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Age matters: dynamics of earthworm casts and burrows produced by the anecic *Amynthas khami* and their effects on soil water infiltration

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Abstract

By creating vertical and continuous burrows, anecic earthworms accelerate the transfer of water in soils. However, the degradation mechanisms and lifespan of burrows and the consequence of changes in burrow characteristics for water infiltration remain poorly known. In this study, the dynamics of the degradation and hydraulic properties of burrows made by the anecic earthworm *Amynthas khami* in a clayey soil were investigated in a meadow and in a woodland in North Vietnam. We selected three categories of surface casts, namely, (i) fresh (a few days old), (ii) dry (> 1 month old) and (iii) degraded by rain (older than the dry casts), as proxies of the age of burrows. The physical and chemical properties of casts were measured and compared to the surrounding soil aggregates without visible earthworm activity (control). Soil cores were sampled below casts and control and the 3D structure of burrows was characterized using X-ray tomography. Then, water infiltration was measured in the saturated soil cores. Fresh and degraded casts had a lower water stability than control aggregates, whereas higher values were found in dry casts. Water infiltration was twice higher in columns below fresh and dry casts than in the control. However, below degraded casts, the positive effect on water infiltration was reduced or disappeared in some cases. The degradation of burrows led to significant increase in the specific surface area, decrease in their minimum diameter and increase in the abundance of cracks connected to burrows. Our results indicate that anecic burrows persist at least for months below degraded casts but that aging due mainly to physical processes reduces water infiltration. This study highlights the importance of taking into account the lifetime of burrows in the soil when assessing the effect of earthworms on soil structure and water transfer.

Key words: macrofauna, biopores, bioturbation, hydraulic conductivity

1. Introduction

Earthworms belong to one of the most important groups of soil fauna worldwide and are good examples of soil bioturbators due to their ability to modify their habitat physically (Bottinelli et al., 2015). Among earthworms, anecic and epi-anecic species *sensu* Bouché (1971) produce continuous and vertical burrows connected to the soil surface where they eject their dejections, the so-called casts. The role of anecic burrows in water infiltration in soil may become more important as the soil approaches water-saturated conditions (Pitkänen and Nuutinen, 1998). The activities of anecic species may therefore help to mitigate the deleterious effect of heavy rains by increasing soil water infiltration (Andriuzzi et al., 2015) and reducing runoff (Jouquet et al., 2008b) especially in tropical humid ecosystems, where the erosion of soil is a major issue.

The morphological characteristics of burrows measured by X-ray computed tomography and image analyses such as burrow continuity, connection to other macropores, size and length are important determinants of water infiltration (Bastardie et al., 2005; Bottinelli et al., 2017; Capowiez et al., 2015). Hence, any changes in these properties may influence the ability of burrows to transfer water. Several mechanisms influencing burrow degradation have been described: breakdown by tillage (Pelosi et al., 2017), compaction by machinery or livestock (Jégou et al., 2002; Ligthart and Peek, 1997), and infilling by earthworm casts (Capowiez et al., 2014). Comparatively, there is dearth of information on the influence of water seepage (Van Den Berg and Ullersma, 1991) and the formation of desiccation cracks (Kretzschmar, 2004) on the degradation of burrows. However, degradation by these two last mechanisms could have significant effects on the hydraulic functionality of abandoned anecic burrows, especially in soils presenting a high shrinkage capacity.

In North Vietnam, the anecic earthworm *Amynthas khami* (Megascolecidae family) produces large casts on the soil surface (Jouquet et al., 2008a). These casts are water stable and may last for months to years under some conditions. Our first observations revealed that casts stop growing after one month and no new cast is produced at the base of the cast, which indicates that the burrow below is no more used by the earthworm. Casts produced by *A. khami* therefore offer a unique opportunity to study burrow dynamics during aging by using the degradation stage of casts as a proxy of the age of burrows. This study aimed to evaluate the effect of aging on the temporal dynamics of the morphology of earthworm burrows and their ability to transport water. We hypothesized that aging leads to a decrease in soil water infiltration and this decrease is more important in soil with a greater shrinkage capacity.

