

Using X-ray tomography to quantify earthworm bioturbation non-destructively in repacked soil cores

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3	Using X-ray tomography to quantify earthworm bioturbation
4	non-destructively in repacked soil cores
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22 Abstract

23 X-ray tomography is used increasingly to study the macroporosity resulting from earthworm 24 activity. However, macropores are not the only features visible on the images; other zones 25 resulting from bioturbation by earthworms can be detected due to differences in greylevels. 26 Four different soil cores were incubated with two earthworm species at two different densities 27 (4 or 8 adults of the endogeic species Allolobophora chlorotica or 2 or 4 adults of the anecic species Aporrectodea nocturna). A fifth core without earthworms was used as a control. After 28 29 six weeks, the cores were analysed by X-ray tomography using a medical scanner. The 3D 30 earthworm burrow systems were reconstructed and a new and specific algorithm was used to 31 determine other bioturbated zones (BZ) that were physically influenced by the earthworms. 32 Expected differences in the structure of the burrow systems between the endogeic and anecic 33 species were observed: the A. chlorotica burrows were narrower and more numerous, more 34 branched and less vertical. When the earthworm density doubled, the volume and length of 35 the A. chlorotica burrow system increased whereas no increase was observed for A. nocturna. 36 The BZ, which were located in the upper section of the cores, represented almost the same 37 volume as the macropores. These zones tended to be located further from the burrows in the 38 A. chlorotica cores: 50% of of the voxels corresponding to BZ were at a distance greater than 39 4 and 5.5 mm from the closest macropore for A. nocturna and A. chlorotica, respectively. 40 Three processes may have contributed to form these zones, which are characterised by 41 increased soil density: (i) soil compaction around the burrows during burrow creation, (ii) cast 42 deposition in the burrows (burrow backfilling) and (iii) crushing of casts on the burrow walls 43 (so called cutanes). The longer distance between BZ and macropores in A. chlorotica cores 44 suggests that the proportion of burrow backfilling is higher for the endogeic species compared 45 to the anecic species. If we assume that BZ located further than 10 mm from any burrow are

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- 46 actually burrows backfilled with casts, the volume of burrow backfilled in our study ranged
- 47 from 14 to 18 % for *A. chlorotica* and from to 8 to 10% for *A. nocturna*.

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- Keywords: burrow system bioturbation cast production Aporrectodea nocturna -
- Allolobophora chlorotica macropore earthworm behaviour

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52 **1. Introduction**

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54 Like benthic organisms, which greatly influence the undersea landscape and sediment 55 biogeochemistry (Dorgan et al., 2006), earthworms play a highly important role in many 56 physical, chemical and biological processes that occur in soil (Meysman et al., 2006). Despite 57 this recognised importance for which earthworms are considered as soil ecosystem engineers 58 (Jones et al., 1994), quantitative estimations of soil bioturbation are scarce (Meysman et al., 59 2006). Bioturbation, simply defined as 'soil or sediment physical displacement by soil or sea 60 organisms' (Wilkinson et al., 2009), is the sum of the different physical actions made by 61 organisms inhabiting, temporarily or permanently, soils or sediments. In terrestrial 62 ecosystems, the part that earthworms play in bioturbation is mainly represented by the 63 creation of burrows with various sizes and shapes and the production of casts with various 64 physical properties (Blanchart et al., 1997) and at different locations (Whalen et al., 2004). 65 Indeed the influence of earthworms on soils is mainly physical and these influences are 66 closely associated with their behaviour. However, even though earthworm behaviour was first studied more a century ago (Darwin, 1881), overall our knowledge is still poor. This is mainly 67 68 because these animals are concealed by the soil in which they live which makes direct 69 observations extremely difficult. The consequences of the limited knowledge of earthworm 70 behaviour are (i) the scarcity of models compared to those available in aquatic biology 71 regarding sediment mixing and burrowing (Meysman et al., 2006; Jarvis et al., 2010); (ii) the 72 extensive use of ecological types (sensu Bouché, 1977), i.e. endogeic versus anecic 73 earthworms, as proxies to describe earthworm behaviour according to the "postulates" of Lee 74 and Foster (1991). Some aspects of these postulates, i.e. permanent vertical and often re-used 75 burrows made by anecics versus discontinuous networks of galleries without preferential 76 orientation made by endogeics, were confirmed by initial tomography studies (Jégou et al.,

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1998; Langmaack et al., 1999; Capowiez et al., 2001). However an increasing number of
more recently published observations suggests that this vision is too simple (Felten and
Emmerling, 2009).

