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École doctorale 129 "Sciences de l'Environnement"
Institut d'Écologie et des Sciences de l'Environnement de Paris

Relationships between functional traits, bioturbation and water transfers of earthworms sampled in northern Vietnam



PhD thesis in soil ecology

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Abstract

Earthworms are known to play a major role in soil structure formation and maintenance through the production of casts and burrows with consequences on soil water transfers. However, most of the literature is based on Lumbricidae species, while the most diverse family, the Megascolecidae, is comparatively understudied. Therefore, this thesis aimed at providing a better understanding of the impact of tropical earthworms from the Megascolecidae family on soil structure and water transfers, particularly using their morpho-anatomical traits as a proxy.

To do so, two experiments were conducted in laboratory conditions. In the first, 23 earthworm species were sampled in northern Vietnam and their morpho-anatomical traits were measured. Earthworms were assigned into four ecological categories based on the morpho-anatomical traits selected for Lumbricidae species. Earthworms were incubated in mesocosms and the whole drilosphere (i.e., volume of surface and belowground casts, empty burrows and lateral soil compaction and bulk density of casts and the lateral compaction) was characterized by X-ray computed tomography. In addition, the soil water infiltration was measured. In the second experiment, the individual and interactive effect of the anecic *Amyntas zenkevichi* and the endogeic *Pontoscolex corethrurus* on soil structure and resultant effects on water transfers were investigated. Burrow systems were characterized using X-ray tomography and soil water transfers were characterized in terms of water infiltration, evaporation, and moisture.

Results of the first experiment showed (i) a good match between the volume of the different parts of the drilosphere and the four ecological categories, whereas differences were less clear for the bulk density of casts or at the vicinity of burrows; (ii) earthworms had no or a positive effect on soil water infiltration without clear distinction between the four ecological categories; (iii) the possible use of both morpho-anatomical traits and machine learning to predict the impact of earthworms on soil water infiltration and bulk density variations of the drilosphere. Results of the second experiments showed that the presence of *A. zenkevichi* and *P. corethrurus* species together had a slight, synergistic, positive effect on soil resistance to water loss by evaporation.

To conclude, this thesis showed that tropical earthworms can be assigned into ecological categories, but links with soil functioning can somehow be wrong. This thesis highlights new avenues of research based on effect traits, that can be used to identify the effects of tropical earthworms on soil structure and water infiltration.

Key words: bioturbation; drilosphere; interspecific interactions; hydraulic properties

Résumé

Les vers de terre, par la production de turricules et de galeries jouent un rôle majeur dans la formation et l'entretien de la structure du sol avec des conséquences sur les transferts d'eau. Cependant, la majeure partie de nos connaissances concerne la famille des Lumbricidae, tandis que la famille la plus diversifiée, les Megascolecidae, est largement sous-étudiée. Par conséquent, cette thèse visait à mieux comprendre l'impact des vers de terre tropicaux de la famille des Megascolecidae sur la structure du sol et les transferts d'eau, en particulier en utilisant leurs traits morfo-anatomiques comme proxy.

Pour ce faire, deux expériences ont été menées au laboratoire. Dans la première, 23 espèces de vers de terre ont été échantillonnées dans le nord du Vietnam et leurs traits morfo-anatomiques ont été mesurés. Les vers de terre ont été classés en quatre catégories écologiques en fonction de des traits morfo-anatomiques utilisés pour les Lumbricidae. Les vers de terre ont ensuite été incubés dans des mésocosmes afin d'y mesurer le volume et la compaction de la drilosphere ainsi que l'infiltration de l'eau. Dans la deuxième expérience, l'effet individuel et interactif du ver anécique *Amyntas zenkevichi* et du ver endogé *Pontoscolex corethrurus* sur la structure du sol et les transferts d'eau ont été mesurés. Les réseaux de galerie ont été caractérisés à l'aide de la tomographie aux rayons X et les transferts d'eau ont été caractérisés en termes d'infiltration d'eau, d'évaporation et d'humidité du sol.

Les résultats de la première expérience ont montré (i) une bonne correspondance entre le volume des différentes parties du drilosphere et les quatre catégories écologiques, alors que les différences étaient moins nettes pour la compaction des turricules ou la compaction autour des galeries; ii) les vers de terre avaient soit un effet positif ou neutre sur l'infiltration de l'eau du sol sans distinction nette entre les quatre catégories écologiques ; (iii) l'utilisation possible des traits morfo-anatomiques pour prédire l'impact des vers de terre sur l'infiltration de l'eau du sol et la compaction de la drilosphere. Les résultats de la deuxième expérience ont montré que la présence des espèces *A. zenkevichi* et *P. corethrurus* ensemble avait un léger effet synergique positif sur la résistance du sol à la perte d'eau par évaporation.

Pour conclure, ce travail montre que les vers de terre tropicaux peuvent être classés en catégories écologiques, mais que les liens avec le fonctionnement du sol ne sont pas toujours cohérents. Cette thèse met en évidence de nouvelles pistes de recherche basées sur les traits d'effet, qui peuvent être utilisés pour identifier les effets des vers de terre tropicaux sur la structure du sol et l'infiltration de l'eau.

Mots-clés : bioturbation ; drilosphère ; interactions interspécifiques ; propriétés hydrauliques

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CHAPTER 1 - GENERAL INTRODUCTION

Save the soil, save the future

Climate change represents a significant threat to ecosystems and human society (IPCC, 2021). Human activities contribute to the accumulation of greenhouse gases, which trap heat energy in the Earth's atmosphere (GISTEMP Team, 2023). The resulting global warming and climate change lead to unforeseen water stresses such as floods and droughts, which have a detrimental impact on agricultural productivity (Bai et al., 2022). These adverse effects further undermine food security and environmental sustainability (Lal et al., 2011). Consequently, there is growing concern about the future viability of soils as a sustainable resource for food production. To address the challenges posed by climate change and meet the global demand for food, a holistic approach to soil and water conservation is urgently needed. Soil hydraulic properties play a crucial role in maintaining the soil-water balance (Ramesh & Iqbal, 2020) and determining the soil's susceptibility to water stresses. Therefore, these factors should be considered as priority targets in current climate change adaptation efforts to reverse soil degradation.

Earthworms: silent and dedicated heroes

Earthworms are vital soil-dwelling creatures found in temperate and tropical regions across the globe. They play a crucial role in the ecosystem as they constitute a significant portion of the soil macrofaunal biomass, ranging from 40% to 90% (Fragoso et al., 1999). Earthworms are considered as ecosystem engineers due to their ability to physically modify their habitat (Jones et al., 1994). Their burrowing and feeding activities create a unique environment known as the drilosphere, consisting of burrows, casts, and compressed zones surrounding their burrows. These structures have a profound impact on the soil's hydraulic properties, influencing the regulation of water through transfer and storage processes (Bertrand et al., 2015; Capowiez et al., 2014b; Hallam & Hodson, 2020). Earthworm burrows serve as preferential pathways for water movement (Bottinelli et al., 2017; Capowiez et al., 2015; Bastardie et al., 2002), enhancing the rate of water infiltration into the soil. Besides altering soil macroporosity (burrows), earthworms affect soil mesoporosity and microporosity leading to changes in water retention in the soil through deposition of cast (Ernst et al., 2009; McDaniel et al., 2015) and creating a

zone of influence around burrow walls (Bastardie, et al., 2005b; Sander & Gerke, 2009). Additionally, earthworm activity affects evaporation through the presence of casts on the soil surface (Liu et al., 2021), and also contributes to the increase of surface roughness, which affect runoff and erosion (Andriuzzi et al., 2015; Blouin et al., 2013a; Jouquet et al., 2008a). Furthermore, earthworms indirectly impact soil structure and hydraulic properties by supporting the growth of plants (van Groenigen et al., 2014) and other soil organisms (Eisenhauer, 2010). The intricate relationship between earthworms and soil hydraulic properties has garnered significant interest, as understanding their influence can potentially be leveraged in mitigating the threats posed by climate change (Andriuzzi et al., 2015).

Earthworms and soil hydraulic properties: Knowledge gaps

Earthworms are classified into 23 families, and till now 5406 valid species have been described (Misirlioğlu et al., 2023). However, the impact of earthworms on soil hydraulic properties has only been studied for some species belonging usually to the Lumbricidae family (Blouin et al., 2013a), while the Megascolecidae family, which is the most abundant family on Earth (with 2051 out of 5358 species) (<http://taxo.drilobase.org>), has been comparatively ignored. Recognizing the fact that it is impossible to study all earthworm species, scientists often evaluate earthworm behaviour and their impact on the soil by assigning them into ecological categories proposed by Bouché (1977) (Bottinelli & Capowiez, 2021). However, the concept of ecological categories has been criticized because earthworms are grouped based on traits that may not necessarily be related to the target soil functions (Bottinelli & Capowiez, 2021; Lal et al., 2011; Neilson et al., 2000; Van Groenigen et al., 2019). As a result, many scientists have attempted to reevaluate or adjust the ecological categories of earthworms based on different characteristics, such as feeding behavior (Briones et al., 2005; Ferlian et al., 2014; Hsu et al., 2023; Neilson et al., 2000), burrowing behaviour (Felten & Emmerling, 2009) or body thickness (Briones & Álvarez-Otero, 2018). Therefore, it is crucial to establish empirical evidence for the relationship between ecological categories and soil hydraulic properties, especially for non-Lumbricidae species, as information on their ecological classification remains limited. Additionally, there is limited information on how competition and mutualism between earthworm species impact their burrowing behaviours and resulting soil hydraulic properties. The functional diversity among earthworms can lead to various types of interactions, including inhibitory, neutral, or facilitative interactions. Consequently, the consequences for soil functioning can be additive, non-additive, or idiosyncratic. Prioritizing

research on these aspects is essential to integrate earthworms, as well as other soil organisms, as tools for sustainable development.

Vietnam: Climate risk and biodiversity hotspot

Vietnam is an agricultural country with nearly half of its labor force involved in agriculture (Anh et al., 2022), and an important contributor to global food security (UN 2021 Food Systems Summit, 2021). However, the agricultural sector in Vietnam is facing a significant threat from climate change (World Bank Group & Asian Development Bank, 2021). In recent years, Vietnam has witnessed numerous extreme weather events, such as floods, storms, and droughts, which have had adverse effects on the economy and human health (Do et al., 2021).

In addition, earthworm diversity in Vietnam is exceptionally rich, with 245 species/subspecies described, and more than 50% of these species can only be found in Vietnam (Lam et al., 2021; Nguyen et al., 2020; Nguyen, et al., 2016a, 2016b, 2021a, 2021b, 2022a, 2022b). Furthermore, it is anticipated that more species will be discovered in Vietnam owing to its diverse habitats and unique geographical locations within the tropical region. This presents opportunities for the application of earthworms in soil conservation efforts in Vietnam. However, research on earthworms in Vietnam is relatively limited and primarily conducted by taxonomists.

The aim of this thesis

The overall aim of this thesis was to provide a better understanding of the impact of tropical earthworms on soil structure and water dynamics.

CHAPTER 2 - LITERATURE REVIEW

2.1. Earthworm classification

The identification of earthworms relies on specific morphological and anatomical features that differentiate various species or families using classification keys. Notable sources for classification keys include Blakemore (2000), Jamieson (1988), Sims & Easton (1972), and Sims & Gerard (1985). Currently, there are approximately 5,406 species and 332 subspecies identified across 382 genera (Misirlioğlu et al., 2023). Earthworms belong to 23 families, with the Megascolecidae family being the largest, housing 2,335 species or subspecies. Following closely behind are Acanthodrilidae with 794, and Lumbricidae with only 689 species or subspecies (Misirlioğlu et al., 2023). Earthworm can be also grouped into guilds according to their ecological strategies, based primarily on differences in burrowing and feeding activities, and their vertical distribution in soil. Örley (1885) categorized earthworms into three distinct groups based on their habitat and behavior: (i) large-bodied species penetrating to 90 – 120 cm deep in the soil; (ii) smaller species living in wood and other vegetable matter; and (iii) species adapted to clayey soil and riparian mud environments. Other authors distinguished British Lumbricidae into surface-dwelling species which are more pigmented and produce no burrows or distinct casts, and soil-dwelling species which are less pigmented and live within a defined burrow system (Evans & Guild, 1948; Graff, 1953; Satchell, 1980). Lee (1959) proposed three main ecological groups for New Zealand Megascolecidae species, which were categorized based on the soil horizons where the earthworms were commonly found. These categories included those inhabiting the litter (small and heavily pigmented), the topsoil (larger, pigmented, and living in permanent burrows), and the subsoil (larger, non-pigmented, and dwelling in deeper soil layers). Similarly, Perel (1975) divided earthworms into two main groups: one group feeds on the soil surface, and the other group feeds within the soil. Bouché (1972, 1977) classified European Lumbricidae based on the morphology, anatomy, diet, and reproduction into three main groups (i.e., epigeic, endogeic and anecic), and four intermediate categories (i.e., epi-anecic, endo-anecic, epi-endogeic, and intermediate). In this classification, the epigeic species are litter feeders living in the litter-soil interface, feeding mainly on fresh litter. They have small body sizes, and they have almost no burrowing activity. The endogeic species are soil feeders living in the upper mineral soil profile. They usually have a medium body size and create an extensive horizontal burrowing network. Anecic species move

throughout the soil profile, feeding mainly on litter in the organic soil layer and bringing their food to deep soil horizons. They have larger body sizes in comparison with the other groups, and they build vertical unbranching burrows. Later, Lavelle (1979) proposed that endogeic earthworm species could be divided into three subcategories based on the soil horizon they inhabit: polyhumic endogeic species ingest soil enriched in soil organic matter content close to the surface; mesohumic endogeic species feed both on mineral and organic particles in the upper soil (10 – 15 cm deep); and oligohumic species feed on soil from the deeper soil layer (30 – 40 cm deep) with poor organic matter content. While there are numerous methods for categorizing earthworms, the ecological classification produced by Bouché (1972, 1977) is the most commonly employed by taxonomists and ecologists. As a result, a simplified version containing only the three main categories, epigeic, endogeic, and anecic has been widely used to investigate how earthworms affect the soil function (Bottinelli & Capowiez, 2021). In the last 50 years, about 10% of the articles dealing with earthworms have cited at least one of the two main references (Bouché, 1972, 1977). However, assigning unknown species to one of the ecological categories proposed by Bouché remains challenging, mostly because Bouché did not really provide explicit assignment rules. In 2020, Bottinelli et al. (2020a) proposed a method to reconstruct and accurately define the three main ecological categories and four additional categories from only 13 morpho-anatomical traits instead of the 23 traits used by Bouché. According to this model, every new species for which the 13 traits are measured or estimated can thus be positioned in the triangle and assigned to an ecological category (assuming that this classification made mainly on Lumbricidae can be extended to other families).

2.2. Earthworm bioturbation: the drilosphere concept

Earthworms modify soil structure by moving surface detritus belowground, making burrows, and ingesting organic matter and mineral particles which are then egested in casts. The soil directly influenced by such activity forms the drilosphere. This concept was first introduced by Bouché (1975) to describe the 2 mm thick layer surrounding earthworm burrows. It was then broadened to encompass any part of the soil influenced by earthworm activity, including the internal micro-environment of the earthworm gut (Lavelle, 1988) and the externally produced earthworm structures such as their casts, middens and burrows (Lavelle, 1988; Brown, 1995; Brown et al., 2000; Schrader et al., 2007), as well as earthworm community and interactions with soil organisms (Lavelle, 1988). Thus, the meaning of this term becomes more ambiguous,

encompassing both the physical structures created by earthworms and the interactions with the surrounding soil. In this thesis, the term “drilosphere” describes the soil impacted by earthworms, which includes casts, middens, burrows, and lateral soil compaction.

- Casts (surface casts and belowground casts) result from the ingestion, digestion and excretion of organic matter and mineral soil, after passing through the earthworms' digestive tract onto the surface or within the soil (Whalen et al., 2004).
- Middens are small mounds that form when surface casts and organic materials accumulate around a burrow opening at the soil surface (Hamilton & Sillman, 1989; Nielsen & Hole, 1964). However, middens have only been observed with a few earthworm species, particularly species belonging to the epi-anecic group, such as *Lumbricus terrestris* (Stroud et al., 2016).
- Burrows or galleries are macropores created by the excavation process, which involves ingesting and casting or pushing the soil aside (Jégou et al., 2000). They can be permanent or temporary, open or filled with casts.
- The lateral soil compaction refers to the compacted zone surrounding burrows that is created by two processes: the pushing of soil aside during burrow formation (Jégou et al., 2000) and the filling of the burrows by casts and mucus (Capowiez et al., 2011; Piron et al., 2012).

While the soil drilosphere is commonly considered a biological and functional hot spot, our current knowledge about the physical structure of the drilosphere remains limited. Most of the research has focused on surface casts and burrows. Surface casts have been extensively studied, especially because they are easily observable and quantified. Thus, the production rate, distribution and properties are easily accessed. For instance, Bottinelli et al. (2021a) estimated that *A. adexilis* produced from 16 to 219 t soil ha⁻¹ year⁻¹ of surface casts in a catchment in Vietnam, a value close to the record measured in Nigeria by Madge (1969) with 250 t soil ha⁻¹ year⁻¹. Conversely, belowground cast production is less known, few publications carried out in the laboratory provide quantification (Francis et al., 2001; Whalen et al., 2004; Felten and Emmerling, 2009; Capowiez et al., 2011, 2014a; Le Couteux et al., 2015). The proportion of belowground casts to the total casts produced varies greatly among species. For instance, Whalen et al. (2004) found that belowground casts produced by *A. caliginosa*, *A. rosea*, and *L. terrestris* accounted for less than 10% of the total production, whereas James (1991) reported a figure of up to 98% for *A. caliginosa*, *O. cyaneum*, and *Diplocardia smithii*. Mariani et al. (2007) observed values ranging from 36% to 53% for the anecic species *Martodrilus sp.* Curry

& Baker (1998) estimated that *A. caliginosa*, *A. trapezoides*, and *A. longa* produced between 15% and 38% belowground casts. By grinding and mixing soil in their digestive tracts, earthworms completely destabilize the soil structure and contribute to the rearrangement of soil particles, thus modifying the bulk density of the cast compared to parent soil, ultimately affecting the bulk density of the resulting casts in comparison to the original parent soil. The surface casts may have different bulk density (Decaëns, 2000; Jouquet et al., 2008a), possibly higher (Decaëns et al., 2001; Oyedele et al., 2006; Le Mer et al., 2022), similar (Sauzet et al., 2021), or lower (Marashi & Scullion, 2003; Larink et al., 2001) than surrounding soil. However, the bulk density of the surface cast varies a lot depending on soil properties. The few studies comparing the porosity of surface casts of different species revealed contradictory results. Le Mer et al. (2022) showed that the volume of pores > 10 µm of freshly emitted surface casts was higher for *L. castaneus*, *E. fetida*, and *L. terrestris* than for *A. caliginosa*, *A. icterica* and *A. nocturna*. Capowiez et al. (2011) showed that the total pore volume in surface casts made by *A. chlorotica* was higher than that of *A. nocturna*. Larink et al. (2001) showed no difference in pore volume in surface casts made by *L. terrestris* and *A. caliginosa*. Similarly, Le Bayon et al. (2021) showed similar total pore volume in surface casts made by *L. rubellus*, *L. terrestris* and *A. chlorotica*. In contrast to the wealth of information regarding the bulk density of surface casts, information about the bulk density of belowground casts is quite scarce. Capowiez et al. (2011) using X-ray tomography, showed higher grey values, indicating denser material, in zones under the physical influence of *Aporrectodea nocturna* and *Allolobophora chlorotica* compared to the surrounding soil. However, the authors were unable to clearly distinguish between belowground casts and lateral soil compaction due to the similarity in grey values between these two components. Similarly, Le Bayon et al. (2021) have also shown that belowground casts formed by *L. rubellus*, *A. chlorotica*, or *L. terrestris* have bulk densities of 1.45, 1.39, and 1.38 g cm⁻³, respectively, which are higher than the 1.27 g cm⁻³ of aggregates in the control treatment without earthworms.

The volume and structure of burrows made by many species have also been described in detail for a long time through manual excavations (Ligthart & Peek, 1997), 2D terraria (Capowiez, 2000; Capowiez & Bérard, 2006; Felten & Emmerling, 2009) and more recently through 3D reconstruction using X-ray tomography (Joschko et al., 1991, 1993; Bastardie et al., 2003, 2005a; Capowiez et al., 2003, 2014b, 2015; Lamandé et al., 2003; Bottinelli et al., 2017). The characteristics of burrows exhibit significant variation among species. However, due to the large number of earthworm species, it is often impractical to study the burrow

systems of each species. Instead, they are typically assessed in a generalized manner based on the ecological categories they are assigned to. Accordingly, anecic species are known to live in permanent vertical burrows (Jégou et al., 2002; Butt et al., 2003; Field and Michiels, 2006; Capowiez et al., 2009), endogeic species make extensive and temporary burrows (Francis et al., 2001), and epigeic species build few and thin burrows close to the surface (Felten & Emmerling, 2009). Within the anecics, two sub-categories can be distinguished: epi-anecic burrow few galleries, whereas strict anecics burrow a larger network of galleries (Bastardie et al., 2005a; Jégou et al., 1998, 2001a).

Comparatively, there is a dearth of information regarding the properties of the lateral soil compaction around the burrows. Earthworms burrow into the soil by pushing soil aside and by ingesting and egesting soil. This bioturbation activity results in a radial and axial displacement of soil (Barnett et al., 2009). Earthworms generally exert significantly higher pressures radially than axially due to the longitudinal muscle contraction (Barnett et al., 2009; Keudel & Schrader, 1999) which causes soil compaction around their burrows. Consequently, the lateral soil compaction is characterized by a higher bulk density in comparison to the surrounding soil (Bastardie, et al., 2005b; Capowiez et al., 2011; Görres et al., 2001; Rogasik et al., 2003, 2014; Schrader et al., 2007). In addition, the structure of this zone lacks homogeneity. The decrease in bulk density extends from the burrow walls into the surrounding bulk soil due to the reduction in compacting forces caused by earthworms decreasing as the distance from burrow walls increases (Rogasik et al., 2014). However, these conclusions come from only a limited number of studies, which have been assessed using only a limited number of Lumbricidae species: *Lumbricus terrestris* (Bastardie et al., 2005b; Binet & Curmi, 1992; Görres et al., 2001; Haas & Horn, 2018; Jégou et al., 2001b; Rogasik et al., 2003, 2014; Schrader et al., 2007), *Aporrectodea caliginosa*, *Allolobophora icterica*, *Aporrectodea nocturna* (Capowiez et al., 2011, 2021) and *Aporrectodea longa* (Barnett et al., 2009).

2.3. The influence of earthworms on soil water transfers

2.3.1. Earthworms and soil water infiltration

As explained previously, earthworms burrow through the soil by pushing soil aside and by ingesting and egesting casts and at the same time displace the soil radially and axially (Barnett et al., 2009). This behavior led to the formation of large macropores (i.e., burrows) but at the same time can increase the local soil compaction within casts (Puche et al., 2022) or at the

vicinity of burrows (Rogasik et al., 2014). Large macropores made by burrows can act as preferential flow pathways for water (Bastardie et al., 2002; Bottinelli et al., 2017; Capowiez et al., 2015; Hallam et al., 2021) while smaller pores between/within casts or at the vicinity of the burrows can influence water infiltration in when soil is non-saturated (Bastardie et al., 2005b; Hallam et al., 2021; Sander & Gerke, 2009). Under unsaturated conditions, the diameter and connectivity of burrows become of utmost importance and the entire burrow system can improve the efficiency of water infiltration. When the burrows are filled with water, the water flows through the burrow walls and through the soil matrix with the flux of water depending not strictly on burrow diameter (Singh et al., 2013) but also characteristics of the vicinity of the burrows.

A literature review carried out in December 2022 and using Web of Science with the following search terms and selecting the timespan “all years”: TS = ((hydraulic* OR infiltration* OR hydrological* OR hydrodynamic* OR water retention* OR moisture*) AND earthworm* NOT vermi*) showed a record track of 826 articles. The articles were screened based on the criterion that soil water infiltration was reported for treatments with and without earthworms. Most of the studies meeting the criteria focused on water infiltration under saturated conditions (details concerning the meta-analysis are provided in supplementary S1). Interestingly, studies focused only on a few well-known earthworm species such as *Lumbricus terrestris*, *Allolobophora chlorotica*, *Aporrectodea caliginosa*, and *Lumbricus rubellus* belonging to the family Lumbricidae (Figure S1.1). The most commonly studied categories were endogeics, followed by anecics and epigeics, with few studies focusing on sub ecological categories (Figure S1.2). Furthermore, this meta-analysis revealed that were very few studies conducted on the interactions between different species (Figure S1.2). The effect of earthworms on water infiltration was calculated using the logarithm response ratio (LRR) between the soil saturated hydraulic conductivity (K_s) with earthworms and without earthworms. On average, LRR was positive (0.75) and the 95% confidence intervals ($CI_{0.95}$) ranged from 0.48 to 0.97 (Figure S1.2). However, the extent of this impact varied depending on the species and ecological category. The overall mean LRR decreased in the following order: anecic > endogeic > epi-endogeic > epigeic > endo-anecic. However, there was no difference between these categories, as indicated by the overlap of $CI_{0.95}$, and there was no significant heterogeneity in the data (Q-test, $p = 0.22$) (Figure S1.2).

2.3.2. Earthworms affect surface runoff and soil erosion

Earthworms affect surface runoff and lead to changes in soil erosion through three processes: (i) Firstly, earthworms modify soil porosity which directly influences soil water infiltration (as mentioned above); (ii) Secondly, earthworms increase surface roughness by depositing casts at the soil surface, acting as a physical brake that affects water runoff velocity and infiltration into the soil (Blanchart et al., 2004; Jouquet et al., 2004, 2008b, 2010; Le Bayon & Binet, 2001); (iii) and finally, they influence, usually positively, the stability of soil aggregates. The higher clay content in casts (Van Groenigen et al., 2019) acts as a binding agent and the higher content of cations such as Ca^{2+} (Oyedele et al., 2006; Van Groenigen et al., 2019) forms bridges between mineral and organo-mineral particles during the formation of aggregates. The internal secretion of mucus by earthworms also acts as a hydrophobic coating preserving the stability of casts (Chenu et al., 2000). Finally, the compression caused by the muscles in the tail region (McKenzie & Dexter, 1987) explains the increasing stability of aggregates. This helps the soil surface to resist to the impact of raindrops and reduces soil erosion. For instance, Wen et al. (2022) found that *Metaphire guillelmi* activity reduced runoff by up to 61.6% compared to control treatments. In Vietnam, casts produced by *Amyntas khami* reduced water runoff by 75% under experimental conditions (Jouquet et al., 2008b). Shuster et al. (2002) also showed that the presence of earthworms can decrease soil erosion by up to 50%. On the other hand, earthworms can produce small and isolated casts that have lower soil structural stability compared to the surrounding soil aggregates (Blanchart et al., 2004). These casts can disintegrate when subjected to raindrop impact, potentially leading to increased soil erosion. For example, surface casts produced by the *Metaphire guillelmi* were completely dispersed, leading to an increase in erosion of up to 169% (Wen et al., 2022). Similarly, Le Bayon et al. (2002) showed that earthworm casts might increase soil detachment during rainstorm events in Brittany. In another example, in laboratory experiment, Jouquet et al. (2013) reported that casts formed by *M. posthuma* increased soil detachment and erosion. Additionally, freshly excreted casts often have low water stability. They are fragile and can be quickly scattered, causing erosion to intensify. As an illustration, when fresh casts of *M. anomala* lacked vegetation cover, they vanished following an 18 mm rainfall event, whereas those shielded by vegetation remained intact for several months (Blanchart, 1990).

