Earthworms promote greater richness and abundance in the emergence of plant species across a grassland-forest ecotone

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Abstract

Aims
Chalk grasslands are subject to vegetation dynamics that range from species-rich open grasslands to tall and encroached grasslands, and woods and forests. In grasslands, earthworms impact plant communities and ecosystem functioning through the modification of soil physical, chemical and microbiological properties, but also through their selective ingestion and vertical transportation of seeds from the soil seed bank. Laboratory experiments showed that seed–earthworm interactions are species specific, but little is known on the impact of seed–earthworm interactions in the field. The overall aim of this study was to better understand seed–earthworm interactions and their impact on the plant community. First we analyzed the composition of seedlings emerging from casts after earthworm ingestion. Then we compared seedling composition in casts to the plant composition of emerging seedlings from the soil and of the aboveground vegetation along four stages of the secondary succession of chalk grasslands.

Methods
Four stages of the secondary succession of a chalk grassland—from open sward to woods—were sampled in Upper Normandy, France, in February 2010. Within each successional stage (×3 replicates), we sampled the standing vegetation, soil seed bank at three soil depths (0–2, 2–5 and 5–10cm) and earthworm surface casts along transects. Soil and cast samples were sieved before samples were spread onto trays and placed into a greenhouse. Emerging seedlings were counted and identified. Effect of successional stage and origin of samples on mean and variability of abundance and species richness of seedlings emerging from casts and soil seed banks were analyzed. Plant compositions were compared between all sample types. We used generalized mixed-effect models and a distance-based redundancy multivariate analysis.

Important Findings
Seedling abundance was always higher in earthworm casts than in the soil seed bank and increased up to 5-fold, 4-fold and 3.5-fold, respectively, in the tall grassland, woods and encroached grassland compared to the soil surface layer. Species richness was also higher in earthworm casts than in the soil seed bank in all successional stages, with a 4-fold increase in the encroached grassland. The plant composition of the standing vegetation was more similar to that of seedlings from casts than to that of seedlings from the soil seed bank. Seedlings diversity emerging from casts in the tall and encroached grasslands tended toward the diversity found in woods. Our results indicate that earthworms may promote the emergence of seedlings. We also suggest that the loss of some plant species in the seed bank and the tall grass vegetation in intermediary successional stages modify the local conditions and prevent the further establishment of early-successional plant species.

Keywords: aboveground–belowground interactions, earthworm casts, seedling emergence, secondary succession, seed bank

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INTRODUCTION

Seed banks play a major role in the dynamics and composition of plant communities (Bakker et al. 1996; Bossuyt and Honnay 2008; Fenner 2000; Luzuriaga et al. 2005). They constitute reserves of non-germinated seeds in the soil or at the soil surface (Csontos 2007). The viability of these seeds depends on seed characteristics as well as on external factors such as light, moisture or temperature (Benech-Arnold et al. 2000; Thompson and Grime 1979). Transient seed banks contain seeds that are germinable for less than a year, whereas seeds from persistent seed banks remain viable for more than a year, up to decades or longer (Thompson and Grime 1979). Seed longevity in the soil is particularly dependent on their size, shape and depth (Bekker et al. 1998; Thompson et al. 1993). Small spherical seeds that are located deep in the soil tend to live longer than large seeds in the soil surface layers (Bekker et al. 1998). The capacity of seeds to remain viable in the soil in a dormant state enables them to survive extreme events such as fire or drought (Thompson 2000) and to germinate under favorable conditions for seedling establishment.

