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**Using X-ray tomography to quantify earthworm bioturbation  
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  
28 species *Aporrectodea nocturna*). A fifth core without earthworms was used as a control. After  
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*.

48

49 Keywords: burrow system – bioturbation – cast production – *Aporrectodea nocturna* –  
50 *Allolobophora chlorotica* – macropore – earthworm behaviour

51

**52 1. Introduction**

53

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  
67 studied more a century ago (Darwin, 1881), overall our knowledge is still poor. This is mainly  
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.,

77 1998; Langmaack et al., 1999; Capowiez et al., 2001). However an increasing number of  
78 more recently published observations suggests that this vision is too simple (Felten and  
79 Emmerling, 2009).

80 Earthworm burrowing behaviour has been studied by a variety of methods so far: resin  
81 or plaster moulds (Shipitalo and Butt, 1999), the careful and time consuming removal of fine  
82 soil layers under field conditions (Ligthart and Peek, 1997) or the use of 2D terraria under  
83 laboratory conditions (Evans, 1947; Schrader 1993; Capowiez 2000). But since the beginning  
84 of the 90s, X-ray tomography has been increasingly applied in soil biology to obtain precise  
85 and non-destructive analysis of earthworm macropores. Authors used either repacked soil  
86 cores with introduced earthworms (Joschko et al., 1991; Jégou et al., 1998; Langmaack et al.,  
87 1999; Capowiez et al., 2001; Bastardie et al., 2003) or natural soil cores (Daniel et al., 1997;  
88 Pierret et al., 2002; Bastardie et al., 2005).

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

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

110 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  
113 authors were able to measure the increase in soil bulk density around some burrows and thus  
114 determined the width and volume of the so-called drilosphere (sensu Bouché, 1975), the  
115 zones under the physical influence of earthworms. Indeed, when looking at images resulting  
116 from X-ray tomography of artificial soil cores, besides macropores (around them but not  
117 exclusively around them), other soil regions are different from the soil matrix and are thus  
118 influenced by earthworms (see for example Fig. 11 in Rogasik et al., 2003). These regions are  
119 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  
121 characterised by either a difference in soil bulk density (due to soil compaction or soil  
122 looseness), water content (water is more dense than air) or mineralogy. To describe not only  
123 porosity (black voids) but also differences in greylevels in images was already successfully  
124 applied to study sedimentary structures (Honeycutt and Plotnik, 2008).

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

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*  
129 *nocturna* and *Allolobophora chlorotica*) at two different densities.

130

## 131 2. Material and methods

### 132 2.1. Earthworms and soil cores

133 The soil used for the experiment was obtained from the first 20 cm of topsoil  
134 (30.2% clay, 48.7% silt and 21.1% sand; 5.1% organic matter; pH = 8.3) in an abandoned  
135 orchard in Montfavet, near Avignon (43°55' N, 4°48' E) in the SE of France. In this  
136 orchard, the earthworm density is about 450 ind. m<sup>-2</sup> and this earthworm community is  
137 dominated by the species *A. nocturna* (76 ind. m<sup>-2</sup>) and *A. chlorotica* (176 ind. m<sup>-2</sup>). These  
138 two species were also chosen because they belong to different ecological types: *A.*  
139 *nocturna* is a true anecic whereas *A. chlorotica* (leucotypic form) is an endogeic species.  
140 Mature earthworms were collected and weighed after washing and gentle drying with filter  
141 paper.

142 Repacked soil cores were prepared using PVC cylinders (35 cm in length and  
143 16 cm in diameter) lined with a mixture of sealing varnish and sharp fine sand to prevent  
144 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  
146 minutes on sieved soil at 23 % moisture content (gravimetric). This treatment resulted in a  
147 soil dry bulk density of 1.1 g.cm<sup>-3</sup>. To minimise variations in soil bulk density between top  
148 and bottom of the cores, the soil was compacted stepwise in 12 layers. Each layer  
149 comprised 600 g of soil. The final thickness of each layer was approximately 2.5 cm.  
150 Before adding a new soil layer, the surface of the previous layer was gently scratched  
151 using a small rake to increase cohesion between layers.



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

163 At the end of the experiment, cores were imaged using a medical X-ray tomograph  
164 (Prospect SX Advantage, General Electrics) at the Bagnols-sur-Cèze hospital to obtain a set  
165 of images 3 mm thick every 3 mm. The settings at which the X-ray beam was operated were  
166 130 mA and 120 kV. For classical medical scanners, the image size is limited to 512 \* 512  
167 pixels. As a consequence, each pixel in our case was approximately 0.4 \* 0.4 mm.

## 168

### 169 2.2. Image transformations and determination of macroporosity

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

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.