2.3.3. Earthworms and soil water storage

Through their burrowing activity, earthworms do not only create water pathways into the soil, but also create pockets of water storage in the soil at deeper soil layers. Indeed, water can be held by the soil matrix through the penetration of water into the earthworm burrow walls. However, increased soil porosity and the presence of water vapor channels can also lead to higher evaporation rates, resulting in a reduction in soil water storage (Ernst et al., 2009). Furthermore, earthworms have been found to increase the formation of water-stable aggregates in the soil (Bossuyt et al., 2005; Buck et al., 2000). The arrangement of these aggregates creates more possibility for water to be held by capillary forces, thereby enhancing soil water storage. Numerous studies have shown that a higher proportion of water-soluble aggregates is associated with greater water retention capacity (Basche et al., 2016; Franzluebbers, 2002; Suzuki et al., 2007; Zibilske & Bradford, 2007). Similarly, Hallam & Hodson (2020) reported that although differences in the magnitude of the impact reported for *L. terrestris* and *A. chlorotica*, a consistent linear regression was described between the percentage of water stable aggregates and water storage capacity. On a smaller scale, the presence of small pores within the cast may enhance the retention of plant-available water more effectively than soil aggregates (Hindell et al., 1997; Lipiec et al., 2015). Additionally, the enrichment in organic matter (Bottinelli et al., 2020b; Le Mer et al., 2022) and in clay in casts (Asawalam & Johnson, 2007; Van Groenigen et al., 2019) can also enhance the water holding capacity of soil by increasing the water absorption capacity of soil. Moreover, when deposited at the soil surface, these casts form a barrier that can improve soil moisture by retarding soil evaporation (Hallam & Hodson, 2020; Liu et al., 2021).

2.4. Trait-based approach to predicting the impact of earthworms on soil functions

Regarding the controversial and highly variable effects of earthworms on water dynamics, recent studies suggest grouping earthworms based on the ecological functions they fulfil. This question is crucial in ecology and in environmental sciences. A common approach relies in assigning species to groups relies on the “traits” (box 1) they share (Brussaard, 1997). Bouché’s ecological groups (Bouché, 1977) are the most widely used. Only based on the morphology and anatomy (box 1) of earthworms, they were first used to understand the impact of the environment on earthworm diversity and abundance, and later, by extension, for understanding the impact of the functional diversity on soil functioning. As a result, since the 2000s, the term

"functional group" has been increasingly used to refer the three main ecological categories (Bottinelli & Capowiez, 2021). However, there are no sharp boundaries between these ecological categories and intermediate forms are numerous (Bottinelli et al., 2020a). Although the authors demonstrated significant variability within the three main ecological categories, such as Bastardie et al. (2005a) for burrowing behaviour, many others have also pointed out unexpected similarities among these categories, e.g., Van Groenigen et al. (2019) for cast fertility or Neilson et al. (2000) for food preference. This underscores the importance of thoroughly testing and validating the connections between ecological categories and soil functioning if we expect meaningful results to ensue.

On the other hand, the functional trait (box 1) approach is currently employed in various fields of environmental studies, such as plant ecology (Cadotte et al., 2008; Lavorel & Garnier, 2002; Wright et al., 2005), animal studies (Archaimbault et al., 2010; Luck et al., 2012; Mims et al., 2010; Moretti et al., 2017; Pey et al., 2014), and research on microbes (Larkin & Martiny, 2017). Functional trait approaches are based on objective relationships between individual properties and soil functioning. The functional trait approach is not hampered by taxonomic difficulties and it can be used with individuals that are difficult to identify (Carmona et al., 2016). In other research fields, notably for plants, functional approaches have brought several new insights to the understanding of how organisms affect their environment (Bardgett, 2017). Conversely, this approach was little applied on soil ecology. Studies measuring functional traits of earthworms and relate them soil functioning are rare. The very limited number of studies showed that (i) the axial and radial pressure on soil generated during earthworm burrowing can be linked with anterior muscle development (Keudel & Schrader, 1999), (ii) the vertical distribution of earthworms can be linked to the size of earthworms (Jiménez & Decaëns, 2000), (iii) the soil structure affected by earthworms is linked to the typhlosole type, gizzard size and septa thickness (Marichal et al., 2017), and (iv) the capacity of earthworms to dig into deep horizons can be related to the thickness of their tegument (Briones & Álvarez-Otero, 2018). However, none of the abovementioned studies explicitly tested the links between earthworm traits and the studied soil processes, except for the study of Marichal et al. (2017). Consequently, it is currently impossible to make general conclusions about the importance of earthworm traits in characterizing soil functions.

Box 1. Key definitions

Traits are defined as morphological, anatomical, physiological, phenological or behavioural characteristics that are measurable at the individual level, from the cell to the whole organism (Violle et al., 2007).

Morphological traits are colour, form, structure, and external characteristics of organisms.

Anatomical traits are the internal structure and organization of organisms.

Functional traits are traits that determine how it influences an ecosystem process (**effect traits**), or how it responds to a change in environmental conditions (**response traits**) (Díaz et al., 2013; Violle et al., 2007).

2.5. Interacting effects of earthworms on soil drilosphere and water transfers

The impact of earthworms on soil water transfers is known to vary considerably between species and/or ecological categories (Capowiez et al., 2015; Ernst et al., 2009). Conversely, the importance of the interspecific interactions on soil water transfers is totally ignored, although coexistence of species in the same habitat can lead to significant changes in their bioturbating activities. In the presence of anecic species, the volume/area of burrows made by endogeic species (Capowiez et al., 2001; Felten & Emmerling, 2009) may decrease and their activity depth (Jégou et al., 2001a) may change, leading to altered interactions. The authors hypothesized that endogeic species use burrows made by anecic species to feed on belowground casts or on litter roped within their burrows, meanwhile burrowing activity of anecic species are not affected. An example of direct interactions is the competition for the resources or habitat when two endogeic species sharing similar ecological niches are present together leading to a decrease in their burrowing activities (Felten & Emmerling, 2009). We consider that future research in earthworm ecology and the need to understand their impact on water transfers are required due to the increasing frequency of flood and drought events.

2.6. Earthworms in Vietnam

Vietnam is a tropical country ranked as the 16th most biodiverse country in the world. Taxonomy reported that Vietnam has 245 species of earthworms belonging to 24 genera and 8 families, with more than 50% species found only in Vietnam (Lam et al., 2021; Nguyen et al.,

2020; Nguyen et al., 2016a, 2016b, 2021a, 2021b, 2022a, 2022b). Among the 8 families, the Megascolecidae is the most dominant, comprising 224 species/subspecies, followed by Ocnerodrilidae (7 species), Octochaetidae (5 species) and Moniligastridae (7 species), Lumbricidae (2 species), Almidae (1 species), Eudrilidae (1 species), and Rhinodrilidae (1 species) (Lam et al., 2021; Nguyen et al., 2020; Nguyen et al., 2016a, 2016b, 2021a, 2021b, 2022a, 2022b). However, research on earthworms in Vietnam is relatively limited and primarily conducted by taxonomists. Only 70 results were found on Web of Science using the keywords "Earthworm* AND Vietnam*". Among these, very few studies deal with bioturbation and the impact of earthworms on soil water dynamics. Indeed, as far as we know, only *Amyntas adexilis* and *Metaphire posthuma* have been studied (Bottinelli et al., 2010, 2021a, 2021b; Jouquet et al., 2008a, 2008b, 2012a, 2012b, 2013; Le Mer et al., 2021). In addition, it is worth mentioning that most of the earthworms found in Vietnam have not yet been assigned into ecological categories.

2.7. Research objectives and thesis outline

2.7.1. Research objectives

The overall aim of this thesis was to provide a better understanding of the impact of tropical earthworms on soil structure and water characteristics. To this end, the thesis focuses on investigating two specific research objectives outlined in Figure 1.

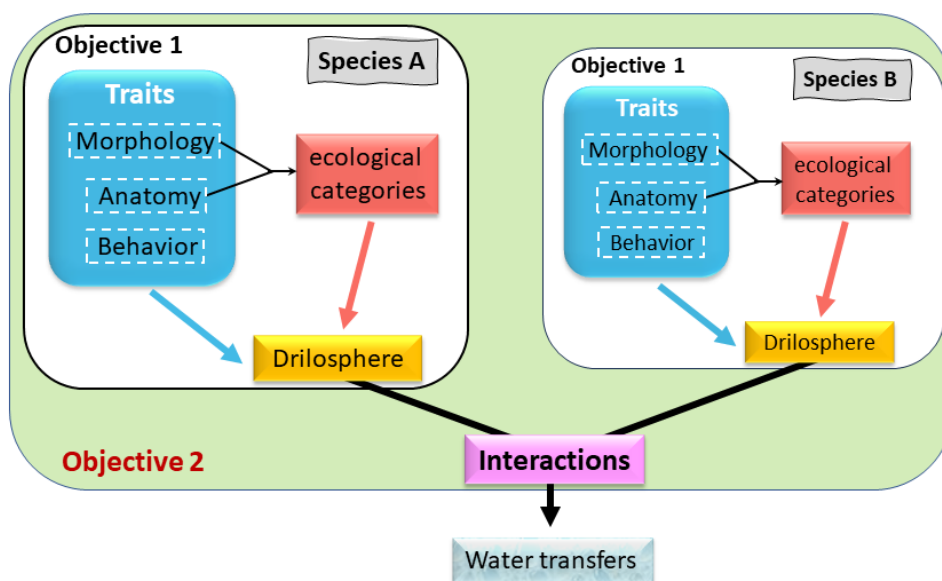


Figure 1. Diagram presenting the two objectives of the thesis. The red and blue arrows correspond to the ecological categories approach and functional traits approach, respectively, employed to predict the impact of earthworms on soil functions.

The primary objective of this thesis was to compare the effectiveness of the “traditional” ecological category approach and the functional trait approach to assess the impact of earthworms on soil structure and water infiltration. We collected a total of 23 earthworm species from northern Vietnam and conducted laboratory experiments to measure their effects on soil porosity, compaction and water infiltration. Additionally, for each species, we measured a range of morphological and anatomical traits that could potentially explain burrowing behaviors. We tested the hypothesis that the functional traits would be a better proxy than the “traditional” ecological categories for assessing the impact of earthworms on soil structure and water infiltration.

The second objective was to explore how species interactions affect their burrowing behavior and soil water. To this end, we used a mesocosm experimental approach that allowed to investigate the interaction between the anecic *Amyntas zenkevichi* and the endogeic *Pontoscolex corethrurus*. We hypothesized that if two species compete for the same resources or habitat, their burrowing activities would likely change, consequently affecting soil water dynamics.

2.7.2. Thesis outline

The manuscript is organized into five chapters followed by the general conclusions and perspectives on the work. The content of each chapter is described as follows:

Chapter 1 provides a general introduction regarding the capacity of earthworms to impact soil water regulation by altering soil physical properties, which is crucial for mitigating extreme events induced by climate change. Furthermore, it addresses the general knowledge gaps regarding earthworms and soil hydraulic properties, particularly in tropical regions.

Chapter 2 gives a comprehensive literature review on the different way to gather earthworms into ecological categories, as well as on how earthworms influence soil structure and water. It outlines the current state of knowledge and highlights relevant knowledge gaps, ultimately leading to the definition of the research objectives for this thesis.

Chapter 3 presents the materials and methods employed in this thesis. It details two mesocosm experiments (incubation of single species or multi-species) conducted under laboratory conditions to address thesis objectives.

Chapter 4 and Chapter 5 respectively present the results and discussions. The assignment of Vietnamese species into ecological categories is first described, followed by the drilosphere structure of individual earthworm species and its impacts on water infiltration. Subsequently, the relationship between drilosphere structure and water infiltration with ecological categories or earthworm traits is examined. Finally, the discussion focuses on the interaction between two earthworm species, which leads to changes in their drilosphere structure and soil water dynamics.

Chapters 3, 4, and 5 are based on the work contained in three research articles (one published and two under review), as detailed in Table S2.

The chapter 6 summarizes the main findings of this thesis, provides the limitations of the study, and prospects for future research.

CHAPTER 3 - MATERIALS AND METHODS

3.1. Earthworm sampling and trait measurements

23 earthworm species were collected by hand-sorting or electric stimulation in soils from different localities in northern Vietnam (Table 1 and Fig. 2). Adult earthworms were first grouped into morphospecies according to their size, colour, pigmentation, and genital markings. For each morphospecies, three individuals were preserved in a 4% formaldehyde solution for identification and quantification of morpho-anatomical traits. Earthworm specimens were identified by two taxonomists from the Institute of Ecology and Biological Resources Vietnam and the Department of Biology, School of Education, Can Tho University, Vietnam. Earthworm's characteristics, including the prostomium shape, clitellum type, setae arrangement, first dorsal pore location, male pores, genital markings, spermathecal pores, septa thickness, intestinal origin, the intestinal caeca, last heart, ovaries and testes, were observed and compared with the relevant literature (Chen, 1933, 1946; Gates, 1972; Pham, 1995; Sims & Easton, 1972; Thai, 1982, 1983, 1984, 1987; Thai & Le, 1993) to identify species. Among the 23 specimens, 16 were identified at the species level, whereas the 7 others were probable new species and were only identified at the genus level (they were named using their genera and sp. 1, 2, and so on).

Table 1: List of collected species and description of the ecological category and locality.

Species	Ecological category	Locality	Land use	Soil type (*)
<i>Amyntas acalifornicus</i>	Unknown	21°17' N 105°49' E	Forest	Acrisols
<i>Amyntas alluxus</i>	Unknow	20°56' N 105°29' E	Forest	Acrisols
<i>Amyntas aspergillum</i>	Anecic ^a	20°57' N 105°29' E	Cropland	Acrisols
<i>Amyntas brevicapitatus</i>	Unknown	21°17' N 105°50' E	Grassland	Acrisols
<i>Metaphire acampanulata</i>	Unknown	20°57' N 105°29' E	Forest	Acrisols
<i>Amyntas leucocircus</i>	Unknown	21°16' N 105°50' E	Cropland	Acrisols
<i>Amyntas morrissi</i>	Epi-endogeic ^b	20°57' N 105°29' E	Forest	Acrisols
<i>Amyntas ophthalmopapillatus</i>	Unknown	20°15' N 105°42' E	Forest	Acrisols
<i>Amyntas robustus</i>	Endogeic ^c	20°37' N 105°35' E	Grassland	Acrisols
<i>Amyntas sp1</i>	Unknown	21°17' N 105°49' E	Forest	Acrisols
<i>Amyntas sp2</i>	Unknown	20°15' N 105°42' E	Forest	Acrisols
<i>Amyntas sp3</i>	Unknown	20°21' N 106°32' E	Cropland	Acrisols
<i>Amyntas sp4</i>	Unknown	20°15' N 105°42' E	Forest	Acrisols
<i>Amyntas sp5</i>	Unknown	20°19' N 105°37' E	Forest	Acrisols
<i>Amyntas sp6</i>	Unknown	20°56' N 105°29' E	Forest	Acrisols
<i>Amyntas tienphucanus</i>	Unknown	21°27' N 105°38' E	Forest	Acrisols
<i>Amyntas triastriatus</i>	Unknown	21°04' N 105°46' E	Garden	Fluvisols
<i>Amyntas vuongmontis</i>	Unknown	21°27' N 105°38' E	Forest	Acrisols
<i>Amyntas zenkevichi</i>	Unknown	20°44' N 106°17' E	Grassland	Cambisols
<i>Metaphire posthuma</i>	Endogeic ^d	21°04' N 105°46' E	Garden	Fluvisols
<i>Metaphire sp7</i>	Unknown	20°19' N 105°38' E	Forest	Acrisols
<i>Polypheretima elongata</i>	Endogeic ^e	20°44' N 106°05' E	Cropland	Cambisols
<i>Pontoscolex corethrurus</i>	Endogeic ^f	20°57' N 105°29' E	Cropland	Acrisols

^a (Liu et al., 2021)

^b (Zhang et al., 2016)

^c (Lin et al., 2016)

^d (Bottinelli et al., 2010)

^e (Rossi et al., 1997)

^f (Marichal et al., 2010)

(*) : World Reference Base (2006) Soil Groups (<https://soilgrids.org/>)



Figure 2. Images of 23 earthworm species collected in northern Vietnam. The black line represents 1 cm.

Traits *a priori* linked to the feeding, vertical distribution or burrowing behaviour were measured on three or four individuals per species. The weight, diameter, length, pigmentation, gradient of coloration and firmness were measured on alive earthworms that were incubated in soil cores (n=4) while the other traits were measured on dead individuals preserved in a 4% formaldehyde solution (n=3). The body's weight, length and diameter are rough estimators of earthworm strength. The diameter and length were measured with a vernier calliper on earthworms placed in cold water to reduce their activity. The pigmentation facilitates homochromy and provides visual protection from predators. This can be an indicator of the vertical location of earthworms. Earthworms were classified as pigmented or non-pigmented. In addition, pigmented species can have a colouration gradient that can be antero-posterior or dorso-ventral (Fig. 3a). The firmness of the tegument indicates the overall earthworm strength. By squeezing the earthworms between two fingers, the firmness was classified as firm or soft. The presence of longitudinal and transversal furrows, which are marks resulting from the contraction of earthworms for locomotion, was different between ecological categories (Bottinelli et al., 2020a) (Fig. 3b). The setae are chitinous structures that help earthworms grip when moving through the soil. The number of setae was counted under a stereomicroscope on segment xxx. The septa separate segments from each other, and their thickness can be associated with the capacity of earthworms to burrow the soil (Marichal et al., 2017). Septa thickness was assessed between septum xiii and xiv and classified as absent, thin or thick (Fig. 3c). The tegument thickness (cuticle + epidermis) (Fig. 3d) is expected to have a key role in determining the degree of earthworm resistance to water loss and therefore might be an indication of the vertical distribution of earthworms (Briones & Álvarez-Otero, 2018). The thickness of the longitudinal and circular muscles was also investigated as an indicator of the strength of earthworms to burrow the soil. The structure of the longitudinal muscle was determined and assigned as intermediate or fasciculated (Fig. 3e). All the measurements were performed on thin sections in the precuticular zone at segment viii. Transverse body sections were processed using the classical haematoxylin and eosin staining method (Feldman & Wolfe, 2014). Selected segments were cleaned and dehydrated using graded ethanol concentrations. Segments were embedded with paraffin and then cut using a Sakura Accu SRM 200CW microtome. For the thickness of the tegument and both muscles, measurements were performed at 5 random locations and averaged. The typhlosole is one or more longitudinal folds of the dorsal intestinal wall, which increases the functional surface of the intestine. According to Perel (1977), greater typhlosole development is found in species that feed on lower-quality diets. When present, the typhlosole was classified as lamelliform, bifid or trifid (Fig. 3f).

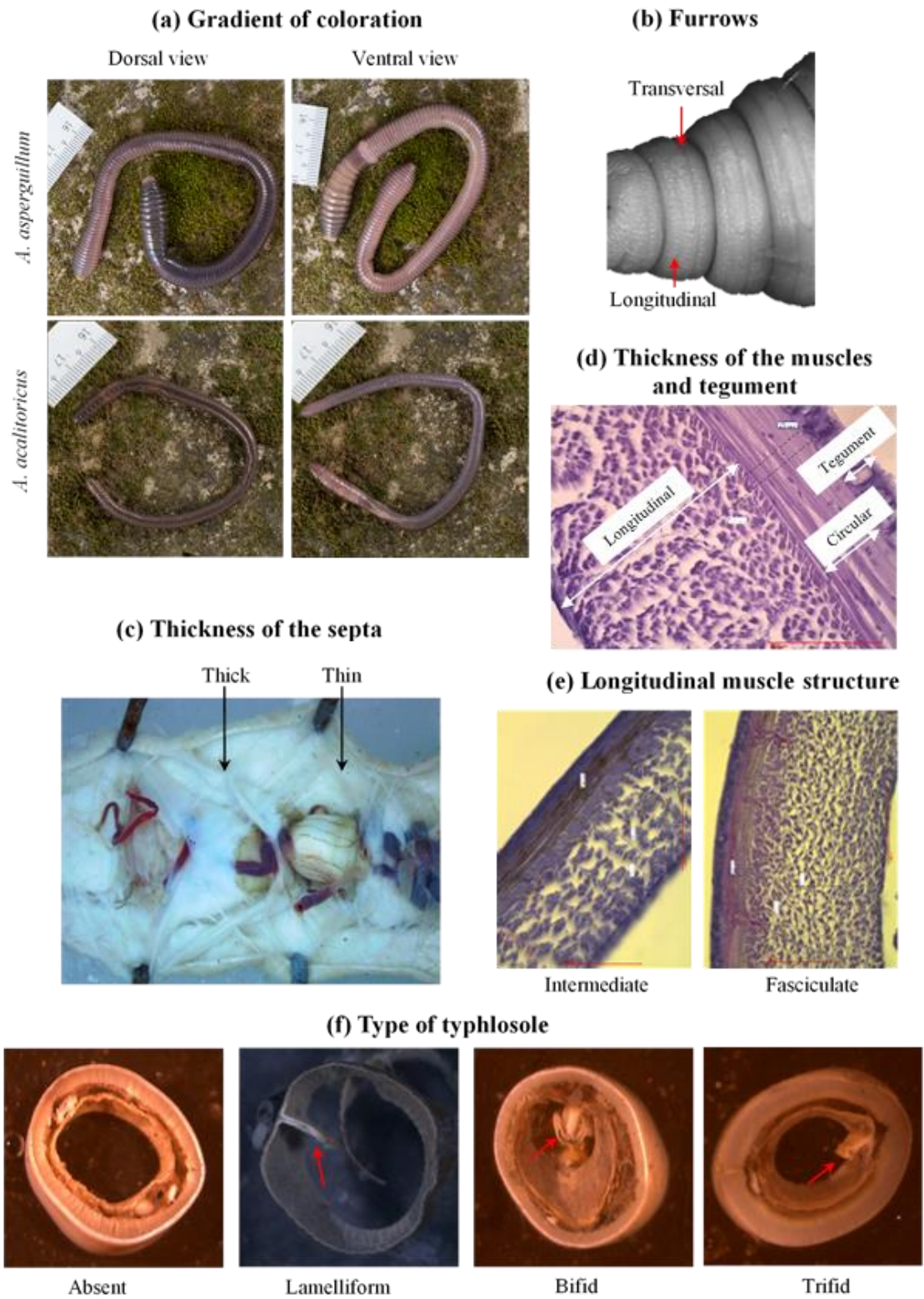


Figure 3. Pictures showing several morpho-anatomical traits measured.

3.2. Soil properties

The soil was taken from the top 10 cm of an Acrisols (20°57' N 105°29' E) and a Fluvisol (21°04' N 105°46' E). The Acrisols was characterized by a soil organic carbon content of 1.7%, a clayey texture (58% clay, 25% silt and 17% sand) and a pH_{KCl} of 4.6 (Doan et al., 2021). The Fluvisol was characterized by a soil organic carbon content of 2.4%, a loamy texture (13% clay, 50% silt and 37% sand) and a pH_{KCl} of 7.1 (Bottinelli et al., 2010). Both soils were sieved at 2 mm and homogenized and kept in plastic bags to prevent evaporation before being used to create mesocosms.

3.3. Experimental design

For addressing the research objectives, two mesocosm experiments under laboratory conditions were carried out:

- (i) Experiment 1 (Exp.1): Investigation of the drilosphere properties and the influence on soil water transfers of the 23 tropical species.
- (ii) Experiment 2 (Exp.2): Determine the interactions between *A. zenkevichi* (anecic species) and *P. corethrurus* (endogeic species) in an assemblage and its effect on water transfers. Both of these species are widely distributed in northern Vietnam (Nguyen et al., 2016b) and are frequently found coexisting at sampling sites in Exp.1. Moreover, despite being classified as an endogeic species, *P. corethrurus* exhibits activity predominantly near the soil surface. As a result, its activity competes for food resources with *A. zenkevichi*, potentially causing changes in the bioturbation behavior of both species and consequently impacting water transfers.

The design of these two experiments is presented in detail in the following sections.

3.3.1. Mesocosms preparation

Mesocosms were made of polyvinyl chloride cores (36 cm in height) filled with sieved soil packed with a hydraulic press to reach a dry bulk density similar to that found in the field (i.e., 1 g cm^{-3} for the Acrisols and 1.2 g cm^{-3} for the Fluvisol) and remoistened at 40% gravimetric water content for the Acrisols and 25% for the Fluvisol. To reduce variations in soil bulk density between the top and bottom of the cores, the soil was compacted stepwise in ten layers

to reach 30 cm height. For Exp.1, the size of cores was 15 cm in diameter, while it was 20 cm for Exp.2.

3.3.2. Earthworm incubation

In Exp.1, one individual or no earthworm as a control were introduced in each core. 21 earthworm species were incubated in the Acrisols, while 2 species (*Amyntas triastriatus* and *Metaphire posthuma*) were incubated using in the Fluvisol. In total, 102 cores corresponding to 4 cores per species and 10 cores without earthworms were be prepared.

In Exp.2, *Amyntas zenkevichi* (2 individuals), *Pontoscolex corethrurus* (6 individuals), or their assemblage (1 individual of *Amyntas zenkevichi* + 3 individuals of *Pontoscolex corethrurus*) (~3 g for each combination) were incubated in the Acrisols. In total, 18 cores corresponding to four cores per monospecific treatment (single species), four cores per bispecific treatment (assemblage) and four cores without earthworms were be prepared.

The food source for earthworms in Exp.1 and Exp.2 was buffalo dung. Buffalo dung was air-dried, grinded, remoistened and added to the top of the cores and was regularly replenished according to the consumption of the earthworms. For Exp.1, the incubation lasted four weeks, while for Exp.2, the incubation lasted 92 days. During the incubation, microcosms were kept at 25 °C in the dark. Every week, soil moisture was maintained by adding water according to the weight loss of mesocosms for Exp.1. For Exp.2, soil columns were watered 11 times at days 13, 24, 31, 37, 45, 51, 58, 65, 72, 79 and 86 by pouring same amount of water in all columns. At the end of the incubation, the earthworms in the mesocosms in Exp.1 were eliminated by adding 10 ml of chloroform. Subsequently, they were left to decompose for two weeks before the column was scanned and its water infiltration was measured. In Exp.2, soil column was scanned and water infiltration was measured immediately after collecting the residual buffalo dung and casts on soil surface at the end of experiment. Earthworms in each column were collected to quantify their weight.

