Biol Fertil Soils (2017) 53:951–955
DOI 10.1007/s00374-017-1235-8

SHORT COMMUNICATION

Earthworm burrowing activity of two non-Lumbricidae earthworm species incubated in soils with contrasting organic carbon content (Vertisol vs. Ultisol)

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Received: 9 June 2017 / Revised: 17 August 2017 / Accepted: 20 August 2017 / Published online: 26 August 2017

Abstract The aim of this study was to investigate the burrowing activity of two earthworm species: the endogeic *Drawida sinica* and one undescribed *Amynthas* species incubated in Vertisol and Ultisol presenting different soil organic C content. Because of their contrasting feeding behaviours, we hypothesised that soil type would have a bigger influence on the burrowing activity of the endogeic than the anecic species. Repacked soil columns inoculated with earthworms for 30 days were scanned using X-ray tomography and the compiled images used to characterise the burrow systems. After scanning, the saturated hydraulic conductivity (K_{sat}) was also measured. The *Amynthas* species burrows were less numerous (30 vs. 180), more vertically oriented (57 vs. 37°), more connected from the surface to the bottom of the columns (73 vs. 5 cm^3) and had a higher global connectivity index (83 vs. 28%) than those of *D. sinica.* The K_{sat} was threefold faster in columns incubated with *Amynthas* and was linked to the volume of percolating burrows (R^2 = 0.81). The soil type did not influence *Amynthas* burrow characteristics. In contrast, there were 30% more *D. sinica* burrows in the Vertisol than in the Ultisol while other burrow characteristics were not affected. This result suggests that these burrows were more refilled with casts leading to shorter and discontinuous burrows. The K_{sat} was negatively related to the number of burrows (R^2 = 0.44) but was not statistically different between the Vertisol and the Ultisol, suggesting a constant impact of this species on the K_{sat}. We found that a decrease in the amount of soil organic C by 50% had only a small influence on earthworm burrowing activity and no effect on the K_{sat}.

Keywords Megascolecidae · Moniligastridae · Preferential flow · Soil structure · Tomography

The original version of this article was revised: the name of the author was incorrectly spelled as "X. Peng". The correct spelling is "X. H. Peng" and the corresponding author of this article was changed from "Nicolas Bottinelli" to "X. H. Peng". These are now presented correctly in this article.

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Introduction

Among soil organisms, earthworms play a major role in shaping soil hydraulic properties (Shipitalo and Le Bayon 2004). Earthworm burrows can act as preferential flow pathways for water (Bastardie et al. 2002; Edwards et al. 1992). The redistribution of fine soil particles and their concentration in casts in the soil may also affect soil porosity and water retention within soil layers (Ernst et al. 2009; McDaniel et al. 2015). The interface between burrows and the soil matrix, the so-called drilosphere (Bouché 1975) can be a potential transfer zone for lateral water transfer between earthworm burrows and the surrounding soil matrix (Bastardie et al. 2005; Sander and Gerke 2009). Thus, understanding the factors governing earthworm burrowing behaviour is of particular interest as their activities influence soil hydraulic properties. However, our knowledge of the effects of earthworms on soil hydraulic properties are still fragmentary since their impact on these processes have only been reported for a few species all belonging to the Lumbricidae family (Blouin et al. 2013), whereas other species and families were comparatively ignored.

Historically, earthworms have been classified into functional groups according to their behaviour and morphology (Bouché 1971). Surface-feeding species (anecic species) produce one to several burrow openings on the soil surface to access litter. In contrast, geophageous species (endogeic species) ingest a mixture of soil–organic particles while burrowing below the soil surface. According to this classification anecic species are expected to enhance water flow to deeper soil depths, whereas endogeic species are expected to favour diffuse infiltration into the topsoil.

There is evidence that soil organic matter (SOM) content controls the burrowing activity of earthworms. It is observed that endogeic earthworms respond to decrease food availability in soil by increasing their consumption rate (Evans 1947; Hughes et al. 1996; Martin 1982). Conversely, anecic species are less dependant from SOM content since their main C resources come from the litter (Curry and Schmidt 2006). Then, any changes in SOM content might impact differently the burrowing activity of anecic and endogeic earthworms and consequently their influence on water transfer.

The aim of this study was to provide a qualitative (3D images), quantitative (burrow characterisation) and functional characterisation (saturated hydraulic conductivity) of the burrow systems made by two Asian earthworm species: the endogeic *Drawida sinica (*Moniligastridae) and one undescribed anecic *Amynthas* species (Megascolecidae). For this, we used two clayey soils presenting different soil organic C content and repacked soil columns. Because of their different feeding behaviour, we hypothesised that burrowing activity of the endogeic species would be more influenced by soil type and especially soil organic C than the anecic species.