178 The greylevel histograms of the 8-bits images of all the cores in which earthworms  
179 were introduced were all bimodal, with one large peak (grey values) corresponding to the soil  
180 matrix and one narrow peak (black values) corresponding to voids and thus to macropores  
181 (see Figure 2 in Capowiez et al., 1998 for an example). In this case, image segmentation  
182 through binarisation is easy and widely applied (Russ, 1995) but to increase the accuracy of  
183 the reconstruction and take into account the 3D information, a segmentation procedure was  
184 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  
187 are too small (less than 100 voxels, i.e. about  $0.5 \text{ cm}^3$ ) are discarded. At this stage, the volume  
188 of macroporosity, and the number of burrows (a burrow is a set of connected voxels) were  
189 computed. The 3D-volumic information on the macropore can be translated into 3D skeletons  
190 by determining all the ultimate eroded points (i.e. centroids) in all images and linking all the  
191 centroids with pores that overlap between two successive images. A burrow is then a set of  
192 connected segments (a segment joins the centroids of two overlapping pores) and the burrow  
193 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  
195 deviation (relative to the vertical direction) of segments. Mean vertical deviation between  
196 cores were compared with a Kruskal-Wallis test followed by a post-hoc comparison test (Zar,  
197 1984). Two other characteristics not easy to compute: burrow continuity and diameter. There  
198 is no easy way to characterise the continuity of the burrow system so we used the method  
199 previously used in Capowiez et al. (2006). Briefly, virtual horizontal planes are defined at  
200 equal distance in the core and the number of pathways connecting two of these planes is

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

$$\text{circularity} = 4 \pi \text{ area} / \text{perimeter}^2$$

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

$$\text{ECD} = 2 (\text{area}_{\text{circ.}>0.6} / \pi)^{0.5}$$

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

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

223  
224 *2.3. Determination of other kinds of soil bioturbation (excluding macropores)*

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  
227 and in most cases cannot be done by direct binarisation thresholding (i.e. the definition of the  
228 minimal and maximal greylevel values). Indeed, the BZ per se did not correspond to a peak in  
229 the greylevel histogram and are merged in the large soil matrix peak leading to a slightly  
230 skewed histogram. At this stage, we tested and developed several procedures applied on each  
231 2D image that gave slightly different results. In our case the best procedure, judged by a  
232 visual estimate, was obtained with the following steps using ImageJ: (i) a smoothing filter  
233 (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  
236 'objects' smaller than 60 pixels. The anisotropic diffusion filter was developed by Christopher  
237 Mei based on the study of Perona and Malik (1990) and was used here with the following  
238 parameters: iteration = 7; k = 40 and lambda = 0.2.

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

249 supported by studies where examples of CT-images were provided (Figure 5 in Jégou et al.,  
250 1998; Figure 7 in Francis et al., 2001; Figure 11 in Rogasik et al., 2003).

#### 251 252 2.4. Physical measurements on surface casts

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

### 259 260 3. Results

#### 261 3.1. Earthworm burrow systems

262 The burrow systems made by *A. nocturna* and *A. chlorotica* looked quite different  
263 (Fig. 2). The most obvious difference was the diameter of burrows but other characteristics  
264 also contributed to make the two burrow systems look different. Burrows made by the anecic  
265 species were more continuous and vertical. The higher earthworm density appears to have had  
266 an effect in the case of *A. chlorotica* but no clear effect was detected for *A. nocturna*. All  
267 these visual impressions were confirmed when the burrow characteristics were quantified  
268 (Table 1). The effect of the increase in earthworm number (2-fold) depended on the species.  
269 The burrow volume and length slightly decreased (about -12%) when the number of *A.*  
270 *nocturna* inside the core doubled whereas a marked increase was observed in burrow length  
271 (2-fold) and volume (1.7-fold) when the number of *A. chlorotica* doubled. The burrows made  
272 by *A. chlorotica* were significantly narrower, more numerous, more branched and tended to  
273 be less vertical. The continuity of the C8, N2 and N4 burrow systems, estimated using a

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).

