



Earthworm burrowing modes and rates depend on earthworm species and soil mechanical resistance

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ABSTRACT

Earthworms drive multiple soil processes, but their specific impact on soil functions differs between earthworm species and ecological categories. A key challenge in modern agriculture is soil compaction due to heavy machinery, but we have limited quantitative knowledge about how the burrowing activity of different earthworm species is affected by compaction. Here, we address this question in a laboratory experiment with 2-D terraria, where we used *Aporrectodea caliginosa* (Savigny, 1826) and *Aporrectodea longa* (Ude, 1885) as representatives of two different ecological categories. We exposed both species to four different soil mechanical resistance levels and monitored their burrowing activity for three days. We quantified burrowing rates and cast production, assessed the burrowing mode, and estimated energy requirements as a function of soil mechanical resistance. The results showed that the burrowing rates of both earthworm species significantly decreased with increasing soil mechanical resistance, but that the impact was species-dependent and lower for *A. longa*. Earthworms changed their burrowing mode towards ingestion when soil mechanical resistance increased, and this shift was more prominent for *A. caliginosa* that primarily burrowed via cavity expansion (i.e. by pushing soil aside) at low soil mechanical resistance. We further show that energy requirement and cast produced per unit burrow length increased with soil mechanical resistance. Our study revealed significant and species-dependent adverse effects of soil mechanical resistance on earthworm burrowing, which in turn has consequences for many soil processes mediated by earthworms, such as water infiltration, soil aeration, nutrient cycling and soil organic matter turnover.

1. Introduction

Earthworm burrowing impacts soil drainage and aeration, soil structure stability, and the resulting macropore networks provide habitat for smaller soil organisms (Bolton and Phillipson, 1976; Francis et al., 2001). Burrowing behaviour is highly influenced by a range of soil properties and soil conditions, including soil bulk density. An increase in soil bulk density due to e.g. soil compaction has been shown to decrease earthworm abundance (Pižl, 1992; Crittenden et al., 2014), and reduce burrowing activity (Rushton, 1986; Capowiez et al., 2021) and cast production (Kretzschmar, 1991). Compaction generally leads to higher soil mechanical resistance, which increases the energy requirement of earthworms for burrowing (Ruiz et al., 2015) and reduces burrow

lengths (Söchtig and Larink, 1992; Stovold et al., 2004). It has been found that compaction adversely affects earthworm activity, as well as soil functions and processes that are directly or indirectly mediated by earthworms (Beylich et al., 2010).

Earthworm species differ in their burrowing activity, and therefore, burrow characteristics and systems are also earthworm specific (Francis et al., 2001). Bastardie et al. (2005) discerned two classes of burrow systems with different morphological characteristics (e.g. connectivity, diameter, continuity and length), which were associated with different earthworm ecological categories or species. Using of this information, it is plausible that changes in soil conditions – e.g. soil mechanical resistance – impact different species differently. Thus, the effects on different soil processes vary, because specific soil processes are closely related to

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earthworm species or specific ecological groups, such as preferential flow through vertical macropores created by anecic species or aggregate production mediated by endogeic earthworms.

When earthworms move through the soil in their search for food or shelter, they can burrow either by ingesting (and egesting) soil, cavity expansion (i.e. pushing the soil aside), or by using and expanding existing cracks (Lee, 1985). It has been assumed that the preference for a certain burrowing mode (ingestion, cavity expansion or crack propagation) varies as a function of soil conditions. It is claimed that in compacted soil, earthworms shift their burrowing mode from cavity expansion to ingestion (Dexter, 1978). Nevertheless, it remains largely unclear if and how the burrowing mode changes depending on soil mechanical conditions. The burrowing mode likely impacts earthworm burrowing rates, as earthworms might burrow faster or slower depending on how they move through the soil. Moreover, we may expect that different burrowing modes require different amounts of energy (Beylich et al., 2010). Changes in earthworm energy requirements

would not only affect burrowing rates, but also earthworm growth rates and population dynamics.

The importance of earthworm bioturbation for many soil processes is well recognized, nevertheless, there is a lack of information regarding how burrows are created (Capowiez et al., 2001). Understanding earthworm burrowing as a function of soil conditions is crucial to gain knowledge of how much soil is “processed” by earthworms under different soil conditions, to establish quantitative links between earthworm activity, available energy resources (i.e. soil organic carbon) and soil processes mediated by earthworms. Considering that soil compaction is a major threat to different soil functions, there is a need to assess how earthworm behaviour is affected by compaction at species level, and to quantify how compaction affects species-specific burrowing rates. Moreover, identifying how burrowing modes and energy requirements of earthworms vary with soil mechanical resistance can provide a better understanding of the temporal dynamics of earthworm burrowing.