2. Materials and methods

2.2. Study site

The field work took place at the 'M-Tropics' long-term observatory catchment located in Dong Cao village in North Vietnam (20° 57'N, 105° 29'E). The climate is subtropical humid with an annual mean temperature and rainfall of 20 °C and 1800 mm, respectively. The dominant soil is an Acrisol (WRB) (Podwojewski et al., 2008). The catchment is covered by woodlands, secondary forests, meadows and fallows. The experiment took place during the dry season in March 2017. We selected two land uses, namely, one meadow and one woodland, presenting a large amount of surface casts produced by *A. khami*. The mass of casts found in meadow compared to those found in woodland was 2800 *versus* 4400 g (oven dry weight) per m^2 and the number of fresh casts was 3 *versus* 2 per m^2, respectively. The soil (0-5-cm depth) in the meadow compared to those in the woodland had an organic C content of 3.7 *versus* 2.5%, a pH (KCl) of 3.8 *versus* 3.9, a clay content of 58 *versus* 50%, a sand content of 16 *versus* 17% and a coefficient of linear extensibility (COLE) of 0.07 *versus* 0.05. According to Grossman (1968), the COLE values indicated that the soil in meadow and in woodland had a high and medium shrinkage capacity, respectively.

Cast sampling and analyses

As a proxy of the age of burrows, we selected three categories of surface casts produced by *A. khami* based on visual inspection: 'A', fresh cast in production and moist in appearance, assumed to be a few days old; 'B', dry cast and assumed to be more than 1 month old; and 'C', dry cast with marked signs of degradation (greenish color due to algae and with cracks at the surface) and assumed to be older than casts 'B' (Fig. 1). Both at the meadow and the woodland, the surface casts of each category and their surrounding topsoil aggregates (0-5-cm depth) without visible earthworm activity (control) were sampled in four 100-m^2 plots situated at 50 m distance from each other. In total, 160 samples were collected: 2 land uses × 4 blocks × 4 (3 types of casts + 1 control) × 5 replicates.

To test that the three categories of casts (fresh, dry and degraded) represented an aging gradient, we measured their water stability, hydrophobicity and organic carbon content assumed to change during the aging of casts (Decaëns, 2000; Decaëns et al., 1999). To work on samples with similar physical properties, we broke the casts and control aggregates into pieces to obtain aggregates between 10 and 13 mm. Water stability of aggregates was measured after oven-drying samples at 40 °C for 2 days.

The mean weight diameter was calculated after immersion of 5 g of soil aggregates in 200 mL of distilled water (10 minutes), vertical sieving in the water through a column of sieves (5, 3.15 and 0.2 mm) and oven-drying at 40 °C (Le Guillou et al., 2012). Aggregate hydrophobicity was assessed by measuring the water drop penetration time (WDPT) on 5 aggregates per plot. It corresponded to the time required for a 13-µL drop of deionized water to penetrate the aggregate that was previously oven-dried at 40 °C for 2 days (Chenu et al., 2000). The total soil organic carbon content was determined by potassium dichromate oxidation titration for aggregates previously crushed to a size of 2 mm.

Burrow sampling and analyses

Soil samples under 'A', 'B' and 'C' casts and in the surrounding soil without visible of surface earthworm activity (control) were manually excavated with a sampler device having cutting edges at its base. The device permits the introduction of one PVC column (16 cm in height, 12.5 cm in diameter) in its inner space. The sampling device was introduced vertically into the soil with a hammer. 'A' and 'B' casts were always represented by a single type, while 'C' casts were either individual casts (called 'C1') or composed of an accumulation of degraded casts (called 'C2'). In the woodland, 'C1' and 'C2' casts were found, whereas in the meadow, only 'C2' casts were present. In total, 72 soil columns were sampled: 2 land uses x 4 blocks x (3 or 4 groups of casts + 1 control) x 2 pseudo-replicates.

Half of the soil columns sampled below 'A', 'B', 'C1' and 'C2' casts (N = 28) were scanned with a X-ray scanner (SIEMENS SOMATOM® Definition Flash) at Bach Maï Hospital (Hanoï, Vietnam) using a voltage of 120 kV and a current of 93 mA. The acquired images were series of cross-sections in DICOM 16-bit grayscale format (resolution of 330 x 330 µm^2, with an image acquired every 600 µm). All subsequent steps of image preparation and processing were carried out with ImageJ and Avizo software. Images were (i) formatted in 8-bit TIFF format; (ii) rendered isotropically (final resolution of 250 x 250 x 250 µm^3); (iii) aligned on the Z axis and (iv) filtered by Median-3D, with a radius of 2 pixels to decrease noise. We manually selected the threshold for segmenting macropores at the 2/3 distance between the peak of the soil matrix and the peak of macroporosity (Capowiez et al., 1998). Then, macropores < 1 mm^3 in size were removed (Fig. 2a). We selected manually central earthworm burrows defined here as round macropores in 2D images, presenting a diameter superior to 3 mm and located below casts. First, central burrows (Fig. 2b and 2c) were selected on 2D images with the plugin "interactive geodesic reconstruction 3D" implemented in MorphoLibJ (Legland et al., 2016) for ImageJ software. Second, on 3D images, pores connected to central burrows were isolated (Fig. 2d and 2e) with the draw tool in the module "volume edit" in Avizo software. For 'A', 'B', and 'C1' columns only one central burrow was found (Fig. 2b). Conversely, 'C2' columns had several central burrows with different ages (Fig. 2c). We therefore measured the properties of all central burrows and averaged their properties. Macropore quantification was performed with BoneJ (Doube et al., 2010) in ImageJ software. Central burrows were characterized by their volume and specific surface with the plugin "particle analyzer" and their diameter with the plugin "thickness" (Table 1).