Seed survival can be impacted by their ingestion by diverse organisms. Provided that seeds are not fully digested, seed ingestion may also lead to seed dispersal (endozoochory) and seedling establishment by triggering seed germination and by reducing seed dormancy (Janzen 1969; Traveset 1998). On the contrary, seed survival can also decrease when seeds are digested or severely damaged. Apart from the observed endozoochory in primates (Norconk and Veres 2011), grazing mammals (Neto et al. 1987) and birds (Barnea et al. 1991; but see Traveset 1998 for a complete review), seed ingestion by invertebrates has also been observed (Darwin 1881; Decaëns et al. 2003; Grant 1983; Vega et al. 2011). However, the number of studies in nature is still limited. Among invertebrates, earthworms have been subjects of recent attention (Clause et al. 2011; Decaëns et al. 2003; Eisenhauer et al. 2009a, 2009b, 2010). Several studies showed the impacts of seed ingestion, digestion and egestion of earthworms on seed bank and plant communities (Eisenhauer et al. 2009b; McRill and Sagar 1973; Willems and Huijsmans 1994; see Forey et al. 2011 for a review). Seed ingestion, in association with earthworm movements, leads to the vertical transportation of seeds, i.e. their burial or surface exposure (Donath and Eckstein 2012; Willems and Huijsmans 1994; Zaller and Saxler 2007). In tropical grasslands, earthworm casts contain a higher seed density of viable seeds than the surrounding soil (Decaëns et al. 2003). Seeds surviving the digestion process are thought to benefit from a partial damage of their seed coat, which favors seed germination and seedling establishment (Ayanlaja et al. 2001; Eisenhauer et al. 2009a; McRill and Sagar 1973). Increased germination and seedling establishment might be further enhanced by cast properties. Specifically, casts tend to have a higher content in mineral nutrients and have particular physical and microbial properties (Clause et al. 2014; Jouquet et al. 2008; Shipitalo and Protz 1989). Hence, due to favorable growth conditions associated with high numbers of viable seeds, casts are potentially important regeneration niches for some plant species (see Decaëns et al. 2003; Milcu et al. 2006). Additionally, earthworms selectively ingest seeds according to their size, shape, texture or oil content (Clause et al. 2011; Eisenhauer et al. 2009a; Janzen 1969; Regnier et al. 2008; Willems and Huijsmans 1994). Some studies suggest that earthworms prefer small seeds (Clause et al. 2011; Eisenhauer et al. 2009a) while others suggest the contrary (Regnier et al. 2008), and that they prefer non-grass seeds (either non-leguminous or leguminous) to grass seeds (Zaller and Saxler 2007).

Mechanisms behind the impact of seed–earthworm interactions via seed ingestion on plant communities are still unclear and few studies have focused on them in a natural context (Decaëns et al. 2003; Eisenhauer et al. 2009b; Willems and Huijsmans 1994). The importance of these interactions still needs to be assessed. The relatively undisturbed nature of species-rich semi-natural chalk grasslands constitutes an opportunity for studying the direct relationship between earthworms, seed banks and aboveground communities via seed ingestion, egestion in casts and the impact on seedling emergence. As earthworm communities vary along the grassland succession (Decaëns et al. 1998), these earthworm–seed relationships are likely to vary along a gradient of secondary succession.

Thus, we aimed at better understanding seed–earthworm interactions and their impact on plant communities across a grassland-forest ecotone. To do so, we analyzed the composition of seedlings emerging from casts after earthworm ingestion and compared it to the plant compositions of emerging seedlings from the soil and of the aboveground vegetation, along four stages of the secondary succession of chalk grasslands.

Two questions led our study: (i) do seeds preferentially germinate from earthworm casts than from the surrounding soil? and (ii) are assemblages of species germinating from earthworm casts similar to those found in the soil seed bank and the standing vegetation along the successional gradient? Overall, we discuss the potential of earthworms as drivers of the plant community assemble.

MATERIALS AND METHODS

Study site

The study site is the natural reserve of Saint-Adrien (1° 7′ 30″ E, 49° 22′ 22″ N) located 15 km south of Rouen (Upper Normandy, France). Yearly average rainfalls and temperatures are 800 mm and 10°C, respectively. This 32 ha site is particularly well documented (Alard et al. 1998; Dutoit and Alard 1995; Dutoit et al. 2004). It is composed of a mosaic of different stages of secondary succession from open grasslands to scrub and woods. Soils are shallow rendzinas (Rendzina, Protorendzina) under grassland communities and deeper...
rendzinas (Brown Rendzina, Drift Brown Rendzina, Brown Calcareous soil) under shrubs and woods (Dutoit et al. 2004).