The main objective of the present study was to quantify earthworm

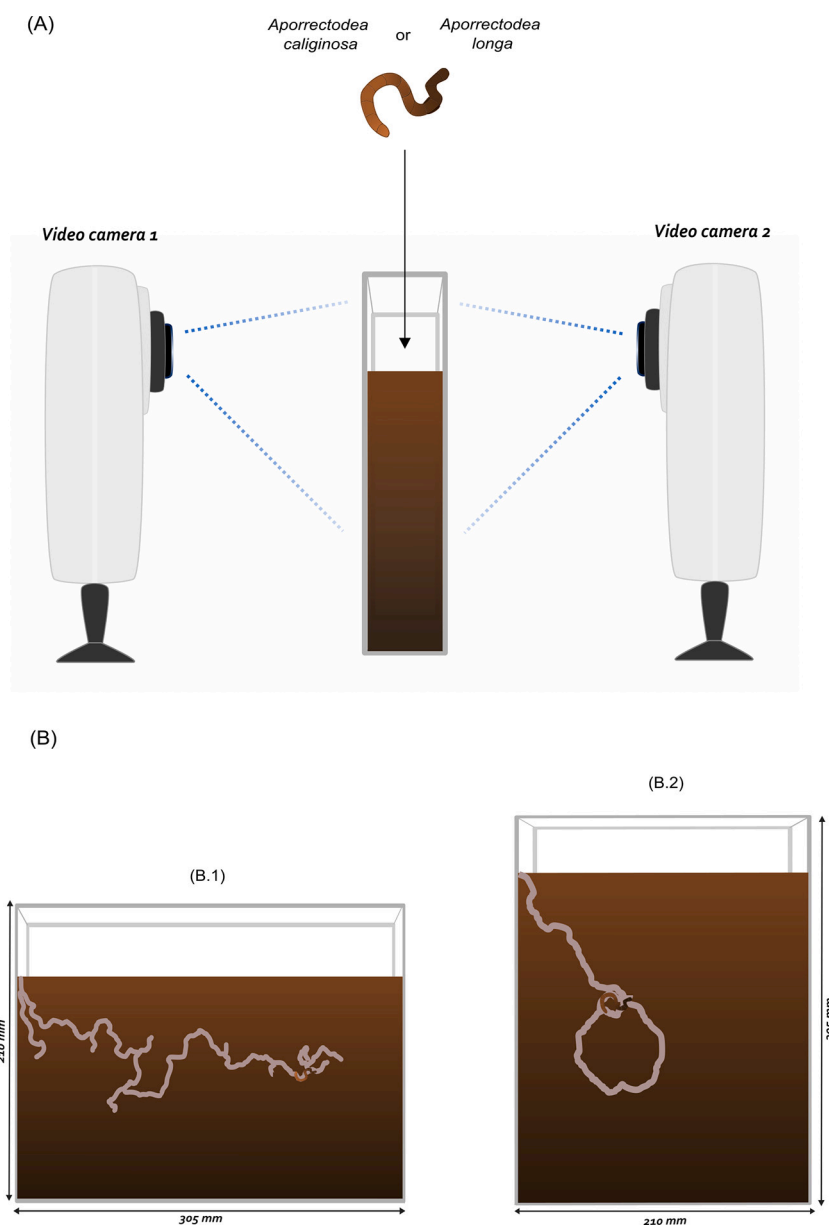


Fig. 1. (A) Lateral view of the experimental set-up. (B) Camera view of the experimental set-up, (B.1) for *A. caliginosa* and (B.2) for *A. longa*. The experimental set-up was located inside a growth chamber. Inside the growth chamber, where there was no light, a constant temperature (15 °C) and constant air humidity (60 %). Note that the figures are not drawn to scale.

burrowing rates as a function of soil mechanical resistance for an endogeic (*Aporrectodea caliginosa* (Savigny, 1826)) and an epi-aneic (*Aporrectodea longa* (Ude, 1885)) earthworm species, using a laboratory 2-D terrarium. Moreover, we determined the earthworm burrowing mode (ingestion versus cavity expansion) and cast production at different levels of soil mechanical resistance, and estimated the energy requirement for burrowing as influenced by soil mechanical resistance. We hypothesized that burrowing rates decrease and energy requirements increase with increasing soil mechanical resistance, that the burrowing mode is dependent on soil mechanical resistance, and that the two earthworm species are differently affected by compaction.

2. Materials and methods

2.1. Experimental design

This study considered two earthworm species and monitored earthworm burrowing activity using a 2-D terrarium. To study the impact of soil mechanical resistance on earthworm burrowing, the soil in the terrarium was prepared to represent different soil bulk densities. Each combination of earthworm species and soil mechanical resistance level was replicated five times. In total, we performed 40 experimental cycles (two earthworm species \times four soil mechanical resistance levels \times five replicates).

For each cycle, one earthworm was placed on the surface of the terrarium, and earthworm activity was recorded for three days with two surveillance cameras (LUPUSNET HD - LE 936 PLUS) on each side of the terrarium (Fig. 1). The 2-D terrarium was placed inside a growth chamber (Model SED-41C8, Percival Scientific Inc.), and kept at a constant temperature of 15 °C and 60 % relative air humidity. Inside the chamber, there was no light, and no diurnal temperature cycles were programmed. To ensure similar day rhythms for the earthworms, experiments were always started at around the same time of day.

The 2-D terraria were made from transparent Plexiglas® and had inner dimensions of 210 mm \times 305 mm \times 3 mm for *A. caliginosa* and 305 mm \times 210 mm \times 5 mm for *A. longa*; i.e. the orientation of the box was changed for the epi-aneic *A. longa* as shown in Fig. 1 as they tend to burrow more vertically than horizontally. The difference in thickness of the boxes is due to the difference in diameter between the two earthworm species; however we ensured that the “relative thickness” of the box (i.e. the ratio of earthworm diameter to terrarium thickness) was similar for both earthworm species (similar to Perreault and Whalen, 2006).

The terraria were carefully filled with soil (silt clay loam, see Section 2.2), layer by layer (seven layers in total), to ensure homogeneous soil bulk density within the terrarium. The experiment involved four different soil bulk densities (1.1, 1.3, 1.5 and 1.7 g cm⁻³). For each soil bulk density, we measured its corresponding soil mechanical resistance using a micro-penetrometer (cone apex angle 15°, velocity 5 mm min⁻¹) in a similar apparatus as described by Ruiz et al. (2015). The mean cone penetration resistance was 117, 579, 949 and 1068 kPa for the four compaction levels. Since earthworms experience mechanical resistance rather than bulk density when they burrow, we correlated earthworm burrowing to soil mechanical resistance in this study.

In total, we used five individuals of each earthworm species. Every individual was used at all four compaction levels, with the sequence of compaction levels randomly assigned. Earthworms were left to rest in optimum conditions for at least two weeks before they were used again.

