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Effects of cast properties and passage through the earthworm gut on seed germination and seedling growth

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ABSTRACT

Success of seed germination and seedling establishment is potentially affected by interactions with earthworms. Two of the possible mechanisms that might explain such impact are the selective ingestion of seeds by earthworms that might break seed dormancy, and germination in their nutrient-rich casts. The aim of this study was to disentangle the effect of seed passage through the earthworm gut and the effect of cast alone, as a germination medium, on the germination and growth of four herbaceous species. We hypothesized that the presence of seeds in casts facilitates their germination and seedling growth and that the passage of seeds through gut favors seed germination. Non-ingested seeds were placed in artificial earthworm casts shaped from cast material of 3 soil types × 3 earthworm species combinations (plus control). Seed germination after seed ingestion and excretion was tested in a Petri dish experiment in the presence of each earthworm species. Contrary to our expectations, we found that passage of seeds through the gut of *Lumbricus terrestris* decreased the germination of *Festuca lemanii* and that all seeds of *Origanum vulgare* and *Urtica dioica* were digested. Total seed germination of non-ingested seeds placed in casts was affected by cast properties, i.e., by the interaction between the soil type and the earthworm species. Seedlings germinating from the control material had a higher relative growth rate than in material from *Allolobophora chlorotica* cast. Our results suggest that seed ingestion alters seed germination success of specific plant species. The cases of lower germination and seedling growth induced by cast effects are discussed with regards to their physical, chemical and microbiological properties.

1. Introduction

Earthworms impact plant communities both through the modification of soil chemical, physical and microbiological properties (Bityutskii et al., 2012; Scheu, 2003; Zhang and Schrader, 1993) and through seed ingestion (see Forey et al., 2011 for a review).

Seed ingestion by earthworms might impact seed germination and seedling growth through two possible different mechanisms: the provision of a nutrient-rich cast substrate that benefits seed germination, i.e., the earthworm cast, and the alteration of the seed coat. The quality of this substrate is closely linked to the earthworm capacity to choose the soil and litter particles that they ingest, which tend to increase cast content in organic matter, and to its capacity to modify soil properties, mainly through an increase in mineralization. Mineralization is then further enhanced by the stimulation of microbial activity (Aira et al., 2003; Chapuis-Lardy et al., 2010; Drake and Horn, 2007; Lavelle et al., 1995). The increased mineralization of the organic matter leads to a higher nutrient availability (Bityutskii et al., 2012) and, generally, to an increased plant growth (Scheu, 2003). The impact of earthworms on plant growth differs with plant species and with soil properties (Eisenhauer and Scheu, 2008; Laossi et al., 2009). Earthworms tend to favor the establishment of grasses over other herbaceous species (Eisenhauer and Scheu, 2008; Laossi et al., 2009). Cast properties depend on both the ingested soil type and the earthworm species (Clause et al., 2014), and seedlings that emerge from casts likely respond to these soil properties. For example, seed germination might respond to cast nitrate (NO_3^-) content (Dong et al., 2012; see Pons, 1989) and seedlings that emerge in enriched-cast might benefit from this enrichment in mineral nitrogen (see Decaëns et al., 2003).

The ingestion of seeds by earthworms alters their germination through the physical damage of the seed coat in the earthworm gizzard or through their partial or total digestion in the earthworm gut (Grant, 1983; McRill and Sagar, 1973). The partial damage of seeds might favor seed germination by breaking seed dormancy (Eisenhauer et al., 2009; Shumway and Koide, 1994). The impact of earthworms on seeds might be driven by their preferences for certain seed species over others (e.g., preference for *Poa annua* over

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Lolium perenne)(McRill and Sagar, 1973). This preference is mainly driven by seed traits, which is the case for small and oil-rich seeds of *Origanum vulgare* and *Urtica dioica* (Aira and Piearce, 2009; Clause et al., 2011; Eisenhauer et al., 2009).

