Biol Fertil Soils (1998) 26:116–121

ORIGINAL PAPER

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Assessment of the burrow system of *Lumbricus terrestris, Aporrectodea giardi,* and *Aporrectodea caliginosa* using X-ray computed tomography

Received: 5 February 1997

Abstract Artificially packed soil columns were inoculated with individuals of different earthworm species (*Lumbricus terrestris*, *Aporrectodea giardi* or *Aporrectodea caliginosa*) and placed under controlled conditions in the laboratory. At the end of the incubation period, which lasted 8 months, three-dimensional reconstructions of the burrow system of each species were obtained using X-ray computed tomography. The particular features of the three burrow systems and the differences between them are discussed in terms of density, orientation and distribution of the burrows and the complexity of the network in relation to variations in soil depth and soil density.

Key words *Lumbricus terrestris* · *Aporrectodea giardi* · *Aporrectodea caliginosa* · Burrow system · X-ray computed tomography

Introduction

Through their influence on the size, form, orientation and connectivity of soil macroporosity, earthworms can strongly affect water, air and solute transport in soil, and probably also affect microorganism development and plant growth (Lee 1985; Edwards et al. 1990; Schrader et al. 1995).

In order to understand the functional importance of earthworm burrows in soil, information about their spatial arrangement is required (Joschko et al. 1993; Ligthart et al. 1993). Most of the techniques generally used to study burrowing activities in the field or in the laboratory are either used to produce two-dimensional (2-D) representations of earthworm burrow nets (e.g. vertical perspex cages (Martin 1982; Schrader 1993)) or only provide information about burrow fragments e.g. using latex, plaster casting, polyurethane casting (Kobel-Lamparski and Lamparski 1987) or thin sections. Finally, when using these techniques it is not always possible to make the accurate and objective measurements which are required in order to characterize soil macroporosity and to understand its role in the soil (Diestel 1993).

X-ray computed tomography provides a new and powerful tool for the non-destructive, accurate, and rapid measurement of soil structural parameters such as density or porosity (Tollner 1991; Porath et al. 1994; Heijs et al. 1995). By providing three-dimensional (3-D) mapping, X-ray computed tomography allows a global representation of the organisation of soil macroporosity owing to the activity of earthworms (Warner et al. 1989; Joschko et al. 1991; Joschko et al. 1993).

Two major groups of burrowing earthworms can be distinguished according to their ecology (Bouché 1977):

1. Anecic species that live in subvertical burrows which are more or less ramified and open at the soil surface.
2. Endogeic species that dig subhorizontal or randomly-oriented burrows.

Because of their respective burrowing behaviour, earthworms of different ecological categories do not influence soil hydraulic properties in the same way (Kretzschmar 1982). Thus there is a need to quantify the impact of each group of earthworm on soil structure.

In this paper we present the first results of a study that aims to understand the burrowing activities of three lumbricid species classed according to the ecological categories determined by Bouché (1972): epianecic, anecic and endogeic. In field studies it is difficult to link each of the species present to a particular burrowing activity. This is

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especially true when the earthworm community consists of several similar-sized species and of individuals of all growth stages (Kretzschmar 1990). Moreover, under field conditions, the influence of interactions between species on individual burrowing activity cannot be controlled. Finally, in the field, it is difficult to assess the age of a burrow system and how it was constructed. Because of all the reasons cited above, for this initial study of the burrow systems of three earthworm species, we decided to undertake an experiment under controlled laboratory conditions.

Materials and methods

Experimental procedure

The experiment was carried out in microcosms made from polyvinyl chloride (P.V.C.) cylinders (20 cm in diameter and 40 cm high). Soil was collected from the deep mineral horizon (between 70 and 80 cm in depth) of a cultivated plot located at the experimental site of Le Rheu (Brittany, France). It was composed of 21.4% clay, 68.2% silt and 10.4% sand, and contained 0.4% organic matter. After air-drying, the soil was sieved to aggregates particles of less than 2 mm, remoistened (20% dry weight 105°C) and introduced into the cylinders. The soil was compacted by packing it in 6 layers; each layer had a bulk density of 1.35 g cm^{-3} (one 3 cm-thick layer at the top of the column and five 5 cm-thick layers below this). The columns were then remoistened by capillary absorption and drained for 24 h to reach a final water suction of approximately 0.1 bar.