Earthworm burrowing behaviour has been studied by a variety of methods so far: resin or plaster moulds (Shipitalo and Butt, 1999), the careful and time consuming removal of fine soil layers under field conditions (Ligthart and Peek, 1997) or the use of 2D terraria under laboratory conditions (Evans, 1947; Schrader 1993; Capowiez 2000). But since the beginning of the 90s, X-ray tomography has been increasingly applied in soil biology to obtain precise and non-destructive analysis of earthworm macropores. Authors used either repacked soil cores with introduced earthworms (Joschko et al., 1991; Jégou et al., 1998; Langmaack et al., 1999; Capowiez et al., 2001; Bastardie et al., 2003) or natural soil cores (Daniel et al., 1997; Pierret et al., 2002; Bastardie et al., 2005).

To study cast production, a great majority of studies focused at the soil surface, which is easily observed (Daniel et al., 1996). Others also attempted to investigate belowground cast production using 2D terraria in the laboratory (Schrader, 1993; Cook and Linden, 1996; Hirth et al., 1996; Whalen et al., 2004). Several field studies were carried out to estimate belowground cast production, in this case identified as 'mammilated vughs', using image analysis of soil slices of resin-impregnated blocks (Vandenbygaart et al., 2000; Jongmans et al., 2003; Bruneau et al., 2004) but automated recognition of the different classes of porosity is still in its infancy. Overall, as mentioned by Whalen et al. (2004), the proportion of casts that are deposited onto the soil surface or within the soil is still largely unknown partly because no satisfactory methods of measuring casts in situ exists. However based on what is known, the proportion of casts produced belowground is not negligible. It is therefore crucial to (i) quantitatively measure soil mixing and its putative influence on associated processes (Covey et al., 2010) and (ii) determine the proportion of burrows that are refilled with casts.

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Indeed, burrow backfilling is an important phenomenon contributing to burrow destruction (Ligthart, 1997) and greatly influences burrow continuity and thus soil transfer properties associated with these burrows (Capowiez et al., 2006). With the exception of a couple of studies (Ligthart, 1997; Francis et al., 2001), the rate of burrow destruction by biological (backfilling) or physical (climate) actions is still largely unknown even though it may be a key parameter for dynamic models of macroporosity based on earthworm behaviour (Bastardie et al., 2002). There is therefore a need for new tools to estimate belowground cast production and some of its consequences such as burrow backfilling.

Recently, Schrader et al. (2007) used classical images from X-ray tomography of soil 111 cores to elucidate more than a simple description of earthworm macropores and studied the 112 lateral compaction of burrows (i.e. cutanes) made by L. terrestris in artificial soil cores. These authors were able to measure the increase in soil bulk density around some burrows and thus determined the width and volume of the so-called drilosphere (sensu Bouché, 1975), the zones under the physical influence of earthworms. Indeed, when looking at images resulting from X-ray tomography of artificial soil cores, besides macropores (around them but not exclusively around them), other soil regions are different from the soil matrix and are thus influenced by earthworms (see for example Fig. 11 in Rogasik et al., 2003). These regions are generally characterised by a difference in image texture or greylevel. Since X-ray tomography 120 is sensitive to changes in density and mineralogy of the material, these regions may be characterised by either a difference in soil bulk density (due to soil compaction or soil looseness), water content (water is more dense than air) or mineralogy. To describe not only porosity (black voids) but also differences in greylevels in images was already successfully applied to study sedimentary structures (Honeycutt and Plotnik, 2008).

The aim of this present study was to develop a new method based on images from Xray tomography to compute quantitative estimations of two kinds of soil bioturbation by

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127 earthworms, i.e. firstly the burrows and then the rest of the drilosphere. To test this approach, 128 four artificial soil cores were incubated with two different earthworm species (Aporrectodea nocturna and Allolobophora chlorotica) at two different densities.