276

### 277 3.2. Bioturbated zones (excluding macropores)

278 The method used to define BZ, based on filtering, was satisfactory (Fig. 1). We  
279 assume that it led to a slight underestimation of the BZ volume since zones that were too  
280 small or less obvious (i.e without a sufficient difference in greylevel) were not selected (see  
281 Figure 1 for some examples). No BZ was ever detected in the control soil core without  
282 earthworms. Overall, the BZ followed some of the macropores (Fig. 4). In the first half of the  
283 cores, these zones were continuous whereas in the second half they were discontinuous. BZ  
284 zones were not homogeneously distributed with depth or along burrows (Fig. 4). There was a  
285 clear tendency for these zones to be more abundant in the upper 1/3 of the cores especially  
286 when the number of earthworms was low (C4 and N2, Fig. 5). There was no visual difference  
287 in BZ between the two earthworm species. Interestingly, the volume of these zones was very  
288 close to the macropore volume (ranging from 80.6 to 90.2% of the latter for the four cores).  
289 BZ tended to be further from the burrows in the *A. chlorotica* cores: 50% of the voxels  
290 corresponding to BZ were at a distance greater than 4 and 5.5 mm from the closest macropore  
291 for *A. nocturna* and *A. chlorotica* respectively (Fig. 6).

292

### 293 3.2. Surface casts

294 For the lower abundance (C4 and N2), both earthworm species produced the same  
295 weight of surface casts (Table 2). When the number of earthworms doubled, the weight of  
296 casts produced by *A. chlorotica* greatly increased (2.5-fold) whereas the increase was only  
297 1.5-fold for *A. nocturna*. As the bulk density of casts was not influenced by earthworm  
298 density, data were grouped for each species. The soil bulk density of casts made by *A.*

299 *nocturna* was significantly higher than those made by *A. chlorotica*. Estimated volumes for  
300 these surface casts ranged between 36 and 92 cm<sup>3</sup> (Table 2).

301

## 302 4. Discussion

### 303 4.1. Earthworm burrow systems

304 Although there were no replicates in this study and thus statistical analysis was not  
305 possible, this descriptive and methodological approach provided interesting information on  
306 the earthworm burrow systems made by the two species. These burrow systems were in  
307 agreement with the postulates of Lee and Foster (1991): anecic earthworms make permanent  
308 homes whereas pathways of endogeic earthworms are temporary. Indeed, endogeic  
309 earthworm burrows are supposed to be temporary since they can be backfilled with casts  
310 leading to less continuous burrows and thus a greater number of burrows (a backfilled burrow  
311 is then split into two). Burrows made by anecic earthworms are permanent and reused to  
312 reach the surface where they feed and are thus more continuous. These results are in  
313 agreement with a previous study (Capowiez et al., 2001) which showed that the burrows  
314 made by *A. chlorotica* were less vertical, more branched and narrower than those made by *A.*  
315 *nocturna*. When we compared the C4 and N4 cores, which were colonised by earthworms at  
316 the same density, we found that values for total burrow length and volume were almost the  
317 same with slight differences in length (7.66 vs 6.24 m) and volume (134.0 vs 164.6 cm<sup>3</sup>) due  
318 partly to the smaller diameter of *A. chlorotica* burrows. When the earthworm abundance  
319 doubled, we observed a striking difference between the two species. For *A. chlorotica*, burrow  
320 length doubled and burrow volume almost doubled (with a clear trend for a increased  
321 colonisation of the lower part of the core; Fig. 2) whereas for *A. nocturna* burrow length and  
322 volume slightly decreased. This absence of a correlation between earthworm abundance and  
323 burrow length was observed previously (Capowiez et al., 2000) in a field study focusing on

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

#### 333 4.2. Bioturbated zones

334 The drilosphere was broadly defined as “distinct zones of soil directly and indirectly  
335 modified by earthworms” (Bouché, 1975). This currently accepted definition is clearly based  
336 on visual criteria and, as such, seems to be well adapted to the case of *Lumbricus terrestris*,  
337 for which the burrow walls were found to be made of two layers differing in appearance, bulk  
338 density, porosity and composition (Jégou et al., 2001; Görres et al., 2001; Schrader et al.,  
339 2007). Jégou et al. (2001) assumed that the outer layer (width = 7 mm) corresponded to soil  
340 compaction during burrow formation whereas the inner layer (width = 3 mm) was formed of  
341 casts deposited and crushed at the burrow walls during earthworm movements.

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



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

361 Earthworms deposit part of their casts inside the burrows, backfilling them  
362 temporarily (presumably for anecic) or permanently (presumably for endogeic earthworms).  
363 Fresh casts have an higher water content than the surrounding soil (Hindell et al., 1994;  
364 Francis et al., 2001) but this difference may disappear with time (Mariani et al., 2007). Under  
365 our study conditions, the surface casts and the soil had mean water contents of 26.2% and  
366 23.5% (on a weight/weight basis), respectively, at the end of the experiment. If the  
367 belowground casts followed the same evolution, these differences may play a minor role in  
368 greylevel differences. The casts may, depending on the earthworm species and land use, have  
369 different bulk densities (Blanchart et al., 1997; Decaëns, 2000; Jouquet et al., 2008). In the  
370 present study, we found that the surface casts made by *A. nocturna* had a significantly higher  
371 bulk density than those produced by *A. chlorotica*. However it is difficult to infer the possible  
372 effects in the resulting CT-images since the structure of the belowground casts may have been

