SHORT COMMUNICATION

Burrow refilling behavior of *Aporrectodea turgida* (Eisen) and *Lumbricus terrestris* L. as revealed by X-ray computed tomography scanning: Graphical and quantitative analyses

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Whalen, J. K., Han L. and Dutilleul, P. 2015. **Burrow refilling behavior of *Aporrectodea turgida* (Eisen) and *Lumbricus terrestris* L. as revealed by X-ray computed tomography scanning: Graphical and quantitative analyses**. Can. J. Soil Sci. **95**: 231–235. Solute and gas transport through earthworm burrows is altered when burrows become refilled. Earthworm burrow refilling was evaluated with non-invasive X-ray computed tomography in undisturbed soil cores. Proportionally, *Lumbricus terrestris* refilled burrows had more air-filled space left around their perimeter than those of *Aporrectodea turgida*, which often were completely refilled.

Key words: Burrowing behavior, computed tomography (CT) scanning, earthworms, soil macroporosity

Whalen, J. K., Han L. et Dutilleul, P. 2015. **Le remplissage des sillons par *Aporrectodea turgida* (Eisen) et *Lumbricus terrestris* L. révélé grâce à la tomographie assistée par ordinateur aux rayons X: analyses graphiques et quantitatives**. Can. J. Soil Sci. **95**: 231–235. Le transport des solutés et des gaz dans les trous de ver de terre se modifie lors du remplissage des sillons. Les auteurs ont évalué le remplissage des trous de ver de terre grâce à la technique non invasive de tomographie aux rayons X assistée par ordinateur dans des carottes de sol non perturbé. Toutes proportions gardées, après remplissage, les trous de *Lumbricus terrestris* contiennent plus d'air en périphérie que ceux d'*Aporrectodea turgida*, qui sont souvent totalement remplis.

Mots clés: Remplissage des sillons, tomographie assistée par ordinateur, vers de terre, macroporosité du sol

Geometrical parameters of macroporosity must be known to model water and solute transport, as well as gas diffusion rates, from structured soils. Earthworm burrows function as soil macropores due to their large diameter (generally >1.5 mm; Wang et al. 1994; Daniel et al. 1997). Quantification of the earthworms' contribution to transport processes requires knowledge of burrow continuity, orientation and interconnectivity through time. The depth and volume of macropores generated by endogeic and anecic earthworm species were determined from one-time measurements of 2-D terrariums (Wang et al. 1994, 2004) or from 2-D images produced by X-ray computed tomography (CT) (Bastardie et al. 2003). However, earthworms can change macropore continuity by refilling burrows (e.g., when ingested soil or worked soil is redeposited in burrows no longer in use). After 28 d, Capowiez et al. (2014) reported that 40 to 50% of burrows were refilled by two endogeic earthworms (*Allolobophora chlorotica* and *Aporrectodea caliginosa*), with less refilling ($\sim$20%) occurring in burrows of two anecic earthworms (*Aporrectodea caliginosa nocturna* and *Aporrectodea caliginosa meridionalis*). The method developed by Capowiez et al. (2014) involved tracing burrowing patterns onto a transparent sheet of plastic wrapped around the outer surface of a repacked soil core. To our knowledge, despite recent attempts in this direction (Capowiez et al. 2011; Rogasik et al. 2014), there has been no clear illustration and quantification of earthworm burrow refilling within undisturbed soil cores using CT scanning data. Therefore, the purpose of our study was: (1) to make such a demonstration, by working at a fine resolution voxel size ($0.3 \times 0.3 \times 0.3$ mm in 3D), (2) to analyze areas and volumes obtained from CT images constructed by helical scanning, and (3) to collect sufficient data on burrow refilling by endogeic and anecic earthworms to perform statistical analyses and obtain significant results.

Experimental Setup

Soil microcosms were rigid-wall polyvinyl chloride (PVC) cylinders (15-cm internal diameter, 15-cm height). They contained intact soil from the 0- to 15-cm layer of a sandy loam (Dystric Gleysol, pH 7.0, 20.9 g organic C kg^{-1}) under corn production at the Macdonald Campus Research Farm, Ste-Anne-de-Bellevue, Québec, Canada (lat. 45°30′N, long. 73°35′W) in July, about 2 mo after the field was harrowed and planted. Microcosms were

Abbreviations: **CT**, computed tomography; **GUI**, graphical user interface; **HU**, Hounsfield unit handled carefully to minimize soil structure disturbance, and were placed in a cold room (4°C) for about 8 wk where they dried gradually to about 50% of field capacity, effectively eliminating live earthworms before the experiment began. Earthworms for this experiment were collected from an adjacent field under clover production by hand-sorting and extraction with 0.5% formaldehyde, and kept in laboratory culture (12°C) in sandy-loam soil with fragmented clover residue for about 4 wk before starting the experiment.