2.2. Soil properties and soil conditions

The soil used was taken from the top 20 cm of an arable field near Uppsala, Sweden (59° 49' 58.4"N, 17° 42' 13.2"E). The topsoil texture is silty clay loam (39.8 % clay, 51.9 % silt, and 8.3 % sand). The soil organic carbon (SOC) content was 2.26 %, and the pH was 5.9 (measured in a 1:2 ratio of soil: deionized H₂O). Visible plant and root

residues were removed before the soil was sieved using a 2 mm mesh, and then oven-dried for 24 h at 105 °C. For the experiments, the soil was moistened to a matric potential of -100 hPa. The amount of water required was calculated on the basis of soil water retention measurements carried out on soil cores placed on a tension plate using the same soil and the four considered bulk densities.

2.3. Earthworms

Experiments were carried out with two earthworm species: *A. caliginosa* and *A. longa*. Earthworms are traditionally classified into three main ecological categories (Bouché, 1977): epigeic, endogeic and anecic. However, earthworm species do not always fit into one category, and therefore, we followed the recent categorization developed by Bottinelli et al. (2020), where *A. caliginosa* is considered 80 % endogeic, 4 % anecic and 16 % epigeic, and *A. longa* is considered 68 % anecic and 32 % endogeic. The two earthworm species used in this study thus represent different ecological strategies: endogeic vs. epi-aneic. Both species are among the most common species in agricultural fields in Sweden (Boström, 1995; Lagerlöf et al., 2002) and Scandinavia (Sveistrup et al., 1997; Rasmussen, 1999). Globally, *A. caliginosa* is found mostly in temperate zones of Europe and North America, but there are also records in Australia, Asia (e.g. China, Japan, Russia) and South America (e.g. Argentina, Chile) (GBIF Secretariat, 2021a). *A. longa* is primarily found in temperate zones of Europe, with some records in the east of North America (GBIF Secretariat, 2021b).

Adult earthworms were taken from a laboratory population kept at the Department of Ecology, Swedish University of Agricultural Sciences. Before the experiments, all earthworms were stored in a growth chamber at 15 °C. In immediate preparation for the experiments, earthworms were kept in Petri dishes with moist filter paper for 48 h to empty their guts, and then their body weights were recorded. The initial average body weight of *A. caliginosa* and *A. longa* was 0.51 g (standard deviation, SD = 0.15 g) and 1.93 g (SD = 0.41 g), respectively. The width on average was of 2.5 mm (*A. caliginosa*) and 4 mm (*A. longa*), while the length was 50 mm (*A. caliginosa*) and 120 mm (*A. longa*). The earthworms were placed inside the 2-D terraria and left to burrow for 69 h. After the experiments, the earthworms' guts were emptied again, and their final body weight was recorded.

2.4. Burrow metrics derived from image analysis

An image was extracted every 5 min from the original film, to create a time-lapse image sequence. The cushion effect (i.e. geometrical distortion) of the images was corrected using bUnwarpJ (Arganda-Carreras et al., 2006) in ImageJ. Tracking of earthworm movement was done using the “Manual tracking” plugin in ImageJ. For the tracking, *x* and *y* coordinates were obtained by following the movements of the earthworms prostomium in each image. The coordinates allowed us to quantify i) the burrow length, ii) the number of changes of direction of an earthworm (i.e. changing from forward to backwards moving, or vice versa), and iii) the total movement of the earthworms. The latter includes both burrowing (i.e. creation of new soil pore space) and movements in already existing burrows. Both burrowing and total movement were quantified by assuming that the earthworms moved along straight lines between coordinates on successive images. For the quantification of burrow length and total movement, the burrow network was recreated from the recorded coordinates, timestep by timestep. The straight line between the coordinates for consecutive time points was considered a new burrow if it ended outside of the existing network. If it ended inside the existing burrow, it was considered movement but not burrowing. The created line was then dilated to the approximate diameter of the earthworm. An earthworm was considered to have changed direction if the distance between the position of the prostomium at time points *t* and *t* - 1 was larger than the distance between the positions at *t* + 1 and *t* - 1. These quantifications were done in R software (R Core

Team, 2018). Hourly averages were calculated for total earthworm movement, burrow length, and burrowing rate (i.e. increment in length divided by increment in time). Quantification of total burrow length was verified with manual measurements at the end of the experiments. The manual measurements were made with a ruler on the final images of the burrows.

2.5. Cast production

At the end of the experiments (i.e. after 69 h), all casts were collected from the surface and from within the burrows. Because the amount of cast for SOC analysis was not always enough for the 40 experimental cycles, we had to pool casts from some experiments, resulting in three replicates for SOC cast analysis. The samples were oven-dried and their weights were recorded. The SOC contents of the casts were measured by dry-combustion on a TruMac CN (LECO Corp.).

2.6. Estimation of burrowing mode

Based on cast production and burrow length, we estimated how much of the burrowing was done by ingestion and how much by cavity expansion. The volume of ingested soil ($V_{\text{ingestion}}$) was calculated as the product of the dry mass of all casts produced (m_{cast}) and the initial soil bulk density (ρ_{soil}). The total volume of the created burrows (V_{burrow}) was calculated from the burrow length (L_{burrow}) and the burrow radius (r_{burrow}) by assuming a cylindrical shape of the burrows. We define the “ingestion factor” ($f_{\text{ingestion}}$) as the fraction of ingested soil volume ($V_{\text{ingestion}}$) to total burrow volume (V_{burrow}):

$$f_{\text{ingestion}} = \frac{V_{\text{ingestion}}}{V_{\text{burrow}}} = \frac{m_{\text{cast}}}{\rho_{\text{soil}} \cdot L_{\text{burrow}} \cdot \pi \cdot (r_{\text{burrow}})^2} \quad (1)$$

An $f_{\text{ingestion}}$ of zero indicates that all burrows were produced by pushing the soil aside (i.e., burrowing by cavity expansion), while an $f_{\text{ingestion}}$ of one indicates that all burrows were created by ingestion.

