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Using morpho-anatomical traits to predict the effect of earthworms on soil water infiltration

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ABSTRACT

Despite the well-known potential of earthworms to modify soil macroporosity and water infiltration through their burrowing activities, it is still difficult to predict which species are more effective in increasing water infiltration. We thus investigated the relationships between soil water infiltration, burrow network properties, morphology and anatomy of a diversity of earthworm species. We sampled 23 earthworm species in northern Vietnam and measured 16 morpho-anatomical traits *a priori* linked to their feeding, vertical distribution or burrowing behaviour. One individual was then incubated in a repacked soil core for four weeks under laboratory conditions, each in four replicates. After incubation, burrow network properties were assessed using X-ray computed tomography, and soil saturated hydraulic conductivity was measured using the falling head method. We found large differences among species belonging to similar or different ecological categories in their effect on soil saturated hydraulic conductivity. We observed neutral to positive effects with an increase of up to 30-fold compared to cores without earthworms. The effect of earthworms on soil hydraulic conductivity was well predicted by burrow network properties (volume and continuity of burrows) or morpho-anatomical traits (body weight, circular and longitudinal muscles thickness) using the random forest regression models ($R^2 > 0.95$ in both cases). Earthworms with a large body weight and circular and longitudinal muscle thicknesses have the highest probability of increasing soil saturated hydraulic conductivity compared to other species. In addition, we showed from the coinertia analysis that body weight and circular and longitudinal muscle thickness were positively associated with the volume and continuity of burrows. Our findings therefore illustrate the power of employing a trait-based approach to predict the effect of non-Lumbricidae earthworms on water infiltration in comparison to the classical ecological category approach. Such trait information could be used to prioritize practices that favour the expression of more desirable earthworm traits to increase soil water infiltration.

1. Introduction

The effect of earthworm burrows on soil water infiltration is variable and depends mainly on the size, orientation, continuity, connectivity and vertical distribution of the burrows (Bastardie et al., 2003; Bottinelli et al., 2017; Capowiez et al., 2015). Given that more than 5300 valid earthworm species have been described (Drilobase, <http://taxo.drilobase.org/>), it seems impractical to study the burrowing behaviour of

all described species. A common approach to assess the effects of earthworms on soil functioning is to group them into ecological categories (Bottinelli et al., 2020; Bouché, 1977; Capowiez et al., 2021). Because ecological categories were initially defined from a collection of traits (i.e., mainly morphological, anatomical, reproductive and dietary) that are not necessarily linked to the burrowing behaviour of earthworms, several studies noted that earthworms assigned to one ecological category can have different bioturbating behaviour (Felten and

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Emmerling, 2009; Zicsi et al., 2011) and diet (Neilson et al., 2000). In addition, the identification of ecological categories by researchers is rarely justified and often leads to incorrect assignments, which is even more evident for non-Lumbricidae species (Bottinelli et al., 2020). A striking example is the case of *Amyntas gracilis*, which has been assigned as anecic, endogeic, epigeic and *epi-endogeic* (Bottinelli et al., 2020). As an alternative to the ecological category approach, trait-based approaches have been developed. An effect trait is an individual property that affects an upper level of organization (e.g., ecosystem processes) (Violle et al., 2007). Unlike the ecological category, trait-based approaches are based on objective relationships between individual properties and soil functioning. In other research fields, notably for plants, trait-based approaches have brought several new insights to the understanding of how organisms affect their environment (Bardgett, 2018). Experience in other research fields led us to believe that using functional trait-based approaches for earthworms is a major opportunity to bring new insights into the understanding of earthworms' impact on soil functioning.

However, studies that measure the effect traits of earthworms and relate them to their burrowing activities and consequently water dynamics in soil are rare. This 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 and Schrader, 1999), (ii) the vertical distribution of earthworms can be linked to the size of earthworms (Jiménez and 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 and Álvarez-Otero, 2018). However, with the exception of the study of Marichal et al. (2017), the links between earthworm traits and the studied soil processes were not explicitly tested in the abovementioned studies. Overall, with the current knowledge, it is impossible to draw generalizable conclusions about the relevance of earthworm traits that are important to characterize burrowing behaviour and to predict water infiltration. There is thus a need for studies that systematically compare earthworm traits in a large set of species and relate these traits to the burrowing behaviour of earthworms. The question is also perhaps even more important in tropical countries where earthworm functional impacts remain poorly studied (Blouin et al., 2013).

Therefore, we investigated a set of 23 earthworm species from northern Vietnam. We compared their burrow systems and effects on saturated soil water infiltration under laboratory conditions. Most species belonged to the Megascolecidae family, and we screened each for a suite of 16 morpho-anatomical traits *a priori* related to their feeding, vertical distribution or burrowing behaviour. With these data, we wanted to determine (i) the usefulness of ecological categories to predict the effect of non-Lumbricidae earthworms on soil water infiltration and (ii) the characteristics of earthworms that have a relevant effect on soil macroporosity and water infiltration.

2. Materials and methods

2.1. Earthworm sampling and trait measurements

In total, 23 earthworm species were collected by hand-sorting or electric stimulation in soils from different localities in northern Vietnam (Table 1). 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

Table 1

List of collected species and description of the ecological category and locality.