2. Material and methods

2.1. Earthworms and soil cores

The soil used 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 abandonedorchard in Montfavet, near Avignon (43°55' N, 4°48' E) in the SE of France. In this orchard, the earthworm density is about 450 ind. m⁻² and this earthworm community is dominated by the species A. nocturna (76 ind. m⁻²) and A. chlorotica (176 ind. m⁻²). These two species were also chosen because they belong to different ecological types: A. nocturna is a true anecic whereas A. chlorotica (leucotypic form) is an endogeic species. Mature earthworms were collected and weighed after washing and gentle drying with filter paper.

Repacked soil cores were prepared using PVC cylinders (35 cm in length and 16 cm in diameter) lined with a mixture of sealing varnish and sharp fine sand to prevent earthworms from crawling along the PVC walls. A hydraulic press was used to compact 145 five cores simultaneously. Cores were compacted by applying a pressure of 270 kPa for 5 Manuscrift d'auteur / Author manuscript 144 148 148 148 148 120 121 121 minutes on sieved soil at 23 % moisture content (gravimetric). This treatment resulted in a soil dry bulk density of 1.1 g.cm⁻³. To minimise variations in soil bulk density between top and bottom of the cores, the soil was compacted stepwise in 12 layers. Each layer comprised 600 g of soil. The final thickness of each layer was approximately 2.5 cm. Before adding a new soil layer, the surface of the previous layer was gently scratched using a small rake to increase cohesion between layers.

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The bottom of each core was sealed and the top was closed using a lid with small holes to prevent significant water losses. Five cores were prepared: (i) two or (ii) four *A. nocturna* individuals; (iii) four or (iv) eight *A. chlorotica* individuals; v) a control without earthworms. The number of individuals introduced in the cores corresponded to densities of approximately 100, 200 and 400 individuals m⁻² respectively. These densities were chosen to take into account (i) the mean weight of each species (2.01 g for *A. nocturna* and 0.42 g for *A. chlorotica* and (ii) the relative densities of each species observed in the field (*A. chlorotica* density is 2.2 fold that of *A. nocturna*). The cores were incubated in a dark room, at 12°C. Water (5 ml per core) was supplied weekly. Food (5 g of dried grass) was added at the top of each core. After 6 weeks, chloroform (10 ml) was applied in each core to kill the earthworms and prevent them from burrowing.

At the end of the experiment, cores were imaged using a medical X-ray tomograph (Prospeed SX Advantage, General Electrics) at the Bagnols-sur-Cèze hospital to obtain a set of images 3 mm thick every 3 mm. The settings at which the X-ray beam was operated were 130 mA and 120 kV. For classical medical scanners, the image size is limited to 512 * 512 pixels. As a consequence, each pixel in our case was approximately 0.4 * 0.4 mm.

2.2. Image transformations and determination of macroporosity

All images were manipulated using ImageJ (available at http://rsb.info.nih.gov/ij/). Images resulting from tomography are in the DICOM format with greylevels expressed in Hounsfield values related to the attenuation of the X-ray beam (Taina et al., 2007). One important step in CT-image processing is the transformation of 16-bits (DICOM format) to 8bits images to save memory and be able to handle images with most classical software packages. This reduction in image depth is done in ImageJ by setting the minimum and

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176 maximum greylevel values that will be kept and transformed into the final 8-bits image. In 177 our case, these values were set to 1000 and 3000 HU.

The greylevel histograms of the 8-bits 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 thus to macropores (see Figure 2 in Capowiez et al., 1998 for an example). In this case, image segmentation through binarisation is easy and widely applied (Russ, 1995) but to increase the accuracy of the reconstruction and take into account the 3D information, a segmentation procedure was proposed by Pierret et al. (2002) and previously applied successfully to this kind of image. In 185 brief, macropores are traced starting from the darkest voxels by studying local variation in 186 mean greylevel when the current voxel is included in the current macropore. Macropores that are too small (less than 100 voxels, i.e. about 0.5 cm³) are discarded. At this stage, the volume of macroporosity, and the number of burrows (a burrow is a set of connected voxels) were computed. The 3D-volumic information on the macropore can be translated into 3D skeletons by determining all the ultimate eroded points (i.e. centroids) in all images and linking all the centroids with pores that overlap between two successive images. A burrow is then a set of connected segments (a segment joins the centroids of two overlapping pores) and the burrow system is the set of burrows in a core. Then it is possible to compute other characteristics for 194 each core: burrow system length, rate of branching (per unit of length) and mean vertical deviation (relative to the vertical direction) of segments. Mean vertical deviation between cores were compared with a Kruskall-Wallis test followed by a post-hoc comparison test (Zar, 1984). Two other characteristics not easy to compute: burrow continuity and diameter. There is no easy way to characterise the continuity of the burrow system so we used the method previously used in Capowiez et al. (2006). Briefly, virtual horizontal planes are defined at equal distance in the core and the number of pathways connecting two of these planes is

