Applied Soil Ecology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Inoculation of an ecosystem engineer (Earthworm: *Lumbricus terrestris*) during experimental grassland restoration: Consequences for above and belowground soil compartments

Estelle Forey[a,*], Matthieu Chauvat[b], Sekou F.M. Coulibaly[a], Estelle Langlois[a], Sebastien Barot[b], Julia Clause[a,c]

[a] *Normandie Univ, UNIROUEN, IRSTEA, ECODIV, 76000, Rouen, France*
[b] *IEES-P (IRD, CNRS, UPMC, UPEC), 7 quai Saint Bernard, 75252, Paris Cedex, France*
[c] *Université de Poitiers, Laboratoire Ecologie & Biologie des Interactions—UMR CNRS 7267, Equipe Ecologie Evolution Symbiose, 5, rue Albert Turpin, TSA, 51106, 86073, Poitiers Cedex 9, France*

ARTICLE INFO

Keywords:
Plant-soil interactions
Collembola
Functional types
Disturbance
Alkaline peat
In situ experiment
Plant productivity

ABSTRACT

Although soil organisms might strongly affect the dynamics and composition of natural vegetation, relatively few studies have tried to in-situ manipulate soil fauna, especially in restoration ecology. The objective of this study was thus to observe the impact of a soil ecosystem engineer (*Lumbricus terrestris* L.) on plant communities as well as on soil organisms (springtails) in a reclaimed floodplain previously devoid of earthworms. Within a randomized factorial design based on buried frames (depth 0.45 m), half of the quadrats (1 m^2) were inoculated with 100 earthworms, the other half served as control. After one year of experiment, earthworm inoculation doubled the plant biomass and favored grass species over forbs. Both abundance and diversity of Collembola (depending on functional groups) were negatively impacted by the presence of earthworms. Using a path analysis we found that this negative impact was probably indirect and due to an earthworm effect on plant community structure and plant functional groups. We suggest in our particular case that vegetation, and more precisely plant biomass and functional traits, may be more influential than soil properties in driving Collembola assemblages. Regarding restoration, we conclude that manipulating earthworms could be an interesting tool for increasing plant productivity but may disfavor soil biodiversity and alter above-belowground linkages.

1. Introduction

Over the last decade, an increasing number of conceptual and empirical studies have stressed the importance of soil fauna and above-belowground linkages in driving communities and ecosystem properties (Bardgett and Wardle, 2003; Wardle et al., 2004). For example, invertebrate soil fauna might enhance both secondary succession and local plant species diversity (De Deyn et al., 2003). The composition and diversity of soil fauna communities also determine ecosystem multifunctionality (Wagg et al., 2014). As a consequence, it has been suggested to consider soil biota and above-belowground linkages to assist conservation and restoration ecology (Kardol and Wardle, 2010).

To date, the manipulation of soil fauna in restoration ecology has concerned earthworms as emblematic soil ecosystem engineers (sensu Jones et al., 1994). Through their feeding and burrowing activities, they influence soil physical properties such as aggregate stability, soil structure, infiltration of water, and aeration of deeper soil layers. Earthworms also modify soil biotic properties such as microbial biomass and activity, nutrient cycling and mineralization, density of other soil invertebrates, plant productivity and community composition, and aboveground food webs (for a complete review see Blouin et al., 2013). The inoculation of earthworms in soils devoid or with a low density of earthworms may be a tool for assisting the rehabilitation of degraded lands (Butt, 1999; Snyder and Hendrix, 2008; Boyer and Wratten, 2010; Jouquet et al., 2014). However, a large majority of these studies focused on earthworm inoculation techniques (choice of earthworm species, density, methods, timing, costs, etc.; for a review see Butt, 2008), and only looked at the restoration of soil properties such as aggregate structure and soil porosity (Fraser et al., 2003; Marashi and Scullion, 2003), soil fertility (Scullion and Malik, 2000; Fraser et al., 2003) or remediation of contaminated soils (Sizmur et al., 2011). In comparison, studies that used earthworm inoculation to assist restoration of the biotic (above and/or belowground) component of an ecosystem are rather scarce (e.g. Curry and Boyle, 1987; Roubickova et al.,

---

* Corresponding author.
E-mail address: estelle.forey@univ-rouen.fr (E. Forey).

https://doi.org/10.1016/j.apsoil.2017.12.021
Received 26 July 2017; Received in revised form 18 December 2017; Accepted 19 December 2017

Please cite this article as: Forey, E., Applied Soil Ecology (2018), https://doi.org/10.1016/j.apsoil.2017.12.021

2009; Mudrak et al., 2012). And only one of them tested the effect of earthworms on both late successional plants and soil fauna (Mudrak et al., 2012). In this 3-month laboratory pot experiment, these authors demonstrated that the inoculation of earthworms could contribute to drive the succession of plant and soil fauna communities (Collembola).

Among belowground organisms Collembola (springtails) constitute a model soil organism group (e.g. Henneron et al., 2017). They are the most abundant soil invertebrates present in almost all terrestrial ecosystems. By interacting with microorganisms through fungal grazing and others mechanisms, these soil microarthropods are recognized to play an important role in litter decomposition, nutrient cycling and plant growing processes (Petersen, 2002; Partsch et al., 2006; Forey et al., 2015). Collembolan species are generally classified into three life-form groups (epedaphic, hemiedaphic and euedaphic) according to their ecology and sensitivity to environmental conditions (Gisin, 1943; Petersen, 2002), which allow to investigate their functional assemblages. Recent studies demonstrated that plant community structure and functions could be strong drivers of collembolan assemblages (Abgrall et al., 2017; Henneron et al., 2017). Indeed, according to the plant functional diversity hypothesis (Balvanera et al., 2006; Eisenhauer et al., 2010b), rich plant communities could favor decomposer diversity due to enhanced microhabitat and substrate heterogeneity (Wardle et al., 2005). Alternatively, the plant mass-ratio hypothesis (Grime, 1998) states that soil fauna assemblages should be driven primarily by traits of the dominant plant species (those contributing most to productivity; Wardle et al., 2005).

