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# The effects of earthworms on the demography of annual plant assemblages in a long-term mesocosm experiment

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ABSTRACT

Earthworms have been shown to influence plant growth, survival and fecundity. They can therefore affect plant demography in plant communities changing their composition. A long term mesocosm experiment was set-up to test the effects of an endogeic (*Aporrectodea caliginosa*) and an anecic (*Lumbricus terrestris*) earthworm species on assemblages of four species of annuals: one grass (*Poa annua*), two forbs (*Veronica persica* and *Cerastium glomeratum*) and one legume (*Trifolium dubium*). The number of individuals and the biomass of each species were investigated. *A. caliginosa* and *L. terrestris* affected the density of *T. dubium* at each of the three monitored census dates. The other plant species responded to *A. caliginosa* and *L. terrestris* at the second and third generations. The presences of *A. caliginosa* and *L. terrestris* reduced the total number of plant individuals from the second to the third generation. At harvest (3rd generation), *T. dubium* and *V. persica* had more and larger individuals in the presence of *A. caliginosa*. When both earthworm species were present, *T. dubium* had few but larger individuals. Our study confirms that earthworms affect plant demography and plant community structure. Our results also show that accurate prediction of long-term effects of earthworms on plant communities cannot be achieved using results on their short-term effects on plant growth. This is due to the poor understanding of the effects of earthworms on plant resource allocation and demography, and also the possibility that earthworms may exert the opposite effect on the short and long-term availability of nutrients.

## Introduction

Earthworms are known to modify soil structure, increase nutrient mineralization, foster the release of plant growth substances, and change soil microbial communities. These effects generally result in positive effect on plant growth (Scheu 2003; Brown et al. 2004). While most studies have focused on the short-term responses of plant individuals to earthworms (Scheu 2003; Brown et al. 2004), some experiments have shown that earthworms may affect interspecific competition and plant community structure (Wurst et al. 2005; Eisenhauer and Scheu 2008; Laossi et al. 2009).

In short-term plant assemblage experiments, earthworms have been shown to affect plant competition by promoting individual plant species against others (Wurst et al. 2005; Laossi et al. 2009). They have also been shown to affect differently seed germination (Decaëns et al. 2003; Milcu et al. 2006) and seed production (Poveda et al. 2005). In a recent study earthworms have been shown to influence seed germination through maternal effects (Laossi et al. 2010a). These demographic effects should lead to changes in the plant community structure and composition in the long-term (Thompson et al. 1993; Eisenhauer and Scheu 2008; Wurst et al. 2008). Furthermore, since the effects of earthworms on plant biomass production and demography are not necessarily correlated (Laossi et al. 2009), long-term effects on community structure cannot be predicted from short-term effects on plant growth.

In this study, we performed a long-term mesocosm experiment investigating the effect of two earthworm species belonging to two different functional groups (endogeic and anecic earthworms), and their interaction, on simple plant communities of four annuals belonging to three functional groups (grasses, forbs, and legumes). These plants and earthworm species have been chosen because their interactions have already been studied in short-term microcosm experiments (Laossi et al. 2009, 2010a,b). In this study we tested four different hypotheses: (1) earthworms exert different effects on the demography of competing plant species, thereby

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changing the structure of their community (frequency of individuals of each species); (2) the long-term effect of earthworms on plant communities cannot easily be predicted from their short-term effects on plant species; (3) earthworms lead to different plant species ranking for their total biomass and their demography; (4) earthworm-induced short-term positive effects on plant biomass, should increase the total biomass of the plant community after several generations.

