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Depth distribution of soil organic matter and burrowing activity of earthworms—mesocosm study using X-ray tomography and luminophores

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Abstract

Earthworms feed on organic matter present at the soil surface or within the soil. Thus, its distribution in the soil profile is likely to greatly influence earthworm behavior and, in turn, their burrow system. To test this idea, two anecic and two endogeic earthworm species were introduced into repacked soil cores (depth = 30 cm) upper half filled with a top soil containing 4% organic matter (0–15 cm) and lower half filled with a deep soil at 2% organic matter (15–30 cm). Earthworm behavior was studied using X-ray tomography combined with luminophores (colored particulate tracers of 63–125 μm size) placed at 0, 3, and 12 cm depth, a method widely used in sediment ecology. We observed that anecic and endogeic earthworms had contrasting reactions to the conditions with only endogeic species burrowing more intensively in the upper part. From a quantitative point of view, only a few percent of luminophores were displaced. However, luminophore displacements also provided qualitative information to complement the tomography: (i) endogeic species and especially *Aporrectodea caliginosa* bioturbated the most soil close to the surface (3 cm depth) and (ii) the two anecic species influenced the luminophore distribution differentially with *Lumbricus terrestris* displacing significantly more luminophores, whatever their initial depth, than *Aporrectodea nocturna* due to intense surface cast activity. Beyond methodological developments, our study found that endogeic earthworms burrow more in zones with higher organic matter contents and this explains why they are mainly found close to the soil surface in non-tilled soils.

Keywords Behavior · *Lumbricus terrestris* · *Aporrectodea nocturna* · *Aporrectodea caliginosa* · *Apporrectodea icterica*

Introduction

Due to their activities, earthworms play many important roles in the soil (aeration, infiltration, organic matter burial). However earthworm behavior (surface activities, burrowing, casting) has not yet been fully described for all species and is generally simplified using ecological categories (anecic, endogeic, or epigeic) despite their limitations (Bottinelli et al. 2020). There is thus a need for a better characterization of the behavior of the common earthworm species. This is an important first step for the development of simulation models either to test assumptions about interactions between earthworms or to estimate the functional consequences of earthworm activities at different temporal or spatial scales (van Schaik et al. 2014).

In the soil, biotic such as intra- and interspecific interactions and abiotic conditions influence earthworm behavior (Uvarov 2017). The major abiotic factors that are known to govern earthworm behavior are organic matter (type and location), soil bulk density, pH, temperature, and soil water content (Hughes et al. 1996; Rushton 1986; Auclerc et al. 2013; Moreau-Valencogne et al. 2013; Le Couteulx et al. 2015; Frazao et al. 2019). If the anthropic influences such as tillage are disregarded, it is obvious that earthworms faced sharp gradients

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in organic matter in the soil with litter at the soil surface and then decreasing and limited concentrations with depth (Lee 1985). Since organic matter is earthworm food and endogeic earthworms do not generally forage at the soil surface, these gradients are assumed to greatly influence endogeic behavior, as showed in 2D terraria by Cook and Linden (1996) and Hughes et al. (1996). It is thus noteworthy that in most studies designed to investigate earthworm behavior in repacked soil cores, a unique and homogenized soil horizon was used (Joschko et al. 1993; Jégou et al. 1998; Bastardie et al. 2005a; Capowiez et al. 2011; Bottinelli et al. 2017).

In soil science, the use of X-ray tomography to describe earthworm burrow systems within the soil has gained popularity due to the suitability and worldwide availability of medical scanners worldwide (Taina et al. 2013). There is also no need to use micro-tomography or industrial scanners to observe earthworm burrows as their diameter is pluri-millimetric (Gutiérrez et al. 2018). In cores sampled in natural soils, the difficulty is to assess which porosity is due to earthworms and which is due to physical perturbation or other soil inhabitants (Bastardie et al. 2005b; Taina et al. 2013; Pelosi et al. 2017). In repacked soil cores, this task is much easier and thus additional information can be gained about what happens in or around these burrows in terms of soil lateral compaction or burrow refilling (Rogasik et al. 2014; Capowiez et al. 2015). A set of very diverse measurements is then available to describe volumes, length, continuity, and branching pattern (Capowiez et al. 2001).

In aquatic ecology, scientists studying benthic biological bioturbation activity (i.e., the mixing of sediments particles by benthic fauna) face the same problem as soil biologists in that the particle displacement of soil or sediments cannot be directly observed since the soil or sediment matrix conceals the animals. Thus, they have also developed indirect tools to characterize particle transport due to benthic activities (Gérino et al. 1998; Schmidt et al. 2007; Lagauzère et al. 2011). One of the most current methods in aquatic ecology is the use of conservative fluorescent particulate tracers, such as luminophores (sand stained with fluorescent colors) (e.g., Mahaut and Graf 1987; Gilbert et al. 2007). Generally, fluorescent luminophores are initially deposited at the sediment surface (e.g., Maire et al. 2008; Hedman et al. 2011), and sometimes at different depths (Gilbert et al. 2007; Foulquier et al. 2010). At the end of the experiment, sediments are sectioned into cm-thick layers and then luminophores are identified and counted under UV light in subsamples for each sediment layer in order to construct tracer profiles (Black et al. 2007; Duport et al. 2006; Lagauzère et al. 2011; Anschutz et al. 2012). From a qualitative point of view, along with species behavioral knowledge, the use of such profiles allowed to define bioturbation functional groups (Kristensen et al. 2012). In the same time, the quantification of benthic bioturbation activities was developed and various models,

from mono-coefficient (Boudreau 1986; Anschutz et al. 2012) to multi-coefficients models (e.g., François et al. 2002; Gérino et al. 2007), were built to analyze the tracer profiles. This destructive method is one of the most widely used, although in recent years, some authors have developed new imaging techniques based on nondestructive screening of the luminophores at high spatial resolution over time (Gilbert et al. 2003; Solan et al. 2004; Lindqvist et al. 2013). To our knowledge, conservative particulate tracers, such as luminophores, have rarely been used to measure the macrofauna (e.g., earthworms) biological bioturbation activities in soil. While McTavish et al. (2019) have recently used fluorescent tracers, they applied it to assess the burial of surface-applied wood ash by *Lumbricus terrestris* in a forest soil.

