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Active dispersal of the endo-anecic earthworm *Aporrectodea longa* (Ude) in an experimental box

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Abstract

We investigated the potential of the horizontal dispersal of the endo-anecic earthworm *Aporrectodea longa* by using an experimental box of 0.7 m^3. Two treatments, one with and another without vegetation cover (*Miscanthus x giganteus*) were compared, each with three replicates and ten earthworm individuals for each replicate. Mean horizontal dispersal of *A. longa* was 7 cm day^{-1} in a range of 8 cm (± 3) day^{-1} in the presence of *Miscanthus* and 6 (± 2) without. We calculated that the horizontal dispersal per year was in the same range (6 to 8 m yr^{-1}) that was within the typical range of several earthworm species from 2.5 to 14 m yr^{-1}. These findings have significant relevance for studies of earthworm population spread and distribution, especially in light of modelling earthworm immigration potential and velocity, triggered for example through regional climate change.

Keywords: Earthworms, *Aporrectodea longa*, horizontal movement, earthworm dispersal, experimental box, *Miscanthus*

1. Introduction

Darwin (1881) already observed that earthworms come to the soil surface for at least three reasons: feeding, reproduction and movement. Earthworms may emerge frequently from the soil or rarely, and may return to their burrows or may re-enter the soil elsewhere (Lee 1985). Possible factors responsible for the horizontal distribution of earthworms are physico-chemical soil properties, such as temperature, moisture, pH, inorganic salts, aeration and soil texture, available food and food quality as well as reproductive potential and the dispersive power of a species (Murchie 1958, in Edwards & Bohlen 1996). Further reasons for the horizontal spread of earthworms might be habitat disturbance, predator pressure, or overpopulation (Mather & Christensen 1988). According to Grigoropoulou & Butt (2010) earthworm dispersal (e.g. *Lumbricus terrestris*) can be affected by population density and resource availability.

There is much evidence that earthworms are rather mobile. For example, horizontal movement can reach several meters per year (Lee 1985). Moreover, Schwert (1980) and Mather & Christensen (1988, 1992) showed that earthworms may migrate quite long distances (up to 19 m) very quickly and sometimes in large numbers. Horizontal movement normally occurs during the night (Butt et al. 2003). Evidence was found for a collective movement, e.g. of *Eisenia fetida,* based on consensual decision (Zirbes et al. 2010).

An adequate knowledge of the horizontal movement potential of earthworm species has become important for example in the light of earthworm invasion into new habitats (Eijsackers 2010, Hendrix 2006, Hendrix et al. 2006) or in the light of global change. All in all, despite the increasing interest in biological invasions by earthworms, little is known about the species-specific behaviour and rate of colonisation.

The present study was conducted to investigate the potential of the horizontal dispersal of the endo-anecic species *Aporrectodea longa* (acc. to Felten & Emmerling 2009), a wide-spread and abundant earthworm species of arable soils in western Germany. Little recent data on the potential dispersal of *A. longa* is available, but is important for population studies in the context of modelling earthworm potential reaction on regional climate change.

2. Materials and methods

2.1. Experimental box

To determine the active movement of *A. Longa*, we used an experimental box of 0.7 m^3 (1 m length × 1 m width × 0.7 m depth). The bottom of the box was filled with a mixture of sand and gravel (approx. 5–10 cm thickness) for water drainage. For this, the box had several water outlets at the corresponding depth. Sandy-silt soil from an agricultural field in the north-west of Trier was sieved < 4 mm and filled into the box so that the natural soil profile (Fluvisol) was nearly reconstituted. Subsoil from 40–60 cm soil depth was filled to at least 30 cm depth and afterwards this soil material was covered with a 30-cm layer of the former Ap-horizon. Soil material was rewetted to a water content of 16 % to 22 % dw in the topsoil and subsoil, respectively, to adjust both horizons approximately to field capacity. We controlled moisture content via Soil Moisture Sensors (ΔT Theta Probe, UMS, Germany) and adjusted water loss before a new run, if necessary. The soil was manually compacted stepwise (every 5 cm) to a bulk density of approx. 1.3 g cm^{-3} dw. Soil chemical properties were as follows: (i) topsoil Ap: pH ($CaCl_2$) 5.9, Nt 0.11 %, TOC 1.50 % and CEC 65.2 $mmol_c$ kg^{-1} dry weight; (ii) subsoil: pH ($CaCl_2$) 5.8, Nt 0.04 %, TOC 0.4 % and CEC 51.7 $mmol_c$ kg^{-1} dry weight.