3.3.3. The consumption of buffalo dung and production of surface cast

At the end of the experiment (both Exp.1 and Exp.2), the casts produced on soil surface were collected and manually eliminate the adhered soil aggregate and organic debris and then air-dried for 1 week. The buffalo dung that was not buried was collected and air-dried. The total

food consumption of buffalo dung corresponded to the air-dried amount of dung added minus the amount remaining on the soil surface at the end of the experiment.

3.4. Image analysis

3.4.1. X-ray photography and image preprocessing

Soil cores and surface casts in Exp.1 and soil cores in Exp.2 were scanned using X-ray computed tomography by Siemens Somatom® Definition Flash at Bach Mai Hospital (Hanoi, Vietnam) and Siemens Somaton go.Top at Medlatec hospital (Hanoi, Vietnam), respectively. The scanner settings were 120 kV and 93 mA, and the acquired images were in DICOM format (16-bit, 512×512 pixels). Images were then transformed into 8-bit images and rendered isotropic with a pixel size of 0.3 mm for Exp.1 and 0.45 mm for Exp.2 using Fiji/ImageJ (Schindelin et al., 2012).

3.4.2. Extraction and quantification the different components of the drilopshere

3.4.2.1. Experiment 1

+ Extraction the different components of the drilopshere

The software Avizo® (Thermo Fisher Scientific) were applied to extract the different parts of the drilopshere and visualized them. Empty burrows and compaction zones (belowground casts and lateral soil compaction) were extracted from the soil matrix with the workflow described in Figures 4, 5 and 6. These parts were combined to represent the whole volume of the subsurface drilosphere (Fig. 7) and skeletons were drawn either from the whole subsurface drilosphere or the empty burrows (Fig. 8).

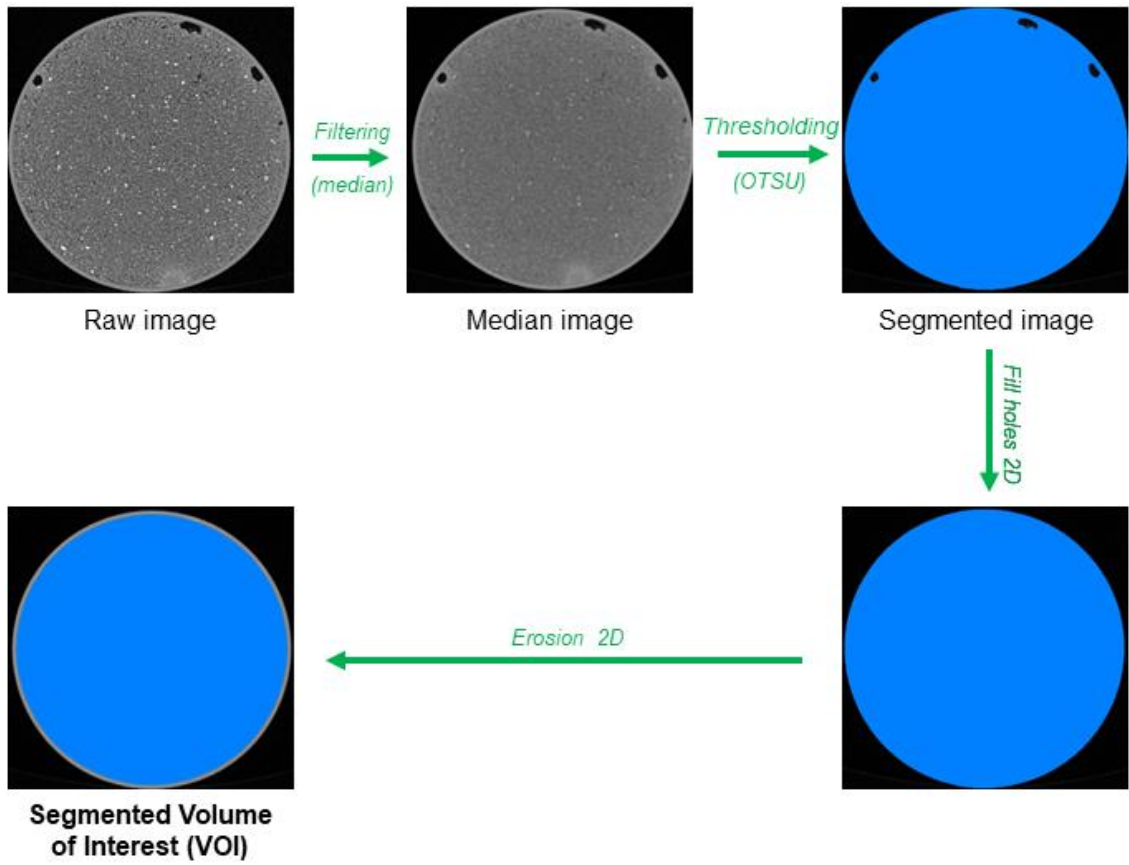


Figure 4. Workflow to get the volume of interest (VOI).

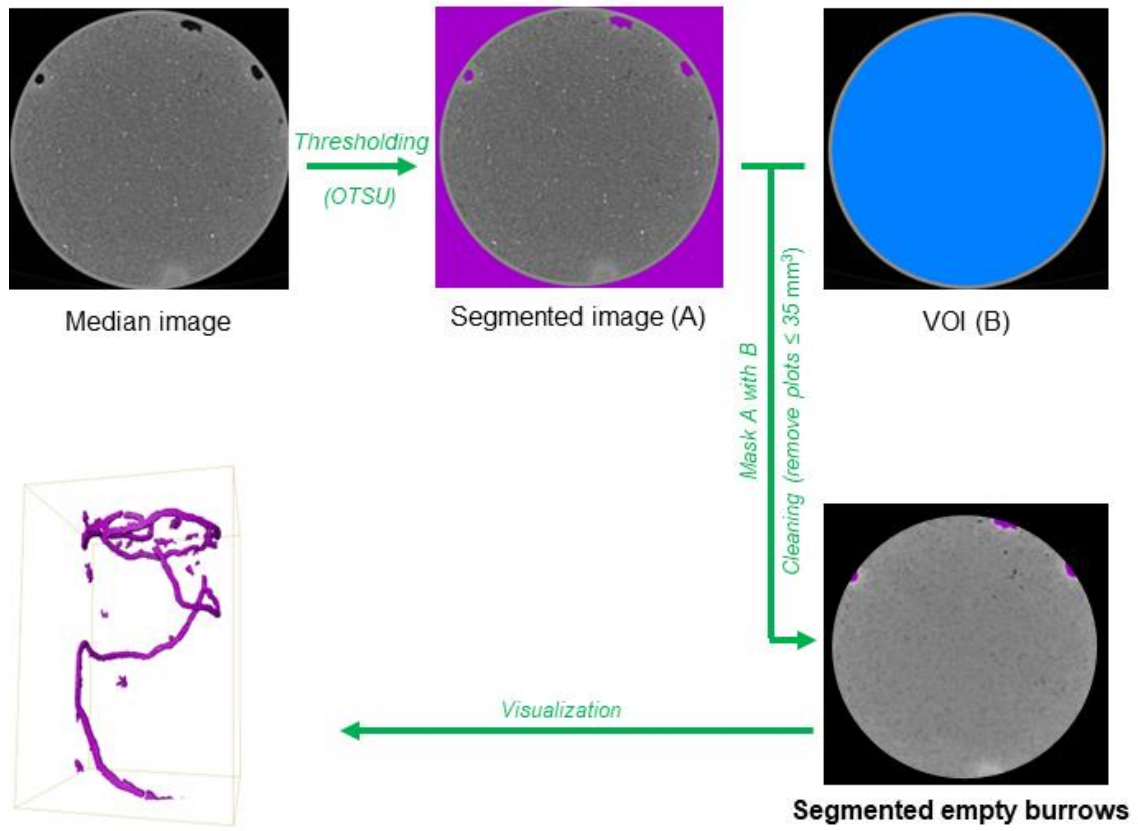


Figure 5. Workflow to separate empty burrows from the soil matrix.

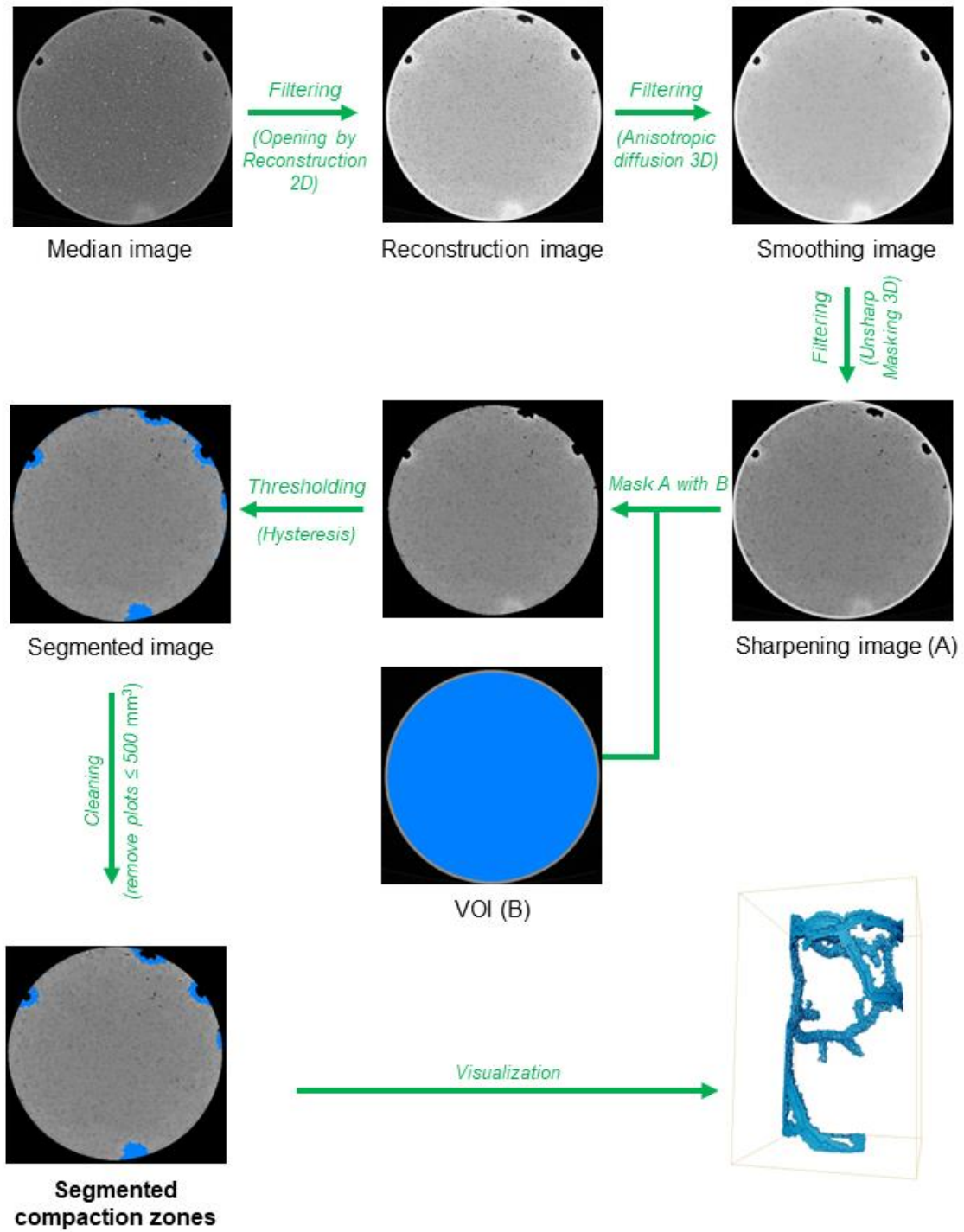


Figure 6. Workflow to separate compaction zones from the soil matrix.

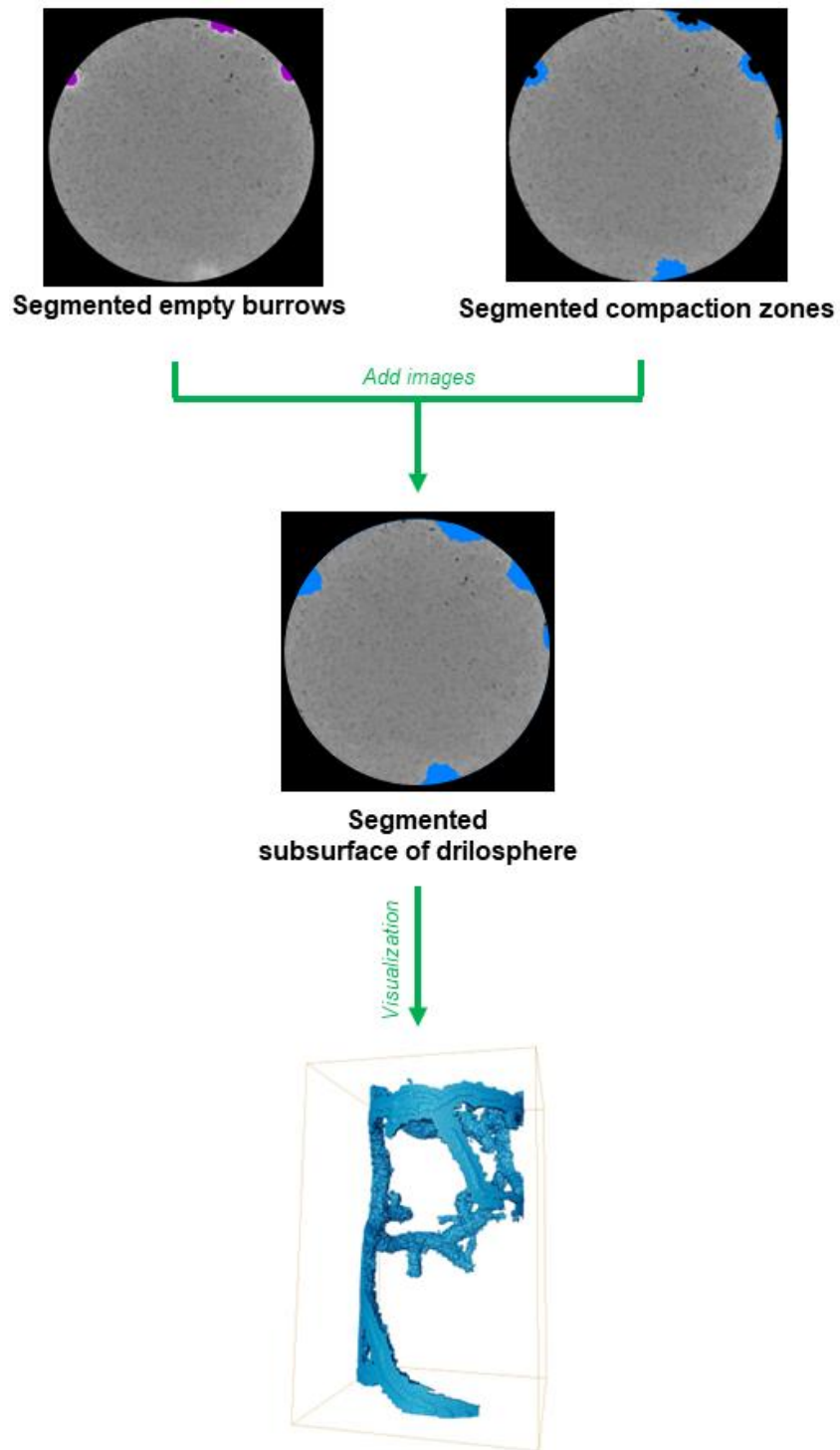


Figure 7. Workflow to separate the whole drilosphere from the soil matrix.

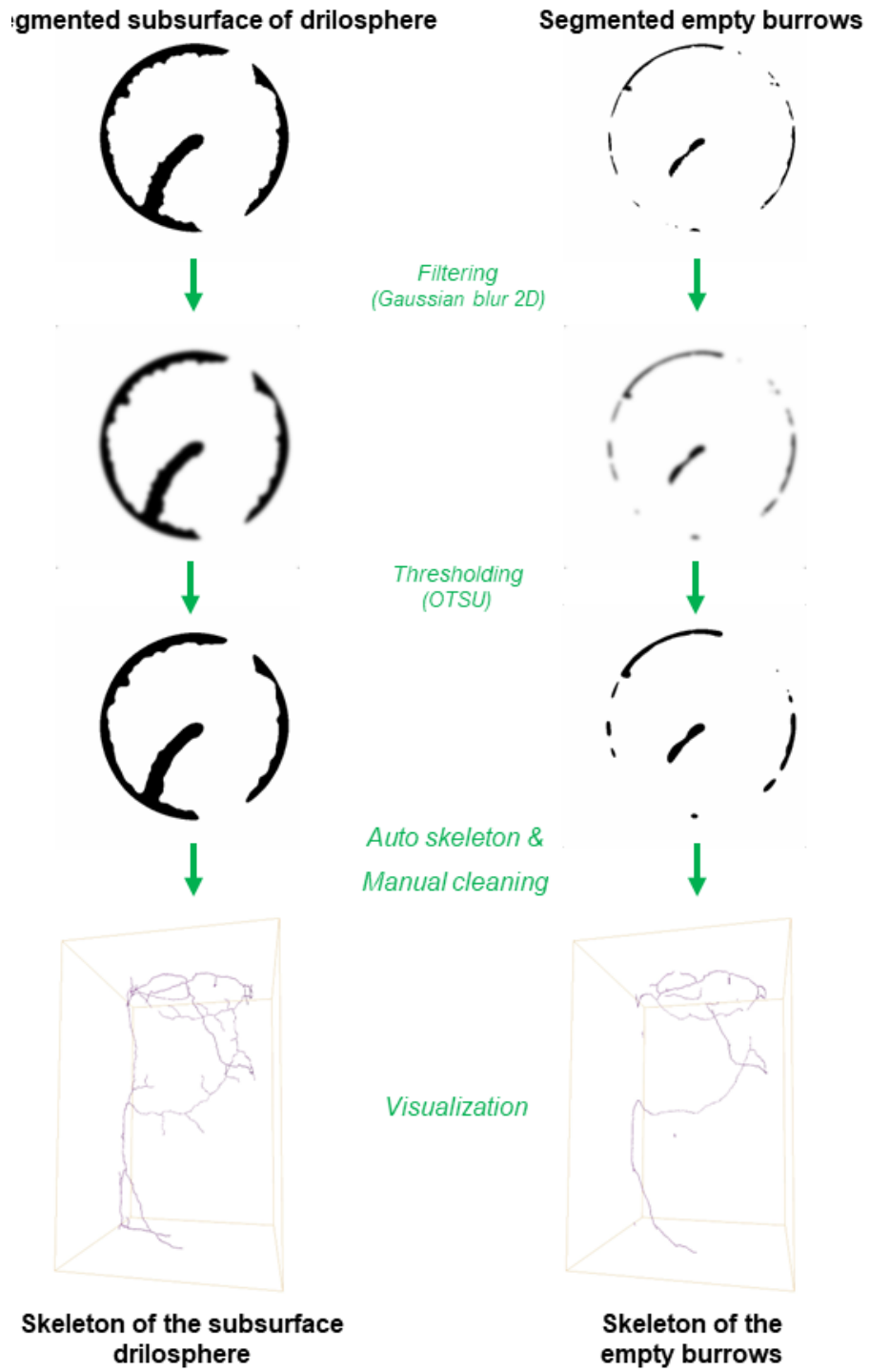


Figure 8. Workflow to calculate skeletons within the whole drilosphere and empty burrows.

+ Quantification characteristics of the drilopshere

The structural properties of the drilopshere were characterised with the software Fiji/ImageJ (Schindelin et al., 2012).

Empty burrows characteristics included volume, number, diameter, continuity and connectivity was calculated using the “MorphoLibJ” plugin (Legland et al., 2016). The mean diameter for each core was calculated as the sum of the volume fraction of burrows multiplied by the diameter of the maximum inscribed sphere within each burrow. To assess burrow system continuity, as no standardized measure exists, were summed as the volume of burrows whose vertical extension (> 60 degrees) was larger than 30% of the core length. The burrow connectivity Γ (Equation 1) reflects the probability of two randomly chosen burrow voxels belonging to the same burrow cluster:

$$\Gamma = \frac{1}{N_p^2} \sum_{k=1}^{N_i} n_k^2 \quad [\text{Equation 1}]$$

where N_p is the number of all burrow voxels p , N_i is the number of all clusters and n_k is the number of burrow voxels in Cluster k . Γ can range from 0 (many unconnected clusters) to 1 when all burrow voxels belong to a single connected cluster.

The volume of the lateral soil compaction was calculated using the “MorphoLibJ” plugin (Legland et al., 2016).

The volume of belowground casts: The length of the skeletons was measured with the plugin “boneJ” (Doube et al., 2010) in Fiji/ImageJ. The length of belowground casts corresponded to the length of the whole subsurface drilosphere minus the length of the burrows. Considering that subsurface casts refilled burrows, they have a cylinder shape. Therefore, the volume of belowground casts was estimated using their length and the diameter of the burrows.

The structural property of the drilopshere: To assess these parameters, the ratio between the grey value of the different drilosphere components (Fig. 9, 10 and 11) and the grey value of the soil matrix were calculated. Finally, the volume of the surface casts was assessed by multiplying their mass to their bulk density. A calibration curve between the soil bulk density and its grey value has been obtained from soil columns (5 cm in diameter and 5 cm in height) manually compacted to obtain a gradient of soil bulk density ranged from 0.9 to 1.5 g cm⁻³ (Fig. 12).

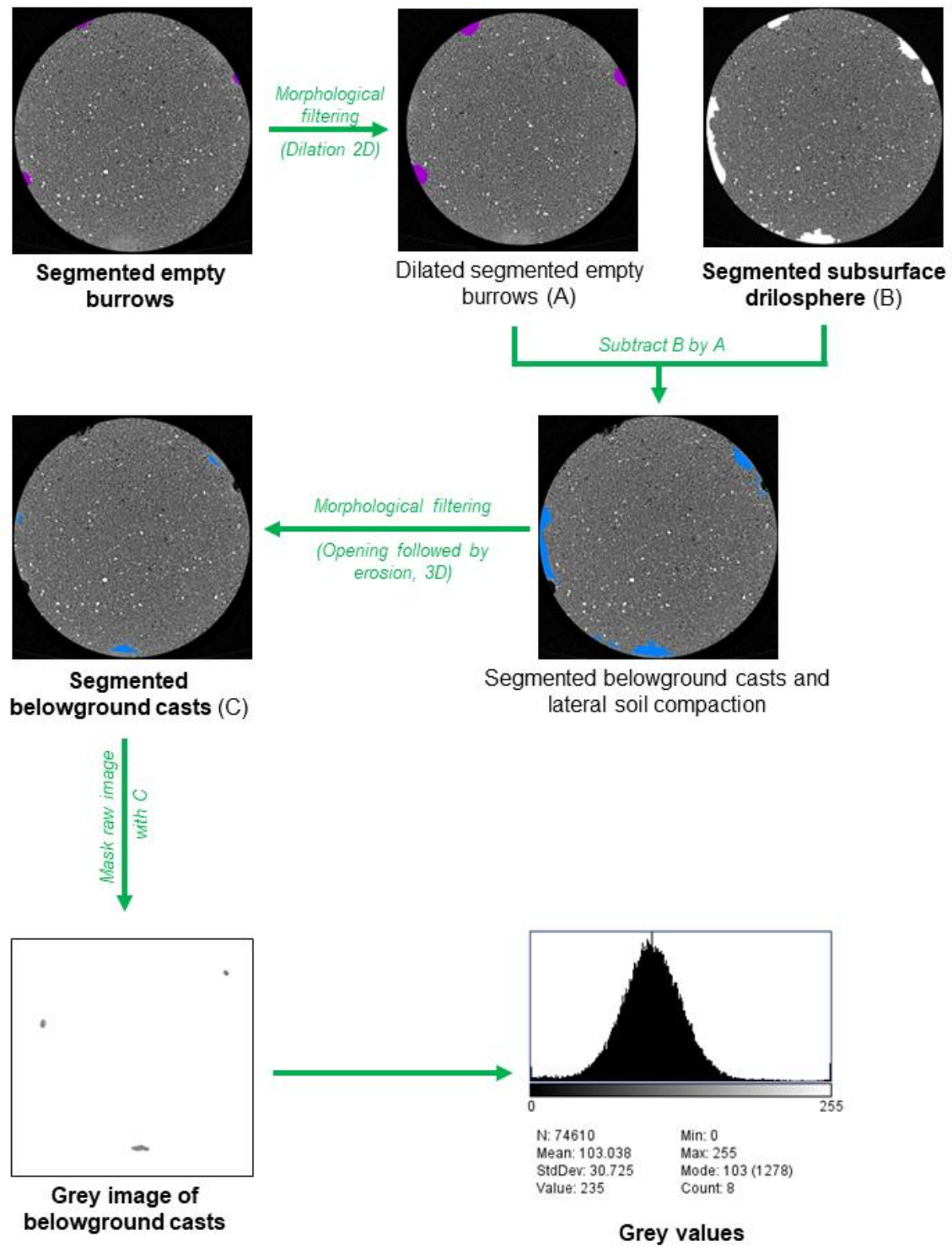


Figure 9. Workflow to measure the grey value of belowground casts.