The objective of our study was to assess the importance and impact of inoculated earthworms (*Lumbricus terrestris* L.) on above and belowground compartments, i.e. on plants and on Collembola. This study was set within a larger restoration project that aimed at using former exploited gravel pits to restore wetland habitats or grasslands to be used as pastures for cattle grazing or mowing (Mchergui et al., 2014). In this reclaimed land, earthworm abundance was around zero. Thus, the inoculation of earthworm as an ecosystem engineer in this site could facilitate ecosystem dynamics to recreate an herbaceous ecosystem. After one year of experimentation, we monitored plant and Collembola communities in plots inoculated or not with *L. terrestris*. We hypothesized that earthworms might (1) indirectly enhance plant productivity through increasing soil fertility, (2) drive plant species assemblages by favouring some functional types (3) and thus directly or indirectly modify Collembola communities with contrasting responses according to their functional group (Fig. 1).

2. Materials and methods

2.1. Study area and restoration project

The experiment was conducted in a currently exploited gravel quarry ('Carrières et Ballastières de Normandie') in Yville sur Seine (49° 29' 05' N; 00° 52' 31" E) located in the floodplain Seine valley in France. Average daily temperatures range from −5 °C in winter to 27 °C in summer. For decades, these gravels have been dug out from pits that are naturally filled with water after their use. Recently, a new project aimed at filling pits with a deep layer of sediments dredged out from the nearby Seine River, which is then covered by alkaline peat to restore local grasslands for grazing or mowing. Thereby, collective industrial objectives of dredging the Seine, exploiting gravels and ecological objectives of restoring grasslands can be met.

After been exploited, a gravel-pit was progressively filled between 2009 and the end of 2011 with sediments dredged from the Seine River (between 7 and 8 m) and covered with a layer of sand (10 cm, to stabilize sediments). Then, to reconstitute the initial soil profile, a layer of peat coming from adjacent wet meadows (between 70 and 80 cm) was added. This is an alkaline alluvial peat naturally deposited during the Holocene (Mchergui et al., 2014). Lastly, the peat was topped with a layer of grassland soil (10 cm). This topsoil corresponded to the a mixture of the organic layers of a soil called "hemic histosoil covered by clay alluvial deposits" (Mchergui et al., 2014) that was present before the exploitation of a new gravel pit. This topsoil was collected in wetlands located less than 500 m away, which correspond to the desired grassland. The potential C and N mineralization of the peat was estimated in aerobic conditions and respectively ranged between 98 and 348 µg d^{-1} g^{-1} and between 4 to 8.1 µg d^{-1} g^{-1} of dry soil.

Vegetation was absent in this site during the first months of the experimentation. Earthworm density observed on another gravel-pit refilled with a comparable process (but without topsoil) was very low (0.27 ± 0.49 ind m^{-2}) after 3 years of monitoring (Grand Port Maritime de Rouen (GPMR), 2013) in comparison to nearby permanent wet grasslands (1082 ± 363 ind m^{-2}). This allowed us to state that earthworms were almost absent from our site just after the refilling

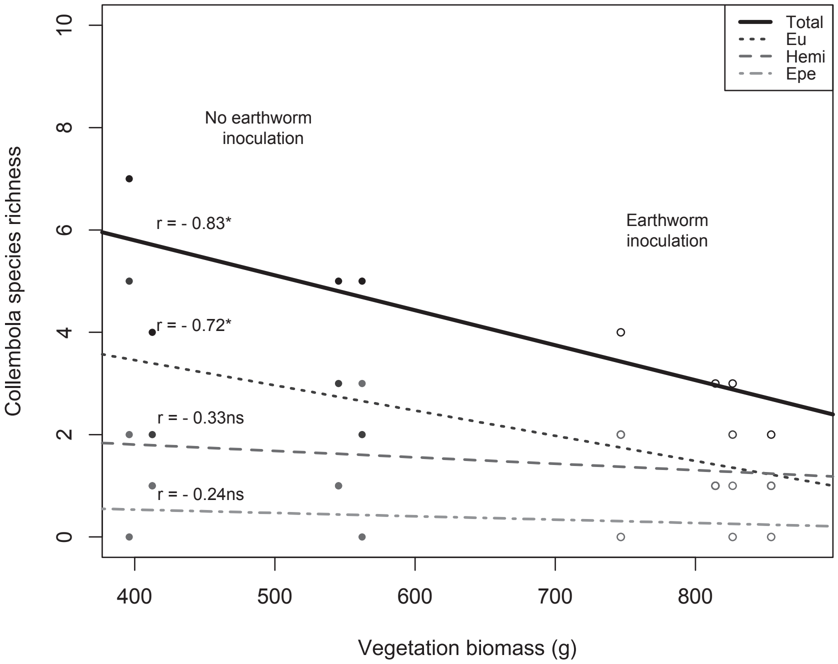


Fig. 1. Impact of plant biomass on total Collembola species richness (Total), and species richness of Euedaphic (Eu), Hemiedaphic (Hemi) and Epedaphic species (Epe) in plots inoculated with earthworms (white points) and plots not inoculated with earthworms (black points). Correlation coefficients (r) and level of significance ($\alpha = 0.05$) are indicated for each relation: *$p < 0.05$, ns: non-significant.

process.

2.2. Earthworms inoculation

In May 2012, two treatments were established corresponding to the inoculation of earthworms (EW) or to the control (C). Each treatment was replicated 10 times in PVC frames (length x width x height = 1 m x 1 m x 0.60 m). The frames were forced into the soil at a depth of 0.45 m (Appendix A) as a compromise to limit the up and down dispersion of earthworms, to limit shading effects of PVC on plant communtities, and to enable the quadrat set-up (see also Eisenhauer et al., 2009). Soil disturbance could not be avoided and was mostly located outside quadrats. At least 2 m separated each quadrat. In September 2012, at the starting time of the experiment (T0), EW quadrats were inoculated with 100 adult individuals of *Lumbricus terrestris* L. Treatments (EW or C) were randomly assigned. Earthworms were purchased at a local fishing bait store.