## Materials and methods

### *Experiment set up*

We set up mesocosms consisting of PVC pots (diameter 50 cm, height 45 cm). Drains at the bottom of pots were covered with 1 mm plastic mesh to prevent earthworms from escaping. Soil was collected at the ecology station of the Ecole Normale Supérieure at Foljuif (France). It was a sandy cambisol supporting a meadow (OM 2.55%, C/N ratio 12.4, C concentration 1.47%, N concentration 0.12%, $NO_3^-$ 14 mg/kg, $NH_4^+$ 20.1 mg/kg and pH 5.22). A total of 20 mesocosms filled with 45 kg of sieved (2 mm) dry soil were placed outdoor in Bondy near Paris (Bondy annual average temperature is 11.9 °C and the average rainfall is 647.3 mm). Before starting our experiment, the mesocosms were watered to field capacity, then watered regularly and weeds germinated from the seed bank were removed. 40 g of dried litter (72 h at 60 °C) of grass leaves were placed at the soil surface and 10 g was mixed with the first cm of soil, prior to the addition of earthworms and seeds. This constituted the main food source for the earthworms at the beginning of the experiment because of the natural scarcity of organic matter in the soil.

We used an anecic earthworm, *Lumbricus terrestris* Linné (1758) (LT) and an endogeic earthworm, *Aporrectodea caliginosa* Savigny (1826) (AC). *L. terrestris* was purchased in a store and *A. caliginosa* was collected in the park of the IRD centre in Bondy (France). Four treatments consisting of three earthworm treatments (AC, LT, AC + LT) and a control (without earthworms) were set up. Five replicates were implemented for each treatment combination. Seven *L. terrestris* individuals (approximately 135 g $m^{-2}$ in total) and 30 *A. caliginosa* individuals (approximately 105 g $m^{-2}$ in total) were introduced in each mesocosm including these species.

Five days after earthworm introduction, seeds (0.5 g for each plant species) of *Veronica persica* Poiret (1808), *Poa annua* Linné (1753), *Trifolium dubium* Sibthorp (1794), and *Cerastium glomeratum* Thuillier (1799) were added to mesocosms. Four weeks later, 25 plants of each species were kept per mesocosm (the other plants were cut down with scissors in small pieces to make sure they died and their biomass left in the mesocosms). Mesocosms were weeded every two weeks during the experiment to remove seedlings of plants species from seed bank (i.e. non-targeted species). The experiment was watered according to plant stage (from 1 l every two days for seedlings to 2 l every day for adult plants) and precipitations (mesocosms were watered to maintain the soil near field capacity when natural precipitation did not do so). Mesocosm position was randomized every month to avoid confounding factors. Plant dead organic matter remained in the mesocosms. The experiment lasted 21 months, which roughly encompass 3 plant generations.

### *Data collection*

#### *Plant assemblages*

Three census dates were chosen to correspond roughly to the flowering of the three plant generations observed: though the life-cycles of all individuals were not fully synchronized, at these date 95% of individuals were flowering. At each census the number of individuals of each plant species was counted. For the first census date, individuals were counted 4 months after sowing (week 13). This allowed us to determine if earthworms affected the survival of the 25 seedlings of each plant species kept in mesocosms. On week 34, the number of individuals (flowering adults) of the second generation was determined. This census was repeated at the end of the experiment (week 92). At week 13 we assessed the effect of earthworms on seedling growth and survival starting from a simple plant community. Afterwards, our two other censuses also integrated the effect of earthworms on plant fecundity, seed size, seed germination, and complicated competitive interactions building up with the age of the plant community. At the end of the experiment, total root biomass was assessed by extracting two soil cores per mesocosm (6 cm diameter × 20 cm depth). The soil cores were separated in two depths (0–10 cm and 10–20 cm) to test for possible earthworm effects on the root profile. Roots were then separated from the soil by washing on a 600 μm mesh, but the roots of the individual plant species were not recognizable. Specific dried (72 h at 60 °C) shoot biomasses and total root biomass, were weighed.

#### *Earthworms*

At the end of the experiment, earthworms were collected by hand-sorting, counted and weighed individually (fresh weight with gut content). The number of cocoons was also counted.

#### *Soil analyses*

Mineral N ($NH_4^+$, $NO_3^-$) contents in 10 g soil were colorimetrically determined from 100 ml of 1 M KCl extracts according to Bremner (1965) using a Autoanalyser III (seal Ananlytical, Bran et Luebbe, Plaisir, France) at the end of the experiment.

