Burrow systems made by *Aporrectodea nocturna* and *Allolobophora chlorotica* in artificial cores: morphological differences and effects of interspecific interactions

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Abstract

Burrow systems of earthworms contained in artificial cores were analysed through X-ray computed tomography and 3D skeleton reconstructions. Gas diffusion experiments were carried out on these cores to characterize soil transfer properties associated with the different burrow systems. Three types of cores were studied: cores in which *Aporrectodea nocturna*, an anecic earthworm was introduced (treatment 1), cores in which *Allolobophora chlorotica*, an endogeic earthworm was introduced (treatment 2) and cores that contained both species (treatment 3). Comparisons of the characteristics of the burrow systems of treatment 1 and 2 show important differences: the burrow system of *A. nocturna* comprises fewer burrows, which are longer, less branched, more vertical and have a lower sinuosity. The burrow system of *A. chlorotica* is characterized by lower continuity, which however did not result in a lower soil diffusivity. To study the burrow systems made by the two species in the same core (treatment 3), a separation that was based on differences in pore diameter between the two species and that takes into account the burrow orientation was designed. This separation was proven to be efficient since it resulted in low percentages of errors (around 10%) when applied to the burrow systems of treatments 1 and 2. Comparison of the burrow systems from treatments 1 and 3 demonstrated that the burrow system of *A. nocturna* was influenced by the presence of *A. chlorotica*: in treatment 3, *A. nocturna* made burrows that were smaller, more vertical and less branched. However, these interactions have to be confirmed under natural conditions. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: X-ray tomography; Interspecific interactions; Earthworm burrow system; 3D reconstruction; Burrow typology; Gas diffusion

1. Introduction

Earthworms, as *ecosystem engineers*, play an important role in many soil ecosystems (Lavelle et al., 1997). This role is achieved inside or in the vicinity of burrows. Moreover, water, gas and solute transfer processes can be enhanced by the presence of earthworm burrows (Kretzschmar and Monestiez, 1992; McCoy et al., 1994). Therefore, observation of burrow systems is important to understand both (i) the actual role of earthworms in the soil ecosystem and (ii) the influence of these burrow systems on soil transfer properties.

The use of X-ray tomography in soil biology enables rapid and accurate observation of earthworm burrow systems in artificial (Joschko et al., 1991, 1993; Jégou
et al., 1998; Langmaack et al., 1999) and in natural conditions (Capowiez et al., 1998; Capowiez et al., in press). However, analysis of natural earthworm burrow systems is difficult since the actual composition (species, density and age structure) of the earthworm community that built the burrow system is ignored.

In a previous study using 2D terraria, I analysed the lengths burrowed and the surface explored by several earthworms of the species *Aporrectodea nocturna* (anecic) and *Allolobophora chlorotica* (endogeic). It was concluded that interspecific and intraspecific interactions can occur between these two earthworm species (Capowiez, 2000). These interactions modify the shape of the burrow system and therefore can have an influence on the physical and ecological processes that take place in the ‘drilosphere’ (Lavelle et al., 1997). However, due to the severe spatial limitations imposed on the earthworms by the 2D terrarium, these interactions had to be confirmed under more natural conditions. An experiment was, therefore, carried out in artificial 3D cores and the burrow systems were reconstructed and analysed at the end of the incubation period using computer-assisted tomography. In this experiment therefore, interactions that affect the length or shape of the burrow system could be studied, but not trophic or other possible interactions.

The two aims of this study were (i) to describe and analyse the earthworm burrow systems made by the two earthworm species *A. chlorotica* and *A. nocturna* in artificial cores and (ii) to determine if there are interspecific interactions between these earthworm species.

