) AND CLOSED (C) PORES.



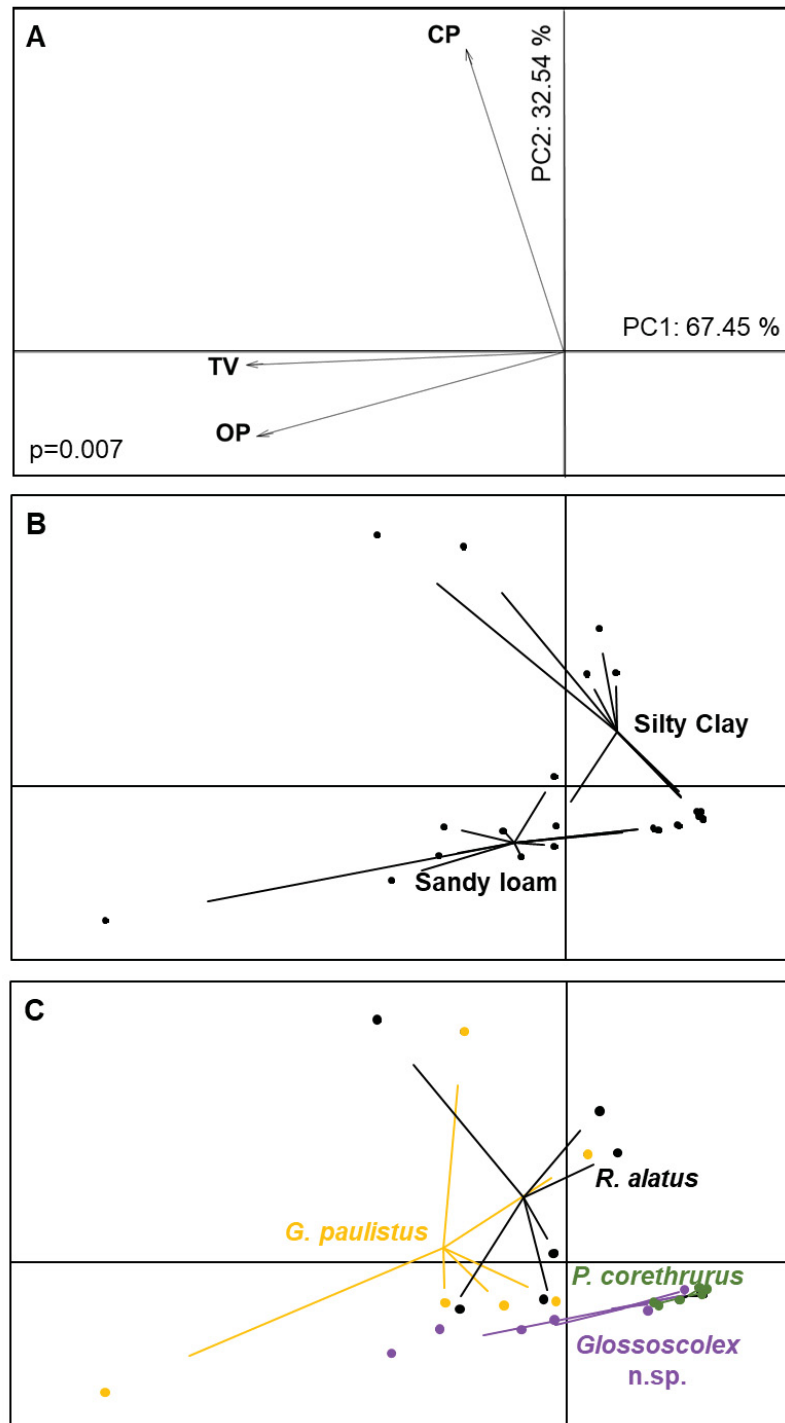
SOURCE: The author (2021).

FIGURE 11. 3D MODEL OF THE RECONSTRUCTION OF THE POROUS NETWORK USING MICRO-CT OF CASTS OF FOUR TROPICAL ENDOGEIC SPECIES (*Rhinodrilus alatus*, *Glossoscolex paulistus*, *Glossoscolex n. sp.*, *Pontoscolex corethrurus*) IN TWO SOILS OF CONTRASTING TEXTURE, USING CTVOX REALISTIC 3D - VISUALIZATION. POROSITY REPRESENTED IN BLUE COLOR.



SOURCE: The author (2021).

FIGURE 12. PRINCIPAL COMPONENT ANALYSIS (PCA) OF THE SOIL PORE VOLUME IN CASTS OF THE FOUR TROPICAL ENDOGEIC SPECIES (*Rhinodrilus alatus*, *Glossoscolex paulistus*, *Glossoscolex* n.sp., *Pontoscolex corethrurus*) IN TWO SOILS OF CONTRASTING TEXTURE (SILTY CLAY, SANDY LOAM), OBTAINED FROM 3D ANALYSIS OF MICRO-CT. TV = TOTAL PORE VOLUME; OP = OPEN PORES; CP = CLOSED PORES (3A). A. CORRELATION BETWEEN POROSITY VARIABLES AND THE FIRST TWO PCA AXES. B. POSITION OF INDIVIDUAL REPLICATES OF THE CASTS PRODUCED IN EACH SOIL TYPE ON THE PLANE DEFINED BY THE FIRST TWO PCA AXES. C. POSITION OF THE INDIVIDUAL REPLICATES OF THE CASTS OF THE DIFFERENT SPECIES ON THE PLANE DEFINED BY THE FIRST TWO PCA AXES.



SOURCE: The author (2021).

3.6 DISCUSSION

Very few authors have explored the porosity of earthworm casts, and only a couple of studies have evaluated pore size distribution using Hg porosimetry (Blanchart *et al.*, 1993; Barros *et al.*, 2004). Fresh and aged casts of *Millsonia anomala* showed a concentration of pores between 5.6 and 29 μm in the sandy soil (75% sand, 8% clay) of the Lamto Savanna of Ivory Coast (Blanchart *et al.*, 1993), while those of *P. corethrurus* showed a concentration of very fine pores (0.01 to 0.1 μm , i.e., micropores) in a heavy clay Latossol (80 % clay of texture) from Manaus in the Brazilian Amazon (Barros *et al.*, 2004). The Hg porosimetry of the latter species casts were similar to those of the control soil. Clearly, cast porosity is highly related to soil texture, with a concentration of much smaller pores in clayey soils, as we observed in the casts of all species in the finer-textured silty clay used in the present study (almost complete absence of pore sizes between 0.1 and 100 μm). On the other hand, in sandy soils most of the pores are of a much larger size, as seen for casts of all species in the present study and for *M. anomala* at Lamto.

The microporosity in casts with high contents of finer-sized particles may result from the process of progressive desiccation that begins in the earthworm hindgut, as described by Barois *et al.* (1993), and continues after casts are egested. In this process, the loss of water leads to the agglomeration of clay particles, which then form very compact casts that contain only intraparticle pores. However, the loss of volume due to desiccation creates cracks in the surface of the casts (>100 μm), which are not taken into account by the Hg porosimetry but are present in the samples (Barros *et al.*, 2004). Intraparticle porosity is responsible for water retention and availability to plants. It causes slower drainage and slower water movement through the soil (Othmer *et al.*, 1991).

Earthworm species had little impact on the intrusion volume and the mean size of the pores responsible for most of the Hg intrusion. Most of the difference in the porosity of these biogenic aggregates was due to soil texture. As clay contents decrease and sand contents increase there is a displacement of the intrusion porosity curve towards larger pores. Mesopores or interparticle pores characterize this type of porosity that is responsible for aerating roots and draining water in the soil and aiding in rapid drainage and water flow (Lu *et al.*, 2014).

When the soil passes through the gut of an earthworm, the clay particles can be rearranged, first by crushing the soil in the gizzard and then in the anterior intestine by increasing water and polysaccharide (intestinal mucus) contents that act as diluting and cementation agents, respectively, superimposing clay particles and decreasing pore space (Barois *et al.*, 1993). This is why casts of *P. corethrurus* were considered to be so compact in the study of Chauvel *et al.* (1999). The 3D micro-CT analyses also showed how casts from the silty clay soil had much lower pore volume compared to those from soil sandy loam.

The lower pore volume of the casts in the silty clay soil was shown clearly in the ACP. Closed porosity (CP) was also closely associated with casts from the silty clay soil and mainly with those of the larger species *R. alatus* and *G. paulistus*. These results also suggest that the size of the earthworm may influence the final porosity of the casts, by the amount of soil they can ingest as well as the force exerted by the gizzards, and by the last segments and the periproct at cast egestion. However, in the silty clay soil it was the smallest species (*P. corethrurus*) that had the highest relative percentage of closed porosity, followed by *R. alatus*. *P. corethrurus*, known to be a compacting species, therefore created casts with lower total porosity and higher proportion of closed pores with low connectivity, also called “rounded porosity” in the literature (Fragoso *et al.*, 1997; Cooper *et al.*, 2016).

Glossoscolex n.sp., like *P. corethrurus* is a mesohumic endogeic species, that feeds on soil, with some selective ingestion; however, this selection did not greatly alter the porosity of the casts. In its native sandy loam soil, this species did not show selective ingestion and generated casts with higher porosity.

3.7 CONCLUSIONS

The four earthworm species studied showed differences in the distribution, volume, and percentage of major pores types (closed, open, total) in their casts. However, the texture of the original soil was the major factor determining porosity, while earthworm species was less important, though size and feeding habit of the earthworms regulated both total porosity and the proportion of open vs closed pores in the casts.

The compacting species *P. corethrurus* showed the largest differences in the percentage of open and closed pores, but its casts generally had statistically different

total porosity than those of the larger species *R. alatus* and *G. paulistus*. As these are only the first earthworm casts that have been evaluated using micro-CT, and only four species have had their casts studied using Hg porosimetry, further research is needed in order to clarify the factors involved in determining cast porosity. As both chemical and microbiological properties of the casts may also be altered by the pore size distribution within them, this kind of study deserves further attention, particularly as bioturbation and ecosystem engineering are major soil processes related to important soil ecosystem services in-situ.

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4 CHAPTER III: SOIL CHEMICAL AND STRUCTURAL PROPERTIES OF ENDOGEIC EARTHWORM CASTS

4.1 RESUMO

Dentre a fauna do solo, as minhocas são provavelmente os organismos mais conhecidos por seus efeitos nas propriedades físicas e químicas do solo e por representarem a maior proporção da biomassa animal do solo. Seus coprólitos são agregados biogênicos enriquecidos em alguns nutrientes devido ao metabolismo das minhocas e alimentação seletiva, e podem afetar o crescimento das raízes das plantas e as populações e atividades microbianas, acelerando a mineralização dos nutrientes e as emissões de gases de efeito estufa. No entanto, apenas uma pequena parte das espécies de minhocas no mundo tiveram seus coprólitos estudados. Assim, o presente estudo teve como objetivo compreender melhor o processo de estruturação dos coprólitos e as mudanças em algumas propriedades físicas e químicas ao longo do tempo nos coprólitos de três espécies de minhocas endogeicas tropicais (*Rhinodrilus alatus*, *Glossoscolex paulistus* e *Glossoscolex* n.sp.) produzidas em dois solos com textura contrastante (argilo siltosa, franco arenosa). Os coprólitos das três espécies apresentaram maior fertilidade química (NH_3^+ , NO_3^- , C orgânico, pH) e os das espécies maiores *R. alatus* e *G. paulistus* apresentaram maior estabilidade dos agregados, indicando efeito positivo dessas minhocas na agregação e fertilidade do solo.

Palavras-chave: Oligochaeta. Micronutrientes. Ciclagem de nutrientes. Efeito das minhocas.

4.2 ABSTRACT

Among the soil fauna, earthworms are probably the best-known animals due to their effects on soil physical and chemical properties and because they represent the largest proportion of soil animal biomass. Their casts are biogenic aggregates enriched in some nutrients due to earthworm metabolism and selective feeding, and may affect plant root growth, microbial populations and activity, accelerating nutrient mineralization and greenhouse gas emissions. However, only a small proportion of the earthworm species in the world have had their castings evaluated. Hence, the present study aimed to better understand the cast structuring process and the changes in some chemical and physical properties over time in the castings of three tropical endogeic earthworm species (*Rhinodrilus alatus*, *Glossoscolex paulistus*, and *Glossoscolex* n.sp.) produced in two soils with contrasting texture (silty clay, sandy loam). The casts of the three species had higher chemical fertility (NH_3^+ , NO_3^- , $\text{C}_{\text{organic}}$, pH) when compared to the original soil, and those of the larger species *R. alatus* and *G. paulistus* had higher aggregate stability, indicating positive effect of these earthworms on soil aggregation and fertility.

Keywords: Oligochaeta. Micronutrients. Nutrient cycling. Earthworms' effect.

4.3 INTRODUCTION

Soil is a source of important environmental services for humans, and a habitat for much of the planet's plant, animal, and microbial life, in addition to being an important source of atmospheric gas emissions and a place of intense nutrient cycling (Stockdale & Watson, 2012).

The quality of the soil as a substrate for plants and as a habitat for animals is highly variable and depends on factors such as organic matter content (OM), pH, texture, moisture and porosity (Doran & Zeiss, 2000). Not only do the intrinsic characteristics of the soil affect animal life, but the activity of soil fauna can also affect the quality of the soil (Lee & Foster, 1991).