Four different successional stages of chalk grasslands were sampled to observe the temporal evolution of seed bank–earthworms interactions: open (O), tall (T) and encroached (E) grasslands and woods (W). Open grasslands are characterized by species-rich herbaceous vegetation dominated by Carex flacca, Festuca lenanii and Teucrium chamaedrys. Tall grasslands (T) are dominated by a grass species: Brachypodium pinnatum and are subject to summer mowing by Prim Holstein cows (3 ind.ha⁻¹). Encroached grasslands (E) are also dominated by B. pinnatum and are encroached with many shrub and ligneous species such as Cornus sanguinea, Crataegus monogyna and Rosa canina. The last stage (W) corresponds to an early forest dominated by maple trees (Acer campestre), common dogwoods (C. sanguinea) and common spindle (Euonymus europaeus).

Regarding earthworm composition, Decaëns et al. (1998) showed that endogeic species dominated in all successional stages. They also showed that density and biomass of anecic species increased in tall and encroached grasslands and those of epigeic species increased in woods (see Supplementary Table S1). Dominant endogeic species are Allolobophora chloretica (Savigny) and Aporrectodea caliginosa (Savigny); dominant anecic species are Lumbricus terrestris (L.) and Ap. giardi (Savigny); dominant epigeic species are Dendrodrilus rubida (Savigny) and Lumbricus rubellus (Hoffmeister) (see Decaëns et al. 2008). Field observations suggest that the relative proportions of ecological groups of earthworm did not significantly change from data by Decaëns et al. (1998) within each successional stage.

Within each of the four successional stages, three 10-m transects were positioned perpendicularly to the slope and were spaced of at least 100 m. Five plots were chosen on each transect. In each plot, casts, soil seed bank and vegetation were sampled. In total, we gathered 300 samples = 3 transects × 4 successional stages × 5 plots × 5 sample origins (i.e. vegetation, casts and 3 soil depths). All transects were located at the center of each successional stage and at least 3 m from any other stage (see Łuczaj and Sadowska 1997 for vascular plants) to avoid any edge effect. They were exposed to similar light and temperature conditions (South oriented).

**Seedling emergence from soil and cast seed banks**
The persistent soil seed bank was sampled in February 2010, when species with transient seed banks (Types I sensu, Thompson and Grime 1979) are not abundant in chalk grassland soils (Davies and Waite 1998). In each plot, four soil subsamples were collected with a soil core (ø 5 cm, every 50 cm). Each soil sample was separated into three depths (Gross 1990): 0–2, 2–5 and 5–10 cm. Subsamples for the four soil cores were pooled to obtain one sample per plot and per depth. The total mean volume sampled per transect was 5967.9 cm³, which is >1200 cm³, the volume needed to describe grassland seed banks (Roberts 1981).

Cast sampling was carried out in February 2010. In each plot, earthworm surface casts were manually collected in a 2×2 m quadrat (one person, 20 min/quadrat). This time period was chosen to sample a sufficient amount of cast while maintaining a constant sampling effort. Casts were easier to sample under low vegetation density with high density of casts (i.e. woods) than under tall vegetation (i.e. tall and encroached grasslands) or low cast density (open grassland). No distinction was made between casts of different earthworm species. Volume of casts sampled was: 75±80 cm³ in open grasslands, 81±45 cm³ in tall grasslands, 67±36 cm³ in encroached grasslands and 114±46 cm³ in woods.

All soil and cast samples were kept in the fridge for 2 weeks (5°C) to help break seed dormancy (Gross 1990), after their volume was measured in a beaker after removing coarse gravels from samples. Samples were then water sieved at 4 mm to remove the coarsest plant fragment and very fine gravels and at 0.2 mm to reduce soil volume (Ter Heerdt et al. 1996). We followed Ter Heerdt et al.’s (1996) germination approach to monitor seed bank species content. Although the total seed content is best assessed by the extraction method (see Weiterová 2008), it is labor-intensive and time-consuming. As our goal was to describe the impact of earthworms on overall chalk grassland plant communities, monitoring germinating seeds with this germination approach was sufficient.