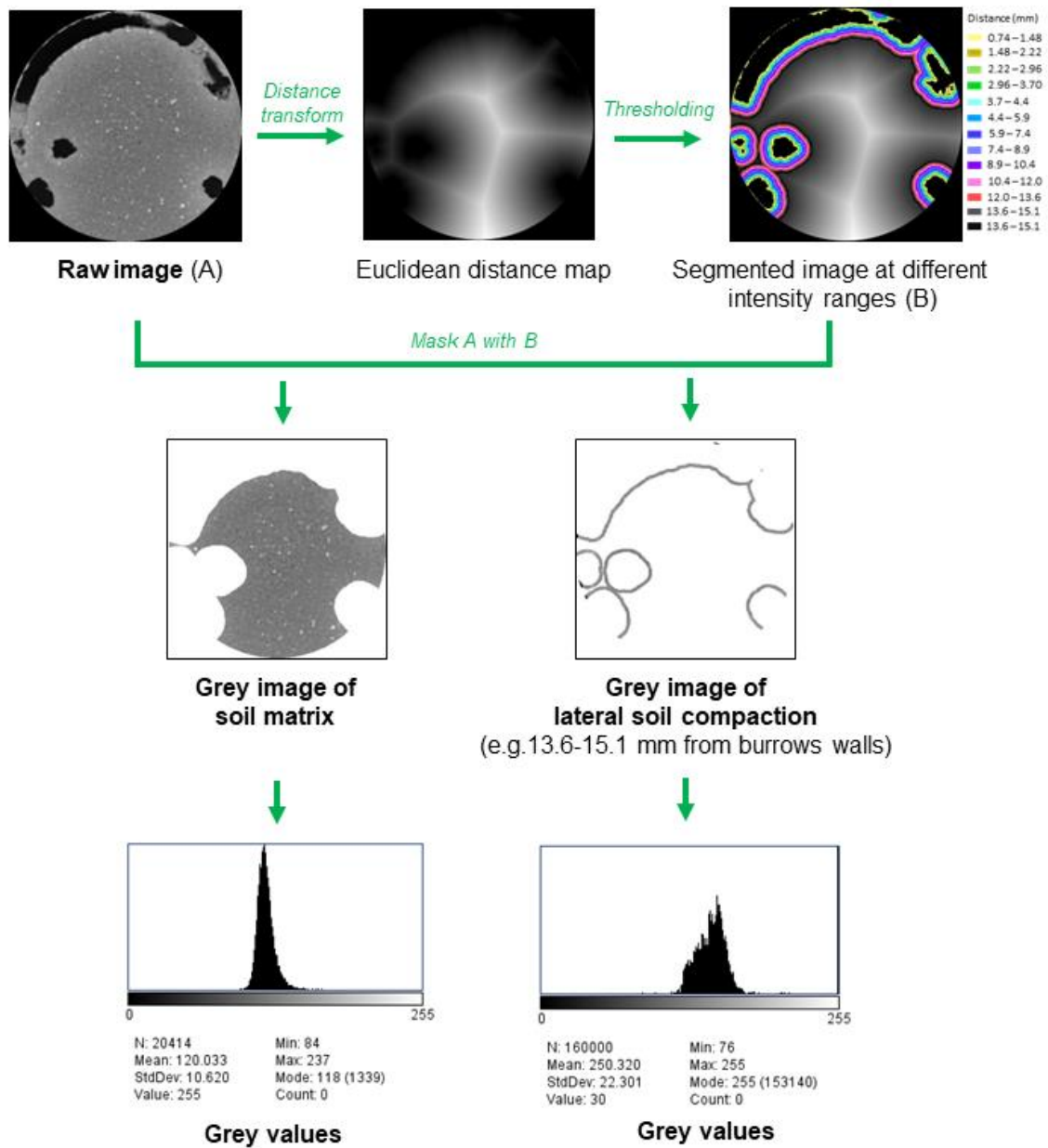


Figure 10. Workflow to measure the grey value of lateral soil compaction and the soil matrix.

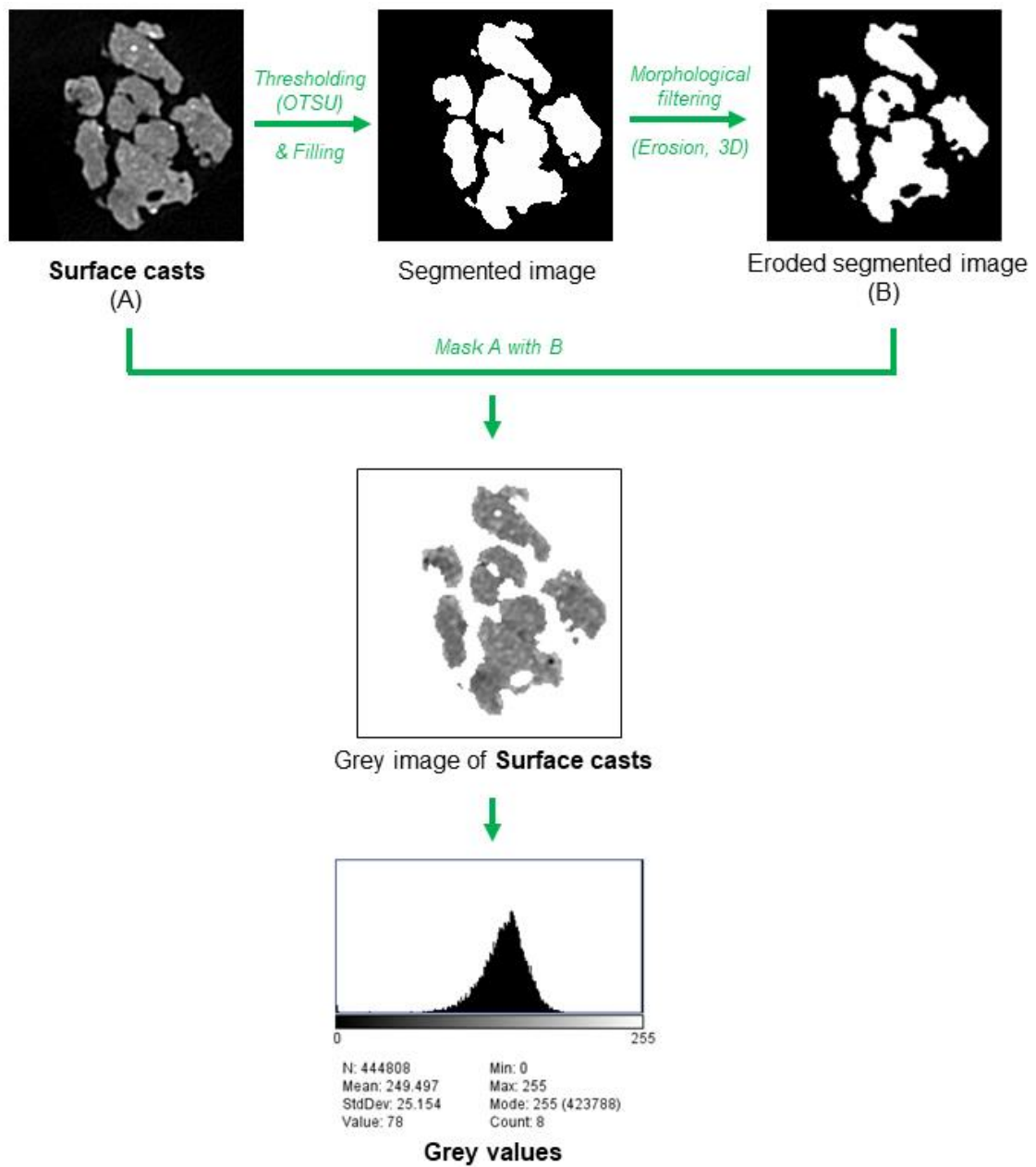


Figure 11. Workflow to measure the grey value of surface casts.

CT images of soil columns
at different bulk density (g cm^{-3})

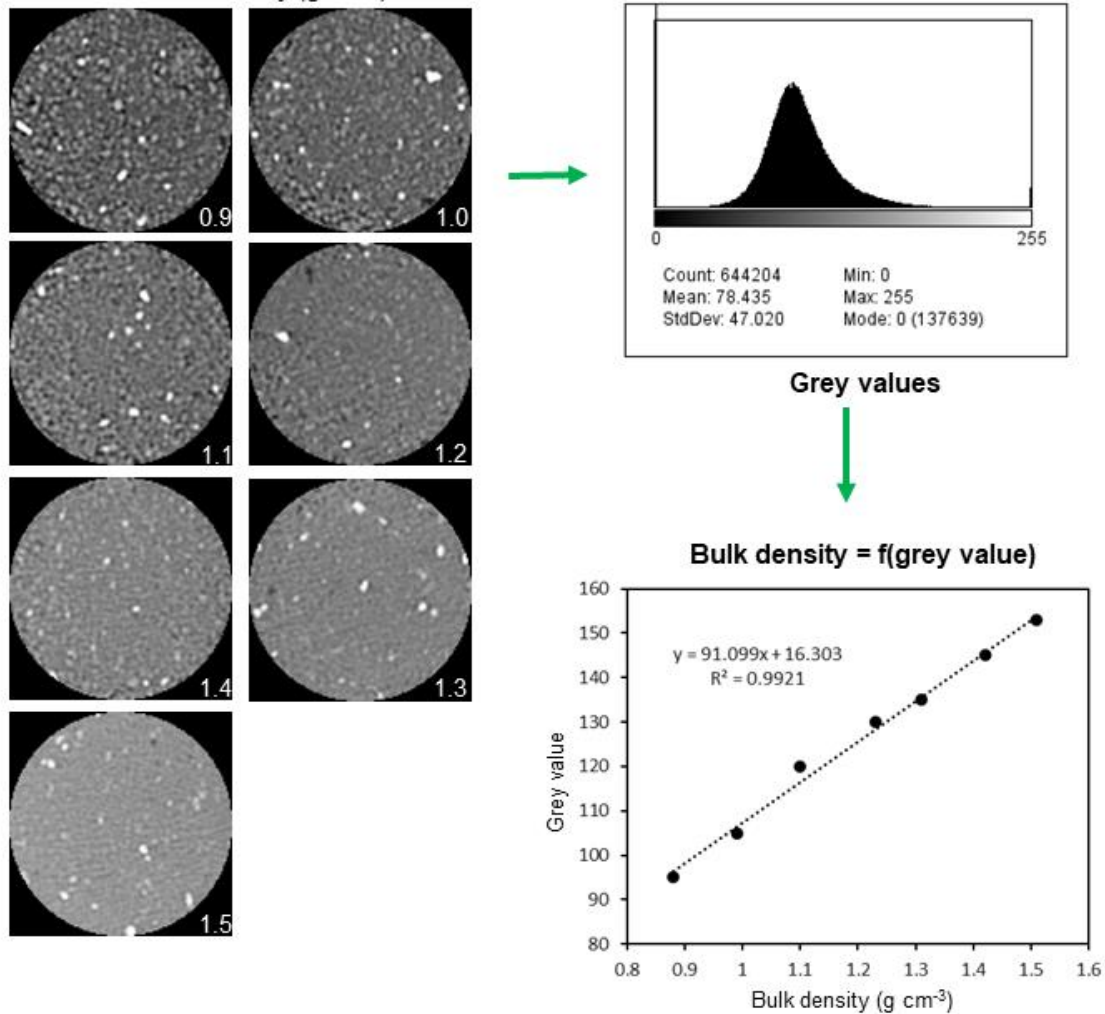


Figure 12. Linear relationship between soil bulk density and grey value of the soil matrix.

3.4.2.2. Experiment 2

+ Extraction the different components of the drilopshere

The software Avizo® (Thermo Fisher Scientific) were applied to extract the different parts of the drilopshere and visualized them. The segmentation was performed with Avizo software (<https://www.thermofisher.com>). Compaction zones were distinguished from the soil matrix due to their slightly brighter grey colours. To accentuate differences, we used the following filters: opening by reconstruction, unsharp masking 3D and median filter (similar to the procedure presented in Fig. 4, 5 and 6). The three phases, empty burrows, compaction zones and soil matrix were segmented by applying the “3 phases auto thresholding”. To isolate belowground casts from the lateral soil compaction within the compacted zones, we first dilated the empty burrows to overlap the compaction around burrows. The volume of belowground

casts corresponded to the volume of compaction zones minus the volume of dilated empty burrows. This methodology led to some artefacts, which have been removed using the module “opening”. The volume of soil lateral compaction corresponded therefore to the volume of compacted zones minus the volume of belowground casts. Finally, only isolated features $> 10 \text{ mm}^3$ were kept for quantification.

+ Quantification the different components of the drilosphere

The “MorphoLibJ” plugin (Legland et al., 2016) and “soilJ” (Koestel, 2018) in Fiji software (Schindelin et al., 2012) were used to quantify the volume of belowground casts, empty burrows and soil lateral compaction. In addition, other properties characterizing empty burrows were computed: number, volume of percolating burrows, continuity, diameter, and vertical distribution (i.e., mean of the burrow barycenters). To assess continuity, as no standardized measure exists, we summed the volume of burrows whose vertical extension (> 60 degrees) was larger than 30% of the core length.

3.5. Water characteristics

+ Experiment 1

At the end of the experiment, the soil saturated hydraulic conductivity (K_s) (Equation 2) was measured with the falling head method:

$$K_s = \frac{al}{A\Delta t} \ln\left(\frac{h_1}{h_2}\right) \text{ [Equation 2]}$$

where a is the cross-section of the water column, A is the cross-section of the soil core without the polyvinyl chloride cylinder, l is the height of the soil sample in the column, and Δt is the recorded time for the water column to drop from the initial to the final height (h_1 and h_2 , respectively). Before starting the measurements, cores were water-saturated for four days by gradually raising the water level each day to avoid possible imprisonment of air bubbles as much as possible. Then, 4 cm of water was added to the top of the core, and the time needed for 2 cm of water to be infiltrated was recorded. The procedure was repeated three times and the final infiltration rate was averaged.

+ Experiment 2

Each column was equipped with FD-probes (model: JXBS-3001-TR; Weihai Jingxun Changtong Electronic Technology Co., Ltd., China) at 7 cm depth to continuously measure (every 5 minutes) the temporal development of the soil volumetric water content. Columns were weighed before adding water and the cumulative soil water evaporation was calculated and converted in mm of water. At the end of the experiment, water infiltration was measured using the single ring method also named the ‘Beerkan’ method following the same protocols described in Capowiez et al. (2015). In brief, the core was located on a device with free drainage and a fixed volume of water (300 ml corresponding to 0.99 cm of water height) was poured into the core head at time zero and the time elapsed for the known volume of water to infiltrate was measured. When the first volume had infiltrated completely, a second fixed volume of water was added. The procedure was repeated for 10 to 15 volumes until a steady state was reached. The final infiltration rate (cm day^{-1}) was then estimated by computing the slope (linear regression) of the relationships between cumulative infiltration and time elapsed at steady state.

3.6. Statistical analysis

Statistical analyses were performed with R (R Core Team, 2010) and figures were produced with the package “ggplot2” (Wickham, 2016).

3.6.1. Assignment of the Vietnamese species into ecological categories

To assign earthworm species into ecological categories, we conducted a principal component analysis (PCA) of mixed data with the package “ade4” (Dray & Dufour, 2007) based on morpho-anatomical traits (pigmentation, colouration gradient, weight, diameter, length, tegument firmness, presence of furrows, typhlosole type and longitudinal muscle structure) as suggested by Bottinelli et al. (2020a). Earthworms were then grouped using k-means clustering based on the projections of the species on the two first axes of the PCA. Differences in categorical traits between each cluster were analysed by Fisher’s exact test and then through pairwise tests with the package “rcompanion”. Differences in quantitative traits were analysed by one-way ANOVA and afterwards through Tukey's multiple comparison test with the package “agricolae” (Mendiburu, 2020).

3.6.2. The drilosphere properties and the influence on soil water transfer of the tropical species and the relationship to traits and ecological categories (Exp.1)

The effect of earthworms on soil saturated hydraulic conductivity (K_s) was expressed as the response ratio (RR), calculated as the ratio of the measure of K_s in soil columns with earthworms and in control columns without earthworms. The grey ratio values of surface and belowground casts, the lateral soil compaction to the soil matrix were used as an indicator of the local bulk density. T-test was used to determine whether the mean of RR_{K_s} or grey ratio values differed significantly from 1, indicating either an increase or a decrease in water infiltration and the local bulk density. Differences in parameters (food ingestion, volume percentage of the whole drilosphere, empty burrows characteristics, grey ratio values, and K_s) between groups or species were analysed by one-way ANOVA and afterwards through Tukey's multiple comparison test with the package "agricolae" (Mendiburu, 2020). Before t-tests and ANOVA, parameters were tested for normality and homogeneity of variance by the Shapiro–Wilk test and Levene's test. Differences among treatments were declared significant at the 0.05 probability level.

Random forest was used with the package "caret" (Kuhn, 2008) to test the capacity of dataset to predict the effect of earthworms on RR_{K_s} based on the 16 morpho-anatomical traits or the 5 burrow network properties. Random forest is a nonparametric method, which consists of a large number of individual tree models trained from bootstrap samples of the data (Breiman, 2001). The optimization of parameters for the model was based on minimizing the root mean square error (RMSE) between the measured and estimated values of RR_{K_s} . The model performance was assessed by leave-one-out cross-validation. The model predictions were evaluated by calculating the coefficient of determination (R^2) and RMSE. In this case, we preliminarily applied recursive feature elimination (RFE) to consider only the most discriminating predictors applied to the final model. Associations between explanatory and response variables were evaluated with accumulated local effects (ALE) plots (Apley & Zhu, 2020) from the "iml" package (Molnar et al., 2018). The outcomes from the ALE plot showed the net effect on RR_{K_s} above or below the average over the range of feature variables. In addition, coinertia analysis from the "ade4" package was applied between matrices of morpho-anatomical traits and burrow network properties, allowing us to examine the relationship between the two datasets. The level of association between the two datasets was measured with the RV matrix correlation coefficient.

In addition, random forest regression was also used (the package “caret”) (Kuhn, 2008) to identify traits influencing grey ratios of the different parts of the drilosphere. The traits tested were the surface food ingestion, the longitudinal and circular muscle thickness, pigmentation, antero posterior and dorso ventral gradients, length and diameter of the body. We then calculated the Shapely value with the package “kernelshap” (Michael et al., 2023), a widely used approach to explain the black box in machine learning, which can help to measure the net contribution of traits to grey ratio values and radius of the lateral compaction and their contribution direction.

3.6.3. The interactive effects of ecologically distinct earthworm species on soil drilosphere properties and water dynamics (Exp.2)

Differences in bioturbating behaviours, soil water infiltration and cumulative water evaporation were analysed by one-way ANOVA and afterwards through Tukey's multiple comparison test with the package “agricolae” (Mendiburu, 2020). Repeated two-way ANOVA was performed with the package “lme4” (Bates et al., 2015) to test the significant difference in gravimetric soil moisture at the 11 watering events between single and mixed species and control without earthworm. Pairwise comparisons between all treatments within each day were done with the package “emmeans” (Lenth et al., 2023). To test for evidence of interaction between species, we compared the observed values in the mixed species columns to that expected without interaction. Since the mass of earthworms was equal to 3 g in all columns, the expected values in the mixed species columns without interaction corresponded to the average values of single species columns for each property. We defined earthworm interaction as any deviation in the experimental results when compared to the expected results using t-test. Before t-test and ANOVA, parameters were tested for normality and homogeneity of variance by the Shapiro–Wilk test and Levene’s test, respectively. To explore the dissimilarities of surface and belowground activities between single and mixed specie columns, we used a between-class analyses (BCA) and Monte Carlo permutation tests (1000 permutations) with the package “ade4” (Dray & Dufour, 2007). Spearman correlations were calculated to study the relationships between all the properties measured in columns incubated with earthworms (N = 14).

CHAPTER 4 – RESULTS

4.1. Assignment of the Vietnamese species into ecological categories

Using the 11 morpho-anatomical traits proposed by Bottinelli et al. (2020) from the French Lumbricidae species (Bottinelli et al., 2020a), the projection of the data from the 23 species onto the two factorial planes of the PCA showed an organization following roughly a triangle shape (Fig. 13).

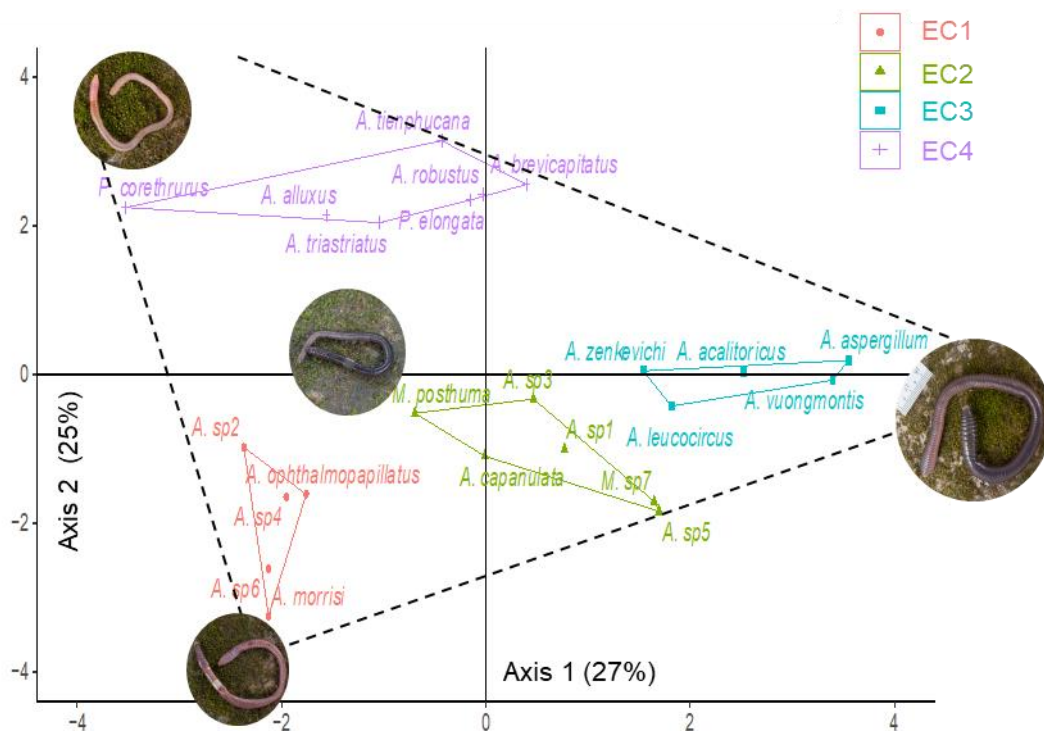


Figure 13: Score plot from the principal component of analysis (PCA) followed by k-means clustering based on 11 morpho-anatomical traits (pigmentation, colouration gradient, weight, diameter, length, tegument firmness, presence of furrows, typhlosole type and longitudinal muscle structure) to assign the 23 earthworm species into ecological categories (EC). Earthworm images are *A. morrisi*, *A. aspergillum*, and *P. corethrurus* located at three angles of triangle and *M. posthuma* in the middle.

Using k-means clustering, four ecological categories (EC) could be identified: EC1 (*A. sp2*, *A. sp4*, *A. sp6*, *A. morrisi*, and *A. ophthalmopapillatus*), EC3 (*A. aspergillum*, *A. acalifornicus*, *A. vuongmontis*, *A. leucocircus*, and *A. zenkevichi*) and EC4 (*A. alluxus*, *A. brevicapitatus*, *A. robustus*, *A. tienphucanus*, *A. triastriatus*, *P. corethrurus*, and *P. elongata*), representing each pole, and EC2 (*A. sp5*, *M. sp7*, *A. acampanulata*, *A. sp1*, *A. sp3* and *M. posthuma*) was located between the three poles.

The 11 traits characterizing the four ECs are presented in Table 2 and Table S1 (details are presented in Supplementary S2). The structure of the longitudinal muscle, the presence of the longitudinal furrow and the type of typhlosole were not significantly different among the four clusters. Earthworms from EC1 have the smallest size and lightest weight. They were all pigmented, with a gradient along the body. The epithelium was not rigid, and few have transversal furrows. Earthworms from EC3 were the largest and heaviest. They were all pigmented with a gradient along the body. The epithelium was mostly rigid, and all have transversal furrows. Earthworms from EC4 were of medium size and weight. They were all nonpigmented, with no gradient along the body. The epithelium was soft, and all have transversal furrows. Finally, earthworms from EC2 were of medium size and weight. They were all pigmented, with a gradient along the body. The epithelium was either soft or rigid, and half of the species have transversal furrows. The five additional morpho-anatomical traits were presented in Table 2 and Table S1. Trait values for each EC were similar except for the thickness of the longitudinal muscle, which was the largest for species belonging to EC3, smallest for species from EC1 and intermediate for the other ECs.

If we assume that the ecological categories defined by Bouché (1977) can be applied to non-Lumbricidae species, Cluster 1 (EC1) gathered epigeic species, since individuals were pigmented, have a small body size and a soft epithelium, and few of them have transversal furrows. Cluster 4 (EC4) gathered endogeic species, since individuals were unpigmented, have a medium body size, have a soft epithelium, and all individuals have transversal furrows. Cluster 3 (EC3) gathered anecic species, since individuals were pigmented, have a large body size, have a rigid epithelium, and all have transversal furrows. Finally, Cluster 2 (EC2) gathered intermediate species. They were all pigmented with a medium body size. Their epithelium was either rigid or soft, and 50% of the individuals have transversal furrows. Among the species collected, some have already been studied and assigned by other researchers: *A. aspergillum* has been identified as anecic (Liu et al., 2021), *A. morrisoni* as epi-endogeic (Zhang et al., 2016), *P. corethrurus* as endogeic (Marichal et al., 2010), *A. robustus* as endogeic (Lin et al., 2016), *P. elongata* as endogeic (Rossi et al., 1997) and *M. posthuma* as endogeic (Bottinelli et al., 2010). Results of the thesis confirm these assignments, except for *A. morrisoni* that was assigned as epigeic and *M. posthuma* as intermediate. Because the assignment proposed here was based on PCA followed by cluster analysis, it was expected that with a larger dataset, the assignment of the studied species might change slightly, and other ecological categories, such as epi-endogeic, endo-anecic or epi-anecic, could be differentiated.

Table 2: Morpho-anatomical traits characterizing the four ecological categories (EC). Values given in the table were the means across all species in each EC, and standard deviations were in parentheses. Values followed by different letters were significantly different within each trait. The number of asterisks for the significance level indicates the p value range (***) $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, and ns for $p > 0.05$).

Morpho-anatomical traits	EC1	EC2	EC3	EC4	p value
Length (mm)	69 (13) c	118 (31) b	169 (28) a	110 (25) b	***
Diameter (mm)	3 (0) c	4 (1) bc	6 (1) a	4 (2) ab	*
Weight (mg)	540 (200) c	2347 (980) b	5456 (3730) a	2053 (1080) b	***
Pigmentation (yes)	100% a	100% a	100% a	0% b	***
Antero-posterior gradient (yes)	100% a	67% a	60% a	0% b	**
Dorso-ventral gradient (yes)	40% ab	100% a	100% a	0% b	***
Epithelium rigidity (yes)	0% b	50% ab	80% a	0% b	**
Muscle structure	Inte. (80%)	Inte. (50%)	Fasc. (60%)	Fasc. (88%)	ns
Longitudinal furrow (yes)	0%	0%	0%	15%	ns
Transversal furrow (yes)	17% b	50% ab	100% a	100% a	**
Typhlosole type	Lame. (60%)	Lame. (83%)	Lame. (80%)	Lame. (71%)	ns
Longitudinal muscle thickness (μm)	179 (104)	196 (67)	237 (95)	147 (51)	ns
Circular muscle thickness (μm)	51 (10) c	102 (43) ab	142 (45) a	70 (18) bc	**
Tegument thickness (μm)	26 (7)	34 (7)	39 (9)	32 (8)	ns
Septa thickness	Thin (100%)	Thin (50%)	Thin (80%)	Thin (57%)	ns
Number of setae	59 (11)	57 (8)	65 (9)	59 (26)	ns

4.2. Buffalo dung ingestion

Buffalo dung ingestion varied from 0.1 to 25 g per individual for 28 days (Fig. 14). Largest values were found for the anecic *A. aspergillum* and the intermediate *A. sp1* whereas lowest values were found for the anecic *A. vuongmontis* and the endogeic *A. brevicapitalus*. On average, anecic and intermediate species ingested significantly more dung than endogeic species while epigeic species had intermediate values ($p < 0.01$).