The soil fauna contributes largely to soil structure (Sarr *et al.*, 2001) and the decomposition and mineralization of OM (Blanchart *et al.*, 1997). Among the most important and most widely recognized soil animals are the earthworms, frequently heralded as good indicators of soil quality. Earthworms are among the organisms best-known for their effects on soil physical and chemical properties and represent the largest proportion of animal biomass in most soils (Lee, 1985). These organisms are also known as “ecosystem engineers” because they form biogenic structures (pores, galleries, burrows and casts), that act directly and indirectly on the regulation of resources available to other organisms (Jones *et al.*, 1994).

When living in an environment poor in organic matter, earthworms supply their biological needs by ingesting large amounts of soil from which they extract the necessary nutrients. As a result, when worms feed, they ingest organic and mineral material. Their casts often contain higher levels of organic C, inorganic P, and available Ca, Mg, K, Na as well as mineral-N than the original uningested soil (Hulugalle and Ezumah, 1991; Santos *et al.*, 1996; Quadros *et al.*, 2002; Fiuza *et al.*, 2011). In fact, the impact of earthworms on soil fertility in the casts was recently reviewed by van Groenigen *et al.* (2019). However, the impact of earthworms on these properties depends on the ecological category of the earthworm, as well as the substrate upon which they feed (van Groenigen *et al.*, 2019). Endogeic earthworm species, that live in the soil mineral layer have an important role in N mineralization and plant nutrient absorption (Rizhia *et al.*, 2007; Eriksen-Hemel & Whalen, 2007; Lubbers *et al.*, 2011), and as they do not preferentially ingest plant litter like epigeics and endogeics, tend to

have smaller, often non-significant effects on major soil chemical fertility attributes (van Groenigen *et al.*, 2019).

However, endogeic earthworms also often preferentially ingest more fine sand, silt, and clay-sized particles, and those richer in organic matter (Harris *et al.*, 1966) than the bulk original soil (Shipitalo & Protz, 1988; Trigo & Díaz Cosín, 1992). This preferential ingestion not only can affect nutrient availability but also soil physical properties, as differences in cast texture and susceptibility to erosion may also occur, particularly in casts of different ages (Blanchart *et al.*, 2004).

Hedde *et al.* (2013) showed how the effects of earthworms on aggregate stability depend on the characteristics of the soil type and the earthworm species, since there are both compacting and decompacting species (Blanchart *et al.*, 1997). According to Lavelle (1978), earthworms are the major agent responsible for macroaggregation in the top 15 cm of the soil profile. Geophagous species therefore have a major contribution to regulating soil structure (Mariani *et al.*, 2007), soil macroaggregation (Blachart *et al.*, 1997) and pore formation (Pagenkemper *et al.*, 2015).

Although there are many studies that have evaluated the fertility and structure of casts, few have done so using various species and different soil types simultaneously, and few have assessed chemical attributes in casts of different ages. As the earthworm cast stabilization processes is still not fully understood, and there can be important relationships between the cast's physical and chemical properties in different soil types and ages, the present study was undertaken to better understand the process of cast structuring in different endogeic species and elucidate how earthworms of different species affect the chemical properties during cast ageing in soils of different textures.

4.4 MATERIAL AND METHODS

4.4.1 SOILS AND SPECIES USED

Two soils with contrasting texture were collected from the Brazilian Cerrado biome. A silty clay Cambisol (4% sand, 44% clay, and 52% silt) from the Paraopeba National Forest (19°15'17.2"S, 44°24'04.9"W, altitude 725 m), and a sandy loam Acrisol (74% sand, 13% clay and 13% silt) from a >50 yr old pasture at the Santa Rosa

Ranch, in Assistência district (22°30'35.7"S, 47°36'42.1"W, altitude 515 m), near Rio Claro – SP. Three earthworm species were maintained in both soils: *Rhinodrilus alatus* Righi, 1971, *Glossoscolex* n.sp. and *Glossoscolex paulistus* Michaelsen, 1926 (Figure 1, Chapter I). The first species is a large earthworm (around 50-60 cm long) widely harvested for fish bait from its native habitat in the Paraopeba-MG region (Drummond *et al.*, 2013), while *G. paulistus* is another large species (around 25 cm long) occurring mainly in the region around Araras and Rio Claro-SP, and also widely collected for fish bait (Righi, 1972; Abe & Buck, 1985). On the other hand, *Glossoscolex* n.sp. is a relatively long (22 cm), but thinner unpigmented species that has not yet been formally described, and which is only known to occur at the Santa Rosa Ranch.

The experiment was undertaken in an incubator (Full Gauge Imperial III Incubator), in the dark at a temperature of 22 ± 1 °C. Adult individuals of *R. alatus* and *G. paulistus* were placed individually in plastic boxes (with removable lids) measuring 24 x 17 x 10 cm (width, length, and height, respectively). For *Glossoscolex* n.sp. three individuals were added to each pot. Due to the different sizes of each species, 500 g of soil were used for *R. alatus* and *G. paulistus* while only 250 g were used for *Glossoscolex* n.sp. The moisture was kept around 70% of the container capacity value. Boxes with similar quantities of the uningested control soil were treated similarly. All treatments were replicated five times. The experiments with each soil were conducted separately, due to limited incubator space.

The fresh casts (<24 h-old) were manually removed from the surface of the soil, and similar amounts of uningested soil were taken from the control boxes. Around 100 g of the castings were then incubated in small plastic boxes (11 x 11 x 3.5 cm) with loose lids for another day (48h) or until they reached the age of 4, 8, 16 and 32 days, in order to perform analyses for routine soil fertility, including pH, Ca, K, Mg, P (Melich-1), CEC, Base saturation, Al, and H+Al, as well as micronutrient (Fe, Cu, Mn, Zn) and inorganic nitrogen (N-NH_4^+ and N-NO_3^-) contents. Other analyses (P_{sol} , total C, N, H, and aggregate stability) were performed only on fresh casts (<24 h-old). All samples were dried in an oven at 40 °C before analyses described below.

4.4.2 TEXTURE, ROUTINE SOIL FERTILITY AND MICRONUTRIENTS

The soil particle size analysis results from the control soils and casts of all species are provided in Table 1 (chapter 1). Routine chemical and micronutrient

analyses (available) were performed on casts collected at 24 and 48h, and after 4, 8, 16, and 32 days on all five replicates for each species and soil type, as well as the controls following methods described in Hue and Evans (1986): pH in CaCl_2 0.01M (10 g with 1:2.5 soil/solution ratio); nonexchangeable potential acidity ($\text{H}+\text{Al}$) with 10 g soil and pH 7 0.5 mol L^{-1} Ca acetate; exchangeable Ca^{2+} , Mg^{2+} and Al^{3+} with 10 g soil and 1 mol L^{-1} KCl; and available P, Mn^{2+} , Fe^{3+} , Zn^{2+} and Cu^{2+} and exchangeable K^{+} and Na^{+} measured using 10 g soil and 0.05 mol L^{-1} H_2SO_4 and 0.025 mol L^{-1} HCl (Mehlich-1).

4.4.3 INORGANIC NITROGEN (N-NH_4^{+} AND N-NO_3^{-})

Ammonium (N-NH_4^{+}) was determined by ultraviolet absorption spectrophotometry at 640 nm, after sample (10 g soil) preparation by the addition of phenol, sodium nitroprusside and an oxidizing solution (sodium citrate and sodium hypochlorite) based on the Berthelot reaction (Mulvaney, 1996). Nitrate (N-NO_3^{-}) was determined using the ultraviolet absorption spectrophotometer (UV-VIS) with samples (10 g) previously prepared with 10% H_2SO_4 solution by the Griess-Ilosvay method, with zinc as a reducing agent and read at 210 nm (Heinzmann *et al.*, 1984).

4.4.4 Soluble-P

The extraction of P_{sol} was performed by shaking 1 g of soil in 25 mL of CaCl_2 0.01 mol L^{-1} (soil:solution= 1:25), for 1 hour. After stirring, the samples were centrifuged and filtered at 2 μm , and P determined using an optical emission spectrometer with inductively coupled plasma (ICP-OES VARIAN 720-ES).

4.4.5 CNHS BY COMBUSTION

For total C, N, H and S analysis, samples were manually ground and passed through a 212 μm mesh and encapsulated in aluminum foil. The samples (0,025 g) were analyzed by thermal conductivity and a CNHS elemental analyzer (Elementar Vario El III).

4.4.6 AGGREGATE STABILITY

The aggregate stability analysis was carried out using the wet sieving method. The casts (50 g) were initially saturated with water at room temperature and then sieved. The soil retained in each sieve was then dried in an oven at 60 °C and weighed, and the geometric mean diameter (GMD) and weighted mean diameter (WMD) were determined, according to Kemper and Chepil (1965).

4.4.7 STATISTICAL ANALYSIS

The data were submitted to the Shapiro-Wilk normality test. To assess potential differences between casts and control soil and incubation times, means were compared by ANOVA considering a factorial design (3x2) where the effect of the three earthworm species was analyzed in the two soils with different textures. Factors with significant P values (<0.05) were tested using post-hoc Tukey test at 5 % probability.

A Principal component analysis (PCA) was performed using all data from the <24 h old casts, including chemical properties and aggregate stability. The significance of the PCA model was assessed using Monte Carlo permutations (P <0.05) and the ADE-4 package (Dray & Dufour, 2007) in R.

4.5 RESULTS

4.5.1 SOIL FERTILITY ANALYSIS, MICRONUTRIENTS AND TEXTURE

The soil particle size analysis results from the control soils and casts of all species are provided in Table 1 (chapter 1). Overall, the earthworm casts had similar textural class than the original soil, except for the casts of *Glossoscolex* n.sp. (silty clay loam). Nonetheless, all species showed preferential particle ingestion, with *R. alatus* and *G. paulistus* ingesting more clay and silt and less sand in the sandy loam soil, and *Glossoscolex* n.sp. ingesting less clay and more silt in the silty clay soil.

Soil fertility and micronutrient analyses revealed significant differences between the earthworm species and the incubation times (Tables 5 and 6). In the silty clay, *R. alatus* casts showed significant differences for most of the attributes analyzed, except for Mn and C_{org} (Table 5). Fresh casts were often significantly different from other ages,

and were higher for Mg, Fe, Cu and Zn than most of the other incubation times. On the other hand, pH, H+Al, P and CEC were generally lower in the fresh than older casts. Conversely, in the sandy loam fresh casts of this species tended to have higher pH, Na and P contents, but lower Al, Cu, Fe and Zn contents than the other age classes, therefore showing results opposite to those in the silty clay.

In the silty clay, aged (16 and 32 d-old) *G. paulistus* casts had the highest pH, Na and Cu contents, but the lowest P and H+Al contents, while fresh casts (<24 h -old) had the highest P and Fe contents. In the sandy loam, these older casts also had the highest pH and Na but also K contents, and the lowest H+Al contents. Opposite to the results in the silty clay, Cu and Mn contents were the lowest.

Fresh castings of the *Glossoscolex* n. sp. in the silty clay had the highest Al, C_{org} and P contents, as well as the lowest H+Al, and a trend for lower Ca and Cu contents and Base saturation compared with the other cast age classes. In the oldest casts (16 and 32 d), P contents and CEC were lowest. Conversely, in the sandy loam fresh casts had few significant differences with other age classes, and the oldest casts (16 and 32 d) had the lowest contents of K, Na, P, Cu and Fe.

In the control soil, significant differences between incubation periods were also detected, with lowest Al, Ca, Mg, Na, Cu, Mn, Sum of Bases and CEC at 32 days in the sandy loam. Conversely, in the silty clay, Ca, Mg, Mn, Zn, Sum of Bases and CEC were highest in the oldest-age incubation (32 d).

When the comparison was made between the earthworm species and control soil at each incubation time interval (Table 6), significant differences were detected independent of the age of the cast in both soil types. In the following paragraphs we describe some of the main results of these analyses, separately for each soil type.

In the silty clay at 24 h, the control soil has the highest values for most parameters measured, including Ca, Mg, Cu, Mn, Fe, Zn, SB, and CEC. However, the casts of all species had significantly higher C_{org} contents, and those of both *Glossoscolex* species higher Al than the control and *R. alatus* casts. On the other hand, casts of *R. alatus* had higher contents of Mg, Cu, Fe, Zn and SB than those of the other two species.

At 48 h, *R. alatus* casts had the highest levels of Al, Ca, Mg, C_{org}, SB, and CEC. On the other hand, the control soil had higher values of pH, Mn, Fe and Zn than any of the earthworm casts. For the micronutrients, Zn, Cu and Fe contents were lower in the casts of both *Glossoscolex* species than those of *R. alatus*. At 96 h, the control soil

had the highest micronutrient (Cu, Fe, Zn) contents, while casts of both *Glossoscolex* species had higher H+Al, Mg, and CEC contents, while those of *R. alatus* had higher pH.

At 8 days, the control soil had the highest pH, but the lowest contents of Al, H+Al, Ca, Mg, P, C_{org}, SB and CEC, which were significantly different from the earthworm casts. At 16 d, the control soil had significantly higher contents of all micronutrients and lower P content than all the casts. Finally, at 32D, the control soil once again had the highest micronutrient contents, as well as higher values for Ca, Mg, SB, and CEC than the casts. Conversely, casts of *R. alatus* had the highest P contents, while those of this species and *G. paulistus* had higher pH, and those of *Glossoscolex* n.sp. had higher H+Al contents.

