

Morphological and functional characterisation of the burrow systems of six earthworm species (Lumbricidae)

Yvan Capowiez¹ · Nicolas Bottinelli² · Stéphane Sammartino³ ·
Eric Michel⁴ · Pascal Jouquet^{2,5}

Received: 30 March 2015 / Revised: 11 June 2015 / Accepted: 16 June 2015 / Published online: 27 June 2015
© Springer-Verlag Berlin Heidelberg 2015

Abstract Earthworm burrow systems are generally described based on postulated behaviours associated with the three ecological types. In this study, we used X-ray tomography to obtain 3D information on the burrowing behaviour of six very common anecic (*Aporrectodea nocturna* and *Lumbricus terrestris*) and endogeic (*Aporrectodea rosea*, *Allolobophora chlorotica*, *Aporrectodea caliginosa*, *Aporrectodea icterica*) earthworm species, introduced into repacked soil cores for 6 weeks. A simple water infiltration test, the Beerkan method, was also used to assess some functional properties of these burrow systems. Endogeic worms make larger burrow systems, which are more highly branched, less continuous and of smaller diameter, than those of anecic worms. Among the anecic species, *L. terrestris* burrow systems are shorter (9.2 vs 21.2 m) with a higher number (14.5 vs 23.5) of less branched burrows (12.2 vs 20.2 branches m⁻¹), which are also wider (7.78 vs 5.16 mm) than those of *A. nocturna*. In comparison, the burrow systems made by endogeic species appeared similar to each other. However, *A. rosea* burrows were short and narrow, whereas *A. icterica* had a longer burrow system

(15.7 m), more intense bioturbation intensity (refilled macropores or soil lateral compaction around them) and thus a greater number of burrows. Regarding water infiltration, anecic burrow systems were far more efficient due to open burrows linking the top and bottom of the cores. For endogeic species, we observed a linear relationship between burrow length and the water infiltration rate ($R^2=0.49$, $p<0.01$). Overall, the three main characteristics significantly influencing water infiltration were burrow length, burrow number and bioturbation volume. This last characteristic highlighted the effect of burrow refilling by casts.

Keywords Water infiltration · Macropores · Bioturbation · *Aporrectodea icterica* · *Lumbricus terrestris* · *Aporrectodea caliginosa*

Introduction

It is paradoxical that although earthworms are increasingly recognised as the main soil ecosystem engineers in temperate natural and agro-ecosystems, existing data on their precise effects on soil functioning are mainly qualitative. Indeed, earthworms influence many soil functions through their physical (production of burrows and casts), chemical (modification of C and N cycles for example) and biological (interactions with other soil biota, from microorganisms to plants) actions (Bottinelli et al. 2015). Their physical influence on soil is the most obvious since it results in visible signs either above (casts and burrow entrances) or below the soil surface (burrows and cast refilling or surrounding them). These physical effects were also the first recognised evidence of their activities as studied experimentally by Darwin 1881 (i.e. the burial of stones).

✉ Yvan Capowiez
capowiez@avignon.inra.fr

¹ INRA, UR1115 'Plantes et Systèmes de culture Horticoles', Site Agroparc, 84914 Avignon cedex 09, France

² IRD, UMR 242 iEES Paris, 32 avenue Henri Varagnat, 93143 Bondy, France

³ UAPV, UMR 'EMMAH', Site Agroparc, 84914 Avignon cedex 09, France

⁴ INRA, UMR 'EMMAH', Site Agroparc, 84914 Avignon cedex 09, France

⁵ IFCWS, Civil Engineering Department, Indian Institute of Science, 560012 Bangalore, Karnataka, India

However, because most of the physical consequences of earthworm activity are hidden in an opaque medium, quantitative data remain scarce. This is despite the seminal study of Joschko et al. (Joschko et al. 1993), around 20 years ago, in which X-ray tomography was used for the first time to study earthworm burrows. This lack of quantitative knowledge is evident in the recent major textbooks on soil ecology (Lavelle and Spain 2001; Shipitalo and Le Bayon 2004) and even in specialised textbooks on the multi-scale architecture of soil porosity (Ritz and Young 2011). In all these books, the physical effects of earthworms on soil structure are classified according to the three ecological types but no quantitative data on burrow characteristics is given. Indeed, it appears that nothing has evolved since Lee and Foster's review (1991) in which they associated a particular burrowing behaviour with each ecological type. In brief, epigeic earthworms were described as rarely burrowing, whereas anecic earthworms make permanent and vertically orientated burrows up to 1 m in depth and endogeic burrows are refilled and lack preferential orientation. However, further more recent studies suggested that this clear-cut separation is not always true as far as burrowing behaviour is concerned (Bastardie et al. 2005a; Ernst and Emmerling 2009; Felten and Emmerling 2009). Overall, at present, students and young scientists are only provided with limited information on these important topics.

This surprising lack of knowledge is in sharp contrast with the situation in the field of sediment ecology where a typology of bioturbation behaviours (biodiffusor reworkers, upward conveyors) and thus burrow morphologies has been established (Gerino et al. 2003). This has enabled the development of a simulation model (Needham et al. 2004), tools which are still scarce in soil biology (Bastardie et al. 2002). Sediment bioturbation is under the influence of a wide variety of species from different phylums (annelids, crustaceans, gasteropods, etc.) which may have favoured the typology proposed. In comparison, the variety of soil organisms that act as soil bioturbators is limited to earthworms even if some coleopterans (Nahmani et al. 2005; Badorreck et al. 2012) and ants and termites, in tropical climates, can play a role (Jouquet et al. 2014).