This species was chosen firstly because this is an anecic earthworms feeding on litter and creating deep burrows in the soil, thus causing mixing of soil layers. Secondly, this species is easily purchasable for restoration purposes. Finally, this anecic species was recorded on adjacent wet grasslands with similar mesic histosol (alkaline peat). We also carefully read the recommendation of (Butt, 2008) that constitute one of the few available papers on species choice recommendation in restoration ecology. The main concern was the low survival of *L. terrestris* recorded in several studies, which encouraged us to specifically increase the number of earthworms to inoculate. Thus, prior to our experiment, the survival of *L. terrestris* was tested in five mesocosms (Ø8 cm x 10 cm long PVC tubes) filled with the peat and without any addition of litter. In each mesocosms (12 °C, darkness), five individuals of *L. terrestris* were inoculated. After 4 weeks, all individuals were recovered (100%).

The number of inoculated earthworms in comparative field trials is highly variable (Butt, 1999) and there is no clear recommendation about the density to inoculate. For example, densities of inoculated earthworms in natural grasslands varied from 22 ind m^{-2} in Wales to 200 ind m^{-2} in Romania (Lee, 1985), but these studies did not concern peat soils. We decided to inoculate 100 mature earthworms per m^2 (about 420 g of fresh weight) as a compromise to simulate an event of colonization, to prevent mortality following introduction and to keep a realistic achievement for ecosystem management on the industrial site.

2.3. Field sampling

After one year (T1), in September 2013, plant species richness and total cover were recorded within each quadrat. We also estimated the percentage cover of each plant species present. Then, each plant species was assigned to one of the following functional types: grasses or forbs. Leguminous species (only two species recorded) were included in the ‘forb’ functional type for further analyses. To estimate the cover proportion of grasses over forbs, we calculated the relative percent cover of each species per quadrat and the cumulative sum per plant functional type. These relative covers were used to assess a grass to forb ratio. Lastly, total plant biomasses were randomly sampled in five quadrats of each treatment, oven-dried (5 days, 60 °C) and weighed. At the beginning of the experimentation, plant cover was around zero and consequently, biomasses gave estimation of plant productivity.

Within each quadrat inoculated or not, surface soil (10 cm depth) was sampled for chemical analyses and Collembola were sampled with a steel cylinder (Ø 5 cm, 10 cm depth). Each Collembola sample (n = 20) was individually placed into a plastic container, transported to the laboratory in cool boxes and stored for at most 24 h at 4 °C before further processing. Earthworms at the end of the experiment were harvested in the same quadrats used for total plant biomasses (n = 5). They were collected by hand-sorting soil monoliths of 50 cm × 100 cm × 50 cm, then counted and identified (Bouché, 1972).

2.4. Laboratory analyses

Soil moisture was determined after oven drying (105 °C, 24 h). NH_4^+ and NO_3^- were extracted with a 0,2 M K_2SO_4 solution (100 mL of solution and 20 g of dry soil) and measured by colorimetry with an AA3 auto-analyzer (BRAN + LUEBBE, Norderstedt, Germany). Total C and N (C and N) concentrations were measured with a CHN pyrolysis micro-analyzer (NF ISO 10,694 and NF ISO 13878, ThermoScientific, France). Due to high levels of organic matter, total C was considered to be mostly composed of organic C. pH was measured with combined ThermoScientific glass electrodes (1:5 ratio, ISO 10390).

Collembola were extracted by the dry-funnel method (Tullgren, 1918) and stored in 70% ethyl alcohol. Identification to species level followed several keys (Gisin, 1960; Hopkin, 2007). Finally, all species were allocated to one of three different life forms, i.e. epedaphic, hemiedaphic, euedaphic (Gisin, 1943). These life forms are closely related to dispersal ability and various functional attributes such as reproduction, mobility, and metabolic activity (Petersen, 2002).

2.5. Data analyses

Statistical analyses were achieved with the R-software (R Development Core Team, 2008). The ‘vegan’ package was used to calculate plant and Collembola species richness and evenness. Univariate analyses were performed to detect significant effects of earthworm inoculation on soil, plant and Collembola variables. Normality and homoscedasticity of data were tested (Shapiro-Wilk and Levene, $\alpha = 0.05$) to decide whether parametric or non-parametric tests had to be used using respectively Student's t tests or Kruskal-Wallis. For counted data (abundance and species richness of Collembola), we used a generalized linear model assuming a Poisson error structure. We assessed relationships between total plant cover, grass cover, forb cover or plant biomass and the Collembola species richness or evenness by calculating Pearson correlation coefficients.

To estimate direct and plant-mediated indirect effects of earthworm inoculation on total and euedaphic Collembola species richness, we used path analysis with the ‘lavaan’ R package. Since earthworm inoculation had no significant impact on soil properties, the latter were not considered in our models. The earthworm inoculation treatment was converted to 0 (C) and 1 (EW) and was treated as a binary exogenous variable. Binary exogenous variables are handled by the ‘lavaan’ package. We fitted the model by testing for the overall goodness of fit using the maximum likelihood (ML) estimation procedure (Appendix B). Adequate model fits are indicated by a non-significant axitest ($p > 0.05$), low Akaike Information Criterion (AIC) and low Root Mean Square Error of Approximation (RMSEA) (Grace, 2006). Results were interpreted by using standardized path coefficients (SPC) of the model and P values. Path coefficients are analogous to partial correlation coefficients. They describe the direction and the strength of a relationship between two variables.

3. Results

3.1. Earthworms communities

After one year of experiment, *L. terrestris* density dropped by 85% in our inoculated plots and only one individual of *L. terrestris* was recorded in the five control plots. No difference in earthworm density between the two treatments was recorded for earthworms that might have naturally colonized the site via cocoons from the spread topsoil (excluding *L. terrestris* individuals; $F = 3{,}60$ $p = 0.057$). We found 14.4 mature earthworms per m^{-2} with a total species richness of 3 (without counting *L. terrestris*). In addition to *L. terrestris*, six other earthworm species were recorded, among which two epigeic species (*Lumbricus rubellus, Lumbricus castaneus*) and four endogeic species (*Apporrectodea caliginosa, Octolasion lacteum, Allolobophora chlorotica, Apporectodea*

Table 1
Impact of earthworm inoculation (EW) on plant communities. Mean and standard deviation are given (n = 20, except for plant biomass n = 10). Student *t*-tests or Kruskal tests were used. Bold values show significance at *p* = 0.05.