The earthworm species studied were *Lumbricus terrestris* L., *Aporrectodea giardi* Ribaucourt, and *Aporrectodea caliginosa* Savigny. *L. terrestris* is considered as an epianecic species (Bouché 1972); it shows anecic behavior when the quantity of litter decreases during the winter, spring or summer and epigeic behavior (surface activity and diet mainly constituted of fresh organic matter) when litter is available in the autumn. *A. giardi* and *A. caliginosa* are respectively considered as anecic and endogeic species (Bouché 1972). They constitute the major part of earthworm communities in Brittany's agroecosystems (Binet 1993). Adult earthworms were collected from the same plot as the soil using the formalin method (Raw 1959) and were subsequently washed several times with water after.

Three treatments were set-up, and each was inoculated with one of the selected earthworm species. Owing to the great difference in the mean weight of individuals of each species, it was not possible to introduce the same weight of worms in all the microcosms. Three replicates per treatment were established (Table 1). A layer of rye (0.011 g g^{-1} introduced worm day^{-1}) containing 2.96% nitrogen and 42.07% carbon was placed on the soil surface. As the organic matter content of the soil was very low, this litter constituted the main source of organic matter. To remoisten the soil and the litter, each microcosm received 100 ml distilled water three times a week. They were kept at 12°C with 12 h light day^{-1} for 246 days (from 16 June 1994 to 16 February 1995).

X-ray computed tomography scanning

At the end of the incubation period, the casts produced this time were collected from the soil surface. All the columns were stored at 4°C until being subjected to a computed tomography (CT) scan at Pontchaillou University Hospital (Rennes).

Each column was placed on its side in the CT unit (Siemens Somatom Plus) set at 120 kV and 165 mA s^{-1}. The X-ray source and an array of detectors rotated through 360° around the soil core. X-rays passed through the column and were measured by the detector located on the opposite side. Each transmission measurement of this type provided a 2-D image of one slice of the core.

Two techniques were used:

1. A sequential technique with a slice thickness of 1 mm (1 replicate per treatment).
2. A spiral technique with a slice thickness of 3 mm (2 replicates per treatment); successive slices overlap by 2 mm.

The second technique was much faster and cheaper than the first.

Each 2-D image corresponded to a 512 pixels matrix where the voxel (volume element) size was $0.41 \times 0.41 \times 1$ mm, or $0.41 \times 0.41 \times 3$ mm^3 according to the scanning procedure.

After conversion of the data, 3-D reconstructions of the burrow systems were made using the 2-D digitalized slices with Voxtool software (Advantage Window, UNIX). In the 3-D images (Figs. 1–4), white areas indicate material with a low attenuation value or a low density, such as water, organic matter or porous regions, while black areas correspond to the soil matrix. Although only one example of the three radiographs obtained for each treatment is presented here, the comments take into account the observations made on all the 3-D images.

Two-dimensional images were produced at 10 mm intervals along the lenght of the soil column. In these images (Fig. 5), white areas correspond to the soil matrix and black areas to the burrows.

After the scanning procedure, all the columns were sectioned vertically into two equal parts. Earthworms, cocoons and casts were collected from one half of each column. One half column per treatment was impregnated with polyester resin and cut into 2-cm-thick sections.

Results

At the end of the experiment, all (in the case of epianecic and anecic species) or almost all (in the case of endogeic species) the litter had disappeared from the soil surface and, in most cases, a positive weight gain of the earthworms was observed. Moreover, numerous cocoons or young individuals were collected in all the treatments. The burrowing activity of the three species could be studied in terms of burrow density, connectivity (i.e. number of branching nodes in the burrow system), orientation and distribution in relation to variations in soil depth and soil density.

Table 1 Characteristics of the three different treatments

Treatments			Earthworms			Litter Total weight[a] (g)
	Ecological types	Species	Number	Weight		
				(g)	(g m^{-2})	
1	Epianecic	*Lumbricus terrestris*	2	7.1	226	19.36
2	Anecic	*Aporrectodea giardi*	2	8.5	270	23.24
3	Endogeic	*Aporrectodea caliginosa*	6	2.9	92	7.97

[a] Total weight of litter distributed over the incubation period of 246 days. Each microcosm received 0.011 g litter g^{-1} introduced worm day^{-1}