2.7. Estimation of energy requirement

We estimated the energy requirement of earthworms from the amount (mass) of ingested soil (m_{cast}), the organic carbon content in the soil (i.e., before ingestion; SOC) and in the casts (i.e., after egestion; OC_{cast}), and by assuming an energy density (w) for soil organic carbon of 35 kJ per g of soil organic carbon (Bölscher et al., 2017). We assumed that SOC was initially uniformly distributed in the soil. The energy required by earthworms (E_{req}) is then given as:

$$E_{\text{req}} = E_{\text{intake}} - E_{\text{cast}} = m_{\text{cast}}(SOC - OC_{\text{cast}})w \quad (2)$$

2.8. Statistical analyses

The homoscedasticity and normality of the data were verified using Bartlett and Shapiro-Wilk tests. The data, except the $f_{\text{ingestion}}$, were log-transformed due to variance heterogeneity and/or deviations from normality. Total burrow length, total earthworm movement, cast production, cast production per burrow length, $f_{\text{ingestion}}$, number of direction changes, energy requirement per day, and energy need per burrow length, were analysed with a two-way ANOVA with earthworm species and soil mechanical resistance as factors, to see if there was any interaction effect. In addition, analyses were also made separately for each species with a one-way ANOVA. For the analysis of the burrowing rate data, as it did not fulfil the homoscedasticity and normality assumptions even after log-transformation, a non-parametric Kruskal-Wallis test was performed with soil mechanical resistance and species as factors. Moreover, a Tukey test was done to make pairwise comparisons between soil mechanical resistance levels for total burrow length, total movement, number of direction changes, energy requirement per day, and energy need per burrow length. Linear regressions were used to analyse

how burrowing properties changed as a function of soil mechanical resistance. All statistical calculations were performed using the R software (R Core Team, 2018), R stats package version 3.6.2. For the figures, the ggplot2 package version 3.3.3 was used.

3. Results

3.1. Characteristics of earthworm burrow systems

For *A. caliginosa*, the final burrow system after 69 h of burrowing displayed an intricate pattern with many dead-ends (Fig. 2). Visual inspection of the image sequences indicated this was due to an “exploratory behaviour” of *A. caliginosa*, meaning that the individuals “poked” the surrounding soil with the prostomium and sometimes moved backwards. In contrast, *A. longa* created a burrow system with one main burrow that did not have any dead-end branches. However, *A. longa* created more loops than *A. caliginosa*. Also, *A. longa* did not show the same degree of “exploratory behaviour” as *A. caliginosa*.

In general, *A. caliginosa* burrowed and also moved more than *A. longa*. However, this difference decreased with increasing soil mechanical resistance, and earthworm movement was similar for both species at the highest soil mechanical resistance level. The total length of created burrows was significantly different between earthworm species ($p < 0.01$, Two-way ANOVA test) and soil mechanical resistance levels ($p < 0.01$, Two-way ANOVA test), while the total earthworm movement was only significantly different between soil mechanical resistance ($p = 0.01$, Two-way ANOVA test) but not between earthworm species (Table 1). Note that not all earthworm movements resulted in the creation of a new burrow, and therefore, the total earthworm movement is always larger than the total burrow length. The total earthworm movement decreased significantly with increasing soil mechanical resistance for *A. caliginosa* ($p = 0.03$, Two-way ANOVA test), but not for *A. longa* ($p = 0.32$, Two-way ANOVA test). The number of direction changes decreased with increasing soil mechanical resistance (Table 1).

3.2. Burrowing rates

For both earthworm species, burrowing rates fell markedly after the first few hours of burrowing, and this trend was more pronounced at lower soil mechanical resistance levels (Fig. 3). At higher soil mechanical resistance levels, burrowing rates were more stable throughout the whole duration of the experiments (Fig. 3). Some fluctuations in burrowing rates were recorded throughout the experiments, but no diurnal cycles were observed.

Average burrowing rates differed significantly between earthworm species ($p < 0.01$, Kruskal-Wallis test). Burrowing rates of *A. caliginosa* were significantly higher than for *A. longa*, at all soil mechanical resistance levels ($p < 0.01$, Kruskal-Wallis test). For example, at the lowest soil mechanical resistance (117 kPa), the burrowing rate of *A. caliginosa* (13.5 cm d^{-1}) was more than twice as high as for *A. longa* (6.5 cm d^{-1}). Average burrowing rates significantly decreased with increasing soil mechanical resistance for both earthworm species ($p < 0.01$, Kruskal-Wallis test). Burrowing rates for *A. caliginosa* were more strongly reduced with increasing soil mechanical resistance than for *A. longa* (Fig. 3). The burrowing rates were reduced by 57 % and 45 % for *A. caliginosa* and *A. longa*, respectively, when the mechanical resistance increased from 117 kPa (lowest soil mechanical resistance) to 1068 kPa (highest soil mechanical resistance).

3.3. Cast production

Total cast production was different for the two earthworm species ($p < 0.01$, Two-way ANOVA test), but was not influenced by soil mechanical resistance ($p = 0.34$, Two-way ANOVA test; Fig. 4). On average, *A. longa* produced about twice as much casts per day than *A. caliginosa*, at all soil mechanical resistance levels. Although the amount of casts

