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Quantitative estimates of burrow construction and destruction, by anecic and endogeic earthworms in repacked soil cores

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ABSTRACT

Although the role of earthworms in soil functioning is often emphasised, many important aspects of earthworm behaviour are still poorly understood. In this study we propose a simple and cost-effective method for estimating burrow system area and continuity, as well as a new and often neglected parameter, the percentage of burrow refilling by the earthworms own casts. This novel parameter is likely to have a huge influence on the transfer properties of the burrow system. The method uses standard repacked soil cores in PVC cylinders and takes advantages of clay shrinkage and the fact that earthworms were previously shown to prefer to burrow at the PVC/soil interface. In this way, after removing the PVC cylinders off dry cores, the external section of the burrow system made by earthworms along the soil walls could be easily described. We applied this method to characterise the burrow systems of four earthworms species: two anecics (*Aporrectodea caliginosa nocturna* and *Aporrectodea caliginosa meridionalis*) and two endogeics (*Aporrectodea caliginosa icaliginosa* and *Allolobophora chlorotica*). After one month the burrow's area generated by both anecic species were much larger (about 40 cm^2) than the endogeic burrow's area (about 15 cm^2). *A. nocturna* burrow system continuity was higher than that of *A. meridionalis* and both anecic burrow systems were more continuous than those made by the endogeic earthworms. This was partly explained by the far larger proportion of the burrow area that was refilled with casts: approximately 40% and 50% for *Al. chlorotica* and *A. caliginosa*, respectively compared with approximately 20% for the anecic burrows. We discuss whether these estimates could be used in future models simulating the dynamics of earthworm burrow systems by taking into account both burrow creation and destruction by earthworms.

1. Introduction

The important role of earthworms in soil functioning has resulted in their status as soil 'ecosystem engineers' (Jones et al., 1994; Jouquet et al., 2006). In particular, earthworms are known to be able to modify soil transfer properties for water, nutrients or pollutants by creating burrows (McCoy et al., 1994). These modifications are dependent on the ecological type of earthworm under consideration (Shipitalo and Le Bayon, 2004) in link with different burrowing behaviour (Lee and Foster, 1991). However, to date, many important aspects of their behaviour and some important effects resulting from this behaviour are not well characterised. The main reason for this lack of knowledge is the difficulty to directly observe their behaviour since most earthworms (excluding epigeics) are hidden in the soil most of the time (excluding the foraging, dispersion and reproduction phases of *Lumbricus terrestris*). This results in a surprising absence of models in soil biology (Barot et al., 2007) especially compared with marine biology where a number of simulation models based on processes (bioturbation by different organisms) and the resulting structures (burrow morphology) are available to simulate O_2 exchange in sediments for example (Koretsky et al., 2002).

Some methods are now available and widespread to study the underground behaviour of earthworms such as X-ray tomography (Joschko et al., 1993; Capowiez et al., 1998; Bastardie et al., 2003) or radio- or lead-labelling of earthworms (Capowiez et al., 2001; Bastardie et al., 2005; Caro et al., 2012). However these methods still require some technical skills in image analysis and this limits the amount of published data on earthworm behaviour and thus prevents estimation of its variability. There is therefore a need for a fast, cost-effective and easy methodology that could be used to study differences between earthworm species and the possible influence of biotic and abiotic factors on this behaviour. 2D terrariums have some of these advantages (Schrader, 1993) but are generally limited to short-term observations. This method is also likely to overestimate interactions between earthworms since due to the limited

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space available avoidance between individuals is clearly difficult (Capowiez, 2000).

Although the link between soil water transfer and macropores in general, and earthworm burrows in particular, has been widely accepted for decades (Bouma, 1991; McCoy et al., 1994), some aspects of the so-called macropore bypass are still largely unknown (Nimmo, 2012). The important parameters involved in this relationship are burrow density, distribution with depth and morphology (orientation, continuity topology, and tortuosity). It is however generally accepted that burrow continuity is the crucial factor (Ela et al., 1991, 1992; Trojan and Linden, 1992; McCoy et al., 1994). This characteristic cannot be estimated with classical field observation based on successive 2D planes (unless methylene blue dye is used). Even in 3D, burrow continuity is notoriously difficult to characterise and there is no standard definition of this parameter. For example, for Langmaack et al. (1999), burrow continuity was simply estimated by the longest vertical distance of any burrow whereas Capowiez et al. (2006) computed the number of continuous pathways (burrows) between successive vertical planes across cores.

