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Burrowing and casting activities of three endogeic earthworm species

affected by organic matter location

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Abstract

Earthworms are crucial for production and maintenance of soil structure and their activities can strongly impact soil functioning (e.g. water regulation, nutrient dynamics). This laboratory study investigated the bioturbation activity of three endogeic species, A. chlorotica, A. icterica and A. caliginosa, as affected by different locations of organic matter (OM) in the soil profile: OM scattered on the soil surface (surface-OM) or homogeneously mixed into the soil (mixed-OM). Microcosms, each containing a combination of one species (three individuals) and one OM location, were subjected to controlled environmental conditions (temperature, humidity and day/night cycle) for 60 days. At the end of the experiment, microcosms were cut into multiple horizontal cross-sections every centimetre and bioturbation activities were analysed based on the number of burrows, the burrowed area and the percentage of burrowed area totally refilled with casts.

Results showed that regardless of species, there was significantly fewer burrows and a greater percentage of burrowed area refilled with casts under mixed-OM than under surface-OM. A. chlorotica and A. caliginosa had a significantly greater burrowed area under mixed-OM than under surface-OM. Regardless of OM location, as depth increased, burrow number and area decreased for A. chlorotica and generally increased for A. icterica. In contrast, burrowing activity of A. caliginosa was affected by OM location as depth increased: under...
mixed-OM, burrow number decreased but burrowed area remained constant, whereas under surface-OM, burrow number remained constant and burrowed area increased.

These results improve understanding of effects of endogeic species on soil structure and highlight effects of OM location on earthworm bioturbation. Especially this study gives information about the burrowing activity of *A. icterica* which has so far been little documented, and also informs about refilled burrows which is a major parameter for soil functioning.

Keywords: soil structure, behaviour, *Allolobophora chlorotica*, *Allolobophora icterica*, *Aporrectodea caliginosa*

1. Introduction

Earthworms have been described as soil engineers (Jones et al., 1994; Lavelle et al., 1997) because of their ability to modify their own environment and notably the soil structure via their bioturbation activity which consists of burrowing and producing casts. This ingestion-egestion of soil strongly affects soil structure (Dexter, 1988; Lee and Foster, 1991) depending on the context, earthworms increase porosity (Pérès et al., 2010; Lamandé et al., 2011; van Schaik et al., 2014) or increase bulk density (Blanchart et al., 1997). Consequently, earthworms affect several soil functional properties and ecosystem services, such as soil moisture, water infiltration and water regulation, soil organic matter (OM) availability, nutrient cycling and primary production (Jouquet et al., 2006; Capowiez et al., 2009; Blouin et al., 2013; Crittenden et al., 2014). To understand the functional links between earthworms and soil structure, scientists have focused on one aspect of bioturbation (*i.e.* burrows) (Pérès
et al., 2010; Lamandé et al., 2011; van Schaik et al., 2014) but without integrating other aspects of its complexity, especially casts.

Earthworm bioturbation results from complex interactions and can be affected by various parameters, such as the location of OM, which is a food resource for earthworms and affects earthworm foraging activity (Jeanson, 1968; Martin, 1982; Pérès et al., 2010). However, this aspect needs further study, especially in relation to cast production.

Bioturbation properties of anecic earthworms are well described: these dwelling earthworms build a relatively permanent burrow system, vertically oriented, bring soil from the depth to the soil surface and cover their burrow walls with their casts (Kretzschmar, 1990; Kretzschmar and Aries, 1990; Daniel et al., 1997; Jégou et al., 1999, 2001; Shipitalo and Butt, 1999; Bastardie et al., 2003; Nuutinen and Butt, 2003).