### 2. Material and methods

#### 2.1. Preparation of the artificial cores

The soil used for the experiment was obtained from the 20 cm topsoil (30.2% clay, 48.7% silt and 21.1% sand; 5.1% organic matter) of our study site, an abandoned peach orchard in Montfavet, near Avignon (SE of France). In this site, the earthworm community is dominated by three earthworm species: *Aporrectodea nocturna, Allolobophora chlorotica* and *Lumbricus terrestris*. For this experiment, the first two species were chosen since they belong to different ecological types: *A. nocturna* is a truly anecic species whereas *A. chlorotica* (leucotypic form) is an endogeic species (Bouché, 1972). Mature earthworms were collected and placed on damp filter paper for a few hours until needed.

Artificial cores were prepared as follows. PVC cylinders (length = 35 cm and diameter = 16 cm) were lined inside with sealing varnish and sharp fine sand to prevent earthworms crawling along the walls instead of burrowing in the soil. A hydraulic press was used to compact five cores simultaneously. Based on the experiment of Kretzschmar (1991), it was decided to apply a pressure of 270 kPa to soil of 23% moisture content. This author showed that an important animal activity (assessed by cast production) was reliably observed with these parameter values. To prevent great variations in compaction between the two extremities of the cores, the soil was added in 12 layers to each core. Each layer consisted of 600 g of soil and was pressed for 10 min (this was shown to be sufficient time to reach equilibrium). The final width of the layers was about 2.5 cm. The surface of the previous layer was gently scratched using a small rake to increase cohesion between layers before adding a new layer.

A total of 10 cores was set up. The treatments were as follows: (i) four individuals of *A. nocturna* were introduced to three cores (treatment 1 or T1), (ii) eight individuals of *A. chlorotica* were introduced to three other cores (treatment 2 or T2) and (iii) two individuals of *A. nocturna* and four individuals of *A. chlorotica* were introduced to the remaining four cores (treatment 3 or T3). The number of individuals of each species introduced into the cores reflected the differences in the mean weight of the species (2.01 ± 0.1 g for *A. nocturna* and 0.42 ± 0.04 g for *A. chlorotica*) and the relative densities observed in the study site (the density of *A. chlorotica* was 1.3 times that of *A. nocturna*). These 10 cores were placed in a dark room (at 12°C) for 2 months. Food (1 g of dried ground litter collected at our study site) and water (5 ml) were supplied once a week.

#### 2.2. Burrow systems analysis

After about 60 days, cores were analysed using X-ray tomography (Prospeed SX Advantage, General Electric) at Bagnols sur Cèze hospital to obtain a
set of images 2 mm thick every 3 mm with a resolution of 0.4 mm per pixel. The settings for the X-ray beam were 160 mA, 140 kV and a duration of 1 s. For technical and economical reasons, only 8 out of 10 cores were processed: two for T1 (cores 001 and 003), three for T2 (cores 004 to 006) and T3 (cores 007 to 009). Three-dimensional skeleton reconstructions were obtained by the method presented in detail in Capowiez et al. (1998). In this case, however, pores touching the edge of the core were not removed. The general principle is to vertically project all pores of an image on the next successive image to determine pore continuity between two successive images. The reconstructions are based on ‘segments’ that are the basic connection between two pores on two successive images. A burrow is a set of connected segments (the burrow may be branched or not) and the burrow system is the set of all burrows in a core. Skeleton reconstruction allows computation of several characteristics of the burrows (mean burrow length, branching intensity and segment mean angle relative to the vertical axis) and of the burrow systems (burrow system length, number of burrows). On these burrow systems, a new characteristic was computed: the sinuosity of the burrow system. This parameter is the mean angular deviation between consecutive segments (Fig. 1). By convention, this value is computed considering that each segment is oriented ‘top–down’ (which is the more probable way of creation). This value indicates the way by which an animal explores its environment (Bovet and Benhamou, 1988).