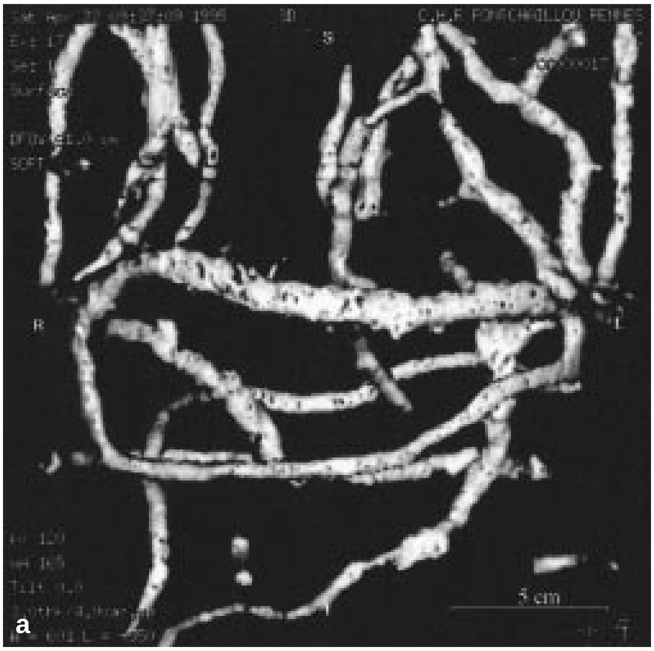

Fig. 1 a Side view of the 3-D reconstruction of the burrow system of *lumbricus terrestris* produced by using the spiral technique

Fig. 2 Side view of the 3-D reconstruction of the burrow system of *Lumbricus terrestris* produced by using the sequential technique

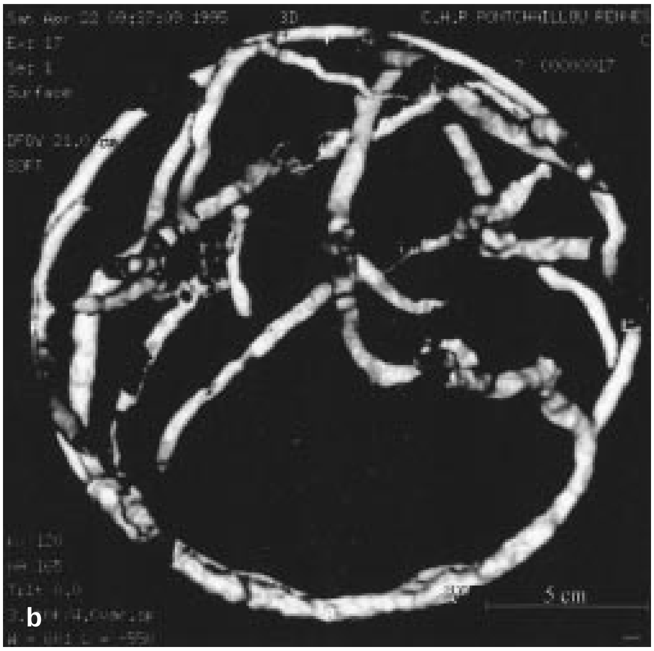

Fig. 1 b The 3-D reconstruction of the burrow system of *Lumbricus terrestris* produced by using the spiral technique viewed from above

The burrow system of *L. terrestris*

The burrows of *L. terrestris* (Figs. 1a, b, 2) reached, at most, 18 cm in depth, but the burrowing activity of this species was maximal between 0 and 13 cm depth. The number of burrows per column was generally limited and sometimes as low as one to three (Fig. 2). No more than five or six branching nodes were observed for the whole burrow system. In the first 8 cm of the soil column, the burrows were orientated vertically, but further down they were mainly horizontal. The diameter of the burrows was assessed to vary between 0.5 and 1 cm.

Owing to the compaction procedure (by packing the cores with layers of soil of 3 and 5 cm), the upper part of each layer was slightly more dense than the rest of the soil, and the boundaries between two successive layers were marked by a thin crack. *L. terrestris* tended to follow this thin crack, so that the burrows were horizontal in these areas. This was especially observed at depths of 8 and 13 cm (Fig. 1a). The 3-D images clearly showed that the worms often had difficulty in passing through the upper, most dense part of the soil layers, so that the number and density of burrows tended to decrease and their orientation became more horizontal with soil depth. These changes in earthworm burrowing activity included a tendency to burrow along the vertical P.V.C. wall.