In the present study, our aim was to provide qualitative (3D images) and quantitative (burrow characterisation) data of the burrow systems made in repacked soil cores by six earthworm species frequently found in agricultural fields. We then used a simplified water infiltration test to provide more insight into their possible functional role.

Materials and methods

Earthworms and soil

The soil for the experiment was obtained from the first 20 cm of topsoil (30.2 % clay, 48.7 % silt and 21.1 % sand; 5.1 %

organic matter; pH=8.3) in an abandoned orchard in Montfavet, near Avignon (43° 55' N, 4° 48' E) in the South-East (SE) of France.

Eight earthworm species are currently found in this orchard, and six were chosen (excluding epigeic worms) for this study. Only adult earthworms were used, and the mean (\pm SE) individual weights were 0.69 g (\pm 0.06) for *Aporrectodea caliginosa*, 0.62 g (\pm 0.05) for *Allolobophora chlorotica*, 0.35 g (\pm 0.04) for *Aporrectodea rosea*, 0.92 g (\pm 0.03) for *Aporrectodea icterica*, 3.42 g (\pm 0.05) for *Aporrectodea nocturna* (sometimes named *A. caliginosa nocturna* or *Nicodrilus nocturnus*) and 4.32 g (\pm 0.09) for *Lumbricus terrestris*. Earthworms were stored for less than 24 h in a dark chamber at 12 °C before being introduced into the soil cores.

The soil was sieved at 2 mm and stored for a few days in a dark chamber at 12 °C. Twenty-eight repacked soil cores were prepared using PVC cylinders (30 cm in length and 16 cm in diameter). Cores were compacted using a hydraulic press by applying a pressure of 180 kPa for 3 min on sieved soil at 18 % moisture content (gravimetric). This treatment resulted in a soil dry bulk density of 1.26 g cm⁻³. To minimise variations in soil bulk density between the top and bottom of the cores, the soil was compacted stepwise in 12 layers of 600 g of soil and of approximately 2.5-cm height. Before adding a new soil layer, the surface of the previous layer was gently scratched using a small rake to increase cohesion between layers. The top of each core was closed using a lid with small holes to prevent significant water loss. Before the introduction of the earthworms, 100 ml of water was poured into the soil.

The experimental set-up was four replicate soil cores for each earthworm under consideration (six species) or left empty as controls. To take differences in biomass into account, two and four earthworms were introduced per core for anecic and endogeic species, respectively. After 6 weeks, 10 ml of chloroform was poured into each core to kill the earthworms.

3D burrow system reconstruction and characterisation

At the end of the experiment, cores were scanned using a medical X-ray tomograph (BrightSpeed Exel 4, General Electric) at the INRA centre in Nancy to obtain a set of images 1.25 mm thick every 1.25 mm in which pixel size was 0.4 mm. The X-ray beam was operated at 130 mA and 50 kV. Images (16-bits DICOM format) were transformed into 8-bit images using ImageJ by setting the minimum and maximum grey-level values to -1000 and 2000 HU, respectively.

The grey-level histograms of the 8-bit images of all the cores in which earthworms were introduced were all bimodal, with one large peak (grey values) corresponding to the soil matrix and one narrow peak (black values) corresponding to voids and macropores (Capowiez et al. 1998). In this case, image segmentation is easy (Russ 1995) and the binarisation threshold was manually set to 185. At this stage, the number

of burrows (a burrow is a set of connected voxels) was computed and the mean burrow diameter was estimated based on their area in 2D images assuming macropores are circular and by excluding macropores whose circularity was below 0.6. The circularity of a macropore is given by the following formula: $\text{circularity} = 4 \pi \text{ area} / \text{perimeter}^2$. The burrows were translated into 3D skeletons by determining all the ultimate eroded points (i.e. centroids) in all images and linking with segment centroids belonging to overlapping pores between two successive images. Based on skeletons, we computed other characteristics for each core: the branching intensity (number of triple points per meter), and verticality (angular deviation from vertical direction) of burrows. The continuity of a burrow system has no standardised definition. We computed the number of burrows with a vertical length greater than 8.5 and 17 % of the core depth (i.e. 2.5 and 5 cm, respectively) to obtain two estimates of the burrow system continuity.

The volume of bioturbation inside the core was also estimated following the method described by Capowiez et al. (2011). In brief, bioturbated zones in images can be assessed due to their intermediate grey-level values using a specific algorithm. This bioturbation volume corresponds to macropores which have been either partially or totally refilled with casts or casts that have been crushed along the burrow walls. For *L. terrestris*, these zones are called cutanes (Rogasik et al. 2014). The intensity of bioturbation was expressed as the percentage of the total macroporosity (i.e. burrow volume and volume of bioturbation) since we already demonstrated for *A. chlorotica* that these volumes (burrows and casts) increased together (Capowiez et al. 2014).

