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Original article

Competitive interactions affect the growth of *Aporrectodea caliginosa* and *Lumbricus terrestris* (Oligochaeta: Lumbricidae) in single- and mixed-species laboratory cultures

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Abstract

Negative interactions between earthworms may arise from high earthworm population densities. Under high populations in the field, niche separation or migration away from competitive pressure may help to regulate a multi-species population to a given level. This may not be possible in laboratory experiments, leading to an increase in competitive interactions which may alter earthworm growth rates and affect decomposition and nutrient mineralization processes. The objective of this experiment was to determine how growth rates of the endogeic earthworm *Aporrectodea caliginosa* Sav. and the anecic earthworm *Lumbricus terrestris* L. are affected by increasing population density and container size in both single- and multi-species cultures. Earthworm growth responses were compared in 1-L cylindrical pots containing disturbed soil and in 2.3-L PVC cores containing undisturbed soil. The relationship describing intra- and inter-specific competition was not affected by container type for both species. Nonetheless, decreasing the container size restricted the growth of *L. terrestris* in both single- and multi-species cultures, but only restricted the growth of *A. caliginosa* in multi-species cultures. For both species, a population density greater than one individual per litre reduced earthworm growth rates significantly, while weight loss in monocultures occurred when there were more than 10 *A. caliginosa*, and more than three *L. terrestris* per litre. Growth rates of both species were restricted in all population density treatments including the lowest of 0.9 individuals per litre. Further work is needed to find the population density at which growth rates are not affected and which may be used as an appropriate population in laboratory pot experiments to measure the effects of earthworms on soil processes and plant growth.
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Keywords: Earthworms; Population density; Growth rate; Container shape

1. Introduction

It has been well established that there is a positive correlation between earthworm numbers and soil fertility in many agricultural ecosystems [23]. However, the bulk of research showing the beneficial effects of earthworms on plant growth, organic matter (OM) decomposition and nutrient cycling was conducted in pot-scale experiments (for example see Refs. [4,27,29,43]). Earthworm population densities in pots are often much larger than field population densities. In a recent review describing the optimal levels of

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abiotic and biotic factors for successful laboratory cultures of soil dwelling earthworms, population density was identified as a limiting factor for earthworm growth and production [34].

Competitive interactions occur as earthworm population density increases in single- and multi-species cultures or as container size decreases, since greater niche overlap occurs when more individuals are present. Earthworm growth rates decline when there is more competition between earthworms [1,33]. When earthworm growth is reduced, earthworms consume less food to increase their body mass. This may consequently decrease organic matter decomposition and nutrient mineralization rates, and may influence earthworm burrowing activities and community structure [15,26]. In multi-species experiments conducted in small pots, competitive effects between earthworms may be overstated compared to field situations, where spatial avoidance can occur [19]. This was demonstrated by Capowiez and Belzunces [15], who showed that earthworms will often avoid the burrows of earthworms from a different ecological group. Therefore, interactions between earthworm species should be interpreted in light of their ability to avoid competition within experimental containers. Yet our knowledge of how the competitive interactions between earthworms are influenced by decreasing niche overlap is poorly understood.

The objective of this study was to determine how inter- and intra-specific competition between *Aporrectodea caliginosa* and *Lumbricus terrestris* are affected by different container sizes. We expect that the reduction in growth of both earthworm species as population density increases in single- and multi-species cultures will be greater in small containers than larger ones.

2. Materials and methods

2.1. Collection of earthworms and soils

Earthworms were collected in the autumn of 2005 from neighbouring fields under alfalfa (*Medicago sativa* L.) and soybean (*Glycine max* (L.) Merr.) production at Macdonald Campus Farm of McGill University, Ste-Anne-de-Bellevue, Québec, Canada. Specimens of *A. caliginosa* were collected by hand sorting, and only juvenile earthworms were kept for the experiment. Specimens of *L. terrestris* were collected by dilute formalin (0.5%) extraction [38], and sorted into adult and juvenile age classes. The earthworms were reared for 3–8 weeks at room temperature (20 °C) in the original field soil moistened to near field capacity before the experiments began.