The burrow system of *A. giardi*

In common with the observations of Saussey (1981), the diapause of *A. giardi* lasted from middle of June (the beginning of the experiment) to the middle of August. The duration of the diapause was determined according to fluctuations in cast production and food consumption. In spite of this period of inactivity, the burrow system of this species (Figs. 3a, b) was much more developed than that of *L. terrestris*. The network often reached the bottom of the column and maximal activity was observed between 4 and 23 cm. The number of reticulations exceeded 15 per 5 cm-thick soil layer. The diameters of the burrows were smaller

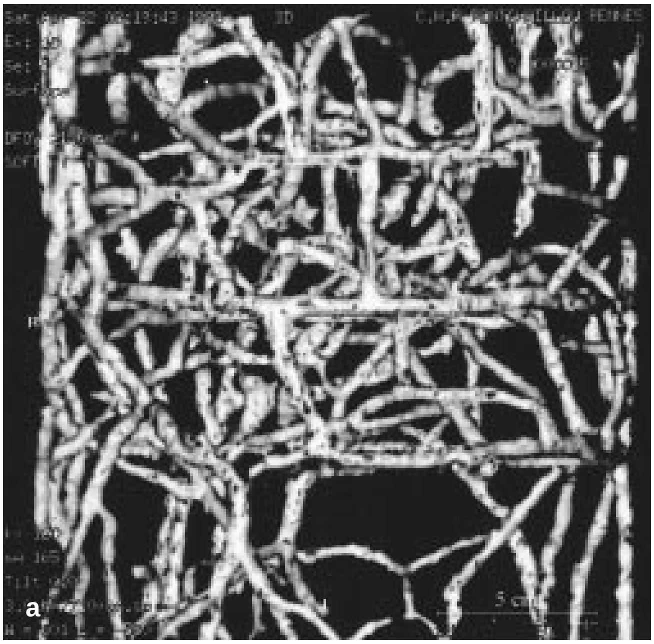

Fig. 3 a Side view of the 3-D reconstruction of the burrow system of *Aporrectodea giardi* produced by using the spiral technique (only part of the system is shown)

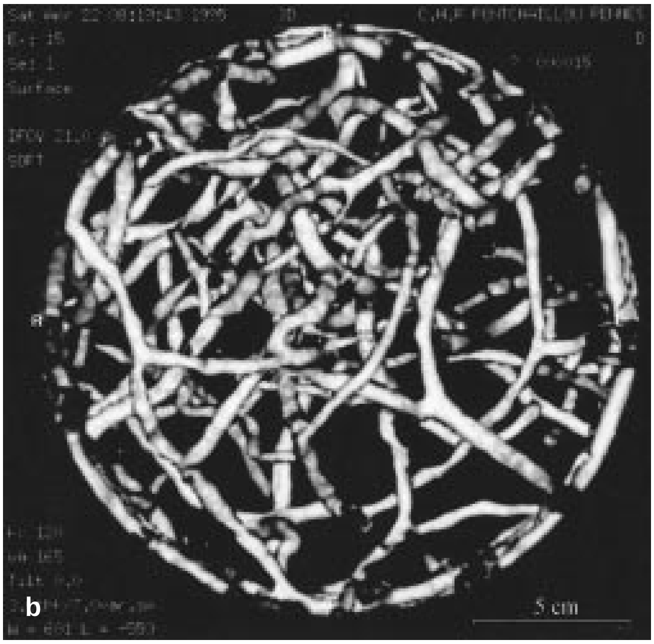

Fig. 3 b The 3-D reconstruction of the burrow system of *Aporrectodea giardi* produced by using the spiral technique viewed from above (only part of the system is shown)

(between 0.35 and 0.5 mm) than those of the burrows of *L. terrestris*. No dominant burrow orientation could be distinguished from the images. In columns containing *A. giardi*, the effect of the compacted soil was also clearly reflected in the pattern of the burrow system (Fig. 3a); it locally influenced burrow orientation but did not prevent the extension of the network into the lower soil layers.

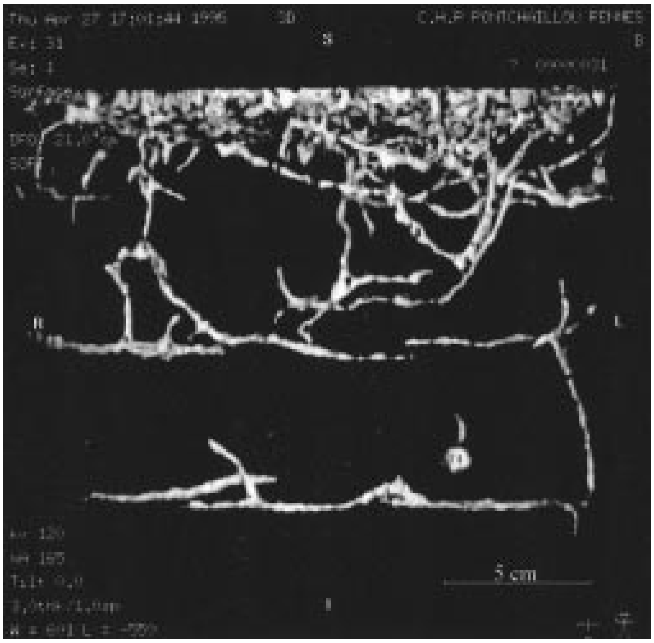

Fig. 4 Side view of the 3-D reconstruction of the burrow system of *Aporrectodea caliginosa* (produced by using the spiral technique)