#### *Statistical analyses*

Data on the four plant species were first analysed altogether with a MANOVA using SAS GLM procedure (sum of squares type III, SS3) (SAS 1990) (Table 1). When the MANOVA documented a significant earthworm effect, data were analysed separately using an ANOVA for each plant species (Table 2). This allowed us to determine which plant species responded to earthworms. Effects of treatments and interactions between treatments were tested on the number of individuals of each species at the first, second and third census date. In this case, the Bonferroni correction was applied to take into account that these measurements were repeated three times on the same experimental unites. Effects of treatments were also tested on measurements made only at the end of the experiment: specific shoot biomasses, average individual plant biomass, total mesocosm shoot biomass, the total root biomass, the total mesocosm plant biomass and mineral nitrogen availability. To determine the direction of significant effects in ANOVAs, we used post hoc multiple comparison tests based on least square means (LSmeans, LSmeans SAS statement). The residuals of each model were analysed to test for normality and homogeneity of variances. All tests were achieved with a significance level $p = 0.05$.

## Results

At the end of the experiment a total of 70 earthworms were recovered (20% of the introduced earthworms), comprising 22 individuals (20 juveniles) of *L. terrestris* – 14 in LT treatment and 8 in LT + AC treatments – and 48 individuals (31 juveniles) of *A. caliginosa* (27 in AC treatment and 21 in AC + LT treatment). On average, 5 cocoons per mesocosm were found in treatments with earthworms (all earthworm treatments).

**Table 1**
MANOVA table of *F*-values (Pillaï's trace) on the effects of earthworm treatments (*A. caliginosa* – AC, *L. terrestris* – LT, *A. caliginosa* + *L. terrestris* – AC × LT) on plant average individual biomass, shoot biomass per plant species and number of individuals at weeks 13, 34 and 92.

| Dependant variable | Independent variable | d.f. | *F*-value | *P*-value |
|---|---|---|---|---|
| Average individual biomass | AC | 1 | 186.26 | **<0.001** |
| | LT | 1 | 55.50 | **<0.001** |
| | Plant species | 3 | 657.09 | **<0.001** |
| | AC × LT | 1 | 18.46 | **<0.001** |
| | AC × plant species | 3 | 25.30 | **<0.001** |
| | LT × plant species | 3 | 40.85 | **<0.001** |
| | AC × LT × plant species | 3 | 75.96 | **<0.001** |
| Shoot biomass | AC | 1 | 0.52 | 0.4760 |
| | LT | 1 | 0.28 | 0.5985 |
| | Plant species | 3 | 130.46 | **<0.001** |
| | AC × LT | 1 | 0.05 | 0.8298 |
| | AC × plant species | 3 | 3.24 | **0.0295** |
| | LT × plant species | 3 | 0.95 | 0.4210 |
| | AC × LT × plant species | 3 | 1.34 | 0.2716 |
| Number of individuals (week 13) | AC | 1 | 0.97 | 0.3275 |
| | LT | 1 | 0.25 | 0.6200 |
| | Plant species | 3 | 18.86 | **<0.001** |
| | AC × LT | 1 | 0.12 | 0.7335 |
| | AC × plant species | 3 | 2.97 | **0.0387** |
| | LT × plant species | 3 | 2.98 | **0.0380** |
| | AC × LT × plant species | 3 | 0.68 | 0.5683 |
| Number of individuals (week 34) | AC | 1 | 1.11 | 0.2969 |
| | LT | 1 | 174.06 | **<0.001** |
| | Plant species | 3 | 169.96 | **<0.001** |
| | AC × LT | 1 | 0.03 | 0.8605 |
| | AC × plant species | 3 | 68.80 | **<0.001** |
| | LT × plant species | 3 | 33.91 | **<0.001** |
| | AC × LT × plant species | 3 | 105.58 | **<0.001** |
| Number of individuals (week 92) | AC | 1 | 8.30 | **0.0055** |
| | LT | 1 | 35.65 | **<0.001** |
| | Plant species | 3 | 37.38 | **<0.001** |
| | AC × LT | 1 | 4.26 | **0.0433** |
| | AC × plant species | 3 | 7.33 | **0.0003** |
| | LTxplant species | 3 | 22.16 | **<0.001** |
| | AC × LT × plant species | 3 | 4.16 | **0.0096** |

Significant effects are given in bold.