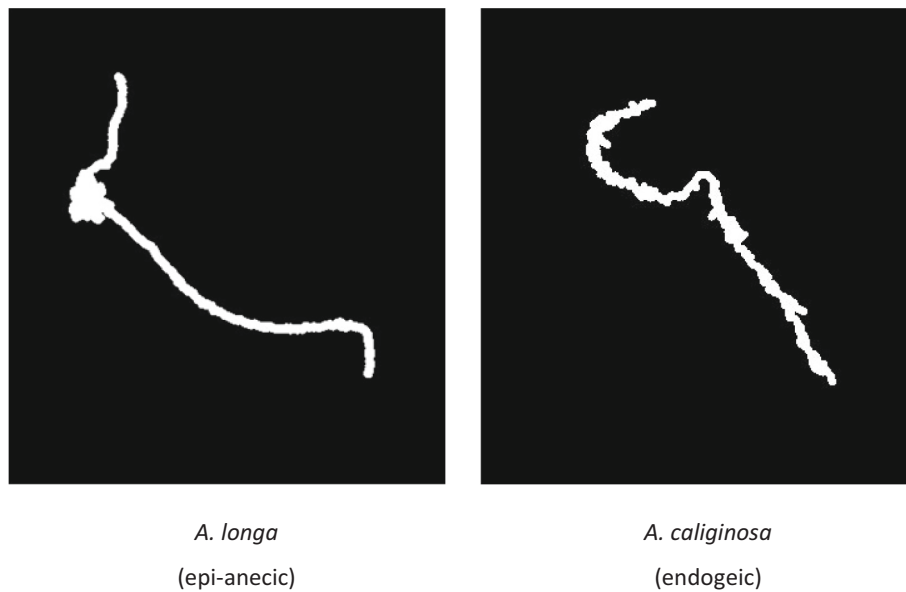


Fig. 2. Typical burrow systems for *A. longa* and *A. caliginosa* (size: 349 × 380 pixels).

Table 1

Mean values (SE) of total earthworm movement and total burrow length during 69 h for *A. caliginosa* and *A. longa* at four levels of soil mechanical resistance. Different letters within a row indicate significant differences between soil mechanical resistance levels ($p < 0.05$). $n = 5$ for all measurements.

		Soil mechanical resistance (kPa)			
		117	579	949	1068
<i>A. caliginosa</i>	Total movement (m)	1.78 ^a (0.12)	1.37 ^{ab} (0.20)	1.28 ^{ab} (0.19)	0.92 ^b (0.04)
	Burrow length (m)	0.45 ^a (0.04)	0.27 ^{ab} (0.05)	0.27 ^{ab} (0.05)	0.18 ^b (0.03)
	Number of direction changes	255 ^a (24)	224 ^a (37)	238 ^a (32)	163 ^a (9)
	<i>A. longa</i>	Total movement (m)	1.42 ^a (0.24)	1.22 ^a (0.19)	0.97 ^a (0.24)
Burrow length (m)	0.24 ^a (0.05)	0.22 ^a (0.01)	0.13 ^a (0.03)	0.13 ^a (0.02)	
Number of direction changes	186 ^a (32)	126 ^a (27)	116 ^a (31)	103 ^a (11)	

produced was related to the length of the burrows created by the earthworms, we found that for both earthworm species, cast production per burrow length increased significantly ($p < 0.01$, Two-way ANOVA test) with soil mechanical resistance (Fig. 4). For both earthworm species, the cast produced per unit length of burrow doubled between the lowest and highest soil mechanical resistance level (Fig. 4).

3.4. Burrowing mode

In general, over the 69 h, burrowing by ingestion was more common for *A. longa* than for *A. caliginosa* (Fig. 5). For *A. caliginosa*, $f_{ingestion}$ (Eq. (1)) increased from 0.34 (about one-third of the burrows were created by ingestion and two-thirds by cavity expansion) at the lowest soil mechanical resistance (117 kPa) to 0.66 (two-thirds of the burrows were due to ingestion and one third due to cavity expansion) at the highest level of soil mechanical resistance (1068 kPa). For both earthworm species, there was a significant effect of soil mechanical resistance on the ingestion factor ($p < 0.01$, Two-way ANOVA test).

3.5. Estimates of energy requirement

The estimated energy requirement (Eq. (2)) per day revealed a

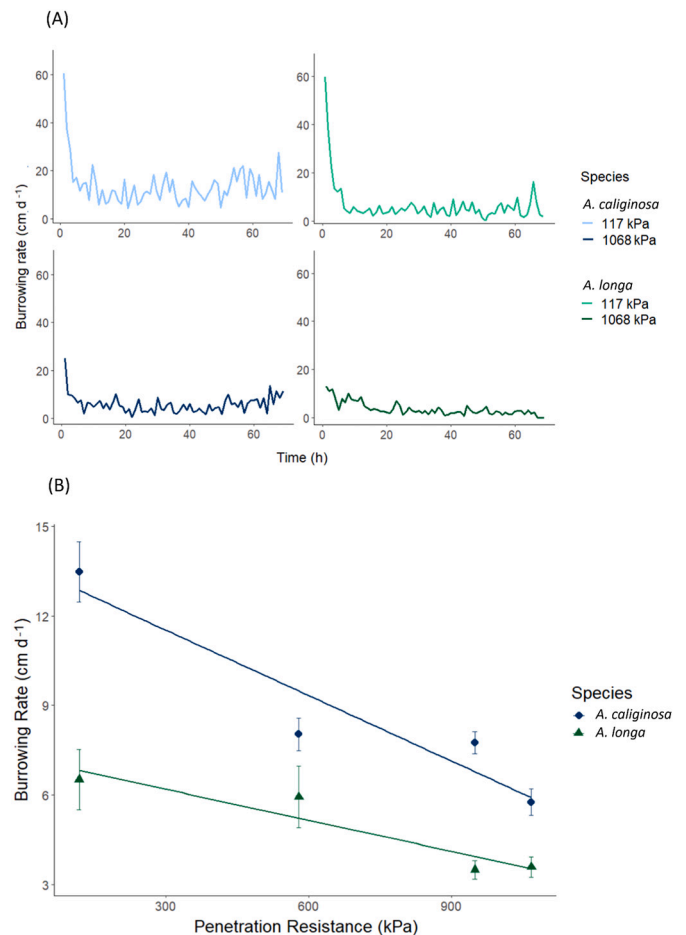


Fig. 3. (A) Burrowing rates as a function of time at two different levels of mechanical resistance for (left) *A. caliginosa* and (right) *A. longa*. (B) Average burrowing rate as a function of soil mechanical resistance (*A. caliginosa*: $R^2 = 0.89$, $p < 0.01$; *A. longa*: $R^2 = 0.89$, $p < 0.01$). Error bars indicate standard error ($n = 5$).