The burrow system of *A. caliginosa*

Although the total depth of the burrow system of *A. caliginosa* (Fig. 4) was 13 cm, the maximal burrowing activity was in the top 7 cm–8 cm. In this zone, the network was well-developed, with at least 15 ramifications in the first 8 cm. The number of branching nodes of this zone exceeded those of any other in the core. As in the case of *L. terrestris*, the burrows of *A. caliginosa* tended to follow the border between successive layers. These zones also corresponded to a decrease in the number of burrows, a tendency for the earthworms to burrow along the P.V.C. wall, and a change in burrow orientation. In the upper part of the column, the burrows did not seem to show any one specific orientation, but they tended to be horizontal below 7–8 cm (Fig. 4).

White zones were observed around some burrows (Figs. 5a, b). They could be attributed to: i) scanning procedure artefacts corresponding to the trace of the same pore in the next slice or, ii) compacted areas resulting from the method used by earthworms to construct their burrows. These two possibilities will be analysed in the discussion.

Discussion

The positive weight gain of earthworms during the experiment, their very low mortality and evidence of reproductive activity indicated that the microcosm environment did not have a negative impact on the earthworms' biology.

The observations made on the 3-D images showed that the burrow system of the three species differed in terms of density, maximal depth, and degree of reticulations. The pattern of the burrow system of *L. terrestris* (few burrows and

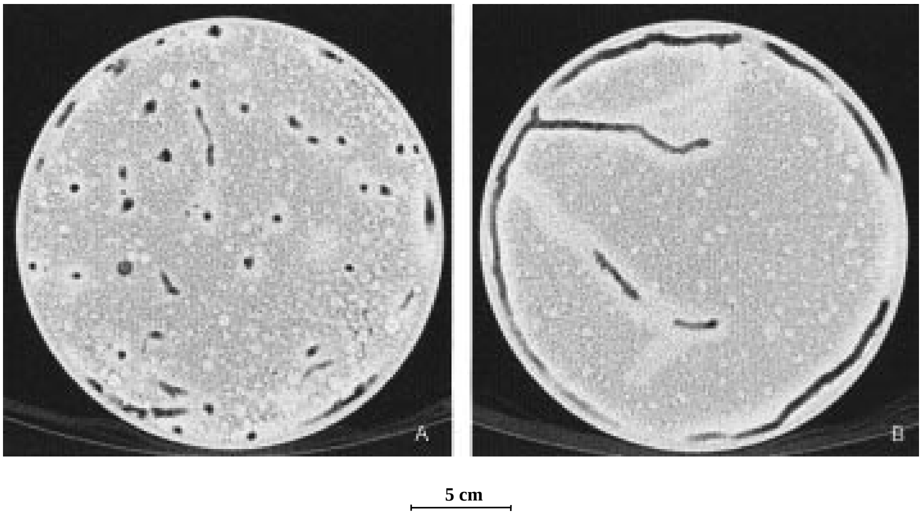

Fig. 5 Burrow system of *Aporrectodea caliginosa*: 2-D images of horizontal sections made at depths of 6 cm (A) and 14 cm (B)

reticulations, and of shallow depth) was in accord with the findings of previous studies which showed that this species lives in continuous permanent burrows (Lee and Foster 1991; Schrader et al. 1995). In contrast, in spite of an inactivity period of 2 months, the burrow system of the other anecic species, *A. giardi,* was well-developed in terms of the density of burrows, degree of reticulations and extension in relation to soil depth. This network does not correspond to the permanent burrow system usually described for anecic species (Lee and Foster 1991). The volume, number of reticulations and extension of the burrow system of *A. giardi* were much greater than those of the other two species, and are therefore supposed to have a more significant influence on soil properties and soil function. Such differences between the burrow systems of the two anecic species are difficult to interpret. They could have been induced by experimental conditions such as food quantity or quality, temperature and humidity.