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

^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).

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; Thai, 1982, 1983, 1984, 1987; Thai and Le, 1993; Pham, 1995; Sims and Easton, 1972) 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).

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 weight, length and diameter of the body 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 nonpigmented. In addition, pigmented species can have a colouration gradient that can be antero-posterior or dorso-ventral (Fig. 1a). The epithelium rigidity indicates the overall earthworm strength. By squeezing the earthworms between two fingers, the epithelium was classified as rigid 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., 2020) (Fig. 1b). 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. 1c). The tegument thickness (cuticle + epidermis) (Fig. 1d) 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 and 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 fasciculate (Fig. 1e). 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 and Wolfe, 2014). Selected segments were cleaned and dehydrated using graded ethanol concentrations. Segments were embedded with paraffin and then cut using a

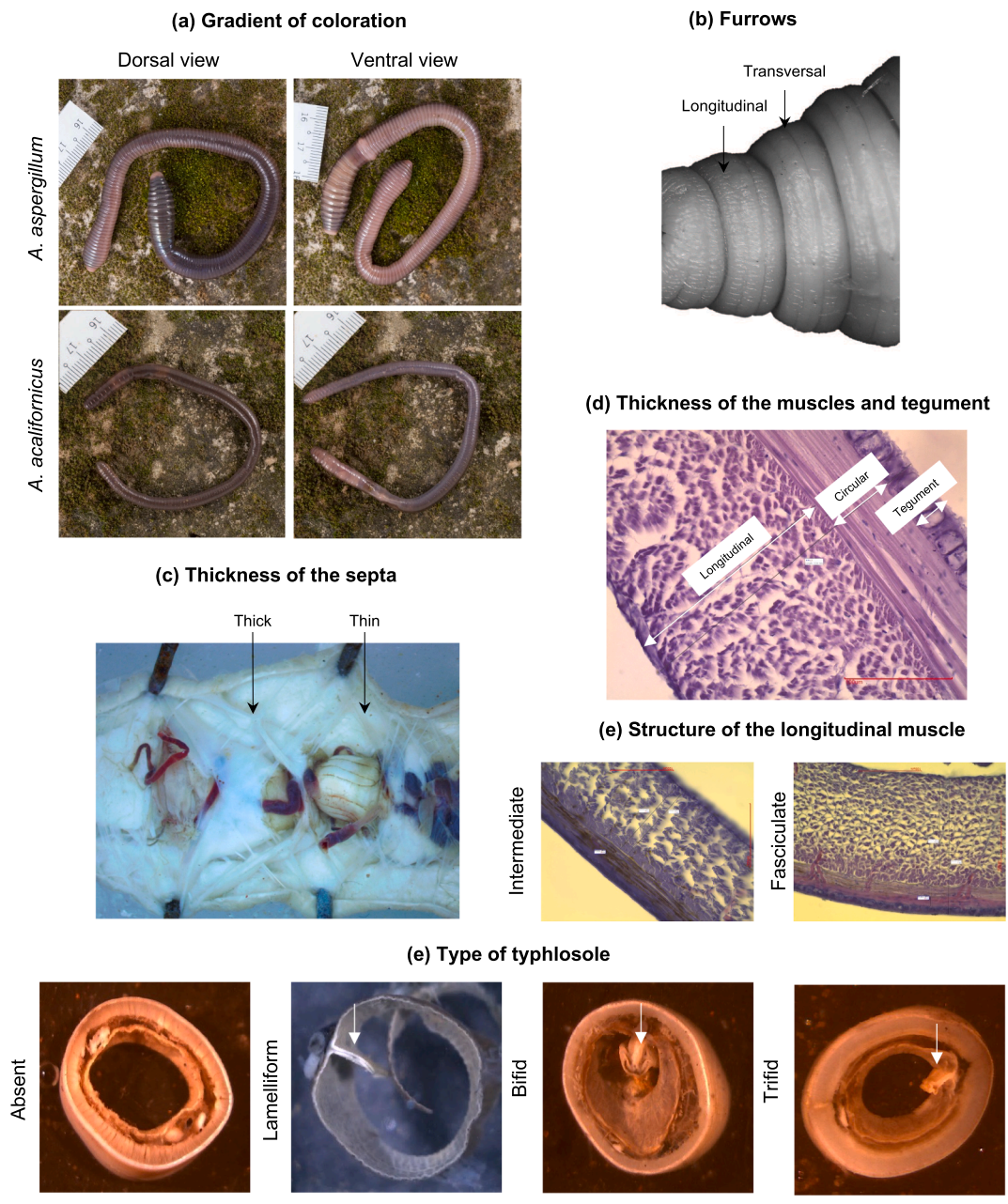


Fig. 1. Pictures showing several morpho-anatomical traits measured in this study: (a) colouration gradient. *A. aspergillum* is pigmented with an antero-posterior and dorso-ventral colouration gradient. *A. acalifornicus* is pigmented with only a dorso-ventral gradient; (b) presence of longitudinal and transversal furrows; (c) septa thickness; (d) thickness of the tegument, longitudinal and circular muscles; (e) structure of the longitudinal muscle; (f) four typhlosole types.

