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Earthworm-mediated maternal effects on seed germination and seedling growth in three annual plants

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ABSTRACT

Many ecological studies have pointed out maternal effects in plants and shown that plant maternal environment influences germination of their seed and subsequent seedling growth. However, few have tested for maternal effects induced by soil macroorganisms. We tested whether two earthworm species (*Aporrectodea caliginosa* and *Lumbricus terrestris*) trigger such maternal effects on seed germination and seedling growth of three plant species (*Veronica persica, Poa annua* and *Cerastium glomeratum*). Our results show that, through maternal effects, *A. caliginosa* enhanced seed germination (*V. persica* and *P. annua*) and seedling growth (*C. glomeratum* and *P. annua*) while *L. terrestris* reduced seed germination only in *V. persica*. In some cases, the increase in germination rates of seeds produced in the presence of earthworms was associated with a reduction of nitrogen content in seeds. These results show that earthworms induce maternal effects in plants and that the size and direction of these effects depend on the combination of plant and earthworm species.

1. Introduction

The phenotype of plant individuals is determined not only by their genotype and environment but also by maternal effects, i.e. the direct contribution of the maternal phenotype to the phenotype of its offspring. Indeed, the tissues immediately surrounding the developing embryo and endosperm are all maternal. These tissues, the integuments of the ovule and the wall of the ovary form the seed coat, the fruit and accessory seed structures such as the hairs, awns and barbs. They are important determinants of seed dormancy, dispersal, and germination traits (Roach and Wulff, 1987). Variation in these traits, such as germination rates, seed survival or seedling growth rate, can carry over to influence the earliest life stages through a transgenerational plasticity (Weiner et al., 1997; El-Keblawy and Lovett-Doust, 1998). Such effects may also influence later stages. Indeed, a small initial advantage – for instance earlier germination – for some seedlings may be maintained or amplified by competition (Weiner, 1990; Miao et al., 1991).

Several studies have shown that properties of the maternal abiotic environment such as light intensity (Galloway and Etterson, 2007; Contreras et al., 2008), temperature (Alexander and Wulff, 1985) and availability of soil nutrients (Wulff and Bazzaz, 1992; Wulff et al., 1994) lead to maternal effects. These characteristics have in turn been shown to influence seed germination and subsequent seedlings growth (Roach and Wulff, 1987). Soil macroorganisms often influence plant growth either directly or indirectly, through modifications of soil properties (Lavelle and Spain, 2001). They are thus likely to cause maternal effects in plants as any biotic or abiotic environmental feature impacting plant growth and resource allocation (Roach and Wulff, 1987). Yet, so far, it seems that no study has focused on the existence of such a maternal effect mediated by soil macroorganisms. Such an effect would constitute a new and original example of aboveground–belowground interaction. We have tested for the existence of such an interaction in the particular case of earthworms.

Although some studies have reported negative effects of earthworms on plants in North America (Hale et al., 2006, 2008), earthworms are generally known to affect positively plant growth, through five main mechanisms (Brown et al., 2004): (1) increased

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mineralization of soil organic matter, which increases nutrient availability (Bohlen et al., 2002, 2004), (2) change in soil structure (Brown et al., 2004), (3) production of plant growth regulating substances (Nardi et al., 1988; Muscolo et al., 1999), (4) stimulation of symbionts (Doube et al., 1994) and (5) biocontrol of pests and diseases (Blouin et al., 2005). These mechanisms affect not only plant biomass but their resource allocation, as well (Scheu, 2003; Laossi et al., 2009). Earthworms, thus, modify the shoot/root ratio (Scheu, 2003; Eisenhauer and Scheu, 2008), and the allocation to seed production. They modify the number of seeds produced (Poveda et al., 2005a; Laossi et al., 2009) and seed size (Poveda et al., 2005b). They have been shown to modify the C/N of vegetative organs (Eisenhauer and Scheu, 2008) and have thus also been shown to impact the seed nutrient content (Atlavinyte and Vanagas, 1982). Since earthworms are likely to affect various seed traits, they should lead to maternal effects. They should, in other words influence the germination of seeds produced by plants grown in their presence or the growth of seedlings germinating from these seeds. However, no study has ever tested the existence of such an earthworm-mediated maternal effect in plants. We thus tested the effect of an anecic (*Lumbricus terrestris* L.) and an endogeic (*Aporrectodea caliginosa* (Savigny)) earthworm species and the effect of the two species in combination on the germination of seeds and subsequent seedling growth. This has been tested in three annual plants (grassland species) belonging to two functional groups (*Poa annua* L. a grass; *Cerastium glomeratum* Thuill. and *Veronica persica* Poiret, two forbs).