All sieved samples were spread over a layer of moist gauze added to 3 cm vermiculite in a 34×61 cm tray. All trays were placed in a non-heated greenhouse for germination, and samples were watered regularly to keep optimal moisture levels. Trays were regularly randomly moved. Species were identified at the seedling stage with Muller’s seedling determination key (Muller 1978) and counted before they were removed from the sample. Seedlings were then eliminated. Seedlings that could not be identified were grown further until identification was possible. After the first 2 months, samples were carefully turned over in order to facilitate the emergence of new seedlings. Seedlings that died during the experimentation and could not be identified were only added to the density data (26% of the total density).

**Sampling of standing vegetation**
The *in situ* aboveground vegetation (vascular plants) was sampled in June 2010 in each plot, i.e. five 2×2 m quadrats in each transect. The cover-abundance index of Braun-Blanquet (1964) was used to quantify the expressed vegetation: (i) cover < 5%; (ii) 5% < cover < 25%; (iii) 25% < cover < 50%; (iv) 50% < cover < 75%; (v) cover >75%. The ‘+’ code was used for species represented only by a few individuals. Species were identified with the nomenclature of Provost (1998).

**Data analysis**
A generalized linear mixed modeling (GLMM) approach was used to test the effect of the origin of samples (OS: standing vegetation, cast or soil layers a, b and c), the successional stage (S: O, T, E, W) and their interaction on the abundance and
RESULTS

Differences in seedling abundance and species richness between soil and casts

Totally, 3701 seedlings from 57 species were observed in the soil and cast seed banks (51 species in soil and 44 in casts). Four species—Plantago media, Polygonum aviculare, Ranunculus repens, Thesium humifusum—were found in the soil seed bank only and Arenula pratensis was found in the cast seed bank only (Supplementary Tables S2 and S3). C. flaccus was the dominant species in the cast and in the soil seed banks, where it represented 35.4, 43.6, 56.3 and 61.4% of seedlings emerging in the cast seed bank. B. pinnatum, C. sanguinea, Sesleria albicans, C. flaccus, T. chamaedrys, Genista tinctora, Anthericum ramosum and F. lenanii constituted 50.8% of seedlings emerging in the cast seed bank. Species richness was higher in all samples in all stages of the secondary succession except in open grasslands (Fig. 1a; Supplementary Table S4). Seedling abundance in casts compared to the soil surface layer increased 5-fold in tall grasslands, 4-fold in woods and 3.5-fold in encroached grasslands (Fig. 1a; Supplementary Table S4). The 2-fold difference was not significant in open grasslands. Overall and in casts, seedling abundance was higher in tall and encroached grasslands than in the two other successional stages (GLMM: $\chi^2(3) = 18.62, P = 0.0003$ within casts). Seedling abundance was the lowest in woods (Fig. 1a).

Species richness of emerging seedlings was significantly influenced by the abundance of emerging seedlings in samples and by an interaction between the successional stage and the OS in soil samples ($P < 0.001$ and $P = 0.007$, respectively; GLMM: $\chi^2(18) = 186.06, P < 0.001$; Table 1). Seedling abundance was significantly higher in casts than in all soil samples in all stages of the secondary succession except in open grasslands (Fig. 1a; Supplementary Table S4). Seedling abundance in casts compared to soil surface layer increased 5-fold in tall grasslands, 4-fold in woods and 3.5-fold in encroached grasslands (Fig. 1a; Supplementary Table S4). The 2-fold difference was not significant in open grasslands. Overall and in casts, seedling abundance was higher in tall and encroached grasslands than in the two other successional stages (GLMM: $\chi^2(3) = 18.62, P = 0.0003$ within casts). Seedling abundance was the lowest in woods (Fig. 1a).