373 modified. Indeed, once the casts are deposited in burrows, depending on earthworm behaviour  
374 and thus whether the burrows are reused, they can be crushed along the burrow walls to form  
375 so-called cutanes (Jégou et al., 2001). This in turn will increase the soil bulk density of the  
376 burrow walls (Schrader et al., 2007). Overall it is difficult to disentangle all these processes  
377 (changes in soil mineralogy or texture, increases in water content and soil bulk density) based  
378 solely on the greylevel images provided by tomography. Thus three processes probably  
379 contributed to the formation of our selected BZ: (i) soil compaction during burrow creation,  
380 (ii) cast deposition inside burrows and (iii) cast crushing along the burrow walls. These three  
381 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  
384 burrows based on the postulates of earthworm behaviour. Indeed, using 2D terraria, Capowiez  
385 (2000) was able to show that the rate of burrow reuse was significantly higher for *A. nocturna*  
386 than *A. icterica* suggesting that belowground casts made by the anecic species may have a  
387 greater probability of being transformed into cutanes. This is in agreement with our  
388 observations: the burrow systems made by *A. chlorotica* consisted of a larger number of  
389 burrows (since burrow backfilling creates two burrows) and, at least for C4, were less  
390 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*.  
392 This may indicate that more of the BZ made by *A. chlorotica* were indeed burrows backfilled  
393 with casts whereas for *A. nocturna* there was a greater proportion of cutanes around the  
394 burrows. In conclusion, the detected BZ are a good estimation of the extent of the drilosphere  
395 but we can not attribute all the BZ to belowground cast deposition since some of these zones  
396 may have been caused by soil compaction during burrow formation. Using tomography only,

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

399         Despite this limitation, it was possible to quantitatively compare our estimations to  
400 those previously published. A review of the literature highlights three kinds of estimates: (i)  
401 drilosphere volume; (ii) the proportion of belowground casts; and (iii) the rate of burrow  
402 backfilling. Studies quantifying drilosphere volume are rare. Based on measurements of  
403 compaction around burrows, Schrader et al. (2007) estimated that the drilosphere volume was  
404 53 cm<sup>3</sup> for one individual of *L. terrestris* after 70 days of incubation in an artificial soil core  
405 (height = 30 and diameter = 15 cm). Our observations for *A. nocturna* are in relative  
406 agreement: the drilosphere volume was between 30 (core N4) and 75 cm<sup>3</sup> (core N2) per  
407 individual for almost the same duration and soil bulk density. The proportion of below and  
408 aboveground casts was previously determined only in laboratory experiments (often with  
409 loose soil). The results were highly variable ranging from less than 10% in 2D terraria for *A.*  
410 *caliginosa*, *A. rosea* and *L. terrestris* (Whalen et al., 2004) to 98% of belowground casts with  
411 *A. caliginosa*, *O. cyaneum* and *Diplocardia smithii* (James, 1991). Mariani et al. (2007)  
412 reported values between 36 and 53% for the anecic *Martodrilus sp.* Curry and Baker (1998)  
413 estimated between 15 and 38% belowground casts for *A. caliginosa*, *A. trapezoides* and *A.*  
414 *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  
416 belowground casts, we computed that in our study between 70 and 85% of the casts were  
417 made belowground, which is a value in the high range of those previously published.  
418 Obviously more studies on this important parameter need to be carried out, possibly based on  
419 new and standardised methods. Information on the rate of burrow backfilling is even scarcer.  
420 Schrader (1993) computed a rate of 50-60% of burrows backfilled by *A. longa* whereas Hirth  
421 et al. (1996) estimated this rate to be between 66 and 85% for *A. caliginosa* and *A. rosea*, both

422 of these studies were made in 2D terraria. After successive scans of the same soil cores every  
423 20 days and analysis of the CT-images, Francis et al. (2001) estimated that 72-85% of the  
424 burrows made by *A. caliginosa* were backfilled (but the precise computations that were made  
425 were not presented). In our case, if we assume that BZ are backfilled burrows when they are  
426 at a distance greater than 10 mm from burrows, we computed a rate of backfilling from 14 to  
427 18 % for *A. chlorotica* and from 8 to 10% for *A. nocturna* at the end of the six week  
428 incubation. These values, based on the arbitrary threshold of 10 mm, appear to be low  
429 compared to those previously published.