Four soil microcosms were brought to the laboratory (20°C) for initial CT scanning (using the Helical Scan option to collect 3-D spatial data continuously; see below). Within 6 d of the initial CT scanning, 2 cm of soil was carefully removed from the surface to provide space for applying water, earthworms and litter, and soils were moistened to 75–80% of field capacity. Six adults of *Aporrectodea turgida* (average total fresh weight: 6.4 ± 0.5 g) were placed on the surface of two microcosms and three adults of *Lumbricus terrestris* (average total fresh weight: 17.8 ± 0.8 g) on the surface of the two others, and were sprayed with 2–3 mL of distilled water to re-humidify their body surface before entering the soil. Thereafter, 1 g of ground alfalfa was added on the soil surface and moistened with 2–3 mL of distilled water. Earthworms remained 28 d in the microcosms. Then, all surviving earthworms were expelled with 80 mL of 0.5% formaldehyde applied to the soil surface, while microcosms were placed 1 d in a fume hood to evaporate residual formaldehyde prior to new CT scanning. At this point, soil moisture content was approximately 70% of field capacity based on the mass of undisturbed soil-filled core.

The high-resolution X-ray CT scanner in our study was used by Lafond et al. (2012). The CT scanning parameter values were: 100 mA (tube current), 120 kV (tube voltage), 1 mm (X-ray beam width), 18 cm (field-of-view diameter), and 1.2 (zoom factor). The Helical Scan option enabled 0.3-mm-thick CT images to be constructed without a gap along the vertical axis of a cylinder (z-axis). Each CT image (x–y plane) consisted of one 512×512 matrix of CT numbers. In each CT scanning session, 500 cross-sectional CT images covering 15 cm vertically were thus constructed continuously for each microcosm.

Following Lafond et al. (2012), after trying several thresholds for CT numbers, we considered a voxel with dimensions $0.3 \times 0.3 \times 0.3$ mm to be (part of) a "pseudo-macropore" if the associated CT number was below -500 Hounsfield unit (HU), that is, mid-way between the calibrated values for pure air (-1000 HU) and pure water (0 HU); the threshold of -500 HU theoretically describes space with 50% air + 50% water, for example. Thus, the term "pseudo-macropore" is justified because CT scanning does not measure porosity directly and soil pores are not restricted to perfect cubes or parallelepiped rectangles.

Using functions from the MATLAB 2013a Graphics toolbox (The MathWorks, Inc., Natick, MA), we developed a graphical user interface (GUI) to visualize each of the two sets of 500 CT images produced per soil column scanned (1) at the beginning and (2) at the end of the experiment. This was essential to determine whether an earthworm burrow that was present at the end already existed in the initial scan, or not. Another MATLAB 2013a function was used to generate five pseudo-random numbers (from 1–100, 101–200, 201–300, 301–400, and 401–500), to locate five refilled earthworm burrows per microcosm in the CT images numbered by the pseudo-random numbers or as close as possible to these (called sites 1–5), and as many open earthworm burrows contained in the same CT images, to be used as controls for later paired comparisons in terms of soil pseudo-macroporosity.

Thus, earthworm burrow refilling after 28 d was evaluated at five "sites" per microcosm, by comparing soil pseudo-macroporosity inside and at the immediate periphery of a refilled burrow (which did not exist at the beginning of the experiment), with that of an open burrow (which was created during the 28 d but not refilled) used as "control". Given the two microcosms used per earthworm species, this led to 40 observations or sets of measures in a given dimension (2D or 3D). The limits of the "smallest rectangle" in 2D (in one CT image) and of the "smallest parallelepiped rectangle" in 3D (over five successive CT images centered on the CT image containing the "smallest rectangle") were drawn around the refilled and open earthworm burrows in our customized GUI (see examples in Fig. 1a and 2a). Thereafter, the histograms of CT numbers for the "smallest parallelepiped rectangles" were produced with MATLAB 2013a built-in functions for the refilled burrow and the companion open burrow (see Fig. 1b, c and 2b, c, bottom panels).