In this paper, we describe a simple and cost-effective method for quantitative estimation of several key characteristics of earthworm burrow systems (burrow area, percentage of burrow refilling and burrow continuity). The method was used to characterise the burrows of four earthworm species currently found in the south of France. The earthworms belonged to different ecological types (anecic and endogeic) since it is often assumed that the behaviour of the ecological types are different (Lee and Foster, 1991) and sometimes postulated that these behaviours are homogeneous within ecological types.

2. Materials and methods

2.1. Earthworms and repacked soil cores

The soil for the experiment was obtained from the first 20 cm of topsoil (30.2% clay, 48.7% silt and 21.1% sand; 5.1% organic matter; $pH_{H_2O} = 8.3$) in an abandoned orchard in Montfavet, near Avignon (43°55′ N, 4°48′ E) in the SE of France. Adult earthworms were sampled by hand in the same orchard and stored for less than 24 h in a dark chamber (at 12 °C) before being introduced into the soil cores. In this orchard, the earthworm density was about 450 individuals m^{-2} and the most abundant earthworms were two endogeic species, *Allolobophora chlorotica* (yellow form) and *Aporrectodea caliginosa* and two anecic species, *Aporrectodea caliginosa nocturna* and *Aporrectodea caliginosa meridionalis* (Bouché, 1972). The size and diameter of the two endogeic species was 50–80 mm and 3–7 mm for *Al. chlorotica* and 60–80 mm and 3.5–4.5 mm for *A. caliginosa* (Bouché, 1972). The two anecic species have a marked dark pigmentation and are typically for the south of France. They ranged in size and diameter from 85 to 110 mm and 2.5 to 4 mm for *A. meridionalis* and 90 to 180 mm and 4 to 5 mm for *A. nocturna* (Bouché, 1972).

The soil was sieved at 2 mm and stored for a few days in a dark chamber at 12 °C. Soil cores were prepared using PVC cylinders (35 cm in length and 16 cm in diameter). The PVC walls were not lined with sand and varnish (i.e. no attempt was made to prevent earthworms from burrowing along the PVC walls). A hydraulic press was used to compact five cores simultaneously by applying a pressure of 270 kPa for 4 min on sieved soil at 20% moisture content (gravimetric). This treatment resulted in a soil dry bulk density of 1.1 g cm^{-3}. To minimise variations in soil bulk density between the top and bottom of the cores, the soil was compacted stepwise in 12 layers of 600 g of soil. The final thickness of each layer was approximately 2.5 cm. Before adding a new soil layer, the surface of the previous layer was gently scratched using a small rake to increase cohesion between layers. The bottom of each core was sealed and the top was closed using a lid with small holes to prevent significant water loss and earthworm escape. Before the introduction of the earthworms, 100 ml of distilled water was gently poured into the soil. A total of 24 cores were used: six cores for each species. No litter was added to the soil surface to increase earthworm burrowing (Hughes et al., 1996). For each species, three adult earthworms were introduced into each core corresponding to a density of approximately 200 individuals m^{-2}. Average earthworm weight was 2.46, 0.85, 0.62 and 0.41 g for *A. nocturna*, *A. meridionalis*, *A. caliginosa* and *Al. chlorotica*, respectively. At the end of the incubation period (4 weeks), casts at the soil surface were sampled, dried and weighed. Then the earthworms were killed by pouring 15 ml of chloroform into each core. This ensured that no earthworm activity could occur inside the soil cores after the incubation period.