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. 1f).

2.2. Soil and mesocosms

21 earthworm species were incubated with a soil collected in an Ultisol (20°57' N 105°29' E) while two species (*Amyntas tristriatus* and *Metaphire posthuma*) were incubated using a soil collected in a Fluvisol (21°04' N 105°46' E). The Ultisol is characterized in 0–10 cm depth 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 is characterized in 0–10 cm depth 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). Soils were sieved at 2 mm, homogenized and kept in plastic bags to prevent evaporation. Mesocosms were made of polyvinyl chloride (PVC) cores (height: 36 cm, diameter: 15 cm) 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 Ultisol and 1.2 g cm^{-3} for the Fluvisol) and remoistened at 40 % gravimetric water content for the Ultisol 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. In each core, we introduced one individual or no earthworm as a control. In total, we prepared 102 cores corresponding to four cores per species and 10 cores without earthworms. Buffalo manure was air-dried, grinded, remoistened and added to the top of the cores and was regularly replenished according to the consumption of the earthworms (i.e., from 2 to 30 g air-dried). The incubation lasted four weeks, and microcosms were kept at 25 °C in the dark. Soil moisture was maintained by adding water every week according to the weight loss of mesocosms. At the end of the incubation, 10 ml of chloroform was applied to each core to sacrifice the earthworm.

2.3. Burrow networks and soil water infiltration

Burrows within the soil cores were analysed by X-ray computed tomography using a medical scanner (Siemens Somatom® Definition Flash) at Bach Mai Hospital (Hanoi, Vietnam). The scanner settings were 120 kV and 93 mA, and the acquired images were in DICOM format (16-bit, 512×512 pixels). Image processing was performed with Fiji/ImageJ (Schindelin et al., 2012). Images were transformed into 8-bit images and rendered isotropic with a pixel size of 0.3 mm. To reduce scatter and noise, images were filtered using a 3D median filter with a radius of 2 pixels. To exclude the PVC, a cylindrical region of interest with a circular diameter of 145 mm was used. Prior to image analysis, automatic segmentation was performed according to Otsu's method to separate the image into macropores and the soil matrix. Then, macropores below 200 voxels, corresponding to 10 mm^3 , were discarded to remove interaggregates resulting from packing. The MorphoLibJ plugin (Legland et al., 2016) was used to calculate the following burrow characteristics: volume, number, diameter, continuity and connectivity. 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, we summed the volume of burrows whose vertical extension ($>60^\circ$) was larger than 30 % of the core length. The burrow connectivity Γ (Eq. (1)) reflects the probability of two randomly chosen burrow voxels belonging to the same burrow cluster:

$$\Gamma = \frac{1}{N_p^2} \sum_{k=1}^{N_i} n_k^2 \quad (1)$$

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 soil saturated hydraulic conductivity (K_s) (Eq. (2)) was measured with the falling head method:

$$K_s = \frac{al}{A\Delta t} \ln\left(\frac{h_1}{h_2}\right) \quad (2)$$

where a is the cross-section of the water column, A is the cross-section of the soil core without the PVC 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.

2.4. Statistical analyses

Statistical analyses were performed with R (R Core Team, 2022), and figures were produced with the package “ggplot2” (Wickham et al., 2016). To assign earthworm species into ecological categories, we conducted a principal component analysis (PCA) of mixed data with the package “ade4” (Dray and Dufour, 2007) based on morpho-anatomical traits (pigmentation, colouration gradient, weight, diameter, length, epithelium rigidity, presence of furrows, typhlosole type and longitudinal muscle structure) as suggested by Bottinelli et al. (2020). 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). 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. We used t tests to compare the mean RR_{K_s} of each species to the value 1. Differences in RR_{K_s} and burrow network properties between clusters were analysed by one-way ANOVA and afterwards through Tukey's multiple comparison test. Before t tests and ANOVA, parameters were tested for normality and homogeneity of variance by the Shapiro–Wilk test and Levene's test, respectively. Differences among treatments were declared significant at the 0.05 probability level. Random forest regression was used with the package “caret” (Kuhn, 2008) to test the capacity of our dataset to predict 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). We preliminarily applied recursive feature elimination (RFE) to consider only the most discriminating predictors. The optimization of parameters for the final 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. Associations between explanatory and response variables were evaluated with accumulated local effects (ALE) plots (Apley and 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. Finally, 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.

3. Results

3.1. Assignment of the Vietnamese species into ecological categories

The projection of the 23 species onto the two factorial planes of the PCA from the 11 morpho-anatomical traits used to identify ecological categories of French Lumbricidae species (Bottinelli et al., 2020) showed an organization following roughly a triangle shape (Fig. 2). Using k-means clustering, four ecological categories (EC) could be identified: EC1 (*A. sp2*, *A. sp4*, *A. sp6*, *A. morrissi*, and *A. ophthalmopapillatus*), EC3 (*A. aspergillum*, *A. acalifornicus*, *A. vuongmontis*, *A. leuocircus*, 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*, *M. campanulata*, *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. 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 are all pigmented, with a gradient along the body. The epithelium is not rigid, and few have transversal furrows. Earthworms from EC3 are the largest and heaviest. They are all pigmented with a gradient along the body. The epithelium is mostly rigid, and all have transversal furrows. Earthworms from EC4 are of medium size and weight. They are all nonpigmented, with no gradient along the body. The epithelium is soft, and all have transversal furrows. Finally, earthworms from EC2 were of medium size and weight. They are all pigmented, with a gradient along the body. The epithelium is either soft or rigid, and half of the species have transversal furrows.