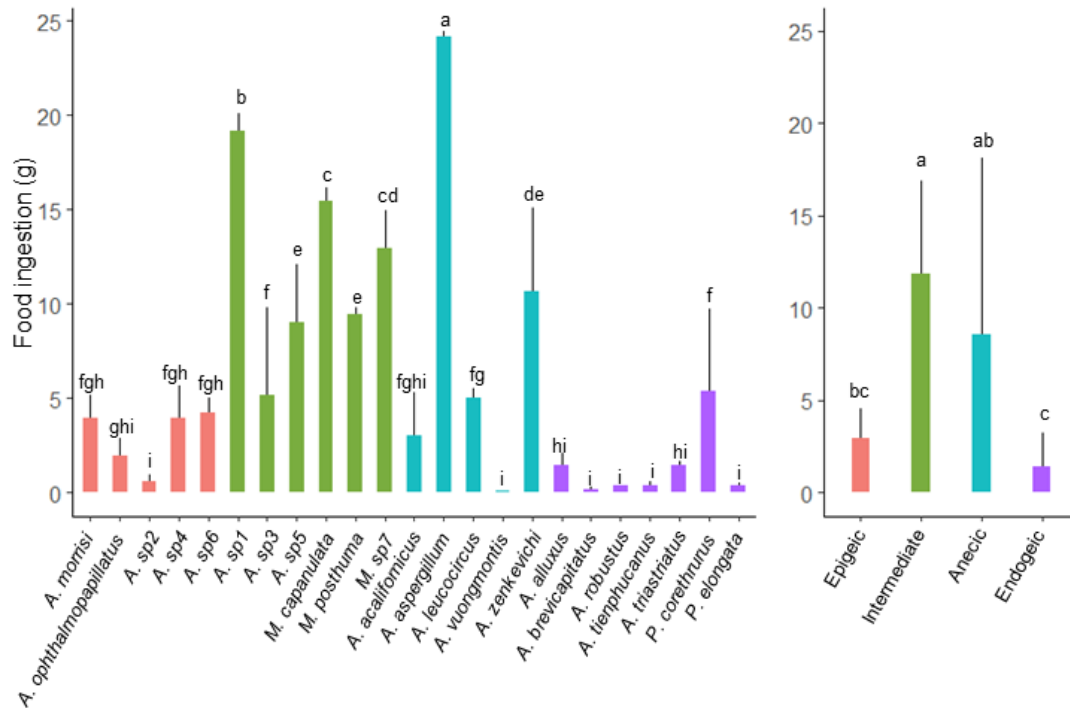


Figure 14: Average values with standard deviation of buffalo dung ingestion for the 23 earthworm species and ecological categories. Different letters indicated statistically significant differences between species or ecological categories.

4.3. The empty burrow systems and its relationship to earthworm traits, ecological categories, and soil saturated hydraulic conductivity

4.3.1. The characteristics of empty burrow networks

Representative empty burrow networks for each species were shown in Figure 15. Species belonging to epigeic group (EC1) produced few and thin burrows located close to the surface of the core. Conversely, burrow networks were more variable for the other groups. For anecic group (EC3), *A. aspergillum*, *A. acalifornicus*, and *A. zenkevichi* produced few, wide and continuous burrows distributed from the top to the bottom of the core, while the burrows of *A. vuongmontis* and *A. leucocircus* were slightly more discontinuous. For two out of seven species assigned to endogeic group (EC4) (*P. corethrurus* and *A. brevicapitatus*), burrows were quite similar to those of species belonging to epigeic, whereas for the five other species, the number of burrows was much larger, distributed from the top to the bottom of the soil core. Species belonging to intermediate (EC2) produced burrows similar to those produced by species in epigeic except for *M. posthuma*, which produced burrows similar to those produced by species in endogeic.

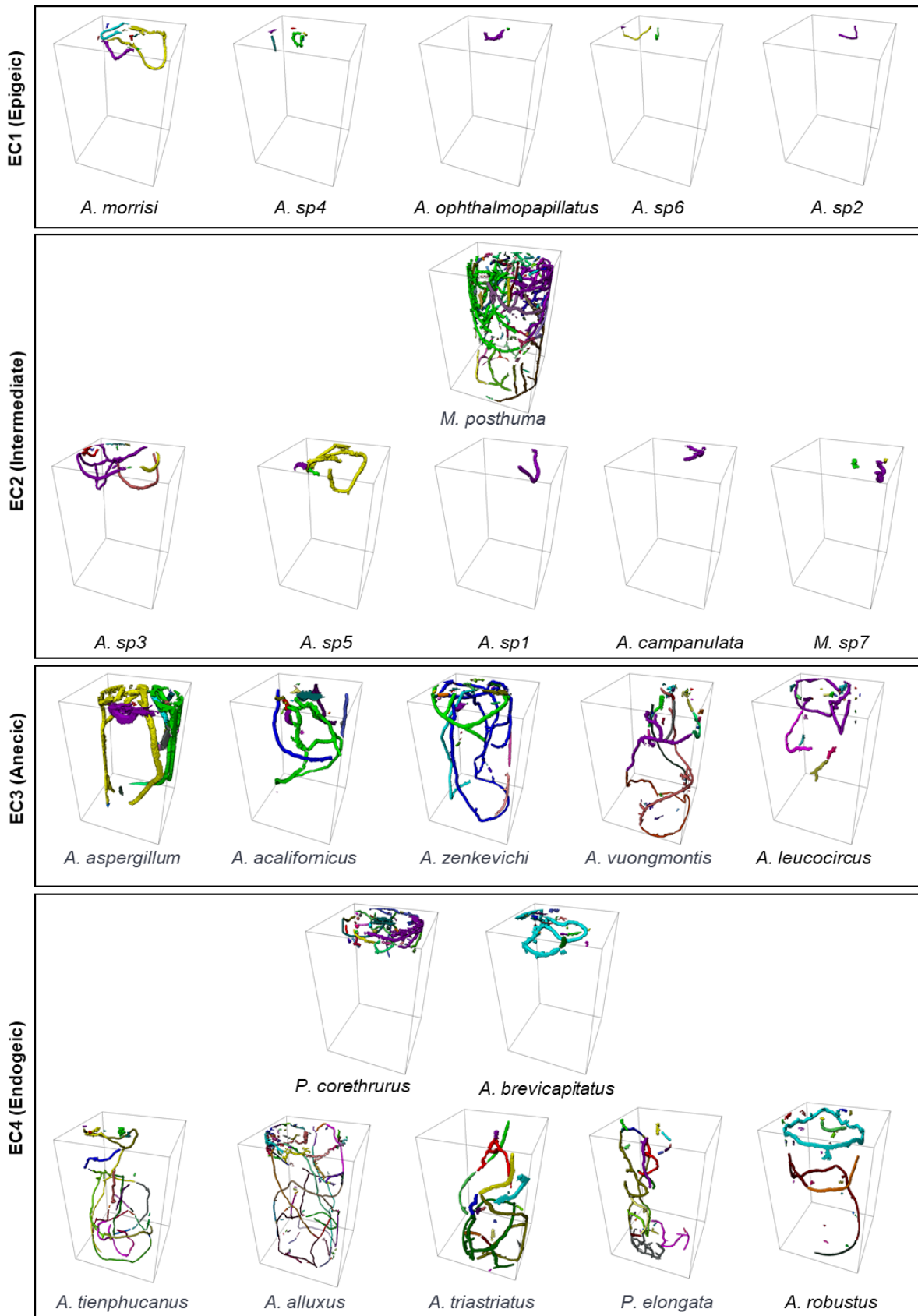


Figure 15. Examples of 3D reconstructions of the burrow systems made by earthworms in repacked soil columns (30 cm in height and 15 cm in diameter) after 4 weeks.

Overall, the quantification of the empty burrow characteristics via image analysis confirmed the large variability of burrow networks found within intermediate, anecic and endogeic earthworms (Fig. 16). Earthworms from the epigeic and intermediate groups made fewer burrows (number and volume percentage), and these burrows were less wide and continuous but more connected than those made by species assigned to anecic and endogeic. Species from intermediate group can be differentiated from those belonging to epigeic group by the volume percentage of the burrows, which was significantly larger. The burrow network of species assigned to anecic and endogeic groups could be differentiated only based on the diameter of the burrows, which was larger for burrows made by species belonging to anecic group.

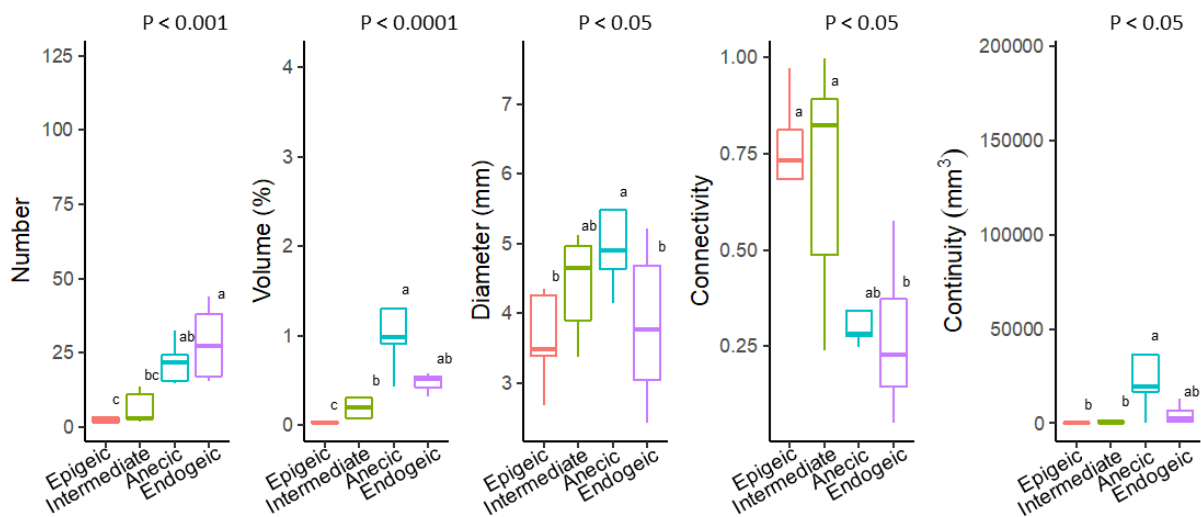


Figure 16: Burrow network properties (number, volume, diameter, connectivity, and continuity) of the four ecological categories. Boxplots show the interquartile range and median, and whiskers show the 1.5 x interquartile range. P values were calculated using one-way ANOVA. Different letters indicate significant differences (at the 0.05 significance level) among ecological categories.

4.3.2. The impact of earthworms on soil saturated hydraulic conductivity

The response ratio of soil hydraulic conductivity (RR_{K_s}) was significantly larger than 1 for 10 species (2 intermediate, 2 anecic and 6 endogeic), whereas it was not different from 1 for the other 13 species (5 epigeic, 4 intermediate, 3 anecic and 1 endogeic) (Fig. 17a). The mean RR_{K_s} was the highest for species assigned to anecic, the lowest for those assigned to epigeic and intermediate for those assigned to intermediate and endogeic (Fig. 17b). However, the results of the ANOVA showed that RR_{K_s} measured for species assigned to anecic and endogeic groups

were not significantly different but were both higher than those measured for species assigned to epigeic group.

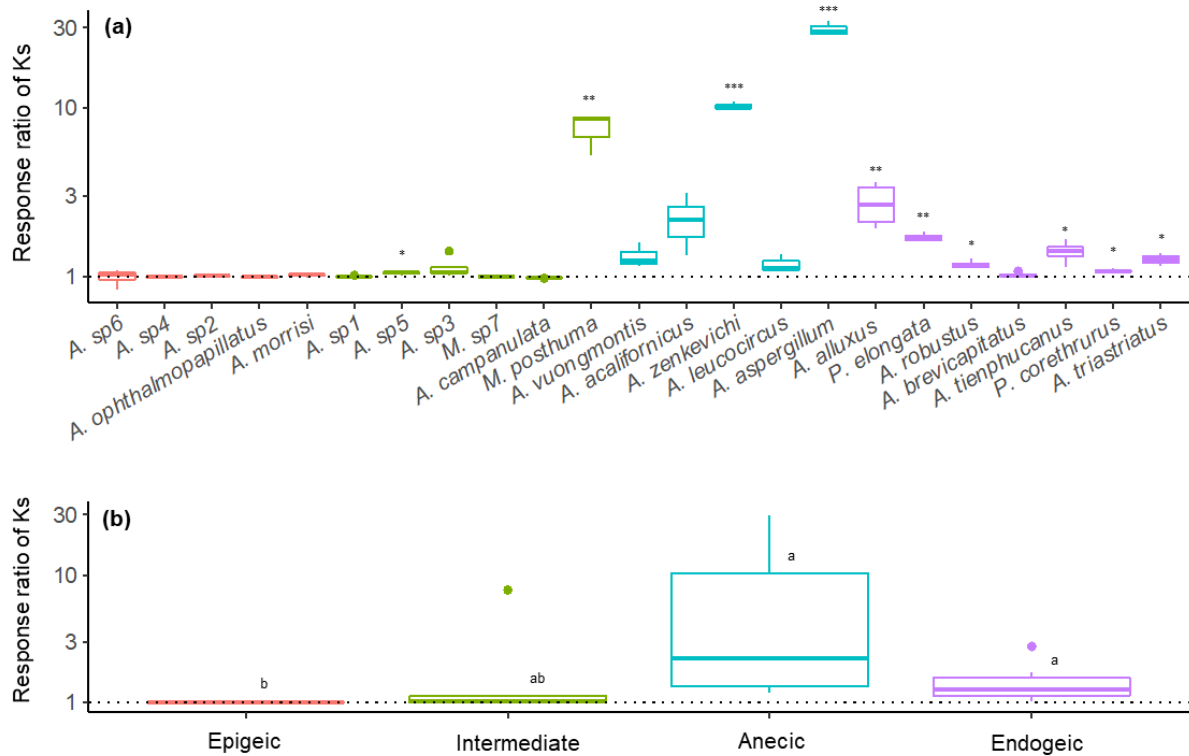


Figure 17. Response ratio of soil saturated hydraulic conductivity (K_s) among earthworm species (a) and among the four ecological categories (b). Boxplots show the interquartile range and median, and whiskers show the 1.5 x interquartile range. Asterisks indicate a significant difference between the mean of the response ratio of soil saturated hydraulic conductivity and the reference value of 1 (** $p < 0.01$; *** $p < 0.001$; * $p < 0.05$). Different letters indicate significant differences (at the 0.05 significance level) among ecological categories.

4.3.3. Relationships between saturated hydraulic conductivity, traits and burrow networks

The random forest regression models combined with RFE performed well in predicting values of RR_{K_s} using morpho-anatomical traits (R^2 of 0.97, RMSE of 1.17) (Fig. 18a) or burrow network properties (R^2 of 0.96, RMSE of 1.31) (Fig. 18b). The selected morpho-anatomical traits were the body weight and thickness of the circular and longitudinal muscle. Regarding burrow properties, only the volume and continuity of burrows were used to predict RR_{K_s} . To further investigate the specific relations between RR_{K_s} and the predictors, ALE analysis were conducted for the selected predictors of the random forest models. The ALE plots for the morpho-anatomical traits (Fig. 19a) showed that RR_{K_s} increased with increasing body weight and circular and longitudinal muscle thickness. Similarly, the ALE plots for the burrow networks (Fig. 19b) showed that RR_{K_s} increased with increasing burrow volume and continuity.

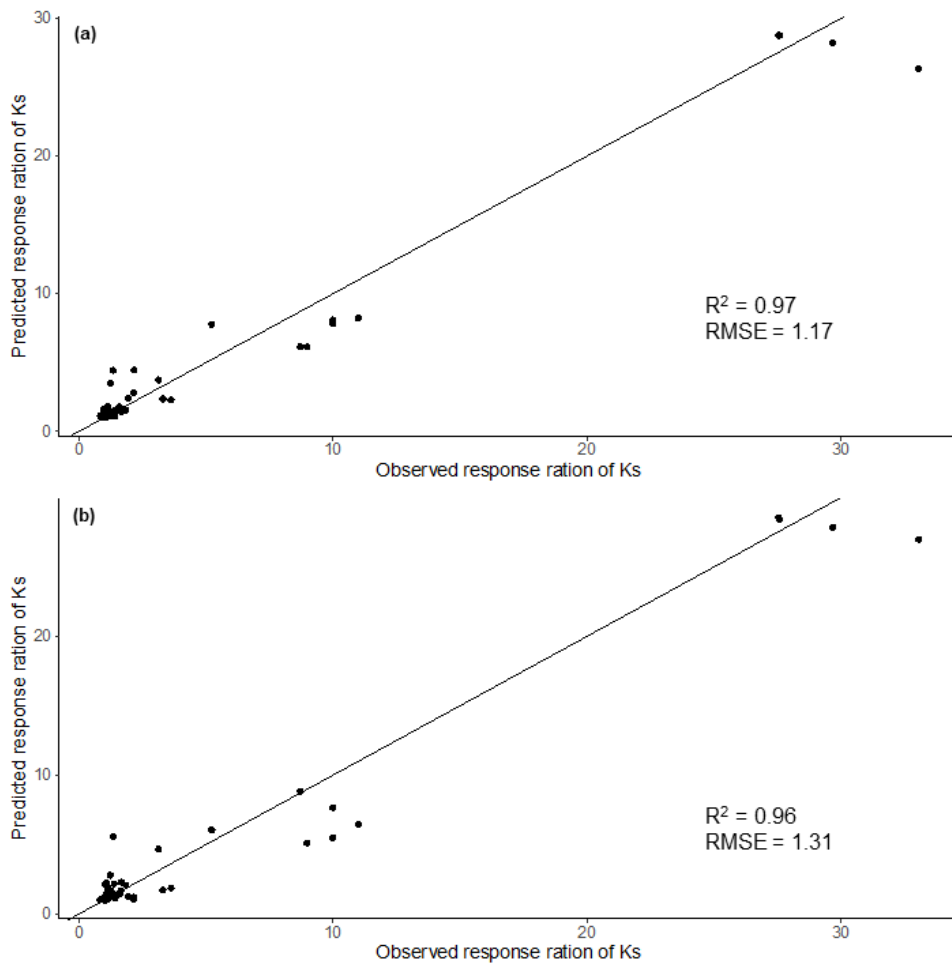


Figure 18. Predicted vs. measured response ratio of soil water infiltration (K_s) derived from random forest models using morpho-anatomical traits (a) and burrow network properties (b). Abbreviation: R^2 , coefficient of determination; RMSE, root-mean-square error. The black line corresponds to $y = x$.

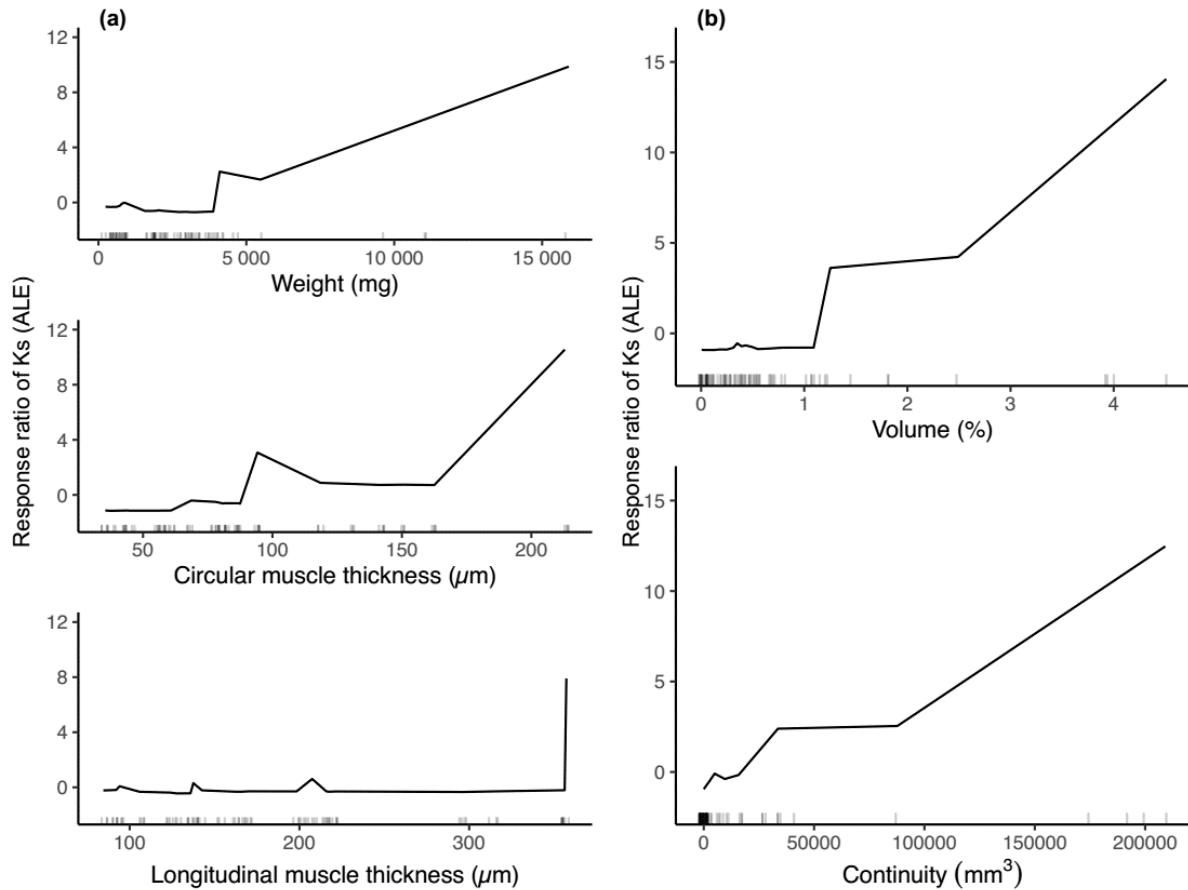


Figure 19. Accumulated local effects (ALE) plots of the predicted response ratio of soil hydraulic conductivity (K_s) for (a) the three selected morpho-anatomical traits and (b) the two selected burrow network properties. Grey markings on the x-axis show the distribution of each variable.

A coinertia analysis was performed to analyse the covariation between morpho-anatomical traits and burrow properties. The two projected inertias represented 97% of the total coinertia (Fig. 20), presenting moderate covariation ($RV = 0.47$, $p < 0.01$). The volume, continuity and diameter of burrows covaried positively with body weight, circular and longitudinal muscle thickness, length, diameter and the presence of a rigid epithelium. The number of burrows covaried positively with the absence of pigmentation, the presence of a bifid typhlosole and the presence of longitudinal furrows. The connectivity of burrows covaried positively with the absence of a transversal furrow, the absence of a typhlosole and the presence of an antero-posterior colouration gradient.

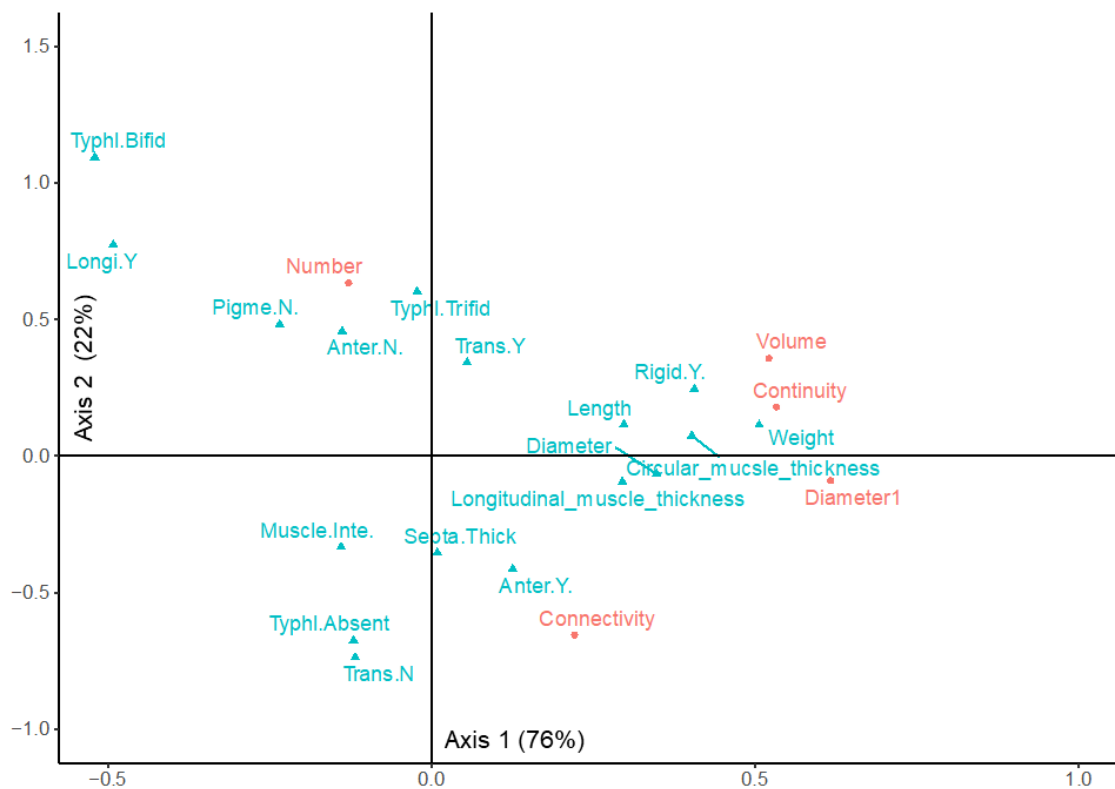


Figure 20. Projections of the morpho-anatomical traits (blue) and burrow properties (red) resulting from the coinertia analysis. For clarity, only morpho-anatomical traits with high scores were depicted on axis 1 and 2.

4.4. Structural properties of the drilosphere and its relationship to earthworm traits and ecological categories

Only 21 species among the 23 were used to evaluate their drilosphere characteristics because the two species *Amyntas triastriatus* and *Metaphire posthuma* were incubated in a different soil which might alter drilosphere properties.