In the sandy loam at 24 h, the control soil had significantly higher Al, Ca, Mg, Zn, SB and CEC contents than the earthworm casts, while the castings of *R. alatus* had the highest P. At 48H, the control soil had significantly higher Ca, Mg, Zn, SB and CEC compared with the casts, while *G. paulistus* casts had highest H+Al and Mn contents and lowest C_{org}, while those of *R. alatus* had the lowest Mg, SB and CEC values.

At 96 h and at 8 days the control soil and *G. paulistus* casts had the highest Ca, Mg, SB and CEC values, while those of *Glossoscolex* n.sp. had the highest micronutrient (Cu, Mn, Fe) contents. At 16 days, the castings of *Glossoscolex* n.sp. had the lowest contents of Ca, Mg, P, C_{org}, SB and CEC, compared to the remaining treatments. Finally, at 32 days *R. alatus* casts had the highest values of Ca and Mg, as well as Mn, Zn and Fe, SB and CEC compared with the remaining treatments, and the control soil had the lowest Ca, Mg, SB and CEC values.

TABLE 5. SOIL CHEMICAL PROPERTIES OF CASTS OF THREE TROPICAL ENDOGEIC EARTHWORM SPECIES AND CONTROL SOILS OF CONTRASTING TEXTURE AT DIFFERENT INCUBATION STAGES. *SB = SUM OF BASES; CEC = EFFECTIVE CATION EXCHANGE CAPACITY.

Species	Time	pH CaCl ₂	Al	H+Al	Ca	Mg	K	Na	P	C _{org}	Cu	Mn	Fe	Zn	SB	CEC
mg dm ⁻³ cmolc dm ⁻³ cmolc dm ⁻³																
Silty Clay																
<i>R. alatus</i>	24H	3.9 C	1.0 B	8.3 BC	1.4 AB	1.1 A	0.03 A	0.02 B	4.3 BC	27.6 ns	1.9 A	4.5 ns	14.9 A	1.6 A	2.6 A	3.6 BC
	48H	3.4 D	2.0 A	11.7 A	1.4 AB	1.2 A	0.02 B	0.01 C	3.3 C	27.6 ns	1.7 A	1.7 ns	9.3 B	0.9 AB	2.6 A	4.6 A
	96H	4.1 B	1.3 B	8.8 B	1.5 A	0.4 B	0.03 A	0.03 A	8.5 A	30.4 ns	0.1 B	1.5 ns	7.3 B	0.1 B	2.1 AB	3.4 BC
	8D	3.4 D	2.0 A	11.3 A	1.3 AB	1.0 A	0.02 B	0.01 C	3.9 C	28.2 ns	1.7 A	1.6 ns	8.7 B	0.8 AB	2.3 A	4.3 AB
	16D	4.4 A	0.9 B	6.6 C	0.9 B	0.4 B	0.04 A	0.02 B	5.3 BC	28.7 ns	0.2 B	1.4 ns	8.6 B	0.2 B	1.3 B	2.3 D
	32D	4.3 AB	1.1 B	7.6 BC	1.5 A	0.6 B	0.04 A	0.02 AB	6.5 AB	29.5 ns	0.1 B	1.7 ns	8.4 B	0.0 B	2.1 AB	3.2 C
<i>G. paulistus</i>	24H	3.8 B	1.9 A	10.7 B	1.3 ns	0.4 ns	0.03 ns	0.03 AB	7.7 A	27.8 AB	0.1 B	1.4 ns	7.7 A	0.1 AB	1.8 ns	3.6 ns
	48H	3.7 BC	1.7 A	12.7 A	1.1 ns	0.4 ns	0.03 ns	0.02 BC	7.0 AB	24.4 B	0.1 B	1.5 ns	6.7 AB	0.1 AB	1.6 ns	3.3 ns
	96H	3.6 BC	1.8 A	12.3 AB	1.2 ns	0.9 ns	0.02 ns	0.02 BC	6.2 ABC	30.2 A	0.1 B	1.5 ns	6.8 AB	0.3 AB	2.1 ns	3.9 ns
	8D	3.5 C	1.9 A	13.7 A	1.2 ns	0.8 ns	0.03 ns	0.02 C	6.5 ABC	30.2 A	0.2 B	1.6 ns	5.8 AB	0.1 AB	2.1 ns	4.0 ns
	16D	4.3 A	1.6 A	7.1 C	1.5 ns	0.7 ns	0.03 ns	0.04 A	4.8 BC	28.1 AB	0.2 AB	1.6 ns	4.9 B	0.1 B	18.5 ns	20.1 ns
	32D	4.3 A	1.1 B	7.3 C	1.2 ns	0.5 ns	0.03 ns	0.04 A	4.5 C	28.9 A	0.3 A	1.4 ns	5.1 B	1.0 A	1.8 ns	2.9 ns
<i>G. n. sp.</i>	24H	3.8 ns	1.9 A	10.8 B	1.3 DC	0.4 ns	0.03 ns	0.03 ns	7.7 A	26.8 A	0.1 B	1.4 ns	7.7 A	0.1 ns	1.8 B	3.7 AB
	48H	3.8 ns	1.8 A	11.8 AB	1.1 C	0.4 ns	0.03 ns	0.02 ns	7.0 AB	24.4 AB	0.1 B	1.5 ns	6.7 AB	0.1 ns	1.6 B	3.4 AB
	96H	3.7 ns	1.7 A	12.3 AB	1.7 A	0.5 ns	0.03 ns	0.03 ns	7.4 AB	26.2 AB	0.1 B	1.5 ns	7.2 A	0.1 ns	2.2 A	4.0 A
	8D	3.7 ns	1.6 AB	12.5 A	1.6 AB	0.6 ns	0.03 ns	0.03 ns	7.1 AB	24.7 AB	0.5 A	1.5 ns	6.8 A	0.1 ns	2.2 A	3.8 AB
	16D	3.8 ns	1.6 AB	12.5 A	1.6 AB	0.5 ns	0.03 ns	0.03 ns	6.7 AB	23.3 B	0.5 A	1.6 ns	6.8 A	0.1 ns	2.1 A	3.8 AB
	32D	3.7 ns	1.5 B	13.1 A	1.6 AB	0.5 ns	0.03 ns	0.03 ns	5.5 B	23.6 AB	0.5 A	1.6 ns	4.9 B	0.2 ns	2.1 A	3.3 B
Control	24H	3.8 B	1.6 ns	12.3 ns	5.6 B	3.3 B	0.03 ns	0.02 ns	3.6 ns	18.7 ns	2.2 A	14.5 A	24.5 A	3.5 A	9.0 B	10.6 B
	48H	3.9 A	1.5 ns	9.7 ns	0.8 C	0.4 C	0.03 ns	0.02 ns	3.8 ns	22.9 ns	1.7 B	2.1 B	12.3 B	1.0 B	1.2 C	2.8 C
	96H	3.9 A	1.5 ns	8.9 ns	0.8 C	0.4 C	0.03 ns	0.02 ns	3.6 ns	25.4 ns	1.7 B	1.6 B	9.1 B	0.8 B	1.2 C	2.7 C
	8D	3.9 A	1.5 ns	9.2 ns	0.8 C	0.4 C	0.03 ns	0.02 ns	3.1 ns	23.9 ns	1.7 B	1.6 B	8.9 B	0.8 B	1.2 C	2.7 C
	16D	3.9 A	1.5 ns	9.0 ns	8.3 B	5.2 AB	0.03 ns	0.02 ns	3.6 ns	23.4 ns	2.3 A	16.3 A	19.2 A	3.1 A	13.6 B	15.1 B
	32D	3.9 A	1.7 ns	9.9 ns	13.1 A	6.6 A	0.02 ns	0.02 ns	3.2 ns	24.1 ns	2.5 A	20.1 A	23.9 A	3.8 A	19.7 A	21.4 A

TABLE 5. CONTINUATION...

Sandy loam																
		0.0 B	3.4 ns	6.5 B	3.6 B	0.01 A	0.03 A	8.2 A	22.9 ns	0.2 B	16.8 ns	15.4 B	0.3 C	10.2 C	10.2 C	
R. alatus	24H	5.4 A														
	48H	5.0 C	0.0 A	3.2 ns	1.3 C	0.01 A	0.02 B	4.1 B	25.0 ns	2.3 A	16.3 ns	19.2 AB	3.1 B	2.5 D	2.5 D	
	96H	5.3 AB	0.0 AB	3.2 ns	1.9 C	0.01 A	0.02 B	5.0 B	25.0 ns	2.5 A	20.1 ns	23.9 A	3.8 AB	3.5 D	3.5 D	
	8D	5.3 ABC	0.0 AB	3.2 ns	8.2 B	0.01 A	0.02 B	3.7 B	23.7 ns	2.5 A	19.0 ns	23.0 AB	3.8 AB	14.3 BC	14.3 BC	
	16D	5.3 ABC	0.0 AB	3.4 ns	11.5 A	7.6 A	0.01 A	4.0 B	23.8 ns	2.5 A	18.5 ns	23.5 A	3.9 AB	19.1 AB	19.1 AB	
	32D	5.1 BC	0.0 AB	3.6 ns	11.7 A	7.6 A	0.01 B	4.2 B	27.7 ns	2.7 A	19.1 ns	25.8 A	4.5 A	19.3 A	19.4 A	
G. paulistus	24H	5.1 BC	0.1 ns	3.5 AB	6.6 BC	4.3 BC	0.01 C	2.3 B	19.8 AB	2.3 AB	29.4 AB	30.1 ns	2.4 BC	10.9 BC	11.0 BC	
	48H	4.9 C	0.1 ns	3.7 A	2.8 C	0.00 B	0.00 C	0.9 B	16.2 B	2.2 AB	31.8 A	30.9 ns	1.6 C	4.9 C	4.9 C	
	96H	5.4 AB	0.0 ns	3.1 BC	12.5 A	7.7 A	0.00 AB	0.03 BC	4.5 A	26.4 A	2.7 A	20.7 BC	25.1 ns	4.4 A	20.3 A	20.3 A
	8D	5.5 AB	0.0 ns	3.1 BC	11.0 AB	7.0 AB	0.01 AB	0.03 ABC	4.8 A	23.3 AB	2.1 AB	17.5 C	19.3 ns	3.5 AB	18.1 AB	18.1 AB
	16D	5.6 A	0.0 ns	2.9 C	8.7 AB	5.3 AB	0.01 A	0.06 AB	5.4 A	25.5 A	1.4 BC	16.0 C	19.0 ns	2.3 BC	14.0 AB	14.0 AB
	32D	5.6 A	0.0 ns	2.8 C	8.6 AB	4.6 BC	0.01 AB	0.06 A	4.4 A	24.2 A	0.3 C	10.5 C	13.9 ns	1.3 C	13.3 AB	13.3 AB
G.n.sp.	24H	5.5 AB	0.0 ns	3.0 AB	6.1 ns	4.6 ns	0.02 AB	0.05 ABC	5.7 A	27.6 A	3.4 AB	28.8 AB	40.0 A	1.8 ns	10.7 ns	10.8 ns
	48H	5.5 AB	0.0 ns	2.8 B	6.7 ns	4.4 ns	0.01 AB	0.06 AB	6.9 A	23.9 A	4.2 AB	21.6 AB	40.4 A	2.2 ns	11.2 ns	11.2 ns
	96H	5.5 AB	0.0 ns	2.9 AB	5.8 ns	4.6 ns	0.01 AB	0.06 AB	6.4 A	26.8 A	3.5 AB	36.5 A	42.8 A	1.5 ns	10.4 ns	10.4 ns
	8D	5.5 AB	0.0 ns	2.8 B	6.0 ns	4.7 ns	0.02 A	0.07 A	7.2 A	24.7 A	5.6 A	29.8 AB	41.0 A	0.8 ns	10.9 ns	10.9 ns
	16D	5.3 B	0.0 ns	3.4 A	5.1 ns	3.4 ns	0.01 B	0.02 C	2.1 C	16.9 B	1.4 B	29.2 AB	26.5 AB	1.3 ns	8.5 ns	8.6 ns
	32D	5.7 A	0.0 ns	2.7 B	7.5 ns	4.6 ns	0.01 B	0.04 BC	4.1 B	24.3 A	2.0 AB	13.7 B	12.5 B	2.2 ns	12.2 ns	12.2 ns
Control	24H	5.5 A	0.1 A	4.7 ns	13.5 A	6.5 A	0.01 ns	0.06 A	4.3 ns	25.2 ns	2.5 A	19.0 A	23.0 ns	3.8 ns	20.0 AB	20.2 AB
	48H	5.6 A	0.1 AB	3.0 ns	13.6 A	7.1 A	0.01 ns	0.06 A	4.2 ns	21.5 ns	2.5 A	18.5 A	23.5 ns	3.9 ns	20.7 A	20.8 A
	96H	5.6 A	0.1 AB	3.0 ns	11.1 B	6.9 A	0.01 ns	0.06 AB	4.8 ns	22.9 ns	2.6 A	18.7 A	23.2 ns	4.0 ns	18.1 B	18.2 B
	8D	5.7 A	0.1 AB	3.0 ns	11.4 B	7.2 A	0.01 ns	0.06 A	4.3 ns	24.6 ns	2.6 A	19.7 A	23.2 ns	4.1 ns	18.6 B	18.7 B
	16D	5.6 A	0.1 AB	3.1 ns	11.1 B	7.2 A	0.01 ns	0.06 A	4.4 ns	26.6 ns	2.7 A	19.6 A	21.1 ns	3.7 ns	18.4 B	18.5 B
	32D	5.0 B	0.1 B	3.5 ns	1.4 C	1.2 B	0.01 ns	0.05 B	3.9 ns	24.1 ns	2.0 B	12.3 B	22.2 ns	3.3 ns	2.6 C	2.7 C

SOURCE: The author (2021).