Plant communities	Control	EW	t/X^2	*p value*
Plant biomass (g m^{-2})	479 ± 87	824 ± 50	**7.07**[a]	**0.001**
Plant cover (%)				
Total	72 ± 17	88 ± 15	**4.24**[b]	**0.039**
Grasses	32 ± 14	45 ± 10	**−2.78**[a]	**0.012**
Forbs	68 ± 14	55 ± 10	0.87[a]	0.398
Forbs/Grasses ratio	2.13 ± 0.96	1.32 ± 0.52	2.15[a]	**0.047**
Species richness (nb m^{-2})				
Total	13.4 ± 2.6	11.9 ± 3.2	−1.13[a]	0.273
Grasses	3.3 ± 0.9	3.6 ± 1.3	−1.96[a]	0.066
Forbs	10.1 ± 2.3	8.3 ± 2.4	0.13[b]	0.723
Diversity index				
Shannon index	2.25 ± 0.26	2.20 ± 0.32	0.41[b]	0.520
Eveness	0.87 ± 0.06	0.89 ± 0.05	0.87[a]	0.394

[a] Student t-test.
[b] Kruskal test.

Table 2
Impact of earthworm inoculation (EW) on Collembola communities. Mean and standard deviation are given (n = 20). Generalized linear model with poisson distribution were used for the statistical analysis of abundance and species richness. Student *t*-tests were used for diversity indexes. Bold values show significance at *p* = 0.05.

Collembola communities	Control	EW	t value	*p value*
Abundance (ind m^{-2})				
Total	10426 ± 4892	8840 ± 4831	−0.69	0.499
Epedaphic	737 ± 726	283 ± 517	−1.52	0.146
Hemiedaphic	5270 ± 3038	2720 ± 883	**−2.42**	**0.028**
Euedaphic	4420 ± 1803	5836 ± 4402	0.38	0.384
Species Richness				
Total	6.22 ± 1,79	3.66 ± 1,22	**−3.54**	**0.003**
Epedaphic	1.00 ± 1.00	0.44 ± 0.72	−1.35	0.196
Hemiedaphic	2.22 ± 0.83	1.22 ± 0.44	**−3.18**	**0.006**
Euedaphic	3.00 ± 1.11	2.00 ± 1.00	−2.00	0.062
Simpson index	0.74 ± 0.04	0.68 ± 0.08	**−2.13**	**0.048**
Shannon index	1.63 ± 0.18	1.31 ± 0.23	**−3.23**	**0.005**
Eveness	0.73 ± 0.11	0.82 ± 0.08	1.94	0.071

rosea). Earthworm communities (without counting *L. terrestris*) were quite similar between the two treatments (see Appendix C), but we noted that *O. lacteum* was slightly more abundant in EW than in C plots (respectively 4.4 ± 2.6 and 0.8 ± 1.8 ind m^{-2}).

3.2. Effect of earthworm inoculation on chemical properties

Earthworm inoculation (EW) had no effect on any of the measured soil properties compared to the control (C) one year after the inoculation (Appendix D). In our soils, pH was slightly alkaline (pH = 7.6), Ammonium and nitrate were respectively around 6.8 and 5.5 g kg^{-1}, N_{tot} was 9.3 g kg^{-1}, and C/N was 13.8.

3.3. Effect of earthworm inoculation on plant communities

The presence of earthworms led to a significant increase of plant cover (+21%) and plant biomass (+58%) after one year of the experiment (Table 1). This positive effect strongly depended on plant functional type: only grass species had a significant increase of plant cover (+69%, Table 1). As a consequence, we observed a lower proportion of forbs compared to grasses (forbs/grasses ratio) in EW than C (Table 1). No changes in plant species richness and diversity indexes (Eveness or Shannon) were observed between treatments (Table 1).

3.4. Effect of earthworm inoculation on Collembola communities

Total species richness (SR) of Collembola was markedly reduced (−41.1%) following earthworm inoculation (t = −3.53, p = 0.003; Table 2). More precisely, SR of the hemiedaphic life form was constantly lower in EW compared to C (Table 2), and the same effect was seen for the Shannon and Simpson indices. Only evenness did not differ between treatments (Table 2). Total abundance of Collembola did not differ between C and EW (Table 2). Nevertheless, when considering collembolan life forms, abundance of hemiedaphic was reduced by 48.3% in EW (Table 2).

3.5. Links between earthworms, plants and Collembola

Collembola evenness was not impacted by earthworm treatment but was significantly positively correlated with plant biomass (R = 0.77), and to a lesser extent, positively correlated with the grass cover and plant cover (R = 0.49 and R = 0.36) and negatively correlated with the forb cover (R = −0.45).

Model selection for the path analysis (Appendix B) showed that the negative impact of earthworms on Collembola was not direct but mediated through the positive effect of earthworm inoculation on plant biomass (path analysis; SPC = 0.94, $p < 0.001$), and a negative impact of plant biomass on Collembola (path analysis; SPC = −0.83, $p < 0.001$; Appendix E). Results of path analysis on the impact of earthworm inoculation on euedaphic Collembola population (Appendix B) showed a similar indirect effect (path analysis; SPC = −0.68, *p* = 0.005; X^2 (1) = 0.46, *p* = 0.5; RMSEA = 0.0, *p* = 0.5).

4. Discussion

4.1. Earthworms inoculation and natural earthworm colonization

Although earthworm inoculation largely doubled plant productivity, the density of retrieved *L. terrestris* dropped by 85% in our inoculated plots after one year of experiment. This strong collapse in earthworm number might be due to particularly high temperature events recorded in August just before the sampling of earthworms in September. Indeed, until mid-July we noted high earthworm activities (e.g cast production at the soil surface) in earthworm plots (EW) suggesting that earthworms were still alive. Earthworm death in August and the subsequent mineralization of their biomass probably only marginally benefited plant growth. Indeed, at this time of year (July) plants have already reached their maximum biomasses. The positive effect of earthworms on plant biomass was thus very likely due to earthworm activities before their death. Curry and Schmidt (2006) reported that topsoil-dwelling species established better than deep-burrowing species (such as *L. terrestris*) in a reclaimed cutaway peat soils in Ireland. Another explanation to our low recovery of *L. terrestris* could be that our anecic individuals were still aestivating at the time of sampling and could not be caught by digging at a depth of 0.50 m. They might also have escaped drought by burrowing deeper than 0.5 m. For technical reasons it was not possible to sample earthworms deeper or to use deeper frames for earthworm inoculation. Hence it cannot be fully excluded that more earthworms survived.