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summed. Finally the number of equidistant planes is increased from two (the top and the bottom of the core) to nine (the core is divided into eight parts). The rationale behind the estimation of the burrow diameter is that less vertical macropores have bigger areas. So for each pore in each image, we computed the area and the circularity according to the following equation:

circularity = 4 π area / perimeter²

The circularity ranging from 1 for perfectly circular pores to 0 for very elongated pores. By studying the relationships between pore area and pore circularity, we determined that the median pore area was constant in each core for pores whose circularity was greater than 0.6 (whereas the mean area was not). Thus we decided to compute the Equivalent Circular Diameter (ECD) according to the following equation:

ECD = 2 (area _{circ.>0.6} /
$$\pi$$
)⁰

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where area _{circ.>0.6} was the median area of the pores whose circularity was greater than 0.6. Differences in median diameter between treatments were assessed using a non parametric test followed by an post-hoc comparison test (Zar, 1984).

For the sake of 3D rendering, we defined a point of observation and computed the distance between this point and each voxel of the macropores. By projecting these values on a vertical 2D image, keeping the minimum values (meaning that the nearest macropores hide all the macropores behind them) then subsequent translating distance into a gradient colour map (here yellow for the minimum and blue for the maximum), we can visualise the resulting earthworm burrow systems in 3D.

2.3. Determination of other kinds of soil bioturbation (excluding macropores)

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225 Although bioturbated zones (BZ), excluding voids (i.e. macropores), could be easily 226 visually distinguished in each image (Fig. 1), identifying these zones is not straightforward and in most cases cannot be done by direct binarisation thresholding (i.e. the definition of the minimal and maximal greylevel values). Indeed, the BZ per se did not correspond to a peak in the greylevel histogram and are merged in the large soil matrix peak leading to a slightly skewed histogram. At this stage, we tested and developed several procedures applied on each 2D image that gave slightly different results. In our case the best procedure, judged by a visual estimate, was obtained with the following steps using ImageJ: (i) a smoothing filter (radius 2); (ii) filtering the image using anisotropic diffusion (implemented as a plugin in 234 ImageJ and available at http://rsbweb.nih.gov/ij/plugins/anisotropic-diffusion.html); (iii) 235 binarisation thresholding (min=0 and max =145); (iv) opening (radius 1) and (v) removal of 'objects' smaller than 60 pixels. The anisotropic diffusion filter was developed by Christopher Mei based on the study of Perona and Malik (1990) and was used here with the following parameters: iteration = 7; k = 40 and lambda = 0.2.

However, we found that to obtain meaningful results the critical step was the initial adapted transformation of 16-bits into 8-bits images. It was more important than the precise definition of the parameters for each step, or even the choice of the filtering method. Overall, we are aware that no single or general protocol can be provided to detect BZ in images derived from different experimental set-ups (depending on the soil and scanner characteristics). We are quite confident that, in most cases, filtering these zones, possibly through texture analysis, is possible except perhaps in soil that is too sandy or compact. Indeed, for dense materials, depending on the size of the core, the power limits of the medical scanner may be reached leading to poorly dynamical greylevel histograms (i.e. overrepresented by bright colours). In most cases however, these zones are clearly visible as

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supported by studies where examples of CT-images were provided (Figure 5 in Jégou et al.,
1998; Figure 7 in Francis et al., 2001; Figure 11 in Rogasik et al., 2003).

2 2 4 Physical measurem

2.4. Physical measurements on surface casts

For each soil core, at the end of the incubation period all surface casts were collected. These casts were weighed, dried (at 110°C for 48h) and weighed again to determine their water content and dry weight. The bulk density of the casts was computed on six cast samples (of about 1 g each) for each soil core using the paraffin method (Pansu et al., 1998). The total volume of the surface casts in each core was estimated using the measured bulk densities. The difference in soil bulk density between species was then assessed using a Student t-test.