Our previous experiment that combined three earthworm species with three soil types showed that cast properties interactively depend on soil types and on earthworm species (Clause et al., 2014). Casts of anecic *Lumbricus terrestris* had higher NH_4^+ and P contents than casts of endogeic *Aporrectodea caliginosa* and *Allolobophora chlorotica* in the Luvisol, Rendosol and Histosol soils. On the other hand, casts of *L. terrestris* had a lower C:N ratio than the two endogeic species in the Luvisol. Casts of *A. chlorotica* had a higher Mg content in the Rendosol but not in the two other soil types. These differences in cast properties between soil types and earthworm species likely impact the response of seeds and seedlings that germinate in casts. Only one attempt has been made at assessing the respective impacts of ingestion and cast properties (Eisenhauer et al., 2009). It showed that seed ingestion by earthworms mostly increased seed germination, although the effect varied with plant species, and that earthworm casts alone—from one type of soil—primarily decreased the germination (Eisenhauer et al., 2009).

The aim of this study was to disentangle the effect of seed ingestion from the effect of earthworm casts as a germination substrate. To do so, germination and seedling growth of four plant species were monitored in artificial casts made of soil coming from 12 treatment combinations: 3 soil types × (casts from 3 earthworm species + no-worm control casts) (see Clause et al., 2014). The effect of ingestion was tested through the comparison between seeds that were ingested and excreted, and seeds that were not ingested by earthworms. Thereby, we tested whether (i) cast substratum obtained from various earthworm species and soil types facilitates seedling growth when seeds have not been ingested but does not alter germination rate, (ii) seedling growth is greater in casts having higher nutrient contents, and (iii) seed ingestion by earthworms increases seed germination success.

2. Methods

2.1. Soil characteristics

Parental soils and cast materials are the same as in Clause et al. (2014). Soils were collected from the top layer (0–20 cm) of three different permanent grasslands in Upper-Normandy, France. Climate is temperate oceanic with a mean annual rainfall of 800mm and a mean temperature of 10 °C. The soil from Saint-Adrien (N49°22′22″, E1°07′41″) is a rendzic Leptosol (IUSS, 2006; hereafter Re) supporting vegetation dominated by *Brachypodium pinnatum* (L.) P. Beauv., *Festuca lemanii* Bastard and Carex flacca Schreb. The soil from Yvetot (N49°36′37″, E 0°44′15″) is a NeoLuvisol-Luvisol (IUSS, 2006; hereafter Lu) supporting a vegetation dominated by *Agrostis capillaris* (L.), *L. perenne* (L.) and *Ranunculus acris* (L.). The soil from Yville-sur-Seine (N49°25′11″, E0°52′54″) is a Histosol (IUSS, 2006; hereafter Hi), where the vegetation is dominated by *Poa trivialis* (L.), *L. perenne* and *A. capillaris*. All soils were hand-sieved within two days after collection with a 5-mm-mesh sieve and air-dried for a week. Microcosms were cylindrical pots (13.5 × 11 cm) filled with 750 g of one type of soil watered with 115 mL water (see Clause et al., 2014 for further details).

2.2. Earthworms and cast mate rial

We used the anecic *L. terrestris* L. and the endogeic *A. chlorotica* (Sav.) and *Aporrectodea ros*ea (Sav.) that are commonly found in grassland ecosystems of North–West France (Decaëns et al., 2008). Anecic earthworms feed on plant litter and contribute to the incorporation of soil organic matter into deeper soil layers via their vertical movement and endogeic species mostly feed on soil organic matter (Lee, 1985). *A. chlorotica* individuals (AC; 0.32 ± 0.08 g, average fresh weight) were hand-sampled in April 2011 in grasslands outside the university campus of Mont-Saint-Aignan. *A. rosea* individuals (AR; 0.23 ± 0.04 g) were hand-sampled in alluvial deposits near the Seine River and *L. terrestris* individuals (LT; 5.23 ± 0.73 g) were purchased in a fishing bait store. After voiding their guts for 24h on moist filter paper in Petri dishes, three adult individuals from a single species were added to each microcosm leading to a total of twelve treatments: 3 soil types × (casts from 3 earthworm species + no-worm control casts). Each treatment was replicated five times and all 60 microcosms were kept in darkness at 17 °C for the length of the experiment.