Natural earthworm colonization of epigeic and endogeic species from the spread topsoil was observed. One year after reconstitution of the experimental site, we found about 14.4 ind m^{-2} with a total species richness of 3 (without counting *L. terrestris*). Nevertheless, this density was still low compared to nearby permanent wet grasslands with 1082 ± 363 ind m^{-2}.

4.2. Impact of earthworm inoculation on plants

The inoculation of *L. terrestris* had a very strong and positive effect on plant productivity. Both plant cover and biomass nearly increased by 20% and 60% respectively (Table 1). Curry and Boyle (1987) also reported an increase of plant yield (25% the second year, and 49% the third year) when they inoculated earthworms in a restoration experiment following an industrial peat extraction. Strangely, soil chemical measurements did not reflect any increase in soil fertility. An explanation would be that plants quickly absorbed soil nutrients mineralized by inoculated earthworms (through the consumption of litter) so that soil content in mineral nutrients did not increase. Earthworm impact on plants is generally positive but depends on plant functional type (Scheu, 2003; van Groenigen et al., 2014). Grass species were more favored by the presence of earthworms than forbs. Other studies reported a more pronounced and positive effect of earthworms on graminoid species (i.e. grasses) over other functional types (Wurst et al., 2005; Eisenhauer and Scheu, 2008; Laossi et al., 2009). Generally it is suggested that grasses benefit more from a higher availability of N in soil due to earthworm activities, maybe because of their high root densities, which would increase their competitive ability against other plant types such as legumes. Additionally, earthworms might directly impact plant communities through the ingestion and/or digestion of seeds (Milcu et al., 2006a; Forey et al., 2011). The passage of seeds through the earthworm gut potentially damages them, altering their germination and seedling establishment depending on seed traits (Clause et al., 2011). *L. terrestris* is recognized to have a very high seed ingestion rate (86%) compared to other earthworm species such as *Allolobophora chlorotica* (15%) and could act as an important selection pressure for plants by selecting small or large oil-rich seeds, such as forb and legume seeds (Clause et al., 2017) and digesting them.

Despite the overall positive effect of earthworms on plant productivity and differences in the responses of plant functional groups to earthworms, earthworm inoculation had no effect on plant diversity indices. Nevertheless, we can hypothesize that the strong positive effect of earthworms on graminoids and on plant productivity could favor grasses and lead to significant changes in plant diversity indices between treatments within a few years.

4.3. *Impact of earthworm inoculation on Collembola*

Earthworm inoculation had a strong deleterious impact on soil Collembola assemblages and mainly on hemiedaphic species (species richness and density). (Milcu et al., 2006b) also found that *L. terrestris* reduced the total density of Collembola (20%) and particularly the density of *Folsomia candida* (46%), a hemiedaphic species. Several descriptive or experimental studies have already suggested the detrimental effect of earthworms on microarthropods, including Collembola (for a review, see Eisenhauer, 2010). Several mechanisms have been suggested to explain these negative impacts. The burrowing activity of earthworms might trigger a physical stress factor for Collembola populations (Maraun et al., 2003). By feeding and reducing the amount of litter, earthworms might also compete with CollemboIa for resources or habitat. Our results on Collembola life forms did not support this hypothesis as Collembola living at the soil surface (epedaphic) were not affected by the earthworm inoculation. Lastly, indirect effect of earthworms through their impact on the structure and composition of microbial community structure and composition may negatively impact fungivorous Collembola.

4.4. *Importance of plant community for Collembola assemblages*

Collembola community characteristics also appeared to be related to plant community structure. Indeed, Collembola evenness was not impacted by earthworm treatment but was significantly positively correlated with plant biomass, grass cover and plant cover and negatively correlated with the forb cover. These results support the plant abundance hypothesis stating that the overall plant biomass affects either negatively or positively Collembola communities (e.g. Bokhorst et al., 2012). Relationships between Collembola evenness and plant also revealed that the response of Collembola communities to plant cover depends on the identity of the plant functional type (Salmon, 2004; Sabais et al., 2011). These results are in line with the plant mass-ratio hypothesis according to which plant community identity and composition would drive collembolan assemblages by controlling dominant plants traits (e.g. Wardle et al., 2005). For example, grass cover but not for cover had a beneficial effect on evenness. The well-developed root system of grass species might contribute to the build-up of plant root-associated microorganisms such as fungi and bacteria (Carpenter-Boggs et al., 2003; Eisenhauer et al., 2010a). Therefore, the rhizosphere of grasses likely improves food availability in comparison to forb or leguminous species that have less developed root systems, and thereby favor diverse food resources and diverse Collembola species (i.e. high Collembola evenness).

To disentangle the direct effect of earthworms on Collembola richness from their indirect effect via their impact on plants, we used a path analysis. This analysis suggested that earthworms had an indirect effect on Collembola through a modification of vegetation structure (plant biomass and cover), which itself directly impacted Collembola richness and evenness. Recently, (Abgrall et al., 2017) suggested that plant functional traits play a key role in structuring Collembola assemblages and are more influential for Collembola communities than abiotic factors. However, we cannot exclude that soil parameters such as physical structure were not partially influenced by earthworm inoculation and played a role in their impact on Collembola.

5. Conclusion

Deliberate or accidental (e.g. biological invasion) introductions of new species into ecosystems provide rare opportunities to understand their importance and their impact on both local biota and ecosystem processes. Here, we demonstrated that the inoculation of earthworms in a restoration context might provide very fruitful opportunities to study their influences on plants, belowground fauna and also consequences for ecosystem services such as primary productivity. In this *in situ* experiment, earthworms quickly doubled plant yield one year after inoculation but had no impact on plant diversity and a negative impact on collembolan diversity. Longer monitoring is necessary to confirm these first results. Nevertheless, this study demonstrates that the introduction of a single soil species might impact both the above and belowground compartments and their interactions in the very short term (1 year). This study also stresses the strong influence of feedbacks between plant community characteristics (biomass and functional type) and soil biotic assemblages. We therefore finally recommend using restoration experiments manipulating keystones species to further analyze such feedbacks.