Material and methods

Soils, microcosms and earthworms

Topsoils (0–25 cm depth) were collected from a Ultisol in a garden close to the Ecological Experimental Station of Red Soil, Jiangxi Province, of China (N 28° 12′ 19″ E 116° 55′ 56″) and a Vertisol from Huaiyuan County, Anhui Province of China (N 31° 48′ 33″ E 117° 10′ 30″). Both soils had loamy-clayed texture but the coefficient of linear extensibility index was higher for the Vertisol than the Ultisol (0.05 vs. 0.03). The pH_{H20} was lower in the Ultisol than in the Vertisol (5.3 vs. 7), and the soil organic C content was twofolds higher in the Ultisol than in the Vertisol (15.4 vs. 8.3 g kg^{-1}). Soils were air-dried, crushed and sieved to between 0.3 and 1 mm for the Ultisol and between 0.4 and 1 mm for the Vertisol to obtain the same bulk density when moistened. Microcosms were constructed using PVC cylinders (20 cm in length and 10 cm in internal diameter). Soil was compacted manually stepwise in five layers of 3 cm height. Once constructed, water was added dropwise from the top of the columns to reach 70% of the field capacity. The final dry bulk density was checked by measuring the height of the soil and resulted to 1.15 g cm^{-3} for both soils. Mature specimens of *Drawida sinica* (Moniligastridae) and one undescribed *Amynthas* species (Megascolecidae) were collected in the Ultisol. *D. sinica* was assumed to be endogeic because of its relatively small size (0.5 g) and pink colour whereas the *Amynthas* species was assumed to be anecic due to its large size (5.2 g) and antero-dorsal brownish pigmentation. The earthworms were kept at 25 °C for 1 week in each type of soil prior to the start of the experiment and were supplied with rice straw. Monospecific earthworm species were introduced at the surface of the cylinders at a rate similar to those found in the field, i.e. three *D. sinica* and one *Amynthas* species per column. We also used rice straw as the organic matter resource for earthworms in the microcosms. The rice straw was air-dried before being ground to a maximum width of 5 mm and was added at 2 g dry weight per microcosm at the soil surface. To remoisten the soil and litter, each microcosm received 100 ml distilled water every week. In total, 24 microcosms were set up: (two earthworm species + one control without earthworm) × two types of soil × four replicates. Microcosms were kept at 25 °C in the dark for 30 days. At the end of the incubation period, surface casts and rice straw were weighed, after being oven-dried for casts and air-dried for straws. Then, chloroform (10 ml) was applied to each column to kill the earthworms and prevent them from burrowing.

Belowground activity and water infiltration

Columns (soil within the PVC) were scanned with a medical computed tomographic scanner (Discovery CT750 HD, GE, USA). The scanner was run with the excitation voltage of 120 kV and a current of 600 mA. Resolutions were 0.6 mm in the vertical direction and 0.3 mm in the horizontal direction. Each column was scanned horizontally with 512 pixels × 512 pixels. Images were analysed using Fiji software (Schindelin et al. 2012). Segmentation of the images was performed with the Otsu method. Macropores that were smaller than a set size (less than 537 voxels, i.e. 27 mm^3), which represented on average less than 2% of the porosity, were discarded. For each soil column, the following burrow characteristics were calculated: volume of all burrows and those connected from the top to the bottom of the columns (here referred to as percolating burrows), burrow number, orientation and connectivity index. The orientation of each burrow was represented by the angle between the maximum Feret diameter (i.e. the longest distance between two parallel lines enclosing the burrow) and the XY plane. The mean orientation of burrows was calculated as the sum of the volume fraction of burrows multiplied by the orientation. The global connectivity index (%) was calculated as the ratio of the largest burrow volume and the total burrow volume (Dal Ferro et al. 2013).

Columns were saturated by capillarity, and the saturated hydraulic conductivity was determined using a constant-head device (a Marriot bottle) with similar apparatus to that reported by Bastardie et al. (2003).

Statistical analysis

Burrow system characteristics were log-transformed to gain homoscedasticity when required. We used two-way ANOVA and post hoc LSD tests with species and soil type as factors. Relationships between burrow characteristics and saturated hydraulic conductivity were built up using regression analysis with the procedure glmulti to select best-fit models according to Akaike's information criteria (AIC). The routine fits all combinations of linear models up to a maximum of predictors and selects the one with the lowest AIC value. All statistical calculations were carried out using RStudio software (2015).

Results

Surface activity

On average, the *Amynthas* species reduced litter by 40% compared to the control soil and produced on average 360 mg g worm^{-1} day^{-1} of casts regardless of the soil type ($p > 0.05$ for both parameters). In contrast, *D. sinica* did not incorporate litter into the soil and did not produce surface casts.