Statistical Analysis

Two types of analysis of variance (ANOVA) were applied. For a given earthworm species (i.e., for *A. turgida* only, or *L. terrestris* only), a repeated-measures ANOVA (ANOVAR) was performed on the paired percentages (1 pair = 1 refilled burrow and the companion open burrow; see Table 1), to assess statistically the difference in pseudo-macroporosity between refilled burrows and companion open burrows. Data underwent arcsine-square-root transformation to improve the normality of their distribution prior to ANOVAR. To compare the burrow refilling behavior between the two earthworm species while taking into account the differences in size of the *A. turgida* and *L. terrestris* earthworms and their burrows, classical ANOVA was performed on ratios (1 ratio = number of pseudo-macropores in a refilled burrow divided by the number of pseudo-macropores in the companion open burrow). All ANOVAs were performed on the 2-D and 3-D data, using SAS software,

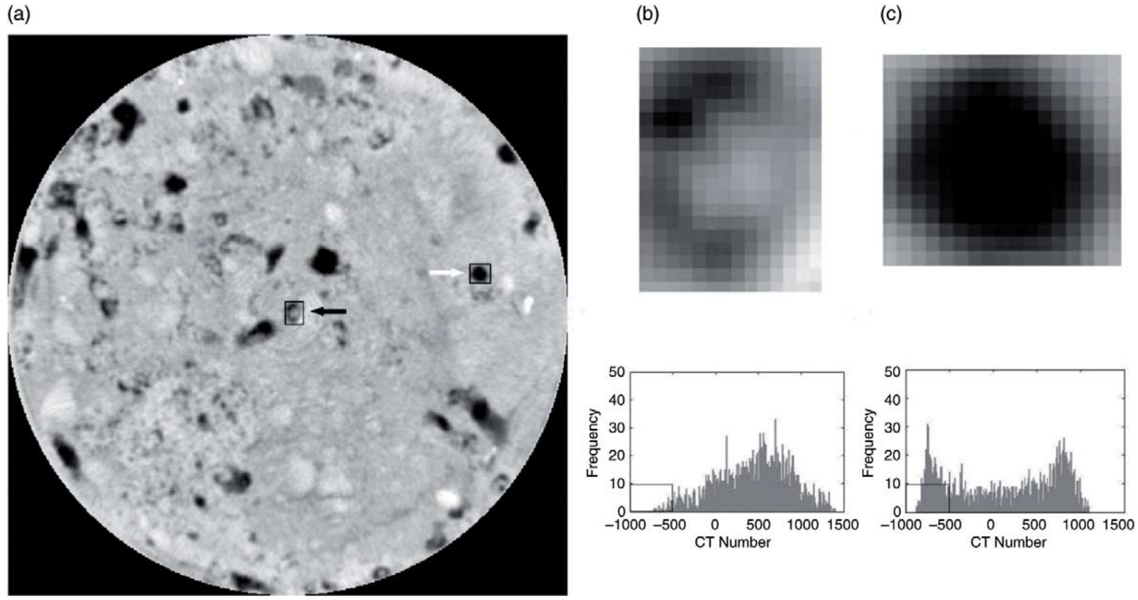

Fig. 1. (a) Illustration by X-ray computed tomography (CT) scanning of the burrowing behavior of *Aporrectodea turgida* (Eisen), with in the same CT image (see black and white arrows): one refilled burrow (black arrow; the frame corresponds to the ‘‘smallest rectangle’’ containing the considered section of the burrow in 2D), and the open burrow used as control (white arrow; similar meaning of the frame). (b), top panel. Zoom in of the refilled burrow; darker gray tone = less dense material. (b), bottom panel. Histogram of the CT numbers for all voxels of the ‘‘smallest parallelepiped rectangle’’ containing five slices of the refilled burrow. (c), top and bottom panels. Zoom-in of the open burrow and histogram of the CT numbers for all voxels of its ‘‘smallest parallelepiped rectangle’’.