### *Plant demography*

Earthworm treatments affected plant abundances at each generation and these effects depended on plant species (Table 1 and Table 2). The significant results of MANOVA tests (Table 1) allowed us to analyse separately the abundance of each plant species (Table 2).

At the beginning of the experiment, each plant species represented 25% of the individuals in the assemblage in every treatment. After 13 weeks, the abundance of *T. dubium* was significantly reduced by *A. caliginosa* (−19%) and *L. terrestris* (−18%) (Fig. 1a and Table 2).

At the second census date (week 34), the presence of *A. caliginosa* and the presence of *L. terrestris* increased the abundances of *P. annua* (+40% and 4.5 fold, respectively) and *C. glomeratum* (+13% and + 32%, respectively) but reduced the abundance of *T. dubium* (−56% and −47%) and *V. persica* (−85% and −33%) (Fig. 1b and Table 2). The treatment with both earthworm species (AC + LT) increased the abundance of *C. glomeratum* (4 fold increase) and *V. persica* (3.7 fold increase). In *A. caliginosa and L. terrestris* treatments *P. annua* and *C. glomeratum* dominated the assemblage in abundance (50% and 40% in AC treatment and 65% and 25% in LT treatment, respectively). *T. dubium* and *V. persica* represented 7% and 2% of all individuals in the presence of *A. caliginosa* and 4% and 5% in the presence of *L.*

**Table 2**
ANOVA table for the effects of earthworms (*A. caliginosa* – AC, *L. terrestris* – LT, *A. caliginosa* + *L. terrestris* – AC × LT) on the specific number of individuals in first (week 13), second (week 34) and third (week 92) census date within plant assemblages. The same model is analysed independently for the four plant species. Total d.f. = 19.

| | d.f. | *Poa annua* (*F*-value) | | | *Trifolium dubium* (*F*-value) | | | *Cerastium glomeratum* (*F*-value) | | | *Veronica persica* (*F*-value) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Number of individuals (week 13) | Number of individuals (week 34) | Number of individuals (week 92) | Number of individuals (week 13) | Number of individuals (week 34) | Number of individuals (week 92) | Number of individuals (week 13) | Number of individuals (week 34) | Number of individuals (week 92) | Number of individuals (week 13) | Number of individuals (week 34) | Number of individuals (week 92) |
| AC | 1 | 021 | 108.29$^{***}$ | 12.28$^{**}$ | 6.14$^{*}$ | 6.74$^{*}$ | 2.41 | 1.25 | 52.58$^{***}$ | 12.95$^{**}$ | 3.00 | 10.10$^{*}$ | 0.04 |
| LT | 1 | 0.38 | 119.10$^{***}$ | 3.73 | 5.26$^{*}$ | 3.82 | 58.86$^{***}$ | 2.28 | 82.63$^{***}$ | 1.53 | 0.12 | 22.78$^{***}$ | 65.95$^{***}$ |
| AC × LT | 1 | 1.17 | 231.72$^{***}$ | 1.48 | 0.00 | 0.72 | 19.38$^{***}$ | 0.52 | 40.75$^{***}$ | 0.22 | 0.33 | 33.04$^{***}$ | 2.55 |
| $r^2$ | | 0.10 | 0.97 | 0.52 | 0.48 | 0.41 | 0.83 | 0.20 | 0.91 | 0.48 | 0.17 | 0.87 | 0.82 |