4.4.1. Volume of casts, empty burrows, and lateral soil compaction

The drilosphere corresponded to the volume occupied by surface casts, belowground casts, empty burrows and the compacted layer around burrows. It occupied a negligible to a significant proportion of the soil volume, ranging from 0.1 to 21.2 % (Fig. 21a). The largest volume was measured for the anecic *A. aspergillum* whereas the lowest volume was measured for the epigeic *A. sp6*. On average, the volume of the drilosphere was the largest for anecic species (8.5%), intermediate for endogeic and intermediate species (2.5 and 1.5%) and the

lowest for epigeic species (0.5%) ($p < 0.001$) (Fig. 21b). The volume of surface casts was the highest for intermediate species, lowest for endogeic and intermediate for anecic and epigeic species ($p < 0.001$). The volume of belowground casts ($p < 0.001$) and burrows ($p < 0.001$) was the highest for anecic species, intermediate for endogeic and the lowest for intermediate and epigeic species. The volume of the lateral soil compaction was the highest for anecic and endogeic species and the lowest for epigeic and intermediate species ($p < 0.001$). In terms of proportion (Fig. 21c), casts and soil lateral compaction represented more than 70% of the whole drilosphere regardless the ecological category. The activity of epigeic and intermediate species occurred at the soil surface (production of casts) and belowground (production of burrows and compaction around burrows) in similar proportions. Conversely, the activity of endogeic and anecic species dominated belowground (production of burrows and casts, and compaction around burrows).

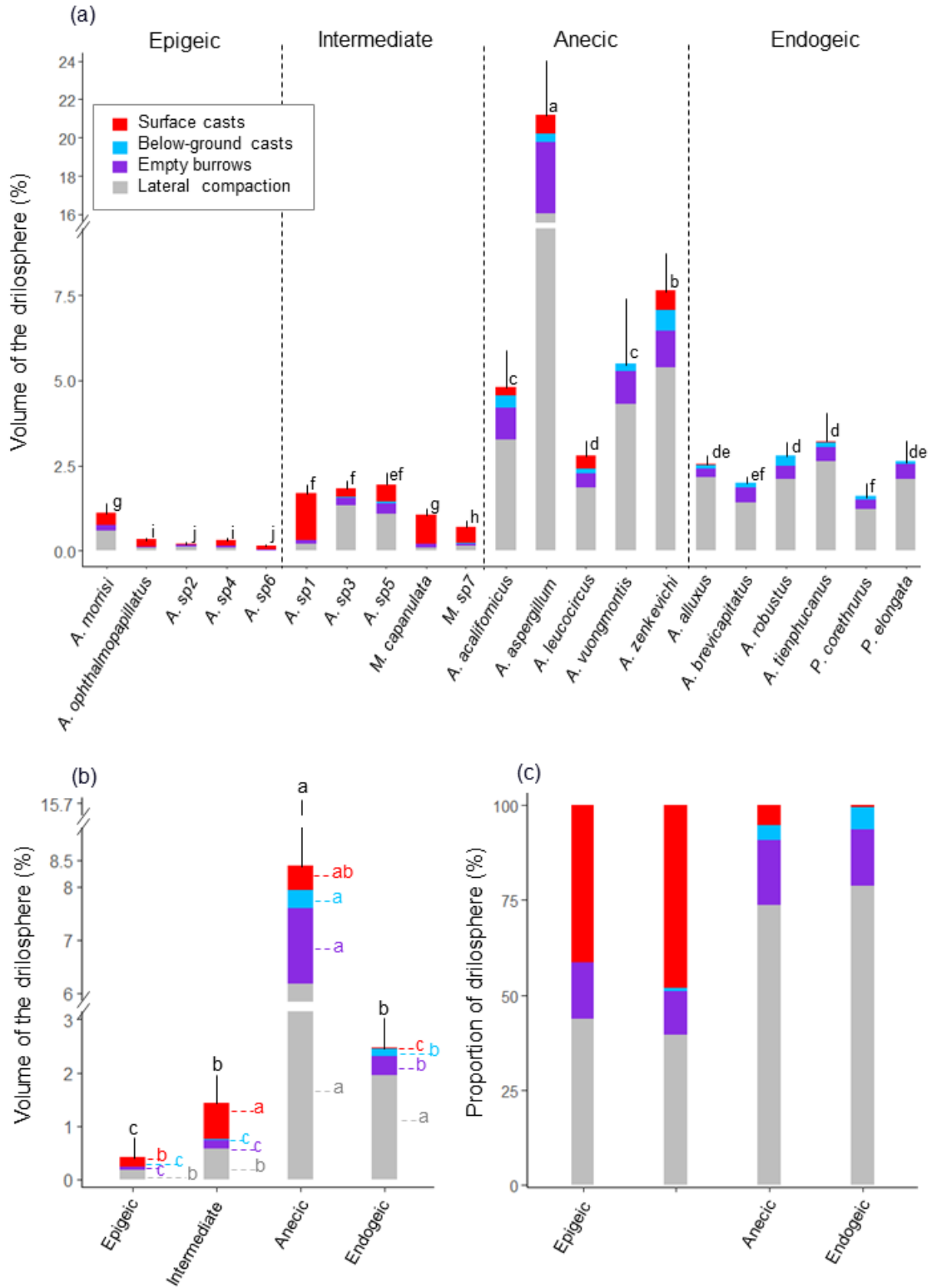


Figure 21. (a) Volume percentage of the drilosphere (ratio of the volume of the different components of the drilosphere to the volume of soil) for the 21 earthworm species and (b) ecological categories; (c) volume proportion of the drilosphere (ratio of the volume of the different components of the drilosphere to whole volume of the drilosphere). Different letters indicated statistically significant differences between species or ecological categories of the volume percentage of the whole drilosphere.

4.4.2. Compaction of the drilosphere

The grey ratio values measured in dry surface casts, wet belowground casts and burrow walls were equal or superior to 1 (Fig. 22). In general, intermediate species showed large variability in comparison to the other ecological categories. For dry surface casts (Fig. 22a), grey ratio values were superior to 1 for 12 species out of 16 with a maximum of 1.45 for the endogeic *P. elongata*. Grey ratio values were higher than 1 for anecic, endogeic and epigeic species without statistical difference between them ($p > 0.05$). Grey ratio values of dry surface casts for intermediate species were not statistically different from 1. For wet belowground casts (Fig. 22b), grey ratio values were superior to 1 for all the species (values ranged from 1.1 to 1.2). Grey ratio values were higher than 1 for the four ecological categories. Anecic and endogeic species had larger grey ratio values than epigeic species while intermediate species had intermediate values ($p < 0.05$). For the burrow walls (Fig. 22c), grey ratio values were superior to 1 for 17 species out of 21 with a maximum of 1.35 for the intermediate *A. sp5*. Grey ratio values were higher than 1 for the four ecological categories. Anecic and endogeic species had larger grey ratio values than epigeic while intermediate species had intermediate values ($p < 0.05$). Grey ratio values measured in the lateral soil compaction were superior to 1 close to the burrows walls and decreased exponentially as function of distance from the burrows (Fig. 23a). However, for *A. aspergillum* grey ratio values followed a sigmoidal relationship with distance. The radius of the lateral soil compaction varied considerably between species (from 0 to 11 mm) (Fig. 23b). intermediate species showed large variability in comparison to the other ecological categories. Largest radius was found for the anecic *A. aspergillum* and smallest radius was found for the intermediate *M. acampanulata*. The radius of the lateral soil compaction was wider for anecic, shorter for epigeic, and intermediate for endogeic and intermediate species ($p < 0.01$).

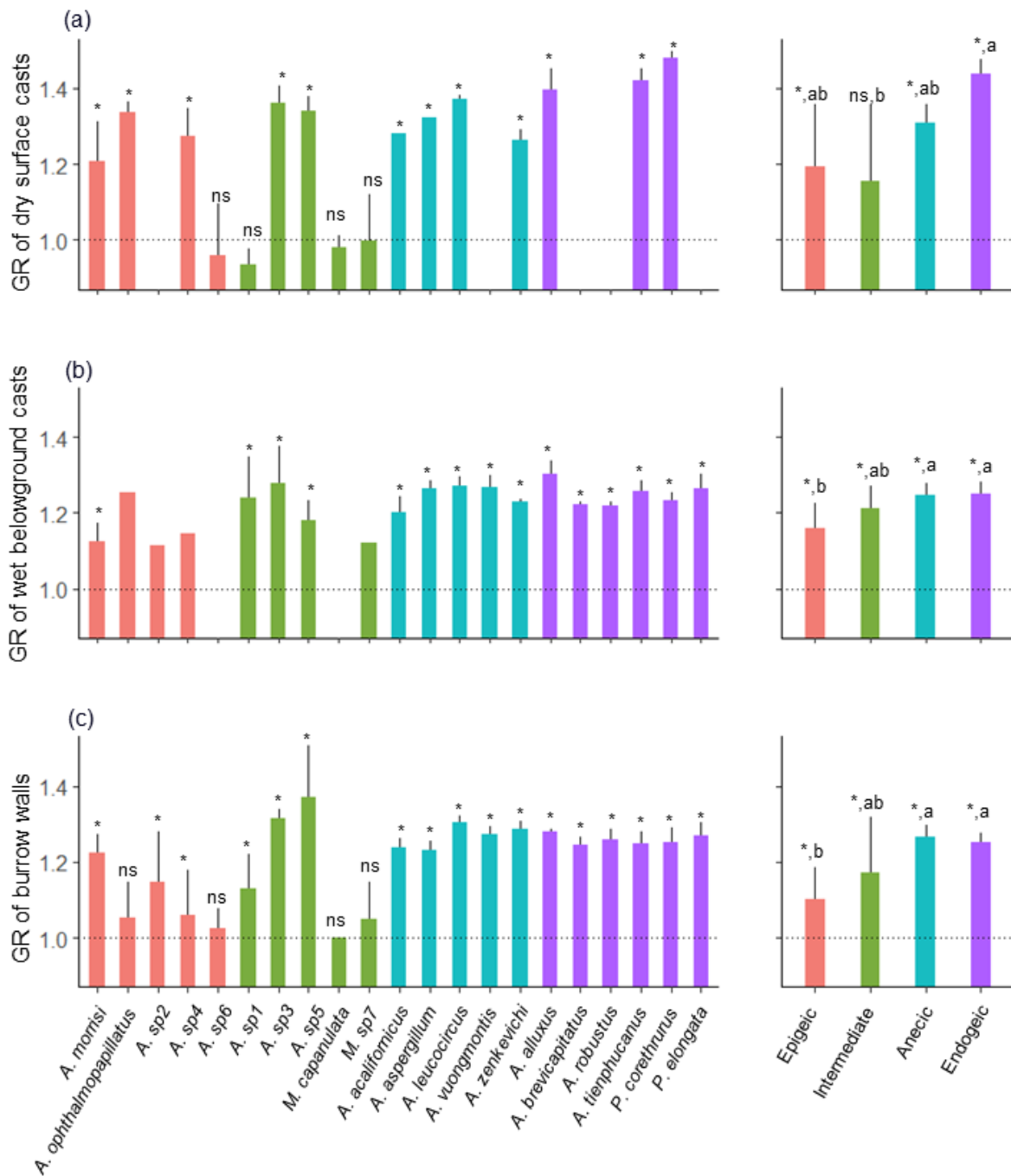


Figure 22. Grey ratio values (GR) of (a) the surface casts, (b) belowground casts and (c) burrow walls to the soil matrix of 21 earthworm species and ecological categories. Asterisks indicated a statistically significant difference and “ns” if non-significant difference with the value 1. Different letters indicated statistically significant differences ecological categories.

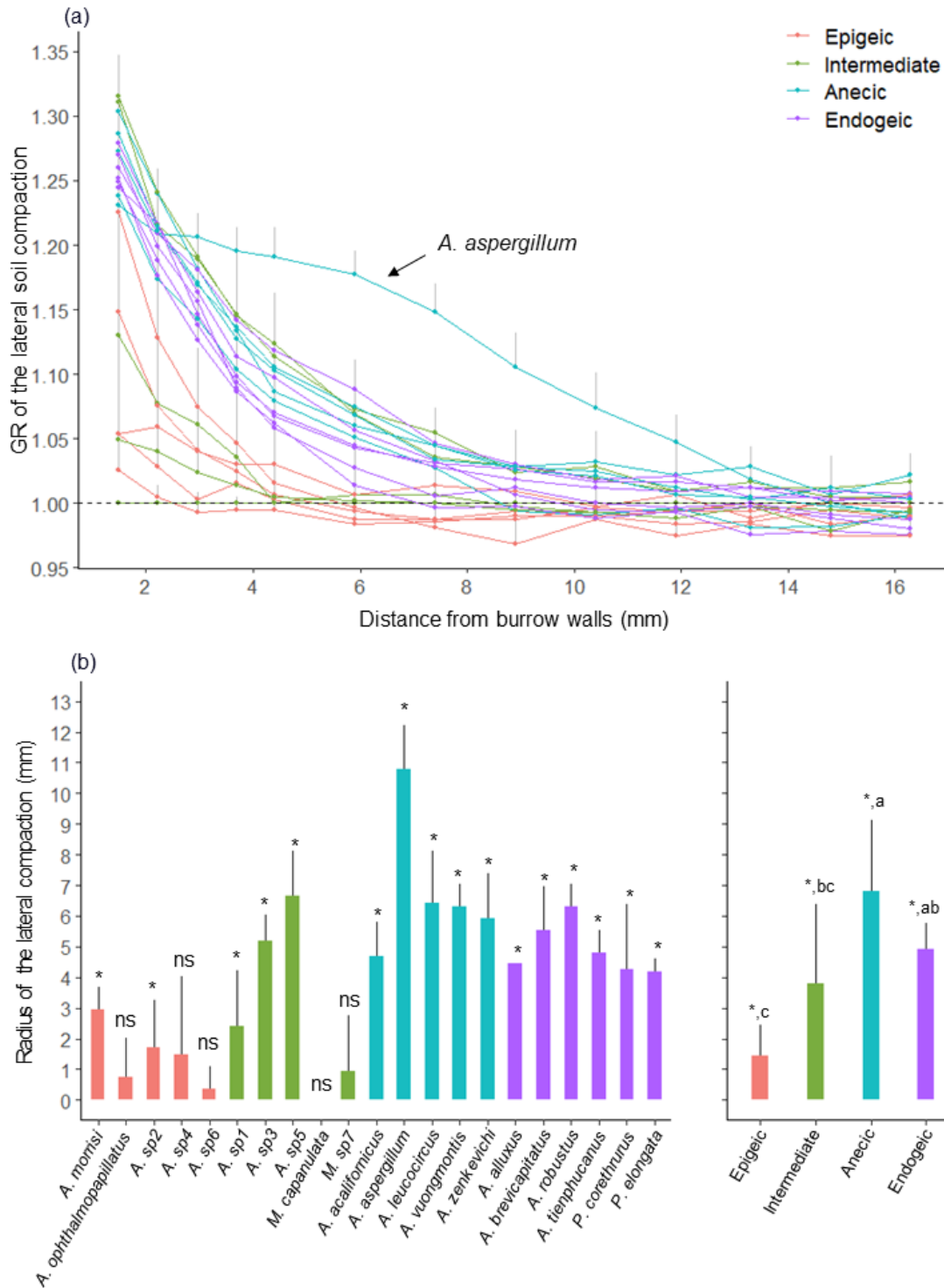


Figure 23. (a) Grey ratio values (GR) as a function of the distance from the burrow walls to the soil matrix of the 21 earthworm species grouped into ecological categories. The horizontal dashed line represents the threshold value to assess radius of the lateral compaction; (b) the radius of the lateral soil compaction for the 21 species and ecological categories. Asterisks indicated a statistically significant difference and “ns” if non-significant difference with the value 0. Different letters indicated statistically significant differences between ecological categories.

4.4.3. Relationships between morpho-anatomical traits, food ingestion and compaction of the drilopshere

The random forest regression models performed reasonably to predict grey ratio values of surface casts ($R^2 = 0.70$), burrow walls ($R^2 = 0.60$) and the radius of the lateral compaction ($R^2 = 0.65$) but did not for the grey ratio values of belowground casts ($R^2 = 0.15$) (Fig. 24).

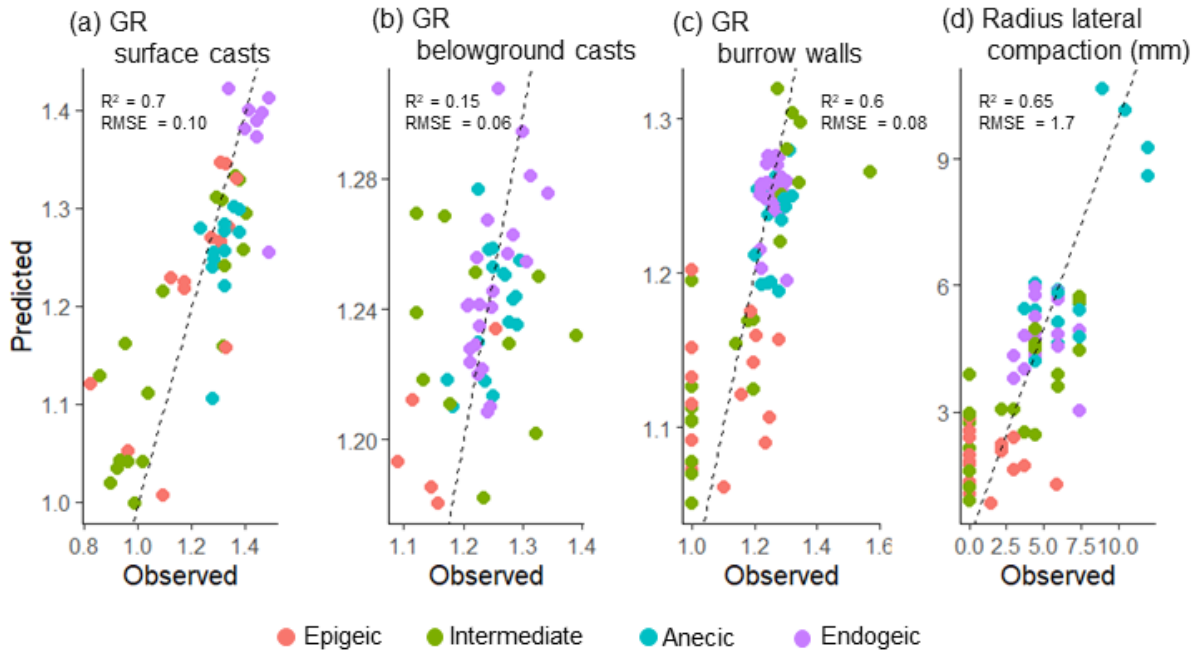


Figure 24. Predicted vs. observed grey ratio values (GR) of (a) surface casts, (b) belowground casts, (c) burrow walls and (d) radius of the lateral soil compaction of leave-one-out cross-validation derived from random forest regression. R^2 and RMSE (root-mean-square error) were presented. The black dashed line corresponds to $y = x$

We used the SHAP method to increase the model interpretability. For grey ratio values of surface casts (Fig. 25a), food consumption was the most important variable, followed by the thickness of longitudinal muscle and length of the body. The food consumption showed a negative relationship with grey ratio values of surface casts. The points representing an individual sample with low values (in blue) were mainly concentrated on the right side, while the points with high feature values (in red) were mostly on the left side. Conversely, the length of the body showed a positive relationship with grey ratio values of surface casts. In the case of the thickness of the longitudinal muscle, there was not clear positive or negative relationship with grey ratio values as the red and blue points were mixed, indicating nonlinear relationships. For grey ratio values of burrow walls (Fig. 25b), food consumption was the most important trait, followed by the diameter and the length of the body, and the thickness of the longitudinal

muscle. The food consumption showed negative relationship with grey ratio values of burrows walls whereas the diameter and length of the body showed positive relationships. Again, the thickness of the longitudinal muscle showed nonlinear relationship. For the radius of the lateral soil compaction (Fig. 25c), the diameter of the body was the most important trait, followed by the food consumption, length of the body and the thickness of the two muscles. The diameter and length of the body showed positive relationships with the radius of the lateral soil compaction of burrows walls whereas other traits showed nonlinear relationships.

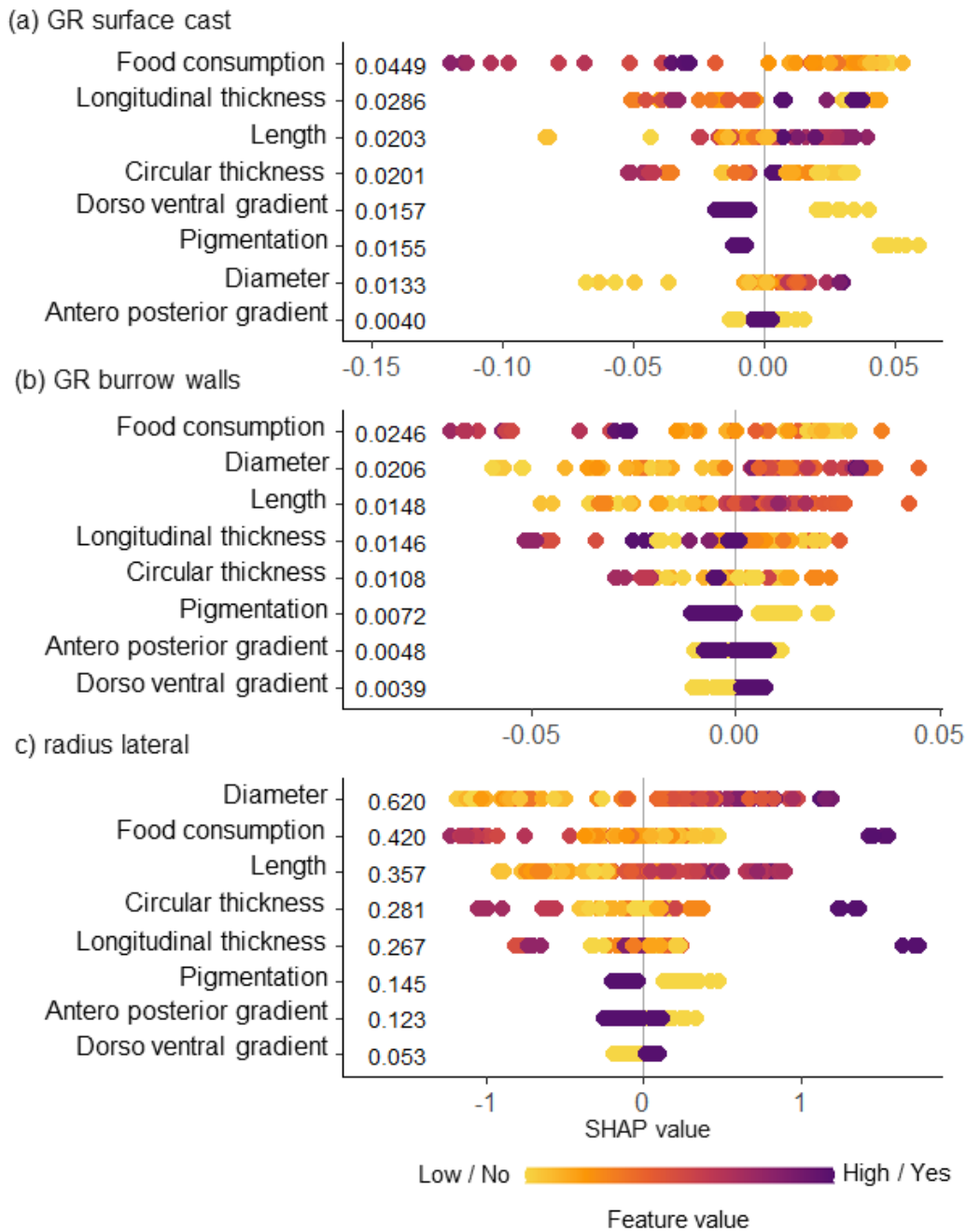


Figure 25. SHAP summary plots indicating the importance of traits and their influence on (a) surface cats, (b) burrow walls and (c) radius of the lateral soil compaction. The colors denote the feature magnitude (purple = high or presence of the categorical trait, yellow = low or absence of the categorical trait); SHAP values were shown on the x-axis, and the feature names were shown to the left of each row. Each point on the graph represents an individual sample and their color represent the actual value of that trait. The higher absolute value on the x-axis, the stronger impact on model prediction, with a positive SHAP value indicating a positive influence and a negative value indicating a negative influence. Coloring using feature values also makes it possible to visualize the general correlation between feature values and SHAP values.

4.5. Interactions of two ecologically different earthworms and its influence on water dynamics

All the earthworms were recovered at the end of the experiments. The total mass of *A. zenkevichi* decreased by 25% whereas the total mass of the *P. corethrurus* increased by 16%. Total mass change was not statistically different between single and mixed species treatments ($p > 0.05$).

4.5.1. Surface and belowground activity

Food ingestion ranged from 19.8 to 29.5 g after 92 days of incubation and was similar in treatments with single and mixed species treatments (Table 3). Similarly, no difference was found between single and mixed species treatments for the production of surface casts which ranged from 84 to 151.7 g after 92 days of incubation (Table 3). For both, values of ingestion of dung and cast production measured in mixed species treatments were significantly higher by 44% and 53%, respectively, than the expected values without interaction.

Table 3: Means (SD) of surface and belowground activity in columns with single species and mixed species after 92 days of incubation. The expected values in the mixed species columns without interaction corresponded to the average values of single species columns for each property. Values for single and mixed species were compared with one way ANOVA (p-value¹). Values bearing different letters were statistically significantly different. Comparison between mixed species columns and expected value without interaction was tested with t test (p-value²). The number of stars for the significance level indicates the p-value range (*** p < 0.001, ** p < 0.01, * p < 0.05, and ns p > 0.05).

Bioturbation behaviour	<i>P. corethrurus</i>	<i>A. zenkevichi</i>	Mixed	p-value ¹	Mixed_exp	p-value ²
Food ingestion (g)	21.1 (15.2) a	19.8 (9.6) a	29.5 (5.6) a	ns	20.5	**
Surface casts (g)	84 (65.7) a	113.8 (80.3) a	151.7 (43.5) a	ns	98.9	**
Empty burrows (cm ³)	52.1 (20.7) a	68.4 (25.8) a	58.1 (7.9) a	ns	60.3	ns
Burrow diameter (mm)	3.1 (0.2) b	3.9 (0.2) a	3.6 (0.2) a	***	3.5	ns
Number of burrows	84.5 (26.6) a	24.75 (11.5) b	66.5 (16.5) a	**	54.6	ns
Continuity of burrows (cm ³)	0 (0) c	41.2 (19.3) a	14.5 (1.2) b	***	20.6	**
Percolating burrows (cm ³)	0 (0) b	16.6 (8.8) a	9.2 (8.1) a	**	8.3	ns
Vertical distribution (cm ³)	4.9 (1.2) b	14.9 (3.0) a	5.6 (1.5) b	***	9.9	**
Belowground casts (cm ³)	29.8 (13.0) a	3.5 (1.2) b	26.2 (11.6) a	***	16.7	ns
Soil lateral compaction (cm ³)	92.0 (79.6) b	243.3 (97.2) a	172.1 (25.2) ab	*	167.6	ns

Burrows in columns with single and mixed species were shown in Figure 26. *A. zenkevichi* made few continuous burrows distributed from the top to the bottom of the column. In comparison, *P. corethrurus* made numerous burrows concentrated close to the surface. In the columns with mixed species, burrows looked like the addition of burrows made by both species.