All soil columns (N = 72) were water-saturated for 4 days by gradually raising the water level each day to avoid possible imprisonment of air bubbles as much as possible. Water infiltration rate (L min^{-1}) was determined using a constant head device with an apparatus similar to that reported by Bastardie et al. (2003). The value of the hydraulic gradient (the difference between water levels imposed on the inflow and outflow divided by the column length) was 4 and the outflow was recorded during 3 min.

Statistical analyses

Prior to analysis, the data were tested for homogeneity of variance and normality and log-transformed when required. For each land use, One-way ANOVAs for randomized complete block design were performed to assess differences in soil physico-chemical properties, macropore properties and water infiltration between the different treatments. LSD post-hoc multiple comparison tests were performed to assess the statistical significance of differences between means. Spearman correlations were calculated to study the relationships between the aging of burrows and water infiltration in 'A', 'B', 'C1' and 'C2' columns (N = 28). Differences were considered significant only when P values were lower than 0.05. All statistical analyses and plots were carried out with R software using "car", "agricolae" and "ggplot2" packages.

Results

Properties of earthworm casts as influenced by aging

The properties of casts changed according to their degradation stage (Table 2). The mean weight diameter of aggregates was lower in 'A' casts than in control (on average 1.1-fold) but differences were only significant in the meadow. 'B' casts had similar mean weigh diameter of aggregates than in control. From 'B' to 'C' casts, the mean weight diameter of aggregates decreased (on average 1.5-fold) to reach values lower than in control. Organic carbon content was higher in casts than in control (on average 1.5-fold) without significant difference between types of casts. The water drop penetration time of aggregates was higher in all the cast categories than in control (on average 26-fold) and it increased from ‘A’ to ‘C’ casts (on average 3-fold).

3D properties of central earthworm burrows

Three of the four replicates of the ‘A’, ‘B’, ‘C1’ and ‘C2’ central burrows are shown in Fig. 3. ‘A’ burrows had similar diameters and none were degraded. ‘B’ burrows showed more variability, some of which were not degraded, whereas others had cracks connected to them. ‘C1’ burrows all showed marked signs of degradation, characterized by cracks directly connected to burrows and other biopores connected to cracks. ‘C2’ burrows were similar to ‘C1’ burrows except that several burrows were present in each column. The quantification confirmed the visual impressions (Table 3). The 3D properties of the ‘A’ and ‘B’ central burrows were not significantly different at the two land uses. The comparison of ‘A’ and ‘C2’ burrows revealed a similar pattern of degradation with similar intensity. The degradation was characterized by an increase in the specific surface area (on average 2.5-fold), an increase in the volume of macropores connected to the central burrows (from 0 to 22458 mm^3 in the meadow and from 2598 to 37886 mm^3 in the woodland) and a decrease in the minimum diameter (on average 2.5-fold). However, results were not statistically significant in meadow for the minimum diameter. Comparison between ‘C1’ and ‘C2’ burrows in the woodland showed only a decrease in the volume percentage of burrows (2-fold).

Water infiltration

‘A’ and ‘B’ columns had similar and higher water infiltration rate (on average 2-fold) than control (Fig. 4). In the woodland, water infiltration rate decreased between 'B' to 'C1' columns (1.4-fold) to reach similar value than control. Between 'C1' to 'C2' columns water infiltration rate increased to reach higher value than control. In the meadow, water infiltration rate was always higher under casts than in control and it decreased significantly from 'A' to 'C2' columns. Water infiltration rate in 'A', 'B', 'C1' and 'C2' columns was statistically significantly correlated ($P < 0.05$) with the minimum diameter ($r = 0.47$) and the specific surface area ($r = -0.47$) of the burrows.