Bioturbation properties of endogeic earthworms, however, are less well known, despite existing studies (Bolton and Phillipson, 1976; Capowiez et al., 2001; Jégou et al., 2001; Felten and Emmerling, 2009), these earthworms are reported to burrow through the soil, creating horizontal and randomly oriented burrows considered to be temporary structures (Bouché, 1972). Only few data exist about their casting activity in soil and especially the proportion of burrows refilled with casts which is an important parameter for soil functioning (Schrader, 1993; Francis et al., 2001; Perreault and Whalen, 2006; Capowiez et al., 2014). Additionally, some endogeic species have received attention, e.g. *A. chlorotica* (Capowiez et al., 2001, 2014) and *A. caliginosa* (Schrader, 1993; Francis et al., 2001; Jégou et al., 2001; Perreault and Whalen, 2006; Capowiez et al., 2014), but other species are not well documented such as *A. icterica* which has been only assessed once, through its burrow network (Bastardie et al., 2005a).

A study of earthworm bioturbation encounters several challenges, of which difficulty in accessing burrows and casts due to soil opaqueness is one. Several authors have used
transparent 2D-terrariums (Schrader, 1993; Whalen et al., 2004; Perreault and Whalen, 2006; Felten and Emmerling, 2009). Their relative thinness, however, may influence earthworm behaviour and therefore this 2D approach appears less relevant than 3D microcosms (Capowiez et al., 2001, 2014). Several authors have used 3D X-ray tomography (Joschko et al., 1989; Jégou et al., 1997; Capowiez et al., 1998), which is relevant for burrow network assessment but this does not allow direct assessment of cast production (Joschko et al., 1993). As an alternative, the study by Hirth et al., 1996 is particularly interesting because it used cross sections of cylindrical microcosms to analyse both burrowing and casting by endogeics. Another challenge to studying earthworm bioturbation is correctly identifying which species produced observed burrows and casts, especially in natural conditions, in which several species bioturbate the soil (Capowiez et al., 1998; Pérès, 2003; Bastardie et al., 2005b). Thus, despite their artificiality, microcosms remain necessary to describe bioturbation activity of a species (Bastardie et al., 2005b).

The aim of this study was to assess under controlled conditions, burrowing and casting activities of three endogeic earthworms (i) as a function of OM location in the soil profile, and (ii) as a function of soil depth. The destructive method used is based on soil cross sections of microcosms and was used to observe, classify and quantify bioturbation, i.e. number of burrows, burrowed area and percentage of total burrowed area totally refilled with casts.
2. Materials and Methods

Our experimental system takes benefits from previous studies such as Jégou et al. (2001) for the design of microcosms and from Hirth et al. (1996) for the assessment of burrowing activity.

2.1. Experimental system

2.1.1. Microcosm characteristics

Twenty four microcosms were built using PVC cylinders (20 cm in length and 15 cm in internal diameter). They were cut lengthwise into two equal halves to facilitate their final opening. A 500 µm nylon mesh was placed at the upper and lower openings to retain earthworms.

2.1.2. Soil and organic matter features

The soil was collected from an arable field in Le Rheu, Brittany, France (N 48°09, W 1°81) and was a silt loam soil (FAO, 1988) with 16% sand, 69% silt, 15% clay. Soil organic matter content (2%) and pH$_{H_2O}$ (6.1) were in accordance with the values observed in cultivated soils in Brittany (“BDAT,” 2002). Soil was air-dried before being passed through a 2 mm sieve to remove biostructures already present.

We used ryegrass (Lolium perenne L.) from an unmown and untreated grassland as the OM resource for earthworms. This OM was oven-dried for 48h at 60°C before being ground to a maximum width of 1 mm. OM was supplied at 20.7 g dry weight (dw) per microcosm, i.e. 100 g of soil with 0.6 g dw of OM, corresponding to a non-limiting food resource for earthworms (Curry and Schmidt, 2007). Two OM treatments were defined: OM mixed with all the soil (mixed-OM treatment, 12 microcosms) and OM evenly scattered on the soil surface (surface-OM treatment, 12 microcosms).
2.1.3. Microcosm filling

Microcosms were filled with five layers of soil. Each layer had a bulk density of 1.3 g.cm$^{-3}$: it was made of 690 g dw of soil that was packed down to obtain a 3-cm-high layer. Thus, soil in the columns was 15 cm deep. The bulk density of each layer has been recorded in some cultivated fields (Peigné et al., 2009; Bottinelli et al., 2013) and in other microcosm experiments (Jégou et al., 1999). Additionally, the use of dry soil and thin layers prevented inter-layer smoothing when soil was packed down. Once constructed, microcosms were re-moistened by capillary absorption and freely-drained for 48 h to reach field capacity which was kept by re-wetting the surface every two weeks.