In this study, our aims were (i) to determine whether different soil layers with different organic matter contents influence the behavior of four common earthworm species, two anecic and two endogeic, using X-ray tomography; and (ii) to assess whether luminophores could also be used in soil biology to estimate the range and intensity of soil bioturbation by earthworms.

Material and methods

Soil and earthworm sampling

Soil and earthworms were collected from an abandoned apple orchard in Montfavet, near Avignon (SE of France). Soil was manually collected with a shovel and carefully separated into top soil (0–15 cm depth) and deep soil (15–30 cm depth). Each soil layer was then sieved to 3 mm and homogenized excluding roots and litter. The main characteristics of each soil layer were determined by the Laboratoire d'Analyse des Sols (INRA https://www6.hautsdefrance.inra.fr/las) using standardized protocols and are presented in Table 1. The two

Table 1 Main characteristics of the soils sampled at different depths in the abandoned orchard and used to fill the soil cores

	Top soil (0–15 cm)	Deep soil (15–30 cm)
Clay (%)	19.7	18.8
Silt (%)	50.8	50.8
Sand (%)	29.5	30.4
pH (H$_2$O)	8.18	8.38
CEC (cmol+ kg^{-1})	8.48	6.09
Organic matter (%)	3.99	2.07
C/N	10.8	10.2
N (g kg^{-1})	2.15	1.18
P (g kg^{-1})	0.069	0.039
K (Olsen) (g kg^{-1})	0.174	0.186

soil layers were quite similar regarding soil texture, pH, and K content. However, CEC and organic matter, as well as the N and P contents, varied greatly. Even if the C/N ratio was similar, organic matter and N content were halved with depth. In the same orchard, four earthworm species belonging to two different ecological types, anecic (*Aporrectodea nocturna* and *Lumbricus terrestris*) and endogeic (*Allolobophora icterica*, *Aporrectodea caliginosa*), were collected by hand-sorting of topsoil.

Experimental procedure

Twenty-five experimental units were prepared using PVC cylinders (length, 35 cm; internal diameter, 16 cm) filled with soils. To prevent significant variations in compaction between the two extremities of the cores, 10 layers of soil were gradually added to each core. Each layer consisted of 900 g of soil at 19.5% water content pressed down with a hydraulic press (pressure 125 kPa for 5 min) leading to a final layer width of about 3 cm and a soil bulk density of 1.14 g cm^3. The surface of the each compacted layer was then gently scratched with a small rake to increase cohesion between layers before adding a new layer. In each core, five top soil layers were added after five deep soil layers leading to a final soil of 15 cm deep soil and 15 cm top soil (see Appendix Fig. A1).

In order to quantify soil bioturbation by earthworms, discrete layers (about 1 mm thick) of fluorescent inert tracers (luminophores; 10 g of 63–125 µm tracers) were homogeneously spread after compaction on the soil layers at 12 cm deep (pink color) and 3 cm deep (green color). In each core, a layer of orange tracers (10 g) followed by 2 g of wheat straw was homogeneously deposited at the soil surface (see Appendix Fig. A2). Preliminary tests showed that the four species were able to ingest large quantities of luminophores within the 63–125-µm range size.

To mimic monospecific communities, about 6 g of each earthworm species were added at the surface of soil cores so as to calculate bioturbation per g of earthworm irrespective of the species. Six cores were prepared for each species and only one core for a control (without earthworms), giving a total of 25 soil cores. Adult earthworms were cleaned with water, gently dried on a filter paper, weighted, and placed on the core surface, i.e., 2 *L. terrestris*, 3 *A. nocturna*, 8 *A. icterica*, and 11 *A. caliginosa*. Individual mean earthworm weight was 3.2, 2.1, 0.54, and 0.79 g for *A. nocturna*, *L. terrestris*, *A. icterica*, and *A. caliginosa*, respectively.

All cores were then incubated at 16 °C and 60% humidity in a controlled dark room. During the experiment, the cores were weighed every 5 days to determine water loss which was then compensated by adding distilled water. After 26 days of incubation, chloroform (10 ml) was applied on each soil core to kill the earthworms and avoid the creation of new burrows. Then, earthworm casts were carefully collected at the surface

of each soil, dried, weighed, and homogenized. Care was taken to manually remove any luminophores adhering to the casts. Cores were scanned using a medical scanner at the French National Institute for Agricultural Research (INRA, Nancy, France). Finally, the cores were vertically sectioned into 1-cm thick layers from the surface down to 15 cm depth, and into 2-cm thick layers down to 30 cm. The soil from each layer was removed separately, dried, weighed, and homogenized (see Appendix Fig. A3).

3D burrow system reconstruction

The medical scanner (BrightSpeed General Electrics) was set at 50 mA and 120 kV. The final resolution of the images was $0.4 \times 0.4 \times 1.25$ mm^3 (the last value is the spacing between images). These images were first transformed into 8-bit images (selecting grey levels between − 1000 and 2000 Hounsfield values) and then binarized using a simple threshold (70) since the grey level histogram showed two well-separated peaks (pores and soil matrix). The resulting 3D burrow system (macropores smaller than 0.5 cm^3 were removed) was then characterized using ImageJ and adapted macros. We computed the burrow volume and the estimated burrow diameter (taking into account only very circular pores, i.e., those whose circularity computed in ImageJ was higher than 0.8). The burrow system was then skeletonized using a simple overlap rule between pores in successive 2D images to obtain the burrow length and then the rate of burrow branching. We also computed the percentage of the burrow volume in the two core halves (0–15 and 15–30 cm).