2.3. Separation of the burrows in T3

Although the two species studied have different size and diameter, pore areas in the T1 and T2 cores were not clearly separated (results not shown). Indeed, the macropore area in a horizontal image depends both on the diameter of the earthworm and on the orientation of the macropore: the less vertical the macropore, the larger its area in the image (Jégou et al., 1999). Therefore, we plotted the distribution of the burrow segments in a ‘space’ defined by the two following variables: (i) segment orientation and (ii) the minimum of the two areas of the pores at the extremities of each segment (bearing in mind that a segment links two connected pores between two successive images). The minimum of the two pore areas was used instead of the mean because it gave a better separation (see below how the quality of the separation was assessed). The last step in this separation was to find in this space artificial boundaries that give the best separation between the two sets of segments (S1 and S2, one where segments probably belong to the smaller earthworm species, i.e. *A. chlorotica* and the other where segments probably belong to the larger species, i.e. *A. nocturna*). To assess the quality of a separation, we applied it to the T1 and T2 cores. The percentages of ‘misclassified’ segments provided an estimation of the efficiency of the separation. Boundaries for the separation are then assessed through an optimization procedure that minimizes the sum of the errors for the separation of all the cores from T1 and T2. This optimization procedure was programmed using Splus software (Mathsoft). At the end of this procedure, two corrections ‘by continuity’ were applied to the two sets of segments:

1. if the four segments (two on each side) around a segment are of the same type, then the central segment is allocated to the same type,
2. if 80% of the segments of one burrow are of one type then all the segments of this burrow are allocated to this type.

2.4. Study of intra-specific interaction

This study is based on the two sets of segments separated as described above. Two kinds of analysis were used to address the following questions: (i) are the shape and the length of the burrows of each species modified by the presence of the other species? and (ii) are the two sets of segments distributed independently of each other in the cores?

The first question was studied by analysing whether the characteristics of the two sets of burrows are the same in T2 or T1 as in T3 (some of these characteristics have to be divided by 2 before the comparisons since the number of earthworms is twice greater in T1 and T2 than in T3 for each species).

The second question was studied by analysing the K-functions (Ripley, 1977) between the two sets of segments: we computed the mean number of segments of the other type at an increasing distance from the segment of the studied type. The distribution of the mean number of segments of the other type around segments of the studied type was then compared to simulated burrow systems where no spatial interaction occurs between the two sets of segments (principle of the Monte Carlo tests (Crowley, 1992)). These simulations were achieved by iteratively adding randomly chosen burrows from one core of T1 and one core of T2. The iterative process was stopped when the total burrow length reached the density of the studied T3 core. The simulation was accepted only if the relative proportions of burrow length of each species were close to those observed in the T3 cores (±10%). For each T3 core, six cores were simulated (two cores T1 x three cores T2). To increase the number of simulations, 90°, 180° and 270° rotations were applied to one set of segments in all the simulated cores. Therefore, 24 simulated cores were available for each T3 core enabling us to make comparisons at a level of significance of about 4% (i.e. 1/24). Comparisons between the observed distribution of the K-functions and simulated ones (that represent a distribution without spatial interaction between the two sets of segments) were made graphically by observing the position of the K-function for the observed core relative to the hull, made by the minima and maxima of the K-functions for the simulated cores.

2.5. Diffusion measurements

To characterize the soil transfer properties related to the burrow systems, gas diffusion experiments were carried out. The procedure used was described by Kretzschmar and Monestiez (1992): butane was injected at the top of the cores at the beginning of the experiment and gas samples were taken regularly at the bottom of the cores. Butane concentrations in these samples were estimated using a gas chromatograph (Chrompack). Core diffusion coefficients were computed by fitting the changes in butane concentration over time using a ‘finite-element’ model and a least-square estimation method (Marquardt, 1963). Gas diffusion was measured twice for each core.

The influence of burrows on soil transfer properties is thought to be linked to continuity of these burrows (Bouma, 1991; McCoy et al., 1994). In order to relate gas diffusion with this characteristic of burrow systems, continuity of the burrows systems was estimated by the procedure already used in Capowiez (2000). A varying number of virtual horizontal planes (each separated by the same distance) were defined in the cores. Then, the number of different and continuous pathways made by the observed burrows between two successive planes was counted. Finally the total number of pathways for each core as a function of the number of virtual planes was computed.