Species richness of emerging seedlings was significantly influenced by the abundance of emerging seedlings in samples and by an interaction between the successional stage and the OS ($P < 0.001$ and $P = 0.007$, respectively; GLMM: $\chi^2(16) = 400.53, P < 0.001$; Table 1). Species richness was much higher in casts than in all soil samples in all stages of the secondary succession (Fig. 1b; Supplementary Table S4). Species richness was four times higher in casts than in the soil surface layer in encroached grasslands (Fig. 1b; Supplementary Table S4). It was also more than three times higher in casts in tall grasslands and twice in open grasslands (Fig. 1b; Supplementary Table S4). Overall, species richness was higher in all samples in tall and encroached grasslands than in the two other successional stages. Casts alone followed the same pattern but the difference was only significant between casts of encroached grasslands and of woods (Fig. 1b; Supplementary Table S4). Species richness decreased with soil sample depth.
The role of specific earthworm–seed interactions

Effects of sample origin and successional gradient on plant composition from standing vegetation, soil seed bank and cast samples

The db-RDA showed that the OS contributed more to the similarity of plant composition than the stage of the secondary succession, and that both factors interacted significantly ($F_{(12,259)} = 2.4, P < 0.001$; Table 2).

The graphical representation of the db-RDA showed that plant composition varied between sample origins (Fig. 2 and Table 2). The vegetation samples were discriminated from the rest of the samples along the axis 1 (10.0% of total inertia, $P < 0.001$; Fig. 2). Plant composition was closer between casts and the soil surface layer than between casts and the other soil layers b and c. Plant composition in casts was overall closer to the plant composition in the standing vegetation than the soil seed bank was (Fig. 2). Axis 1 was strongly influenced by the abundance of

DISCUSSION

Higher seedling abundance and species richness in casts that in the soil seed bank

In all stages of the secondary succession, more seedlings of more species emerged from casts than from the soil seed bank.

Table 1: abundances and species richness of seed bank communities differ between soil samples and along successional stages

<table>
<thead>
<tr>
<th>Selected model</th>
<th>Null model</th>
<th>Formula*</th>
<th>AIC</th>
<th>df</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seedling abundance</td>
<td>1773</td>
<td>$S \times OS$</td>
<td>1617</td>
<td>18</td>
<td>186.06b</td>
</tr>
<tr>
<td>Species richness</td>
<td>1304</td>
<td>$S \times OS + \sqrt{\text{Abundance}}$</td>
<td>936</td>
<td>16</td>
<td>400.53b</td>
</tr>
</tbody>
</table>

Akaike information criterion (AIC) values and associated residual degrees of freedom (df) are shown for the GLMM assessing the variation in seed abundance and plant species richness of emerged seedlings among successional stage (S), origin of samples (OS: cast, soil layers a, b and c) and their interaction.

*Offsets and olre are not indicated.

*Levels of significance with $\alpha < 0.001$ of models compared to a null model with no factor (null model = 1 + offset (Log(volume of sample) + random (transect identity) + random (olre)).

Table 2: effect of successional stage, sample origin and their interaction on the plant composition of emerged seedlings and of the standing vegetation

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>Inertia</th>
<th>% constrained inertia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>410.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage (S)</td>
<td>3</td>
<td>23.4</td>
<td>23.2$^a$</td>
</tr>
<tr>
<td>Origin of samples (OS)</td>
<td>4</td>
<td>43.2</td>
<td>42.7$^a$</td>
</tr>
<tr>
<td>$S \times OS$</td>
<td>12</td>
<td>34.3</td>
<td>34.0$^a$</td>
</tr>
<tr>
<td>Residuals</td>
<td>250</td>
<td>309.9</td>
<td></td>
</tr>
</tbody>
</table>

Contributions of each factor are indicated as a percentage of the constrained inertia (=variance) and resulted from the Monte-Carlo permutation test ($n = 9999$) on the db-RDA. df, degrees of freedom.

$^a$Levels of significance with $\alpha < 0.001$.

*B. pinnatum* in the vegetation of all grasslands and the abundance of *C. flacca* in the vegetation of open and tall grasslands.