Quantification of luminophore displacement in soil and casts

In order to quantify tracers from each soil layer and cast sample, a weighted subsample was homogeneously spread in a black dish (4×4 cm), which was placed inside a dark box ($50 \times 40 \times 40$ cm) and illuminated with two UV light tubes (Philips TL 8W/08 T5/BLU; emission peak at 365 nm). One photo was taken for each subsample, using a digital camera (Olympus C-2500L) fixed at the top of the box. Finally, the surface (pixels number) occupied by each colored tracer was quantified with an image analysis software (Image-Pro® Plus, Media Cybernetics; Gilbert et al. 2007) (see materials and methods in the Supplementary text for the detailed description: Fig. A.4). The data obtained was used to produce tracer repartition profiles and calculate tracers found in casts. The data were also used to calculate a particle displacement coefficient (PDC) for each core using a newly developed model. This PDC model allowed the vertical upwards and downwards cumulative displacement of particles to be calculated during a defined time by taking into account both the proportion of particles displaced (p, expressed as a ratio from the

total quantity in the soil) and the distance of displacement of particles from the initial deposit layer (d, expressed in cm) (see Appendix Fig. B). For each soil layer, except the initial deposit layer, a p*d value was calculated (unit, cm). Then, all the values within the soil column were summed and this sum was divided by the time period of bioturbation experiment (unit, day). To facilitate PDC value readability, the obtained value was multiplied by 1000 giving a final PDC unit of 10^{-3} cm day^{-1}.

$$PDC = 10^3 \sum p_i d_i t^{-1} \left(10^{-3} cm\ d^{-1}\right),$$
$$with \sum p_i = 1$$

where the index i refers to the layer number.

This formal measure of particle transport has been designed to be as general and simple as possible, thus allows comparing the transport intensity in very different situations. This data-based analysis can be calculated when profiles of tracers are available with a time reference, without any extra assumptions.

Statistical analysis

To analyze differences regarding characteristics of the burrow systems and cast production of the four earthworm species, we used a one-way ANOVA after log-transformation when heteroscedasticity was met. To analyze the effect of using two soil layers in the mesocosms, we visually compared the percentage of the burrow system found in the upper half of the core to the values found in a previous study using the same soil but only using top soil, richer in organic matter (Capowiez et al. 2015).

Due to the lower number of replicates (i.e., number of cores) and the apparent non-normality of the data, bioturbation values (particle displacement coefficient and percentage of luminophores found in surface casts) were analyzed with a Kruskal-Wallis test. In this case, each luminophore color was tested independently.

Results

Casts and 3D burrow systems

At the end of the incubation period, *L. terrestris* had produced a significantly higher amount of surface casts than the other three species (Table 2). One representative burrow system per species is shown in Fig. 1. We observed clear visual differences between the burrows of the two anecic and the two endogeic species. The endogeic species burrowed a lot in the upper half of the core (made with top soil) and made only a few burrows in the lower part of the core. In contrast, the anecic species burrowed in both parts of the core.

The quantitative characterization of the burrow systems made by each species showed significant differences between endogeic and anecic species (Table 2). The burrow systems of both endogeic species were longer (for a same total earthworm weight) than those of the anecic species. In addition, the burrows were more abundant, more branched, and had a larger diameter than those of anecic species. We also found that the endogeic burrow systems were more concentrated in the upper half of the cores and this was significantly different for *A. nocturna* (Table 2).

The results of this study were compared with those obtained under exactly same conditions but in homogenized soil (only top soil was used; Capowiez et al. 2015). This confirmed that both endogeic species modified their burrowing behavior when faced with a sharp gradient in soil organic matter content (Fig. 2). The percentages of burrow volume made by *A. caliginosa* and *A. icterica* in the upper halves of the cores were clearly reduced by approximately 20% whereas this percentage remained constant for anecic earthworms.

Luminophore displacement

Tracers deposited at different depths of the soil columns were both displaced within the soil and retrieved in the casts (Table 3 and Fig. 3). Overall, the amount of tracers displaced

Table 2 Mean (and SE) values for the main characteristics of the burrow systems and surface cast production made by the four earthworm species in the repacked soil cores. Values bearing different letters in each line are significantly different

|	A. caliginosa	A. icterica	A. nocturna	L. terrestris
Number of earthworms per core	11	8	3	2
Burrow volume (cm^3)	103.8^a (15.8)	113.9^a (25.4)	65.2^b (19.4)	48.8^b (17.5)
Number of burrows	17.6^a (3.4)	13.7^a (2.7)	11.3^a (5.9)	2.0^b (1.1)
Burrow length (m)	11.93^a (5.75)	7.95^{ab} (2.98)	3.99^{bc} (1.56)	1.83^c (0.91)
Burrow branching (m^{-1})	0.042^a (0.006)	0.037^a (0.002)	0.022^b (0.002)	0.016^b (0.008)
Burrow diameter (mm)	2.85^b (0.48)	3.07^b (0.62)	4.18^{ab} (1.24)	6.78^a (2.03)
Percentage of volume in the upper half (%)	85.7^a (2.5)	73.6^a (6.3)	41.7^b (11.6)	69.6^a (6.0)
Surface cast production (g of dry soil)	9.51^b (1.51)	7.41^b (1.13)	8.47^b (3.5)	23.60^a (3.24)
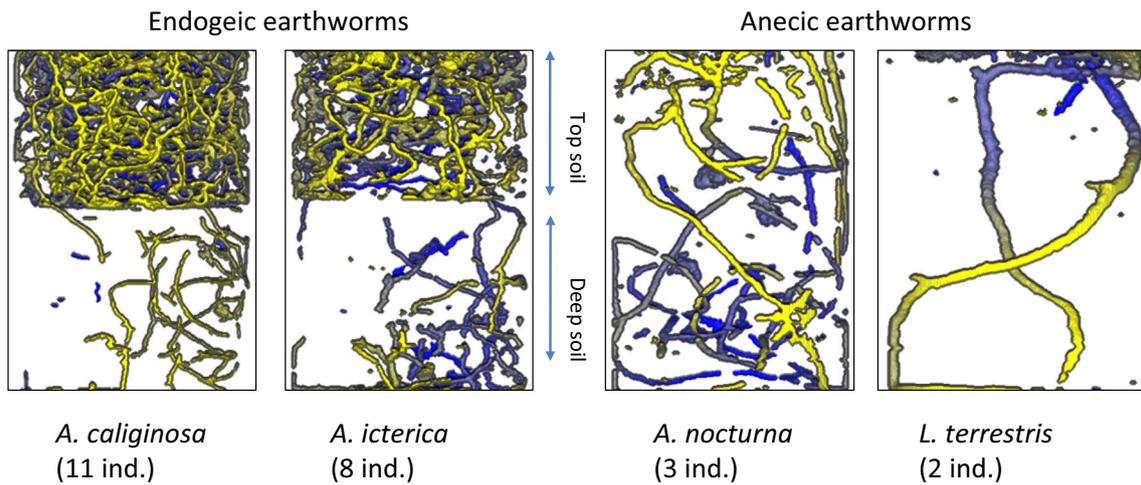
Fig. 1 Examples of burrow systems of the four species in the repacked soil cores containing two soil layers (0–15 and 15–30 cm at 4 and 2% organic matter content, respectively). Colors are provided for 3D rendering (yellow and blue in the foreground and background, respectively)