2.6. Statistical analysis

When possible (sufficient number of data and normality of the data), type I-ANOVA with multiple comparisons (Newman and Keuls procedure (Zar, 1984)) was used to compare the treatments. When this was not possible, only qualitative results are presented. The results of each diffusion experiment were compared using a Kruskal Wallis rank test.

3. Results

3.1. Differences between the burrow systems

The 3D skeleton reconstructions are presented in Fig. 2. The burrow systems built by the two earthworm
species are very different. The *A. nocturna* burrows appear to be more continuous and less branched than those of *A. chlorotica* while *A. nocturna* burrows generally have a greater diameter (majority of dark segments) than those of *A. chlorotica* (equal number of dark and light segments). However, even in the burrow systems of *A. chlorotica*, segments with high diameter can be found, therefore, a separation based only on the pore diameter would have been inefficient.

In two cores (004 and 008), layers without burrows are observed in the bottom part of the core. In these cases, observation of the images corresponding to these layers reveals that the pores are very often filled with casts (X-ray tomography allows us to distinguish between casts and soil matrix based on differences in density and therefore in greylevels, (Capowiez, 1999)). The absence of burrows in these layers is, therefore, due to: (i) totally backfilled pores, (ii) partially backfilled pores whose areas therefore become smaller than the threshold used for pore selection (Capowiez et al., 1998) and (iii) the removal of burrows that are less than three segments long (Capowiez et al., 1998).

The characteristics of burrows and burrow systems are presented in Table 1. No statistical difference in total burrow lengths was observed between treatments but characteristics related to the shape and the continuity of the burrow systems strongly differ between treatment 1 and 2. Compared to that of *A. chlorotica*, the burrow system of *A. nocturna* has fewer burrows which are longer, less branched, more vertical and had lower sinuosity. The burrow systems in the
Table 1

Characteristics of burrow systems for the eight studied cores

<table>
<thead>
<tr>
<th></th>
<th>A. nocturna</th>
<th>A. chlorotica</th>
<th>A. nocturna + A. chlorotica</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>001 003</td>
<td>004 005 006</td>
<td>007 008 009</td>
</tr>
<tr>
<td>Total length (m)</td>
<td>8.22 5.43</td>
<td>5.45 11.42 5.87</td>
<td>7.91 9.73 8.76</td>
</tr>
<tr>
<td>Number of burrows</td>
<td>59 46</td>
<td>139 176 140</td>
<td>108 94 103</td>
</tr>
<tr>
<td>Mean burrow length (mm)</td>
<td>139 118</td>
<td>39 65 42</td>
<td>73 104 85</td>
</tr>
<tr>
<td>Rate of burrow branching (m$^{-1}$)</td>
<td>19.34 19.33</td>
<td>55.26 48.26 57.38</td>
<td>38.32 41.71 37.01</td>
</tr>
<tr>
<td>Segment mean angle (%)</td>
<td>33.63 a 34.70 a</td>
<td>39.54 b 42.88 c 39.67 b</td>
<td>37.94 b 44.64 c 37.78 b</td>
</tr>
<tr>
<td>Sinuosity (%)</td>
<td>18.59 a 17.43 a</td>
<td>33.38 d 29.19 c 33.05 d</td>
<td>24.41 b 33.44 d 25.67 b</td>
</tr>
</tbody>
</table>

*Means followed by the same letter are not statistically different at the 95% significance level.

3.2. Separation of the segments in the T3 cores

The distributions of the segments in the space defined by the segment orientation and the minimum pore area are rather different for the two species (Fig. 3). However, nearly horizontal segments cannot be attributed to one species unless their corresponding pore area is small. It was then decided to draw boundaries that cut the reference space into three parts: part 1 contains the segments from the smaller species (A. chlorotica), part 2 contains the segments from the larger species (A. nocturna) and part 3 contains sub-horizontal segments and is called the ‘neutral zone’.