Discussion

Water stability of earthworm casts as influenced by aging

The water stability of earthworm casts has been widely documented, including the stability of casts produced by *A. khami* (Jouquet et al., 2008a), but this study showed that the water stability of casts is highly variable in the field depending on their age. Fresh and degraded casts ('A' and 'C' casts) had lower water stability than control aggregates, whereas higher values were found for dry casts ('B' casts). The decrease in water stability of fresh casts can be attributed to the intense remolding that occurs during the passage of soil through earthworms destroying the preexisting soil microstructure (Marinissen and Dexter, 1990; Shipitalo and Protz, 1988), whereas the increase in stability of dry casts results from hardening with age (Hindell et al., 1997), the development of fungi or the production of polysaccharides of microbial origin (Marinissen and Dexter, 1990; Shipitalo and Protz, 1989). The decrease in water stability of degraded casts is consistent with Decaëns et al. (2000), who found that the aging of casts produced by the anecic earthworm *Martiodrilus carimaguensis* promoted the formation of cracks and colonization by soil macrofauna, resulting in a decrease in bulk density and water stability. Finally, considerable changes in the properties of casts (water stability and hydrophobicity) confirmed that the visual appearance of casts can be used as a proxy of cast age.

Morphology and hydraulic functionality of burrows as influenced by aging

We observed that burrows of *A. khami*, once abandoned, persist at least several months in clayey soils. This result is in line with the study of Potvin and Lilleskov (2017), who found in a rhizotron facility that 67% of the burrows produced by the epi-anecic earthworm *Lumbricus terrestris* persisted for more than 7 years in soils dominated by sand. However, it seems unlikely that burrows made by *A. khami* would last as long as those of *L. terrestris*. Indeed, *A. khami* individuals abandon their burrow after one month once the cast connected to the entrance of the burrow become dry and hard, which imped the earthworm to burrow anymore. Conversely, *L. terrestris* individuals are known to build permanent burrows associated with middens (Butt et al., 2003). The midden is a collection of organic and inorganic materials plus earthworm casting around the burrow entrance, which is regularly maintained by *L. terrestris.* Also, the impact of the soil texture on the degradation dynamics of the burrows is unknown. On one hand, the degradation might be faster in clayey soils than is sandy soils since the former produce cracks when drying. On the other hand, clayey soils are probably better able to withstand pressures exerted by the action of infiltrating water during flooding events.

Even though burrows were found below degraded casts (‘C1’ and ‘C2’), our study revealed significant changes in their morphology characterized by an increase in the specific surface area and the volume of macropores connected to them, and a decrease in the minimum diameter compared to those of younger burrows. No infilling by earthworm casts clogging the burrows was observed in the images, and no compaction or tillage happened (as evidenced by the presence of surface casts). Cracks but no biopores were found directly connected to burrows, indicating that the formation of cracks after successive wetting/drying cycles had a strong influence on the aging of burrows. Because the degradation of burrows was similar between the two land uses, we suggest, contrary to our hypothesis that the difference in COLE between the two soils (0.07 *versus* 0.05) was not sufficient to alter differently the dynamics of burrows. Another mechanism that might explain the changes in the morphology of burrows and the formation of cracks is the collapse of the burrow wall due to the seepage force generated by the flow of water (Van Den Berg and Ullersma, 1991). This collapse could explain the significant changes in the specific surface area and the minimum diameter.

One functional consequence of the aging of burrows was evidenced by the change in water infiltration rate. Our study confirmed the findings of previous field studies showing the efficiency of the burrows of anecic earthworms in transporting water when the soil was saturated (Andriuzzi et al., 2015; Pitkänen and Nuutinen, 1998). However, this positive effect disappeared in degraded burrows (‘C1’) in woodland and decreased significantly in the presence of multiple degraded burrows (‘C2’) in meadow. The changes in water infiltration were partly explained by changes in the morphology of the burrows, such as the increased specific surface area and the decreased minimum diameter. These results confirmed our hypothesis that changes in the 3D properties of burrows would have a large effect on the functionality of burrows. Water infiltration rate of several degraded burrows in the woodland (‘C2’) was similar than that in ‘A’ columns despite clear differences in the morphology of the burrows between ‘A’ and ‘C2’ columns. This apparent contradiction could be explained by the fact that water infiltration was measured for 72 columns, whereas only half of the columns were analyzed by X-ray tomography. It also shows that the presence of several central burrows, even degraded ones, can have a positive influence on water infiltration.

Conclusions

Our results provide evidence that earthworm burrows produced by the anecic earthworm *A. khami* can persist at least for months in clayey soils. However, the morphology of burrows changed during aging, mainly due to physical processes, and the positive influence of the burrows on water infiltration is reduced or even disappeared in some cases. Further research is needed to assess the timescale of the degradation dynamics of burrow systems of different earthworm species in a range of pedo-climatic conditions.