Acknowledgments

The authors want to thank the members of the ECODIV lab for field assistance, Fabrice Bureau and Audrey Boigné for fruitful discussions, as well as Marthe Akpa-Vinceslas for coordinating soil analyses. We thank the editor and the two anonymous reviewers for their comments on this paper. Lastly, we would like to thank the “Région Haute-Normandie” and the SCALE Network for providing funds for this research. We are grateful to CBN for the use of their site.

Appendix A

Study site with 20 quadrats in a peat-filled pit in Yville-sur-Seine, Upper-Normandy, France. 10 quadrats (frames) were randomly inoculated with 100 earthworms (EW) per m^2 and 10 quadrats were used as controls (Ctrl).

Appendix B

Results of path analysis on the impact of earthworm inoculation and plant biomass on Collembola richness. SPC: standardized path coefficient. R^2 : proportion of variance explained by the model. RMSEA: Root Mean Square Error of Approximation. SRMR: Standardized Root Mean Square Residual. See also figure 2 for a visual representation of relationships between variables.

	Total Collembola			Epedaphic Collembola			Hemiedaphic Collembola			Euedaphic Collembola		
	SPC	*p*	R^2	SPC	*p*	R^2	SPC	*p*	R^2	SPC	*p*	R^2
Plant biomass ~ Earthworm inoculation *(direct)*	0.94	< 0.001	0.88	0.94	< 0.001	0.88	0.94	< 0.001	0.88	0.94	< 0.001	0.88
Collembola richness ~ plant biomass *(direct)*	−0.83	< 0.001	0.69	−0.24	0.489	0.06	−0.31	0.352	0.10	−0.72	0.003	0.53
Collembola richness ~ Earthworm inoculation *(indirect)*	**−0.78**	**< 0.001**	–	−0.22	0.491	–	−0.29	0.355	–	**−0.68**	**0.005**	–
X^2 ; df ; *p*	0.005; 1; 0.94			0.090 ; 1 ; 0.76			0.277 ; 1 ; 0.60			0.46; 1; 0.50		
RMSEA ; p_{RMSEA}	0.00; 0.94			0.00 ; 0.77			0.00 ; 0.60			0.00; 0.50		
SRMR	0.002			0.012			0.020			0.018		
AIC	131.1			122.48			128.93			131.50		

Appendix C

Means and standard deviations (m ± SD) of earthworm species abundance and richness (ind m^{-2}) at the end of the experiment ($n = 5$). Juvenile earthworms (no clitellum) could not be identified, but their total abundance are given. Kruskal tests were used. Bold values show significance at $p = 0.05$

At the beginning of the experimentation, i.e. before *L. terrestris* inoculation, earthworm density and species richness per m^2 were null in the experimental site.

	Earthworm communities (ind m$-^2$)			
Earthworm species:	Control treatment	Earthworm treatment	X^2	*p value*
Allolobophora chlorotica	1.6 ± 1.7	2 ± 1.4	0.21	0.650
Apporrectodea caliginosa	1.6 ± 1.7	2.8 ± 5.2	0.01	0.734
Apporectodea rosea	0.8 ± 1.1	2.8 ± 3.3	1.03	0.309
Lumbricus castaneus	3.6 ± 3.8	2.4 ± 4.3	0.97	0.324
Lumbricus rubellus	0.6 ± 3.3	1.6 ± 2.6	0.45	0.502
Lumbricus terrestris	0.4 ± 0.9	16.4 ± 6.1	**7.25**	**0.007**
Octolasion lacteum	0.8 ± 1.8	4.4 ± 2.6	**4.70**	**0.030**
Total density				
without *L. terrestris*	11.4 ± 4.8	17.2 ± 5.2	3.60	0.057
with *L. terrestris*	11.6 ± 4.8	33.6 ± 8.2	**6.98**	**0.008**
with *L. terrestris* and juveniles	25.2 ± 4.15	55.2 ± 8.4	**6.86**	**0.008**
Total species Richness	3.4 ± 1.3	4.6 ± 0.9	2.79	0.094

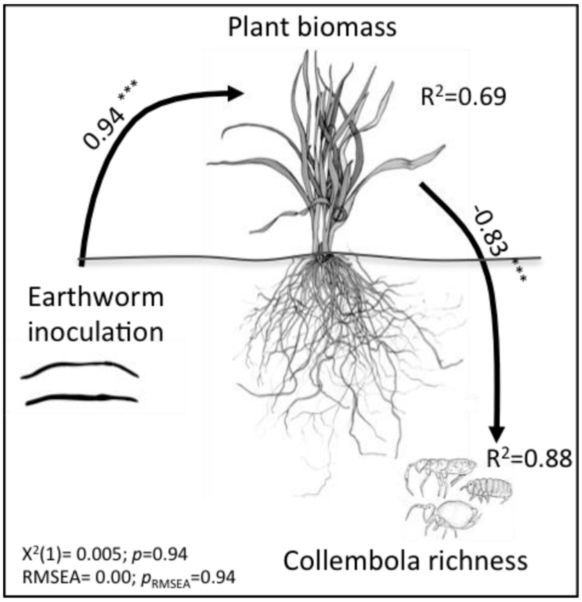
Appendix D

Impact of earthworm inoculation on soil properties. Mean and standard deviation are given (n = 20). Student *t*-tests or Kruskal tests were used. Bold values show significance at $p = 0.05$.

Soil variable	Control	EW	t/X^2	*p value*
Soil moisture	(%)	65 ± 11	62 ± 6	0.21^b
pH		7.6 ± 0.1	7.6 ± 0.1	0.03^a
$NH_4{}^+$	(g kg^{-1})	7.8 ± 2.2	5.9 ± 3.0	0.20^b
$NO_3{}^-$	(g kg^{-1})	7.2 ± 2.9	3.9 ± 1.8	0.14^b
Ntot	(g kg^{-1})	9.5 ± 1.4	9.2 ± 1.2	-0.47^a
Ctot	(g kg^{-1})	136 ± 20	132 ± 17	-0.50^a
Corg	(g kg^{-1})	131 ± 20	127 ± 17	-0.50^a
C/N		13.8 ± 0.2	13.8 ± 0.2	-0.30^a

Appendix E

Indirect impact of earthworm inoculation on Collembola total species richness via its impact on plant biomass obtained from the path analysis (see Appendix B). Standardized Path Coefficient (SPC) between variables and levels of significance ($^{***}p < 0.001$) are indicated. R^2 is the proportion of variance explained. RMSEA: Root Mean Square Error of Approximation.