LEGEND: capital letters mean significant differences between incubation times within each treatment, for each of the variables evaluated. ns = not significant.

TABLE 6. SOIL CHEMICAL PROPERTIES OF CASTS OF THREE TROPICAL ENDOGEIC EARTHWORM SPECIES AND CONTROL SOILS OF CONTRASTING TEXTURE AT DIFFERENT INCUBATION TIMES. SB = SUM OF BASES; CEC = EFFECTIVE CATION EXCHANGE CAPACITY.

Time	Treatment	pH CaCl ₂	Al	H+Al	Ca	Mg	K	Na	P	C	Cu	Mn	Fe	Zn	SB	CEC
						cmolc dm ³			mg dm ³	g dm ³		mg dm ³	mg dm ³		cmolc dm ³	
Silty clay																
24h	<i>R. alatus</i>	3.9 ns	1.0 C	8.3 ns	1.4 B	1.1 B	0.03 ns	0.02 B	4.3 B	27.6 A	1.9 B	4.5 B	14.9 B	1.6 B	2.6 B	3.6 B
	<i>G. paulistus</i>	3.8 ns	1.9 A	10.7 ns	1.3 B	0.4 C	0.03 ns	0.03 A	7.7 A	27.8 A	0.1 C	1.4 B	7.7 C	0.1 C	1.8 C	3.6 B
	<i>G.n.sp.</i>	3.8 ns	1.9 A	10.8 ns	1.3 B	0.4 C	0.03 ns	0.03 A	7.7 A	26.8 A	0.1 C	1.4 B	7.7 C	0.1 C	1.8 C	3.7 B
	Control	3.8 ns	1.6 B	12.3 ns	5.6 A	3.3 A	0.03 ns	0.02 B	3.6 B	18.7 B	2.2 A	14.5 A	24.5 A	3.5 A	9.0 A	10.6 A
48h	<i>R. alatus</i>	3.4 C	2.0 A	11.7 A	1.4 A	1.2 A	0.02 ns	0.01 B	3.3 B	27.6 A	1.7 A	1.7 B	9.3 B	0.9 B	2.6 A	4.6 A
	<i>G. paulistus</i>	3.7 B	1.7 AB	12.7 A	1.1 B	0.4 B	0.03 ns	0.02 A	7.0 A	24.4 AB	0.1 B	1.5 B	6.7 C	0.1 C	1.6 B	3.3 B
	<i>G.n.sp.</i>	3.8 B	1.8 AB	11.8 A	1.1 B	0.4 B	0.03 ns	0.02 A	7.0 A	24.4 AB	0.1 B	1.5 B	6.7 C	0.1 C	1.6 B	3.4 B
	Control	3.9 A	1.5 B	9.7 B	0.8 C	0.4 B	0.03 ns	0.02 A	3.8 B	22.9 B	1.7 A	2.1 A	12.3 A	1.0 A	1.2 B	2.8 C
4d	<i>R. alatus</i>	4.1 A	1.3 B	8.8 B	1.5 A	0.4 BC	0.03 ns	0.03 A	8.5 A	30.4 ns	0.1 B	1.5 ns	7.3 B	0.1 B	2.1 A	3.4 B
	<i>G. paulistus</i>	3.6 C	1.8 A	12.3 A	1.2 B	0.9 A	0.02 ns	0.02 B	6.2 AB	30.2 ns	0.1 B	1.5 ns	6.8 B	0.3 B	2.1 A	3.9 A
	<i>G.n.sp.</i>	3.7 BC	1.7 A	12.3 A	1.7 A	0.5 B	0.03 ns	0.03 A	7.4 A	26.2 ns	0.1 B	1.5 ns	7.2 B	0.1 B	2.2 A	4.0 A
	Control	3.9 AB	1.5 AB	8.9 B	0.8 C	0.4 C	0.03 ns	0.02 B	3.6 B	25.4 ns	1.7 A	1.6 ns	9.1 A	0.8 A	1.2 B	2.7 C
8d	<i>R. alatus</i>	3.4 C	2.0 A	11.3 B	1.3 B	1.0 A	0.02 B	0.01 C	3.9 B	28.2 AB	1.7 A	1.6 ns	8.7 A	0.8 A	2.3 A	4.3 A
	<i>G. paulistus</i>	3.5 C	1.9 AB	13.7 A	1.2 B	0.8 B	0.03 A	0.02 B	6.5 A	30.2 A	0.2 C	1.6 ns	5.8 B	0.1 B	2.1 A	4.0 AB
	<i>G.n.sp.</i>	3.7 B	1.6 BC	12.5 AB	1.6 A	0.6 C	0.03 A	0.03 A	7.1 A	24.7 BC	0.5 B	1.5 ns	6.8 B	0.1 B	2.2 A	3.8 B
	Control	3.9 A	1.5 C	9.2 C	0.8 C	0.4 D	0.03 A	0.02 B	3.0 C	23.9 C	1.7 A	1.6 ns	8.9 A	0.8 A	1.2 B	2.7 C
16d	<i>R. alatus</i>	4.4 A	0.9 B	6.6 C	0.9 B	0.4 B	0.04 A	0.02 C	5.3 B	28.7 A	0.2 B	1.4 B	8.6 B	0.2 B	1.3 ns	2.3 ns
	<i>G. paulistus</i>	4.3 A	1.6 A	7.1 C	1.5 B	0.7 B	0.03 AB	0.04 A	4.8 BC	28.1 AB	0.2 B	1.6 B	4.9 B	0.1 B	18.5 ns	20.1 ns
	<i>G.n.sp.</i>	3.8 C	1.6 A	12.5 A	1.6 B	0.5 B	0.03 AB	0.03 B	6.7 A	23.3 B	0.5 B	1.6 B	6.8 B	0.1 B	2.1 ns	3.8 ns
	Control	3.9 B	1.5 A	9.0 B	8.3 A	5.2 A	0.03 B	0.02 C	3.6 C	23.4 B	2.3 A	16.3 A	19.2 A	3.1 A	13.6 ns	15.1 ns
32d	<i>R. alatus</i>	4.3 A	1.1 ns	7.6 C	1.5 B	0.6 B	0.04 A	0.02 BC	6.5 A	29.5 A	0.1 C	1.7 B	8.4 B	0.0 B	2.1 B	3.2 B
	<i>G. paulistus</i>	4.3 A	1.1 ns	7.3 C	1.2 B	0.5 B	0.03 B	0.04 A	4.5 BC	28.9 AB	0.3 B	1.4 B	5.1 B	1.0 B	1.8 B	2.9 B
	<i>G.n.sp.</i>	3.7 C	1.5 ns	13.1 A	1.6 B	0.5 B	0.03 AB	0.03 AB	5.5 AB	23.6 B	0.5 B	1.6 B	4.9 B	0.2 B	2.1 B	3.3 B
	Control	3.9 B	1.7 ns	9.9 B	13.1 A	6.6 A	0.02 B	0.02 C	3.2 C	24.1 B	2.5 A	20.1 A	23.9 A	3.8 A	19.7 A	21.4 A

CONTINUATION...

		Sandy loam													
<i>R. alatus</i>	5.4 A	0.0 B	3.4 ns	6.5 B	3.6 B	0.01 B	0.03 AB	8.2 A	22.9 AB	0.2 B	16.8 B	15.4 B	0.3 C	10.2 B	10.2 B
<i>G. paulistus</i>	5.1 B	0.1 AB	3.5 ns	6.6 B	4.3 AB	0.00 C	0.01 B	2.3 C	19.8 B	2.3 A	29.4 A	30.1 AB	2.4 B	10.9 B	11.0 B
<i>G. n. sp.</i>	5.5 A	0.0 B	3.0 ns	6.1 B	4.6 AB	0.02 A	0.05 A	5.7 AB	27.6 A	3.4 A	28.8 AB	40.0 A	1.8 B	10.7 B	10.8 B
Control	5.5 A	0.1 A	4.7 ns	13.5 A	6.5 A	0.01 B	0.06 A	4.3 BC	25.2 AB	2.5 A	19.0 AB	23.0 AB	3.8 A	20.0 A	20.2 A
<i>R. alatus</i>	5.0 B	0.0 AB	3.2 B	1.3 C	1.1 D	0.01 A	0.02 B	4.1 B	25.0 A	2.3 B	16.3 B	19.2 B	3.1 AB	2.5 D	2.5 D
<i>G. paulistus</i>	4.9 B	0.1 AB	3.7 A	2.8 C	2.1 C	0.00 B	0.00 B	0.9 C	16.2 B	2.2 B	31.8 A	30.9 AB	1.6 B	4.9 C	4.9 C
<i>G. n. sp.</i>	5.5 A	0.0 B	2.8 D	6.7 B	4.4 B	0.01 A	0.06 A	6.9 A	23.9 A	4.2 A	21.6 B	40.4 A	2.2 B	11.2 B	11.2 B
Control	5.6 A	0.1 A	3.0 C	13.6 A	7.1 A	0.01 A	0.06 A	4.2 B	21.5 A	2.5 B	18.5 B	23.5 B	3.9 A	20.7 A	20.8 A
<i>R. alatus</i>	5.3 B	0.0 B	3.2 A	1.9 C	1.6 C	0.01 AB	0.02 B	5.0 ns	25.0 ns	2.5 B	20.1 B	23.9 B	3.8 A	3.5 C	3.5 C
<i>G. paulistus</i>	5.4 B	0.0 B	3.1 A	12.5 A	7.7 A	0.00 B	0.03 AB	4.5 ns	26.4 ns	2.7 B	20.7 B	25.1 B	4.4 A	20.3 A	20.3 A
<i>G. n. sp.</i>	5.5 C	0.0 B	2.9 B	5.8 B	4.6 B	0.01 A	0.06 A	6.4 ns	26.8 ns	3.5 A	36.5 A	42.8 A	1.5 B	10.4 B	10.4 B
Control	5.6 A	0.1 A	3.0 AB	11.1 A	6.9 A	0.01 AB	0.06 AB	4.8 ns	22.9 ns	2.6 B	18.7 B	23.2 B	4.0 A	18.1 A	18.2 A
<i>R. alatus</i>	5.3 B	0.0 B	3.2 A	8.2 AB	6.1 AB	0.01 B	0.02 B	3.7 C	23.7 ns	2.5 B	19.0 B	23.0 B	3.8 A	14.3 AB	14.3 AB
<i>G. paulistus</i>	5.5 AB	0.0 B	3.1 AB	11.0 A	7.0 A	0.01 B	0.03 B	4.8 B	23.3 ns	2.1 B	17.5 B	19.3 B	3.5 A	18.1 A	18.1 A
<i>G. n. sp.</i>	5.5 AB	0.0 B	2.8 B	6.0 B	4.7 B	0.02 A	0.07 A	7.2 A	24.7 ns	5.6 A	29.8 A	41.0 A	0.8 B	10.9 B	10.9 B
Control	5.7 A	0.1 A	3.0 AB	11.4 A	7.2 A	0.01 B	0.06 AB	4.3 BC	24.6 ns	2.6 B	19.7 B	23.2 B	4.1 A	18.6 A	18.7 A
<i>R. alatus</i>	5.3 ns	0.0 B	3.4 ns	11.5 A	7.6 A	0.01 ns	0.01 B	4.0 A	23.8 AB	2.5 ns	18.5 ns	23.5 ns	3.9 A	19.1 A	19.1 A
<i>G. paulistus</i>	5.6 ns	0.0 B	2.9 ns	8.7 A	5.3 B	0.01 ns	0.06 A	5.4 A	25.5 A	1.4 ns	16.0 ns	19.0 ns	2.3 AB	14.0 A	14.0 A
<i>G. n. sp.</i>	5.3 ns	0.0 B	3.4 ns	5.1 B	3.4 C	0.01 ns	0.02 AB	2.1 B	16.9 B	1.4 ns	29.2 ns	26.5 ns	1.3 B	8.5 B	8.6 B
Control	5.6 ns	0.1 A	3.1 ns	11.1 A	7.2 AB	0.01 ns	0.06 AB	4.4 A	26.6 A	2.7 ns	19.6 ns	21.1 ns	3.7 A	18.4 A	18.5 A
<i>R. alatus</i>	5.1 B	0.0 B	3.6 A	11.7 A	7.6 A	0.00 B	0.01 C	4.2 ns	27.7 A	2.7 ns	19.1 A	25.8 A	4.5 A	19.3 A	19.4 A
<i>G. paulistus</i>	5.6 A	0.0 B	2.8 B	8.6 B	4.6 B	0.01 A	0.06 A	4.4 ns	24.2 B	0.3 ns	10.5 B	13.9 BC	1.3 C	13.3 B	13.3 B
<i>G. n. sp.</i>	5.7 A	0.0 B	2.7 B	7.5 C	4.6 B	0.01 A	0.04 B	4.1 ns	24.3 B	2.0 ns	13.7 AB	12.5 C	2.2 BC	12.2 C	12.2 C
Control	5.0 B	0.1 A	3.5 A	1.4 D	1.2 C	0.01 A	0.05 AB	3.9 ns	24.1 B	2.0 ns	12.3 AB	22.2 AB	3.3 AB	2.6 D	2.7 D

SOURCE: The author (2021).

