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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