References

Abgrall, C., Chauvat, M., Langlois, E., Hedde, M., Mouillot, D., Salmon, S., Winck, B., Forey, E., 2017. Shifts and linkages of functional diversity between above and belowground compartments along a flooding gradient. Funct. Ecol. 31, 350–360.

Balvanera, P., Pfisterer, A.B., Buchmann, N., He, J.-S., Nakashizuka, T., Raffaelli, D., Schmid, B., 2006. Quantifying the evidence for biodiversity effects on ecosystem functioning and services. Ecol. Lett. 9, 1146–1156.

Bardgett, R.D., Wardle, D.A., 2003. Herbivore-mediated linkages between aboveground and belowground communities. Ecology 84, 2258–2268.

Blouin, M., Hodson, M.E., Delgado, E.A., Baker, G., Brussaard, L., Butt, K.R., Dai, J., Dendooven, L., Peres, G., Tondoh, J.E., Cluzeau, D., Brun, J.J., 2013. A review of earthworm impact on soil function and ecosystem services. Eur. J. Soil Sci. 64, 161–182.

Bouché, M.B., 1972. Lombriciens de France: Écologie et Systématique. INRA Ann. Zool. Ecol. Anim. Publ. France 671 pp.

Bokhorst, S., Phoenix, G.K., Bjerke, J.W., Callaghan, T.V., Huyer-Brugman, F., Berg, M.P., 2012. Extreme winter warming events more negatively impact small rather than large soil fauna: shift in community composition explained by traits not taxa. Global Change Biol. 18, 1152–1162.

Boyer, S., Wratten, S.D., 2010. The potential of earthworms to restore ecosystem services after opencast mining - a review. Basic Appl. Ecol. 11, 196–203.

Butt, K.R., 1999. Inoculation of earthworms into reclaimed soils: the UK experience. Land Degrad. Dev. 10, 565–575.

Butt, K.R., 2008. Earthworms in soil restoration: lessons learned from United Kingdom case studies of land reclamation. Restor. Ecol. 16, 637–641.

Carpenter-Boggs, L., Stahl, P.D., Lindstrom, M.J., Schumacher, T.E., 2003. Soil microbial properties under permanent grass, conventional tillage, and no-till management in South Dakota. Soil. Tillage Res. 71, 15–23.

Clause, J., Margerie, P., Langlois, E., Decaens, T., Forey, E., 2011. Fat but slim: criteria of seed attractiveness for earthworms. Pedobiologia 54, S159–S165.

Clause, J., Forey, E., Eisenhauer, N., Seal, C.E., Soudey, A., Colville, L., Barot, S., 2017. Seed selection by earthworms: chemical seed properties matter more than morphological traits. Plant. Soil. 413, 97–110.

Curry, J.P., Boyle, K.E., 1987. Growth rates, establishment, and effects on herbage yield of introduced earthworms in grassland on reclaimed cutover peat. Biol. Fertil. Soils 3, 95–98.

Curry, J.P., Schmidt, O., 2006. Long-term establishment of earthworm populations in grassland on reclaimed industrial cutaway Peatland in Ireland. Finnish Peatl. Soc. 57, 65–70.

De Deyn, G.B., Raaijmakers, C.E., Zoomer, H.R., Berg, M.P., de Ruiter, P.C., Verhoef, H.A., Bezemer, T.M., van der Putten, W.H., 2003. Soil invertebrate fauna enhances grassland succession and diversity. Nature 422, 711–713.

Eisenhauer, N., 2010. The action of an animal ecosystem engineer: identification of the main mechanisms of earthworm impacts on soil microarthropods. Pedobiologia 53, 343–352.

Eisenhauer, N., Milcu, A., Nitschke, N., Sabais, A.C.W., Scherber, C., Scheu, S., 2009. Earthworm and belowground competition effects on plant productivity in a plant diversity gradient. Oecologia 161, 291–301.

Eisenhauer, N., Scheu, S., 2008. Earthworms as drivers of the competition between grasses and legumes. Soil Biol. Biochem. 40, 2650–2659.

Eisenhauer, N., Sabais, A.C.W., Schonert, F., Scheu, S., 2010a. Soil arthropods beneficially rather than detrimentally impact plant performance in experimental grassland systems of different diversity. Soil Biol. Biochem. 42, 1418–1424.

Eisenhauer, N., Bessler, H., Engels, C., Gleixner, G., Habekost, M., Milcu, A., Partsch, S., Sabais, A.C.W., Scherber, C., Steinbeiss, S., Weigelt, A., Weisser, W., Scheu, S., 2010b. Plant diversity effects on soil microorganisms support the singular hypothesis. Ecology 91, 485–496.

Forey, E., Barot, S., Decaens, T., Langlois, E., Laossi, K.R., Margerie, P., Scheu, S., Eisenhauer, N., 2011. Importance of earthworm-seed interactions for the composition and structure of plant communities: a review. Acta Oecol. 37, 594–603.

Forey, E., Coulibaly, S.F.M., Chauvat, M., 2015. Flowering phenology of a herbaceous species (*poa annua*) is regulated by soil Collembola. Soil Biol. Biochem. 90, 30–33.

Fraser, P.M., Beare, M.H., Butler, R.C., Harrison-Kirk, T., Piercy, J.E., 2003. Interactions between earthworms (*Aporrectodea caliginosa*), plants and crop residues for restoring properties of a degraded arable soil. Pedobiologia 47, 870–876.

Gisin, H., 1943. Oekologie und Lebensgemeinschaften der Collembolen im Schweizerischen Exkursionsgebiet Basels. Rev. Suisse Zool. 50, 131–224.