Water infiltration experiment

To study water infiltration in soil cores, we used the single ring method also named the ‘Beerkan’ method (Braud et al. 2005). In brief, the core was located on a device with free drainage and a fixed volume of water (150 ml corresponding to 0.75 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 about 17 to 20 known volumes until an apparent steady state of infiltration was reached (i.e. the time elapsed between two volume additions was constant; Fig. 1). We then estimated the final infiltration rate (L min^{-1}) by computing the slope (linear regression) of the relationships between cumulative infiltration and time elapsed at steady state (Capowiez et al. 2009). Additionally, the time before breakthrough at the bottom of the core was noted. It should be noted that this method was used here for comparison purposes and to mimic heavy rainfalls. Since border effects were

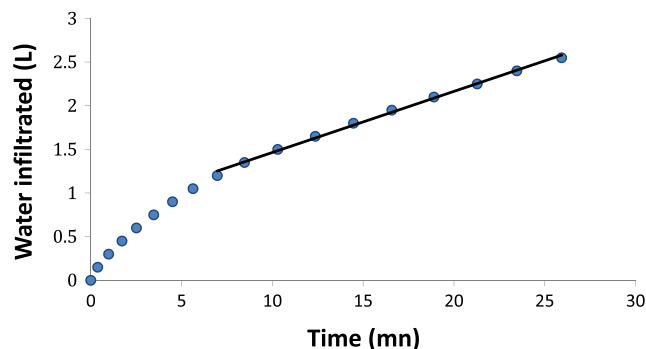


Fig. 1 Results from a water infiltration test in a repacked soil core containing *A. icterica* burrows. The water infiltration rate is computed by linear regression on the last ten points corresponding to the steady state (see plain line)

likely to occur along the PVC core (but in the same way for all the cores), absolute values of infiltration had no real meaning.

Data analysis

The significance of the differences in burrow characteristics for each species and the two parameters assessed using the infiltration test (infiltration rate and breakthrough time) was tested using permutation tests due to the small number of replicates. For this, we used some functions of the ‘RVAideMemoire’ packages in R and adjusted the p values to take into account the effects of the number of modalities (7). No statistical analysis was carried out for the number of burrows with a vertical length greater than 5 cm because the distributions were too skewed (zero-inflated distributions).

The burrow system characteristics were analysed by an ordination method (principal component analysis) using ‘ade4’ packages in R applied to the data from the 24 cores, thus excluding cores without earthworms.

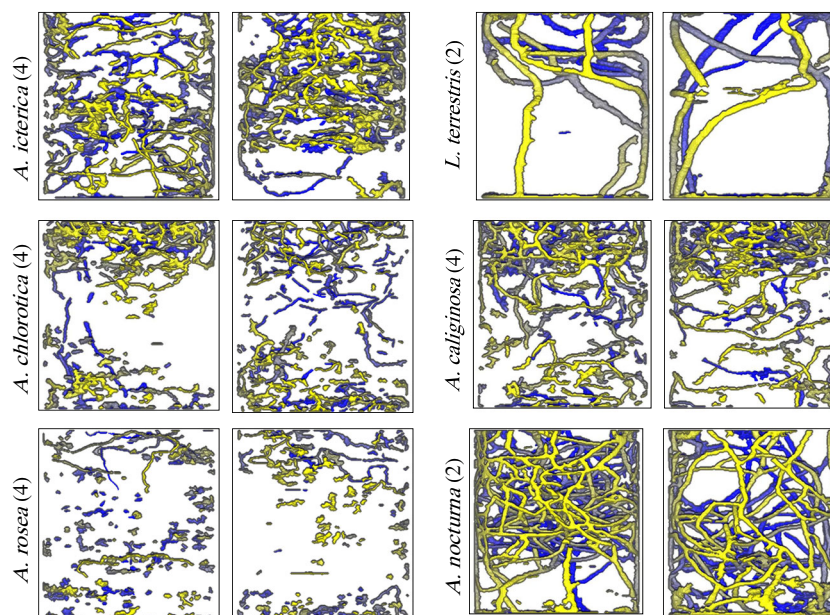
To study the relationships between water infiltration rate and the burrow system characteristics, we first used a multiple linear regression and then applied model selection using the ‘MuMIn’ package in R which enables to test all the possible combinations of factors.

Results

Characterisation of the 3D burrow systems

Representative burrow systems for each earthworm species are shown in Fig. 2. The *L. terrestris* burrow system was significantly different to the others due to the burrow diameter and marked verticality especially in the bottom part of the cores. In contrast, *A. rosea* made very disconnected and tiny burrows, which has no preferential orientation. The burrow systems of the other four species were quite similar except the relatively low burrow continuity for *Al. chlorotica* and

Fig. 2 Examples of 3D reconstructions of the burrow systems made by earthworms in repacked soil cores (30 cm in height and 16 cm in diameter) after 6 weeks. Two and four earthworms were incubated in each soil core for anecic and endogeic species, respectively. Colours are provided for 3D rendering (*yellow* in the foreground and *blue* in the background) (colour figure online)



A. rosea and the larger burrow diameter and continuity for *A. nocturna*.

The quantification confirmed the visual impressions (Table 1). Both anecic species made significantly less burrows, and these burrows were wider and less branched ($p < 0.05$). They also tended to make more continuous burrows (especially *L. terrestris*). We did not observe a significant difference in burrow length or bioturbation volume between anecic and endogeic species. Some significant differences were observed between the two anecic species. *L. terrestris*'s burrowing activity was twofold lower than that of *A. nocturna*, and the resulting burrows were significantly wider and more continuous ($p < 0.05$).