The boundaries were chosen to give the minimal error percentages assessed by applying the separation criteria described in Section 2.3 to the T1 and T2 cores and then applying two corrections by continuity. The efficiency of separation was estimated at between 97 and 87% depending on the core (Table 2). The percentages of segments that are in the ‘neutral zone’ was very low (always less than 5%) and these are ignored in the following.

Table 2

Efficiency of the separation assessed by the percentages of error (misclassified segments) made on the cores from treatment 1 and treatment 2

<table>
<thead>
<tr>
<th></th>
<th>A. nocturna</th>
<th>A. chlorotica</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>001 003</td>
<td>004 005 006</td>
</tr>
<tr>
<td>After the separation</td>
<td>11.7 18.6 14.8 15 12</td>
<td></td>
</tr>
<tr>
<td>Continuity correction 1</td>
<td>8.36 15.2 16.3 13.3 9.72</td>
<td></td>
</tr>
<tr>
<td>Continuity correction 2</td>
<td>2.8 9.12 13.3 10.2 8.68</td>
<td></td>
</tr>
</tbody>
</table>

*The effect of the two corrections by continuity is also presented.

3.3. Study of the interspecific interactions in T3

The separation procedure distinguished between three sets of segments in the T3 cores and enabled us to study the modifications in the length or in the shape of the burrow systems made by each species when the other species was present. To ensure that the separation into three zones had no effect on the comparisons, we applied it to the burrow systems of T1 and T2 cores as well. Moreover, the values for the total length and number of burrow were divided by 2 in the T1 and T2 cores since there were twice as many earthworms of each species in these cores than in T3 cores. Comparisons between T1 and T3 for A. nocturna show that this species made less burrows and that these burrows were more vertical, smaller and less branched (Table 3). Comparisons between T2 and T3 for A. chlorotica show that this species seems to make longer burrows (Table 3).

No meaningful difference was found in the spatial distribution of the two sets of segments between the observed and the simulated cores. Segments of the burrow systems made by A. chlorotica are randomly distributed around segments of A. nocturna. In contrast, segments of the burrow systems made by A. nocturna are not randomly distributed around A. chlorotica segments. But in one core, we find less segments than expected under the assumption of no spatial interaction and in the two remaining cores we find more segments (Fig. 4). There is, therefore, no evidence of a clear spatial interaction between the two species.
3.4. Diffusion measurements

Soil diffusion coefficients were of the same magnitude in all the cores. In the first set of measurements, we observed that these coefficients were significantly larger for the cores that contained both species of earthworms ($P = 0.03$; Table 4). Unfortunately this trend was not found in the second repetition ($P = 0.15$; Table 4). The continuity was lower in the cores that contained A. chlorotica (T2) whatever the number of virtual planes (Fig. 5). No difference was observed between the T1 and the T3 cores.

4. Discussion

4.1. Differences between the burrow systems

Horizontal burrows were absent from our artificial cores in contrast to similar experiments using the same method (Jégou et al., 1998). This indicates that our protocol based on relatively small soil layers and surface scratching is satisfactory and prevents earthworms from burrowing horizontally between the artificial soil layers. However, this method still suffers from spatial limitations. This is evidenced by the existence of a border effect (we found a greater number of burrows near the walls of the cores) which was greater for A. nocturna (T1) than for A. chlorotica (T2) (results not shown).

The burrow systems of the two species studied are clearly different and these differences are in agreement with the postulated characteristics of burrow systems from endogeic and anecic species (Lee and Foster, 1991). The burrows made by the anecic species were more vertical, longer, less branched and less sinuous than those made by the endogeic species. These characteristics have consequences for burrow system continuity with A. nocturna burrows being more continuous than those of A. chlorotica. In spite of these differences, no difference was found in gas relative diffusivity coefficients for the burrow systems made by the two species.