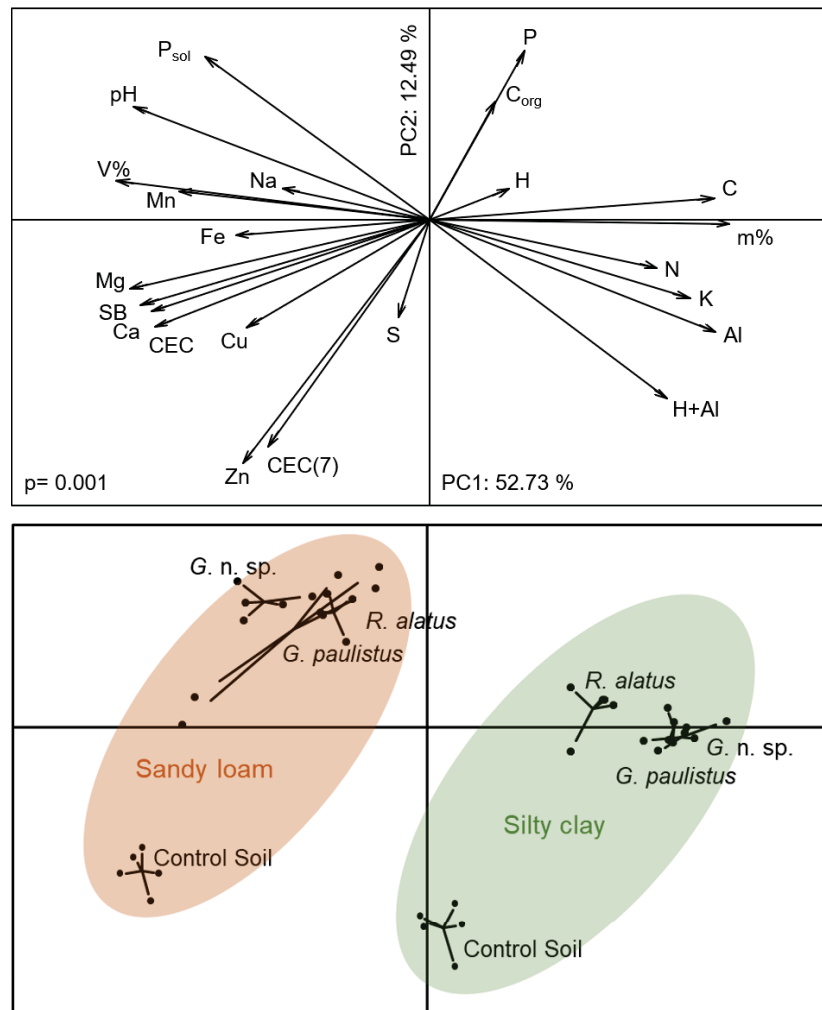
LEGEND: capital letters mean significant differences between each treatment, for each of the variables evaluated within each of the incubation periods/times.
 ns = not significant.

Total N, H and S analyses revealed no differences between casts and control soil in either of the two soil types (Table 7). On the other hand, P in solution was significantly higher in *Glossoscolex* n. sp. than *G. paulistus* casts in their native sandy loam soil. Furthermore, total C contents were higher in both *Glossoscolex* species casts than the control soil.

TABLE 7. TOTAL N, C, H AND S AND P IN SOLUTION (P_{sol}) IN CONTROL SOILS AND CASTS OF THREE TROPICAL ENDOGEIC EARTHWORM SPECIES PRODUCED IN TWO SOILS OF CONTRASTING TEXTURE.

Species	N		C		H		S		P _{sol.}	
Silty clay										
<i>R. alatus</i>	0.19	ns	2.15	AB	0.91	ns	0.01	ns	0.38	ns
<i>G. paulistus</i>	0.19	ns	2.56	A	0.92	ns	0.01	ns	0.68	ns
<i>Glossoscolex</i> . n. sp.	0.19	ns	2.62	A	0.90	ns	0.01	ns	0.60	ns
Control	0.19	ns	1.80	B	0.86	ns	0.01	ns	0.46	ns
Sandy loam										
<i>R. alatus</i>	0.15	ns	1.50	ns	0.44	ns	0.01	ns	1.15	AB
<i>G. paulistus</i>	0.16	ns	1.61	ns	0.43	ns	0.01	ns	0.97	B
<i>Glossoscolex</i> . n. sp.	0.13	ns	1.48	ns	0.39	ns	0.01	ns	1.42	A
Control	0.14	ns	1.40	ns	0.39	ns	0.01	ns	1.09	AB

FIGURE 13. PRINCIPAL COMPONENT ANALYSIS (PCA) USING ALL THE SOIL CHEMICAL VARIABLES IN THE UNINGESTED CONTROL SOIL AND CASTS OF THREE TROPICAL ENDOGEIC EARTHWORM SPECIES (*Rhinodrilus alatus*, *Glossoscolex paulistus*, *Glossoscolex n.sp.*) IN TWO SOILS OF CONTRASTING TEXTURE (SILTY CLAY, SANDY LOAM). CEC = CATION EXCHANGE CAPACITY; CEC (7) = CATION EXCHANGE POTENTIAL AT pH7; m% = SATURATION BY Al; V% = BASE SATURATION; SB = SUM OF BASES; P_{sol} = P IN SOLUTION (CaCl₂). A. CORRELATION BETWEEN SOIL CHEMICAL VARIABLES AND THE FIRST TWO PCA AXES. B. POSITION OF INDIVIDUAL REPLICATES OF THE CONTROL SOILS AND CASTINGS PRODUCED IN EACH SOIL TYPE ON THE PLANE DEFINED BY THE FIRST TWO PCA AXES.



SOURCE: The author (2021).

4.5.2 AGGREGATE STABILITY

Earthworm casts 24 h-old showed significantly higher proportions of larger aggregates than control soils, particularly those larger than 4 mm in both soil types (Table 8, Figure 14). Mass of the medium-sized aggregates retained in the 2 mm sieve were also significantly higher in casts of *Glossoscolex n. sp.* than in the other treatments in the silty clay soil. In this soil casts of this species also had more

aggregates >1 mm mesh, similar to the control soil. In the sandy loam control aggregates of this size were proportionately more abundant than in all of the earthworm casts.

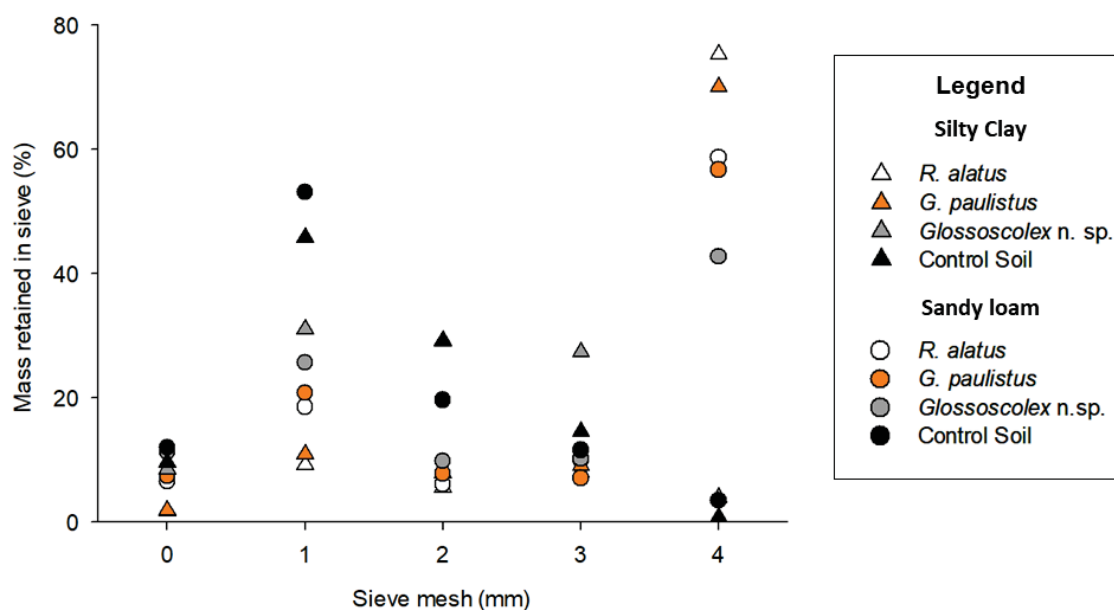
TABLE 8. AGGREGATE STABILITY (WEIGHT OF SOIL IN G RETAINED IN SIEVES of 4 mm, 2 mm, 1 mm, 0.1 mm, AND 0.053 mm) AND MEAN WEIGHT-DIAMETER (MWD) OF THE CASTS OF THREE TROPICAL ENDOGEIC EARTHWORM SPECIES AND THE CONTROL SOIL IN TWO SOILS OF CONTRASTING TEXTURE.

Species	Sieves					Total (g)	MWD	
	4 mm	2 mm	1 mm	0.1 mm	0.053 mm			
Silty Clay								
<i>Rhinodrilus alatus</i>	36.1 A	3.8 B	2.7 B	4.4 C	0.9 B	47.9 ns	3.2 A	
<i>Glossoscolex paulistus</i>	33.5 A	4.3 B	3.8 B	5.2 C	0.9 B	47.8 ns	3.1 A	
<i>Glossoscolex</i> n. sp.	1.7 B	12.1 A	12.9 A	13.7 B	3.8 A	44.3 ns	1.0 B	
Control Soil	0.3 C	6.2 B	12.4 A	19.5 A	4.1 A	42.5 ns	0.7 C	
Sandy loam								
<i>Rhinodrilus alatus</i>	29.0 A	4.9 ns	3.0 C	9.2 C	3.2 C	49.3 ns	2.6 A	
<i>Glossoscolex paulistus</i>	27.5 A	3.4 ns	3.8 C	10.1 C	3.6 B	48.4 ns	2.5 A	
<i>Glossoscolex</i> n. sp.	20.9 B	5.0 ns	4.8 B	12.5 B	5.6 A	48.8 ns	2.1 B	
Control Soil	1.6 C	5.3 ns	9.0 A	24.4 A	5.5 A	45.8 ns	0.6 C	

SOURCE: The author (2021).

LEGEND: capital letters represent statistically significant differences between treatments for each of the variables, within each soil type. ns = not significant.

FIGURE 14. STABILITY OF AGGREGATES IN CASTS OF THREE TROPICAL ENDOGEIC EARTHWORM SPECIES (*Rhinodrilus alatus*, *Glossoscolex paulistus*, *Glossoscolex n.sp.*) AND CONTROL SOIL (PERCENTAGE MASS RETAINED IN DIFFERENT SIEVES), IN TWO SOILS OF CONTRASTING TEXTURE: (SILTY CLAY, SANDY LOAM).



SOURCE: The author (2021).

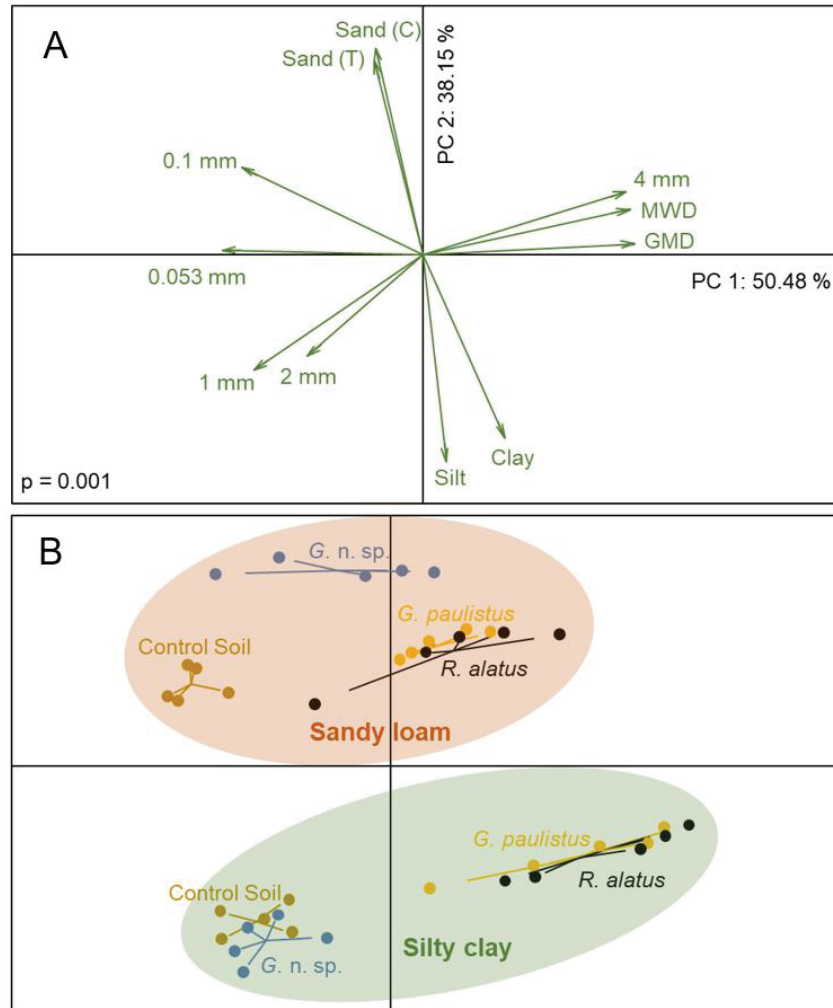
Proportions of the smaller aggregates retained in the 0.1 mm sieve were larger in the control than worm casts in both soils and the smallest (micro-aggregates) retained in the 0.053 mm sieves were more prevalent in the control and *Glossoscolex* n. sp. casts than in the casts of the other two earthworms. In both soils, casts of the larger species *R. alatus* and *G. paulistus* also had a higher mean weight diameter (MWD).