2.2. Earthworm treatment

Clitellate earthworms were extracted by hand-sorting in the field and were stored in the experimental top soil in a separate box for one week prior to the experiment. After this period earthworms were washed with water and carefully dried on cellulose paper. Two experimental treatments were carried out: (i) in the first treatment, the soil surface was left without any vegetation and (ii) secondly, we planted six young seedlings of *Miscanthus* (*Miscanthus x giganteus*) in a circle of equal distance from the centre. The soil coverage of *Miscanthus* plants was estimated to be approx. 25 %. We used this plant because of its importance in our investigation area. Each treatment was repeated threefold. We used 60 individuals in total, divided into 10 individuals for each replication of each treatment. Earthworms were placed on the soil surface in the centre of the box. Only earthworms which were vital and entered the soil immediately were used for the investigations. The experiment was performed under constant conditions in terms of temperature (16°C), relative humidity (70 %) and illumination cycle (12 h dark/12 h illuminated). A preliminary experiment was conducted in order to receive results of the dispersal behaviour of this species based on an area of 1 square meter under laboratory conditions. According to this, each run was stopped after five days to exclude that earthworms reach the walls of the box. Subsequently, earthworms were extracted from the soil by applying Allyl isothiocyanate (AITC) as a chemical expellant (Zaborski 2003), and the distance from the point of discovery of an individual to the centre of the box was noted. Afterwards, the top soil was thoroughly aerated and prepared for the following replicate. We kept three days between two replicates as a minimum.

2.3. Earthworm observation (video documentation)

In order to observe above-ground movement of earthworms during the night, a CCA video camera together with two IR radiators were installed approx. 160 cm above the soil surface on a tripod. The video camera started automatically at 18:00 and was active until 06:00 of the following day. Video records were stored on a PC with a resolution of one picture per minute.

2.4. Statistical analyses

All results are presented as means ± S.D. of the three replicates per treatment (- *Miscanthus* vs. + *Miscanthus*). For statistical comparison of the two treatments, results of all individuals was used. Treatment comparison was performed with a non-parametric Mann-Whitney-U-test ($p < 0.05$) using the SPSS 17.01 software package.

3. Results

In sum, 24–25 of the 30 earthworms were extracted from the soil within the three replicates of both treatments. This represents a recapture rate of 80 to 83 %.

Mean dispersal of *A. longa* was 7 cm per day, with a range of 6 to 8 cm. There were marked differences between the two treatments. In the first experiment without any vegetation cover (- *Miscanthus*), mean movement of *A. longa* varied between 28 (± 13) cm and 31 (± 14) cm (Tab. 1). The variability in horizontal dispersal of all individuals within the replicates was very high, with a range of 3 to 50 cm. The calculated daily movement was on average 6 cm (± 2). In the treatment with *Miscanthus* seedlings (+ *Miscanthus*), mean movement was higher and varied between 32 (± 14) cm and 42 (± 12) cm. The calculated daily movement was on average 8 cm (± 3) (Tab. 1). By comparison of the two treatments, with or without *Miscanthus*, it was clearly found that the mean horizontal movement of *A. longa* individuals was significantly enhanced in the *Miscanthus* treatment by approx. 35 % ($p = 0.08$; see Fig. 1).

4. Discussion

Based on the experimental design of the present study, we investigated the potential horizontal movement of *A. longa* in a laboratory experiment. A comparison with field studies or field observations is therefore limited. However, there is much published data from field investigations of the potential spread of various earthworm species. In most cases an earthworm's dispersal was studied when they were introduced into new habitats, such as reclaimed land, cut-over peat soils or polder soils (van Rhee 1969, Curry & Boyle 1987, Marinissen & van der Bosch 1982, Hoogerkamp et al. 1983). Graff (1961), Stockdill (1982) and Mather & Christensen (1988) investigated rates of spread in grassland and arable soils. Most investigations were conducted as recapture studies; however, Mather & Christensen (1988) observed specifically the nightly above-ground movement of earthworms. It can be summarized from literature data that the variability in dispersal of the investigated species and in the various land-use types is quite small, in a range of 2.5 to 14 m yr^{-1} (Tab. 2) and may increase to a maximum of 19 m (Mather & Christensen 1988). Compared to these results,