In comparison, the burrow systems of the four endogeic earthworm species appeared visually more similar with no significant differences regarding burrow verticality and branching intensity (Table 1). One difference, obviously associated with the small size of *A. rosea*, was that its burrow system was approximately twofold shorter than those of the other endogeics ($p < 0.05$). The *A. caliginosa* and *Al. chlorotica* burrow systems shared a lot of characteristics (length, number, branching intensity). Some characteristics also allowed *A. icterica* to be differentiated from these two species. *A. icterica* built significantly longer burrow systems, and overall, its burrow systems were more continuous ($p < 0.05$). This species also caused a significantly higher volume of bioturbation in the soil cores (Table 1 and Fig. 3; $p < 0.01$). For *A. icterica*, this bioturbated volume represented

45 % of the macropore volume compared to 20 % for *A. caliginosa* and *Al. chlorotica*.

PCA of the burrow system characteristics clearly separated almost all the species except *A. rosea* and *Al. chlorotica* (Fig. 4). The first axis, explaining 48 % of the variability, opposed endogeic species to anecic species and was correlated to a contrast in burrow diameter and verticality on one side and the number of burrows on the other side. The second axis (explaining 21 % of the variability) opposed *A. icterica* to *A. rosea* and *Al. chlorotica*, *A. caliginosa*, which had intermediate values. This axis was correlated to the contrast in branching intensity (positive values) and burrow continuity and bioturbation intensity (negative values).

Water infiltration and burrow systems

The water infiltration rate in the soil cores where anecics had burrowed was much higher than those of endogeic worms (greater than 10 L mn^{-1}), and we observed breakthrough after only 10 s on average (Table 1). For soil cores that had contained endogeic earthworms, *A. caliginosa* and *Al. chlorotica* burrows resulted in significantly higher water infiltration rates than those of *A. rosea* ($p < 0.05$), *A. icterica* having intermediate values. The breakthrough time was higher in *A. icterica* cores than those with *A. rosea* (the two other species had intermediate values).

Model selection was applied only on the endogeic soil cores and revealed that burrow length had a positive and

Table 1 Means (+SE) for each species and control cores (without earthworms) of the burrow system characteristics and results of the water infiltration tests carried out within these cores

Treatment	No. of earthworm	Burrow length (m)	No. of burrows	Diameter (mm)	Branching intensity (m ⁻¹)	Continuity1 (no. burrows >50 mm ³)	Continuity2 (no. burrows >25 mm ³)	Verticality (°)	Intensity of bioturbation (%)	Infiltration rate (L mm ⁻¹)	Breakthrough time (s)
<i>A. caliginosa</i>	4	13.1 c (1.4)	124.5 b (1.6)	3.65 c (0.07)	29.2 a (1.1)	0.5 (0.3)	4.5 b (0.3)	63.5 b (0.7)	23.6 bc (4.3)	5.15 b (0.72)	383 b (99)
<i>Al. chlorotica</i>	4	10.6 c (1.0)	126.7 b (16.4)	3.28 d (0.05)	32.3 a (1.8)	0.0 (0.0)	1.0 cd (0.4)	63.1 bc (1.6)	19.2 c (2.0)	4.41 b (0.72)	420 b (87)
<i>A. icterica</i>	4	15.7 b (0.7)	180.7 a (30.2)	3.52 cd (0.07)	28.2 a (1.2)	1.0 (0.4)	6.0 a (0.4)	64.3 ab (0.2)	44.8 a (3.1)	3.76 b (0.17)	304 b (20)
<i>A. rosea</i>	4	6.4 d (0.5)	121.2 b (4.9)	2.95 e (0.00)	37.2 a (2.4)	0.0 (0.0)	0.7 d (0.6)	63.9 ab (1.9)	30.9 b (0.8)	2.32 c (0.17)	564 c (45)
<i>A. nocturna</i>	2	21.0 a (1.4)	23.5 c (2.6)	5.16 b (0.07)	20.2 b (1.0)	1.0 (0.0)	1.5 c (0.3)	66.1 ab (0.4)	18.2 c (0.6)	11.03 a (1.42)	10 a (1)
<i>L. terrestris</i>	2	9.2 cd (1.0)	14.5 c (2.8)	7.78 a (0.6)	12.2 c (2.1)	2.5 (0.9)	3.2 b (0.6)	68.8 a (0.6)	15.5 c (2.6)	12.42 a (0.84)	11 a (1)
No earthworm	0	0.02 e (0.0)	1.3 d (0.3)	1.93 f (0.02)	11.1 c (3.6)	0.0 (0.0)	0.0 d (0.0)	60.0 c (0.3)	–	1.99 c (0.07)	642 c (33)

Letters indicated significant differences (at the 0.05 significance level) between earthworm species. Each burrow system characteristic was tested separately

^aNo. of burrows whose vertical extension is greater than

significant effect and the number of burrows, and the bioturbation volume had a negative and significant effect on the water infiltration rate (Table 2; $p < 0.05$ in both cases). When water infiltration was plotted as a function of the burrow length, the positive and linear relationship was clear ($R^2 = 0.49$ and $p < 0.01$) but we also found that *A. icterica* burrows did not strictly follow the same trend as other endogeic species (Fig. 5). When *A. icterica* was excluded from the linear regression, R^2 increase to 0.95 ($p < 0.001$).