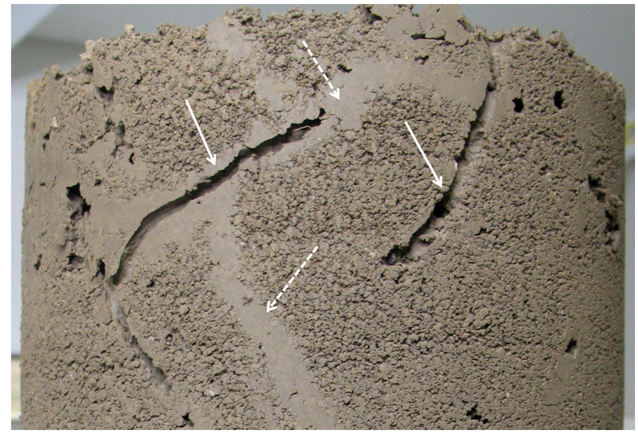

Fig. 1. Photo of a section (height = 10 and width = 16 cm) of the burrow system made by *A. meridionalis* along the soil core wall. Arrows and broken arrows indicate open burrows and refilled burrows, respectively.

2.2. Quantification of earthworm burrows and refilling

The cores were placed on absorptive tissue and left to completely dry in the laboratory for two months at between 20 and 25 °C. Due to the type of clays found in this soil (montmorillonite), the soil cores inside the PVC shrank by a few mm. It was thus possible to remove the PVC around the soil core with minimal disturbance to the cores. Since the cores were not lined with sand, a large proportion of the burrows were made along the PVC walls (Kretzschmar, 1990). Using a transparent sheet of plastic placed around the soil core, the open and refilled burrows (Fig. 1) on the outside surface of the cores were traced with different colour pens (red and blue, for open and refilled macropores, respectively). Burrow refilling was assessed with a metal pin to distinguish genuine refilled burrows from burrows with casts crushed along the burrow walls.

A small ruler was fixed to the transparent sheet and the burrow traces drawn on the transparent plastic sheet were digitised using a colour scanner (resolution = 200 dpi). Images were processed using ImageJ (http://rsb.info.nih.gov/ij/). The RGB coloured images were first separated into three different images (one red, one green and one blue). The red and blue images were scaled in cm using the ruler and filtered by removing all objects that were smaller than 2000 pixels (0.32 cm^2). On each image, the number of objects, their area and their vertical extension were computed. Finally three characteristics of the burrow system were computed: the percentage of burrow refilling (area of the refilled burrows divided by the total area of burrows refilled or not * 100), the mean area of open burrows (excluding refilled areas) and the maximal continuity of the open burrow system (the largest vertical extension). We chose to compute the latter two characteristics on only the open sections of the burrow systems because we were interested in the potential efficiency of these burrows regarding water infiltration. To investigate whether burrow refilling was constant as a function of depth, the percentage of burrow refilling was also computed for each third of the cores.

2.3. Statistical analysis

Characteristics of the burrow system were log-transformed to gain homoscedasticity when required. Percentages were transformed by the arcsin function. Normality was visually checked (by Q–Q plots). Since the data did not diverge too far from the assumption of normality and the experimental design was balanced ($n = 6$ for each species), an analysis of variance was performed (Zar, 1984). Each parameter (burrow area, percentage of burrow refilling and maximal vertical continuity) was analysed with a one-way ANOVA with earthworm species as the main factor. All computations were made using R.

3. Results

3.1. Surface casts and burrow refilling

The average surface casts per earthworm weighed 8.23 g, 11.23 g, 10.31 g and 3.16 g (dry weight) for *A. nocturna*, *A. merdionalis*, *A. caliginosa* and *Al. chlorotica*, respectively. Because surface casts (weight) and subsurface casts (area) were computed on a different basis, it was not possible to compute the percentage of surface casts made by each species.

The percentage of burrow refilling (based on the relative area of open and refilled burrows) was very similar for the two anecic species (approximately 20%) (Fig. 2A) and was almost constant with depth (Fig. 3). The intensity of burrow refilling was significantly higher for both endogeic species, close to 50% for *A. caliginosa* and approximately 40% for *Al. chlorotica* and was observed to decrease with depth (Fig. 3).

3.2. Other characteristics of the burrow systems

The mean burrow area produced by each individual earthworm was significantly higher for both anecic species (approximately 40 cm^2) than for either of the endogeic species (between 15 and 20 cm^2) (Fig. 2B).