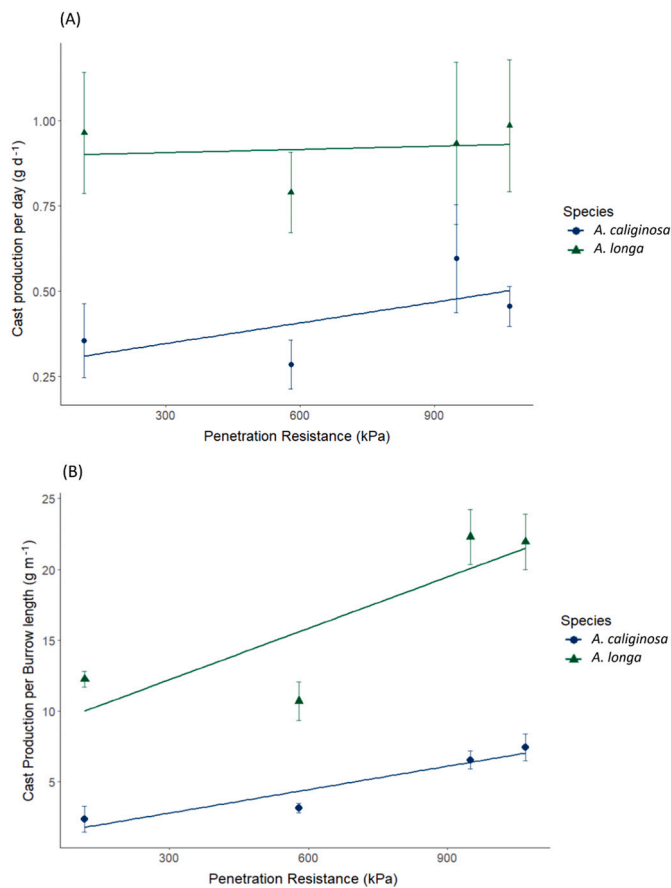


Fig. 4. (A) Cast production per day as a function of mechanical resistance (*A. caliginosa*: $R^2 = 0.89$, $p < 0.01$; *A. longa*: $R^2 = 0.89$, $p < 0.01$). (B) Cast production per metre of burrow as a function of soil mechanical resistance (*A. caliginosa*: $R^2 = 0.41$, $p = 0.22$; *A. longa*: $R^2 = 0.023$, $p = 0.92$). Error bars indicate standard error ($n = 5$).

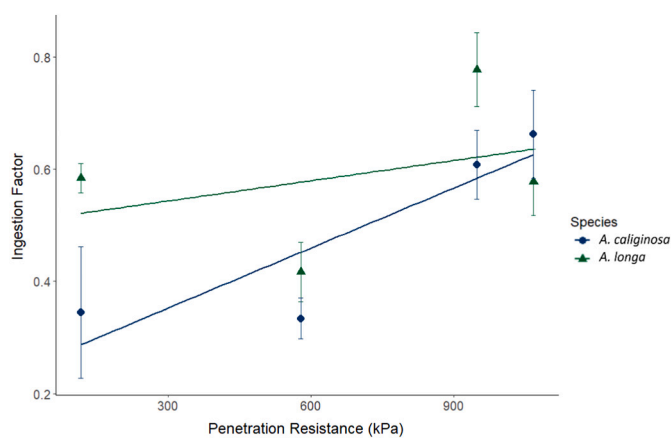


Fig. 5. Ingestion factor as a function of soil mechanical resistance (*A. caliginosa*: $R^2 = 0.78$, $p < 0.01$; *A. longa*: $R^2 = 0.12$, $p = 0.36$). Error bars indicate standard error ($n = 5$).

significant difference between earthworm species ($p < 0.01$, Two-way ANOVA test). On average, across all soil mechanical resistance levels, daily energy used by *A. longa* and *A. caliginosa* was $4.4 \times 10^{-2} \text{ kJ d}^{-1}$ (SD = 0.002) and $1.9 \times 10^{-2} \text{ kJ d}^{-1}$ (SD = 0.005), respectively. Our data show that the daily energy used increased by 10 % for *A. longa* and 80 % for *A. caliginosa* when soil mechanical resistance increased from 117 kPa

Table 2

Mean values (SE) of energy requirement per day (kJ d^{-1}) for *A. caliginosa* and *A. longa* at four levels of mechanical resistance. Different letters within a row indicate significant differences between soil mechanical resistance levels ($p < 0.05$). $n = 5$ for all measurements.

	Soil mechanical resistance (kPa)			
	117	579	949	1068
<i>A. caliginosa</i>	0.015 ^a (0.015)	0.017 ^a (0.015)	0.027 ^a (0.009)	0.017 ^a (0.006)
<i>A. longa</i>	0.042 ^a (0.035)	0.045 ^a (0.029)	0.046 ^a (0.048)	0.042 ^a (0.032)

to 949 kPa (Table 2). Energy demand at 1068 kPa was slightly lower than at 949 kPa for both earthworm species (Table 2). Also, there was no significant impact of soil mechanical resistance on energy requirement per day for either of the species (*A. caliginosa*: $p = 0.51$; *A. longa*: $p = 0.97$, Two-way ANOVA test).

The energy used per burrow length for *A. longa* was four times higher ($9.6 \times 10^{-1} \text{ kJ m}^{-1}$) than for *A. caliginosa* ($2.2 \times 10^{-1} \text{ kJ m}^{-1}$). On average, the concentration of organic carbon of the casts was 2.12 % and 2.09 % for *A. caliginosa* and *A. longa*, respectively (the initial soil organic carbon concentration was 2.26 %; see Section 2.2). For both earthworm species, the energy requirement per metre burrow significantly increased with increasing soil mechanical resistance (*A. caliginosa*: $p < 0.01$; *A. longa*: $p = 0.02$, Two-way ANOVA test; Fig. 6).