3. Results

3.1. Earthworm burrow systems

The burrow systems made by *A. nocturna* and *A. chlorotica* looked quite different (Fig. 2). The most obvious difference was the diameter of burrows but other characteristics also contributed to make the two burrow systems look different. Burrows made by the anecic species were more continuous and vertical. The higher earthworm density appears to have had an effect in the case of *A. chlorotica* but no clear effect was detected for *A. nocturna*. All these visual impressions were confirmed when the burrow characteristics were quantified (Table 1). The effect of the increase in earthworm number (2-fold) depended on the species. The burrow volume and length slightly decreased (about -12%) when the number of *A. nocturna* inside the core doubled whereas a marked increase was observed in burrow length (2-fold) and volume (1.7-fold) when the number of *A. chlorotica* doubled. The burrows made by *A. chlorotica* were significantly narrower, more numerous, more branched and tended to be less vertical. The continuity of the C8, N2 and N4 burrow systems, estimated using a

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274 method to determine the number of pathways between virtual horizontal planes, was quite 275 similar and much higher than the continuity of the C4 burrow system (Fig. 3).

3.2. Bioturbated zones (excluding macropores)

The method used to define BZ, based on filtering, was satisfactory (Fig. 1). We assume that it led to a slight underestimation of the BZ volume since zones that were too small or less obvious (i.e without a sufficient difference in greylevel) were not selected (see Figure 1 for some examples). No BZ was ever detected in the control soil core without earthworms. Overall, the BZ followed some of the macropores (Fig. 4). In the first half of the cores, these zones were continuous whereas in the second half they were discontinuous. BZ zones were not homogeneously distributed with depth or along burrows (Fig. 4). There was a clear tendency for these zones to be more abundant in the upper 1/3 of the cores especially when the number of earthworm species. Interestingly, the volume of these zones was very close to the macropore volume (ranging from 80.6 to 90.2% of the latter for the four cores). BZ tended to be further from the burrows in the *A. chlorotica* cores: 50% of the voxels corresponding to BZ were at a distance greater than 4 and 5.5 mm from the closest macropore for *A. nocturna* and *A. chlorotica* respectively (Fig. 6).

3.2. Surface casts

For the lower abundance (C4 and N2), both earthworm species produced the same weight of surface casts (Table 2). When the number of earthworms doubled, the weight of casts produced by *A. chlorotica* greatly increased (2.5-fold) whereas the increase was only 1.5-fold for *A. nocturna*. As the bulk density of casts was not influenced by earthworm density, data were grouped for each species. The soil bulk density of casts made by *A*.

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nocturna was significantly higher than those made by *A. chlorotica*. Estimated volumes for
 these surface casts ranged between 36 and 92 cm³ (Table 2).

4. Discussion

4.1. Earthworm burrow systems

Although there were no replicates in this study and thus statistical analysis was not possible, this descriptive and methodological approach provided interesting information on the earthworm burrow systems made by the two species. These burrow systems were in agreement with the postulates of Lee and Foster (1991): anecic earthworms make permanent homes whereas pathways of endogeic earthworms are temporary. Indeed, endogeic earthworm burrows are supposed to be temporary since they can be backfilled with casts leading to less continuous burrows and thus a greater number of burrows (a backfilled burrow is then split into two). Burrows made by anecic earthworms are permanent and reused to reach the surface where they feed and are thus more continuous. These results are in agreement with a previous study (Capowiez et al., 2001) which showed that the burrows made by A. chlorotica were less vertical, more branched and narrower than those made by A. nocturna. When we compared the C4 and N4 cores, which were colonised by earthworms at the same density, we found that values for total burrow length and volume were almost the same with slight differences in length (7.66 vs 6.24 m) and volume (134.0 vs 164.6 cm³) due partly to the smaller diameter of A. chlorotica burrows. When the earthworm abundance doubled, we observed a striking difference between the two species. For A. chlorotica, burrow length doubled and burrow volume almost doubled (with a clear trend for a increased colonisation of the lower part of the core; Fig. 2) whereas for A. nocturna burrow length and volume slightly decreased. This absence of a correlation between earthworm abundance and burrow length was observed previously (Capowiez et al., 2000) in a field study focusing on