Figure 26. 3D reconstructions of the burrow systems made by *A. zenkevichi*, *P. corethrurus* and mixed species in repacked soil cores (30 cm in height and 20 cm in diameter) after 3 months.

The quantification of burrow properties showed significant differences between the two species (Table 3): burrows of *A. zenkevichi* were statistically significantly less numerous, wider, more continuous and percolating, and deeper. No significant difference in the volume of all empty burrows was observed. The compacted zones were isolated into belowground casts and soil lateral compaction around burrows. *P. corethrurus* made significantly more belowground casts than *A. zenkevichi* whereas the volume of lateral soil compaction was significantly larger for *A. zenkevichi*. Mixing species led to properties similar to *A. zenkevichi* for the diameter and the volume of percolating burrows, similar to *P. corethrurus* for the vertical distribution, the number of burrows and the volume of belowground casts and intermediate between the two species for the volume of lateral compaction and continuity of burrows. The comparison of the measured properties in the columns with mixed species and the expected values without interaction showed that burrows were closer to the surface and less continuous.

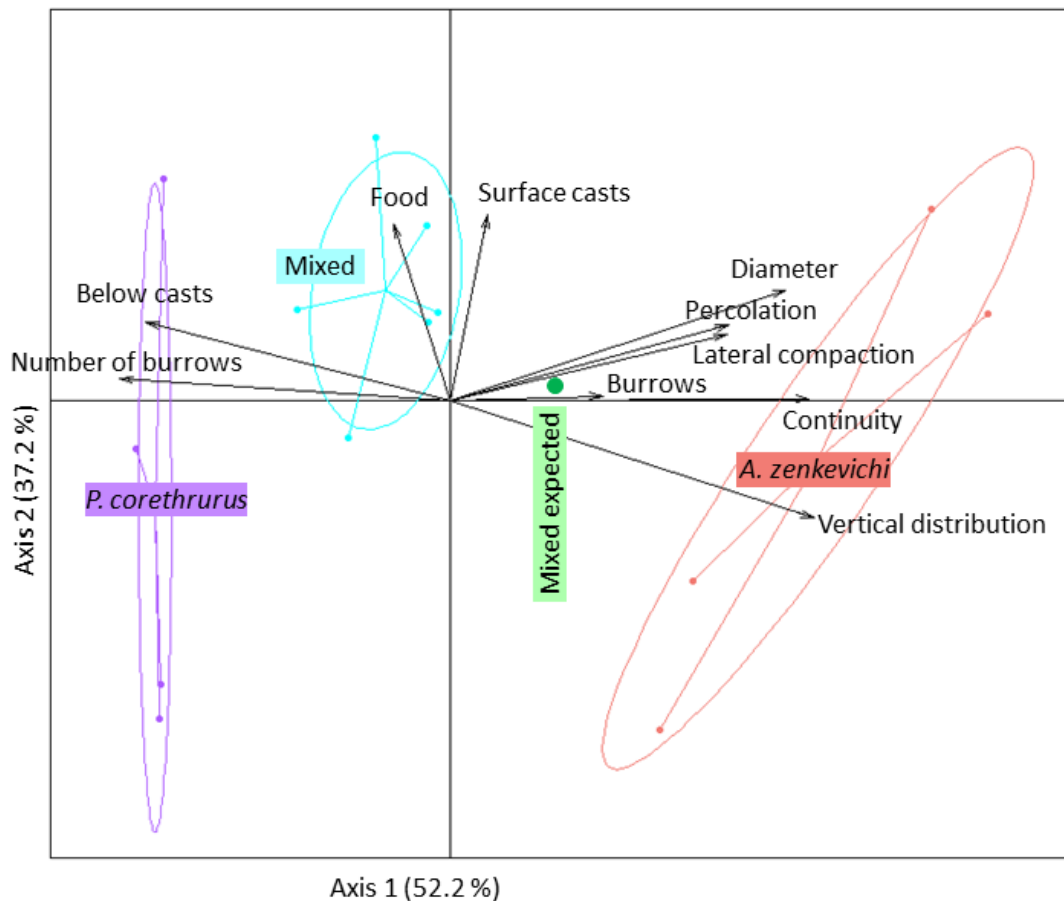


Figure 27. Biplot showing the Between-Class Analysis from variables describing the surface and belowground activity in single species and mixed species columns. The expected value of the projection of mixed species without interaction was added for comparisons.

BCA generated with the properties describing the surface and belowground activities separated columns with *A. zenkevichi*, *P. corethrurus* and the mixed species (Fig. 27). The first and second axis, explained 52.2 and 37.2% of the variability, respectively. The first axis opposed columns with *P. corethrurus* to those with *A. zenkevichi*, those with mixed species, which had intermediate values. The projection of the columns with mixed species were slightly offset toward the localization of *P. corethrurus* and did not overlap with the expected location of the mixed species columns without interaction. This axis was correlated to the belowground activity, mainly the number of burrows and volume of belowground casts (negative values) and the vertical distribution, the volume of percolating burrows, continuity, diameter, and lateral soil compaction (positive values). The second axis opposed columns with the mixed species to the expected mixed species value without interaction and was positively correlated to the surface activity.

4.5.2. Water transfers

The water infiltration rate (Fig. 28a) varied from 645 to 2,266 cm day⁻¹ and was the highest in soil columns with *A. zenkevichi*, intermediate with *P. corethrurus* and the mixed species and the lowest in the control columns without earthworm ($p < 0.05$). Water infiltration in columns with mixed species was similar than that calculated without interaction ($p > 0.05$). Water infiltration was better correlated to the volume of percolating burrows ($r = 0.7$) than empty burrows ($r = 0.6$) (Fig. 29). The cumulative water evaporation (Fig. 28b) varied from 55 to 62 mm throughout the experiment's duration and tended to be the highest in columns with earthworms, but results were not statistically significant ($p > 0.05$). The cumulative water evaporation in the column with mixed species was 4 mm lower than that calculated without interaction ($p < 0.05$). Water evaporation was negatively correlated to surface casts ($r = -0.9$) and in a lesser extent to the volume of empty burrows and soil lateral compaction ($r = -0.6$ in both cases) (Fig. 29). Soil moisture recorded after each watering events represented by the different peaks on the Figure 28d was significantly affected by earthworms ($p < 0.05$) and sampling dates without interaction ($p < 0.01$). The mean soil moisture after pouring water (Fig. 28c) ranged from 37.8 to 39% and was the lowest in soil columns with *P. corethrurus*, intermediate with *A. zenkevichi* and the mixed species and the highest in the control columns without earthworm ($p < 0.05$). Soil moisture in the column with mixed species was 0.6% higher than that calculated without interaction ($p < 0.05$). Soil moisture was negatively correlated to water evaporation ($r = -0.7$) and positively correlated to surface casts ($r = 0.6$) (Fig. 29).

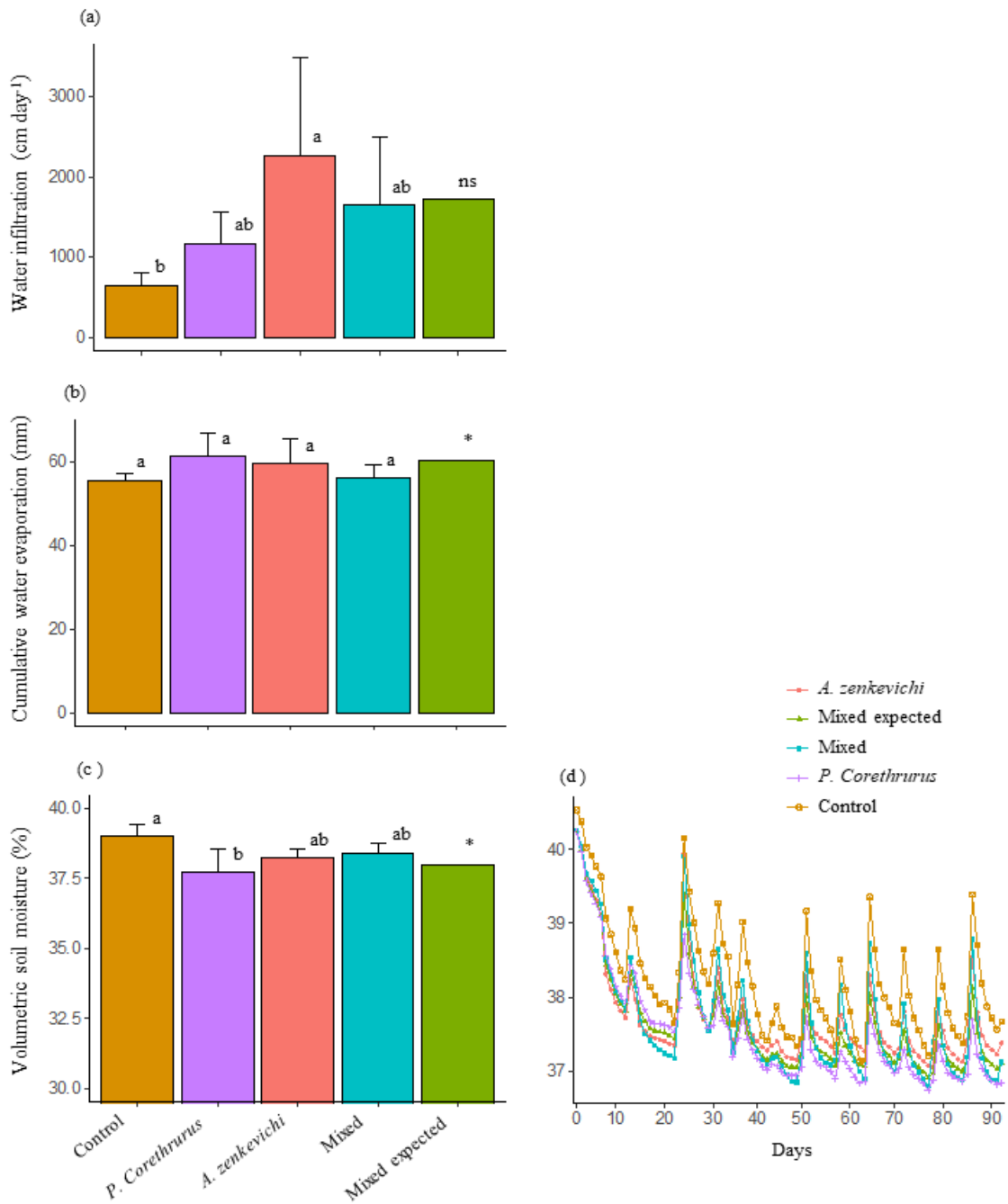


Figure 28. Means + SD of water infiltration rate (a), cumulative water evaporation (b), soil volumetric moisture (c) and soil volumetric moisture dynamics (d). Values for single and mixed species were compared with one way ANOVA. Values bearing different letters were statistically significantly different. Comparison between mixed species columns and expected value without interaction was tested with t test. The number of stars for the significance level indicates the p-value range (* $p < 0.05$)

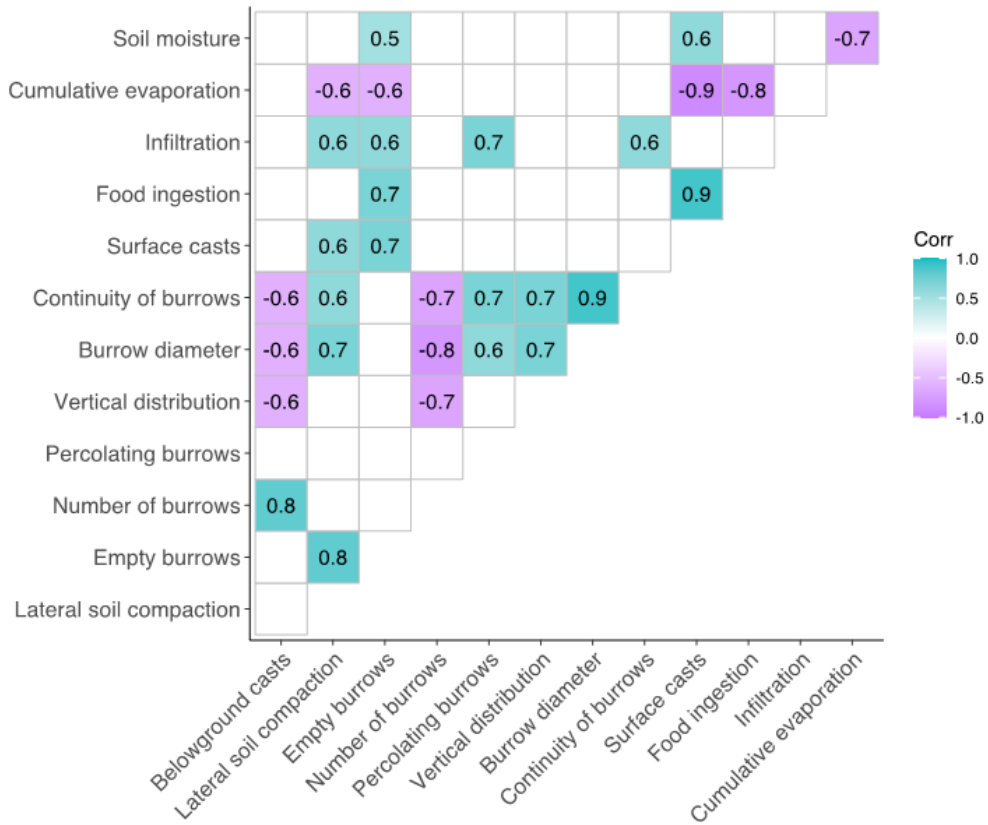


Figure 29. Spearman's correlation coefficient for all the properties measured in columns incubated with earthworms (N = 14)

CHAPTER 5 – DISCUSSION

5.1. The relationship between the drilosphere properties, ecological categories, traits and the soil water infiltration

5.1.1. Relationships between ecological categories, burrow networks and water infiltration

Epigeic, anecic and endogeic earthworms have contrasting living habitats and feeding and burrowing behaviours (Lee, 1985). Thus, it is therefore assumed that species belonging to different ecological categories, either assessed by Bouché (1972) or determined by morpho-anatomical traits, have different effects on soil functioning. As a consequence, many researchers prefer using the term functional group instead of ecological category, even if ecological categories are not designed to be functional (Bottinelli & Capowiez, 2021).

Overall, the effect of earthworms on saturated hydraulic conductivity (K_s) was either neutral or positive, with an increase of up to 30-folds in comparison to columns without earthworms. Our results support the general finding that epigeic species build few and thin burrows close to the surface (Felten & Emmerling, 2009), and consequently have no effect on K_s . Conversely, we could not confirm the general assumption that anecics had a larger effect on K_s than endogeics (Bottinelli et al., 2017; Capowiez et al., 2015). Although K_s was generally larger for anecic than endogeic species, and that their burrows were wider and more continuous, the results were not significantly different because of the large variability within each respective category. Regarding the intermediate category, the species had a similar behaviour than epigeic species, except for *M. posthuma*, which had one of the largest effects on K_s while it made burrows relatively similar to those made by some endogeic species. To our knowledge, our study is the first to report information on the burrowing activities of many Megascolecidae species. We showed a lack of consistency between the ecological categories measured from the morpho-anatomical traits, and the relative effects on soil architecture and K_s . Our findings are also in agreement with other studies on surface cast production and litter consumption (Zicsi et al., 2011), diet through the analysis of ^{15}N - and ^{13}C -enrichment of earthworms (Neilson et al., 2000) and burrow networks (Felten & Emmerling, 2009). Therefore, it can be assumed that (i) grouping earthworms into four ecological categories, instead of the seven proposed by Bouché (1972), did not allow the identification of earthworm's impact on K_s , (ii) the model proposed by Bottinelli et al. (2020a) was not appropriate to identify non-Lumbricidae

earthworm ecological categories and/or (iii) the selected morpho-anatomical traits used to assign earthworms to ecological categories could not explain the burrowing behaviour of earthworms. Thus, the next section of this discussion aims at exploring the relationships between morpho-anatomical traits, burrow network properties and K_s .

5.1.2. Relationships between morpho-anatomical traits, burrow networks and water infiltration

Among the few studies discussing the link between the morphology or anatomy of earthworms and their capacity to influence soil properties (Briones & Álvarez-Otero, 2018; Jiménez & Decaëns, 2000; Keudel & Schrader, 1999; Marichal et al., 2017), only one carried out by Marichal et al. (2017) explicitly tested this link in different fields in Brazil. Their findings revealed a weak ($RV = 0.16$ from coinertia analysis) but significant covariation between the type of typhlosole, size of gizzard and thickness of septa and soil structure properties. Our study, carried out under laboratory conditions, confirms the possibility of linking morpho-anatomical traits and soil structure. We showed a moderate covariation between morpho-anatomical traits and burrow network properties ($RV = 0.47$). In addition, we showed the usefulness of measuring effect traits to predict the capacity of earthworms to increase K_s (R^2 of 0.97 from the random regression model). Among the 16 traits studied, we identified the body weight and the thicknesses of the circular and longitudinal muscles as the three most important traits for the prediction of K_s . In general, the larger the body weight and muscle thicknesses, the larger the effect on K_s . Hence, we demonstrated that the use of only three morpho-anatomical traits could be related to changes in the burrow networks and in turn on K_s values.

5.1.3. Volume properties of the drilosphere and its relationship to ecological categories and traits

Studies reporting the influence of earthworm activity on the soil structure were often limited to the observation of the surface casts or empty burrows. With few exceptions (Capowiez et al., 2011; Jégou et al., 2000), the whole soil drilosphere encompassing together surface and belowground casts, burrows and soil lateral compaction around burrows has never been addressed. Here, we showed for the first time that casts and lateral soil compaction around burrows occupied together more than 70% of the whole drilosphere regardless the ecological category. This finding provided evidence that empty burrows were only the tip of the iceberg and the rest of the drilosphere should not be omitted when one wants to assess the influence of

earthworms on soil structure. On average, the three main ecological categories have contrasting bioturbating behaviours while intermediate species had similar behaviours than epigeic species. However, due to the generally larger body size of intermediate species, the volume of their drilosphere was greater than that of epigeic species. The activities of epigeic and intermediate species were evenly distributed between soil surface and belowground, while the activity of endogeic and anecic species was higher belowground. Differences of behaviour between anecic and endogeic species were mainly due to the surface casting activity which occurred almost exclusively for anecic species. As a result, the calculated proportion of belowground and surface casts represented 90% for endogeic species and 52% for anecic species. This finding confirms the study of Capowiez et al. (2011) who found that 85 and 70% of the casts were made belowground by the endogeic *A. chlorotica* and the anecic *A. nocturna*, respectively. Contrary to our expectation, the volume of belowground casts was larger for anecic than for endogeic species, although the percentage of burrow backfilled was slightly higher for endogeic (27%) than for anecic species (23%). We showed that both categories produced belowground casts resulting in similar rate of backfilling. Consequently, this does not confirm that anecic burrows are more permanent, more reused than those made by endogeic species as usually claimed when considering only the three main ecological categories. Similarly, the calculated ratio between the volume of the soil lateral compaction to the volume of empty burrows and belowground casts showed similar values for anecic (3.3) and endogeic species (4.0), indicating that under our experiment conditions both anecic and endogeic species dug rather by pushing radially and by ingesting the soil. Among the 21 species, one species was clearly wrongly assigned. *A. vuongmontis* was classified as an anecic species based on its morpho-anatomical traits, while it did not show any surface activity (no dung consumption and no production of surface casts). Therefore, this species could be described as hypoenogeic (i.e., species living exclusively belowground), at least under our experimental conditions. In conclusion, our study shows that the universality of the Bouché's ecological category classification can be questionable, as it was made mainly for Lumbricidae and seems not to be completely adapted to tropical species.

5.1.4. Compaction of the drilosphere and its relationship to ecological categories and traits

The drilosphere was generally more compacted than the soil matrix, with the exception of surface casts of some epigeic and intermediate species. This obviously indicates that earthworms compact more the soil than they loosen it since casts and lateral soil compaction

occupied the largest part of the drilosphere. Both anecic and endogeic had similar and larger effects on the drilosphere compaction than epigeic species. Conversely, intermediate species, gathered together species having little and large effects on the compaction of the drilosphere. This finding highlights another limitation of the Bouché's ecological category approach to assess the effect of earthworms on soil structure.

Grey ratio values of surface casts were larger than 1, except for a few epigeic and intermediate species, indicating an increase of compaction for most of the species. The maximum grey ratio value was 1.45 for the endogeic *P. corethrurus*, a value of the same order of magnitude as observed by Blanchart et al. (1993) and De Vleeschauwer and Lal. (1981) comparing bulk densities of endogeic surface casts and topsoils. On average, grey ratio values decreased gradually in the following order: endogeic > anecic > epigeic, intermediate. Our results confirmed the results of Le Mer et al. (2022), who showed that the volume of pores > 10 μm of freshly emitted surface casts was the highest for epigeic species and the lowest for endogeic species. Our study revealed that variations in grey ratio values of surface casts were reasonably predicted by morpho-anatomical traits and feeding behavior ($R^2 = 0.70$), with the primary factor explaining these changes being the consumption of surface dung. It can be assumed that dung ingested increased the organic matter content in casts and thus decreased their bulk densities. This result was in line with Decaëns et al. (2000) who found that surface casts collected in pasture had lower bulk density than those from savanna due to their enrichment in organic debris. We also found that the thickness of the longitudinal muscle played a significant role in the capacity of earthworms to compact surface casts, probably in the tail region as hypothesized by Blanchart et al. (1993). However, the relationship was nonlinear and not easy to interpret.

Grey ratio values of belowground casts were larger than 1 with a maximum of 1.30 for the endogeic *A. alluxus*, indicating that belowground casts were more compacted than the soil matrix. This result was expected since belowground casts were isolated from the soil matrix by their lighter grey color. The increase of grey ratio values of belowground casts, when compared to the soil matrix, was lower and exhibited less variation between species than the observed for surface casts. This phenomenon might be ascribed to the difference in soil moisture (i.e., the shrinking of clayey aggregates during drying was well known to increase bulk density). Indeed, while the properties of surface casts were measured after air-drying, belowground casts were measured directly in soil, without pre-drying procedure. On average, grey ratio values of belowground casts decreased gradually in the following order: endogeic, anecic > intermediate

> epigeic. However, neither food ingestion nor morpho-anatomical traits were considered important to predict grey ratio values variations of belowground casts. If we hypothesized that carbon enrichment was similar between surface and belowground casts (Jégou et al., 1998), it can be assumed that soil moisture variations between casts from different species impeded relationships with functional traits.

Grey ratio values of burrow walls were generally higher than the soil matrix (with a maximum of 1.38 for the intermediate *A. sp5*), except for few epigeic and intermediate species, indicating an increase in compaction for most of the species. Our results were comparable with Rogasik et al. (2014) who measured that *L. terrestris* increased the bulk density of the burrow walls by 30% compared to the soil matrix. Similarly, Capowiez et al. (2021) found that the burrow walls of *A. caliginosa* and *A. chlorotica* increased the compaction up to 10% in comparison to the soil matrix. Similarly, the radius of the lateral soil compaction varied according to species and ecological categories, but variations were more important than those measured on burrow walls. The widest soil lateral compaction reached 11 mm, and it was measured with the the anecic *A. aspergillum*. This value was in agreement with the 22 mm found for *L. terrestris* by Schrader et al. (2007) but measured from the center of the burrow, having a diameter of 9 mm.

Grey ratio values of burrow walls and the radius of the soil lateral compaction decreased gradually in the following order: anecic, endogeic > intermediate > epigeic. Here we clearly showed that endogeic and anecic species had similar effects on lateral soil compaction around burrows probably because both dug more by pushing the soil radially and ingesting it. Variations between species were reasonably predicted using morpho-anatomical traits and feeding behavior (R^2 ranged between 0.60 to 0.65). Barnett et al. (2009) investigated the soil displacement during the burrowing of *L. terrestris* and *A. longa*. They assumed that differences in longitudinal muscular thickness and body mass differences explained the amount of soil displaced by the two species. Here, we confirmed partially their hypothesis. We found that the larger was the earthworm (diameter and length) and the larger its effect on the lateral compaction. However, not only the longitudinal muscles but also the circular muscles were involved but in not a linear way. Interestingly, we found that the increase of dung ingestion decreased burrow wall compaction, which might indicate the presence of casts enriched in organic matter crushed on the burrow walls, decreasing locally the compaction of burrow walls. Finally, it is worth mentioning that pigmentation and color gradients, both widely used to assign ecological categories were poorly related to the compaction of the drilosphere.

5.2. The impact of interactions between two ecologically distinct earthworm species on water dynamics

5.2.1. Differences between *A. zenkevichi* and *P. corethrurus*

The characterization of the burrows made by the two species was already described in section 4.3.1. The burrow quantification in this experiment confirms the findings obtained from the first experiment. Burrows of *A. zenkevichi* were continuous and well connected from the surface to the bottom of the columns. Conversely, *P. corethrurus* burrows were discontinuous and located in the top 5 cm of the columns. In addition, we found that the percentage of burrows backfilled by casts was larger in columns with *P. corethrurus* (37%) than those with *A. zenkevichi* (5%). This last result was comparable with the study of Capowiez et al. (2011) in which the percentage of backfilled burrows represented 18% in columns with the endogeic *A. chlorotica* and 10% in columns with the anecic *A. nocturna*. We also showed that the ratio between the volume of soil lateral compaction to the volume of burrows (empty burrows and burrows backfilled by belowground casts) was much larger in columns with *A. zenkevichi* (3.4) than those with *P. corethrurus* (1.1). This result suggests that *A. zenkevichi* by pushing radially the soil when burrowing had more impact on soil compaction than *P. corethrurus* who ingested soil and backfilled their burrows. Both species ingested buffalo dung and produced surface casts at similar rates. This finding was at first glance rather surprising since *P. corethrurus* is a geophagous species (Lavelle et al., 1987; Topoliantz & Ponge, 2003) and burrow soil to find patches of soil organic matter. Our results could be explained by the very low organic carbon content of the soil (0.3%) forcing *P. corethrurus* to find organic matter at the soil surface. This assumption confirms the important feeding and behavioural plasticity of *P. corethrurus*. Altogether, our results were consistent with the well-acknowledged distinction between burrow systems of anecic and endogeic (Capowiez et al., 2015), but they suggest that *P. corethrurus* should be classified as an epi-endogeic species *sensu* Bouché (1977) in our experimental conditions.