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Figure captions

Figure 1: Examples of earthworm casts used as proxies of the age of burrows: ‘A’ fresh cast in production and humid in appearance, known to be a few days old; ‘B’ dry cast assumed to be more than 1 month old; and ‘C’ dry cast with marked signs of degradation (greenish color due to algae with cracks at the surface) and assumed to be older than 'B’ cast.

Figure 2: Illustration of the central earthworm burrows characterized in 3D in columns under ‘A’, ‘B’, ‘C1’ and ‘C2’ casts. ‘A’, single burrow, known to be a few days old; ‘B’, single burrow, assumed to be more than 1 month old; and ‘C1’ /’C2’, one/several burrows, assumed be older than ‘B’ burrow.

Figure 3: Three-dimensional visualization (using X-ray tomography) of central earthworm burrows in 3 replicates of 1000 cm^3 soil columns sampled along an increasing aging gradient from ‘A’ to ‘C2’ in a meadow and a woodland: ‘A’, single burrow, known to be a few days old; ‘B’, single burrow, assumed to be more than 1 month old; and ‘C1’/’C2’, one/several burrows, assumed be older than ‘B’ burrow.

Figure 4: Box plot of water infiltration rate (n = 4) measured in columns without visible of surface earthworm activity (control) and under ‘A’, ‘B’, ‘C1’ and ‘C2’ casts. ‘A’, single burrow, known to be a few days old; ‘B’, single burrow, assumed to be more than 1 month old; and ‘C1’ /’C2’, one/several burrows, assumed be older than ‘B’ burrow. Values bearing different letters are statistically significantly different. The number of stars for the significance level indicates the p-value range (*** $P < 0.001$)

Table 1: Descriptions of quantified 3D properties measured on central earthworm burrows.

3D property	Definition
Volume percentage (%)	Ratio of the volume of the central burrows to the volume of soil
Specific surface area (mm^{-1})	Ratio of the central burrows surface area to the volume of soil
Mean diameter (mm)	Mean diameter of the central burrows
Minimum diameter (mm)	Minimum diameter of the central burrows
Volume connected (mm^3)	Volume of macropores connected to the central burrows

Table 2: Average values (n = 4) of physical and chemical properties of the topsoil aggregates without visible earthworm activity (control) and 'A', 'B' and 'C' casts in the meadow and woodland. 'A', fresh cast in production and humid in appearance, known to be a few days old; 'B', dry cast with a rounded shape, assumed to be more than 1 month old; and 'C', dry cast with marked signs of degradation (greenish color due to algae with cracks at the surface) and assumed to be older than 'B' cast. Water stability of aggregates as quantified by the mean weight diameter (MWD), total organic carbon (OC) and hydrophobicity represented by the water drop penetration time (WDPT). Values bearing different letters are statistically significantly different within each land use. The number of stars for the significance level indicates the p-value range (*** P <0.001 and ** P <0.01).

Land use	Aggregate	MWD (mm)	OC (%)	WDPT (s)
Meadow	Control	8.5 a	3.7 b	76 d
	A	7.7 b	4.5 a	2459 b
	B	8.6 a	4.8 a	436 c
	C	5.6 b	5.2 a	6098 a
	p-value	***	**	***
Woodland	Control	8.6 a	2.5 b	83 c
	A	7.8 a	3.6 a	745 b
	B	8.6 a	3.6 a	639 b
	C	5.8 b	3.8 a	2604 a
	p-value	***	***	***

Table 3: Average values (n = 4) of morphological characteristics of the central burrows under 'A', 'B', 'C1' and 'C2' casts. 'A', single burrow, known to be a few days old; 'B', single burrow, assumed to be more than 1 month old; and 'C1' /'C2', one/several burrows, assumed be older than 'B' burrow. Volume percentage (VP), specific surface area (SSA), mean diameter (MeD), minimum diameter (MiD) and volume of macropores connected to the central burrows (VC). Values bearing different letters are statistically significantly different within each land use. The number of stars for the significance level indicates the p-value range (*** P <0.001; ** P <0.01; * P <0.05 and ns for P >0.05).