2.1.4. Earthworm introduction

Three endogeic species, according to Bouché (1972), were collected from an arable field: *Allolobophora chlorotica* (Savigny, 1826), *Allolobophora icterica* (Savigny, 1826) and *Aporrectodea caliginosa* (Savigny, 1826). These species are commonly found in cultivated soil in France (Cluzeau et al., 2012). All earthworms were sub-adults or adults and were acclimated to the soil for one week before being introduced into microcosms (Fründ et al., 2010).

Experiments involved use of earthworm monocultures: one endogeic species employing three individuals per microcosm and corresponding to 170 earthworms.m$^{-2}$ and a mean fresh biomass (± standard deviation) of 0.8±0.1 g for *A. chlorotica*, 2.1±0.1 g for *A. icterica* and 1.3±0.1 g for *A. caliginosa* per microcosm. Earthworm biomass and density values were consistent with those found in cultivated fields in Brittany (Pélosi et al., 2014). The experimental design had two OM locations with three earthworms species replicated four times (2x3x4=24 microcosms). Earthworms were placed on the soil surface and allowed to burrow down. Then, microcosms were placed on a raised grid in a climatic chamber at 10°C with a day/night cycle corresponding to the external one (18/6h in June and July).
Microcosms were maintained for 60 days after earthworm inoculation, at which time they were microwaved to stop earthworm activity by killing them *in situ* (5 minutes, 400 Watts). They were then slowly oven dried at 45°C for one week to help cutting cross-sections and to prevent their smoothing.

### 2.2. Bioturbation assessment

Each microcosm was cut from top into eleven cross sections every centimetre corresponding to sections $z_0$ to $z_{10}$. The surface of each section was lightly brushed using a paintbrush and blown using a compressor at its lowest pressure to remove dust and studied with the following procedure:

1. Outlines of burrows were identified with the naked eye and traced with pen on a transparent sheet of plastic placed on the surface. Casts that completely obstructed the burrows were similarly recorded.
2. After digitizing the drawings (resolution: 600 ppi), they were analyzed with Fiji software (Schindelin et al., 2012) and a homemade script in the Jython programming language (http://www.jython.org/). Each burrow was identified and described by its total area and the area occupied by casts. The percentage of burrow refilled with casts (area occupied by casts divided by the burrowed area) was calculated.

### 2.3. Statistical analysis

Data analysis was performed using R software (R. Core Team, 2013). If the normality of residues (Shapiro test) and the heteroscedasticity (Bartlett test) were verified, we used multi-way ANOVA and post-hoc LSD Tukey’s tests with species, depth and OM location as factors. Otherwise, the Kruskall-Wallis test checked for factor effect, and pairwise Wilcoxon tests with Bonferroni correction were used as post-hoc tests. Linear regressions were calculated to test the relation between bioturbation and depth. If linear regression was not significant, the *nls*
function of R was used to estimate parameters of a non-linear model. The goodness of fit of the non-linear model was assessed with a Pearson test of correlation between estimated and observed values. Significance threshold was set at $\alpha = 5\%$.

3. Results

At the end of the experiment, some OM remained on the surface of the surface-OM treatment, which suggested that excess OM had been applied. We observed that surface casts were still being produced at the end of the experiment which suggests that earthworms were still active.