within the soil was low. The maximum range of tracers spread (upward plus downward transport) from the respective initial deposition layers was 6.3 cm, which was observed for the green tracers in cores incubated with *A. caliginosa* (Fig. 3). The quantity of tracers transported due to cast production reached up to 14.1% (orange tracers; *L. terrestris*).

More specifically, orange tracers deposited at the soil surface were displaced within the soil by all species except *A. icteria* (Fig. 3). Tracer transfers through cast production were observed
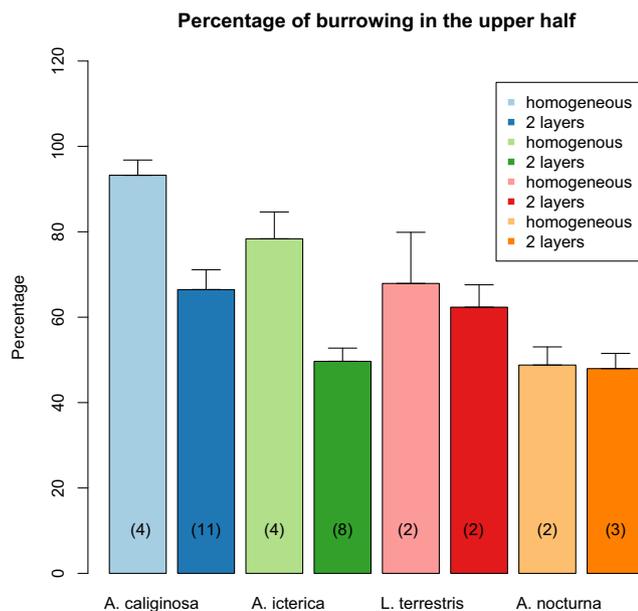

Fig. 2 Percentage of the burrow system found in the upper half (0–15 cm) of the repacked soil cores depending on the earthworm species and the soils used (a homogeneous soil layer or two layers). Soil cores were either (i) homogeneously filled with a soil with a higher content in organic matter (4%) or (ii) filled in two halves with the lower half containing a soil less rich in organic matter (2%) and the upper part was filled with a soil rich in organic matter (4%). The number between brackets indicated the number of earthworm per soil core

for all species with *L. terrestris* being the most efficient. Green tracers located initially at 3-cm depth within the soil column were displaced by all species but the activities of the two endogeic species resulted in relatively larger quantities being displaced over a bigger range (Fig. 3). Very few green tracers were found in the casts of the different species. The deeper tracers (pink; 12-cm deep) were very weakly mobilized by the earthworms and over very short total ranges (Fig. 3). Only a few pink tracers were found in the casts, with the exception of *L. terrestris* casts which contained 2.6% pink tracers.

Overall, regardless of the initial tracer positions, calculation of the particle displacement coefficients suggests that the anecic *A. nocturna* was the least efficient species at displacing tracer. The anecic *L terrestris* was the most efficient (mean PDC, 3.61 10^{-3} cm day^{-1}) at mobilizing the surface orange tracers (Table 3). On the other hand, intermediate tracers (3-cm deep) were displaced with the highest intensities due to the activity of the two endogeic species. Deep tracers (12 cm) again showed that *L. terrestris* is the most efficient bioturbator (mean PDC, 12.13 10^{-3} cm day^{-1}) but almost exclusively through surface cast production.

Discussion

Anecic and endogeic earthworm burrow systems

The differences between the burrows systems of anecic and endogeic species have been well documented since the seminal study of Joschko et al. (1993). Many authors found that anecic species produce fewer more continuous burrows that are wider and more vertical than those of endogeic species (Jégou et al. 1998; Pierret et al. 2002; Bastardie et al. 2005a; Bottinelli et al. 2017). In the present study, the burrow systems of the two endogeic species (*A. caliginosa* and *A. icterica*) were very similar

Table 3 Mean (and SD) values of bioturbation in the repacked soil cores depending on the earthworm species incubated in the cores. Bioturbation was estimated using the computation of particle displacement coefficient (PDC) and the percentage of luminophores found in the surface casts. Values bearing different letters in each row are significantly different and written in italics

