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# Behavioural flexibility in *Lumbricus terrestris* burrowing

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ABSTRACT

*Lumbricus terrestris* is an epi-anecic earthworm, normally occupying a 1–2 m deep, vertical burrow. Some observations suggest that population persistence in much shallower burrows could be possible in a mild and humid climate. This was further investigated at an ex-industrial site in NW England, with a topsoil less than 0.15 m deep, above inert subsoil formed from semi-weathered Leblanc waste. *L. terrestris* were collected from an adjacent woodland soil and introduced into unoccupied areas. After four days, settlement and survival were studied by targeted sampling of half of the individuals, and depth of burrows were measured by resin casting. After 14 months, the second half of inoculated areas were studied and after another four years a further general survey occurred. After four days, 41 % of targeted worms were recovered, with 0.11 m mean burrow depth and burrows ending at the subsoil interface. After 14 months, all age classes of *L. terrestris* were present and burrow depth had not changed. After five years, adult, juvenile and hatchling *L. terrestris* were present, demonstrating establishment of a breeding population. In a parallel laboratory experiment, with site topsoil and subsoil in Evans' boxes, *L. terrestris* avoided subsoil and constructed U-shaped burrows. The results show that through flexible burrow construction, *L. terrestris* can survive above highly constraining subsoil conditions. This is likely to be only possible where severe droughts are uncommon, and topsoil does not freeze in winter.

Burrowing behaviour has a key role in adaptation of many soil- and sediment-dwelling animals. In soil communities, earthworm burrowing has been studied extensively due to its importance for soil structure [1]. Environmental conditions affect earthworm burrowing through variation of soil temperature and moisture [2] with deeper burrowing during drought and frost [3], resource availability [4], soil density [5], texture [6] and water table [7]. However, studies explicitly addressing flexibility of earthworm burrowing behaviour in terms of potential variability within earthworm ecological categories have seldom been addressed [8].

Epi-anecic earthworms are unique in their burrowing behaviour, as they dig a vertical home burrow which opens at the soil surface. A well-known example is *Lumbricus terrestris* L., burrows of which we have measured to depths of 0.7 m in temperate conditions [9] and to 1 m in boreal field soil [7]. The purpose of the present study was to investigate the possible flexibility of *L. terrestris* burrowing depth where subsoil conditions likely restrict typical behaviour. This was considered useful, thinking for instance of the usage of *L. terrestris* inoculation for improvement of shallow artificial topsoils above restricting subsoil conditions.

The field study was conducted at Nob End, an 8.8 ha field site, extensively surveyed floristically [10] and for selected soil fauna [11], at an unmanaged grassland area with sparse ground cover (53.552056, −2.379028). The topsoil (pH 8) extended to max. depth of 0.18 m, with orange, weathered Leblanc waste subsoil below to 1.2 m. The Leblanc process was used in industrial production of sodium carbonate and produced large amounts of hydrogen chloride and calcium sulphide waste piled in heaps [11]. Preliminary observations suggested potential for *L. terrestris* presence despite likely unfavourable subsoil properties. Before experimentation, sampling for earthworms at the site was conducted using a suspension of 5 g Coleman's of Norwich dry mustard powder per litre of water. This produced numerous adult *Aporrectodea caliginosa* Sav. and a single juvenile *L. terrestris*, while an extensive search revealed no *L. terrestris* middens (mean juvenile *L. terrestris* abundance was recorded as $<2\ m^{-2}$).

In March 2010, *L. terrestris* (N = 180) were collected for the experiment with mustard extraction from a near-by woodland site [11] with no signs of Leblanc waste in the subsoil, based on auger samples (dia. 3 cm) to a depth of 1.5 m, but considerable brick debris was present. The individuals were washed in water on emergence and kept overnight in

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field soil under ambient conditions prior to inoculation next day. Burrow depth at the collection site was studied by resin casting [9]. After one day, to allow burrows to dry, polyurethane resin was poured into the burrows and left for a further day to harden and then exposed from the side, measured *in situ* and dug out intact. Based on four completely cast burrows, the mean (±se) vertical depth at the collection site was of 0.32 ± 0.06 m (Fig. 1). These burrows were not typically vertical in shape as found, for example, by Shipitalo and Butt [9], but rather contorted where they passed around brick and other impervious objects in the soil.

Five areas for inoculation were selected, each separated by 10 m at the Leblanc subsoil area, approximately 300 m away from the collection site. Each area contained two 0.3 m × 0.3 m grids, 2 m apart (plots A and B) marked with sixteen equidistant points (0.1 m apart in a square). A shallow (2 cm) hole was made at each point and a total of 160 healthy, *L. terrestris* (2 (A and B) x 16 holes x 5 replicates) were individually introduced into the holes, and observed until fully burrowed down, with grid co-ordinates recorded to permit relocation.