The soil was a sandy-loam mixed of the Chicot series, classified as a frigid Typic Endoquent. It had a pH (H_2O) of 6.3, the organic C content was 30.2 g kg^{-1}, and contained 580 g kg^{-1} sand, 300 g kg^{-1} silt and 120 g kg^{-1} clay. Soils were air-dried to about 10% gravimetric moisture content (−200 kPa matric potential) before use. The earthworm food was a composted cattle manure (sieved <4 mm mesh). The organic C content was 383 g kg^{-1} and the total *N* content was 19.9 g kg^{-1} (Carlo Erba Flash EA NC Soils Analyzer, Milan Italy).

2.2. Experimental setup

Two separate experiments were conducted to evaluate intra-specific competition in single-species cultures and inter-specific competition in multi-species cultures. In each experiment two different shaped containers were used. Small plastic pots with disturbed soil were used as standard laboratory cultures, and larger PVC cylinders with an undisturbed soil core were used to simulate more realistic field soil conditions. The 1-L cylindrical plastic pots (11 cm diameter, 14 cm height) with perforated lids contained about 415 g of air dry soil packed to a bulk density of 1.23 ± 0.05 g cm^{-3} (S.D.) and moistened to 25% gravimetric moisture (about −10 kPa matric potential). The soil cores (10 cm diameter, 30 cm height and a volume of 2.3 L), taken from the same field site, were obtained by hammering a PVC tube into the ground above a visible earthworm burrow and digging out the core. Fine plastic mesh (<1.5 mm) was secured with elastic bands on both ends of the core to prevent soil loss. Undisturbed soil cores were kept in a cold room at −4 °C for 4–6 weeks to kill any earthworms that may have been collected in the core. Each core contained about 3.35 ± 0.2 kg (S.D.) of dry soil with a bulk density of 1.42 ± 0.08 g cm^{-3} (S.D.) and moistened to 25% gravimetric moisture (about −10 kPa matric potential). Approximately 15 g (5 g dm) of fresh manure was placed on the surface of the soil in each pot or core, moistened with 2–3 mL of water and incubated for 2–3 days before the experiment began. The manure application rate was equivalent to about 6 t ha^{-1}, which is similar to that used in enclosure studies (7 t ha^{-1}) to provide a reasonable quantity of surface residue and similar to conventional agricultural practices [8].

2.3. Single-species experiment

The single-species experiment was a factorial randomised incomplete design with two earthworm species (*A. caliginosa* and *L. terrestris*), two container types (pots and cores) and 3–4 population density treatments. In pots, the populations were 2, 4, 8, and 16 earthworms per litre of soil with eight replicates per treatment. In cores, the populations were about 0.9, 3.5, and 7 earthworms per litre of soil with five replicates per treatment. In the pots, juvenile earthworms with a mean mass of 0.32 ± 0.07 and 1.38 ± 0.43 g (S.D.) for *A. caliginosa* and *L. terrestris*, respectively, were washed and placed on moistened paper to void their guts for 24 h [18]. The next day the earthworms were washed, gently blotted dry with paper towels and weighed (gut-free fresh weight). We added 1, 2, 4, or 8 earthworms to each pot, which corresponds to population densities of 2, 4, 8, and 16 earthworms per litre of soil, respectively. The earthworms were then remoistened with approximately 3 ml water and the pot covered with a perforated lid. In the cores, juvenile earthworms with a mean mass of 0.30 ± 0.11 and 2.63 ± 0.95 g (S.D.) for *A. caliginosa* and *L. terrestris*, respectively, were selected. Earthworms were weighed (gut-free fresh weight), then 2, 8 or 16 earthworms were placed on the soil surface, which corresponds to population densities of 0.9, 3.5 and 7 earthworms per litre, moistened with 3 mL of water and the core was covered with fine plastic mesh (<1.5 mm). Pots and cores were placed into a controlled climate incubator at 15 °C in the dark for 28 days. Then, earthworms were removed from the pots by hand sorting and placed into an empty pot with moistened paper to void their guts for 24 h, after which the gut-free fresh weight was determined.