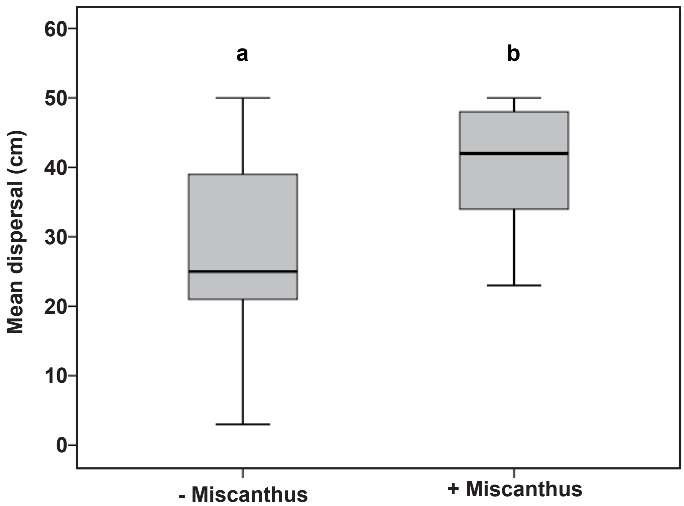

Fig. 1 Box plots of the mean dispersal (in cm) of *Aporrectodea longa* individuals in two treatments, with (+ Miscanthus) and without (- Miscanthus) vegetation with three replicates and ten earthworms each in an experimental box of 0.7 m^3. Significant differences between both treatments are marked by different letters (Mann Whitney *U*-test; $p < 0.05$).

which were conducted from field studies, the estimate of the mean dispersal of *A. longa* (7 m yr^{-1}) from the presented laboratory study is in line with the above mentioned range. It is therefore suggested that the mean horizontal spread of earthworms might be mainly in this order of magnitude. These findings have significant relevance for studies of earthworm population spread and distribution, especially in the light of modelling earthworm immigration potential and velocity triggered for example through regional climate change.

In the presented experiments a total of ten individuals were placed in the centre of the experimental box. By this, a potential overpopulation might have triggered horizontal spreading of individuals away from the overpopulated spot. However, no clear pattern of dispersal was found within the six replicates during each five days (see Tab. 1). The range of overall dispersal was 3 to 50 cm, meaning that some earthworms did not at all feel the need to spread. In some cases however, this range was much smaller, from 23 or 30 cm to 50 cm. All in all, it remains unclear if there was any interference within the various individuals. Interestingly, earthworms normally were found individually rather than nearby each other. We emphasize that individual dispersal largely resulted from gradual spread (Mather & Christensen 1988).

In order to calculate the presented results of the dispersal of *A. longa* on a yearly basis, we estimated an average of 193 days with a mean air temperature >10°C for the region of Trier, Germany, based on the last ten years (German Weather Service); data for the period 2001 to 2010). We assumed that earthworms will be active at mean daily temperatures > 10°C (Daugbjerg 1988, Mather & Christensen 1988). Moreover, *A. longa* has been reported to go into obligatory diapause from as early as May and usually comes out of diapause in September or October (Edwards & Bohlen 1996). As a consequence we subtracted a minimum of 93 days from the active period per year. Based on these assumptions, the mean potential dispersal of *A. longa* was 7 meters per year, with a range of 6 (- Misc.) to 8 (+ Misc.) and thus, this was in the range of other observations (a brief overview is given in table 2).

Tab 1 Summary of the dispersal of *Aporrectodea longa* individuals of the three runs (replicates 1–3) during five-day term experiments each in an experimental box of 0.7 m^3.