Differences of plant composition varied along the secondary succession (Fig. 2 and Table 2). Samples from woods were discriminated from the other stages along the axis 2 (3.3% of total inertia, $P < 0.001$). The difference between sample origins was always higher in the tall grasslands than in encroached grasslands, followed by open grasslands and woods (Fig. 2). In open grassland, the plant composition did not differ between casts and any soil layer. It differed between casts and soil layers b and c in all other successional stages. Plant composition in casts in tall and encroached grasslands shifted towards the plant composition of casts and of vegetation in woods (Fig. 2).

Figure 1: seedling abundance (A) and seedling species richness (B) in soil (Soil layers a, b, c) and cast (Casts) seed banks along the secondary succession. This graph shows mean effects (±SE) of the stage × samples of different origins. Different lowercase letters indicate significant differences between samples within stages. Different capital letters in superscript indicate significant differences between casts of different stages (Tukey’s HSD, $\alpha = 0.05$).

Table 1: abundances and species richness of seed bank communities differ between soil samples and along successional stages

Table 2: effect of successional stage, sample origin and their interaction on the plant composition of emerged seedlings and of the standing vegetation
that their specific selection and ingestion of seeds might not suggest that earthworms do aggregate seeds in their casts, but rounding soil. These seeds were also less viable. Therefore, we in Colombian grasslands contained more seeds than the surrounding soil in all stages. This result that takes the sample volume of casts and soil samples, and the seedling abundance into account (see Materials and Methods) suggests that many seeds survived gut passage. A higher seed abundance in casts partially explained this higher species richness. Several in situ and experimental studies showed a specific seed selection by earthworms (Aira and Piecarch 2009; Asshoff et al. 2010; Clause et al. 2011; Eisenhauer and Scheu 2008a; Eisenhauer et al. 2009a, 2009b; McRill and Sagar 1973; Milcu et al. 2006). Thus, we expected the richness of seedling species emerging from casts to be lower than the surrounding soil due to a seed selection. On the other hand, vertical movements of earthworms may increase the probability for earthworms to encounter a higher species richness of seeds.

The high richness of seeds in earthworm casts in our results can also be explained by the diversity of earthworm species in chalk grasslands of Upper Normandy (Decaëns et al. 1998; Margerie et al. 2001; Supplementary Table S1). Seed–earthworm interactions do not only depend on seed species, but also on earthworm species that may select specific seed species (Asshoff et al. 2010; Clause et al. 2011; Eisenhauer et al. 2009a). The higher seedling abundance and species richness was found in tall and encroached grasslands, which were associated with a higher density of anecic earthworms than in other successional stages (Supplementary Table S1; Decaëns et al. 1998). Anecic earthworms ingest soil and produce a large quantity of casts at the soil surface due to their vertical movement (Lee 1985). They also ingest a larger range of seed sizes than other ecological groups due to their bigger size, although this result varies among studies (Clause et al. 2011; Eisenhauer et al. 2009a). When they cannot directly ingest seeds, they gather them in their middens—small mounts of casts—at the soil surface for further consumption (Eisenhauer and Scheu 2008b). The distinction between casts of each earthworm species is difficult and differences in the proportions of anecic, endogenic and epigeic earthworms (Supplementary Table S1) likely explained the heterogeneity of ingestion patterns. Therefore, although microcosm studies showed a high selective seed ingestion by earthworms (Asshoff et al. 2010; Clause et al. 2011; Eisenhauer et al. 2009a, 2009b; McRill and Sagar 1973), our in situ patterns show a diluted selection and effect on seeds.

**Variations of plant composition along the succession and potential impact of earthworms**

We found that the plant composition was more similar between casts and the standing vegetation than between vegetation and the soil surface layer a, suggesting that earthworms contribute to the emergence of plants in the
vegetation. However, the discrimination of the standing vegetation compared to that of the casts and the soil seed banks in all grasslands in the db-RDA suggests that seedlings that emerged from the seed bank in the greenhouse fail to emerge in the field. This is likely due to the vegetative reproduction of the abundant grass species sampled in the vegetation (B. pinnatum, F. lemanii, Carex spp. and Briza media), the first being described as a social species due to its high ability to expand mostly by vegetative means and dominate species-poor tall grassland successional stage. The abundance and the height of these grasses reduce light penetration to the ground and may limit the germination of seeds whose light requirements are not fulfilled (Saar et al. 2012). This impact of a tall vegetation was supported by the high difference of plant composition between sample origins along the secondary succession. Plant composition between sample origins differed more in the tall grassland than in the three other successional stages, especially in the open grassland and woods.