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

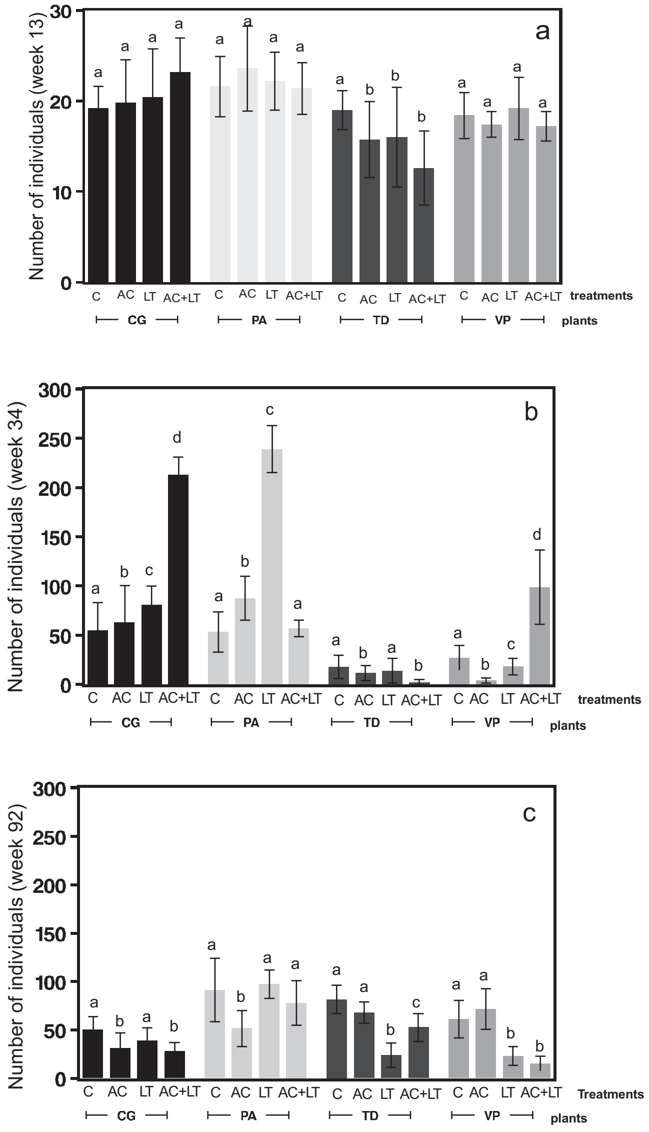

**Fig. 1.** Effects of earthworms on the number of individuals of plant species in the assemblages at the first (a), second (b) and third (c) census date. C, control; AC, *A. caliginosa*; LT, *L. terrestris*; AC + LT, both earthworm species; TD, *T. dubium*; VP, *V. persica*; CG, *C. glomeratum*; PA, *P. annua*. Letters over the bars represent difference within plant species.

*terrestris*, respectively. In the presence of both earthworm species, *V. persica* (30%) and *C. glomeratum* (55%) individuals dominated the plant assemblage while *T. dubium* (0.6%) and *P. annua* (15%) had few individuals.

At the third census date, the presence of *A. caliginosa* reduced the abundance of *P. annua* (−31%) and *C. glomeratum* (− 33%) while *L. terrestris* reduced the abundance of *V. persica* (−61%) and *T. dubium* (−70%). When both earthworm species (AC + LT) were present, the abundances of *T. dubium* (−37%) and *V. persica* (−82%) were lower (Fig. 1c and Table 2). In the presence of *A. caliginosa*, *P. annua* represented 23% of individuals and *C. glomeratum* 14%, whereas *T. dubium* (31%) and *V. persica* (32%) were more abundant. However, in the presence of *L. terrestris*, *T. dubium* (14%) and *V. persica* (13%) were less abundant than *P. annua* (48%) and *C. glomeratum* (25%).

From the second to the third census date, the presence of earthworms reduced the total number of individuals in the plant assemblage by 37% with greater negative effect in the presence of *L. terrestris* (LT treatment −40% and AC + LT treatment −37%). At the end of the experiment (week 92), the total number of plant individuals in the control treatment was 85% higher than at the second census date.

### *Plant biomass*

*A. caliginosa* increased the average individual shoot biomass in *P. annua* (+23%), *T. dubium* (+53%) and *V. persica* (+35%). This effect was amplified for *P. annua* (+115%) and *T. dubium* (+136%) in the treatment with both earthworms species (AC + LT treatment) while the presence of *L. terrestris* alone, reduced the individual shoot biomass in *T. dubium* (−73%) and *V. persica* (−47%). Moreover, the association of both earthworm species increased the average individual shoot biomass in *C. glomeratum* (+375%) and decreased it in *V. persica* (−50%) (Table 3).