## 431 5. Conclusions

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

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449

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- 614

615 **Legends for figures**

616 Fig. 1 A CT-image of the soil core with 8 individuals of *A. chlorotica* (diameter= 16 cm) after  
617 analysis with the medical scanner (width = 3mm). A: raw image after 8bit-transformation  
618 ; B: the same image with the selected bioturbated zones coloured in white ('ab' = around  
619 burrows and 'rm' = refilled macropores).

620  
621 Fig. 2 Three-dimensional rendering of the burrow systems after six weeks of incubation in the  
622 four soil cores (colours range from soft to dark grey as a function of the distance from  
623 the point of observation): C4 and C8 (4 and 8 individuals of *A. chlorotica*), N2 and N4 (2  
624 and 4 individuals of *A. nocturna*).

625  
626 Fig 3 Burrow system continuity in the four soil cores estimated as the number of pathways  
627 connecting two successive planes in function of the number of virtual horizontal equi-  
628 distant planes in the core (C4 and C8 mean 4 or 8 individuals of *A. chlorotica*, and N2  
629 and N4 mean 2 or 4 individuals of *A. nocturna*).

630  
631  
632 Fig. 4 Three-dimensional rendering of the bioturbated zones after six weeks of incubation in  
633 the four soil cores (colours range from soft to dark grey as a function of the distance  
634 from the point of observation): C4 and C8 (4 and 8 *A. chlorotica*), N2 and N4 (2 and 4 *A.*  
635 *nocturna*).

636  
637 Fig. 5 Relative proportion of burrow and bioturbated zone volumes in the upper (1/3) and  
638 lower (2/3) parts of the 4 soil cores (C4 and C8 mean 4 or 8 individuals of *A. chlorotica*,  
639 and N2 and N4 mean 2 or 4 individuals of *A. nocturna*).

640

641 Fig.6 Cumulative distribution of the distance of each voxel of the bioturbated zone to the

642 nearest macropore (C4 and C8 mean 4 or 8 individuals of *A. chlorotica*, and N2 and N4

643 mean 2 or 4 individuals of *A. nocturna*).

# Postprint

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Table 1

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 cm<sup>3</sup>. Values bearing different letters are significantly different at the 5% level.

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

## Postprint

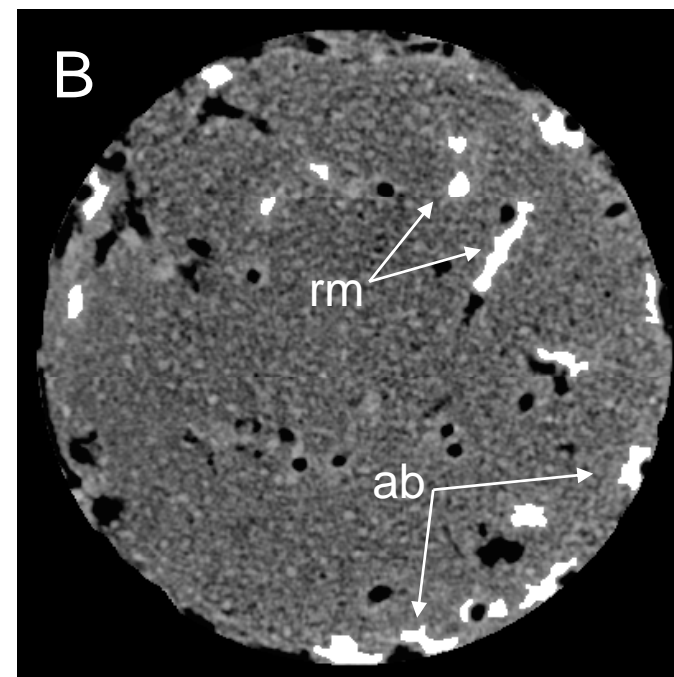
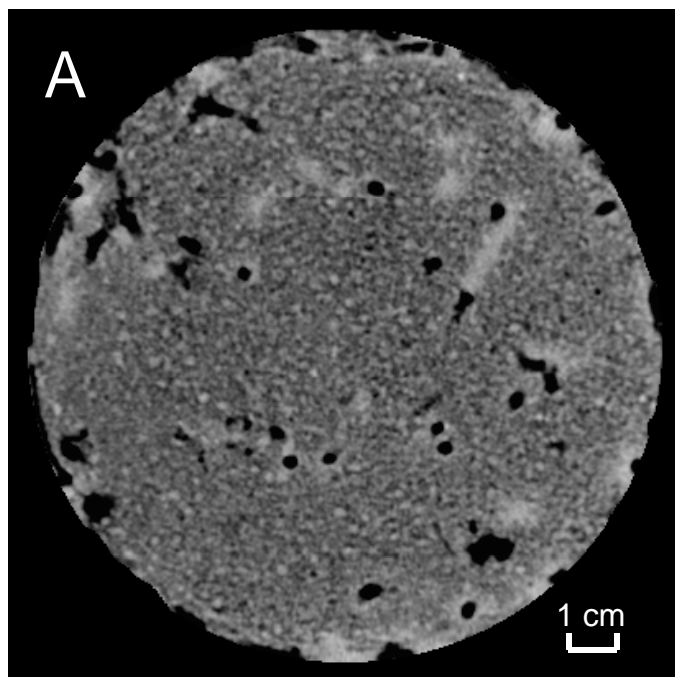
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Table 2