For *R. alatus* in the silty clay, 75% of the mass of casts was retained in the 4 mm mesh, followed by the 0.1 mm mesh with 9%. On the other hand, in the sandy loam, about 58% was retained in the largest mesh and 18% in the 0.1 mm mesh. For *G. paulistus* in the silty clay, 70% of the mass was retained in the 4 mm mesh, followed by 10% in the 0.1 mm mesh. For the sandy loam, 56% remained in the 4 mm mesh and 20% in the 0.1 mm mesh. Hence, for these larger species, the proportion of aggregates of the two larger size-classes represented 74-84% of the total.

For *Glossoscolex* n. sp. in silty clay, the mesh with the greatest retention was 0.1 mm (30%), followed by 1 mm and 2 mm, with 29 and 27%, respectively. In the sandy loam, 42% was retained in the 4 mm mesh, followed by 25% in the 0.1 mm sieve. In both control soils the sieve with the largest retention was 0.1 mm, with about 45% retained in the silty clay and 53% in the sandy loam.

The PCA (Figure 15A) revealed significant relationships between the soil physical variables and the treatments (Monte Carlo $p=0.001$). The first axis (PC1) explained 50.5% of the data variation, separating mainly control soils from earthworm casts due to the higher aggregate stability values and amounts retained in the largest (4 mm) and smallest sieves (0.053) which were higher in the worm casts and the control soil, respectively. The second axis (PC2) explained 38.2% of the data variation, and mainly separated the two soil types by their texture.

FIGURE 15. PRINCIPAL COMPONENTS ANALYSIS (PCA) OF PHYSICAL PROPERTIES AND STABILITY OF AGGREGATES IN EARTHWORM (*Rhinodrilus alatus*, *Glossoscolex paulistus*, *Glossoscolex n.sp.*) CASTS AND CONTROL SOIL WITH TWO DIFFERENT TEXTURES. * WHERE: SIEVE MESHES (4 mm, 2 mm, 1 mm, 0.1 mm, 0.053 mm), MWD: WEIGHTED AVERAGE DIAMETER; GMD: GEOMETRIC MEDIUM DIAMETER. A. CORRELATION BETWEEN SOIL PHYSICAL VARIABLES AND THE FIRST TWO PCA AXES. B. POSITION OF INDIVIDUAL REPLICATES OF THE CONTROL SOILS AND CASTINGS PRODUCED IN EACH SOIL TYPE ON THE PLANE DEFINED BY THE FIRST TWO PCA AXES.



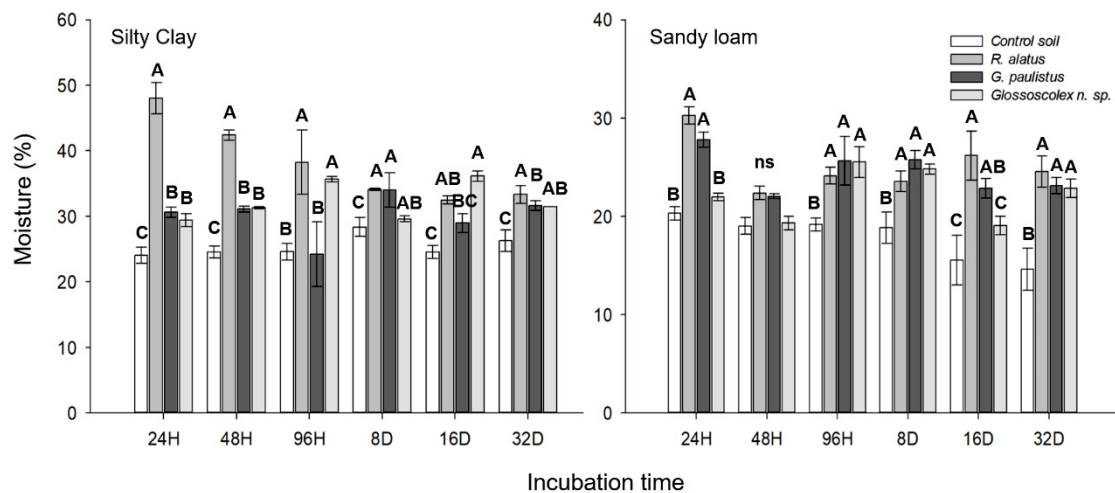
SOURCE: The author (2021).

4.5.3 MOISTURE

The gravimetric soil moisture in the silty clay showed that *R. alatus* casts had higher moisture when compared to the other species, followed by *G. paulistus* for most incubation times, with the exception of 96 h and 16 days where *Glossoscolex n.sp.* cast moisture was higher (Figure 16). Moisture in casts was higher than in the control soil for most species on most occasions. The only exceptions were *G. paulistus* casts at 96h and 16 d.

In the sandy loam, casts of *R. alatus* and *G. paulistus* had significantly higher moisture than the control soil at all sampling dates, except at 48h, when no significant differences were observed between any of the treatments. Casts of *Glossoscolex* n.sp. had higher moisture than the control at 96 h, 8 and 32 d.

FIGURE 16. GRAVIMETRIC SOIL MOISTURE IN EARTHWORM (*Rhinodrilus alatus*, *Glossoscolex paulistus*, *Glossoscolex* n.sp.) CASTINGS AND CONTROL SOIL WITH TWO DIFFERENT TEXTURES AT EACH OF THE INCUBATION TIMES.



SOURCE: The author (2021).

LEGEND: capital letters represent statistically significant differences between species and control soil for each incubation time within the same texture. (ns = not significant).

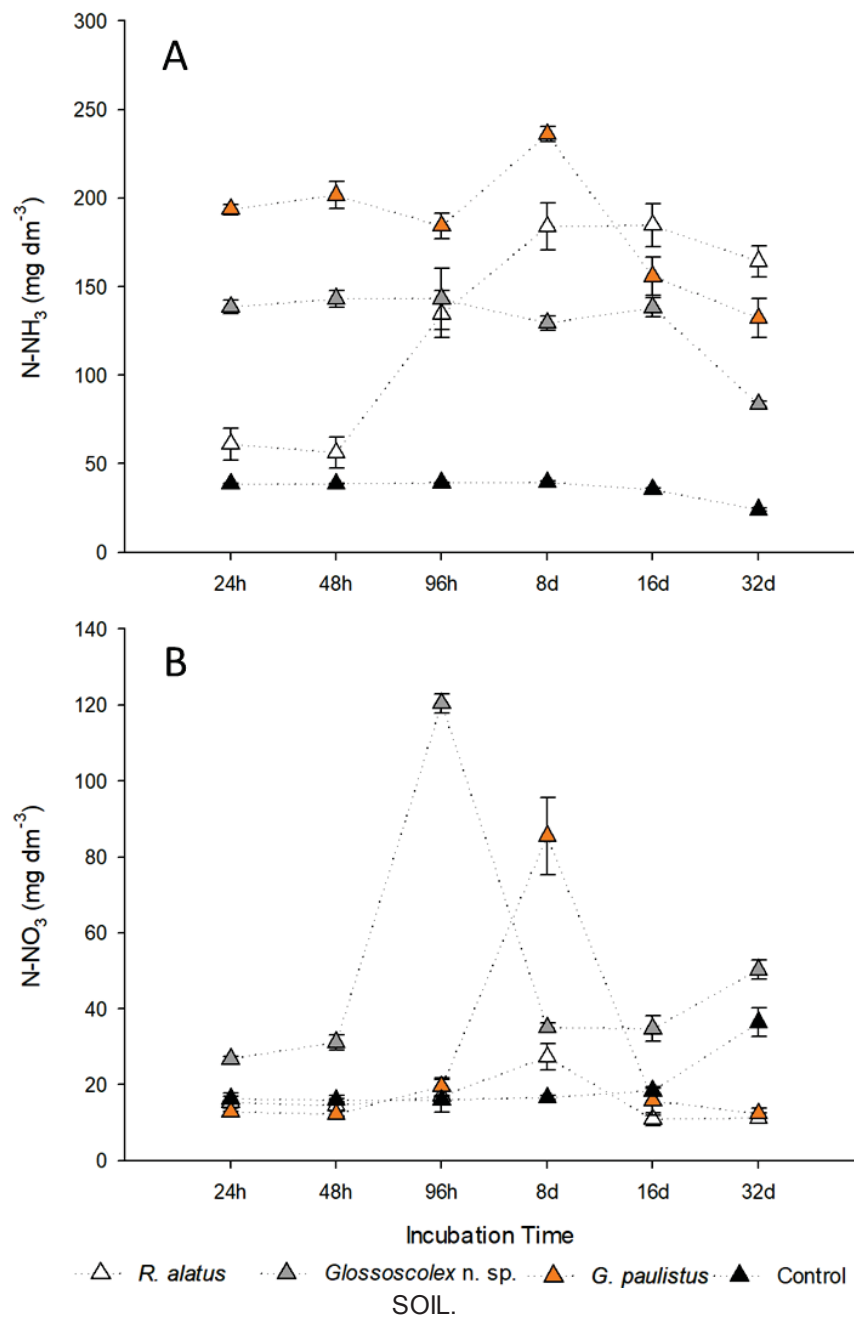
4.5.4 INORGANIC-N (N-NH_4^+ and N-NO_3^-)

Total soil mineral-N contents were generally significantly higher in earthworm casts than in the control soils (Figure 17 and 18), particularly in the silty clay soil, where ammonium contents were especially high in the casts (56-236 mg dm^{-3}). In the casts of *R. alatus* in the silty clay, NH_4 contents increased as the casts aged up to 8 days and then remained more stable (and high). Conversely, in the casts of both *Glossoscolex* species and the control soil, NH_4 contents were lowest at 32 d, although absolute values were still higher in casts than the control soil.

Nitrate contents in the silty clay did not progressively increase in the earthworm casts, indicating slow nitrification, although a few large peaks of NO_3 were observed: at 8 days in the casts of both larger species (*G. paulistus* and *R. alatus*) and at 4 days in *Glossoscolex* n.sp. casts, where they reached the highest value (120 mg dm^{-3}). In

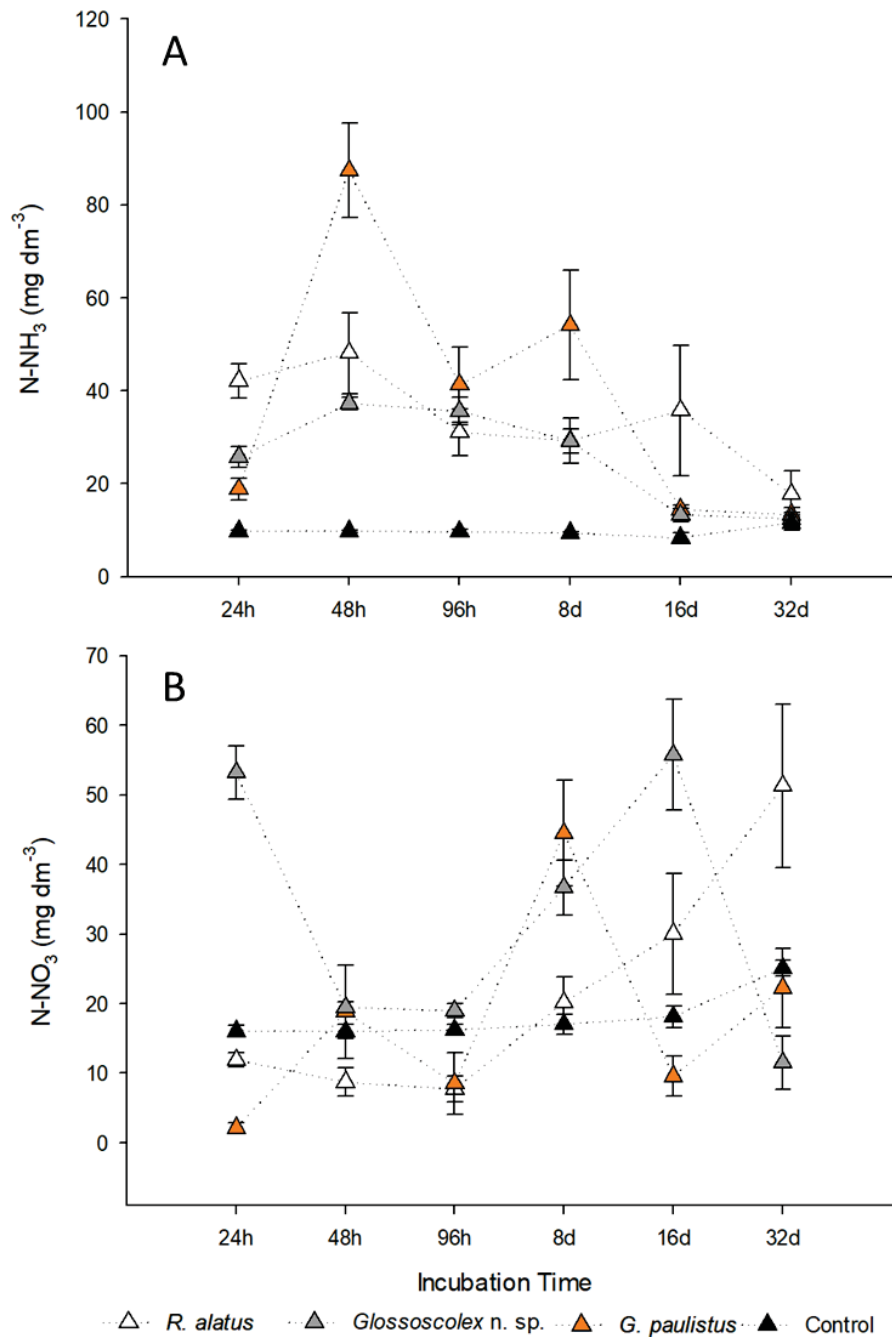
the control soil they remained constantly low and only increased significantly at 32 days. In general, ammonium (N-NH_4^+) contents were much higher than nitrate in the silty clay soil at almost all sampling dates and practically all the treatments, only being higher in the control soil at 32 d (values are found in supplementary material 3).