2.4. Multi-species experiment

The multi-species experiment was designed as a factorial randomised complete design with two container types (pots and cores), and four population treatments with different ratios of *A. caliginosa* to *L. terrestris* (Table 1). There were eight replicate pots and five replicate cores for each treatment. In the pots, the initial mean mass of *A. caliginosa* and *L. terrestris* was 0.33 ± 0.07 and 1.29 ± 0.42 g (S.D.), respectively. In the cores, the initial mean mass of *A. caliginosa* and *L. terrestris* was 0.29 ± 0.09 and 2.25 ± 0.70 g (S.D.), respectively. As in the single-species experiment, earthworms were weighed (gut-free fresh weight) before they were placed in the pot or core, and then incubated at 15 °C in the dark for 28 days for determination of growth rates.

2.5. Calculation of earthworm growth rates

Earthworm growth rates are commonly reported as either absolute growth rates or relative growth rates, and while these measurements may be useful for laboratory experiments in which the growth of an age-specific cohort is followed to maturity, they assume that earthworm growth through time is a continuous linear function [45]. It has been well established that earthworm growth through time follows a logistic curve [21,37]. As an earthworm approaches maturity, a greater proportion of the energy from food resources is likely used in the formation of sexual organs and reproduction rather than the formation of new tissues [21]. Instantaneous growth rates (IGR), which assume that growth proceeds logistically rather than linearly, are better able to account for these factors by calculating the change in an individual's growth during an infinitely short time interval [22,36]. The IGR was calculated using Eq. (1).

$$\mathrm{IGR} = \ln\left(W_f/W_i\right)/\Delta t\left(d^{-1}\right) \tag{1}$$

where W_i and W_f are initial and final earthworm mass (g), respectively, and Δt is the growth interval measured in days [10].

Table 1
Earthworm population density (individuals l^{-1}) and biomass (g gut-free fresh weight l^{-1}) for each population ratio treatment in the multi-species experiment

Container	Population ratio	Earthworm density (individuals l^{-1})			Earthworm biomass ($g\,l^{-1}$)		
	A. caliginosa: *L. terrestris*	*A. caliginosa*	*L. terrestris*	Total	*A. caliginosa*	*L. terrestris*	Total
Pot	1:1	2	2	4	0.6	2.6	3.2
	1:2	2	4	6	0.6	5.2	5.8
	2:1	4	2	6	1.2	2.6	3.8
	2:2	4	4	8	1.2	5.2	6.4
Core	1:1	0.4	0.4	0.9	0.2	1.1	1.3
	1:2	0.4	0.9	1.3	0.2	2.2	2.4
	2:1	0.9	0.4	1.3	0.3	1.1	1.4
	2:2	0.9	0.9	1.8	0.3	2.2	2.5

The soil volume was 0.5 l and 2.3 l in pots and cores, respectively.

2.6. Statistical analysis

In the single-species experiment, the relationship between the dependent growth rate (IGR) of each species and the earthworm population density in two container types were evaluated using regression analysis. In the multi-species experiment, the effects of container type, the number of *A. caliginosa*, the number of *L. terrestris*, and all two-way interactions on the growth rate (IGR) of each species were evaluated using a three-way ANOVA and normality of data was evaluated with the PROC UNIVARIATE function of SAS software [39]. Regression lines were fitted using the PROC REG function, and comparisons of regression intercepts and slopes were evaluated using the PROC GLM function of SAS software [39]. Mean growth rates in different treatments were compared using Tukey's means comparison test ($P = 0.05$).

3. Results

3.1. Single-species cultures

Earthworm mortality in both experiments was low (<2%) for both species. The initial body weights of earthworms of both species were not statistically different between containers. Also, no cocoons hatched in the cores, suggesting that freezing the cores at −4 °C for 4–6 weeks was effective at removing any viable cocoons. Growth rates in both experiments were normally distributed and had equal variances.