Four days after inoculation, short-term persistence, and survival (% recovery from point of inoculation) was evaluated. For each area, either plot A or B was randomly selected. The grid was re-laid and the mustard vermifuge injected directly into the burrow openings, from a 50 ml syringe, and emerging worms collected over a period of 20 min. The other plot, with half of the inoculated earthworms was left untouched. General condition, and mass of emerging *L. terrestris* were recorded and the animals removed from the area, washed, and released nearby. Thirty-three healthy, *L. terrestris* from eighty inoculated (41 %), with adult mean (±se) mass of 4.1 ± 0.22 g were recovered (Fig. 2A). Of the 33 emerging worms (Fig. 2B), 12 burrows (36 %) were successfully cast with resin. Measurements of resin infilling of the burrows was done on exposure by digging and gave a mean depth of 0.11 m (max. 0.14 m; Fig. 1). The burrows did not enter the subsoil and most were "J"-shaped with a bend at the topsoil-subsoil interface. Three replicate subsoil samples of 750 $cm^3$ were collected using known volume cans from between depths of 0.2 m and 0.4 m, showing a mean bulk density of 0.61 g $cm^{-3}$ so the subsoil did not present a physical barrier to *L. terrestris* burrowing.

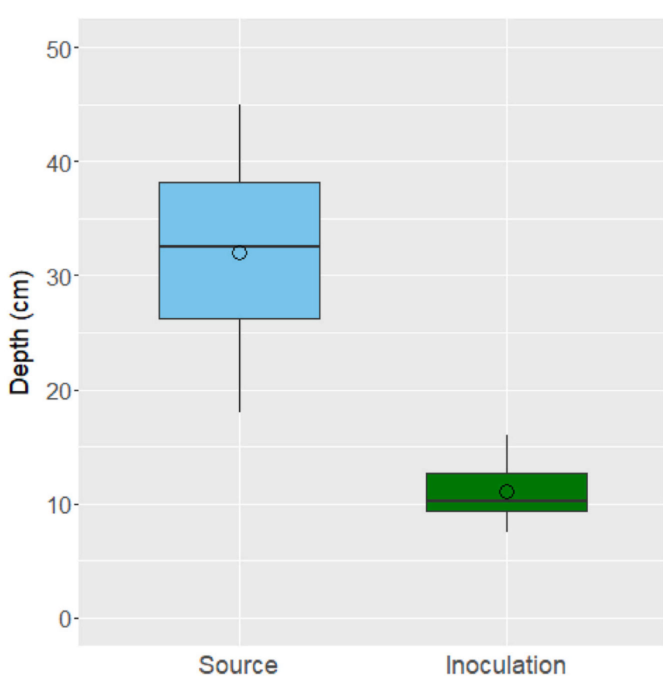


**Fig. 1.** Boxplots of *Lumbricus terrestris* burrow depths from the brown earth collection site (Source) and the inoculation area above Leblanc waste at Nob End, UK. Box lower and upper margins indicate the 1st and 3rd quartiles, respectively, line inside the box is the median, circle the mean and whisker ends the minimum and maximum values.

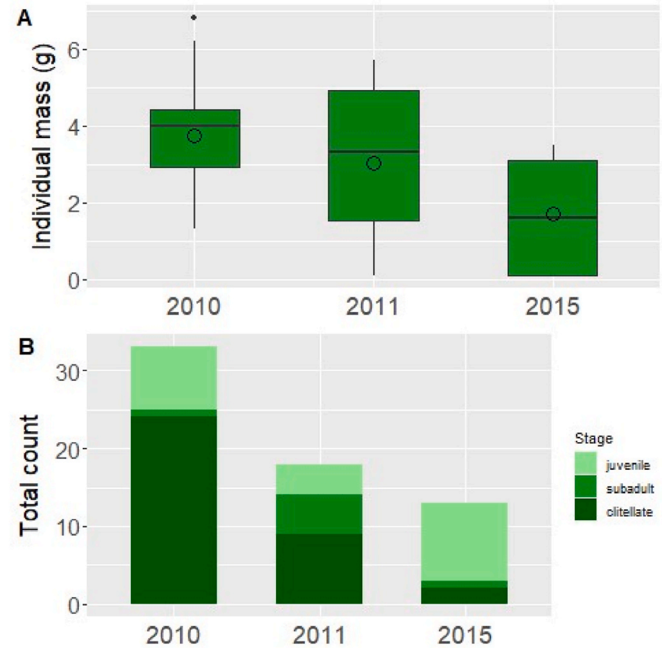


**Fig. 2.** Masses (A; boxplot symbols as in Fig.1, the dot indicates an outlier value) and total counts (B) of all recovered *Lumbricus terrestris* from those inoculated into an experimental site at Nob End in March 2010.
(Note: the area sampled in 2015 was twice that of 2010 and 2011.).