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	<i>A. chlorotica</i>		<i>A. nocturna</i>	
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 casts in g cm <sup>-3</sup> (mean + SD) (n=6)		1.24 <sup>b</sup> (+ 0.16)		1.45 <sup>a</sup> (+ 0.11)
Estimated volume of the surface casts (cm <sup>3</sup> )	36.6	92.0	36.8	55.4

Fig.1





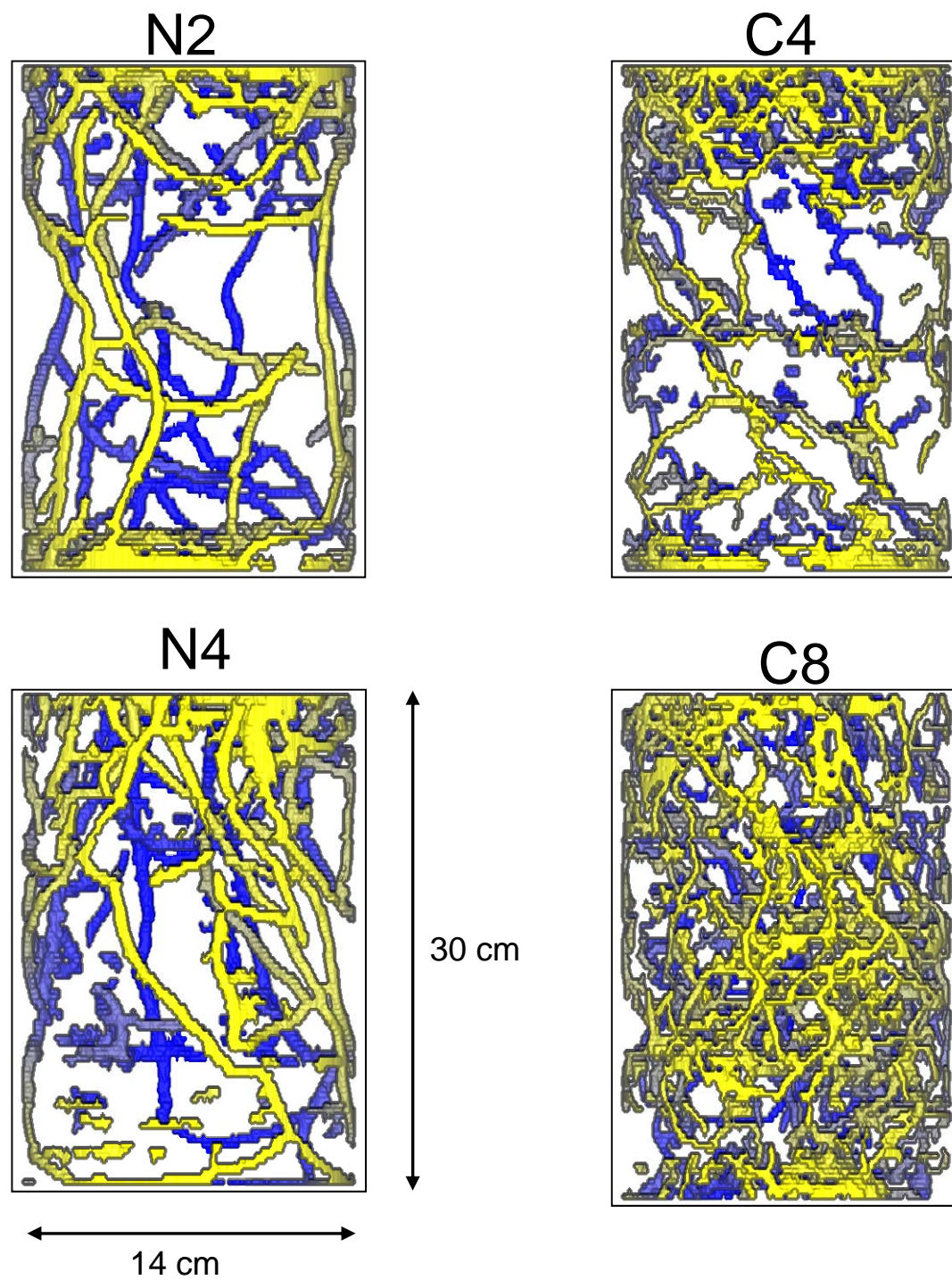