Discussion

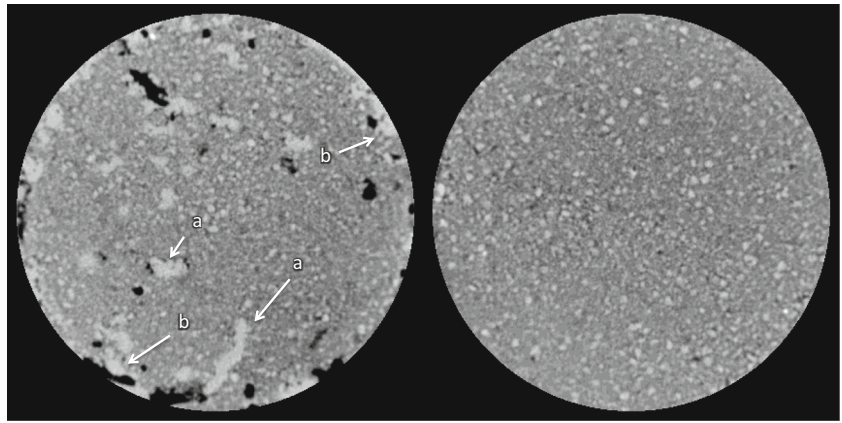
3D burrow morphology

The use of repacked soil cores to study earthworm burrow systems has some drawbacks. The space provided to earthworms is limited: with a medical scanner, the core diameter cannot be larger than 20 cm, and for the sake of feasibility, core length is generally about 30 cm. This could lead to some artefacts. However, most of the earthworms do live in the first 30 cm of soil at the exception of *L. terrestris* whose burrow depth can exceed 1 m. However, *L. terrestris* is known to have behavioural plasticity and, for example, can live as an epigeic earthworm in forest soils (Lavelle 1988). Thus, despite these limitations, we are quite confident that the burrow systems made by the six earthworm species are realistic. For example, it was striking that *L. terrestris* did not burrow extensively in the repacked soil cores but rather had a typical behaviour and made only a few vertical burrows.

The morphology of the burrow systems made by the two anecic species was already described (Jégou et al. 1998; Langmaack et al. 1999; Capowiez et al. 2003). From a visual assessment, the burrow systems described in this study are in agreement with these previously published results. Significant differences were revealed for some burrow system characteristics between the two studied anecic species (burrow length, diameter and bioturbation intensity; $p < 0.05$). These observations illustrate that the term anecic can hide variability in behaviours. Indeed, as Bouché 1972 and Bastardie et al. (2005a) later demonstrated, *L. terrestris* is more of an epi-anecic earthworm rather than a typical anecic earthworm. This means that this species builds a real burrow, in effect a shelter for protection from predation and unfavourable conditions (i.e. drought), that is intensively reused and, under natural conditions, reaches soil depths of sometimes more than 1 m (Shipitalo and Butt 1999). *A. nocturna* and *N. giardi*, in contrast, build more extensive burrow systems which are more highly branched and more or less reused (Bastardie et al. 2005a; Auclerc et al. 2013). Thus, casts sometimes refill these burrows, which reduce the burrow system continuity.

Knowledge of endogeic burrow systems is more limited, but 3D information can be found for *A. caliginosa* (Jégou et al.

Fig. 3 2D images (16 cm in diameter) of a soil core where *A. icterica* (left) burrowed or a control core with no burrows (right). Arrows indicate different zones of bioturbation corresponding to either refilled macropores (a) or compacted areas around macropores (b)



1998; Francis et al. 2001; Dittbrenner et al. 2011), *Al. chlorotica* (Capowiez et al. 2003), *A. icterica* (Capowiez et al. 2006) and *Octalasion cyaneum* (Bastardie et al. 2005a). The present study provided information for *A. rosea*. Beyond the visual similarities and despite a limited number of replicates, our quantifications revealed that *A. rosea* and *A. icterica* are significantly different from the other two endogeic species ($p < 0.05$). These differences (diameter, burrow length) are associated with the small size of *A. rosea*. However, we found that *A. icterica* had the most intense burrowing behaviour producing the longest burrow length, number of burrows, and bioturbation intensity. These three

factors are linked since bioturbation includes refilled macropores, which often cut an existing burrow in two. Thus, overall, we were able to estimate some burrow system characteristics that clearly separate anecic and endogeic species. We further demonstrated obvious differences between the two studied anecic species whereas we found limited differences among the endogeic burrow systems. In this study, tomography was not used in a dynamic manner with regular scans over time (Capowiez et al. 2014), but instead provided snapshots of the macroporosity created by the six earthworm species under homogeneous soil conditions and similar earthworm abundance for anecic and endogeic species. Since, the

Fig. 4 Projection on the first two axes of the principal component analysis of the soil cores based on the characteristics of their burrow systems. Ellipses correspond to 67 % confidence intervals around the species barycentre. Burrow system characteristics ('length' for burrow length (m), 'burrows' for number of burrows, 'diameter' for burrow diameter (mm), 'branching' for branching intensity (m^{-1}), 'continuity1' and 'continuity2' for the number of burrows whose vertical extension was greater than 50 and 25 mm, respectively, 'verticality' for burrow verticality ($^{\circ}$), and 'bioturbation' for intensity of bioturbation (%)) were also projected on the same plan (coordinates were multiplied by two for the sake of readability)