Land use	Column	VP (%)	SSA 10^{-3} (mm^{-1})	MeD (mm)	MiD (mm)	VC (mm^3)
Meadow	A	0.3 a	2.2 b	4.6 a	3.1 a	0 b
	B	0.2 a	1.9 b	4.4 a	3.7 a	14630 ab
	C2	0.3 a	5.6 a	4.0 a	1.8 a	22458 a
	P-value	ns	**	ns	ns	*
Woodland	A	0.2 b	1.8 b	4.3 a	3.8 a	2598 b
	B	0.3 b	2.1 b	4.6 a	3.1 a	2401 ab
	C1	0.4 a	4.2 a	3.7 a	1.5 b	39458 ab
	C2	0.2 b	4.2 a	3.9 a	1.4 b	37886 a
	P-value	**	***	ns	**	*

Fig. 1

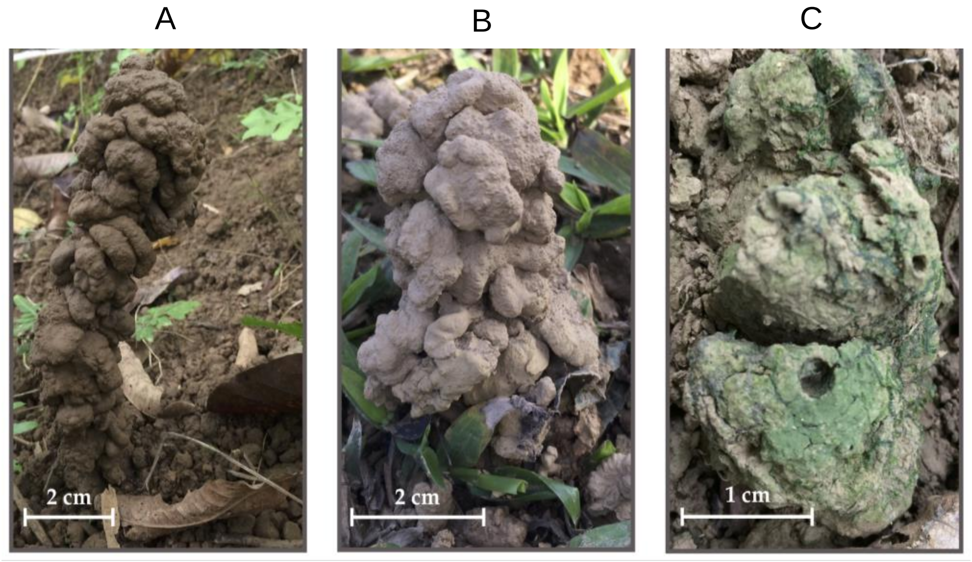


Fig. 2

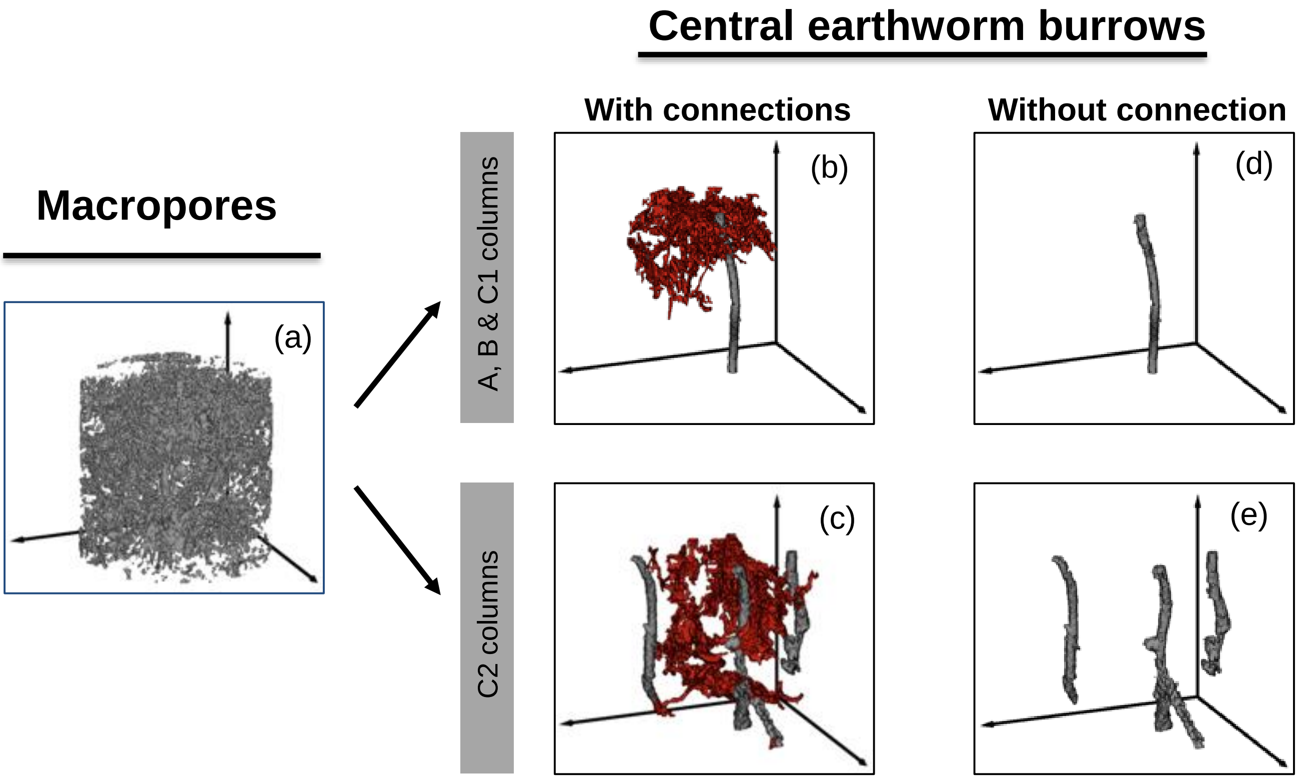

Central earthworm burrows
With connections
Without connection
Macropores
(a)
A, B & C1 columns
C2 columns
(b)
(c)
(d)
(e)