Continuity of the burrow system was assessed by computing the maximal vertical extension of open burrows in each core. Burrows of *A. nocturna* showed the most continuity (with a mean maximal vertical extension of 12 cm), significantly higher than those of *A. meridionalis* (9 cm). Overall, burrow system continuity was significantly higher for the anecic species than for the endogeic species, with a mean value of 6 cm for *A. caliginosa* and *Al. chlorotica* (Fig. 2C).

4. Discussion

Earthworm behaviour has not yet been extensively studied, with, for example, less than 20 publications in the last decade reporting the characterisation of earthworm burrowing behaviour. The putative behaviours were first summarised in Lee and Foster (1991) but in their review, only general differences between anecic and endogeic were described. However, as far as burrowing is concerned, several recent studies revealed that ecological type is a poor predictor of behaviour and thus of the resulting burrow system (Bastardie et al., 2005; Felten and Emmerling, 2009). These two

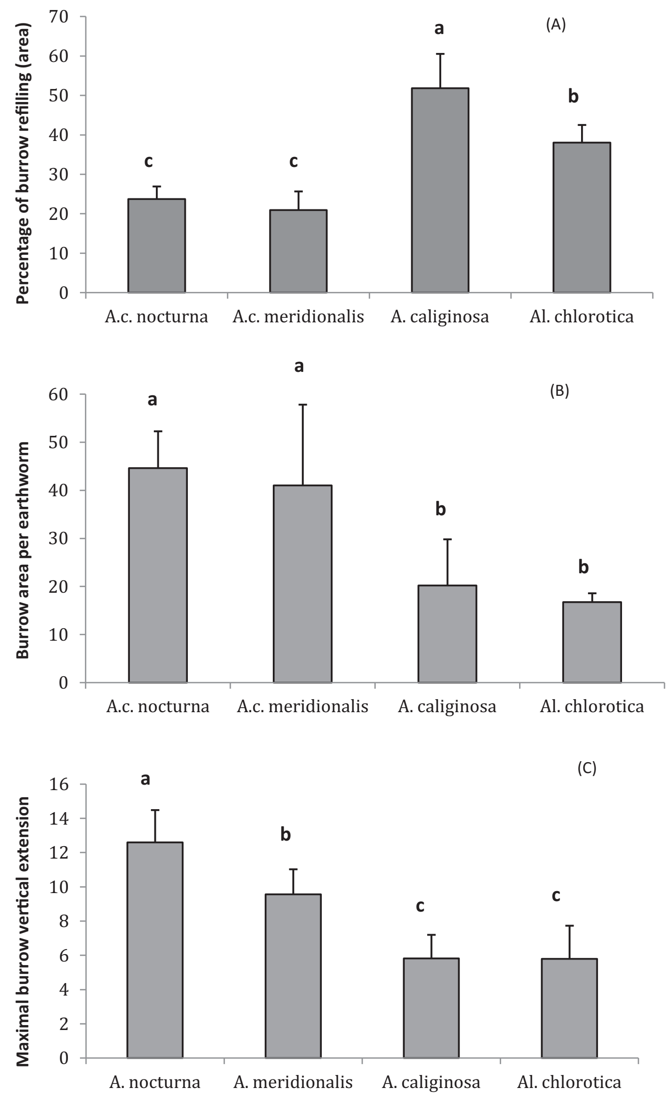

Fig. 2. Characteristics of the burrow systems (means and standard deviations) estimated along the external core walls: percentage of the burrow area refilled with casts (A), burrow area (B) and maximal vertical burrow continuity (C). Different letters above the bars indicate that the results are significantly different at the 5% level.

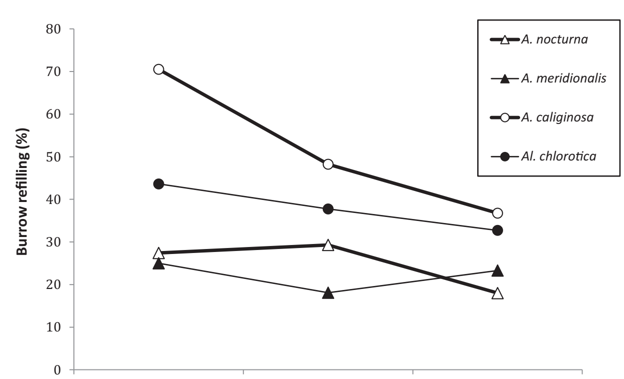

Fig. 3. Distribution with depth of the intensity of burrow refilling for the four earthworm species.

last studies concluded that some species have clearly intermediate positions between the three ecological types.