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the invasion of a Swiss meadow by *A. nocturna*. In that case, the earthworm abundance, highly dominated by *A. nocturna* in the colonised section of the meadow, increased from 273 to 386 earthworms m⁻² but no subsequent significant increase in burrow length was observed. These observations may be explained by (i) reduced burrowing behaviour due to interactions between earthworms (Uvarov, 2009) as previously observed for *A. nocturna* in 2D terraria by Capowiez and Belzunces (2001) or (ii) shared burrows made by other earthworms. Felten and Emmerling (2009) observed this behaviour in 2D terraria for *A. caliginosa* and *Octolasion tyrtaeum*.

4.2. Bioturbated zones

The drilosphere was broadly defined as "distinct zones of soil directly and indirectly modified by earthworms" (Bouché, 1975). This currently accepted definition is clearly based on visual criteria and, as such, seems to be well adapted to the case of *Lumbricus terrestris*, for which the burrow walls were found to be made of two layers differing in appearance, bulk density, porosity and composition (Jégou et al., 2001; Görres et al., 2001; Schrader et al., 2007). Jégou et al. (2001) assumed that the outer layer (width = 7 mm) corresponded to soil compaction during burrow formation whereas the inner layer (width = 3 mm) was formed of casts deposited and crushed at the burrow walls during earthworm movements.

In the present study, we were able to automatically select and measure BZ made by *A*. *chlorotica* and *A. nocturna*, mainly but not exclusively around macropores, thus corresponding to the so-called drilosphere. The protocol used to detect these zones was robust (small deviations in the parameters did not produce large variations in the resulting zone selection) and satisfactory (visual agreement, as shown in Figure 1, and no detection of BZ in the control soil core without earthworms).

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At this stage, the exact nature of these zones is still an open question. These zones were characterised by higher greylevel values indicating that the material is more dense than the surrounding soil. This suggests that these regions are characterised by one or several of the following features: (i) higher soil bulk density; (ii) higher water content; (iii) higher proportion of minerals prone to interact with X-rays. Earthworms influence the mineralogy of egested material (Needham et al., 2004); however this process alone is unlikely to produce such a marked difference in the greylevels of the tomography images. For example, it is known that egested material has a lower proportion of sand (Lee, 1985; Oyedele et al., 2006), which should lead to less dense zones in the resulting images. Earthworms burrow in the soil either by ingesting or pushing aside soil particles (Lee and Foster, 1991). The latter causes radial soil displacement, precisely described by Barnett et al. (2009) for *A. longa* and *L. terrestris*, and results in an increase in soil bulk density in the vicinity of burrows, which Schrader et al. (2007) observed for *L. terrestris* in CT-images.

Earthworms deposit part of their casts inside the burrows, backfilling them temporarily (presumably for anecic) or permanently (presumably for endogeic earthworms). Fresh casts have an higher water content than the surrounding soil (Hindell et al., 1994; Francis et al., 2001) but this difference may disappear with time (Mariani et al., 2007). Under our study conditions, the surface casts and the soil had mean water contents of 26.2% and 23.5% (on a weight/weight basis), respectively, at the end of the experiment. If the belowground casts followed the same evolution, these differences may play a minor role in greylevel differences. The casts may, depending on the earthworm species and land use, have different bulk densities (Blanchart et al., 1997; Decaëns, 2000; Jouquet et al., 2008). In the present study, we found that the surface casts made by *A. nocturna* had a significantly higher bulk density than those produced by *A. chlorotica*. However it is difficult to infer the possible effects in the resulting CT-images since the structure of the belowground casts may have been

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modified. Indeed, once the casts are deposited in burrows, depending on earthworm behaviour and thus whether the burrows are reused, they can be crushed along the burrow walls to form so-called cutanes (Jégou et al., 2001). This in turn will increase the soil bulk density of the burrow walls (Schrader et al., 2007). Overall it is difficult to disentangle all these processes (changes in soil mineralogy or texture, increases in water content and soil bulk density) based solely on the greylevel images provided by tomography. Thus three processes probably contributed to the formation of our selected BZ: (i) soil compaction during burrow creation, (ii) cast deposition inside burrows and (iii) cast crushing along the burrow walls. These three processes may often occur in a temporal sequence.