We collected casts from each microcosm once to twice a week for 180 days. This frequency was chosen in order to collect fresh cast material during the whole experiment. Casts had to be collected manually from the entire microcosm in order to obtain sufficient cast material for chemical and physical analyses (see Clause et al., 2014). The resulting repeated disturbance of the microcosms was also applied to microcosms with no earthworm (controls). Cast material from each microcosm and control soils were air-dried, analyzed (see below) and stored for 6 months before they were used for the present experiment (darkness, room temperature). All data on chemical properties of casts were obtained at this stage, i.e., prior to the shaping of artificial casts, and not at the end of the experiment due to the scant amount of material. These chemical properties were: contents in NO_3^-, NH_4^+, $CaCO_3$, total carbon and nitrogen content, C:N ratio, contents of organic carbon and other mineral nutrients (Na, P, Mn, K), pH and CEC (cation exchange capacity)(see Clause et al., 2014 for details).

2.3. Experimental set-up

Fifty μL, 40 μL or 70 μL of distilled water was added to 0.10 of dry cast or control material collected from Re, Lu and Hi microcosms. Different water contents were added to the cast or control material to reach humidity levels approximating those measured in Clause et al. (2014) (Re: 37 ± 11%; Lu 30 ± 10%; Hi: 83 ± 15%). Pellets were shaped manually to form artificial casts. Artificial casts will be further referred to as casts.

A single seed of one of four species was added to three pellets from each microcosm leading to a total of 720 pellets (60 microcosms × 4 seed species × 3 repetitions). The four species were *F. lemanii* L. (Flem; Poaceae), *O. vulgare* L. (Ovu; Lamiaceae) and *Trifolium repens* L. (Trep; Fabaceae), and *U. dioica* L. (Udi; Urticaceae). All pellets were randomly placed in trays filled with sterilized soil (100 °C, 1 h, repeated after 24) and covered with cheesecloth (c. 77 pellets per tray). Trays were randomly placed in a controlled chamber (24 °C, 16/8h, day/night) for three months. Trays were watered every day and randomly moved within the chamber every three days. Germination was monitored every three days for a month and then once a week. Two months after the start of the experiment, artificial casts were flattened to facilitate the germination of seeds that had not germinated. At the end of the experiment, all seedlings were removed and their shoot, root and total biomasses were measured. Seedling relative growth rate (RGR) was calculated as follow: RGR (day^{-1}) = (ln(W1) − ln(W0))/(T1–T0), where W1 is the seedling weight at the end of the experiment (mg), W0 is the weight of the introduced seed (mg), T1–T0 is the number of days between the seed germination and the end of the experiment. The shoot:root ratio was calculated.

2.4. Effect of seed ingestion

In parallel with the experiment on artificial casts, tests were carried out using Petri dishes to assess the impact of seed ingestion by earthworms. We compared germination of ingested and non-ingested seeds. Twenty seeds of *F. lemanii* (length: 5.54 ± 0.15, width: 0.87 ± 0.02, height: 0.58 ± 0.01 mm), *O. vulgare* (l:0.84 ± 0.01, w:0.57 ± 0.01, h:0.40 ± 0.01 mm), *T. repens* (l:1.13 ± 0.01, w:1.06 ± 0.01, h:1.03 ± 0.02 mm) or *U. dioica* (l:1.08 ± 0.02, w:0.72 ± 0.01, h:0.37 ± 0.02 mm) were placed into a Petri dish (8 cm diameter). Each Petri dish contained three sheets of filter paper moistened with 3 mL of distilled water and 1g of sieved soil (see Eisenhauer et al., 2009). One individual of *L. terrestris* (4.13 ± 0.71 g) or one individual of *A. chlorotica* (0.26 ± 0.05 g) was placed into each Petri dish (24 h, 15 °C, darkness) after voiding their guts. Each treatment (two earthworm species × four seed species) was replicated ten times. Thereafter, earthworms were removed and their gut voided in a Petri dish with moist filter paper (48 h, 15 °C, darkness). Casts were broken down. Seeds that transited through the earthworm gut and that were found in casts were counted as ingested and excreted. Seeds that were not found in casts were considered as non-ingested. For each earthworm individual, the difference between the number of introduced seeds (20) and the numbers of excreted seeds and of non-ingested seeds was taken as the number of totally digested seeds. We did not make the distinction between seeds that were crushed in the gizzard and chemically digested seeds. All seeds that were not recovered will be referred to as digested hereafter. Ingested seeds are either excreted or digested seeds. The level of digestion of excreted seeds after their passage through earthworm gut was not evaluated. The sorted seeds were placed in a controlled chamber (24 °C, light/dark, 18/6 h) and their germination was monitored for 14 days.