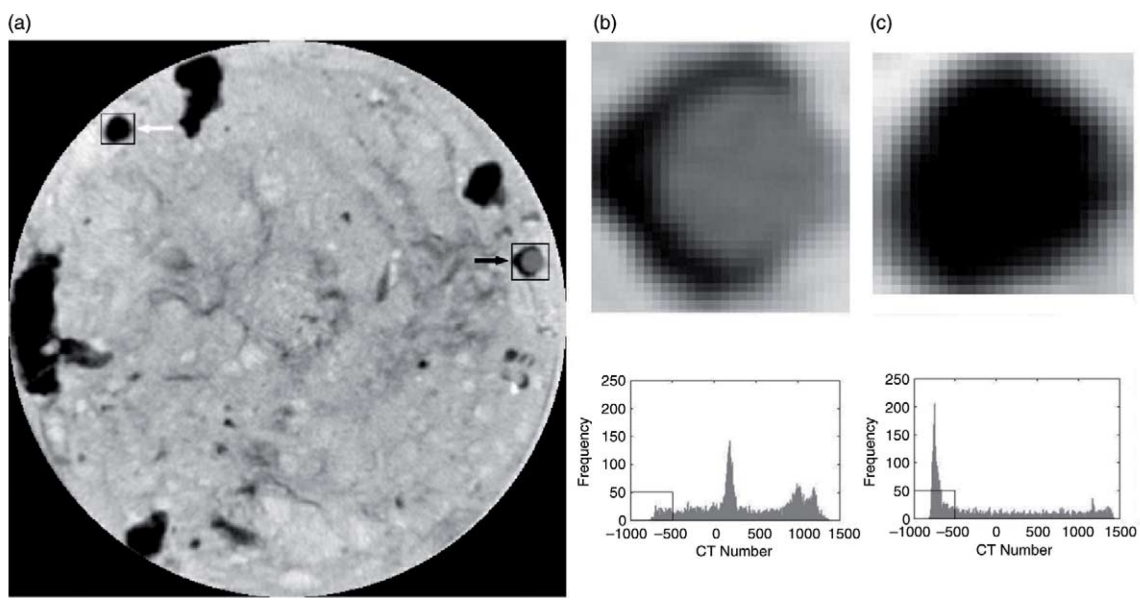

Fig. 2. (a) Illustration by X-ray computed tomography (CT) scanning of the burrowing behavior of *Lumbricus terrestris* L., with in the same CT image (see black and white arrows): one refilled burrow (black arrow; the frame corresponds to the ‘‘smallest rectangle’’ containing the considered section of the burrow in 2D), and the open burrow used as control (white arrow; similar meaning of the frame). (b), top panel. Zoom in of the refilled burrow; darker gray tone = less dense material. (b), bottom panel. Histogram of the CT numbers for all voxels of the ‘‘smallest parallelepiped rectangle’’ containing five slices of the refilled burrow. (c), top and bottom panels. Zoom-in of the open burrow and histogram of the CT numbers for all voxels of its ‘‘smallest parallelepiped rectangle’’.

Table 1. Descriptive statistics (counts and percentages calculated per site per microcosm; below, means and standard errors computed over sites per microcosm), compiled from 2-D and 3-D CT scan data for five refilled burrows randomly sampled (sites 1–5) and the corresponding controls (open burrows) at same depth, in each of two soil microcosms (values separated by a slash (/) in columns 3, 4 and 5 of this table correspond to the refilled burrow at one of the five sites in microcosm 1, value on the left of the slash, and to the refilled burrow at the same site number in microcosm 2, value on the right of the slash) for the two earthworm species; the threshold used here to define a voxel to be a soil pseudo-macropore from CT scan data (CT numbers) is −500 HU; see text for the definition of the "smallest rectangle" in 2D and the "smallest parallelepiped rectangle" (covering 5 CT images) in 3D