5.2.2. Interspecific interactions

We attempted to separate burrows made by the two species within mixed-species columns using the diameter of burrows of each species, as proposed by Capowiez et al. (2001) and Jégou

et al. (2001a). However, this method proved ineffective since the burrow diameters of the two species were relatively similar (3.1 vs. 3.9 mm). We, therefore, compared the measured values of the mixed species columns to the expected value without interaction. Using BCA, we explored the dissimilarities of surface and belowground activities between single and mixed species columns and showed a slight offset, indicating that interactions occurred. The surface activity increased (ingestion of dung and cast production increased by 44% and 53%, respectively) and the burrow systems were more similar to the burrow systems of *P. corethrurus* (i.e., less continuous and less deep). These findings do not confirm the indirect interactions between endogeic and anecic species (Capowiez et al., 2001; Felten & Emmerling, 2009; Jégou et al., 2001a) that could lead either to a decrease in the burrowing activity of endogeic species or an increase in the depth of their burrows. Our results suggest rather an increase of the activity of *P. corethrurus* at the expense of *A. zenkevichi*. However, further investigations into these relationships are necessary in order to gain a comprehensive understanding of the findings presented. At this stage, it was impossible to say whether these interactions were linked to spatial competitions or to trophic interactions between species since both species were active at the soil surface.

5.2.3. Consequences on water transfers

The contrasting burrowing strategies influenced water infiltration rates, whereas the interactions did not. *A. zenkevichi* significantly increased water infiltration rate by 351% compared to the columns without earthworm. In contrast, the impact of *P. corethrurus*, despite having a mean effect that was 184% higher, did not show a significant difference from columns without earthworms. The divergent burrowing strategies had a noticeable effect on water infiltration rates, whereas the interactions did not. Specifically, *A. zenkevichi* significantly boosted water infiltration rates by 351% when compared to columns without earthworms. In contrast, the impact of *P. corethrurus*, although having a 184% higher mean effect, did not show a significant difference from columns without earthworms.

This result was associated with the large volume of percolating burrows and therefore confirms the important role of anecic species in transferring water by gravity when the soil is saturated with water (Capowiez et al., 2015). Even if interactions led to some changes in burrow properties, the volume of percolating measured was similar than that expected without interaction, which explain therefore why interactions did not change water infiltration rate. The

effect of earthworms on water evaporation and soil moisture was very low. *P. corethrurus* increased soil water evaporation by 10% during all the experiment and decreased soil moisture by 3% compared to the columns without earthworm, whereas *A. zenkevichi* had marginal effects. The impact of earthworms on soil moisture can be influenced by several processes having antagonist effects. In one hand, empty burrows can increase gas exchange and thus enhances the loss of water vapor from soil. On the other hand, compacted zones (casts and lateral compaction) can have more micropores (Görres et al., 2001; Lipiec et al., 2015), thus increasing soil water holding capacity (McDaniel et al., 2015). In addition, cast produced on soil surface can be seen as a mulch acting as a physical barrier that could prevent water and heat exchange between the soil and the atmosphere (Liu et al., 2021). The small effects of earthworms on soil moisture and water evaporation were line with Ernst et al. (2009) who showed that both the epi-anecic *L. terrestris* and the endogeic *A. caliginosa* had the tendency to dry the soil in a 100 days laboratory experiment. Our results are, however, in apparent contradiction with Ma et al. (2020) who found that the anecic *A. aspergillum* increased soil moisture up to 26% and decreased soil water evaporation up to 21% in a 100 days semi-field experiment. In our study and that of Ernst et al. (2009), soil columns were regularly watered to promote earthworm activities whereas in Ma et al. (2020) soil columns were left to dry. Since the soil holding capacity was strongly related to soil moisture (i.e., higher was soil moisture and lower was the capacity of pores to hold water), we assume that different results were partly linked to the high soil moisture content under our laboratory conditions, promoting therefore water evaporation from burrows, particularly those close to the soil surface made by *P. corethrurus*. It can be expected that earthworms would reduce soil water evaporation and increase soil moisture at dryer soil states. We found that soil water evaporation was strongly negatively correlated to the surface casts as it was found by Liu et al. (2021). Because the production of surface casts was similar between both species there was not difference in soil water evaporation between them. Conversely, the increase in casting activity due to interactions decreased significantly by 8% soil water evaporation.

CHAPTER 6 – CONCLUSIONS AND PERSPECTIVES

In conclusion, this study demonstrated that earthworm's functional traits can be seen as a promising avenue for studying the impact of earthworms on several key ecosystem processes, such as water dynamics in soil. As far as we are aware, this study is the first to look at Megascolecidae earthworms, and we demonstrated an interest in using the trait-based approach for predicting the impact of earthworms on water.

We showed that earthworms can be assigned into ecological categories, based on the traits selected for Lumbricidae. However, due to the limited number diversity of species collected in our study, only four categories could be distinguished. Since the work of Bouché (1972) identified seven categories when working with more than 125 species, one could expect a higher number of categories with more studied Megascolecidae species. However, we also showed that this classification did not allow us to predict water dynamics in soil. As a first perspective, we propose utilizing the functional classification made by Capowiez et al. (2024), which grouped 50 earthworm species from Europe into six functional groups based on their bioturbating behaviors. Firstly, we could examine whether the tropical species studied in this thesis align with the six established groups. Secondly, it would be intriguing to juxtapose the morphology and anatomy of individuals within each group between European and tropical species. For example, do epigeic species exhibit similar sizes? Are endogeic/hypo-endogeic species uniformly unpigmented? Such a comparison would enable us to evaluate the feasibility of employing a generalized model based on morphology and anatomy to identify the six functional groups. Given that only 21 species were investigated in this thesis, which is insufficient to develop a classification model capable of identifying functional groups based on their morpho-anatomy, we are presently examining earthworm species collected in south of Vietnam. We anticipate that studying a minimum of 100 species will allow us to propose such a model.

Using machine learning statistical analyses, we demonstrated that the impact of earthworms on water infiltration in soil could be well predicted from only three effect traits (body weight, circular and longitudinal muscles thickness). Therefore, our study opens the question of the possibility to predict the impact of earthworms on water dynamic and soil compaction not in function of the Bouché's ecological categories they are assigned but based on particular morpho-anatomical traits. Another perspective of our research is to investigate, under field conditions, the correlation between the presence of these specific traits and soil

water dynamics. This could be achieved by assessing trait profiles by calculating community-weighted means for each trait and measuring soil hydraulic conductivity across various fields. This endeavor will enable us to determine the feasibility of using traits as a tool for ecosystem assessment.

Another striking conclusion of this study is that we demonstrated that the drilosphere is likely to be often underestimated because the soil volume surrounding the burrows is almost never taken into account, while it constitutes a significant proportion of the drilosphere. Indeed, while burrows are macropores facilitating the rapid movement of water and air within the soil, casts and lateral soil compaction tend to be denser than the surrounding soil. This implies a reduction in macroporosity and potentially changes in the distribution of other pore sizes. Consequently, these findings underscore the importance of quantifying the impact of earthworms on water dynamics, particularly under non-saturated conditions, and on other soil functions such as organic matter storage.

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Support information

Supplementary 1.

Earthworms increase soil water infiltration at saturation condition: a meta-analysis

+ Method:

A literature review carried out in December 2022 and using Web of Science with the following search terms and selecting the timespan “all years”: TS = ((hydraulic* OR infiltration* OR hydrological* OR hydrodynamic* OR water retention* OR moisture*) AND earthworm* NOT vermi*) showed a record track of 826 articles. The articles were screened based on the criterion that soil water infiltration was reported for treatments with and without earthworms. Most of the studies that fit the criteria evaluated the impact of earthworms on water infiltration under saturated conditions. Therefore, at the end, twenty-six studies fitted our meta-analysis selection criteria.

For each study, the information that was collected concerned the soil properties (soil type, soil texture, organic carbon, pH), earthworms (species, family, ecological categories, density, biomass), experimental details (laboratory or field experiment, natural soil or repacked soil, experimental duration, treatment, replication, initial bulk density) and the results (mean and SD of soil hydraulic conductivity for with and without earthworm treatments). In case information on the earthworm ecological categories and families is missing, earthworms were categorized into ecological groups based on author statements and the World Earthworm Database “Drilobase” (www.drilobase.org). When data were only presented graphically, values were estimated using the image analysis software ImageJ. If mean or SD values were missing, they were estimated with the methods proposed by Wan et al. (2014) using mean, min, max and number of observations. For studies that did not report minimum and maximum values, the missing SD values were approximated by multiplying the reported mean by the average coefficient of variation for control treatments and experimental treatments (Bai et al., 2013; Fu et al., 2013). In the case of studies that reported data on long-term experiments with different sampling dates, only results at the end of the experiment were considered.

The magnitude of the earthworm-induced effect on soil water infiltration in each study was calculated as the logarithm of the response ratio R (LRR) (Hedges, 1999):

$$LRR = \ln \left(\frac{E}{C} \right)$$

where E and C were the mean soil water infiltration at saturation condition in earthworm treatments and control treatments without earthworms, respectively. A positive effect size (LRR > 0) means earthworms increased soil water infiltration, and a negative effect size (LRR < 0) means earthworms reduced soil water infiltration. When studies reported results for different treatments or habitats, response ratios were calculated using earthworm and control treatments with the same setup.

The analysis was carried out using R (R Core Team, 2019) and the metafor package (Viechtbauer, 2010). Final models were fitted using a restricted maximum-likelihood estimator (REML).

+ Results:

After a systematic literature review, twenty-six articles covered 87 pairwise comparisons (observations) including 75 observations from single-species experiments and 12 from mixed-species experiments were extracted. 65 out of the 87 observations came from studies carried out in laboratory conditions (all with repacked soil) and the remaining (22 studies) corresponded to field experiments. 18 different species belonging to 6 families and 1 unidentified species were represented. In addition, most of the studies focused on Lumbricidae earthworms while other families were almost totally ignored (Figure S1.1). The most commonly used species were *Lumbricus terrestris* (12 observations), *Allolobophora chlorotica* (12 observations), *Aporrectodea caliginosa* (21 observations), and *Lumbricus rubellus* (9 observations) (Figure S1.2). In single-species experiments, the most earthworm species used were endogeic earthworms (64 observations), followed by anecic (16 observations) and epigeic (9 observations) earthworms, and only few experiments used sub ecological categories including endo-anecic earthworms (1 observation) or epi-endogeic earthworms (1 observation) (Figure S1.2).

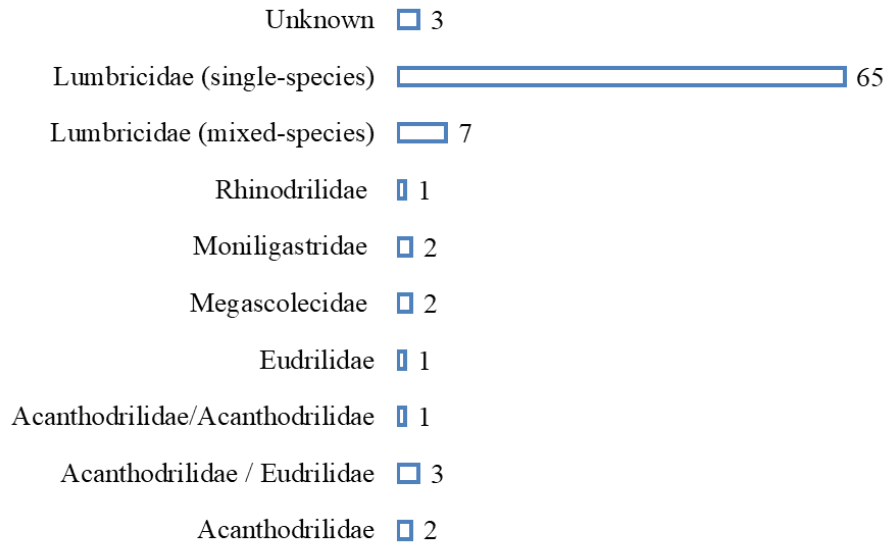


Figure S1.1. Distribution of observations by families in the data sheet

This meta-analysis shows that earthworms have in general a positive effect on soil water infiltration at saturation, as the mean LRR value was 0.75 ($CI_{0.95} = 0.50$ to 1.00) (Figure S1.2). Only a very limited number of studies showed negative LRR values. The impact of single- or multi-species varied significantly, as evidenced by the heterogeneity in the data structure. This heterogeneity is reflected in the results of Cochran's Q-test: $Q = 10,832$, $p < 0.0001$, $\tau^2 = 1.4$, $I^2 = 99.85\%$ for single-species experiments and $Q = 236$, $p < 0.0001$, $\tau^2 = 0.21$, $I^2 = 99.34\%$ for multi-species experiments. Overall, in single species experiments, earthworms increased soil water infiltration compared to control treatment (overall mean LRR = 0.87, $CI_{0.95}$: 0.58 to 1.15), while in multi-species experiments, the effects were similar to control treatment (overall mean LRR = 0.12, $CI_{0.95}$: -0.15 to 0.4). Regarding ecological categories, although the overall mean LRR decreases in the order Anecic > Endogeic > Epi-endogeic > Epigeic > Endo-anecic, there were no significant differences between groups as shown by the overlap of $CI_{0.95}$. Additionally, there were no significant heterogeneity in data (Q-test, $p = 0.22$).

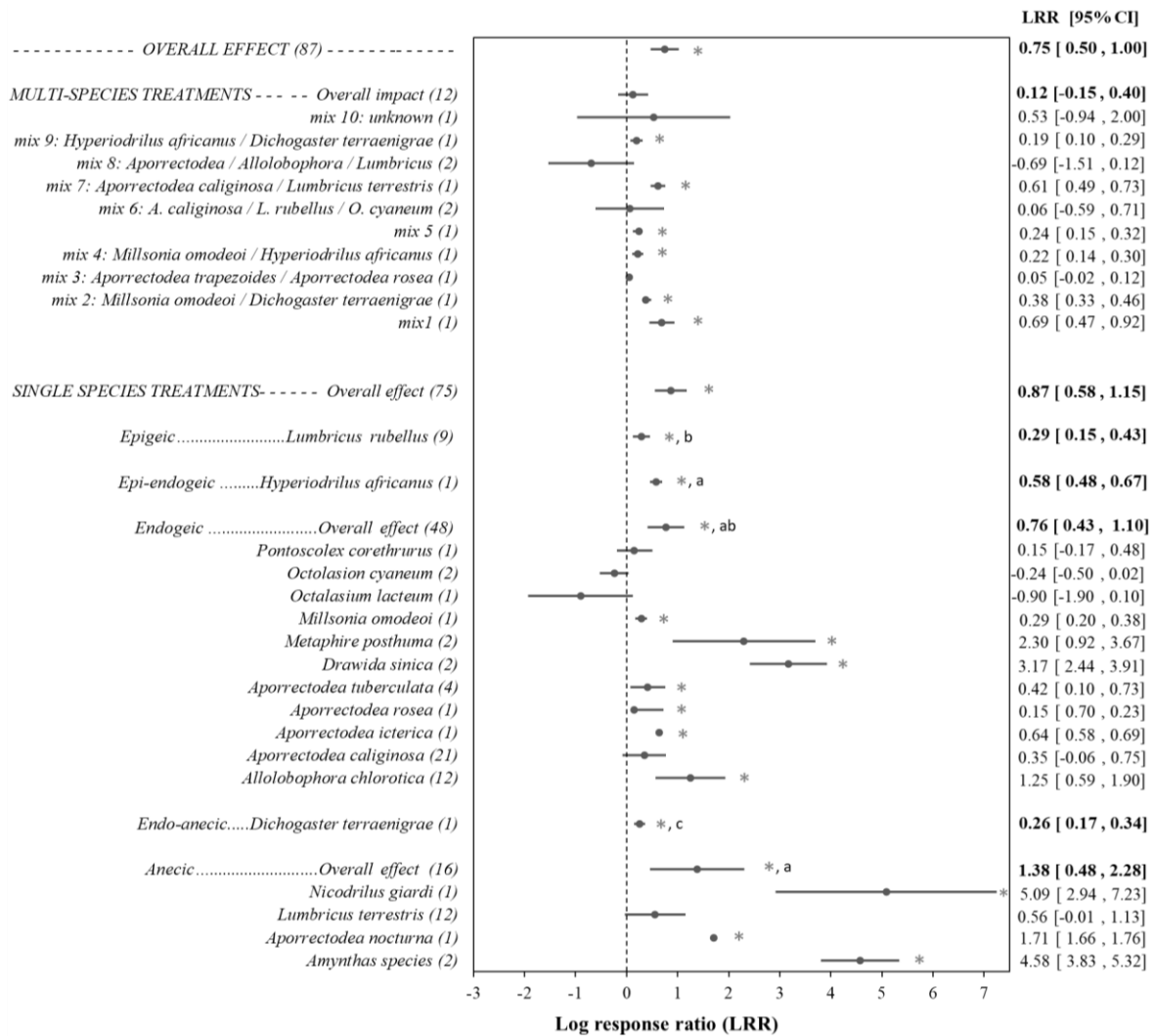


Figure S1.2. The transformed response ratios (effect size) pertaining to earthworm effects on soil water infiltration at saturation condition. Forest plots show the mean effect, error bars denote the 95% confidence intervals (CI_{0.95}), and within parentheses the sample sizes. Earthworm effects were considered significant when confidence intervals did not overlap with zero (indicated by asterisks). Different letters denote significant differences between categories; categories are considered to be significantly different when their CI_{0.95} do not overlap. Mix 1: *Lumbricus terrestris*, *Allolobophora longa* / *Aporrectodea rosea*, *Allolobophora chlorotica*, *Aporrectodea caliginosa* / *Lumbricus castaneus*; mix 5: *Millsonia omodeoi* / *Hyperiodrilus africanus* / *Dichogaster terraenigrae*.

This meta-analysis has revealed that the available information regarding the effects of single-species and multi-species earthworms, as well as their ecological categories, on soil water infiltration under saturated conditions is currently highly fragmented.

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Supplementary 2.

Table S1: Morpho-anatomical traits characterizing the 23 species of earthworms collected in northern Vietnam.

Species	Length (mm)	Diameter (mm)	Weight (mg)	Pigmentation	Antero-posterior gradient	Dorso-ventral gradient	Epithelium rigidity	Muscle structure
<i>A. acalifornicus</i>	137.33 (0.58)	6.46 (0.27)	4986.67 (430.16)	Yes	No	Yes	Yes	Intermediate
<i>A. alluxus</i>	120.00 (4.00)	2.94 (0.04)	916.67 (35.12)	No	No	No	No	Intermediate
<i>A. aspergillum</i>	201.33 (51.03)	7.19 (0.22)	12156.67 (3297.79)	Yes	Yes	Yes	Yes	Fasciculated
<i>A. brevicapitatus</i>	127.67 (14.57)	6.04 (0.03)	3713.33 (165.03)	No	No	No	No	Fasciculated
<i>M.acampanulata</i>	115.00 (8.72)	2.93 (0.24)	2003.33 (191.40)	Yes	Yes	Yes	No	Intermediate
<i>A. leucocircus</i>	153.33 (13.32)	4.44 (0.20)	2226.67 (411.99)	Yes	Yes	Yes	Yes	Fasciculated
<i>A. morrisi</i>	66.00 (8.72)	2.11 (0.36)	573.33 (80.21)	Yes	Yes	Yes	No	Intermediate
<i>A. ophthalmopapillatus</i>	85.33 (11.55)	3.09 (0.30)	813.33 (41.63)	Yes	Yes	No	No	Fasciculated
<i>A. robustus</i>	93.33 (3.51)	6.36 (0.29)	3053.33 (66.58)	No	No	No	No	Fasciculated
<i>A. sp1</i>	128.33 (1.53)	5.05 (0.51)	2880.00 (141.77)	Yes	Yes	Yes	No	Fasciculated
<i>A. sp2</i>	53.00 (5.29)	2.93 (0.12)	416.67 (28.87)	Yes	Yes	No	No	Intermediate
<i>A. sp3</i>	118.00 (2.00)	3.99 (0.17)	1873.33 (70.24)	Yes	Yes	Yes	No	Fasciculated
<i>A. sp4</i>	77.00 (3.00)	2.70 (0.21)	590.00 (20.00)	Yes	Yes	No	No	Intermediate
<i>A. sp5</i>	138.00 (24.06)	4.73 (0.46)	3246.67 (228.98)	Yes	Yes	Yes	Yes	Intermediate
<i>A. sp6</i>	60.33 (1.53)	2.58 (0.10)	296.67 (51.32)	Yes	Yes	Yes	No	Intermediate
<i>A. tienphucanus</i>	140.00 (5.00)	4.56 (0.15)	2113.33 (196.55)	No	No	No	No	Fasciculated
<i>A. triastriatus</i>	90.33 (2.89)	3.28 (0.34)	1973.33 (155.67)	No	No	No	No	Fasciculated
<i>A. vuongmontis</i>	205.00 (7.94)	4.49 (0.27)	4133.33 (399.54)	Yes	No	Yes	Yes	Intermediate
<i>A. zenkevichi</i>	156.33 (2.08)	5.17 (0.22)	4173.33 (144.68)	Yes	Yes	Yes	No	Fasciculated
<i>M. posthuma</i>	62.67 (11.50)	2.06 (0.77)	810.00 (26.46)	Yes	No	Yes	Yes	Fasciculated
<i>M. sp7</i>	152.00 (12.12)	4.63 (0.27)	3153.33 (143.64)	Yes	No	Yes	Yes	Intermediate
<i>Polypheretima elongata</i>	135.00 (8.66)	4.45 (0.02)	2080.00 (130.77)	No	No	No	No	Fasciculated
<i>Pontoscolex corethrurus</i>	77.33 (7.57)	1.93 (0.29)	696.67 (106.93)	No	No	No	No	Fasciculated

Table S1 (Continuation)

Species	Longitudinal furrow	Transversal furrow	Typhlosole type	Longitudinal muscle thickness (μm)	Circular muscle thickness (μm)	Tegument thickness (μm)	Septa thickness	Number of setae
<i>A. acaliformicus</i>	No	Yes	Lameliform	205.43 (85.02)	118.53 (43.59)	46.53 (17.14)	Thin	59.33 (1.15)
<i>A. alluxus</i>	No	Yes	Bifid	94.07 (27.47)	68.63 (10.97)	25.67 (6.77)	Thin	46.33 (0.58)
<i>A. aspergillum</i>	No	Yes	Lameliform	357.23 (125.82)	212.93 (36.85)	40.80 (7.20)	Thin	75.67 (5.51)
<i>A. brevicapitatus</i>	No	Yes	Lameliform	105.83 (35.74)	58.33 (21.26)	24.47 (6.05)	Thin	62.33 (1.15)
<i>M. acampanulata</i>	No	Yes	Lameliform	296.67 (11.81)	141.67 (2.89)	42.43 (2.72)	Thin	49.00 (1.00)
<i>A. leucocircus</i>	No	Yes	Lameliform	314.30 (39.70)	130.87 (38.40)	44.83 (4.60)	Thick	60.00 (1.00)
<i>A. morrisi</i>	No	No	Absent	165.83 (49.39)	60.83 (7.64)	30.57 (0.90)	Thin	50.67 (5.86)
<i>A. ophthalmopapillatus</i>	No	No	Absent	84.50 (19.83)	37.60 (11.59)	23.63 (3.59)	Thin	60.00 (1.00)
<i>A. robustus</i>	No	Yes	Lameliform	215.83 (84.64)	81.67 (48.50)	33.97 (2.55)	Thick	62.67 (3.79)
<i>A. sp1</i>	No	No	Lameliform	162.50 (21.79)	87.50 (48.67)	29.80 (14.03)	Thick	52.67 (2.08)
<i>A. sp2</i>	No	Yes	Lameliform	154.17 (47.19)	56.67 (20.21)	17.70 (3.78)	Thin	72.00 (3.00)
<i>A. sp3</i>	No	Yes	Lameliform	92.03 (20.99)	43.83 (5.40)	22.60 (0.61)	Thin	49.33 (1.53)
<i>A. sp4</i>	No	No	Lameliform	356.25 (103.75)	56.27 (11.25)	36.27 (1.65)	Thin	66.00 (1.00)
<i>A. sp5</i>	No	No	Lameliform	198.33 (23.63)	80.83 (8.78)	37.00 (2.75)	Thick	62.00 (2.65)
<i>A. sp6</i>	No	No	Lameliform	135.83 (23.76)	45.00 (2.50)	21.07 (2.20)	Thin	45.67 (0.58)
<i>A. tienphucanus</i>	No	Yes	Bifid	142.53 (32.79)	78.10 (8.19)	27.83 (2.10)	Thin	81.67 (1.53)
<i>A. triastriatus</i>	No	Yes	Lameliform	221.67 (117.69)	86.67 (20.82)	29.43 (6.69)	Thick	78.67 (8.50)
<i>A. vuongmontis</i>	No	Yes	Trifid	170.50 (13.85)	150.50 (5.07)	41.40 (3.06)	Thin	75.00 (3.46)
<i>A. zenkevichi</i>	No	Yes	Lameliform	137.50 (0.00)	95.00 (10.90)	23.47 (4.80)	Thin	57.00 (3.00)
<i>M. posthuma</i>	No	Yes	Lameliform	207.50 (87.50)	94.17 (33.94)	35.37 (8.49)	Thin	61.67 (6.35)
<i>M. sp7</i>	No	No	Absent	217.50 (82.65)	162.50 (64.08)	37.97 (6.21)	Thick	67.33 (2.52)
<i>Polypheretima elongata</i>	No	Yes	Lameliform	124.17 (6.29)	79.17 (7.64)	46.57 (5.69)	Thick	75.67 (0.58)
<i>Pontoscolex corethrurus</i>	Yes	Yes	Lameliform	127.50 (18.87)	35.43 (7.54)	33.90 (4.48)	Thin	8.00 (0.00)

Table S2. References and the co-authors of the submitted papers.

No	Paper title	Journal	Status	Co-authors
1	Using morpho-anatomical traits to predict the effect of earthworms on soil water infiltration	Geoderma	Volume 429, 1 January 2023, 116245	Q.V. Pham ^{1,2,3} , T.T. Nguyen ⁴ , D.H. Lam ⁴ , Y. Capowiez ⁵ , A.D. Nguyen ⁶ , P. Jouquet ¹ , T.M. Tran ² and N. Bottinelli ^{1,2}
2	Local variations in soil compaction in the drilosphere of a wide range of earthworms and links with ecological categories and functional traits	Soil Biology and Biochemistry	Under review	Q.V. Pham ^{1,2,3} , Y. Capowiez ⁵ , A.D. Nguyen ⁶ , P. Jouquet ¹ , T.T. Nguyen ⁴ , D.H. Lam ⁴ , T.M. Tran ² and N. Bottinelli ^{1,2}
3	Interaction between anecic and endogeic earthworms can lead to synergistic effects on soil functioning	Applied Soil Ecology	Under review	Q.V. Pham ^{1,2,3} , H. X. Dang ¹ , A.D. Nguyen ⁶ , Y. Capowiez ⁵ , P. Jouquet ¹ , T.M. Tran ² and N. Bottinelli ^{1,2}

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