It is known that anecic and endogeic earthworms have distinct influences on soils (Jégou et al., 1998; Brown et al., 2000). They are therefore likely to have different effects on plant growth. Endogeic earthworms keep moving inside the soil to feed on soil organic matter while anecic feed on plant litter at the soil surface and tend to stay in the same burrow. Anecic earthworms fragment plant litter and incorporate it into the soil where it can subsequently be ingested by endogeic earthworms. Such an interaction can lead to higher mineralization and plant growth (Jégou et al., 1998; Brown et al., 2000). Earthworm effects on plant growth vary with the identity of plant species and with the functional groups to which they belong (Brown et al., 2004; Eisenhauer et al., 2009). The germination of the seeds of plant species belonging to different functional groups is thus likely to be affected differently by earthworms through maternal effects. The general purpose of this study was to determine whether earthworms induce maternal effects and to make a first assessment of the frequency of these effects using more than one plant and earthworm species. We tested the existence of maternal effects based on results of a previous experiment (Laossi et al., 2009) in which we found that *L. terrestris* but not *A. caliginosa* enhanced the growth of *P. annua* and *V. persica* and increased seed mass of *V. persica*. No significant earthworm effect was found for *C. glomeratum* (Laossi et al., 2009). We thus expected that earthworm positive effects on biomasses would lead to maternal effects and conversely that the absence of effect on biomasses leads to an absence of maternal effect. We thus made three hypotheses: (1) *L. terrestris* but not *A. caliginosa* induces maternal effects. *L. terrestris* enhances through these maternal effects (2) seed germination and (3) seedling growth of *P. annua* and *V. persica* but not *C. glomeratum*.

2. Materials and methods

2.1. Experiment setup

Seeds used in the present experiment come from a previous experiment (Laossi et al., 2009) which used monocultures (4 plants per microcosm) of *P. annua, V. persica* and *C. glomeratum*. Monocultures of parent plants were grown in a greenhouse according to four earthworm treatments: no earthworm (control), in the presence of *A. caliginosa* (AC), in the presence of *L. terrestris* (LT) or in the presence of both earthworm species (LT + AC). There were five replicates for each earthworm treatment; there were thus 20 microcosms for each plant species. Microcosms consisted of PVC pots (diameter 18 cm, height 17 cm) filled with 3 kg of soil that were daily watered during 7 weeks with 12.5 ml and with 25 ml from week 8 to week 15- the end of the experiment. The soil used for both studies was a sandy cambisol supporting a wet meadow at the ecology station of the ENS – Ecole Normale Supérieure – at Foljuif in France (Laossi et al., 2009);organic matter content 2.55%, C/N 12.4, carbon content 1.47%, total nitrogen content 0.12%, pH 5.22. The soil was air-dried, sieved (2 mm), homogenized and maintained four months without earthworms before initiating the experiments.

Sixty Petri dishes were each filled with 10 g of soil and placed in a greenhouse. Seeds produced in the different earthworm treatments can therefore be considered to be grown in a common environment that has not been influenced by earthworm activities. Each Petri dish received 10 seeds of one of the three plant species. These seeds were randomly sampled among the seeds collected on the four conspecific plants of each microcosm of the first experiment. Two weeks after sowing, the number of seedlings was counted to determine the germination rate (no difference in time of germination between treatments was found within plant species) and the height of seedlings was measured. Petri dishes were watered every day (3 ml) during the experiment to maintain the moisture of the soil. Petri dish position within the greenhouse was randomized every 2 days. Nitrogen concentration in mother seed was measured using a ThermoFinnigan Flash EA 1112 elemental analyzer (ThermoFinnigan, Milan, Italy).

2.2. Statistical analyses

Data were analysed with ANOVAs using SAS GLM procedure (Sum of squares type III, SS3) (SAS, 1990). A full model was first used to test all possible factors (“AC”, “LT” and “plant species”) and all interactions between these factors (Table 1). To determine the direction of significant effects, we used multiple comparison tests based on least square means (LSmeans, LSmeans SAS statement). The residuals of each model were analysed to test normality and homogeneity of variances. All tests were conducted with a significance level $\alpha = 0.05$.

3. Results

The statistical analysis (Table 1 and LSmeans comparisons) showed that both earthworm species induced maternal effects in

Table 1

General ANOVA table for the effects of earthworms (*A. caliginosa* – AC and *L. terrestris* – LT) and plant species on the seed germination, on the height of seedlings and N content of seeds. Significant *P*-values are presented in bold. Total df = 59.

	df	Seedling height		Seed germination		Seed *N* content	
		F	*P*	*F*	*P*	*F*	*P*
AC	1	15.63	**<0.001**	62.16	**<0.001**	17.07	**<0.001**
LT	1	0.00	0.97	2.16	0.15	12.8	**<0.001**
AC*LT	1	1.67	**<0.0001**	11.16	**<0.001**	19.43	**<0.001**
Plant species	2	76.68	0.20	28.41	**<0.001**	87.78	**<0.001**
LT*plant species	2	2.92	**0.03**	2.80	0.07	14.91	**<0.001**
AC*plant species	2	3.89	0.06	26.38	**0.002**	6.43	**<0.01**
AC*LT*plant species	2	0.22	0.80	5.48	**0.007**	4.17	**0.02**
r^2		0.79		0.81		0.85	