2.5. Statistical analyses

Statistical analyses were achieved with the R-software (R Core Team, 2013). ANOVAs were performed to test for the impact of earthworm species, soil type and seed species on germination rates and seedling growth. Seeds that did not germinate were not taken into account for the analysis of seedling growth. The effect of microcosm replicates from which the initial material was collected and the effect of trays in which the artificial casts were placed were tested using random effect in Linear Mixed models (Zuur et al., 2009). They were non significant. Continuous data were log-transformed prior to analysis to improve normality and homoscedasticity of residuals. Tests on germination rates were best fitted with a binomial distribution. Tukey-HSD tests ($\alpha = 0.05$) were performed to compare means of seed germination and seedling growth with the glht function of the multcomp R-package (Hothorn et al., 2008). Correlations were tested between seed germination and seed growth parameters and chemical properties of casts with the cor.test R function. Further details on cast physical and chemical data can be found in Clause et al. (2014).

3. Results

3.1. Seed germination rates in artificial casts

Out of the 720 seeds placed into pellets, 34% germinated. *U. dioica*, *O. vulgare*, *T. repens* and *F. lemanii* represented 41.2%, 25.3%, 18.4% and 15.1% of total seed germination respectively. Seed germination rate was impacted by the seed species factor (GLM, $\chi^2 = 351.4$, df = 3, $p < 0.001$, $n = 240$) and by the interaction between earthworm species and soil types (GLM, $\chi^2 = 333.2$, df = 3, $p = 0.006$, $n = 240$). The seed germination rate of *U. dioica* was the highest compared to that of *F. lemanii*, of *O. vulgare* and of *T. repens* (Table 1). The seed germination rate of *O. vulgare* was also significantly higher than that of *F. lemanii* (Table 1). Overall, the seed germination rate was significantly higher in the control from the Luvisol than in the casts of *A. chlorotica* in the Rendosol and the Luvisol and in casts of *A. rosea* in the Histosol (Table 1). Presence in earthworm cast tended to decrease seed germination rates since higher germination rates were found in artificial casts from control soils than from casts (GLM, $\chi^2 = 418.2$, df = 3, $p = 0.033$, $n = 240$; Table 1).

Table 1

Mean comparisons of the germination rate (%) for all samples ($n = 240$) between different earthworm species (EW) and soil types. Data were analyzed with a Generalized Linear Model (GLM) and means were compared with the Tukey HSD test ($\alpha = 0.05$). Seed species are *Festuca lemanii* (Flem), *Origanum vulgare* (Ovu), *Trifolium repens* (Trep) and *Urtica dioica* (Udi). Earthworm factors are Control (C), *Allolobophora chlorotica* (AC), *Aporrectodea rosea* (AR) and *Lumbricus terrestris* (LT). Soil types are Luvisol (Lu), Rendosol (Re), Histosol (Hi).