In single-species cultures, the IGR of *A. caliginosa* ranged from -1.0 to $7.1 \times 10^{-3}\ d^{-1}$, and was greater than the IGRs for *L. terrestris*, which ranged from -6.0 to $1.9 \times 10^{-3}\ d^{-1}$ (Fig. 1). Growth rates were highest when one to two earthworms were present and decreased significantly ($P < 0.01$) as more individuals of the same species were placed in pots or cores (Fig. 1). Logistic equations best described the earthworm growth response when more individuals of a single-species were placed in pots or cores (Table 2). For *A. caliginosa* there was no difference between the intercepts and slopes of the regression equations describing growth in the pots and cores. For *L. terrestris* the slopes were not different, but the intercept of the regression equation describing growth was greater ($P = 0.0028$) in the core than the pot (Table 2).

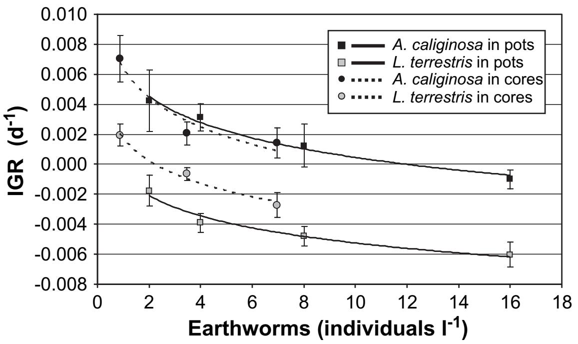

Fig. 1. Influence of increasing earthworm population density on growth rate (IGR) of *A. caliginosa* and *L. terrestris* reared in monocultures in pots and cores. Values shown are mean ± standard error of eight replicates in pots and five replicates in cores. Regression equations are presented in Table 2.

3.2. Multi-species cultures

In the multi-species experiment, *L. terrestris* lost weight in the pots but gained between 1.59 and $6.20 \times 10^{-3}\ d^{-1}$ in the cores (Table 3). Positive growth rates for *A. caliginosa* were observed in cores in all treatments, and in pots when only one *L. terrestris* was present (Table 3). An increase from one to two *A. caliginosa* in either container type did not affect the growth of either earthworm species. However, the number of *L. terrestris* significantly affected ($P < 0.01$) the growth of both species (Table 3). The growth of either species was not affected by two- or three-way interactions between the number of *A. caliginosa*, number of *L. terrestris* or container type.

Table 2
Regression equations fitted through average IGR values, describing the instantaneous growth rate (IGR) for *A. caliginosa* and *L. terrestris* as a function of earthworm population density (individuals l^{-1}) for each container type presented in Figs. 1 and 2

Earthworm	Container	Regression equation	
A. caliginosa	Pot	$IGR = -0.0025 \times Ln(ind\ l^{-1}) + 0.0063$	$R^2 = 0.98$
	Core	$IGR = -0.0028 \times Ln(ind\ l^{-1}) + 0.0064$	$R^2 = 0.95$
L. terrestris	Pot	$IGR = -0.0020 \times Ln(ind\ l^{-1}) - 0.0007$	$R^2 = 0.97$
	Core	$IGR = -0.0022 \times Ln(ind\ l^{-1}) + 0.0017$	$R^2 = 0.98$
Multi-species experiment			
A. caliginosa	With one *L. terrestris*	$IGR = -0.0021 \times Ln(ind\ l^{-1}) + 0.0041$	$R^2 = 0.87$
	With two *L. terrestris*	$IGR = -0.0020 \times Ln(ind\ l^{-1}) + 0.0008$	$R^2 = 0.97$

Table 3
Influence of earthworm numbers and container type on the IGR (±S.E.) of *A. caliginosa* and *L. terrestris* in multi-species cultures[a]