382 In order to investigate this further, it would be interesting to compare freshly made 383 burrows to older ones. Another possibility lies in the comparison of endogeic and anecic burrows based on the postulates of earthworm behaviour. Indeed, using 2D terraria, Capowiez (2000) was able to show that the rate of burrow reuse was significantly higher for A. nocturna than A. *icterica* suggesting that belowground casts made by the anecic species may have a greater probability of being transformed into cutanes. This is in agreement with our observations: the burrow systems made by A. chlorotica consisted of a larger number of burrows (since burrow backfilling creates two burrows) and, at least for C4, were less continuous than the A. nocturna burrow systems. Additionally we observed that a larger 391 proportion of A. chlorotica BZ was found further from the burrows than those of A. nocturna. This may indicate that more of the BZ made by A. chlorotica were indeed burrows backfilled with casts whereas for A. nocturna there was a greater proportion of cutanes around the burrows. In conclusion, the detected BZ are a good estimation of the extent of the drilosphere but we can not attribute all the BZ to belowground cast deposition since some of these zones may have been caused by soil compaction during burrow formation. Using tomography only, Version définitive du manuscrit publié dans / Final version of the manuscript published in : Geoderma, 2011, Vol.162, no.1-2, 124-131, DOI: 10.1016/j.geoderma.2011.01.011

397 it was not possible to distinguish the outer and inner burrow walls as visually defined for L. terrestris by Jégou et al. (2001).

Despite this limitation, it was possible to quantitatively compare our estimations to those previously published. A review of the literature highlights three kinds of estimates: (i) drilosphere volume; (ii) the proportion of belowground casts; and (iii) the rate of burrow backfilling. Studies quantifying drilosphere volume are rare. Based on measurements of compaction around burrows, Schrader et al. (2007) estimated that the drilosphere volume was 53 cm³ for one individual of *L. terrestris* after 70 days of incubation in an artificial soil core (height = 30 and diameter = 15 cm). Our observations for A. nocturna are in relative agreement: the drilosphere volume was between 30 (core N4) and 75 cm³ (core N2) per 406 407 individual for almost the same duration and soil bulk density. The proportion of below and aboveground casts was previously determined only in laboratory experiments (often with loose soil). The results were highly variable ranging from less than 10% in 2D terraria for A. caliginosa, A. rosea and L. terrestris (Whalen et al., 2004) to 98% of belowground casts with A. caliginosa, O. cyaneum and Diplocardia smithii (James, 1991). Mariani et al. (2007) reported values between 36 and 53% for the anecic Martodrilus sp. Curry and Baker (1998) estimated between 15 and 38% belowground casts for A. caliginosa, A. trapezoides and A. longa and cited older studies with rates of approximately 90% in a pasture (Barley, 1959) or 415 more than 50% for A. caliginosa (Boström, 1988). Assuming that all the BZ were belowground casts, we computed than in our study between 70 and 85% of the casts were made belowground, which is a value in the high range of those previously published. Obviously more studies on this important parameter need to be carried out, possibly based on new and standardised methods. Information on the rate of burrow backfilling is even scarcer. Schrader (1993) computed a rate of 50-60% of burrows backfilled by A. longa whereas Hirth et al. (1996) estimated this rate to be between 66 and 85% for A. caliginosa and A. rosea, both

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of these studies were made in 2D terraria. After successive scans of the same soil cores every 20 days and analysis of the CT-images, Francis et al. (2001) estimated that 72-85% of the burrows made by A. caliginosa were backfilled (but the precise computations that were made were not presented). In our case, if we assume that BZ are backfilled burrows when they are at a distance greater than 10 mm from burrows, we computed a rate of backfilling from 14 to 18 % for A. chlorotica and from to 8 to 10% for A. nocturna at the end of the six week incubation. These values, based on the arbitrary threshold of 10 mm, appear to be low compared to those previously published.