Individuals	- Miscanthus		+ Miscanthus	
	distance (cm)	distance (cm day^{-1})	distance (cm)	distance (cm day^{-1})
Replicate 1				
1	17	3.4	30	6.0
2	21	4.2	33	6.6
3	25	5.0	35	7.0
4	25	5.0	37	7.4
5	45	9.0	40	8.0
6	46	9.2	41	8.2
7	50	10.0	44	8.8
8	10	2.0	50	10.0
9	36	7.2	–	–
X (± S.D.)	30.6 (± 14.2)	6.1 (± 2.8)	38.8 (± 8.5)	7.8 (± 1.7)
Replicate 2				
1	17	3.4	27	5.4
2	15	3.0	33	6.6
3	25	5.0	38	7.6
4	35	7.0	43	8.6
5	42	8.4	44	8.8
6	36	7.2	46	9.2
7	25	5.0	49	9.8
X (± S.D.)	27.9 (± 13.1)	5.6 (± 2.6)	31.6 (± 14.3)	6.3 (± 2.9)
Replicate 3				
1	3	0.6	23	4.6
2	14	2.8	28	5.4
3	23	4.6	36	7.2
4	24	4.8	47	9.4
5	34	6.8	48	9.6
6	33	6.6	50	10.0
7	39	7.8	48	9.6
8	46	9.2	50	10.0
9	50	10.0	50	10.0
X (± S.D.)	29.6 (± 15.1)	5.9 (± 3.0)	42.1 (± 11.6)	8.4 (± 2.3)

However, there remains a potential underestimation of the calculated maximum dispersal rates of *A. longa* in the presented experiments. Some individuals were found at a distance of 50 cm from the centre in the edges of the experimental box. It remains possible therefore, that the walls of the experimental box had a limiting effect on the maximum dispersal during the five days even for those individuals found at the edges and not in direct contact to a wall.

Due to the fact that the experiments included a day-and-night illumination cycle, both nightly above-ground movement as well as below-ground dispersal of earthworms can be expected. It is not clear to what extent the movement was horizontal, vertical or diagonal, since only the distance from the centre to the point of discovery was investigated. Our behavioural video observations during each five-day term of the experiments showed at least above-ground movements; however, below-ground dispersal cannot be excluded.

Tab. 2 Summary of the mean horizontal dispersal of different earthworm species (m yr^{-1}) in various habitats, in relation to their ecological classification (ep = epigeic, en = endogeic, an = anecic; sensu Bouché 1977).

Species (ecological class.)	Distance (m yr^{-1})	Environment / soil / vegetation	Reference
Aporrectodea caliginosa (en)	6	polder soil	van Rhee (1969)
	3.5–5	irrigated desert soil	Ghilarov & Mamaev (1966)
	10	pasture	Stockdill (1982)
	9	polder grassland	Hoogerkamp et al. (1983)
	2.5–10	grassland / reclaimed peat	Curry & Boyle (1987)
	7	arable polder soil	Mainissen & van den Bosch (1992)
Allolobophora chlorotica (en)	4	polder soil	van Rhee (1969)
Octolasion cyaneum (en)	8	grassland and arable soils	Graff (1961)
Lumbricus rubellus (ep)	>10	peat soil	Curry & Boyle (1987)
	14	arable polder soil	Marinissen & van den Bosch (1992)
Lumbricus terrestris (an)	9	arable soil	Mater & Christensen (1988)
	4	polder grassland	Hoogerkamp et al. (1983)
Aporrectodea longa (an)	7 (6–8)*	laboratory	this study

* 6 m without vegetation (- *Miscanthus*); 8 m with vegetation (+ *Miscanthus*) with the following assumptions: 193 days > 10°C (German Weather Service, average of the years 2001–2010, Trier region); minus approx. 93 days of obligate diapause for *A. longa* (Edwards & Bohlen 1996).

Dispersal in the presence of vegetation (*Miscanthus x giganteus*) was significantly enhanced by 35 %. This result might be attributed to a stimulating effect of the vegetation in the way that surface-soil humidity might have been increased. Unfortunately, soil moisture was not controlled in the neighbourhood of the plants. Another reason might be that earthworm activity was enhanced through an increase in resource-richness in the *Miscanthus* treatment (Butt et al. 2003). However, earthworm movement was not directly influenced by the *Miscanthus* seedlings in the way that no worm was found directly in the neighbourhood of a plant.

According to the high recapture rate of 80–83 %, it can be assumed that the experimental conditions were favourable for the earthworms. Specifically, we found no evidence that the use of AITC as a chemical expellant affected earthworm activity in any way since we thoroughly aerated the top soil and let three days time before the next run.

5. Conclusions

Earthworms are rather mobile. Data from the literature as well as results from the present laboratory investigation emphasize that the mean horizontal spread of various species ranges between 2.5 and 14 m yr^{-1}. Literature data have been supplemented for the endo-anecic species *A. longa*, for which the mean dispersal rate was estimated to be in the range of 6 to 8 m yr^{-1}. These findings have significant relevance for modelling earthworm immigration potential and velocity triggered for example by climate change in a regional context.

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