The five additional morpho-anatomical traits are 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

Table 2

Morpho-anatomical traits characterizing the four ecological categories (EC). Values given in the table are the means across all species in each EC, and standard deviations are in parentheses. Values followed by different letters are 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	100 % a	100 % a	100 % a	0 % b	***
Antero-posterior gradient	100 % a	67 % a	60 % a	0 % b	**
Dorso-ventral gradient	40 % ab	100 % a	100 % a	0 % b	***
Epithelium rigidity	0 % b	50 % ab	80 % a	0 % b	**
Muscle structure	Inte. (80 %)	Inte. (50 %)	Fasc. (60 %)	Fasc. (88 %)	ns
Longitudinal furrow	0 %	0 %	0 %	15 %	ns
Transversal furrow	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

other ECs.

3.2. Earthworm burrow networks

Representative burrow networks for each species are shown in Fig. 3. Species belonging to 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 EC3, *A. aspergillum*, *A. acalifornicus*, and *A. zenkevichi* produced few, wide and continuous burrows

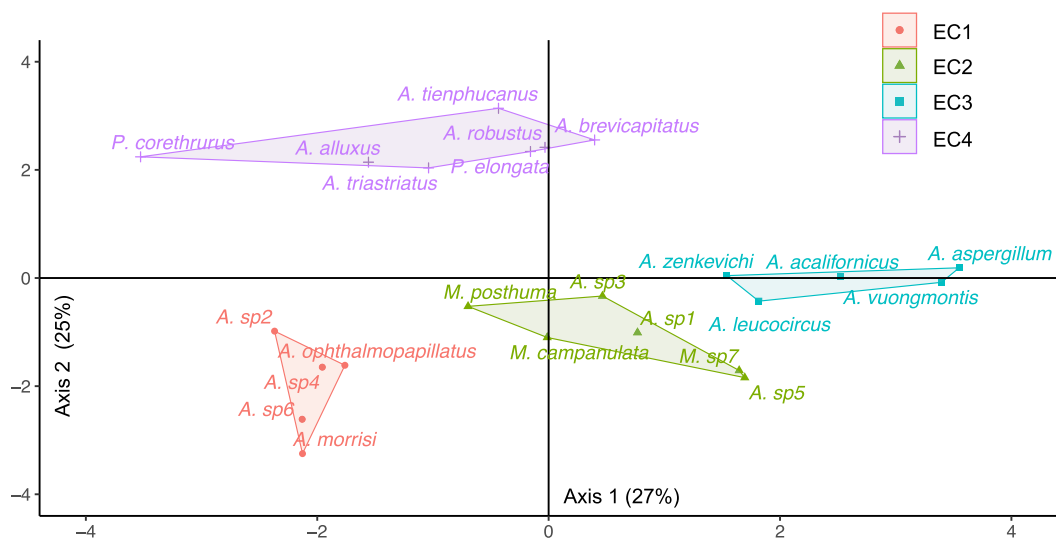


Fig. 2. Score plot from the principal component of analysis (PCA) followed by k-means clustering based on 11 morpho-anatomical traits (pigmentation, colouration gradient, weight, diameter, length, epithelium rigidity, presence of furrows, typhlosole type and longitudinal muscle structure) to assign the 23 earthworm species into ecological categories (EC).