Burrow characteristics

Representative burrow systems for both earthworm species in the Ultisol and Vertisol are shown in Fig. 1. The *Amynthas* burrow system was clearly different from *D. sinica* due to the larger burrow diameter, greater continuity and marked verticality on the top part of the cores. The burrow systems of each species appeared visually similar in both soils. Quantification by image analysis confirmed this first visual inspection (Table 1). The *Amynthas* species produced a significantly higher volume of burrows ($P < 0.01$), which were more vertically oriented ($P < 0.01$), more connected from the surface to the bottom of the columns ($P < 0.01$) and overall more globally connected (connectivity index ($P < 0.01$)) than those of *D. sinica.* In contrast, the number of burrows was significantly smaller than that produced by *D. sinica* ($P < 0.01$). The soil type did not affect the *Amynthas* burrow systems ($P > 0.05$ for every parameter) whereas *D. sinica* produced 30% more burrows in the Vertisol than the Ultisol ($P < 0.05$) with similar other burrow characteristics ($P > 0.05$ in every case).

Saturated hydraulic conductivity

The K_{sat} (Table 1) was the highest in soil columns with *Amynthas* species, intermediate with *D. sinica* and the lowest in the control ($P < 0.01$), in both soils. Soil type did not influence the K_{sat} measured in columns with the *Amynthas* species or *D. sinica* ($P > 0.05$ in both cases). Results of linear regression analysis followed by model selection revealed that the volume of percolating burrows was the best predictor for all columns ($n = 16$, $R^2 = 0.80$, $P < 0.001$) and for *Amynthas* species ($n = 8$, $R^2 = 0.61$, $P < 0.05$). The number of burrows was the best predictor in columns with *D. sinica* ($n = 8$, $R^2 = 0.44$, $P < 0.05$).

Discussion

The investigation of the surface and belowground activities of the two species confirmed their classification in two functional groups. *D. sinica* activity occurred mainly belowground, since rice straw added on the soil surface was not ingested, and no surface casts were found at the end of the experiment. Their burrows were sub-horizontally oriented, hardly ever connected from the top to the bottom of the columns, numerous and characterised by a small global connectivity index. On the other hand, the *Amynthas* species fed at the soil surface and deposited a significant amount of casts. Their burrows were sub-vertically oriented, well connected from the surface to the bottom of the columns with a high global connectivity index. These results are consistent with the well-acknowledged distinction between anecic and endogeic burrowing strategies (Capowiez et al. 2015). These different burrowing strategies had a strong impact on the K_{sat}. The *Amynthas* species significantly increased the water infiltration compared to the control soil whereas *D. sinica* had a smaller effect. This result was associated with the large volume of percolating burrows and therefore confirms the important role of anecic species in transferring water by gravity when the soil is saturated with water (Capowiez et al. 2015).

Our study showed that only the endogeic species *D. sinica* was influenced by soil type, confirming our hypothesis. *D. sinica* produced 30% more burrows in the Vertisol compared to the Ultisol while other burrow characteristics were unaffected. Because the burrow volume was constant, this indicated that burrows were more refilled with casts leading to an increase in shorter and discontinuous burrows. Our findings raise the question of which properties in our experimental conditions contributed to such an increase in casting activity. Soils were different