Treatments			IGR ($\times 10^{-3}$)			
Container	Number of *A. caliginosa*	Number of *L. terrestris*	*A. caliginosa*		*L. terrestris*	
Core	1	1	5.92 ± 2.69	a	2.70 ± 2.16	a
Core	1	2	2.72 ± 1.27	ab	2.42 ± 1.09	a
Core	2	1	4.92 ± 1.08	a	6.22 ± 2.18	a
Core	2	2	1.06 ± 3.15	ab	1.60 ± 1.44	ab
Pot	1	1	1.48 ± 0.97	ab	-3.45 ± 0.48	c
Pot	1	2	-1.03 ± 0.80	b	-4.98 ± 0.77	c
Pot	2	1	1.79 ± 0.77	ab	-2.54 ± 0.53	cb
Pot	2	2	-1.58 ± 0.99	b	-4.76 ± 0.69	c
ANOVA treatment effects[b]						
Number of *A. caliginosa*			n.s.			n.s.
Number of *L. terrestris*			$P = 0.003$			$P = 0.001$
Container			$P = 0.002$			$P < 0.001$

[a] Values in each column for each species and container followed by similar letters are not significantly different by Tukey's HSD test ($P = 0.05$).
[b] Interactions were not significant and are not shown.

Since there was no difference between the slopes and intercepts of the regression lines for *A. caliginosa* in monocultures in pots and cores, regression lines were fitted across all data points from these containers to describe intra-specific competition effects (Fig. 2). This was not done for *L. terrestris* because the regression lines from pots and cores were significantly different. We also fitted regression lines through data points from the multi-species experiments conducted with *A. caliginosa* in pots and cores to describe its growth when one or two *L. terrestris* were present (Fig. 2). Again, logistic growth curves best described the decrease in earthworm growth that occurred when more earthworms were present (Table 2). There was no difference in the slopes of curves describing *A. caliginosa* growth in single-species and multi-species cultures. The intercept of the regression line describing growth of *A. caliginosa* in the presence of two *L. terrestris* differed from the intercept of single-species cultures ($P = 0.019$), and the intercept of multi-species cultures with one *L. terrestris* ($P = 0.029$); however, the intercepts of these regression lines (*A. caliginosa* alone, *A. caliginosa* plus one *L. terrestris*) were not significantly different (Table 2).

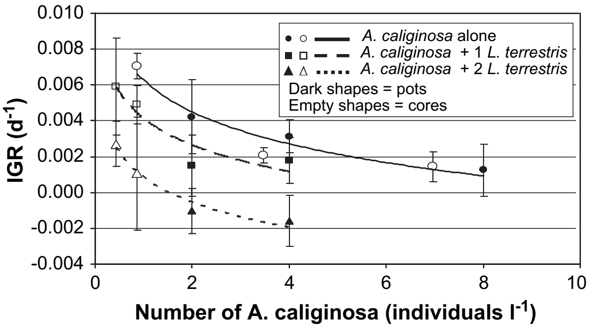

Fig. 2. Regression lines fitted through data from pot and core trials, describing the influence of increasing earthworm population density on the growth rate (IGR) of *A. caliginosa* reared in monocultures and cultures with one and two *L. terrestris*. Values shown are mean ± standard error of eight replicates and five replicates. Regression equations are presented in Table 2.

4. Discussion

4.1. Intra-specific competition effects

The single-species experiment demonstrated that increasing the number of earthworms of the same species in a fixed soil volume reduced growth rates. The regression lines obtained for *A. caliginosa* grown in pots and cores were not significantly different, suggesting that container type did not influence intra-specific competition for this species. Previously, we found that *A. caliginosa* grow poorly in small containers (<0.5 L) [24], but the maximum growth rates reported here ($\text{IGR} = 0.007\ \text{d}^{-1}$) were similar to those reported by Wever et al. [44] ($\text{IGR} > 0.004\ \text{d}^{-1}$) in laboratory pots with fewer than two individuals per litre. The logistic equations fitted to this data predict that *A. caliginosa* will lose weight when population densities of more than 10 individuals per litre are reached.