FIGURE 17. N-INORGANIC (N-NH_4^+ and N-NO_3^-) IN EARTHWORM (*Rhinodrilus alatus*, *Glossoscolex paulistus*, *Glossoscolex n.sp.*) CASTS AND CONTROL SOIL FOR THE SILTY CLAY



SOURCE: The author (2021).

FIGURE 18. N-INORGANIC (N-NH₄⁺ and N-NO₃⁻) IN EARTHWORM (*Rhinodrilus alatus*, *Glossoscolex paulistus*, *Glossoscolex n.sp.*) CASTS AND CONTROL SOIL FOR THE SANDY LOAM SOIL.



SOURCE: The author (2021).

In the sandy loam soil, NH₄ contents tended to be much lower than in the silty clay, ranging from 8-87 mg dm⁻³, while NO₃ contents were not that different, being sometimes higher than in the silty clay. Furthermore, NO₃ contents were higher than NH₄ contents in the control soil on every incubation date, indicating better conditions for nitrification, although there was only a very slight trend for increase in nitrate

contents over time ($R^2 = 0.78$, $p < 0.05$). On the other hand, a significant trend for NO_3 increase was observed in *R. alatus* casts ($R^2 = 0.76$, $p < 0.05$), that reached values higher than NH_4 at 32 days. A peak of NO_3 content was also observed at 8 days in *G. paulistus* casts and at 16 d in casts of *Glossoscolex* n.sp. In the latter species, a trend for decrease in cast NH_4 content over time was observed ($R^2 = 0.75$, $p < 0.05$).

4.6 DISCUSSION

4.6.1 SOIL FERTILITY OF THE CASTS

Higher plant nutrient contents and overall soil fertility have been associated with earthworm activities for centuries (Kevan, 1985), and the role of earthworm casts in soil fertility was recently reviewed by van Groenigen *et al.* (2019). However, very few of the earthworms covered in their review were large species, commonly called minhocú in Brazil. Over 50 of these species are known in Brazil (Brown & James, 2007), but so far, castings of only six of them have been studied (Dadalto & Costa, 1990; Santos *et al.*, 1996; Quadros *et al.*, 2002; Kuczak *et al.*, 2008; Almeida *et al.*, 2009; Fiuza *et al.*, 2011), two of them Amazonian species (*Chibui bari*, Fiuza *et al.*, 2011; and *Rhinodrilus priollii*, Kuczak *et al.*, 2008), and the other four from the Atlantic Forest, Caatinga and Cerrado, although the species of the latter studies were not identified. In the present study, castings of three native species from the Cerrado were compared, in two soil types of contrasting texture, also assessing changes over time, another aspect of cast fertility that has been little studied overall (van Groenigen *et al.*, 2019). So far, only one large earthworm species casts have been studied over time: those of *Martiodrilus carimaguensis*, an anecic worm from the Colombian savanna (Decaëns *et al.*, 1999; Rangel *et al.*, 1999; Decaëns, 2000; Jiménez *et al.*, 2003; Mariani *et al.*, 2007).

The results for the three endogeic species studied here generally confirm those reported for the other large species in Brazil, with increases in pH observed on some occasions, although mainly for older casts (16, 32 d), and not in fresh castings. These changes may be due to ammonia excretion into the gut or to the secretion of calcium carbonate (CaCO_3) from the calcareous glands into the pharynx or esophagus (Lee, 1985; Edwards & Bohlen, 1996; Schrader & Zhang, 1997). All three species evaluated here have calciferous glands (three pairs in *R. alatus*, one pair in both *Glossoscolex*

species), but they are also all holonephridial, i.e., having exonephridia (Righi, 1971; Buck, 1985), in which the ammonia excretion occurs to the exterior surface of the earthworm body and not into the gut. Hence, the pH changes observed may be more due to Ca secretion, and less to NH_3 diffusion into the gut. However, further research on these topics is needed, particularly considering some of the conflicting results observed here (higher pH in the uningested soil on three occasions), and in the literature (De Vleeschauwer & Lal 1981; Haimi & Boucelham, 1991). These pH changes are especially important as they can affect the availability of several other chemical elements in the soil, including nutrient solubility and their availability to plant roots. However, the changes in pH were not necessarily reflected in higher values of Ca and Mg found in the casts, and these were detected only once for the control soil (8 d in the sandy loam). Furthermore, no significant changes in other soluble nutrients were detected, related to pH increases in the present study.

The high NH_4^+ concentration in the casts of all species, particularly in the silty clay soil is somewhat difficult to explain as all of them are exonephridial. Mechanisms of N excretion in these earthworms therefore need additional research, particularly considering that there was no significant difference in the total N contents of the casts compared with the control soils, which could have helped explain higher mineral-N contents via ammonification and nitrification. Furthermore, only on a few occasions was a trend for nitrification of the ammonium detected over time in the casts, indicating suboptimal conditions for this process in the earthworm casts in both soils. N-mineralization is dependent on a variety of factors, several of which can be modified in earthworm castings, such as total N, ammonium and moisture contents, microbial activity and O_2 availability (Wolters, 1991; Elliott *et al.*, 1991).

Previous studies have highlighted how earthworm casts have high amounts of NH_4^+ and NO_3^- compared to the adjacent soil (Lunt and Jacobson, 1944; Parle, 1963; Lavelle *et al.*, 1992; Parkin & Berry, 1994; Decaëns *et al.*, 1999; Kawaguchi *et al.*, 2011), but that NH_4 tends to be high and NO_3 low in fresh casts, and that NO_3 contents tend to increase only in older casts (>16 and often >30 d-old). As the cast age, ammonia is mineralized to nitrate by microbial activity, resulting in lower cast NH_4 contents. However, this process did not occur concomitantly in the casts of any of the species we studied in either of the two soils. Part of this may be due to denitrification processes (N_2 and N_2O emission) another phenomenon frequently observed in earthworm casts (Braga *et al.*, 2016; Elliott *et al.*, 1990; Lubbers *et al.*, 2011; 2013),

which were unfortunately not measured in the present study, although N₂O emission had been detected already for *R. alatus* casts by Santos *et al.* (2021).

Although several studies have reported as much as 2 to 3-fold increases in exchangeable K contents in worm casts (Dadalto & Costa, 1990; Hullugale & Ezumah, 1991; Santos *et al.*, 1996; Quadros *et al.*, 2002; Bartz *et al.*, 2010; Fiuza *et al.*, 2011), few significant effects were observed in the present experiment, with higher contents observed in *R. alatus* casts than control soil and vice versa on two occasions each. Small increases may be due to the release of K by organic materials through fragmentation during digestion. As there was no addition of organic material in the present experiment, this forced the earthworms to feed only on the original soil. Hence, the low K values in the casts were the result of low levels being ingested from the original soil (control).

Higher levels of P in earthworm casts compared to the soil of origin have been reported by several authors (Peixoto & Marochi, 1996; Brossard *et al.*, 1996; Chapuis-Lardy *et al.*, 1998; Quadros *et al.*, 2002), but the reasons for these increases are controversial. The increase may be due to enzymatic activities (acid and alkaline phosphatases) in the earthworm intestines (López-Hernández *et al.* 1993) and casts (Bayon & Binet, 2006) as well as selective ingestion of soil particles rich in clay and organic materials containing high levels of P (Chapuis-Lardy *et al.*, 2011). Given the low pH observed in the present soils, particularly in the silty clay, the action of microbial acid phosphatases are more likely, although pH of the gut of these earthworms has not yet been measured.

Selective ingestion of smaller particles (more clay) by *R. alatus* and *G. paulistus*, and selective ingestion of more silt by *Glossoscolex* n.sp. were detected in the silty clay soil, where higher C contents were also detected on various occasions in the casts. Hence, higher P contents were also detected on various occasions for all species in the silty clay in casts 8 d-old or older, and in fresh casts of *R. alatus* (<24 h) in the sandy loam. In tropical soils P is highly fixed to clay minerals, and only small increases have been observed in P availability in casts, which tends to decrease over time, both in the casts of *M. carimaguensis* (anecic) and *P. corethrurus* (endogeic) (Jiménez *et al.*, 1999; Chapuis & Brossard, 1995). In the present case, a reduction in fresh cast Mehlich-1 P contents compared with ageing casts was only detected for *R. alatus* in the sandy loam and for both *Glossoscolex* species in the silty clay soil.

The increase of C in the casts of the earthworm species (mainly in the casts from soil silty clay) confirms the selection of particles richer in organic matter during soil ingestion. However, this selection by the three endogeic species studied here (≤ 1.2 times) was lower than that reported by Lee (1985) who found increases in C content of the casts from 1.5 to 2 times compared to the adjacent soil. The mechanism for the selection of particles with high levels of C during soil ingestion is still unknown. Earthworms appear to be able to recognize micro-regions within the soil with high C levels, although they also appear to select smaller particles to ingest, specifically when the soil sand contents increase (Barois *et al.*, 1999; Bossuyt *et al.*, 2005). Also, as soil passes through the gizzard and intestine of the earthworm, plant and soil organic matter fractions are fragmented and intimately mixed with the soil (Barois *et al.*, 1993). Furthermore, large amounts of water-soluble mucus are also secreted in the foregut (Trigo *et al.*, 1999), and although most it is reabsorbed in the hindgut, part of it is still released in the casts (Brown *et al.*, 2000). Both these processes can increase cast C content compared to the original uningested soil.

The lack of differences in total C and N and in C_{org} between casts and control soil in the sandy loam appears to indicate that, despite the selective ingestion of finer particles in this soil by both larger species, they appear to be using part of the organic matter from this soil for their metabolic activities, and that they are acting as typical mesohumic endogeics in this soil. This may be also because no additional organic matter food sources were provided to these earthworms, which may actually be more endo-anecic in their behavior in the field, coming more to the soil surface to feed in their natural environments (Abe & Buck, 1985; Drummond *et al.*, 2013).

Many studies have reported increased micronutrient levels in earthworm casts than control soil (Ganeshamurthy *et al.*, 1998; Cheng & Wong, 2002), but these are little known for large earthworm species. Up until now, only *R. alatus* and *G. paulistus* have been studied (Cardoso, 2016; Santos, 2021), although the former author also studied *Glossoscolex* n.sp. casts. Contrary to previous observations, in the present study, micronutrient contents (especially Fe and Zn) were frequently higher in the uningested control silty clay than in the casts of all earthworm species. Increases in cast pH in the silty clay soil may have caused this decrease in available micronutrients in the soil solution and the cation exchange points (Abreu *et al.*, 2007). According to Lindsay (1972), the solubility of Fe decreases approximately a thousand times for each

unit that the soil pH increases, in the pH range of 4 to 9. For Mn the decrease is 100 times for each unit that the soil pH increases.

On the other hand, in the sandy loam, Cu, Mn, and Fe contents increased in the casts of both *Glossoscolex* species, while the values for Zn were close to or below the control. Contents of all micronutrients also tended to be higher in the sandy loam than silty clay soil, indicating higher available/exchangeable (Mehlich-1) pools in the former soil, despite its higher pH. It is known that micronutrients can be retained by the various organic and inorganic components through ionic or molecular adsorption or by precipitation in poorly soluble forms. The capacity of the soil to retain the micronutrients depends on its chemical, physical and biological properties, but among the known factors are texture, pH, moisture, organic matter content, iron oxide, aluminum, manganese content, speciation and potential redox. Generally, the solubility and, consequently, the movement of cationic micronutrients (copper, iron, manganese and zinc) increases with decreasing pH. Hence further work on the mechanisms and reactions involved in micronutrient absorption/desorption in casts are needed, particularly in relation to these parameters, although analysis of parent material contents should also be helpful.