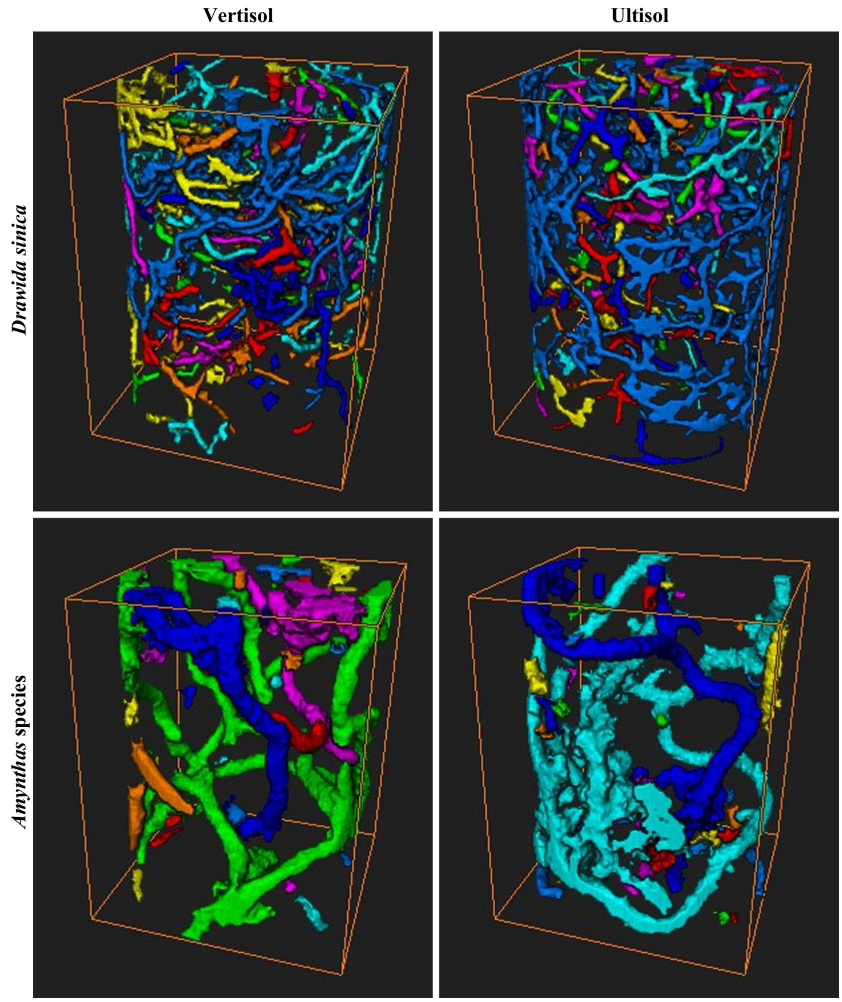

Fig. 1 Examples of 3D reconstructions of the *Drawida sinica* and *Amynthas* species burrow systems in repacked soil cores (15 cm in height and 10 cm in diameter) after 30 days. Different burrow colours indicate distinct individual burrow systems

in terms of pH (5.3 vs. 7) and soil organic C content (8.3 vs. 15.4 g kg^{-1}). Soil organic matter is a source of nutrients for endogeic species, and it is likely that in our experiment, a decrease in soil organic C content led to increased soil ingestion. In contrast, it is unlikely that the neutral pH found for the Vertisol altered *D. sinica* behaviour. Our study therefore confirmed previous reports carried out in 2D microcosms (Evans 1947; Hughes et al. 1996; Martin 1982) showing that endogeic species respond to decreased food availability in Vertisol by increasing soil consumption, leading to more casts in burrows and consequently more discontinuous burrows. Even if the number of burrows of *D.sinica* was negatively related to the K_{sat}, the type of soil did not significantly affect water infiltration, probably because changes were too small.

Table 1 Means + SD burrow characteristics and the saturated hydraulic conductivity values (K_{sat}) for each species and control (columns without earthworms). Percolating burrows are defined as the volume of burrows connected from the top to the bottom of the columns. The global connectivity index (%) was calculated as the ratio of the largest burrow volume and the total burrow volume. Values bearing different letters are statistically significantly different

Earthworm	Soil	Volume (cm^3)	Percolating burrows (cm^3)	Global connectivity (%)	Number	Orientation (°)	K_{sat} (10^{-5} m s^{-1})
Amynthas	Ultisol	86.7 (21.7) a	78.1 (16.6) a	84.3 (15.1) a	23.5 (9.8) c	48.9 (7.4) a	28.5 (15.8) a
	Vertisol	81.7 (26.6) a	68.1 (30.1) a	82.1 (16.6) a	25.3 (7.8) c	52.5 (4.5) a	39.1 (8.4) a
Drawida	Ultisol	51.2 (6.1) b	7.6 (9.1) b	27.7 (7.8) b	138.8 (16.5) b	38.2 (4.5) b	10.1 (3.7) b
	Vertisol	49.1 (7.5) b	1.3 (2.7) b	28.3 (16.2) b	187.1 (22.7) a	34.9 (5.0) b	7.3 (2.4) b
No	Ultisol	–	–	–	–	–	0.3 (0.3) c
	Vertisol	–	–	–	–	–	0.4 (0.4) c

Conclusions

If Lumbricidae earthworms are acknowledged for their positive influence on water infiltration in temperate ecosystems, this study showed that burrows produced by Moniligastridae and Megascolecidae species also increase the K_{sat} in tropical soils. Our study also confirmed the key role played by anecic species in comparison with endogeic ones (in our case *Amynthas* species vs. *Drawida sinica*) and suggested that a 50% decrease in soil organic C has a small impact on earthworm burrowing activity and no effect on the K_{sat}. A better understanding of the way soil properties affect earthworm burrowing activity would help predict the effects of management practices and land use on soil functioning.

Acknowledgments Dr. Bottinelli thanks the Chinese Academy of Sciences Fellowships for Young International Scientists (Code: 2012Y1ZB003) for supporting his stay in China.

Funding information This work was financially supported by the Natural Science Foundation of China (Code: 41371235, 41350110532 and 41471183) and National Key Research and Development Program (2016YFD0300809).

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