3.1. Bioturbation of earthworms as affected by organic matter location

3.1.1. Number of burrows:

The number of burrows per section was significantly ($p < 0.001$) affected by earthworm species, OM location, and by 2-way interactions: species $\times$ OM location, species $\times$ depth, and OM location $\times$ depth.

The number of burrows per section was significantly higher under surface-OM vs. mixed-OM regardless of species (Fig. 1a). It was approximately 2.3, 2.1 and 1.5 times as large under surface-OM vs. mixed-OM for *A. icterica*, *A. caliginosa* and *A. chlorotica*, respectively.

Under surface-OM, the number of burrows per section decreased from *A. icterica* to *A. caliginosa* to *A. chlorotica*, with a significant difference between each pair. Under mixed-OM, *A. icterica* had a significantly higher number of burrows per section than *A. chlorotica*.

3.1.2. Burrowed area per section

Burrowed area was significantly ($p < 0.001$) affected by species, OM location, and the 2- and 3-way interactions (species $\times$ OM location $\times$ depth).
A. chlorotica and A. caliginosa had significantly higher burrowed area under mixed-OM than under surface-OM (Fig. 1b). Their burrowed area was approximately 3.7 and 2.2 times as large, respectively, under mixed-OM vs. surface-OM. Conversely, A. icterica burrowed area was not significantly affected by OM location.

Under surface-OM, the area burrowed per section significantly decreased from A. icterica to A. caliginosa to A. chlorotica.

3.1.3. Percentage of burrowed area refilled with casts

The percentage of refilled area was significantly (p < 0.05 for species × depth and p < 0.001 for other factors) affected by species, OM location, and all 2- and 3-way interactions.

Earthworms refilled their burrows more under mixed-OM than under surface-OM (Fig. 1c). This percentage was approximately 3.1, 1.7 and 2.5 times as high under mixed-OM vs. surface-OM for A. chlorotica, A. icterica and A. caliginosa, respectively.

Under mixed-OM, A. caliginosa had a higher percentage of burrowed area refilled with casts than the two other species. This percentage was not significantly different for the three species under surface-OM.

3.2. Effect of soil depth on bioturbation

Earthworms bioturbated all sections of all microcosms, except one microcosm with A. chlorotica under surface-OM, in which no burrow was found in the two deepest sections. The top section (i.e. $z_0$) of several microcosms could not be analyzed, placing the first analyzed section at a depth of 1 cm (i.e. $z_1$).

3.2.1. Number of burrows as depth increased

Under both OM-location treatments, the number of burrows produced by A. chlorotica decreased as depth increased (Figs. 2a and 2d) whereas it increased as depth increased for A. icterica (Figs. 2b and 2e). A. caliginosa had a varied response: the number of burrows...
decreased as depth increased under mixed-OM, but remained constant as depth increased under surface-OM (Fig. 2f). For all combinations of species and OM location, except A. caliginosa under surface-OM, regressions predicting the number of burrows as depth increased were statistically significant.

3.2.2. Burrowed area as depth increased

A linear relation exists between burrowed area and depth in most treatments (Fig. 3). The burrowing activity of A. chlorotica was concentrated within the first 3 cm under mixed-OM (Fig. 3a). Unlike the mixed-OM treatment, the upper sections under surface-OM did not noticeably differ from others, even though food resources were located at the surface. However, A. icterica and A. caliginosa under surface-OM had burrowed areas at the surface (i.e. z₁) that were higher than those at 1 cm (Figs. 3e and 3f). In both cases, this surface point was excluded from linear regression since its standard error was extremely high, and the surface burrowing could reflect a specific behaviour due to OM location on the soil surface. For both OM locations, burrowing of A. icterica linearly increased as depth increased (Figs. 3b and 3e). There was no significant linear correlation between the area refilled with casts and depth (data not shown).