		Mean ± S.E.[a]
Earthworm (EW)		
	Control (C)	42.2 ± 4.3 a
	L. terrestris (LT)	33.9 ± 3.8 a b
	A. chlorotica (AC)	27.8 ± 4.3 b
	A. rosea (AR)	32.2 ± 4.3 ab
Soil		
	Luvisol (Lu)	40.0 ± 3.7
	Rendosol (Re)	30.0 ± 3.4
	Histosol (Hi)	32.1 ± 3.7
Seed		
	F. lemanii	20.1 ± 3.3 c
	O. vulgare	34.4 ± 4.2 b
	T. repens	25.0 ± 3.4 bc
	U. dioica	56.1 ± 4.2 a
Soil:EW		
	Lu:C	51.7 ± 7.4 a
	Lu:LT	43.3 ± 6.0 a b
	Lu:AC	20.0 ± 7.0 b
	Lu:AR	45.0 ± 7.4 a b
	Re:C	38.3 ± 7.4 ab
	Re:LT	31.7 ± 6.6 ab
	Re:AC	21.7 ± 6.5 b
	Re:AR	28.3 ± 6.5 ab
	Hi:C	36.7 ± 7.2 ab
	Hi:LT	26.7 ± 6.7 ab
	Hi:AC	41.7 ± 8.0 ab
	Hi:AR	23.3 ± 7.7 b

[a] Different lower case letters indicate significant difference, with $\alpha = 0.05$.

3.2. Seedling growth in artificial casts

The seedling relative growth rates (RGR) were significantly impacted by the earthworm species (ANOVA, $F_{(3111)} = 2.78$, $p = 0.044$, $n = 120$) and by the seed species (ANOVA, $F_{(3111)} = 11.73$, $p < 0.001$, $n = 120$; Table 2). Seedlings grew better in controls than in casts of *A. chlorotica* and of *A. rosea* (Table 2). *U. dioica* grew better than the three other seed species *F. lemanii*, *O. vulgare* and *T. repens* (Table 2), especially in the controls (ANOVA, $F_{(3,35)} = 6.68$, $p = 0.001$, $n = 39$; Table 2). The higher RGR in controls compared to casts was only found significant between controls and casts of A. chlorotica for seedlings of *U. dioica* (ANOVA, $F_{(3,71)} = 3.71$, $p = 0.015$, $n = 75$; Table 2).

The shoot growth rate was only influenced by the seed species (ANOVA, $F_{(3116)} = 4.73$, $p = 0.004$, $n = 120$). Shoot growth rates of *U. dioica* and of *F. lemanii* were significantly higher than that of *O. vulgare* ($p = 0.02$ and $p < 0.001$ respectively). Tests within seed species on the growth rate and all tests on the root growth rate and the shoot:root ratio showed no significant effect of seed species, earthworm species or soil types.

3.3. Plant responses and chemical properties of artificial casts

We found few significant correlations between seed germination rates and chemical properties of the analyzed material used

Table 2
Mean comparisons of the Relative Growth Rate (RGR; day^{-1}) of emerging seedlings with different earthworm species/controls (EW), seed species and soil types. Seed species are *Festuca lemanii*, *Origanum vulgare*, *Trifolium repens* and *Urtica dioica*. Earthworm factors are Control, *Allolobophora chlorotica*, *Aporrectodea rosea* and *Lumbricus terrestris*. Soil types are Luvisol, Rendosol, Histosol. Data were analyzed with ANOVA and means were compared with the Tukey HSD test ($\alpha = 0.05$). RGR were calculated on the germinating seeds from Table 1. Samples with $n < 3$ were not considered. n.a.: not applicable.