The burrow system of *A. caliginosa* was rather different. Maximal activity was in the top 7–8 cm, where the number of ramifications reached at least 15 and was higher than the rest of the burrow network. This result is in agreement with the observations of McKenzie and Dexter (1993) who, studying the same species in the field, found that the most complex region of the network was in the top layer of the soil, especially between a depth of 2.5 and 5 cm.

The 3-D images also allowed observation of the effects of the proximity of the P.V.C. wall and of soil density variations (induced by the compaction procedure) on earthworm burrowing behaviour. The variation in soil density could have especially affected the density, distribution and orientation of the burrows of all three species.

The effects of the different compacted layers were easily observed owing to their influence on the pattern of the burrow system of *A. giardi,* but they did not prevent the extension of the network into the lower soil layers. The burrowing of this species seemed to be almost unaffected by soil density variations or the proximity of the P.V.C. wall. In contrast, *L. terrestris* and *A. caliginosa* seemed to be sensitive to high soil density. As the upper part of each layer was slightly more dense than the rest of the soil, these worms tended to make use of the thin crack between two successive layers or along the P.V.C. wall in order to burrow and, in several cases, they were not able to pass through the more compacted area. Similar observations were also made by Kretzschmar (1990) for *Aporrectodea longa,* and our results agreed with those of previous studies (Joschko et al. 1993; Schrader et al. 1995) which showed that the burrow patterns of the endogeic species *Octolasium cyaneum* and *A. caliginosa* are affected by high soil bulk density. However, Schrader et al. (1995) found that the burrow pattern of *L. terrestris* was not affected by high soil density (1.5 g cm^{-3}). The discrepancy between this latter result and ours shows that soil bulk density is not the only parameter influencing the burrow pattern. We can hypothesize that soil texture, and especially silt content which was quite high in the present study (68%), affect the ability of worms to burrow through soil. The combination of these two parameters – soil density and texture – could have determined the extension of the network.

In 2-D images, white zones were observed around some burrows of *A. caliginosa*. They could be attributed to scanning procedure artefacts corresponding to the trace of the same pore in the next slice, or could be considered, as suggested by Lee and Foster (1991), as compacted areas, indicating that the burrows were formed by pushing the soil aside. Although this last explanation seems more plausible and was also proposed by Schrader et al. (1995), especially for low density soils (0.9 g cm^{-3} to 1.2 g cm^{-3}), it needs to be confirmed by: (1) comparison of total cast production and total pore volume, and (2) observations and measurements on the resin-impregnated thick sections. Observations made from more 2-D images are needed to assess whether this feature is peculiar to *A. caliginosa,* or if it appears whatever the species and the depth.

Observations made from thick sections will also allow assessment of whether visual errors (pointed out by Joschko et al. (1991)) were made during the scanning procedure and enable the identification of differences that might be attributable to the two scanning procedures (sequential and spiral).

In conclusion, the use of X-ray computed tomography techniques permitted a global view of the burrow systems (density, maximal depth, network complexity, orientation) of the three species studied. Significant differences were observed between the species, and especially between the two anecic species. *L. terrestris* formed permanent, continuous burrows which showed few ramifications and were mainly located at the top of the soil. In contrast, the formation of burrows by *A. giardi* appeared to be independent of the proximity of the P.V.C. wall and variations in soil density. This species made a dense and complex burrow system extending throughout the whole column. In contrast to *A. giardi*, the burrow systems of *L. terrestris* and *A.caliginosa* seemed to be influenced by variations in soil density.

Despite space limitations leading to “wall effects”, the observations made from microcosms provide useful and complementary information for the study and elucidation of more complex situations in the field. The first results of the study recorded here will be complemented by quantification of the parameters which characterise the burrow system (e.g. volume and diameter in relation to depth, orientation etc.), which is necessary in order to understand the role of macroporosity in the soil.

Acknowledgements This work was supported by the Groupe d’Etudes Pluridisciplinaires Agrobiologie Bretagne (G.E.P.A.B.). The scanner operators of the CHU of Rennes are gratefully acknowledged for giving us the opportunity to scan the soil columns, and for technical assistance. We would like to thank Prof. H. Diestel from the Institut für Landschaftsentwicklung, Technische Universität Berlin, for his advice on the use of X-ray computed tomography and for comments about this manuscript. We gratefully acknowledge Dr. M. Joschko for helpful discussions concerning these results and for valuable comments on this paper. We thank Mr Michel Lefeuvre for establishing the experimental system.

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