Moisture can also affect micronutrient retention as it affects redox reactions. Bingham *et al.* (1976) and Bingham (1985) showed that under reducing conditions, the solubility of cadmium, copper, and zinc decreases, and those for iron and manganese increase. However, moisture contents of the casts were almost always higher than the control soil throughout the incubation dates, and fresh casts were well above (*R. alatus*), near or slightly below (both *Glossoscolex* species) container capacity (36% in the silty clay; 26% in the sandy loam) in both soils. Furthermore, the elements compete with each other and with other organic and inorganic compounds for adsorption sites or available complexing agents. Thus, nutrient retention is affected not only by concentration in the soil solution but also by the concentration of all other system constituents (Doner, 1978; Mattigod *et al.*, 1979).

The PCA highlighted the importance of soil texture in determining the overall chemistry of the control soil and its potential effects on the levels of elements found in the casts. The silty clay soil with its high clay content has a higher capacity for water retention, lower drainage rate and greater capacity for cation exchange. However, the original soil is chemically poor, with low levels of bases and nutrients, and the CEC is saturated with potentially toxic cations (Al^{3+} and H^{+}). Hence, it seems that it is mainly

by selective C and particle ingestion, and possible Ca secretion by the calciferous glands that the earthworms can affect element availability in this soil. On the other hand, in the sandy loam no preferential ingestion of C-rich particles was observed, although higher ingestion of silt and clay were found for two species, but the impacts of the earthworms on overall chemical parameters still remained smaller, being more evident at 32d for the macronutrients and in intermediate aged-casts for the micronutrients.

4.6.2 AGGREGATE STABILITY

Soil aggregation consists of the process where unitary particles come together, forming microaggregates after numerous biogeochemical cycles (Tisdall & Oades, 1982). This formation may be pedogenetic or physicogenic, depending on various soil characteristics, such as clay content and type, polyvalent cations, iron, aluminum, manganese oxides and hydroxides, organic matter, root exudates, compounds from microbial metabolism and compressive action of roots and hyphae (Carvalho, 1991; Bastos *et al.*, 2005). However, soil aggregation in many sites with large macrofauna (particularly earthworm) populations can frequently be primarily biogenic, composed of soil animal feces (Lavelle, 1988). This formation process is faster than the previously ones (Fernandes *et al.*, 2017), and can result in very large aggregates (e.g., giant earthworm casts), filled with smaller, internal micro-aggregates (Six *et al.*, 2004), that are important potential C sequestration-sites in soils (Martin, 1991). The stability of these aggregates is highly variable, and depends greatly on their moisture contents, with fresh casts normally much more fragile and easier to break down, than aged, dried out casts (Shipitalo & Le Bayon, 2004). In fact, the process of cast production and breakdown and its importance to erosion processes has been known since the time of Darwin, having been an important topic detailed in his last book (Darwin, 1881).

In the present case, the biogenic aggregates (earthworm casts) had been previously dried out and therefore undergone a cementation process (Wolters, 1991; Shipitalo & Le Bayon, 2004), but then were then re-wetted (saturated) following standard methodological protocol. Nonetheless, the casts, particularly of the larger species *R. alatus* and *G. paulistus* had a high proportion of aggregates > 4 mm in both soil types, indicating high macro-aggregate stability and lower erosion potential (Blanchart *et al.*, 2004), increasing the chance of contributing to C sequestration in

these casts. This is particularly important in the sandier soil, which naturally had high values for the mesh >0.1 mm diameter, and where maintenance of soil C is more important.

The formation process of macroaggregates begins with reactions that occur in the earthworm gut. Clay platelets are re-oriented (Barois *et al.*, 1993), the gizzard grinds the coarse particles with the finer ingested materials, and the intestinal mucus secreted by the worms acts as a cementing agent, increasing aggregate stability (Trigo *et al.*, 1999). All these earthworm species also have body size >20 cm in length and >1 cm diameter (Brown & James, 2007), with great muscular pressure that is exerted when excreting the casts, causing the clay to be compacted into overlapping layers. This all results in a larger and more physically stable cast, particularly for *R. alatus* and *G. paulistus*. These stable aggregates are essential to provide good soil structure and porous spaces for root development and air and water circulation (Schimiguel *et al.*, 2014).

Numerous studies have shown how earthworms contribute to soil aggregation, but the effects differ depending on soil type (Haynes & Fraser, 1998, Hedde *et al.*, 2013), earthworm species (Blanchart *et al.*, 1997; Marashi & Scullion, 2003; Zhang & Schrader, 1993) or age of the cast (Marinissen & Dexter, 1990; Tomlin *et al.*, 1995). The present study confirms these results for earthworm species and soil type: *Glossoscolex* n. sp. casts had different aggregate stability and size characteristics than the other two species. Furthermore, in the soil sandy loam (its native soil), this earthworm showed mostly aggregates >4mm, while in the silty clay, aggregates were mostly of the 2 to 0.1 mm size, casts that are less stable and more susceptible to degradation. This may be because this species had difficulty in adapting to the silty clay soil, with high mortality rates after a few months of incubation. The casts produced by this species in the silty clay were much smaller and thinner compared to the casts produced by the same species in the sandy loam, where most of the aggregates passed through the >2 mm sieve. Limited adaptability of endogeic species to other soils has been observed previously, and may be due to various factors, including native microbial biome (Gilot-Villenave, 1994; Ortiz-Ceballos *et al.*, 2019).

4.7 CONCLUSION

Casts of three tropical endogeic earthworm species (*R. alatus*, *Glossoscolex paulistus*, and *Glossoscolex* n. sp.) are both chemically and physically different from uningested soil, having in particular, higher contents of NH_4^+ , NO_3^- , organic C, pH and some micronutrients. Furthermore, the dynamics of nutrient availability in casts over time is not uniform, with major differences in several elements between species, soil types and incubation times, that are not easy to explain. Understanding these processes requires further research.

Big earthworm species such as *R. alatus* and *G. paulistus* create large stable casts with a major proportion of macroaggregates compared to smaller species, though differences are evident depending on the original soil texture. Clearly, the effects of earthworms on the chemical and physical properties are dependent on the source soil and its texture, though temporal dynamics are important and need further evaluation in order to clarify mechanisms involved in the improvement of soil structure and fertility by earthworms in tropical soils.

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5 GENERAL CONCLUSIONS

The four tropical endogeic earthworm species studied showed differences in shape, quantity, distribution, volume, and percentage of pores found in their casts. Soil texture was the main factor determining these parameters of cast porosity, although earthworm species were also important. The compacting species *P. corethrurus*, when compared to the other species studied, had the greatest differences in the number of pores and the percentage of open and closed pores in the two soils.

Casts of three large tropical species (*R. alatus*, *G. paulistus*, and *Glossoscolex* n. sp.) are chemically distinct from uningested soil, with higher contents of NH_4^+ , NO_3 , organic C, pH, and some micronutrients. However, the effects of these earthworms on the chemical properties are dependent on the original soil, particularly its texture, and the age of the cast.

The gathering of data on earthworm impacts on soil chemical and physical properties, particularly detailed studies on their casts is important in helping to quantify their overall effects on soil ecosystem processes, and to help value the conservation of native species. In the present case, we show that earthworms tend to be better adapted to their native soils, and that in these they are able to promote important positive impacts on soil porosity, structure, aggregate stability and overall fertility, although the paucity of data on large earthworm species is particularly worrisome, considering their diversification in Brazil and their presence in many natural and disturbed habitats. Further efforts to evaluate the ecology of these species both in laboratory studies and in-situ are needed, in order to promote conservation and sustainable use, and valuation of soil biological ecosystem services.

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7 SUPPLEMENTARY MATERIAL

7.1 SUPPLEMENTARY MATERIAL 1. NUMBER OF COMPUTERIZED MICROTOMOGRAPHY IMAGES USED FOR THE VISOLOG SOFTWARE ANALYSES FOR EACH TROPICAL ENDOGEIC EARTHWORM SPECIES CASTS PRODUCED IN TWO SOILS OF CONTRASTING TEXTURE.

Species	Replicates	Number of images of each casting	
		Silty Clay	Sand Loam
<i>Rhinodrilus alatus</i>	1	937	689
	2	937	794
	3	937	801
<i>Glossoscolex paulistus</i>	1	940	937
	2	940	1011
	3	901	937
<i>Glossoscolex</i> n. sp.	1	834	937
	2	860	963
	3	860	937
<i>Pontoscolex corethrurus</i>	1	960	597
	2	960	940
	3	960	940

SOURCE: The author (2021).

7.2 SUPPLEMENTARY MATERIAL 2. GRAVIMETRIC SOIL MOISTURE IN EARTHWORM
(Rhinodrilus alatus, Glossoscolex paulistus, Glossoscolex n.sp.) CASTINGS AND CONTROL SOIL
 OF TWO CONTRASTING TEXTURES.

Species	Time	Moisture %		Moisture %	
		Silty Clay		Sandy loam	
<i>Rhinodrilus alatus</i>	24H	48.0	Aa	30.3	Aa
	48H	42.4	ABa	22.4	B
	96H	38.3	Bca	24.1	Ba
	8D	34.1	Ca	23.5	Ba
	16D	32.5	Cab	26.2	ABa
	32D	33.3	Ca	24.6	Ba
<i>Glossoscolex paulistus</i>	24H	30.6	Ab	27.8	Aa
	48H	31.1	Ab	22.0	B
	96H	24.2	Bb	25.7	ABa
	8D	34.0	Aa	25.8	ABa
	16D	29.0	ABbc	22.9	ABab
	32D	31.6	Ab	23.1	Aba
<i>Glossocolex n. sp.</i>	24H	29.4	Bb	22.0	ABb
	48H	31.3	ABb	19.3	B
	96H	35.7	Aa	25.5	Aa
	8D	29.6	Bab	24.8	Aa
	16D	36.2	Aa	19.1	Bbc
	32D	31.5	Abab	22.8	ABa
Control Soil	24H	24.0	c	20.3	Ab
	48H	24.5	c	19.0	AB
	96H	24.6	b	19.2	ABb
	8D	28.4	c	18.8	ABb
	16D	24.6	c	15.5	ABc
	32D	26.3	c	14.6	Bb

SOURCE: The author (2021).

LEGEND: capital letters represent statistically significant differences between the sampling dates (incubation times) within each treatment (earthworm species and control soil). ** lower-case letters represent statistically significant differences between the treatments (species and control soil) within each incubation time-period. *** The lack of capital or small letters represents a non-significant value for that analysis.

7.3 SUPPLEMENTARY MATERIAL 3. SOIL MINERAL-N CONTENTS (N-NH₄⁺ = AMMONIUM; N-NO₃⁻ = NITRATE) IN CONTROL SOILS AND CASTS OF THREE TROPICAL ENDOGEIC EARTHWORM SPECIES PRODUCED IN TWO SOILS OF CONTRASTING TEXTURE.

Species	Time	N-NH ₄ ⁺			N-NO ₃ ⁻			N-Mineral		
		mg dm ⁻³			mg dm ⁻³			mg dm ⁻³		
		Silty Clay			Sandy loam					
<i>Rhinodrilus alatus</i>	24H	61.0	Cc		15.3	Ab		76.3	Cc	
	48H	56.3	Cc		14.5	Bb		70.7	Cc	
	96H	134.6	Bb		17.0	ABb		151.6	Bc	
	8D	184.0	Ab		27.2	Ab		211.2	Ab	
	16D	184.7	Aa		10.9	Bb		195.6	ABa	
	32D	164.3	ABa		11.3	Bc		175.6	ABa	
<i>Glossoscolex paulistus</i>	24H	193.7	Ba		12.8	Bb		206.5	Ba	
	48H	201.6	ABa		12.2	Bb		213.9	Ba	
	96H	184.2	BCa		19.5	Bb		203.7	Bb	
	8D	236.1	Aa		85.5	Aa		321.6	Aa	
	16D	155.9	CDab		15.7	Bb		171.5	BCa	
	32D	132.2	Db		12.4	Bc		144.6	Cb	
<i>Glossocolex n. sp.</i>	24H	138.6	Ab		26.8	Ca		165.3	BCb	
	48H	143.2	Ab		31.1	Ca		174.4	Bb	
	96H	143.1	Aa		120.4	Aa		263.5	Aa	
	8D	129.3	Ac		35.0	Cb		164.3	BCc	
	16D	138.4	Ab		34.7	Ca		173.1	Ba	
	32D	83.7	Bc		50.2	Ba		133.9	Cb	
Control Soil	24H	38.6	Ad		16.2	Bb		54.9	ABd	
	48H	38.7	Ac		15.9	Bb		54.6	ABc	
	96H	39.3	Ac		16.0	Bb		55.2	ABd	
	8D	39.5	Ad		16.6	Bb		56.1	ABd	
	16D	35.5	Bc		18.4	Bb		53.8	Bb	
	32D	24.1	Cd		36.4	Ab		60.5	Ac	

SOURCE: The author (2021).

LEGEND: capital letters represent statistically significant differences between the sampling dates (incubation times) within each treatment (earthworm species and control soil). ** lower-case letters represent statistically significant differences between the treatments (species and control soil) within each incubation time-period. *** The lack of capital or small letters represents a non-significant value for that analysis.