The presence of *A. caliginosa* affected the shoot biomass but its effect depended on the plant species (significant AC × plants species, Table 1). At the harvest (week 92), *A. caliginosa* increased the total shoot biomass of *T. dubium* (+47%) and *V. persica* (+40%) (Table 3). *L. terrestris* increased the total shoot biomass of *V. persica* (+280%) but reduced the total shoot biomass of *T. dubium* (−92%). Furthermore, the earthworm mixture increased the total shoot biomass of *T. dubium* (+38%) but decreased that of *V. persica* (−28%) (Table 3 and significant LSmeans). No significant earthworm effect was found on the total shoot biomass of *P. annua* and *C. glomeratum* (Table 3) and on the mesocosm total shoot biomass (Table 3).

Earthworm affected differently the ranking of plant species according to their shoot biomass and their number of individuals. For example, in *A. caliginosa* treatments, *T. dubium* and *V. persica* were the most abundant species (more than 60% of all individuals in the assemblage) but represented a very small shoot biomass (about 30% of the assemblage shoot biomass). Similarly, in the presence of *L. terrestris*, the species ranking was (in decreasing order) *P. annua*, *C. glomeratum*, *T. dubium* and *V. persica*, according to their abundances but was *P. annua*, *V. persica*, *C. glomeratum* and *T. dubium* according to their biomasses. Generally speaking, *P. annua* always presented a higher percentage of the total shoot biomass than its percentage of individuals in the whole plant assemblage.

The treatment with both earthworm species reduced the root biomass by −56% and 32% in the 0–10 cm and 10–20 cm layers, respectively (Table 3 and significant LSmeans comparisons). This effect represented a reduction of 51% in the total root biomass of the mesocosms. The total plant biomass was also reduced by −16% in the presence of *A. caliginosa*, while no significant effect was observed in the *L. terrestris* treatment (Table 3).

### *Soil nutrient content*

As compared to the initial soil of the experiment, we found an increased total organic N in earthworm treatments as well as in the control at the end of the experiment. $NO_3^-$ content increased as compared to the initial situation, but this change was less marked in the presence of earthworms. We also found an increase in $NH_4^+$ in all treatments except the *L. terrestris* treatment ($p < 0.01$). The inorganic form of nitrogen was dominated by $NO_3^-$. $NH_4^+$ (−41% and −72%) and $NO_3^-$ (−28% and −42%) availabilities were lower in the respective presences of *A. caliginosa* and *L. terrestris* ($p < 0.01$ and significant LSmeans comparisons).

## Discussion

As predicted, earthworms affected the relative frequency of the individuals of each plant species at each generation, which reflects earthworm contrasted influence on the demography of the species. At the first census date (week 13) *A. caliginosa* and *L. terrestris* only decreased the abundance of *T. dubium*. Earthworms are known to

**Table 3**
ANOVA table for the effects of earthworms (*A. caliginosa* – AC, *L. terrestris* – LT, *A. caliginosa* + *L. terrestris* – AC × LT) on the specific average individual shoot biomass, specific total shoot biomass of plant assemblages, root production (0–10 cm, 10–20 cm and total root), total shoot biomass and total biomass of plant communities (mesocosm total shoot + mesocosm total root). Total d.f. = 19.

| | d.f. | *P. annua* (*F*) | *T. dubium* (*F*) | *V. persica* (*F*) | *C. glomeratum* (*F*) |
|---|---|---|---|---|---|
| Specific average individual shoot biomass | | | | | |
| AC | 1 | 120.57*** | 158.74*** | 5.53* | 18.09*** |
| LT | 1 | 51.62*** | 0.02 | 75.89*** | 25.71*** |
| AC × LT | 1 | 135.29*** | 47.83*** | 6.03* | 19.54*** |
| $r^2$ | | 0.95 | 0.94 | 0.86 | 0.83 |
| Specific total shoot biomass | | | | | |
| AC | 1 | 0.32 | 26.52*** | 11.40** | 0.02 |
| LT | 1 | 0.03 | 8.71** | 4.08* | 0.02 |
| AC × LT | 1 | 0.09 | 5.91* | 42.93*** | 0.14 |
| $r^2$ | | 0.02 | 0.80 | 0.86 | 0.01 |