Color (and depth) of the luminophores	Bioturbation estimates	L. terrestris	A. nocturna	A. icterica	A. caliginosa
Orange (surface)	PDC (10^{-3} cm day^{-1})	3.6a (1.1)	0.4b (0.4)	0.7b (0.5)	1.0b (0.4)
	In the casts (%)	14.1a (6.1)	0.3b (0.2)	3.6b (1.7)	0.9b (0.6)
Green (3 cm)	PDC (10^{-3} cm day^{-1})	0.5b (0.3)	0.2b (0.3)	0.9ab (0.4)	1.7a (0.4)
	In the casts (%)	0.1 (0.2)	0.0 (0.0)	0.1 (0.1)	0.1 (0.1)
Pink (12 cm)	PDC (10^{-3} cm day^{-1})	12.1a (11.1)	0.2b (0.2)	1.1ab	0.7ab (0.3)
	In the casts (%)	2.6 (1.6)	0.0 (0.0)	0.2 (0.1)	0.1 (0.0)

and no significant differences were observed for any of the burrow characteristics. In contrast, the burrow systems of the two anecic species had obvious differences related to burrow length: *A. nocturna* produced five times more burrows (in length) than *L. terrestris*. This illustrates the possible differences between epianecic species like *L. terrestris* and true anecic species such as *A. nocturna* (Bouché 1972). While *L. terrestris* makes very few, mainly vertically oriented, burrows that are used as a shelter when the earthworm do not forage at the soil surface, *A. nocturna* is also able to feed on soil organic matter but builds a more extensive burrow system (Jégou et al. 1998; Capowiez et al. 2001, 2015). Since the function of the main *L. terrestris* burrow is simply as a shelter, its size and shape are simple and adapted for daily vertical migration (Joyner and Harmon 1961; Bastardie et al. 2005a). Thus, in general, they produce a typical Y-shaped burrow: a main vertical burrow with sometimes another upper part added to increase the foraging area at the soil surface (Shipitalo and Butt 1999). Some close species even used horizontal extensions at the soil surface as shown by Kobel-Lamparski and Lamparski (1987) for *Lumbricus badensis*. Regardless these differences, it was noteworthy that the burrow length per worm was very similar for the four studied species (i.e., about 1 m per worm in 26 days; Table 2).

Influence of organic matter location on burrowing behavior

Organic matter, either found as litter at the soil surface or in different forms within the soil profile (litter buried by anecics or incorporated into soil aggregates, dead roots), is earthworm food and, as such, has a strong influence on their abundance or biomass. This is illustrated, for example, by the general positive effects observed following application of organic amendments to agricultural plots (Lee 1985; Leroy et al. 2008). It is thus striking that only a few previous studies directly focused on its effect on earthworm behavior using 2D terraria (Hughes et al. 1996; Cook and Linden 1996). Studies that used different soil layers with various characteristics provided additional information. For example, Springett and Gray (1997) and Francis and Fraser (1998) used repacked soil cores made of two or three soil layers sampled

at different depths in the field leading to a sharp gradient in soil organic matter. Springett and Gray (1997) observed that *A. caliginosa* mainly burrowed in the first layer whereas *L. terrestris* and *A. longa*, two anecic species, did not show any vertical preference. Francis and Fraser (1998) also observed that *A. caliginosa* burrowed more in the first organic matter-rich layer but this was however not the case for *Octalasion cyaneum*. Mombo et al. (2018) studied the effect of soil Pb pollution on earthworm behavior and used repacked cores with three soil layers (0–5, 5–17, and 17–30 cm) sampled in the field. These authors observed that *A. caliginosa* preferentially burrowed in the first, organic-rich soil layer, except when the Pb concentration in this layer exceeded 10,000 mg kg^{-1}. All these studies are in agreement with our main observation and suggest that earthworm displays contrasting behavior depending on their ecological categories (endogeic vs anecic), with the noticeable exception of *O. cyaneum*.

We compared their burrowing behavior to that observed in previously published study (Capowiez et al. 2015) where earthworm abundance was much lower, especially for endogeics. The higher earthworm abundance in the present study likely increased the competition for food (especially in monospecific assemblages) and thus may have forced earthworms to burrow deeper to avoid spatial competition and find new patches of food within the soil. This trend was clearly illustrated by the study of Ligthart and Peek (1997) during polder colonization by earthworms. They observed that the burrow systems were more vertical when earthworm abundance increased with time. We thus assume that the present experimental conditions (with 11 *A. caliginosa* or 8 *A. icterica* per soil core) likely reduced the difference between this study and the previous report (with 4 *A. caliginosa* or *A. icterica* per soil core).

Luminophores as a complementary method in soil biology

Here, we demonstrated that luminophores can be used to study earthworm bioturbation. We used the same total earthworm biomass per core (6 g) and *L. terrestris* had the highest total soil bioturbation intensity but this was mainly due to soil