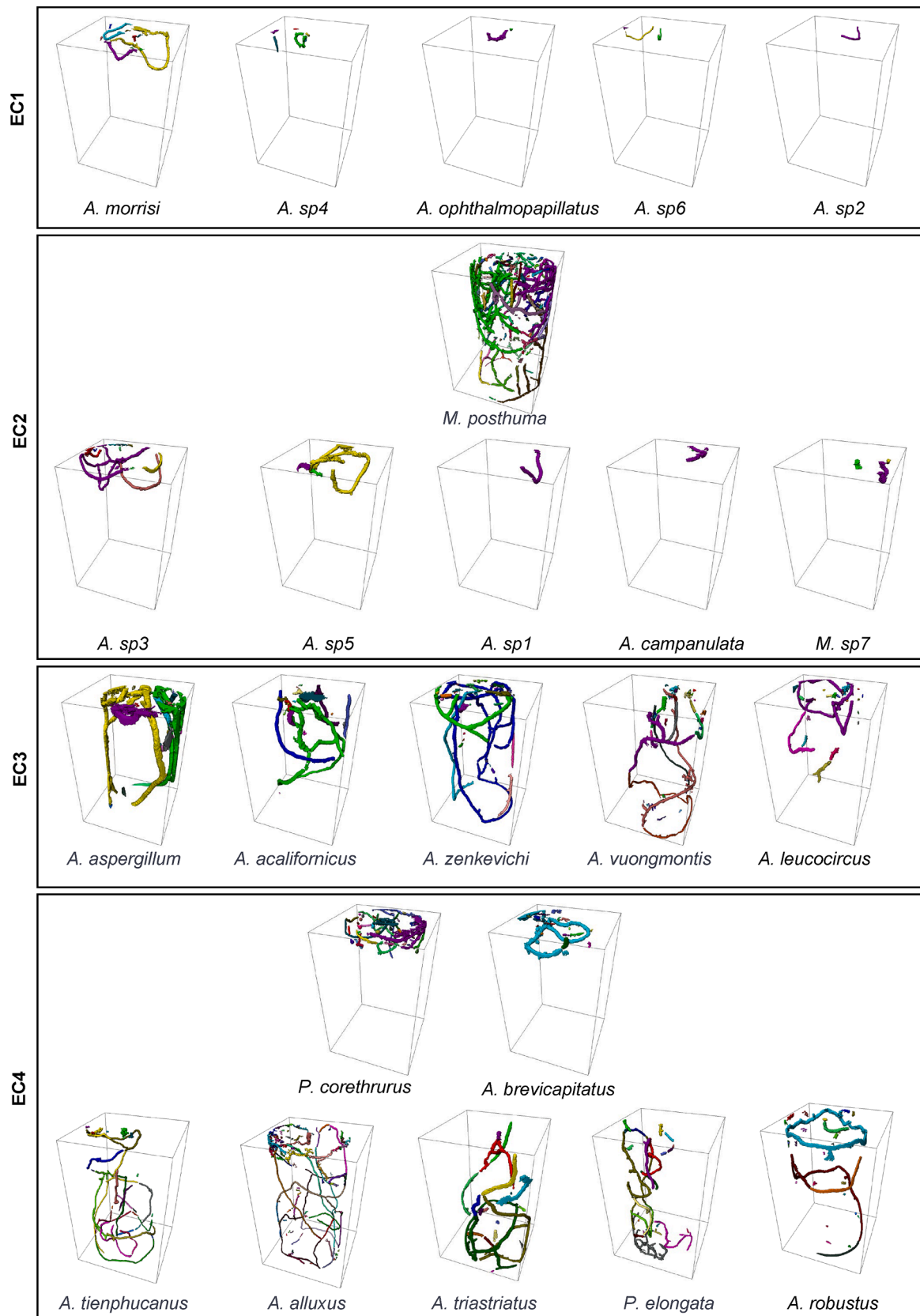


Fig. 3. Examples of 3D reconstructions of the burrow systems made by earthworms in repacked soil columns (30 cm in height and 15 cm in diameter) after 4 weeks. One earthworm was incubated in each soil column. Earthworm species were assigned to four ecological categories (EC).

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 EC4 (*P. corethrurus* and *A. brevicapitatus*), burrows were quite similar to those of species belonging to EC1, 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 EC2 produced burrows similar to those produced by species in EC1 except for *M. posthuma*, which produced burrows similar to those produced by species in EC4.

Overall, the quantification of the burrow characteristics via image analysis confirmed the large variability of burrow networks found within EC2, EC3 and EC4 (Fig. 4). Earthworms from EC1 and EC2 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 EC3 and EC4. Species from EC2 can be differentiated from those belonging to EC1 by the volume percentage of the burrows, which was significantly larger. The burrow network of species assigned to EC3 and EC4 could be differentiated only based on the diameter of the burrows, which was larger for burrows made by species belonging to EC3.

3.3. Relationships between saturated hydraulic conductivity, morpho-anatomical traits and burrow networks

The response ratio of soil hydraulic conductivity (RR_{K_s}) was significantly larger than 1 for 10 species (2 EC2, 2 EC3 and 6 EC4), whereas it was not different from 1 for the other 13 species (5 EC1, 4 EC2, 3 EC3 and 1 EC4) (Fig. 5a). The mean RR_{K_s} was the highest for species assigned to EC3, the lowest for those assigned to EC1 and intermediate for those assigned to EC2 and EC4 (Fig. 5b). However, the results of the ANOVA showed that RR_{K_s} measured for species assigned to EC3 and EC4 were not significantly different but were both higher than those measured for species assigned to EC1.

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) or burrow network properties (R^2 of 0.96, RMSE of 1.31) (Fig. S1). 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, we conducted ALE analysis for the selected predictors of the random forest models. The ALE plots for the morpho-anatomical traits (Fig. 6a) 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. 6b) showed that RR_{K_s}

increased with increasing burrow volume and continuity.

3.4. Covariation between morpho-anatomical traits and burrow networks

We performed a coinertia analysis to analyse the covariation of morpho-anatomical traits and burrow properties. The two projected inertias represented 98 % of the total coinertia (Fig. 7), 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.