| | d.f. | Root biomass 0–10 cm (*F*) | Root biomass 10–20 cm (*F*) | Mesocosm root biomass (*F*) | Mesocosm total shoot biomass (*F*) | Total biomass (*F*) |
|---|---|---|---|---|---|---|
| Root and total biomass | | | | | | |
| AC | 1 | 14.89*** | 0.27 | 10.98** | 0.72 | 4.33* |
| LT | 1 | 3.12 | 3.31 | 3.90 | 0.03 | 3.03 |
| AC × LT | 1 | 6.04* | 4.83* | 7.02* | 0.08 | 3.83 |
| $r^2$ | | 0.60 | 0.34 | 0.58 | 0.05 | 0.41 |

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

negatively affect the competitive ability of legumes relative to non-legumes when grown in multi-specific assemblages (Brown et al. 2004; Eisenhauer and Scheu 2008). In the present experiment, this probably occurred at the beginning of the experiment and could explain the decrease in *T. dubium* density between the seedling stage (week 13) and the final mature stage (week 92).

At the second generation, *L. terrestris* increased the abundance of *P. annua* while the association of both earthworm species favored the abundance of *C. glomeratum* and *V. persica*. This suggests that *L. terrestris* enhanced the competitive ability of *P. annua* against the other plant species. We found similar results in our previous experiment (Laossi et al. 2009) where *P. annua* had access to a greater share of mineral nutrients made available by *L. terrestris*. In this experiment, the treatment with both earthworm species increased the biomass of *V. persica*. This suggests that their interaction induces a higher nutrient availability and can partially explain the positive effect on lower competitors such as *C. glomeratum* and *V. persica*. The higher earthworm density in the treatment with both earthworm species could have increased the nutrient availability to these plants as suggested by Zaller and Arnone (1999) in a field experiment. However, our results showed that earthworms reduced the soil mineral N, so that their death may only have induced a transient fertilization and is not likely to explain the whole dynamics of our plant communities (see below).

At the third census date (week 92), *A. caliginosa* reduced the abundance of *P. annua* and *C. glomeratum* while *L. terrestris* reduced the abundance of *T. dubium* and *V. persica*. The negative effect of *A. caliginosa* on *P. annua* and *C. glomeratum* is difficult to explain. A possible explanation could be that earthworm activity has decreased (due to the decrease in earthworm number) during the 2 years of our experiments, leading to a weakened effect on mineralization and mineral nutrient availability. Two mechanisms could explain the negative effect of *L. terrestris* on the abundances of *T. dubium* and *V. persica*. Firstly earthworms (especially anecic species) have been shown to negatively affect plant survival (Eisenhauer et al. 2010), as found in our study in which earthworm had a negative impact on the survival of *T. dubium* between seedling and adult stages at the first generation. Secondly, we have also found in another experiment with the same plant and earthworm species that earthworms affect seed germination and seedling growth through maternal effects (Laossi et al. 2010a): earthworms modified the size and nutrient content of the seeds produced in their presence, which in turn influences germination rates. The effects of earthworms on plant survival and on seed germination through maternal effects are plausible but not exclusive mechanisms to explain how *L. terrestris* decreased the percentage of *T. dubium* and *V. persica* in our plant assemblages. Additionally, earthworms are known to selectively consume small seeds and to decrease their germination rate as a consequence of physical damages and/or deep burrowing (Decaëns et al. 2003; Milcu et al. 2006). This may be the case for *T. dubium*, which in presence of *L. terrestris* produces smaller seeds (Laossi et al. 2009) with an increased probability of being ingested by earthworms. An opposite outcome was found with the treatment including both earthworm species. It thus seems that *A. caliginosa* compensated the negative effect of *L. terrestris* on *T. dubium* but the mechanisms remain unclear.