Fourteen months after inoculation (May 2011), the five areas still untouched (plot A or B from each area) were sampled with the same methods as previously used, after the soil surface had been searched for middens. No signs of middens were found, but nine adult *L. terrestris* were extracted (11 % recovery), with a mean mass of 4.20 ± 0.32 g. In addition, 5 subadults and 4 juveniles were obtained (Fig. 2B). This time burrow depth was studied by trowel excavation only and based on nine measurements, the mean depth was again 0.11 m. All recovered *L. terrestris* were released close to site of recovery, after washing in clean water and mass determination.

A further investigation was undertaken five years after inoculation (May 2015), with a mustard vermifuge applied across all five re-located inoculation grids (both plots A and B together). Re-sampling across these plots, sampled either 4 or 5 years earlier, was regarded reasonable as it was envisaged that not all individuals may have been obtained by the earlier targeted samplings and that colonisation of the plots by those individuals and production of offspring from cocoons could have occurred. Emerging earthworms were washed, examined, and had masses determined. Middens were again undetectable, but a total of 13 *L. terrestris*, two mature (mean mass 3.27 ± 0.20 g), one subadult and a further ten juveniles and hatchlings were collected (Fig. 2B). All immature specimens were grown to maturity in the laboratory to confirm species identity.

When the field work was started in March 2010, three adult *L. terrestris* (mean mass of 4.97 ± 0.95 g), collected from the woodland site, but not inoculated, were taken to a UCLan laboratory, and set up in separate glass-sided mesocosms (Evans' Boxes; 0.2 x 0.38 × 0.07 m) [12] and kept at 15 °C in darkness. Mesocosms were filled with topsoil (0.15 m) above subsoil (0.15 m) collected from the inoculation site, to mimic the field. These *L. terrestris* were fed periodically with dried grass. After 9 months, the position, shape and dimension of burrows were examined. All 3 earthworms were alive at the end of the experiment, had burrowed extensively within the topsoil but did not enter the subsoil below, the resulting burrow configuration was U- or circle-shaped (Supplementary Fig. 1).

The reduction in recovery rate of *L. terrestris* from the original inoculum was expected in the field, as soil conditions likely led to relatively rapid, over-surface emigration and/or mortality [13]. Studies from both temperate [14] and boreal [15] forests have shown that shallow soil likely limits the distribution of *L. terrestris,* underlining the importance of deep burrowing for population persistence. However, results obtained here, with soil depth as little as 0.11 m, show that persistence in shallow soil is possible, if at low population density. These results concur with findings from an urban soil in Preston, NW England, where *L. terrestris* was found to reside in burrows to 0.2 m when subsoil conditions were unfavourable for deeper construction [16]. In NE France, it was noticed that introduced *L. terrestris* survived for 6 months in burrows with maximum depth of 0.13 m in forest soil where compacted subsoil limited burrowing [17]. The present results suggest that long term population persistence and reproduction at the study site was possible although the mass of adults did show a significant decrease, perhaps reflecting comparatively poor food resource by comparison with the collection site.

Unlike endogeic earthworm species, *L. terrestris* does not construct aestivation chambers during dry or cold conditions but descends to the lower parts of its burrow to avoid, for instance, frozen soil [18]. The present results and those referred to above are from an Atlantic climate where the absence of severe droughts and frosts relieves the necessity of deep burrowing. The use of *L. terrestris* in land improvement and reclamation may often be of interest under such climates and in conditions where subsoil properties restrict deep burrowing. This is the case for instance, at reclaimed landfills where, below a shallow artificial topsoil, there is a deliberately compacted clay which hampers construction of deep burrows [13]. There, the shift to shallow burrowing could be essential for *L. terrestris* population settlement and growth.

The importance of phenotypic flexibility [19] in the behavioural adaptation of *L. terrestris* in the variation of its physical environment has previously been shown in a different context [20]. The present results further underline the general importance of such flexibility in the ecology of this species.

## CRediT authorship contribution statement

**Kevin R. Butt:** Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Validation, Writing – original draft, Writing – review & editing. **Visa Nuutinen:** Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – review & editing.

## 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.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejsobi.2024.103595.

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