4. Discussion

4.1. Identification of ecological categories

One major challenge in earthworm ecology studies is the right assignment of species into ecological categories, particularly for non-Lumbricidae species and species that are not strictly epigeic, anecic or endogeic (Bottinelli et al., 2020). In this study, we collected 23 species (22 Megascolecidae and 1 Rhinodrilidae) in northern Vietnam and assigned them into ecological categories using the 11 morpho-anatomical traits used to identify ecological categories of French Lumbricidae species (Bottinelli et al., 2020). The projection of the species onto the two factorial planes of the PCA showed an organization following roughly a triangle shape. Using k-means clustering, four clusters could be identified, with Clusters 1, 3 and 4 representing each pole and Cluster 2 located between the three poles. If we assume that the ecological categories defined by Bouché (1977) can be applied to Asian species, Cluster 1 gathered epigeic species, since individuals are pigmented, have a small body size and a soft epithelium, and few of them have transversal furrows. Cluster 4 gathered endogeic species, since individuals are unpigmented, have a medium body size, have a soft epithelium, and all individuals have transversal furrows. Cluster 3 gathered anecic species, since individuals are pigmented, have a large body size, have a rigid epithelium, and all have transversal furrows. Finally, Cluster 2 gathered intermediate species. They are all pigmented with a medium body size. Their epithelium is 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),

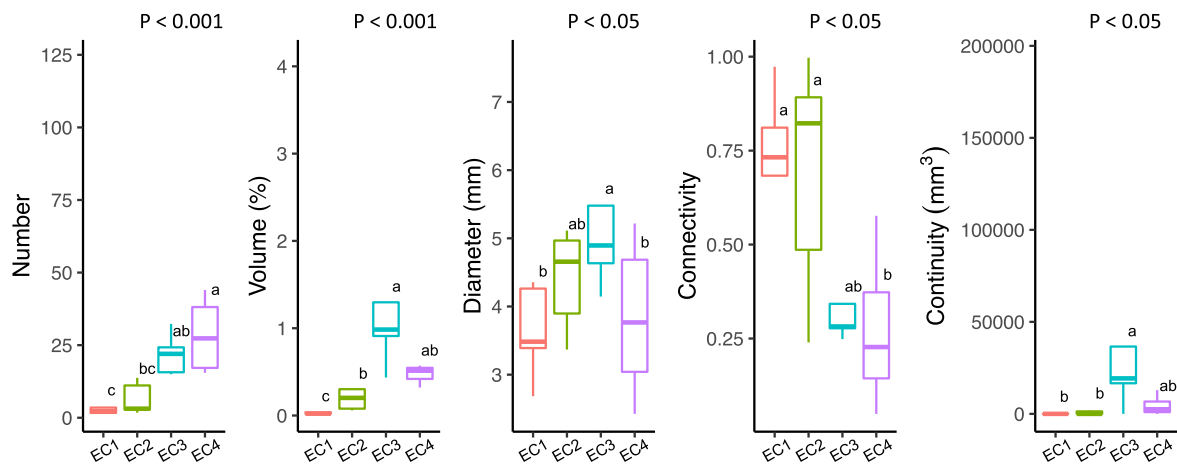


Fig. 4. Burrow network properties (number, volume, diameter, connectivity and continuity) of the four ecological categories (EC). Boxplots show the interquartile range and median, and whiskers show the $1.5 \times$ interquartile range. P values were calculated using one-way ANOVA. Different letters indicate significant differences (at the 0.05 significance level) among ECs.