Although *A. caliginosa* affected negatively the abundance of *P. annua*, it favored this plant species in terms of individual shoot biomass. At the harvest, in the *A. caliginosa* treatment *P. annua* dominated the plant assemblage in terms of biomass production while *V. persica* and *T. dubium* dominated in terms of abundance. The species rankings according to the relative contribution of individuals to biomass or abundance were different and were differently affected by earthworms. This is due to the fact that a species can have the same biomass with few large individuals or with many small individuals. In some cases, the shoot biomass of individuals varied significantly among earthworm treatments (*P. annua* and *C. glomeratum* produced larger individuals in the presence of *A. caliginosa*), while in other cases a given species, such as *P. annua*, can intrinsically have a higher capacity to uptake resources and have larger individuals. This leads to a very general and largely open issue: How do soil organisms affect the relationship between plant individual size and density? This issue is not only theoretical. It must be tackled to predict earthworm effect on the biomass of each species within a community from earthworm effects on the abundances of these species. Indeed, it is very difficult to predict the earthworm effects on the structure of complex plant communities (see above). But it is even more difficult to predict their impacts on the relative contribution of each species to the community biomass, because their demographic effects and their effect on individual biomasses are not necessarily consistent (Neytcheva and Aarssen 2008; Laossi et al. 2010b).

We predicted that earthworms should increase the total biomass of our four species assemblage after several generations. However, the treatment with the two earthworm species in association reduced the total biomass of these assemblages. This shows that earthworms first affect the repartition of resources between plant species and thus the repartition of biomasses between species in the plant assemblage. The negative effect found here on total plant biomass could be due to reduced nitrogen availability. Indeed, our results showed that all earthworm treatments reduced the soil nitrogen availability ($NO_3^-$ and $NH_4^+$) at the end of the experiment. This negative effect on nitrogen availability could occur through leaching via earthworm galleries (Dominguez et al. 2004) and this phenomenon could be more important in the treatment with both earthworm species due to the presence of more galleries. By increasing nutrient losses earthworms should decrease primary productivity on the long-term even if they increase mineralization and plant growth on the short-term (Barot et al. 2007b). Although there are some long term field studies using earthworms (Zaller and Arnone 1999; Eisenhauer et al. 2009), to the best of our knowledge most microcosm or mesocosm experiments using earthworms have a shorter duration than the present experiment (Scheu 2003; Brown et al. 2004; Wurst et al. 2008). It is indeed more difficult to control the number and the activity of earthworms when an experiment lasts more than two or three months and has to rely on the reproduction of earthworms. In our case, the number of earthworms recovered at the end of the experiment was five times smaller than the number of earthworms initially introduced. However, most of them were juveniles and many cocoons were also found (about 5 per mesocosm with earthworms). This suggests that earthworms have been active and reproduced during the experiment and supports the hypothesis that earthworms did have a real impact on soil properties and plants. However, since the number of earthworms fluctuated during the experiment, their level of activity is also likely to have been fluctuating depending on seasonal variations (the mortality of earthworms may have been affected by frost or hot summer days in our case). This could for example explain the fact that, in some cases, earthworm effects on our four species plant assemblage did not add up: their direction varied between the three generation intervals. Such phenomena have probably influenced our experimental results but are realistic because earthworm densities and activities have been shown to be highly variable in space and time in the field (Rossi 2003; Barot et al. 2007a).

## Conclusions

Our results support the hypothesis that earthworms affect plant demography and that this in turn modifies plant community structure. However, new experiments lasting longer than three plant generations are needed to fully explain the underlying mechanisms and assess the sensitivity of plant community structure to earthworm community. Bedsides, our work focuses on plant-earthworm interactions but the issues we have raised are probably very general and are relevant to predict the effect of any soil organism on plant communities (Reynolds et al. 2003; Van der Heijden et al. 2007). Indeed, many soil organisms are likely to affect the structure of plant communities both in terms of species relative biomasses and abundances. Moreover, these effects might be different on the short (a single plant generation) and long-term (several plant generations) because soil organisms may affect in contrasted ways: (1) the capacity of plants to acquire resources, and their demography and (2) the short and long-term nutrient availabilities.

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