The regression slopes of the relationship between earthworm density and growth rates of *L. terrestris* were not different between pots and cores indicating that the container did not affect the intra-specific competition relationship for *L. terrestris.* However, the difference in regression intercepts between the pots and cores indicate that growth of *L. terrestris* was more constrained in pots than cores. Nevertheless, both pots and cores were not very suitable experimental containers for *L. terrestris* because growth rates of *L. terrestris* were negative for all density treatments with the exception of the lowest density treatment in the cores. This supports other research that found that container shape and size restrict the growth of *L. terrestris* [13,46]. Growth rates obtained in cores (IGR $= 0.002\ d^{-1}$) at low population densities (<1 individual per litre) are similar to growth rates reported by Whalen and Parmelee [45] (IGR $= 0.001\ d^{-1}$) and Butt et al. [12] (IGR $= 0.005\ d^{-1}$) for a similar number of earthworms (0.5−3 individuals per litre). The logistic equations fitted to this data predict that *L. terrestris* will lose weight when population densities of more than three individuals per litre are present. Using life-history analysis, Kammenga et al. [30] modelled growth rates for *L. terrestris* and predicted a maximum field population density of four individuals per litre, which was similar to field populations reported by Daniel [20].

The difference in population thresholds between *L. terrestris* (3 individuals per litre) and *A. caliginosa* (10 individuals per litre) that we found are most likely due to their distinctive life histories. Furthermore, *L. terrestris* is territorial in its burrowing habit and commonly inhabits deep semi-permanent vertical burrows, whereas *A. caliginosa* inhabit desultory horizontal burrows in the mineral layers of the soil [25]. Baker et al. [5] also used weight loss as an indicator of earthworm population density thresholds, and found that more individuals of the endogeic earthworm *A. caliginosa* (3 individuals per kilogram of soil) coexisted in monocultures than the anecic *Aporrectodea longa* (2 individuals per kilogram of soil). Interference competition between different species for food and habitat resources can be an important mechanism for population dependent regulation of earthworm growth [40,42]. Furthermore, population size is inversely related to body size for a number of organisms [2,16] including soil fauna [34]. This may explain why small earthworms that consume fewer resources per individual have higher population density thresholds than large earthworms.

4.2. Inter-specific competition effects

Pots and cores were considered to be inappropriate containers for *L. terrestris*, since they lost weight in almost all treatments (Table 3). On the other hand, the appropriate container for *A. caliginosa* depends on the earthworm density and which species are present. Growth curves were similar when *A. caliginosa* was grown alone or grown in a 1:1 ratio with *L. terrestris*, but the intercept of the curve was smaller when more *L. terrestris* were present (1 *A. caliginosa*:2 *L. terrestris*). The earthworm biomass was approximately twice as large in pots and cores with two *L. terrestris* than with one *L. terrestris*. Increasing earthworm biomass in cultures has been shown to negatively affect earthworm maturation and reproduction [11], and these results suggest that increasing earthworm biomass in a confined space can reduce the growth rate. The ratio of 1 *A. caliginosa*:2 *L. terrestris* reduced the population density threshold of *A. caliginosa* to one individual per liter from six to eight individuals per liter when none or one *L. terrestris* was present. These results imply that intra-specific competition has less of an affect on *A. caliginosa* growth rates than inter-specific competition with *L. terrestris*. Furthermore, since there were no significant interaction effects between container type and earthworm species, the container type did not affect the inter-specific competition relationship between *L. terrestris* and *A. caliginosa*.

Our results indicate that heavier earthworms reduce growth rates more than smaller earthworms, at equal population densities. Slower growth rates have been observed for *L. terrestris*, *Allolobophora chlorotica*, *A. caliginosa*, and *A. longa* in multi-species cultures (<4 individuals per litre) when larger earthworms were present, compared to smaller ones [5,11,32,33]. However, in some cases, intra-specific competition may be stronger than inter-specific competition. Lowe and Butt [32] showed that growth rates of *A. longa*, *A. chlorotica* and *Lumbricus rubellus* were lower when paired with the same species than when paired with another species, regardless of the size of the earthworms.

4.3. Factors influencing competitive interactions between earthworms

We found that the slopes of the lines describing earthworm growth for *A. caliginosa* and *L. terrestris* as a function of population density were similar, ranging from -0.0020 to $-0.0028\ d^{-1}$. This suggests a negative feedback (e.g. competition) that becomes more deleterious to earthworm growth as the population density increases and is independent of container size.