The graphical representation of the db-RDA showed that the ingestion and egestion of seeds by earthworms contributed to a differentiation of plant composition along the secondary successional stage of chalk grasslands. The composition of seedlings that emerge from casts tends towards the vegetation of the last successional stage, i.e. woods. Mulrák et al. (2012) showed that earthworms drove the succession of plant communities in an in situ experimental study in post-mining sites. We suggest that the higher similarity of plant composition between sample origins in woods than in grasslands was due to the physical barrier created by trees that prevented the seed rain to reach the wood soil. In those conditions, pools of seeds in woods were not renewed and species were lost when seeds became unviable. The fragmentation of habitats such as grasslands leads indeed to a high extinction rate of species (Saar et al. 2012) and to the loss of grassland seeds species from transient or short-term persistent seed banks (Bakker et al. 1996; Bekker et al. 1998; Bossuyt et al. 2006; Thompson 2000). Other studies showed that the seed bank and the vegetation were the most similar in grasslands and the least similar in forests (Bossuyt and Honnay 2008; Bossuyt et al. 2006; Hopfensperger 2007; Jacquemyn et al. 2011). Thus, we suggest that the high similarity of plant composition between samples of different origins in woods was mostly explained by an absence of similar species in those samples. Surprisingly, earthworm activity uncovered seeds of grassland species such as C. flaccia, Daucus carota, F. lemanii, Hieracium pilosella and Linum catharticum that should rather reduce the similarity between the vegetation and the cast seed bank (Supplementary Tables S2 and S3). This aspect was obscured by the abundance of the seeds of C. sanguinea found in the casts. The presence of seeds of those five species from transient or persistent seed banks (see Thompson et al. 1993) suggests that the physical structure of casts could also play a protective role from environmental conditions and could prevent seed germination (Jouquet et al. 2008; Schrader and Zhang 1997). Although the overall viability of seeds seems to be lower in casts and to differ among seed species due to the passage through the earthworm gut (Decaëns et al. 2003; McRill and Sagar 1973), no study has been done to study the impact of cast age on seed viability. The stimulation of specific microbial and fungal populations and enzyme activities in fresh casts and their temporal variation (Tiwari et al. 1989; Tiwari and Mishra 1993) or the further deterioration of seeds by microbial communities (Aira et al. 2005) might impact seed viability across time.

CONCLUSION

Few in situ studies have been achieved on the impact of seed–earthworm interaction on plant communities via seed ingestion and the emergence of seedlings. Our results support previous findings documenting the aggregation of seeds in casts. Earthworms did ingest a particular composition of seeds from the soil seed bank and had an impact on the emergence of specific seedling species. However, this pattern could not be attributed to specific earthworm species, and patterns were not consistent along the succession. Taken together and considering the number of seedling species emerging from casts, earthworms in our chalk grassland could be considered to have a quite generalist feeding behavior, which shows in the emergence patterns. The high species richness and the higher similarity between casts and vegetation than the similarity between soil and vegetation suggests that earthworms potentially promote the germination and establishment of some species of early chalk grassland succession, and thereby impact the grassland dynamics. The plant composition of standing vegetation differed from that of seedlings emerging from our seed bank in lab conditions. We suggest that the domination of grasses and tall plants in intermediary successional stages prevented a further establishment of species emerging from casts and from the soil seed bank. Further laboratory studies on seed–earthworm species-specific interactions are needed to determine the seeds that earthworms preferentially ingest and their impact on seedling performances. Long-term experimental field studies manipulating earthworm density could help clarify the impact of earthworms on the dynamics of plant communities in the chalk grassland, following the long-term mesocosm experiment of Laossi et al. (2011).

SUPPLEMENTARY MATERIAL

Supplementary material is available at Journal of Plant Ecology online.

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Conflict of interest statement. None declared.

REFERENCES