4. Discussion

4.1. Burrowing behaviour differs between earthworm species

We observed a difference in burrowing behaviour between the two earthworm species. *A. caliginosa* showed a greater exploratory behaviour, i.e. many backwards and forward movements, maybe to find the most suitable spots for foraging. Being an earthworm species with primarily endogeic traits, the many direction changes probably reflect this search for better foraging spaces. However, this exploratory behaviour tended to decrease with increasing soil mechanical resistance, and under high soil mechanical resistance, we observed a significant reduction in the total movement for *A. caliginosa*. A balance between the energy costs and benefits of foraging exists (Stephens and Krebs, 1986), and therefore, the reduction in earthworm movement could mean that exploration for better foraging spots required more energy than could be afforded in soil with high mechanical resistance. *A. longa* showed fewer changes of directions compared with *A. caliginosa*, but a decrease in direction changes with increasing soil mechanical resistance was

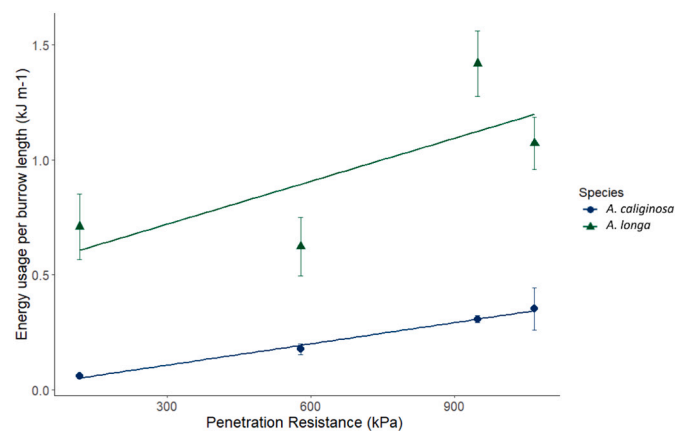


Fig. 6. Energy requirement per metre burrow length as a function of soil mechanical resistance (*A. caliginosa*: $R^2 = 0.99$, $p < 0.01$; *A. longa*: $R^2 = 0.53$, $p = 0.27$). Error bars indicate standard error ($n = 3$).

observed for both earthworm species. *A. longa* had little exploratory behaviour, reflecting a different ecological trait. As, an epi-aneic earthworm *A. longa* naturally feeds on the soil surface, while *A. caliginosa* being an endogeic earthworm forages within the soil (Bottinelli et al., 2020).

The final burrow systems of *A. caliginosa* were longer compared to those created by *A. longa*, and had many dead-end branches, as also reported by Capowiez et al. (2015). We observed that *A. longa* created “loops” in their burrow systems. This is somewhat contradictory to the results from Felten and Emmerling (2009) who reported that burrow systems of *A. longa* were branched. The reasons for the differences could be related to variations in experimental conditions (14 days duration and food provided in Felten and Emmerling (2009) vs. 3 days duration without food provided in our set-up), or reflect that earthworms can behave differently even though they belong to the same species. Our data demonstrate that the burrowing behaviour of an earthworm influences and shapes the characteristics of the burrow system.

4.2. Burrowing rate and burrowing mode are a function of soil mechanical resistance and differ between species

Earthworm burrowing rates decreased with increasing soil mechanical resistance, and the decrease was greater for *A. caliginosa* than for *A. longa* (Fig. 3). In their natural environment, *A. caliginosa* and *A. longa* show different inherent feeding behaviours: *A. caliginosa* burrows primarily to find food, while *A. longa* burrows for shelter and searches for food on the soil surface (Lavelle, 1988; Lowe and Butt, 2002). We expected this difference in feeding behaviour to be reflected in differences of burrowing rates, and this was confirmed by our results indicating higher burrowing rates for *A. caliginosa* than *A. longa*. Under favourable soil conditions, i.e. low soil mechanical resistance, earthworms are little restricted mechanically in their search for food within the soil, and this could be a reason for the higher burrowing rates when soil mechanical resistance is low. Moreover, the energy need for burrowing is lower at low soil mechanical resistance, so earthworms can “afford” to travel relatively long distances to find food.

Earthworms have a hydrostatic skeleton, filled with coelomic fluid and a muscular system composed of longitudinal and circular muscles (Lee, 1985). Depending on which of these muscles earthworms contract, either axial or radial pressure is created. Radial pressure is most relevant for burrowing by cavity expansion (i.e. pushing soil aside) because it allows the earthworm to penetrate the soil in front with lower axial pressure (Keudel and Schrader, 1999). The maximum axial and radial pressures that earthworms can exert vary among earthworm species. *A. caliginosa* can exert relatively high radial pressure but only relatively low axial pressure, while *A. longa* can exert axial pressures twice as high as *A. caliginosa* but can only exert relatively low radial pressures (Keudel and Schrader, 1999; Ruiz and Or, 2018). According to Ruiz and Or (2018), the higher pressures of *A. caliginosa* (endogeic) could provide a mechanical advantage and could extend the activity spectrum of these earthworms, in comparison to anecic worms (*A. longa*). These differences could explain why *A. caliginosa* (high maximum radial pressure) burrows via cavity expansion when soil mechanical resistance is low, while *A. longa* (low maximum radial pressure) burrows via ingestion even at low soil mechanical resistance. The shift from cavity expansion to ingestion for *A. caliginosa* when soil mechanical resistance increased could indicate that soil mechanical resistance exceeded the pressure limit of *A. caliginosa*.

The strong decrease in burrowing rate with increasing soil mechanical resistance, together with the switch in burrowing mode from cavity expansion to ingestion with increasing soil resistance for *A. caliginosa*, may indicate that burrowing by ingestion is slower than burrowing by cavity expansion. This is supported by the generally lower burrowing rates of *A. longa*, which had a higher $f_{\text{ingestion}}$. For *A. longa*, which did not significantly change its burrowing mode, the reduction in burrowing rate with increasing soil mechanical resistance was not as pronounced as

for *A. caliginosa*.