Samples	Explanatory variable	Mean ± S.E.[a]
Total	Earthworm	
	Control	0.055 ± 0.005 a
	L. terrestris	0.053 ± 0.006 a b
	A. chlorotica	0.041 ± 0.005 b
	A. rosea	0.040 ± 0.005 b
	Soil	
	Luvisol	0.045 ± 0.004
	Rendosol	0.049 ± 0.005
	Histosol	0.053 ± 0.005
	Seed	
	F. lemanii	0.032 ± 0.006 b
	O. vulgare	0.041 ± 0.005 b
	T. repens	0.020 ± 0.005 b
	U. dioica	0.057 ± 0.003 a
Control	Seed	
	F. lemanii	0.043 ± 0.014 b
	O. vulgare	0.050 ± 0.008 a b
	T. repens	0.017 ± 0.007 b
	U. dioica	0.069 ± 0.005 a
L. terrestris	Seed	
	F. lemanii	0.032 ± 0.005
	O. vulgare	0.042 ± 0.009
	T. repens	0.026 ± 0.006
	U. dioica	0.065 ± 0.009
A. chlorotica	Seed	
	F. lemanii	n.a.
	O. vulgare	0.028 ± 0.007
	T. repens	n.a.
	U. dioica	0.044 ± 0.006
*A. rosea**	Seed	
	F. lemanii	n.a.
	O. vulgare	n.a.
	T. repens	n.a.
	U. dioica	0.048 ± 0.005
*F. lemanii**	Earthworm	
	Control	0.034 ± 0.014
	L. terrestris	0.032 ± 0.005
	A. chlorotica	n.a.
	A. rosea	n.a.
*O. vulgare**	Earthworm	
	Control	0.050 ± 0.008
	L. terrestris	0.042 ± 0.008
	A. chlorotica	0.028 ± 0.007
	A. rosea	n.a.
*T. repens**	Earthworm	
	Control	0.017 ± 0.007
	L. terrestris	0.026 ± 0.006
	A. chlorotica	n.a.
	A. rosea	n.a.
U. dioica	Earthworm	
	Control	0.069 ± 0.005 a
	L. terrestris	0.065 ± 0.009 a b
	A. chlorotica	0.044 ± 0.006 b
	A. rosea	0.048 ± 0.005 ab

[a] Different lower case letters indicate significant difference, with $\alpha = 0.05$

for the artificial casts. The total seed germination was negatively correlated with the pH and $CaCO_3$ contents ($r = -0.27$, $p = 0.04$ and $r = -0.31$, $p = 0.02$). It was positively correlated with the Mn content ($r = 0.28$, $p = 0.03$). The germination of *F. lemanii* was negatively correlated with contents of NO_3^- and Mg ($r = -0.28$, $p = 0.03$ and $r = -0.29$, $p = 0.03$). The germination of *O. vulgare* was negatively correlated with the contents in $CaCO_3$ and total nitrogen ($r = -0.32$, $p = 0.01$ and $r = -0.27$, $p = 0.04$) and positively correlated with the Mg content ($r = 0.30$, $p = 0.02$). The germinations of *T. repens* and *U. dioica* were not associated with any of the material chemical characteristics. The RGR and the shoot and root growth rates were negatively correlated with the C:N ratio of casts ($r = -0.24$, $p = 0.009$; $r = -0.24$ $p = 0.009$; $r = -0.22$, $p = 0.01$).

3.4. Seed germination after ingestion by earthworms

In the experiment, in which seeds were presented to earthworms to test the impact of seed ingestion only on germination, the difference of germination rate between excreted and non-ingested seeds could only be tested statistically for seeds of *T. repens* and *F. lemanii* in presence of *L. terrestris*. Indeed, *L. terrestris* ingested all the seeds of *O. vulgare* and of *U. dioica* that were presented, and *A. chlorotica* excreted none of the seeds that it ingested (Fig. 1). The germination of all seeds was influenced by the seed species only (GLM, $\chi^2 = 413.3$, df = 3, $p < 0.001$, $n = 94$). The germination of *F. lemanii* seeds that were excreted by earthworms was lower compared to non-ingested seeds (Tukey HSD, $z = 2.17$, $p < 0.03$; Fig. 1).