Fig. 3 Distribution of luminophores at the end of the incubation period for the four earthworms species. Depth of − 1 cm represents casts. For better reading, the *x*-axis is limited to 14 cm, the maximal penetration depth of luminophores, and the *y*-axis to 5%. Values that exceeded the *y*-axis limit are indicated in brackets
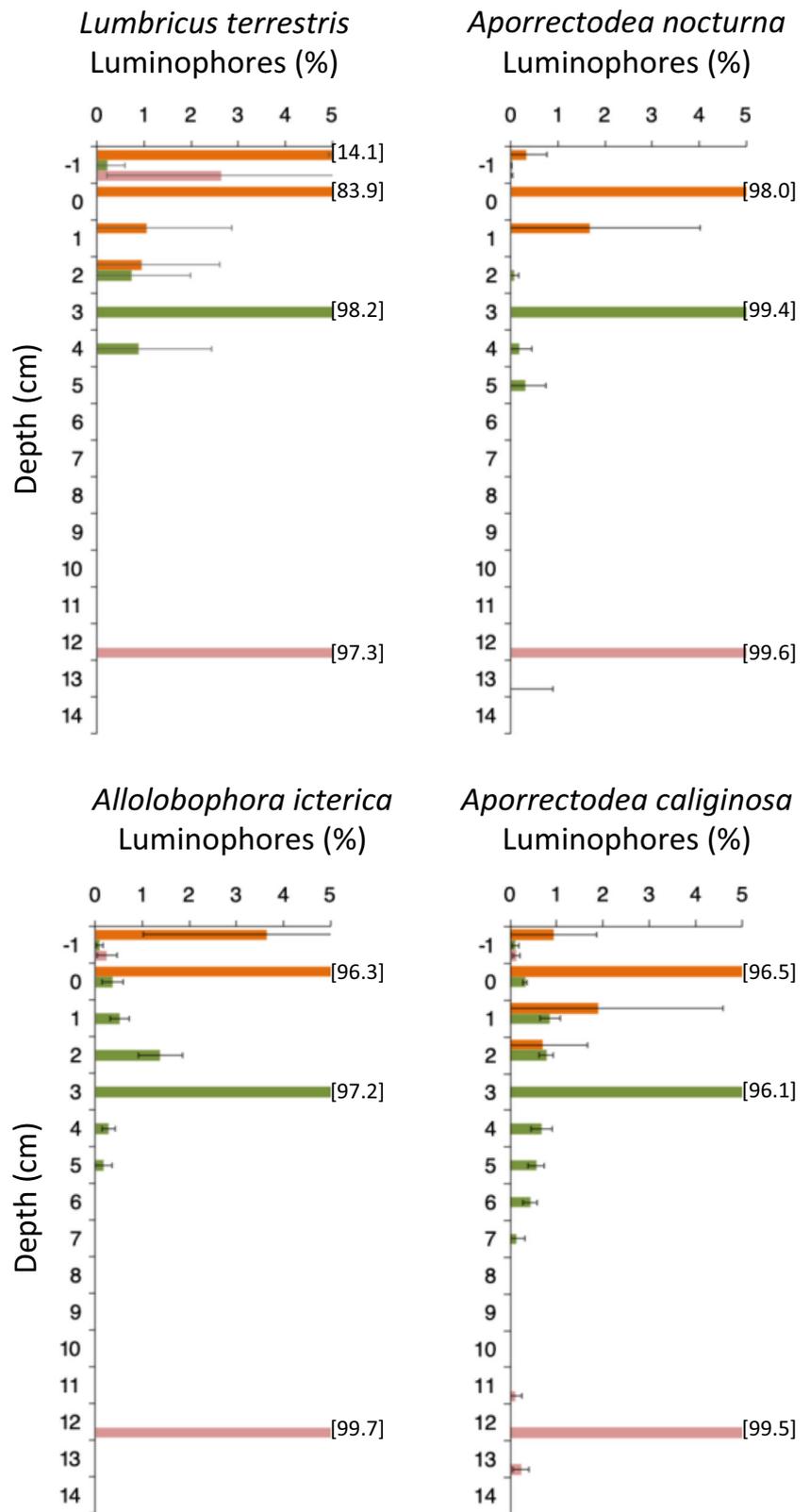
casting at the soil surface. This highlights the increased capacity of *L. terrestris* to mix soil layers of different depths. This species, even in our mesocosm with limited depth (30 cm), was the only one to transfer significant amounts of relatively deep soil onto the soil surface, probably at the initial stages of main burrow formation. This was also associated with far the

highest amounts of surface casts made by this species compared to the other three species. This trend did not correlate to the volume of burrows produced which suggests that *L. terrestris* either preferentially produced casts at the soil surface or burrowed more through ingestion and less by pushing the soil aside, compared to *A. nocturna*, for example.

Beyond displacement rates, the use of luminophores provided data to complement our observations from tomography. The results from the green luminophores located at 3 cm depth showed that the endogeics, and especially *A. caliginosa*, had the highest bioturbation activities in this zone. *A. icterica* had intermediate values between *A. caliginosa* and the two anecic species and this is in agreement in the fact that *A. icterica* generally live and burrow deeper in the soil than *A. caliginosa* (Bernier 1998; Le Couteulx et al. 2015). These findings suggest that luminophores could be useful for detecting the preferential depths of activity for different earthworm species, information, which is not easy to obtain under field conditions (Gerard 1967). Other complementary information was provided by the fact that all species except *A. caliginosa* contributed to the below ground incorporation of surface-deposited orange luminophores. This suggests that *A. caliginosa* had, under our conditions, very limited surface activity.

Overall, the percentage of soil bioturbation remained low especially compared to aquatic organisms such as marine polychaetes. For example, Duport et al. (2006) observed for *Hediste diversicolor* that the average percentage of tracers displaced from the surface deposit layer after 9 days ranged from 24.1 (one worm of 0.33 g) to 37.8% (2.64 g of worm, 8 worms in total); these values correspond to PDC values of 130 and 141 10^{-3} cm day^{-1}, respectively. This may be partly due to the fact that in sediments, hydrodynamic water movements, natural or generated by organism movement, play an important role in the vertical downward transport of particles. In our study, we did not try to mimic rainy periods which could have increased the downward movements of some tracers. Moreover, the cohesion of the particles of deposited tracers with other soil particles could have been strengthened due to the initial soil compaction setup. In order to increase the tracer displacement signal, experiments with longer incubation periods could be carried out, i.e., more than 2 months especially in absence of heavy rain. The use of thicker luminophore deposition layers (several mm) could have also increased the quantity of tracers displaced. Even if, some burrow systems appeared to be dense (such as *A. caliginosa* in the topsoil), the percentage of porosity that it represents is always less than 5% of the soil volume (in our case, it ranged from 1.6 to 3.3% for the four tested species). If bioturbation is mainly driven by soil ingestion and far less by soil being pushed aside when digging, then the degree of soil bioturbation is in agreement (a few percent, see Fig. 3) with the percentage of macroporosity created by the earthworms.

Beyond the quantitative aspect of luminophore results, our findings could also be used, as a very first attempt, to categorize earthworms into bioturbation functional groups. By analogy with aquatic bioturbation classification, the two endogenic species could be classified as biodiffusor species (i.e., with activities that usually result in a constant and random local sediment biomixing over short distances; sensu Kristensen et al. 2012) and more precisely as gallery-diffusors because their activities were not restricted to the surface or subsurface. On the other hand, the two studied anecic species, and especially *L. terrestris*, whose cast production induced non-local vertical particle transfer, could be tentatively classified as conveyor species (Kristensen et al. 2012). These preliminary earthworm bioturbation group definitions and species classifications can now be more precisely discussed and defined more closely based on further experiments.