In a previous study using 2D terraria (Capowiez, 2000) very few differences were found in the shape of the burrow systems of these two species but this was probably due to the high spatial limitations en-
Table 3
Comparisons of the characteristics of the burrow systems of each species after the separation (only the characteristics for which a difference was observed are presented)

<table>
<thead>
<tr>
<th></th>
<th>A. nocturna</th>
<th>A. chlorotica</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1 (n = 2)</td>
<td>T2 (n = 3)</td>
</tr>
<tr>
<td>Total length (m per earthworm)</td>
<td>1.98  1.21  1.13  0.61  1.50</td>
<td>0.57  1.21  0.63  1.26  1.66  1.21</td>
</tr>
<tr>
<td>Number of burrows (per earthworm)</td>
<td>12.2  8   6.5  3  8</td>
<td>13.2  17.2  15  16.7  18.5  17.5</td>
</tr>
<tr>
<td>Segment mean angular deviation (°)</td>
<td>33.5  35.0  31.5  33.1  29.5</td>
<td>39.3  41.8  38.5  39.8  43.2  42.2</td>
</tr>
<tr>
<td>Mean burrow length (mm)</td>
<td>387  278  160  124  173</td>
<td>73  133  70  111  98  105</td>
</tr>
<tr>
<td>Rate of burrow branching (m⁻¹)</td>
<td>11.3  11.9  28.3  26.1  33.4</td>
<td>31.8  27.0  35.1  19.4  30.5  13.7</td>
</tr>
</tbody>
</table>

Fig. 4. Mean number of segments made by one species at an increasing distance from the segments made by the other species (K-functions): solid line is the distribution for the observed core and dotted lines indicates the hull of the distributions for the 24 simulated cores. See text for further explanations.

Table 4

<table>
<thead>
<tr>
<th></th>
<th>A. nocturna</th>
<th>A. chlorotica</th>
<th>Both species</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.001</td>
<td>0.002</td>
<td>0.003</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>0.848</td>
<td>0.671</td>
<td>0.826</td>
<td>0.856</td>
</tr>
<tr>
<td></td>
<td>0.884</td>
<td>0.665</td>
<td>0.838</td>
<td>0.904</td>
</tr>
</tbody>
</table>

The $P$-value of the Kruskall Wallis test is given for the two repetitions.

Table 4

Diffusion coefficients ($10^{-5} \text{m}^2 \text{s}^{-1}$) for the 10 studied cores

- countered by the earthworms in these very artificial conditions. In the 2D terraria the behaviour of these two species was very different: the anecic species often reused their burrows and the endogeic species rarely did so. Three-dimensional reconstructions and 2D terraria, therefore, give us complementary results. While other authors have already noted these profound differences in the shape of the burrow systems between the two ecological types (Evans, 1947; Jégou et al., 1998), these previous studies were not able to quantify the observed structures. We believe that 3D skeleton reconstructions is a tool that allows us to move beyond the descriptive stage of earthworm ecology (Curry, 1998). Other methods using X-ray tomography are available, such as the volume reconstructions developed by Langmaack et al. (1999). This method presents interesting characterisations (such as burrow volumes) but computations of burrow lengths are not straightforward.

It is possible to compare 3D reconstructions from these artificial cores with 3D reconstructions from natural cores obtained from a pre-alpine meadow where *A. nocturna* was the dominant species (Capowiez et al., 1998; Capowiez et al., in press). These comparisons show that burrows in artificial cores are much longer and more branched. Besides, the total burrow length is greater in artificial cores: it ranges from 2.8 to 5 m in natural cores whereas it ranges from 3.6 to 7.6 m in artificial cores (in this case only the first 20 cm are considered). As it can be assumed that the biological erosion of burrows (through backfilling with casts) is the same in the two cases, these differences illustrate the importance of physical erosion of burrows (through trampling for example) under natural conditions.  