Gisin, H., 1960. Collembolen fauna Europas. Mus. D'Histoire Naturelle, Genf.

Grace, J.B., 2006. Structural Equation Modeling and Natural Systems. Cambridge University Press, Cambridge, United Kingdom.

Grand Port Maritime de Rouen (GPMR), 2013. Le Remblaiement Par Des Sédiments de Dragage Et la Restauration écologique de la Ballastière D'Yville Sur Seine (76) – Bilan de L'Expérimentation.

Grime, J.P., 1998. Benefits of plant diversity to ecosystems: immediate filter and founder effects. J. Ecol. 86, 902–910.

Henneron, L., Aubert, M., Archaux, F., Bureau, F., Dumas, Y., Ningre, F., Richter, C., Balandier, P., Chauvat, M., 2017. Forest plant community as a driver of soil biodiversity: experimental evidence from collembolan assemblages through large-scale and long-term removal of oak canopy trees Quercus *petraea*. Oikos 126, 420–434.

Hopkin, S.P., 2007. A Key to the Collembola (Springtails) of Britain and Ireland.

Jones, C.G., Lawton, J.H., Shachak, M., 1994. Organisms as ecosystem engineers. Oikos. 69, 373–386.

Jouquet, P., Blanchart, E., Capowiez, Y., 2014. Utilization of earthworms and termites for the restoration of ecosystem functioning. Appl. Soil Ecol. 73, 34–40.

Kardol, P., Wardle, D.A., 2010. How understanding aboveground-belowground linkages can assist restoration ecology. Trends Ecol. Evol. 25, 670–679.

Laossi, K.R., Noguera, D.C., Bartolome-Lasa, A., Mathieu, J., Blouin, M., Barot, S., 2009. Effects of an endogeic and an anecic earthworm on the competition between four annual plants and their relative fecundity. Soil Biol. Biochem. 41, 1668–1673.

Lee, K.E., 1985. Earthworms: Their Ecology and Relationships With Soils and Land Use. Academic Press, Sydney.

Marashi, A.R.A., Scullion, J., 2003. Earthworm casts form stable aggregates in physically degraded soils. Biol. Fertil. Soils 37, 375–380.

Maraun, M., Salamon, J.-A., Schneider, K., Schaefer, M., Scheu, S., 2003. Oribatid mite and collembolan diversity, density and community structure in a moder beech forest (*Fagus sylvatica*): effects of mechanical perturbations. Soil Biol. Biochem. 35, 1387–1394.

Mchergui, C., Aubert, M., Buatois, B., Akpa-Vinceslas, M., Langlois, E., Bertolone, C., Lafite, R., Samson, S., Bureau, F., 2014. Use of dredged sediments for soil creation in the Seine estuary (France): importance of a soil functioning survey to assess the success of wetland restoration in floodplains. Ecol. Eng. 71, 628–638.

Milcu, A., Schumacher, J., Scheu, S., 2006a. Earthworms (*Lumbricus terrestris*) affect plant seedling recruitment and microhabitat heterogeneity. Funct. Ecol. 20, 261–268.

Milcu, A., Partsch, S., Langel, R., Scheu, S., 2006b. The response of decomposers (earthworms, springtails and microorganisms) to variations in species and functional groupdiversity of plants. Oikos 122, 513–524.

Mudrak, O., Uteseny, K., Frouz, J., 2012. Earthworms drive succession of both plant and Collembola communities in post-mining sites. Appl. Soil. Ecol. 62, 170–177.

Partsch, S., Milcu, A., Scheu, S., 2006. Decomposers (Lumbricidae, Collembola) affect plant performance in model grasslands of different diversity. Ecology 87, 2548–2558.

Petersen, H., 2002. General aspects of collembolan ecology at the turn of the millennium. Pedobiologia 46, 246–260.

R Development Core Team, 2008. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org.

Roubickova, A., Mudrak, O., Frouz, J., 2009. Effect of earthworm on growth of late succession plant species in postmining sites under laboratory and field conditions. Biol. Fertil. Soils 45, 769–774.

Sabais, A.C.W., Scheu, S., Eisenhauer, N., 2011. Plant species richness drives the density and diversity of Collembola in temperate grassland. Acta Oecol. 37, 195–202.

Salmon, S., 2004. The impact of earthworms on the abundance of Collembola: improvement of food resources or of habitat? Biol. Fertil. Soils 40, 323–333.

Scheu, S., 2003. Effects of earthworms on plant growth: patterns and perspectives. Pedobiologia. 47, 846–856.

Scullion, J., Malik, A., 2000. Earthworm activity affecting organic matter, aggregation and microbial activity in soils restored after opencast mining for coal. Soil Biol. Biochem. 32, 119–126.

Sizmur, T., Palumbo-Roe, B., Hodson, M.E., 2011. Impact of earthworms on trace element solubility in contaminated mine soils amended with green waste compost. Environ. Pollut. 159, 1852–1860.

Snyder, B.A., Hendrix, P.F., 2008. Current and potential roles of soil macroinvertebrates (earthworms, Millipedes, and Isopods) in. Ecol. Restor. Restor. Ecol. 16, 629–636.

Tullgren, A., 1918. Ein sehr einfacher Ausleseapparat fur terricole Tierformen. Z. Angew. Entomol. 4, 149–150.

van Groenigen, J.W., Lubbers, I.M., Vos, H.M.J., Brown, G.G., De Deyn, G.B., van Groenigen, K.J., 2014. Earthworms increase plant production: a meta-analysis. Sci. Rep. 4.

Wagg, C., Bendera, S.F., F, Widmerc, van der Heijdena, M.G.A., 2014. Soil biodiversity and soil community composition determine ecosystem multifunctionality. PNAS 110, 5266–5270.

Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setala, H., van der Putten, W.H., Wall, D.H., 2004. Ecological linkages between aboveground and belowground biota. Scienc. 304, 1629–1633.

Wardle, D.A., et al., 2005. How plant communities influence decomposer communities. In: Bardgett, R.D. (Ed.), Biological Diversity and Function of Soils. Cambridge Univ. Press, pp. 119–138.

Wurst, S., Langel, R., Scheu, S., 2005. Do endogeic earthworms change plant competition? A microcosm study. Plant Soil 271, 123–130.