We determined burrowing mode from the ratio of cast volume to burrow volume. Cast production depends greatly on the ecophysiology of earthworm species (Buck et al., 2000) and can provide insights into the burrowing mechanism of earthworm species. Our calculations of the ingestion factor (Eq. (1)) indicate that the burrowing mode i) is a function of soil mechanical resistance, and ii) varies between earthworm species. Some studies have found that surface cast production increases at higher compaction levels (Beylich et al., 2010), yet our results did not reveal any increase in absolute cast production (above and below ground) with increasing soil mechanical resistance. However, cast production per metre burrow increased with increasing soil mechanical resistance, for both earthworm species (Fig. 4). This was expected because earthworms have to ingest more soil to create a unit length of burrow at higher soil mechanical resistance levels. The $f_{\text{ingestion}}$ was larger than 0.5 at the two highest soil mechanical resistances for both earthworm species, which means that the predominant burrowing mode in compacted soils was ingestion. We observed a shift to ingestion of soil between 579 and 949 kPa (Fig. 5), which could indicate a possible threshold in this mechanical resistance range. Our findings are supported by Kemper et al. (1988) and Dexter (1978), who reported that earthworms move through compacted soil mainly by ingestion. Our data suggest that earthworm muscular build could play an important role in determining the burrowing mode of earthworms. Yet the exact reason for the change in burrowing mode remains unclear: it could be forced by mechanical constraints, or could burrowing by ingestion be more energy-efficient than burrowing by cavity expansion in highly compacted soils?.

4.3. Energy requirements per burrow length increased with soil mechanical resistance

We estimated an average daily energy requirement (Eq. (2)) of $1.9 \times 10^{-2} \text{ kJ d}^{-1}$ for *A. caliginosa* and $4.5 \times 10^{-2} \text{ kJ d}^{-1}$ for *A. longa*, averaged across all mechanical resistance levels. Our estimate for *A. caliginosa* is similar to the maintenance costs (including energy requirement for burrowing) obtained from the earthworm energy budget model presented by Johnston et al. (2014), which yields $2.4 \times 10^{-2} \text{ kJ d}^{-1}$ for *A. caliginosa* with 0.5 g body weight at a temperature of 15 °C. The higher absolute energy demand for *A. longa* might explain why *A. longa* had a higher $f_{\text{ingestion}}$, i.e. burrowed primarily via ingestion, already at low soil mechanical resistance levels – *A. longa* is in need of more energy (more food) than *A. caliginosa*.

The energy requirements to create a unit length of burrow doubled for *A. longa* when soil mechanical resistance increased from 117 kPa to 949 kPa, and for *A. caliginosa*, energy consumption per burrow length increased five-fold between 177 kPa and 1068 kPa soil mechanical resistance. This clearly shows that the energy to create a burrow of a certain length increases when the soil mechanical resistance increases, and this was found for both earthworm species. We suggest that analysing the energy requirements concerning different burrowing modes could be a subject for future research. Linking soil mechanical conditions, burrowing mode and energy requirement will allow prediction of earthworm bioturbation under various soil conditions.

In this study, we manipulated soil mechanical resistance by varying soil dry bulk density, while matric potential was kept at -100 hPa for all levels of soil mechanical resistance. For a given soil, mechanical resistance increases with increasing bulk density and decreasing matric potential (e.g. To and Kay, 2005). We could therefore expect similar effects on burrowing rates and energy requirements when soil mechanical resistance increases due to soil drying. However, soil moisture itself has additional direct impacts on earthworm well-being and therefore also on burrowing rates and maintenance costs, since earthworms need to keep their body surface moist by secretion of mucus (Edwards and Bohlen, 1996). Holmstrup (2001), performing laboratory experiments with *A. caliginosa*, showed that earthworms entered diapause at matric

potentials < -200 hPa. In wet soil, oxygen could become limiting for earthworms (Chuang and Chen, 2008), especially in compacted soils where gas transport is restricted (e.g. Berisso et al., 2013).

It is expected that soil compaction and drier soil conditions increase in future due to a continued trend towards heavier farm vehicles and due to climate change, and this would increase soil mechanical resistance (Gao et al., 2012). Therefore, our results suggest the risk of a general decrease in earthworm burrowing together with an increased energy demand of earthworms in arable soil. In the long term, this would negatively affect many soil processes that are mediated by earthworms, including crop yield. The beneficial functions of earthworms for soil processes can only be maintained if soil management is adapted to both reduce soil compaction and increase soil organic matter in arable soils so that earthworms can meet their energy requirements and stay active under future more stressful environmental conditions.

5. Conclusions

We showed that burrowing rates of earthworms decrease with increasing soil mechanical resistance. Burrowing rates were reduced by half when soil mechanical resistance increased from about 100 to 1000 kPa. The impact of compaction on burrowing rates was species dependent, with *A. caliginosa* being more strongly affected than *A. longa*. Soil mechanical resistance also influenced the earthworms burrowing mode, and the impact was different for the two earthworm species studied. *A. longa* burrowed primarily by ingestion at all levels of soil mechanical resistance, while *A. caliginosa* burrowed by cavity expansion at low soil mechanical resistance and by ingestion at high levels of mechanical resistance. Further research is needed to reveal whether earthworms change their burrowing mode as a function of soil mechanical resistance to save energy, or whether they are forced to do so because they cannot exert the pressure needed for cavity expansion. We showed that the energy needed to create a unit length burrow strongly increased with increasing soil mechanical resistance. Our data could be used to parameterize simulation models of earthworm behaviour. The results demonstrate the impact of soil mechanical conditions on earthworm burrowing, and that this effect is species dependent. Further studies with different earthworm species are needed to better understand how soil mechanical conditions affect earthworm burrowing behaviour at the species and ecological group level.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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