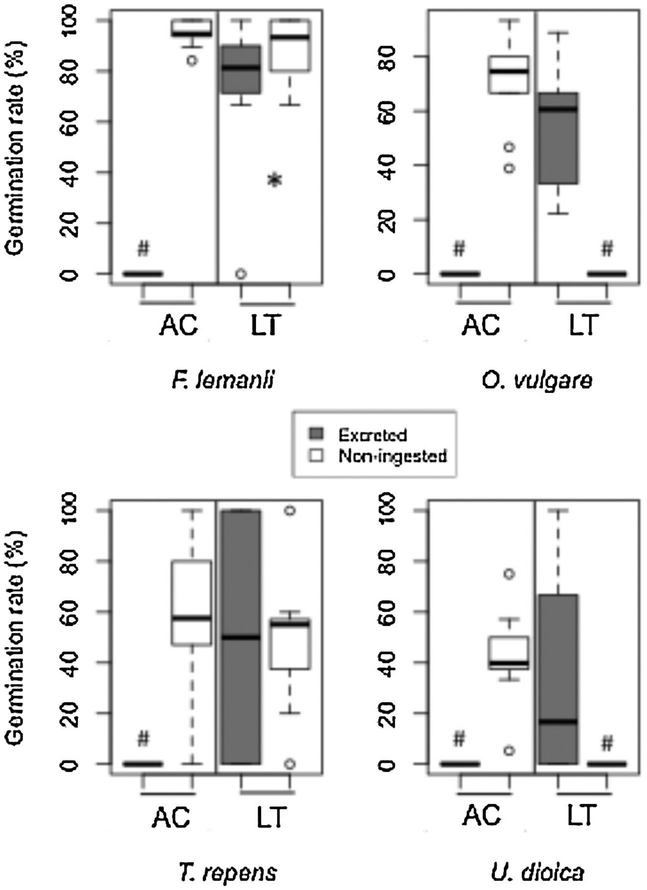

Fig. 1. Impact of seed digestion by *Allolobophora chlorotica* (AC) and *Lumbricus terrestris* (LT) on germination rates of four seed species: *Festuca lemanii*,*Origanum vulgare*, *Trifolium repens*, *Urtica dioica*. Significance is represented by an asterisk (*; $p < 0.05$). Germination was not calculated when no germination occurred due to 100% ingestion (#).

4. Discussion

4.1. Seed germination

We expected seed germination to be affected by seed ingestion but not by cast properties. Overall, seed ingestion by earthworms negatively impacted seed survival. *L. terrestris* ingested all seeds of *U. dioica* and *O. vulgare*, and *A. chlorotica* did not excrete any of the seeds that it ingested. Our ingestion results supported results of previous studies (Clause et al., 2011; Eisenhauer et al., 2009; McRill and Sagar, 1973). They also support the idea that earthworms prefer small and oil-rich seeds, i.e., *O. vulgare* and *U. dioica*. We suggest that earthworms do not only select seeds according to their small size (they are thus easier to process) but that they also select them for nutritive purposes (see Clause et al., 2011). The size and oil content of the seeds are strongly correlated (unpublished data), and we suggest that ingested seeds are sensitive to both physical and chemical damages during the transit through earthworm gut. Many studies have described the impact of earthworms on microbial communities, but there is very little data on the effect of this impact on seed digestion and germination and interactions with the chemistry of seed coats. Thus, it is hard to evaluate how earthworms physically and/or chemically damage specific seeds by ingesting them. Microscopic observations of excreted and non-ingested seeds and the description of microbial enzymatic activities and their impact on seeds might help disentangle the respective impacts of physical and chemical damages on seeds and their germination.

The passage of seeds through the animal gut—here earthworms- did not increase seed germination as found by Traveset et al. (2007). When seeds were excreted, we found that the impact of gut passage on seed germination was low and species-specific. The ingestion and excretion of seeds decreased the germination of *F. lemanii*, and it had no significant effect on the germination of *T. repens*. Eisenhauer et al. (2009) found similar results. The ingestion and excretion of *T. repens, Phleum pratense* and *Bellis perennis*by *L. terrestris* had no impact on germination compared to control treatments. However, germination of *Poa trivialis*, *Plantago lanceolata* and *Medicago varia* seeds increased after their passage through the gut of *L. terrestris* (Eisenhauer et al., 2009). Thus, more ingestion studies using the same seed and earthworm species will allow generalizing on the impact of specific seed-earthworm interactions on seed germination. In addition, patterns of seed germination after gut passage are likely to be determined by seed species, seed quality and the underlying seed properties.