Figure 3

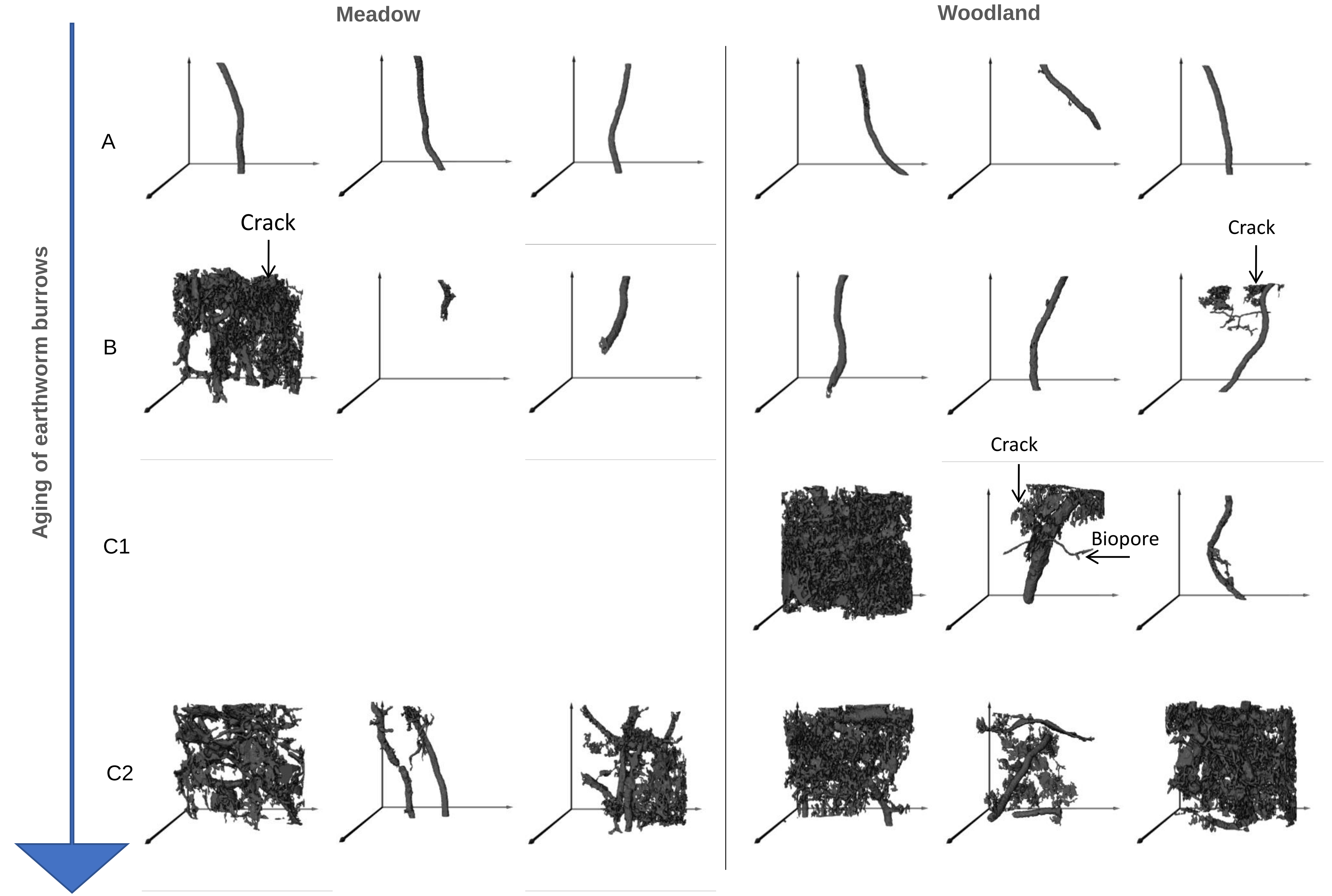
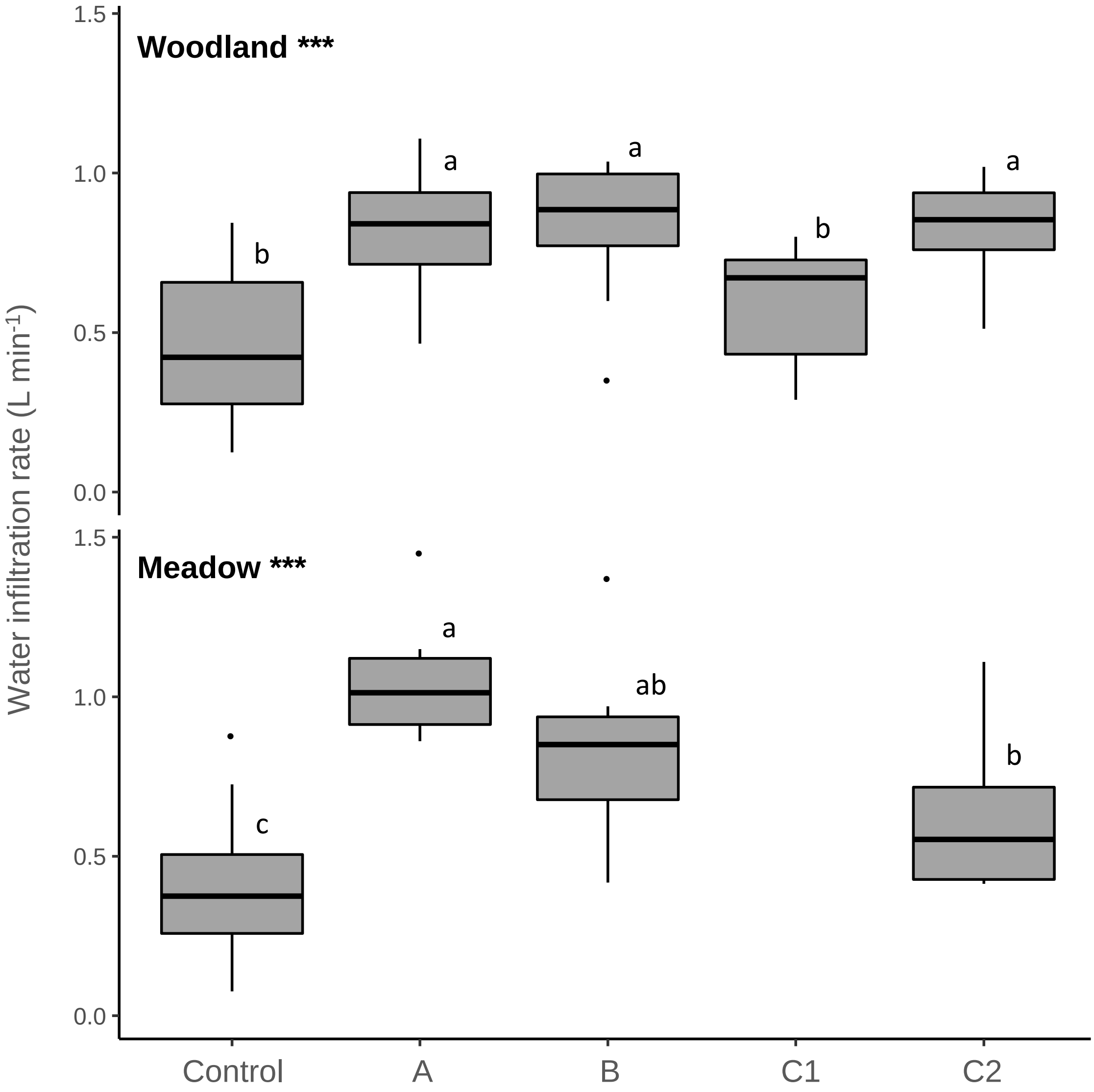

Meadow
Woodland
Aging of earthworm burrows
A
B
C1
C2
Crack
Crack
Crack
Biopore

Fig. 4



Woodland ***
Meadow ***
Water infiltration rate (L min-1)
1.5
1.0
0.5
0.0
b
a
a
b
a
c
a
ab
b
Control
A
B
C1
C2