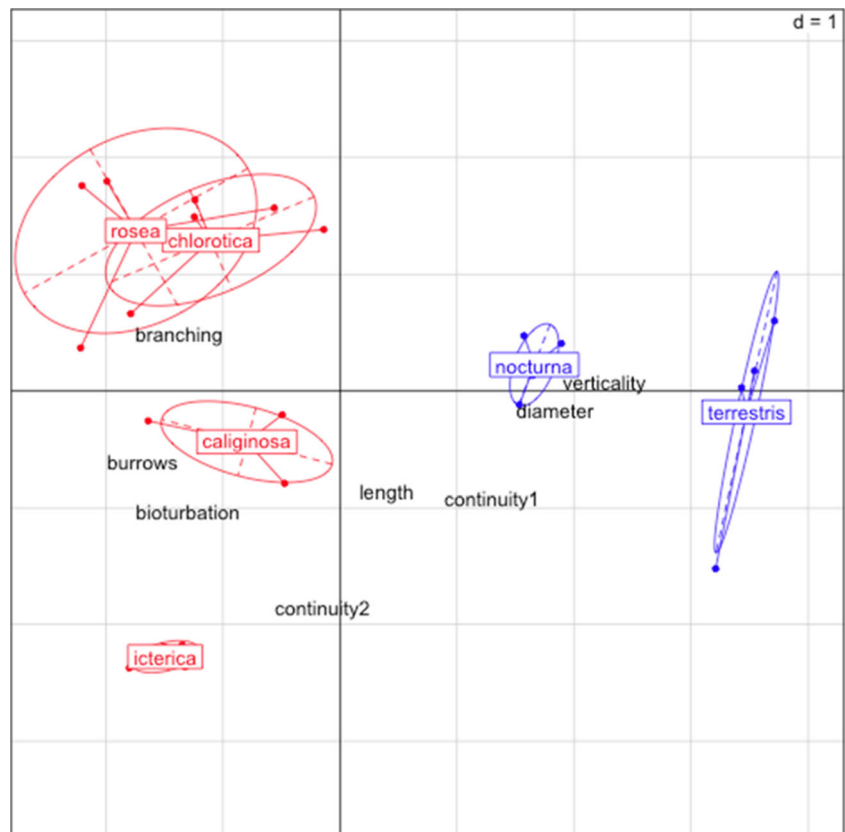


Table 2 Results of linear regression analysis followed by model selection showing only the effect of the variables retained by the model, their standardized effect and significance

Variable ^a	Standardized effect	SE	p Value	R ² of the model
Burrow length	+0.0022	0.0001	0.002	0.97
Burrow number	−0.012	0.0033	0.008	
Volume of bioturbation	−0.029	0.0038	0.0001	

^a Kept after model selection

burrow systems appeared quite stable for each species and quite similar to other published results (Jégou et al. 1998; Langmaack et al. 1999; Francis et al. 2001; Capowiez et al. 2003, 2006; Dittbrenner et al. 2011), the next step will be to study the effect of controlled variations, such as soil modifications (organic matter content or bulk density) or changes to the biotic conditions (earthworm abundance and interactions between species).

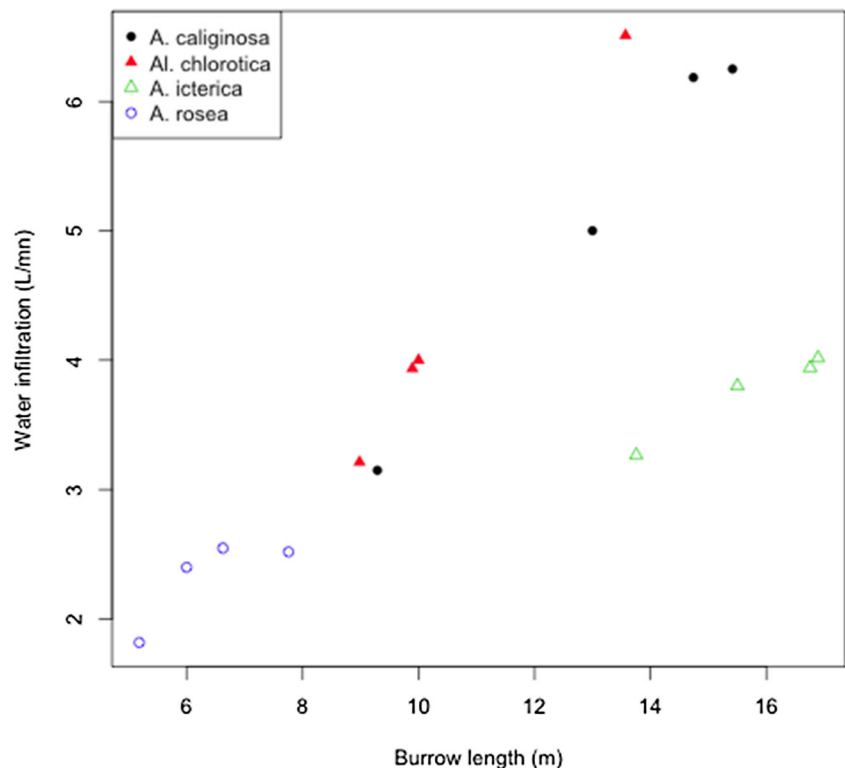
Relationships between burrow systems and water infiltration

One functional consequence of the burrow morphology was studied using ‘Beerkan’ infiltration tests. The water infiltration rates and breakthrough times obtained for anecic burrow

systems were clearly different from those of endogeic worms with far greater infiltration rates. However, these results were expected: obviously continuous macropores, which join both extremities of the soil core, are very efficient at transporting excess water (water sheets of 0.75 cm were applied at regular intervals).