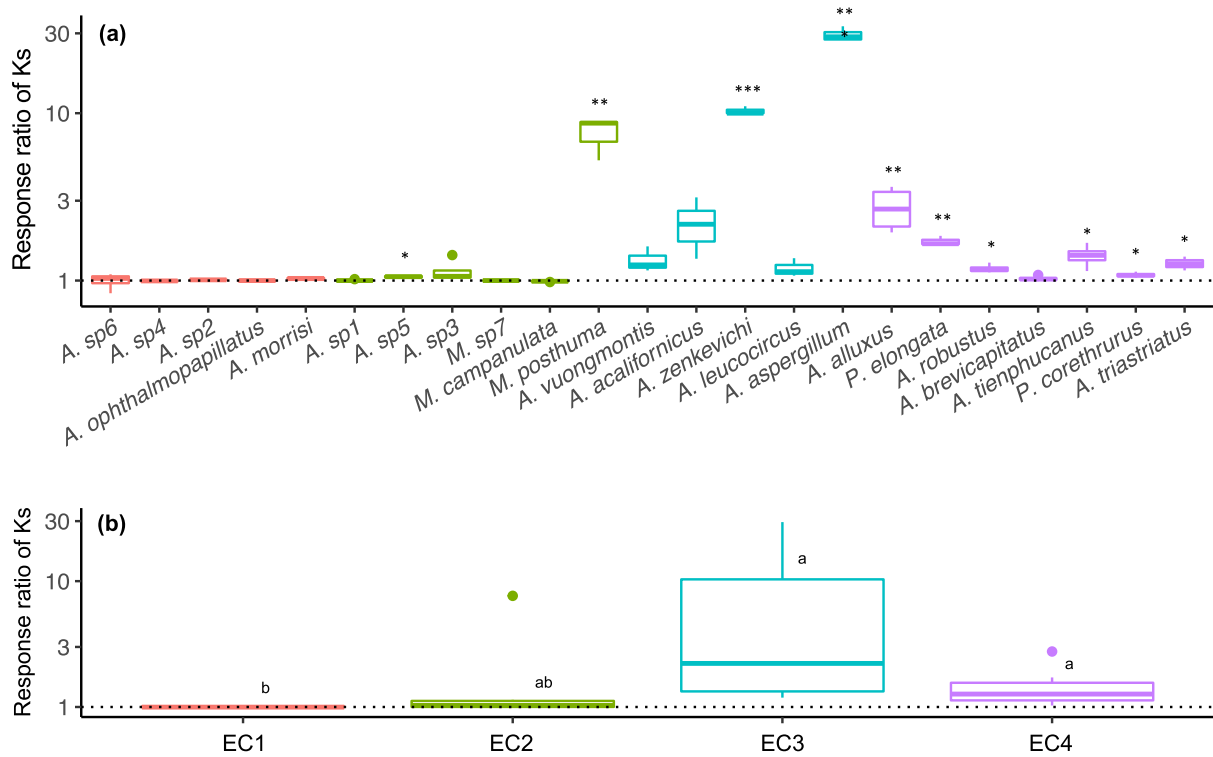


Fig. 5. Response ratio of soil saturated hydraulic conductivity (Ks) among earthworm species (a) and among the four ecological categories (EC) (b). Boxplots show the interquartile range and median, and whiskers show the $1.5 \times$ interquartile range. Asterisks indicate a significant difference between the mean of the response ratio of soil saturated hydraulic conductivity and the reference value of 1 ($*** p < 0.001$, $** p < 0.01$, and $* p < 0.05$). Different letters indicate significant differences (at the 0.05 significance level) among ECs.

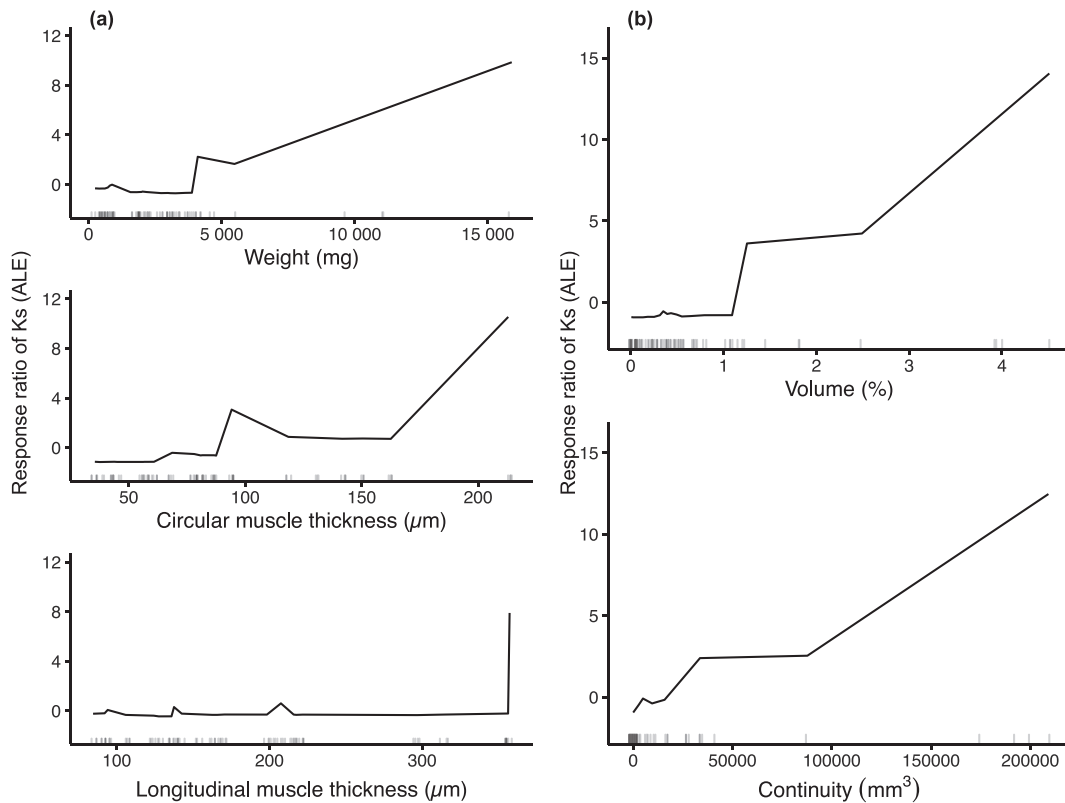


Fig. 6. Accumulated local effects (ALE) plots of the predicted response ratio of soil hydraulic conductivity (Ks) for (a) the three selected morpho-anatomical traits and (b) the two selected burrow network properties. Grey markings on the x-axis show the distribution of each variable.