5. Conclusions

The increasing use of X-ray tomography has enabled rapid and precise descriptions of earthworm burrow systems in artificial or natural soil cores (Bastardie et al., 2005). Based on the images, important factors that may influence burrowing behaviour such as soil compaction (Langmaack et al., 1999), pesticide concentrations (Capowiez et al., 2006) or amendments (Yunusa et al., 2009) could also be investigated. In the present study, we demonstrated that in addition this non-destructive tool can provide information on the 'drilosphere' (i.e. the zones under the physical influence of earthworms) volume and vertical distribution. At this stage, we could not determine the exact contribution of different components (soil compaction, belowground casts or cutanes) to this drilosphere. However the quantitative and spatial information obtained is likely to be useful for a precise description of the influence of different earthworm species or ecological groups and may be a parameter for the future development of models similar to those existing in sediment bioturbation (Jarvis et al., 2010; Covey et al., 2010).

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615 Legends for figures

- Fig. 1 A CT-image of the soil core with 8 individuals of *A. chlorotica* (diameter= 16 cm) after
 analysis with the medical scanner (width = 3mm). A: raw image after 8bit-transformation
 ; B: the same image with the selected bioturbated zones coloured in white ('ab' = around
 burrows and 'rm' = refilled macropores).
 - Fig. 2 Three-dimensional rendering of the burrow systems after six weeks of incubation in the fours soil cores (colours range from soft to dark grey as a function of the distance from the point of observation): C4 and C8 (4 and 8 individuals of *A. chlorotica*), N2 and N4 (2 and 4 individuals of *A. nocturna*).
 - Fig 3 Burrow system continuity in the four soil cores estimated as the number of pathways connecting two successive planes in function of the number of virtual horizontal equidistant planes in the core (C4 and C8 mean 4 or 8 individuals of *A. chlorotica*, and N2 and N4 mean 2 or 4 individuals of *A. nocturna*).
 - Fig. 4 Three-dimensional rendering of the bioturbated zones after six weeks of incubation in the fours soil cores (colours range from soft to dark grey as a function of the distance from the point of observation): C4 and C8 (4 and 8 *A. chlorotica*), N2 and N4 (2 and 4 *A. nocturna*).

Fig. 5 Relative proportion of burrow and bioturbated zone volumes in the upper (1/3) and lower (2/3) parts of the 4 soil cores (C4 and C8 mean 4 or 8 individuals of *A. chlorotica*, and N2 and N4 mean 2 or 4 individuals of *A. nocturna*).

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Fig.6 Cumulative distribution of the distance of each voxel of the bioturbated zone to the nearest macropore (C4 and C8 mean 4 or 8 individuals of *A. chlorotica*, and N2 and N4 mean 2 or 4 individuals of *A. nocturna*).

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Main characteristics of the burrow systems and the bioturbated zones in function of the earthworm species and abundance introduced (after 6 weeks of incubation). No macropore was traced and no bioturbated zone was detected in the control core without earthworm. The volume of the soil core was about 6000 cm³. Values bearing different letters are significantly different at the 5% level.

Species	A. chlorotica		A. nocturna	
Number of earthworms	4	8	2	4
Volume of macroporosity	134.0	232.2	189.0	164.6
(cm ³)				
Burrow length (m)	7.66	15.92	7.06	6.27
Median burrow diameter	3.19 ^b	3.29 ^b	5.01 ^a	5.47 ^a
(mm)				
Vertical deviation (°)	62.4 ^a (+20.1)	59.7 ^{ab} (+20.0)	57.0 ^b (+23.9)	54.0 ^b (+22.4)
(mean + SD)				
Branching rate (m ⁻¹)	2.77 10 ⁻²	2.71 10 ⁻²	1.16 10 ⁻²	1.39 10 ⁻²
Number of burrows	67	102	16	19
Volume of bioturbated	108.0	185.6	146.1	148.1
zones (cm ³)				
Volume of disturbed zone				
whose distance to the	21.8	51.8	21.0	15.3
nearest macropore is > 10				
mm (cm ³)				

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Table 2

Main characteristics of the surface casts depending on the earthworm species and abundance introduced (after 6 weeks of incubation). Values bearing different letters are significantly different at the 5% level

Species	A. chlorotica		A. nocturna		
Number of earthworms	4	8	2	4	
Weight of dry surface cast	29.5	74.2	25.4	38.2	
(g)					
Bulk density of the surface					
<mark>casts</mark> in g cm ⁻³ (mean +	1.24 ^b (+ 0.16)		1.45 ^a (+ 0.11)		
SD) (n=6)					
Estimated volume of the					
surface casts (cm ³)	36.6	92.0	36.8	55.4	

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14 cm

30 cm





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