Thus, a more interesting comparison is the endogeic burrow systems since none had a continuous burrow starting at the top and ending at the bottom of the core. Water is thus obliged to travel some distance in the soil before reaching a new macropore (Allaire-Leung et al. 2000). In this study, we attempted to relate water infiltration to general burrow system characteristics even if not all the burrows may have played a role (Trojan and Linden 1992; Sammartino et al. 2012). Indeed, we were interested in differences in burrowing behaviour between earthworm species assuming that these differences may result in marked differences in the burrow system shape and topology. Only three characteristics were retained during linear analysis and model selection. The first two were burrow length (positively) and burrow number (negatively) indicating that beyond differences in shape, orientation, and diameter, the linear length and the number of macropores created by earthworms primarily influence the water infiltration rate. It is worth noting that the number of burrows is indirectly correlated to burrow continuity: the more burrows, the less continuous they

Fig. 5 Relationship between water infiltration assessed using the ‘Beerkan’ method and the length of the burrow systems created by endogeic earthworms in repacked soil cores



are (for a same burrow length). The third burrow system characteristic, the bioturbation volume, played the greatest role if we compare standardized effects (Table 2). The bioturbation volume, which excluded the macropore volumes in this study, is thus an estimation the amount of casts crushed or not and the lateral compaction along the burrow walls made during either burrow creation and/or re-use. These two phenomena are likely to limit the water transfer from the burrows into the surrounding soil (Bastardie et al. 2005b) in a similar way that crusts at the soil surface decrease water infiltration (Mualem et al. 1990).

Regarding bioturbation volume, we observed that one species, *A. icterica*, caused a far greater amount of bioturbation than other endogeic and anecic species (45 %). It was striking that water infiltration in the cores of this species, which also contained the longest burrow system, was slower (albeit not significantly) than for *A. caliginosa* or *Al. chlorotica* whereas we previously found that burrow length was positively and significantly correlated to water infiltration ($p < 0.05$). Indeed, we believe that the relatively poor water transport efficiency of the *A. icterica* burrow systems is due to lateral compaction or cast crushing rather than to a specific shape or topology of these burrows. Nevertheless, we found that the bioturbation volume did not negatively influence breakthrough time since breakthrough times were the longest for *A. icterica* soil cores compared to soil cores of other endogeic species.

Conclusions

The tools (medical scanner) and software (ImageJ) used in this study are now widely available. Thus, the study of earthworm behaviour and its consequences in terms of burrow systems and soil functioning can now enter a quantitative era. These quantifications are necessary to serve as a basis for modelling the effects of earthworms on soil structure and transfer properties (Schneider and Schröder 2012). In this study, we were able to detect differences between the main characteristics of the burrow system of six common species and their effect on water properties. Comparing anecic and endogeic species is almost a waste of time since the differences are so numerous and obvious. However, we highlighted that compared to *A. nocturna*, *L. terrestris* is more of an epi-anecic species than a purely anecic species as far as burrowing behaviour is concerned. Under our experimental conditions, *A. icterica* is a species characterised by very high values of bioturbation (represented by casts inside the burrows and those crushed along the burrow walls). Thus, for endogeic species, we demonstrated that not only burrow system shape and topology influence water transfer but that the volume of bioturbation around burrows may have been a neglected parameter so far.

References

- Allaire-Leung SE, Gupta SC, Moncrief JF (2000) Water and solute movement in soil as affected by macropore characteristics. 1. Macropore continuity. *J Contam Hydrol* 41:283–301
- Auclerc A, Capowicz Y, Guérolf F, Nahmani J (2013) Application of X-ray tomography to evaluate liming impact on earthworm burrowing activity in an acidic forest soil under laboratory conditions. *Geoderma* 202–203:45–50
- Badorreck A, Gerke HH, Huttli RF (2012) Effects of ground-dwelling beetle burrows on infiltration patterns and pore structure of initial soil surfaces. *Vad Zone J* 11, 10.2136/vzj2011.0109
- Bastardie F, Cannavacciuollo M, Capowicz Y, Dreuzy J-R, Bellido A, Cluzeau D (2002) A new simulation for modelling the topology of earthworm burrow systems and their effects on macropore flow in experimental soils. *Biol Fertil Soils* 36:161–169
- Bastardie F, Capowicz Y, Renault P, Cluzeau D (2005a) A radio-labelled study of earthworm behaviour in artificial soil cores in term of ecological types. *Biol Fertil Soils* 41:320–327
- Bastardie F, Ruy S, Cluzeau D (2005b) Assessment of earthworm contribution to soil hydrology: a laboratory method to measure water diffusion through burrow walls. *Biol Fertil Soils* 41:124–128
- Bottinelli N, Jouquet P, Capowicz Y, Podwojewski P, Grimaldi M, Peng X (2015) Why is the influence of soil macrofauna on soil structure only considered by soil ecologists? *Soil Till Res* 146:118–124
- Bouché MB (1972) *Lombriens de France*. Ecologie et systématique, INRA, Paris
- Braud I, De Condappa D, Soria JM, Haverkamp R, Angulo-Jaramillo R, Galle S, Vauclin M (2005) Use of scaled forms of the infiltration equation for the estimation of unsaturated soil hydraulic properties (the Beerkan method). *Eur J Soil Sci* 56:361–374
- Capowicz Y, Pierret A, Daniel O, Monestiez P, Kretschmar A (1998) 3D skeleton reconstructions of natural earthworm burrow systems using CAT scan images of soil cores. *Biol Fertil Soils* 27:51–59
- Capowicz Y, Pierret A, Moran CJ (2003) Characterisation of the three-dimensional structure of earthworm burrow systems using image analysis and mathematical morphology. *Biol Fertil Soils* 38:301–310
- Capowicz Y, Bastardie F, Costagliola G (2006) Sublethal effects of imidacloprid on the burrowing behaviour of two earthworm species: modifications of the 3D burrow systems in artificial soil cores and consequences on gas diffusion in soil. *Soil Biol Biochem* 38:285–293
- Capowicz Y, Cadoux S, Bouchand P, Ruy S, Roger-Estrade J, Richard G, Boizard H (2009) The influence of tillage type and compaction on earthworm communities and the consequences for macroporosity and water infiltration in crop fields. *Soil Tillage Res* 105:209–216
- Capowicz Y, Sammartino S, Michel E (2011) Using X-ray tomography to quantify earthworm bioturbation non-destructively in repacked soil cores. *Geoderma* 162:124–131
- Capowicz Y, Sammartino S, Michel E (2014) Burrow systems of endogeic earthworms: effects of earthworm abundance and consequences for soil water infiltration. *Pedobiologia* 57:303–309
- Darwin CR (1881) The formation of vegetable mould through the action of worms, with observations on their habits. Murray, London
- Dittbrener N, Moser I, Triebekom R, Capowicz Y (2011) Assessment of short and long-term effects of imidacloprid on the burrowing behaviour of two earthworm species (*Aporrectodea caliginosa* and *Lumbricus terrestris*) by using 2D and 3D post-exposure techniques. *Chemosphere* 84:1349–1355
- Ernst G, Emmerling C (2009) Impact of ecologically different earthworm species on soil water characteristics. *Eur J Soil Biol* 45:207–213
- Felten D, Emmerling C (2009) Earthworm burrowing behaviour in 2D terraria with single- and multiple-species assemblages. *Biol Fertil Soils* 45:789–797