4. Discussion

4.1. Earthworm sensitivity to OM location

Our results showed major differences in the bioturbation of the three species studied, even though all belong to the endogeic group. According to this study, species is a key factor that must be considered when linking earthworms to bioturbation. Additionally, our results highlighted the need to account for effects of OM location on earthworm bioturbation, since it might influence the number of burrows produced, the burrowed area, and the burrowed area refilled with casts. Total burrowed area of A. icterica was noteworthy unaffected by OM
location, even though its behaviour was changed, notably by increasing the number of burrows. However, *A. chlorotica* and *A. caliginosa* were greatly influenced by OM mixed with soil, which led them to increase the area burrowed. These observations are consistent with their food consumption habits: under spruce forest conditions, Bernier (1998) observed that *A. icterica*’s digestive tract contained a higher mineral content than that of other species, including *A. caliginosa*. Conversely, the diets of *A. chlorotica* and *A. caliginosa* are known to be similar (Piearce, 1978). Since OM is a known food resource for the three endogeic species, this study showed that less of it in the soil could lead earthworms to build more burrows supporting previous study under field conditions (Pérès et al., 2010). It should be noted that interspecific competition can affect the burrowing activity of earthworm species. In particular, the burrow system of *A. chlorotica* was unaffected by *Aporrectodea nocturna* (Capowiez et al., 2001) and *A. caliginosa* had a significantly lower burrowing activity in the presence of several other species (Felten and Emmerling, 2009). Thus both OM location and interspecific competition should be included in further studies of earthworm bioturbation activity.

It is worthwhile to note that the measured percentage of burrowed area refilled with casts is far from equal among documented studies. For example, the percentage for *A. caliginosa* in our study (10-35%) was much lower than that of Francis et al. (2001) and Capowiez et al. (2014) (40-85%) but higher than that observed by Perreault and Whalen (2006) (<10%). Differences among studies may stem from the methods used, but also from the influence of food quality, food quantity, temperature and bulk density, which differed among studies. For example, Perreault and Whalen (2006) observed that surface casting of *A. caliginosa* was greater with wetter soil. Our results showed that OM location is another factor that affects the percentage of burrowed area refilled. We observed a lower percentage of burrowed area refilled with casts under surface-OM vs. mixed-OM. There could be several explanations for
this: (i) casts were less stable under surface-OM and were undetectable at the end of the experiment; (ii) casts under the surface-OM treatment had a greater bulk density; (iii) burrows were built by pushing particles; (iv) casts were egested at the soil surface and (v) more casts were crushed against burrow walls under surface-OM vs. mixed-OM. Further studies are needed to explain this difference between the two OM locations and to study the mechanisms of cast production. The measured percentage of burrowed area refilled with casts is an indicator of burrow continuity, notably because reported casts are those that completely obstructed the burrows. Our study suggests that burrows built under mixed-OM are more discontinuous, which can impact on water movements (Allaire-Leung et al., 2000) and burrow lifespan (Capowiez et al., 2014).

4.2. Bioturbation activity as depth increased

We assessed differences in burrow number and burrowed area as depth increased. *A. chlorotica* burrowing activity was concentrated in the top few centimeters which was not observed by Capowiez et al. (2001). This result agrees with an intermediate position of *A. chlorotica* between the ecological categories “endogeic” and “epigeic” (Bouché, 1977; Pérès, 2003). Nevertheless, we observed that this epi-endogeic behaviour was more marked with OM mixed into the soil than with OM on the soil surface. This seems counterintuitive, because OM on the soil surface would induce earthworms to burrow at the surface to feed. But our study does not reveal whether earthworms expressed true epigeic behaviour when OM was on the surface by feeding in the thin organic layer at the top of microcosms. Nevertheless, the effect of *A. chlorotica* on the soil volume was smaller when OM was on the surface. The burrowed area of *A. caliginosa* slightly increased as depth increased when OM was located on the soil surface, which contradicts other studies (McKenzie and Dexter, 1993; Jégou et al., 1997), but no trend was found when OM was mixed into the soil. *A. icterica* is reported to be a typical endogeic earthworm (Bastardie et al., 2005a). This corresponds with
the observation that OM location significantly influenced *A. icterica*, but less than the two other species in our study. Like Francis et al. (2001), we observed no significant decrease in the area refilled with casts as depth increased, which is contrary to results of Capowiez et al. (2014).