Location of refilled burrow	Space dimension	Total number of voxels involved (*)	Number of voxels with CT number value < −500 HU		Percentage,% (column 4 divided by column 3)	Percentage,% for corresponding controls
Aporrectodea turgida (Eisen)						
Site 1	2D	576/255	8/0		1.39/0	27.8/10.4
	3D	3375/1425	62/0		1.84/0	24.5/9.76
Site 2	2D	432/357	0/0		0/0	17.8/8.89
	3D	2375/1955	0/0		0/0	14.7/7.18
Site 3	2D	418/270	0/0		0/0	25.4/13.7
	3D	2185/1350	0/0		0/0	24.1/11.4
Site 4	2D	352/660	6/24		1.70/3.64	26.6/20.8
	3D	2280/3740	28/117		1.23/3.13	22.6/15.6
Site 5	2D	304/272	0/0		0/0	27.2/19.2
	3D	1800/1530	0/0		0/0	25.0/17.1
				Mean (2D)	0.62/0.73	24.96/14.60
				Standard error (2D)	0.38/0.72	1.83/2.35
				Mean (3D)	0.61/0.62	22.18/12.21
				Standard error (3D)	0.39/0.63	1.91/1.84
Lumbricus terrestris L.						
Site 1	2D	775/1650	0/86		0/5.21	49.3/34.4
	3D	4185/8580	0/394		0/4.59	40.6/27.8
Site 2	2D	1377/1638	50/129		3.63/7.88	20.3/41.2
	3D	7420/9000	264/631		3.56/7.01	19.9/35.4
Site 3	2D	1845/2494	250/444		13.5/17.8	20.0/47.7
	3D	9450/13950	1240/2227		13.2/16.0	17.6/43.8
Site 4	2D	2236/1862	82/569		7.53/30.6	42.6/30.6
	3D	12960/9555	385/2781		6.11/29.1	38.7/29.6
Site 5	2D	1089/494	82/0		7.53/0	38.5/59.3
	3D	6300/2700	385/7		6.11/0.26	36.1/51.2
				Mean (2D)	6.44/12.30	34.14/42.64
				Standard error (2D)	2.25/5.41	5.97/5.09
				Mean (3D)	5.80/11.39	30.58/37.56
				Standard error (3D)	2.16/5.12	4.90/4.41

version 9.3(32) (SAS Institute Inc., Cary, NC). The SAS procedures GLM (with and without the REPEATED option) and MIXED were tried, and produced equivalent results.

Difference in Burrow Refilling between *A. turgida* and *L. terrestris*

Using the −500-HU threshold, we demonstrate graphically (Figs. 1 and 2) and quantitatively (Table 1) that burrow refilling is more extensive and complete for *A. turgida* than for *L. terrestris*. The difference in burrow refilling between earthworm species was statistically significant ($P < 0.05$, ANOVA), whether the CT scan data were analyzed in 2D or in 3D, and was not due to variation associated with soil microcosms ($P > 0.05$, ANOVA). Our finding that the endogeic earthworms are more likely to completely refill their burrows, whereas the anecics generally do not, is consistent with the results obtained with another method by Capowiez et al. (2014) for repacked cores. Our histograms of CT numbers suggest that the refilled burrows of *A. turgida* are packed with different materials or materials in different moisture conditions than the refilled burrows of *L. terrestris*. For example, the histogram in Fig. 1b (bottom panel) indicates the presence of a large portion of material with a wide range of densities (from +250 to +750 HU), whereas in Fig. 2b (bottom panel) the histogram indicates the presence of very humid material (see the sharp peak near 0 HU) and a small portion of solid and dense material. Complete burrow refilling by *A. turgida* seems to cause soil disturbance, as the refilled burrow can then exceed the original burrow diameter. This is consistent with endogeic earthworms exerting stronger radial forces than anecic and endogeic species. For instance, *A. caliginosa* exerted radial forces as high as 295 kPa, compared with highest radial force of 65 kPa produced by *L. terrestris* (Keudel and Schrader 1999). By comparison, the air-filled space in the periphery of a burrow refilled by *L. terrestris* can represent a crevice into which it elongated its anterior segments and enlarged the space by expanding the body radially (Seymour 1970; Quillin 2000).

Characteristics of Refilled Burrows versus Open Burrows

As might be expected, refilled burrows of *A. turgida* had significantly ($P < 0.01$, ANOVAR) less free space than open burrows, and the same tendency, very marked, was observed for burrows refilled by *L. terrestris* ($P < 0.01$, ANOVAR). From the numerical information reported in Table 1 [see Mean (2D) and (3D)], it appears that there was a 20- to 40-fold reduction in the pseudo-macroporosity of *A. turgida* refilled burrows and 3.3 to 5.3 times less porosity in the *L. terrestris* refilled burrows, depending on the space dimension and the soil column. This confirms that burrow refilling reduces the available soil macroporosity, more so for endogeic than anecic earthworm species.

The dynamic nature of macroporosity as impacted by earthworm activities, revealed by this work and other literature, has implications for understanding earthworm-mediated effects on gas and liquid transport in the soil profile. Earthworm burrowing, especially of burrows that conduct materials to the soil surface or subsurface, can stimulate gas and liquid transport in the short-term. However, this effect will diminish with time, as conducting burrows are gradually refilled and fall into disuse. The spatio-temporal nature of soil macroporosity as influenced by earthworms remains a rich field for scientific investigation, and will be discussed in future communications.

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