- Francis GS, Tabley FJ, Butler RC, Fraser PM (2001) The burrowing behaviour of three common earthworm species. *Aust J Soil Res* 39:1453–1465
- Gerino M, Stora G, Francois-Carcaillet F, Gilbert F, Poggiale JC et al (2003) Macro-invertebrate functional groups in freshwater and marine sediments: A common mechanistic classification. *Vie et Milieu Life Environ* 53:221–231
- Jégou D, Cluzeau D, Wolf HJ, Gandon Y, Tréhen P (1998) Assessment of the burrow system of *Lumbricus terrestris*, *Aporrectodea giardi* and *Aporrectodea caliginosa* using X-ray computed tomography. *Biol Fertil Soils* 26:116–121
- Joschko M, Müller PC, Kotzke K, Dohring W, Larink O (1993) Earthworm burrow system-development assessed by means of X-ray computed tomography. *Geoderma* 56:209–221
- Jouquet P, Blanchart E, Capowiez Y (2014) Utilization of earthworms and termites for the restoration of ecosystem functioning. *Appl Soil Ecol* 73:34–40
- Langmaack M, Schrader S, Rapp-Bernhardt U, Kotzke K (1999) Quantitative analysis of earthworm burrow systems with respect to biological soil-structure regeneration after soil compaction. *Biol Fertil Soils* 28:219–222
- Lavelle P (1988) Earthworm activities and the soil system. *Biol Fertil Soils* 6:237–251
- Lavelle P, Spain AV (2001) *Soil ecology*. Kluwer Academic Publisher, New-York
- Lee KE, Foster RC (1991) Soil fauna and soil structure. *Austr J Soil Res* 29:745–775
- Mualem Y, Assouline L, Rohdenburg H (1990) Rainfall induced soil seal. A critical review of observations and models. *Catena* 17:185–203
- Nahmani J, Capowiez Y, Lavelle P (2005) Effects of metal pollution on soil macroinvertebrates burrow systems. *Biol Fertil Soils* 42:31–39
- Needham SJ, Worden RH, McIlroy D (2004) Animal-sediment interactions: the effect of ingestion and excretion by worms on mineralogy. *Biogeosciences* 1:113–121
- Ritz K, Young I (2011) *The architecture and biology of soils—life in inner space*. CABI, Oxfordshire
- Rogasik H, Schrader S, Onasch I, Kiesel J, Gerke HH (2014) Micro-scale dry bulk density variation around earthworm (*Lumbricus terrestris* L.) burrows based on X-ray computed tomography. *Geoderma* 213:471–477
- Russ JC (1995) *The image processing handbook*. CRC Press, Boca Raton
- Sammartino S, Michel E, Capowiez Y (2012) A novel method to characterize preferential flow in undisturbed soil cores by using multi-slice helicoïdal CT. *Vad Zone J* 11. doi 10.2136/vzj2011.0100
- Schneider AK, Schröder B (2012) Perspectives in modelling earthworm dynamics and their feedback with abiotic soil properties. *Appl Soil Ecol* 58:29–36
- Shipitalo MJ, Butt KR (1999) Occupancy and geometrical properties of *Lumbricus terrestris* L. burrows affecting infiltration. *Pedobiologia* 43:782–794
- Shipitalo MJ, Le Bayon RC (2004) Quantifying the effects of earthworms on soil aggregation and porosity. In: Edwards CA (ed) *Earthworm ecology*, 2nd edn. St. Lucie Press, Boca Raton, FL, pp 183–200
- Trojan MD, Linden DR (1992) Microrelief and rainfall effects on water and solute movement in earthworm burrows. *Soil Sci Soc Am J* 56:727–733