As germination medium, artificial casts made of earthworm cast material had no impact or decreased germination rates compared to control soil material. Eisenhauer et al. (2009) found similar results. Germination rates were higher in the Luvisol control samples than in other controls or in casts of *A. chlorotica*. Overall, the Luvisol control was the most nutrient-poor material - low NH_4^+, NO_3^-, organic C contents, pH and CEC-, especially in comparison with casts of *L. terrestris*—enriched in NH_4^+, P and K, especially in the Histosol (see Clause et al., 2014). The positive correlation between the Mn content and the germination rate of seeds was unexpected. Indeed, a high Mn content tends to inhibit seed germination and plant growth (Kelley, 1912; Todorovic et al., 2009). Germination is affected at low pH but should not be affected within the pH range that was measured in all samples: 5.8–8.3 (Baskin and Baskin, 2001; Clause et al., 2014). Thus, we believe that there was no direct causal relationship between pH or Mn content and the seed germination. A possible indirect effect is the modification of microbial activity due to manganese-reducing bacteria (Marschner et al., 1991). Seed germination is influenced by nitrate content that can impact seed dormancy (Baskin and Baskin, 2001). Although an increase of nitrate concentration stimulates germination by breaking seed dormancy under certain conditions and for certain seeds, too high concentrations can inhibit germination (Goudey et al., 1988; Pons, 1989). Goudey et al. (1988) reported that the amount of NO_3^- taken up by seeds of *Sinapis arvensis* reached inhibitory levels in a soil containing 26 mg NO_3^- per kg dry weight. NO_3^- contents in our samples were largely over 26 mg NO_3^- per kg dry weight, which might explain the absence of correlation between seed germination and nitrate content.

Higher seedling performances (germination rate and seedling growth) in artificial casts from control soils than from earthworm cast material might be explained by changes in microbiological properties of casts via earthworm activity. The soil of earthworm casts differs from the surrounding soil at least via microbiological properties, stimulated by the selective ingestion of nutritive material in soil aggregates and litter particles (Aira et al., 2005; Chapuis-Lardy et al., 2010; Scheu, 1987). It contains bacteria and fungi that are associated with specific enzymatic activities that may alter the germination of specific seeds (Fujii et al., 2012; Somova et al., 2001).

4.2. Seedling growth

We expected seedling growth to be higher in more fertile samples, i.e., casts that were more nutrient-rich (see Clause et al., 2014). Casts and soil modified by earthworms are known to have a higher content of mineral nutrients than soil with no earthworms and to support greater plant growth (Eisenhauer and Scheu, 2008; Laossi et al., 2010; Zhang et al., 2010). Our results showed that seedlings grew better in the controls, independently from the soil type. Surprisingly, seedlings in casts of *L. terrestris* did not grow better than in the other samples despite their overall initial higher nutrient-content. Seedlings of *U. dioica* grew better than any other species. Poorter and Remkes (1990) also observed a higher relative growth rate of *U. dioica* compared to *O. vulgare*, *T. repens* and *Festuca* sp., which is common in plants that colonize fertile secondary successional disturbed sites (Poorter and Remkes, 1990). Also, as for patterns of seed germination, we believe that microbiological properties of casts are responsible for these unexpected patterns.

5. Conclusion

Our study is a follow-up study of Clause et al. (2014), who showed an interactive impact of soil type and earthworm species on cast physico-chemical properties. The aim of the present study was to disentangle the effect of seed ingestion from the effect of earthworm casts as a germination substrate on four plant species. Shaping artificial casts and sowing seeds in these casts has seldom been carried out. Ingestion and passage through the earthworm gut did not increase the germination success of seeds. Rather, it tended to decrease the germination success of our four plant species through the total digestion of their seeds or damages to their seeds (*O. vulgare* and *U. dioica*), or to decrease the germination rate of excreted seeds (*F. lemanii*). The presence of seeds in casts decreased seed germination and seedling growth as compared to control samples that were less nutrient-rich (mineral and organic). However, effects of the passage through gut or the presence in casts on seed germination and seedling growth were specific of both earthworm species and of seed species.

The role of earthworm-seed interactions in shaping plant communities is increasingly recognized (Forey et al., 2011). Earthworms act as ecological engineers by ingesting and transporting seeds, thereby modifying the soil seed bank. It seems that earthworm-seed interactions via ingestion and excretion in earthworm casts might be detrimental to some plant species, whose germination and establishment successes is reduced by earthworms. The variability of results of experiments testing the impact of earthworms on seed germination stresses the importance of repeating experiments with similar as well as with different seed and earthworm species in order to determine the general rules of seed-earthworm interactions.

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