Figure 2

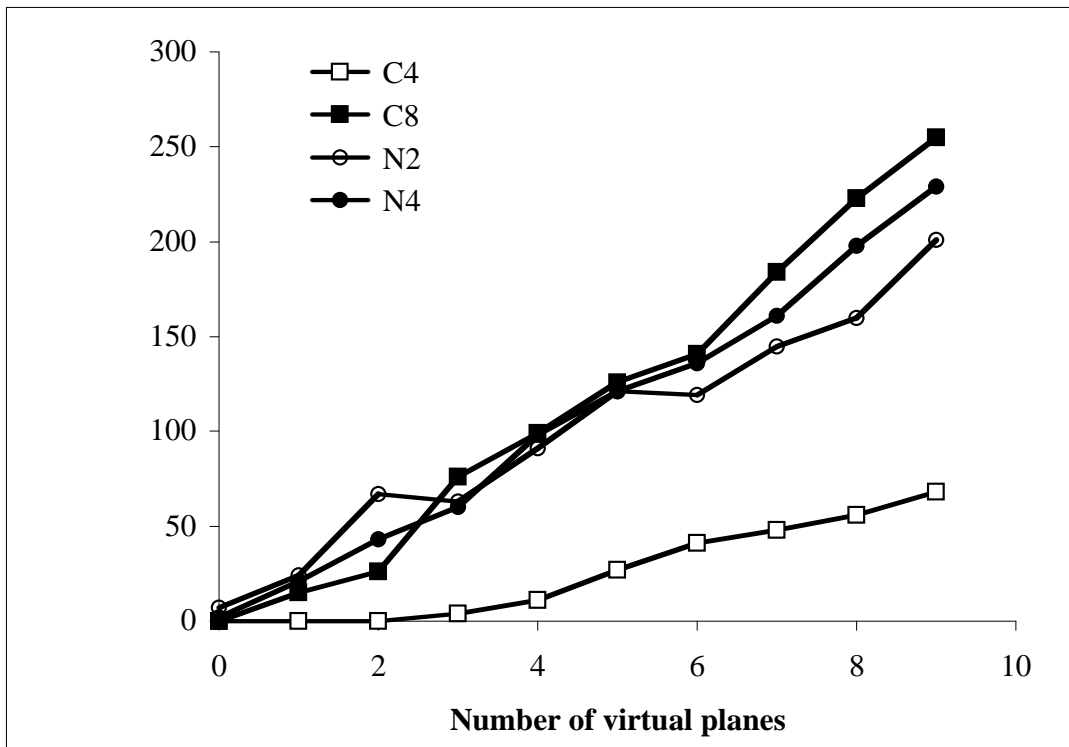


Figure 3

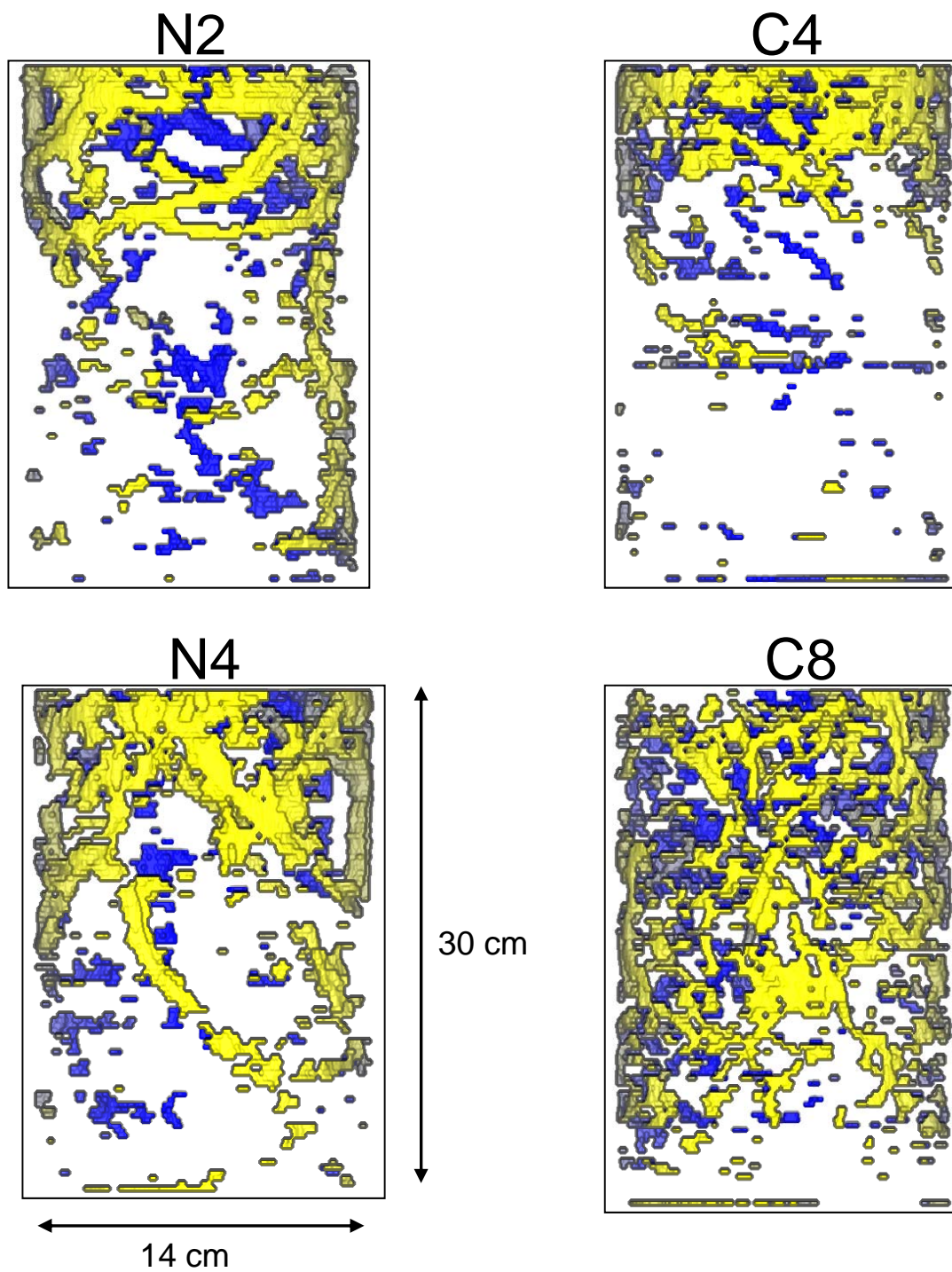


Figure 4

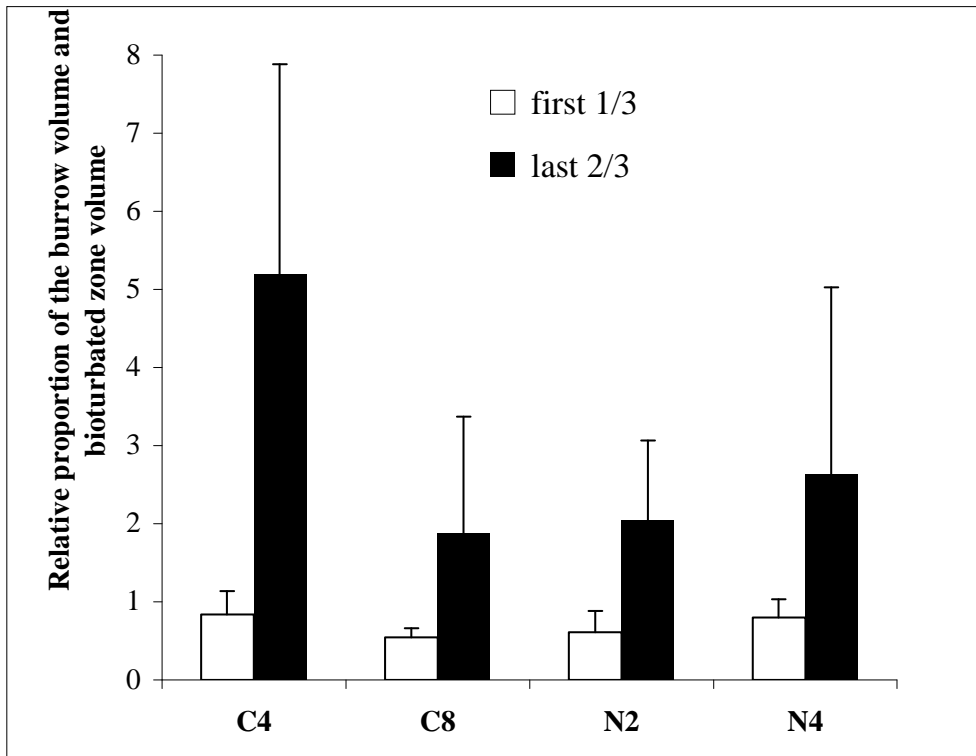


Figure 5