Conclusions

Despite its fundamental importance, the effect of organic matter distribution within the soil on earthworm behavior has been rarely studied. We showed that the two endogeic earthworm species studied preferentially burrowed in the organic matter-rich layer avoiding the organic matter-poor layer below, whereas the two anecic species utilized the profile more uniformly. However, our findings cannot be extrapolated to all the other endogeic or anecic species, especially because the three main ecological categories are not exclusive classes but rather extreme archetypes or poles of a triangular graph in which the earthworm species locate individually (Bouché 1977; Bottinelli et al. 2020). To the best of our knowledge, prior to this study, luminophores had not yet been used to describe and quantify soil or particle displacement by earthworms. Our results suggest that the luminophore technique could be a useful tool by itself but also a significant complement to X-ray tomography methods for understanding earthworm behavior since it provides information on soil layer mixing, bioturbation intensity, and the origin of surface casting. As in aquatic ecology, the use of particle tracers in soil bioturbation studies appears to be a promising tool for the identification of soil bioturbation functional groups.

References

Anschutz P, Ciutat A, Lecroart P, Gérino M, Boudou A (2012) Bioturbation effects by tubificids worms on freshwater sediment biogeochemistry. Aquatic Geochemistry 18:475–497

Auclerc A, Capowiez Y, Guérold F, Nahmani J (2013) Application of X-ray tomography to evaluate liming impact on earthworm burrowing activity in an acidic forest soil under laboratory conditions. Geoderma 202-203:45–50

Bastardie F, Capowiez Y, Renault P, Cluzeau D (2005a) A radio-labelled study of earthworm behaviour in artificial soil cores in term of ecological types. Biol Fertil Soils 41:320–327

Bastardie F, Capowiez Y, Cluzeau D (2005b) 3D characterization of earthworm burrow systems in natural soil cores collected in a 12-year-old pasture. Appl Soil Ecol 30:34–46

Bernier N (1998) Earthworm feeding activity and the development of the humus profile. Biol Fertil Soils 26:215–223

Black KS, Athey S, Wilson P, Evans D (2007) The use of particle tracking in sediment transport studies: a review. J Geol Soc 274:73–91

Bottinelli N, Zhou H, Capowiez Y, Zhang ZB, Qiu J, Jouquet P, Peng XH (2017) Earthworm burrowing activity of two non-Lumbricidae earthworm species incubated in soils with contrasting carbon content (Vertisol vs. Ultisol) Biol Fertil Soils 53:951-955

Bottinelli N, Hedde M, Jouquet P, Capowiez Y (2020) An explicit definition of earthworm ecological categories - Marcel Bouché's triangle revisited. Geoderma 372:114361

Bouché MB (1972) Lombriciens de France. Ecologie et systématique, INRA, Paris

Bouché MB (1977) Stratégies lombriciennes. Ecol Bull 22:122–132

Boudreau BP (1986) Mathematics of tracer mixing in sediments 1. Spatially dependent, diffusive mixing. Am J Sci 286:1161–1198

Capowiez Y, Monestiez P, Belzunces L (2001) Burrow systems made by Aporrectodea nocturna and Allolobophora chlorotica in artificial cores: morphological differences and effects of interspecific interactions. Appl Soil Ecol 16:109–120

Capowiez Y, Sammartino S, Michel E (2011) Using X-ray tomography to quantify earthworm bioturbation non-destructively in repacked soil cores. Geoderma 162:124–131

Capowiez Y, Bottinelli N, Sammartino S, Michel E, Jouquet P (2015) Morphological and functional characterization of the burrow system of sin earthworm species (Lumbricidae). Biol Fertil Soils 51:869–877

Cook SMF, Linden DR (1996) Effect of food type and placement on earthworm (Aporrectodea tuberculata) burrowing and soil turnover. Biol Fertil Soils 21:201–206

Duport E, Stora G, Tremblay P, Gilbert F (2006) Effects of population density on the sediment mixing induced by the gallery-diffusor Hediste (Nereis) diversicolor O.F. Müller, 1776. J Exp Mar Biol Ecol 336:33–41

Foulquier A, Simon L, Gilbert F, Fourel F, Malard F, Mermillod-Blondin F (2010) Relative influences of DOC flux and subterranean fauna on microbial abundance and activity in aquifer sediments: new insights from 13C-tracer experiments? Freshwater Biol 55:1560–1576

Francis GS, Fraser PM (1998) The effects of three earthworm species on soil microporosity and hydraulic conductivity. Appl Soil Ecol 10:11–19

François F, Gérino M, Stora G, Durbec J-P, Poggiale J-C (2002) Functional approach to sediment reworking by gallery-forming macrobenthic organisms: modelling and application with the polychaete Nereis diversicolor. Mar Ecol Prog Ser 229:127–136

Frazao J, de Goede RGM, Capowiez Y, Pulleman MM (2019) Soil structure formation and organic matter distribution as affected by earthworm species interaction and crop residues placement. Geoderma 338:453–463

Gerard BM (1967) Factors affecting earthworms in pasture. Animal Ecology 36:235–252

Gérino M, Aller RC, Lee C, Cochran JK, Aller JY, Green MA, Hirschberg D (1998) Comparison of different tracers and methods used to quantify bioturbation during a spring bloom: 234-Thorium, luminophores and chlorophylla. Estuar Coast Shelf Sci 46:531–547

Gérino M, Frignani M, Mugnai C, Bellucci LG, Prevedelli P, Valentini A, Castelli A, Delmotte S, Sauvage S (2007) Bioturbation quantification and related organisms in the Venice Lagoon. Acta Oecologica-Intern J Ecol 32:14–25

Gilbert F, Hulth S, Strömberg N, Ringdahl K, Poggiale JC (2003) 2-D optical quantification of particle reworking activities in surface marine sediments. J Exp Mar Biol Ecol 285(286):251–263

Gilbert F, Hulth S, Grossi V, Poggiale JC, Desrosiers G et al (2007) Sediment reworking by marine benthic species from the Gullmar Fjord (Western Sweden): importance of faunal biovolume. J Exp Mar Biol Ecol 348:133–144