Our results for the mean burrow area suggest that differences between the burrows made by the different species are more dependent on ecological type than respective weight. *A. nocturna* is far larger and wider than *A. meridionalis* but they produced the same area of open burrows (taking into account that we observed the same percentage of burrow refilling for both species). However, burrows of the larger anecic species (*A. nocturna*) showed significantly more continuity than burrows of the smaller species. For both these parameters, burrows of the two endogeic species had very similar characteristics. Overall burrow continuity, assessed as the maximal vertical extension of the burrows seen on the external core walls, was linked with mean earthworm length.

Some specific consequences of earthworm behaviour were rarely studied and thus remain almost totally unknown. This is the case of burrow refilling with casts. The notable exceptions are the studies of Schrader (1993) and Ligthart (1997). Although Hindell et al. (1994) and Whalen et al. (2004) did report the relative proportion of subsurface casts, burrow refilling was not estimated in these studies. Burrow refilling is a dynamic process that can be seen from two opposite points of view. On one hand, it contributes to the reformation of soil aggregates in the soil profile and on the other hand, it can modify transfer properties of burrows. It thus is surprising that a phenomenon with such crucial implications for soil dynamics has not been studied in more detail. Refilling transforms a continuous pathway into separated burrows and it is one of the main factors leading to burrow disintegration except in the first cm where trampling can also have a major role according to Ligthart (1997). Other physical processes can also contribute to the slow destruction of burrows such dry/wet and thaw/cycles (Drewry, 2006). However as soon as a burrow is disconnected into several smaller burrows, it is likely that its life span is reduced whereas open and continuous burrows are cleaned, repaired, and consolidated by earthworms, especially anecics. Furthermore, their transfer properties are greatly reduced (Allaire-Leung et al., 2000) particularly if the casts that refilled these burrows have a greater soil bulk density as shown by Milleret et al. (2009) for *Al. chlorotica*.

At a first glance, the results presented here about burrow refilling are far from unexpected. It is well known that anecics reuse their burrows intensively, thus producing more continuous burrows compared with endogeics (Jégou et al., 1998; Capowiez et al., 2001). Indeed endogeic earthworm burrows are rather simple pathways, rarely reused and thus regularly refilled with casts (Schrader, 1993, Jégou et al., 1998; Langmaack et al., 1999) since these earthworms produce casts less frequently at the soil surface (Shipitalo and Le Bayon, 2004; Bottinelli et al., 2010; Jouquet et al., 2011). However knowledge of these behaviours is qualitative (for example based on simple images of 3D reconstructions of soil macroporosity) and differences between species inside a same ecological type are still largely unknown (Caro et al., 2013).

The striking result of our study is the low variability in the values measured for the intensity of burrow refilling, burrow area and continuity (see standard deviations in Fig. 1). This is rather unexpected when considering animal behaviour. This suggests that these results could indeed have a mechanistic basis. Regarding burrow refilling, the volume of casts is the volume of soil ingested multiplied by the cast bulk density. The lower values obtained for the anecic thus imply that (i) the casts of endogeic species are less dense; or (ii) anecic casts are crushed more against burrow walls (thus forming cutanes); or (iii) anecics burrow by pushing the soil aside rather than ingesting it. Here again, basic knowledge of earthworm ecology is lacking and this prevents us from favouring one of these assumptions.

5. Conclusions

To date, the dynamics of burrow construction and destruction and the resulting balance between these two processes, is still poorly understood. These gaps in knowledge regarding earthworm behaviour are due to a lack of simple observation methods; we believe that the method presented here can be used by most soil biologists to provide insights into (i) the behaviour of different earthworm species (or life stages), (ii) the variability due to intra or inter-specific interactions and (iii) the variability due to variability in abiotic factors such as soil bulk density, temperature, soil content in organic matter. These data could then be incorporated into existing simulation models (Bastardie et al., 2002; Blanchart et al., 2009) or provide a basis for new models.

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