The differences in soil disturbance between containers did not affect earthworm growth rates. This supports results from a previous experiment where we established that soil disturbance did not affect the growth rates of *A. caliginosa* [24]. The body weights of *L. terrestris* in cores were slightly larger than those in pots because the pot and core trials were run sequentially and field-collected earthworms that were kept in cultures had grown during this time. However, there were no significant differences between the initial body weights of individuals placed in pots and cores. We do not expect significant differences in growth rate responses for *L. terrestris* juveniles for the body weights used in this experiment. Daniel et al. [21] showed that growth rates of *L. terrestris* juveniles respond significantly to differences in body weights when *L. terrestris* weighed less than 0.75 g, but few differences were found when *L. terrestris* weighed between 0.75 and 2 g.

It was expected that at low population densities growth rates would remain level and they would decrease more rapidly with increasing population density. Surprisingly, our logistic regression curves show the opposite relationship as growth rates decreased rapidly at low population densities (1–4 individuals per litre) and levelled out at high population densities. The regression curves show that maximum growth rates were not achieved at the lowest population densities tested in this experiment. We expect an inflection point in the regression curve to occur at population densities lower than those tested in this experiment (<1 individual per litre). At these low population densities the growth rates should remain level and should approach the maximum potential growth rate of each species. The relationship between biomass production of *L. terrestris* fed on activated sludge and population density showed an inflection point at six individuals per litre. Biomass production was level up to this point then decreased in a logistic manner at high population densities similar to the curves obtained in our experiment [28]. To obtain a similar growth curve for our experiment we would need to determine intra- and inter-specific competition at population densities of less than one individual per litre using the same compost as a food substrate.

The interactions between earthworms that lead to slower growth and weight loss in some individuals are probably due to experimental conditions that increase competition for space. Although *L. terrestris* exhibits territorial behaviour [15,25,30], there is no record of territorial competition among *A. caliginosa*. However, there is evidence that container shapes that do not suit an earthworm's burrowing habits may affect growth rates significantly [24,46]. Presumably, earthworms in the field are not subject to the same restrictions, since they can migrate to avoid competition. Therefore, inter-specific interactions between earthworms should be studied in containers that do not affect the growth of earthworms at low population densities. Butt et al. [12] showed that earthworms may be cultured at higher population densities by increasing food quantity and quality. Similarly, inter- and intra-specific competitive effects between earthworms are affected by food particle size [9,33]. This suggests that population dependent regulation of earthworm growth may be a function of food source and quality [17,31]. In a Swiss meadow, the population of *L. terrestris* ranged from 120 to 300 individuals per square meter and was regulated by food availability [20]. Many agricultural practices (e.g. ploughing, manuring, planting cover crops) are known to increase food availability and quality, thus increasing the earthworm population [41] and probably affecting the interactions between earthworms. The quantity and placement of food in the experimental containers probably did not affect growth rates. An excess food was always found at the end of the experiment implying that sufficient food was provided initially. In laboratory microcosms, both endogeic and anecic earthworms have been observed feeding and casting at the surface of the soil [47]. Although these surface activities are atypical of endogeic earthworms in the field, the small size of microcosms probably influences their natural burrowing habits. The growth curves obtained in this study are probably affected by type and quantity of organic substrate used as food; an improvement would be to use standard feed so earthworm growth rates from different studies can be compared.

4.4. Implications for future laboratory and field experiments

The relationship describing intra- and inter-specific competition was not affected by container type for both species. However, decreasing the container size restricted the growth of *L. terrestris* in both single- and multi-species cultures, but did not affect the growth of *A. caliginosa* in multi-species cultures. We have shown that for both species, a population density greater than one individual per litre will reduce earthworm growth rates significantly. Further work is needed to find the population density at which growth rates are not affected and which may be used as an appropriate population in laboratory pot experiments to measure the effects of earthworms on soil processes and plant growth. Our research may be used to improve earthworm inoculation studies, which often suffer from high mortality of earthworms [7] or competition from other colonising species [14]. Furthermore, a better understanding of how competitive interactions between earthworms are influenced by soil temperature, moisture, and food will help to develop laboratory and field microcosm experiments that are more representative of field conditions.

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