Gutiérrez Y, Ott D, Töpperwien M, Salditt T, Sherber C (2018) X-ray computed tomography and its potential in ecological research: a review of studies and optimization of specimen. Ecol Evol 8: 7717–7732

Hedman JE, Gunnarsson JS, Samuelsson G, Gilbert F (2011) Particle reworking and solute transport by the sediment-living polychaetes Marenzelleria neglecta and Hediste diversicolor. J Exp Mar Biol Ecol 407:294–301

Hughes MS, Bull CM, Doube BM (1996) Microcosm investigations into the influence of sheep manure on the behaviour of the geophagous earthworms Aporrectodea trapezoides and Microscolex dubius. Biol Fertil Soils 22:71–75

Jégou D, Cluzeau D, Wolf HJ, Gandon Y, Tréhen P (1998) Assessment of the burrow system of Lumbricus terrestris, Aporrectodea giardi and Aporrectodea caliginosa using X-ray computed tomography. Biol Fertil Soils 26:116–121

Joschko M, Muller PC, Kotzke K, Dohring W, Larink O (1993) Earthworm burrow system-development assessed by means of X-ray computed tomography. Geoderma 56:209–221

Joyner JW, Harmon NP (1961) Burrows and oscillative behavior therein of Lumbricus terrestris. Proceedings of Indiana Academy of Science 71:378–384

Kobel-Lamparski A, Lamparski F (1987) Burrow construction during the development of Lumbricus badensis individuals. Biol Fert Soils 3: 125–129

Kristensen E, Penha-Lopes G, Delefosse M, Valdemarsen T, Quintana CO, Banta G (2012) What is bioturbation? The need for a precise definition for fauna in aquatic sciences. Mar Ecol Prog Ser 446:285–303

Lagauzère S, Coppin F, Gérino M, Delmotte S, Stora G, Bonzom JM (2011) An alternative method of particulate fluorescent tracer analysis in sediments using a microplate fluorimeter. Environ Technol 32:551–560

Le Couteulx A, Wolf C, Hallaire V, Péres G (2015) Burrowing and casting activities of three endogeic earthworm species affected by organic matter location. Pedobiologia 58:97–103

Lee KE (1985) Earthworms. Their ecology and relationships with soil and land use. Academic Press, Sydney

Leroy BLM, Schmidt O, Van den Bossche A, Reheul D, Moens M (2008) Earthworm population dynamics as influenced by the quality of exogenous organic matter. Pedobiologia 52:139–150

Ligthart TN, Peek GJCW (1997) Evolution of earthworm burrow systems after inoculation of lumbricid earthworms in a pasture in the Netherlands. Soil Biol Biochem 29:453–462

Lindqvist S, Gilbert F, Eriksson SP, Hulth S (2013) Activities by Hediste diversicolor during light and dark conditions: experimental quantification of particle reworking using time-resolved imaging. J Exp Mar Biol Ecol 448:240–249

Mahaut ML, Graf G (1987) A luminophore tracer technique for bioturbation studies. Oceanol Acta 10:323–328

Maire O, Lecroart P, Meysman FJR, Rosenberg R, Duchene JC, Gremare A (2008) Methods of sediment reworking assessment in bioturbation research: a review. Aquatic Biology 2:219–238

McTavish MJ, Gorgolewski A, Murphy SD, Basiliko N (2019) Anecic earthworm (*Lumbricus terrestris*) facilitate the burial of surface-applied wood ash. Biol Fertil Soils 56:195–203

Mombo S, Laplanche C, Besson P, Sammartino S, Schreck E, Dumat C, Capowiez Y (2018) Metal soil pollution differentially affects both the behavior and exposure of *A. caliginosa* and *L. terrestris*: a mesocom. Biol Fertil Soils 54:319–328

Moreau-Valencogne P, Bertrand M, Holmstrup M, Roger-Estrade J (2013) Integration of thermal and hydrotime models to describe the development and growth of temperate earthworms. Soil Biol Biochem 63:50–60

Pelosi C, Grandeau G, Capowiez Y (2017) Temporal dynamics of earthworm-related microporosity in tilled and non-tilled cropping systems. Geoderma 289:169–177

Pierret A, Capowiez Y, Belzunces L, Moran CJ (2002) 3D reconstruction and quantification of micropores using X-ray computed tomography and image analysis. Geoderma 106:247–271

Rogasik H, Schrader S, Onasch I, Kiesel J, Gerke HH (2014) Micro-scale dry bulk density variation around earthworm (*Lumbricus terrestris*) burrows based on X-ray computed tomography. Geoderma 213: 471–477

Rushton SP (1986) The effects of soil compaction on *Lumbricus terrestris* and its possible implications for populations on land reclaimed from open-cast coal mining. Pedobiologia 29:85–90

Schmidt S, Jouanneau J-M, Weber O, Lecroart P, Radakovitch O, Gilbert F, Jezequel D (2007) Sediment dynamic at the water-sediment interface of the Thau Lagoon (South France): from seasonal to century time scales using radiogenic and cosmogenic nuclides. Estuar Coast Shelf Sci 72:534–542

Shipitalo MJ, Butt KR (1999) Occupancy and geometrical properties of *Lumbricus terrestris* burrows affecting infiltration. Pedobiologia 43: 782–794

Solan M, Wigham BD, Hudson IR, Kennedy R, Coulon CH et al (2004) In situ quantification of bioturbation using time-lapse fluorescent sediment profile imaging (f-SPI), luminophore tracers and model simulation. Mar Ecol Prog Ser 271:1–12

Springett J, Gray R (1997) The interaction between plant roots and earthworm burrows in pasture. Soil Biol Biochem 29:621–625

Taina IA, Heck RJ, Elliot TR (2013) Application of X-ray computed tomography to soil science: a literature review. Can J Soil Sci 88: 1–20

Uvarov AV (2017) Density-mediated earthworm effects on soil respiration. Polish J Ecol 64:534–546

van Schaik L, Palm J, Klaus J, Zehe E, Schröder B (2014) Linking spatial earthworm distribution to macropore numbers and hydrological effectiveness Ecohydrology 7:401–408