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**Fig. 5.** Continuity of the eight burrow systems assessed by counting the number of different pathways that linked artificial equidistant horizontal planes.
conditions. Moreover, in artificial cores the burrows are recent (the experiment lasted only 2 months) and therefore they are well preserved even in the case of endogeic burrows that are generally thought to be more ‘labile’ in natural conditions since they are rarely reused (Capowiez, 2000).

4.2. Interspecific interaction in T3

To assign segments in the burrow systems from cores of T3 to each species, a criterium based on differences between earthworm diameters is used, but this criterium alone is not sufficient to ensure efficient separation. Knowledge of the 3D structure of the burrow system enables us (i) to modulate this criterium by the orientation of segments and (ii) to propose corrections based on continuity properties of burrows. Finally this separation is characterized by a mean error rate of only 10%. This is the first time, to our knowledge, that such a clear separation is proposed and obtained. It represents an interesting advance that could help us in studying complex natural burrow systems. Moreover, this separation allows the study of modifications of the burrow system of each species due to the presence of the other species. In our study, the structure of the burrow systems elaborated by *A. nocturna* is greatly influenced by the presence of *A. chlorotica*: *A. nocturna* made less burrows which were more vertical, smaller and less branched. Conversely *A. chlorotica* exhibits no significant modification in the structure of its burrow system. These results contrast greatly with our previous study using 2D terraria where we observed that the behaviour of *A. chlorotica* was negatively influenced by the presence of *A. nocturna* (these earthworms made shorter burrows and explored a smaller surface) and that the presence of *A. chlorotica* had no effect on the behaviour of *A. nocturna*. Interactions between earthworm species have not been extensively studied (Curry, 1998), and the main focus was often the effects of such interactions on growth or reproductive output (Abbott, 1980; Elvira et al., 1996; Butt, 1998). To our knowledge, the work of Elton and Koppi (1994) with *Microscolex dubius* and *Aporrectodea trapezoides* is the only previous study that takes the burrowing behaviour of the earthworm species into account. These authors showed that burrow length was always greater when only one species was present. The major difficulty in this kind of experiment focusing on interactions between species, assessed by observation of modifications in the structure of the burrow systems, is that the direction and the nature of the possible interactions are ignored (Capowiez et al., in press). Moreover, in this experiment, the effect of interspecific interactions is intimately tied with a decrease in intraspecific interactions (the number of earthworms of each species in T3 is only half than in T1 or T2). To gain insight into these interactions, it would be necessary to study the possible trophic interactions between these two species. Indeed, some authors have suggested that endogeic species could feed on the casts of anecic species (Shaw and Pawluk, 1986; Bouché, 1987; Bernier, 1998). More studies on these relationships are required to obtain a clear picture from the results presented. At this stage, it is still impossible to say whether these interactions are linked to spatial competition or to trophic interactions (competition or mutualism for example) between earthworm species.

Burrow systems made by the two species together in the same cores (T3) tend to be the most efficient for gas diffusion. These results cannot be explained by the total burrow length of these burrow systems (no significant correlation was found) or by burrow system continuity (T1 cores were found to be as continuous as T3 cores). Further studies are needed to better understand the relationship between soil diffusion improvement and the characteristics of the burrow systems.

5. Conclusions

Using 3D skeleton reconstructions of burrow systems elaborated by the two species *A. nocturna* and *A. chlorotica*, we were able to demonstrate the existence of typical characteristics for each burrow system in these artificial conditions. Moreover we managed to separate the segments created by the two species in cores where the two species were present. These results represent an interesting first step towards an earthworm burrow typology. Previous attempts made under natural conditions (Pérès et al., 1998) were limited because of ignorance of the 3D structure of the burrow system. However, our approach needs to be confirmed on burrow systems under natural conditions to ensure its efficiency. This experiment also provides evidence for the existence of interspecific interactions between the species studied in these artificial condi-
tions. It remains true, however, that examples of such interspecific interactions under natural conditions are rare and often inconclusive (Fragoso and Rojas, 1997; Curry, 1998).

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References