Conclusion

Data on the effect of endogeic earthworms on soil structure are rare in the literature, especially concerning *A. icterica*. Our study provides insights into the burrowing activity of three endogeic species according to organic matter location. Bioturbation is crucial in agriculture particularly in no-till fields which are not mechanically treated to create favourable soil structure and in which structure and soil functioning are strongly affected by biological activity (Capowiez et al., 2009; Peigné et al., 2009; Crittenden et al., 2014).

Our results obtained under controlled conditions have now to be confirmed under field conditions, in which soil heterogeneity can be integrated and interspecific competition between earthworms occur. Among other parameters such as bulk density, OM location may change according to tillage practices: for example, no-till systems keep OM on the soil surface and ploughed systems mix OM into the soil. Our results suggest that the three species do not bioturbate the soil in the same way under these tillage practices because of these differences in OM location. However the combined effect of OM location and other parameters, e.g. bulk density, must be studied to confirm this statement. Moreover, even though these species are endogeic, they do not preferentially burrow the soil at the same depth, but are complementary and thus our results suggest that these species do not occupy the same ecological niche. This is one reason why species diversity within the same ecological category must be maintained or increased. Results of this study will be integrated
into a computer model that simulates impacts of earthworms on soil structure and accounts for tillage practices.

Earthworm ecological categories are still disputed. This study shows that the bioturbation activity of *A. chlorotica* and *A. icterica* agrees with their classification as epi-endogeic and true endogeic, respectively. Recent work has begun to focus on addressing the ecological traits of earthworm species (Lowe and Butt, 2007; Fernández et al., 2010; Pey et al., 2014) and this needs to be continued.

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Captions:
Figure 1. Measured bioturbation parameters of three endogeic earthworm species. Error bars represent one standard error. Bars sharing a letter are not significantly (p > 0.05) different. (a) Mean number of burrows per section; (b) Mean burrowed area per section; (c) Mean percentage of burrowed area refilled with casts.

Figure 2. Mean number of burrows per section by depth of three endogeic earthworm species. Error bars represent one standard error. Linear regressions are given if significant. a) $1.58 \times \text{depth} + 21.04$, $r = 0.63$, $p < 0.001$; b) $-1.52 \times \text{depth} + 7.90$, $r = 0.74$, $p < 0.001$; c) $0.87 \times \text{depth} + 18.09$, $r = 0.57$, $p < 0.001$; d) $1.27 \times \text{depth} + 24.69$, $r = 0.65$, $p < 0.001$; e) $-2.68 \times \text{depth} + 24.74$, $r = 0.69$, $p < 0.001$; f) Regression not significant.

Figure 3. Mean burrowed area per section by depth of three endogeic earthworm species. Error bars represent one standard error. Linear or non-linear regressions are given if significant a) $\text{area} = 3458.67 \times e^{0.36 \times \text{depth} - 0.1}$, Pearson correlation coefficient $= 0.79$, $p < 0.001$; b) $\text{area} = -85.35 \times \text{depth} + 331.76$, $r = 0.60$, $p < 0.001$; c) Regression not significant; d) $\text{area} = 25.84 \times \text{depth} + 397.17$, $r = 0.54$, $p < 0.001$; e) $\text{area} = -78.85 \times \text{depth} + 405.35$ (first layer excluded), $r = 0.69$, $p < 0.001$; f) $\text{area} = -25.67 \times \text{depth} + 364.61$ (first layer excluded), $r = 0.34$, $p < 0.05$. 
Bioturbation activity of endogeic species was affected by organic matter location.

Burrowed area refilled with casts were higher under mixed-OM vs. surface-OM.

*A. icterica* was less affected by OM location than *A. chlorotica* and *A. caliginosa*.

*A. chlorotica* expressed an epi-endogeic behavior.