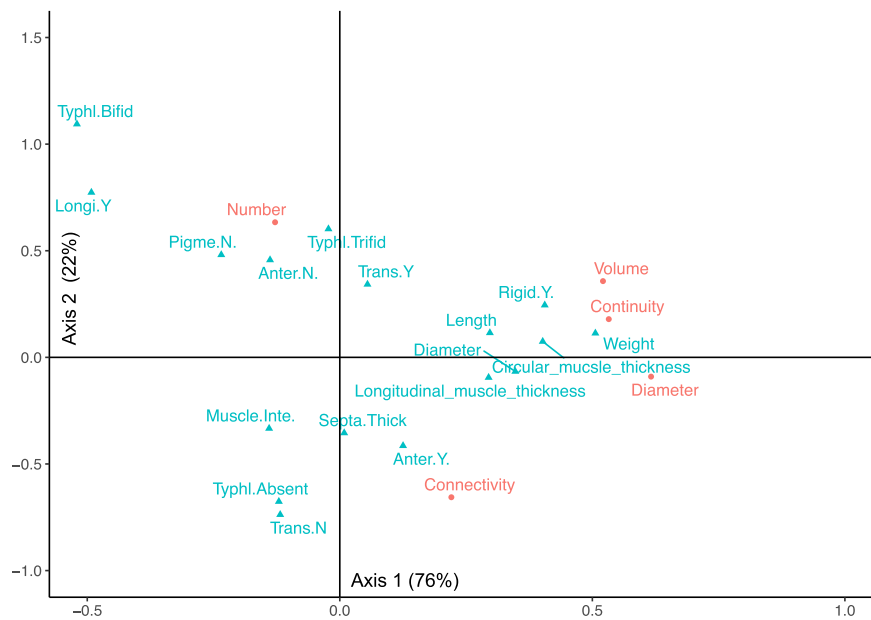


Fig. 7. Projections of the morpho-anatomical traits (blue) and burrow properties (red) resulting from the coinertia analysis. For clarity, only morpho-anatomical traits with high scores are depicted on F1 and F2.

A. morrissi 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). Our results confirm these assignments, except for *A. morrissi* that was assigned as *epigeic* and *M. posthuma* as *intermediate*. Because the assignment proposed here is based on PCA followed by cluster analysis, we expect that with a larger dataset, the assignment of the studied species might change slightly, and other ecological categories, such as *epi-endogeic*, *endo-aneic* or *epi-aneic*, could be differentiated. Additionally, it cannot be excluded that the assignment of species into ecological categories varied according to the soil they inhabit since the intraspecific variability of the body size for instance can be very high (Bouché, 1972).

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

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

Overall, the effect of earthworms on saturated hydraulic conductivity (Ks) was either neutral or positive, with an increase of up to 30-fold 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 and Emmerling, 2009) and consequently have no effect on Ks. Conversely, we could not confirm that anecics had a larger effect on Ks than endogeics, as often observed by other researchers (Bottinelli et al., 2017; Capowiez et al., 2015). Although Ks was generally larger for anecic than endogeic species and 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 similar behaviour

to epigeic species except for *M. posthuma*, which had one of the largest effects on Ks and made burrows relatively similar to those made by some endogeic species. To our knowledge, this is the first report on the burrowing activities of many Megascolecidae species. Our results clearly indicate a lack of consistency among ecological categories based on morpho-anatomical traits and their relative effects on soil architecture and Ks. This is 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 and Emmerling, 2009). It can be assumed that (i) grouping earthworms into four ecological categories instead of the seven proposed by Bouché (1972) was probably not sufficient to accurately identify the effect of earthworms on Ks, (ii) the model proposed by Bottinelli et al. (2020) was not appropriate to identify non-Lumbricidae earthworm ecological categories or (iii) not all of the morpho-anatomical traits were tightly related to the burrowing behaviour of earthworms or other morpho-anatomical traits not used may have been more tightly related to identify ecological categories. Thus, in the next step, we explored the relationships between morpho-anatomical traits, burrow network properties and Ks.

4.3. 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 and Álvarez-Otero, 2018; Jiménez and Decaëns, 2000; Keudel and Schrader, 1999; Marichal et al., 2017), only the one carried out by Marichal et al. (2017) explicitly tested this link in different fields in Brazil and found a weak ($R^2 = 0.16$ 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 ($R^2 = 0.47$). In addition, we showed the usefulness of measuring effect traits to predict the capacity of earthworms to increase Ks (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 Ks. In general, the larger the body weight and muscle thicknesses, the larger the effect on Ks was. 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 Ks values.

5. Conclusions

Our results yield new insights into earthworm effect traits linked to their capacity to burrow the soil and in turn increase soil water infiltration at saturation. We identified the body weight and thickness of the circular and longitudinal muscles as the three most important traits related to the capacity of earthworms to influence soil water infiltration. In an applied context of restoration and agriculture, such trait information could be used to prioritize practices that favour the expression of more desirable earthworm traits to increase soil water infiltration. For future studies, it would be interesting to extend our dataset to other species belonging to the same or different families. It will also be important to extend our research under different environmental conditions to take into account the behavioural plasticity of earthworms. Such an effort would improve the external validity and offer new insights into the mechanisms of earthworm trait contributions to soil architecture and water infiltration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.geoderma.2022.116245>.

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