

Seed selection by earthworms: chemical seed properties matter more than morphological traits

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Abstract

Aims The passage of seeds through the earthworm gut potentially damages seeds, altering seed and seedling performances depending on seed traits. This work was conducted to study to what extent chemical and morphological seed traits determine the seed attractiveness for earthworms.

Methods We tested seed selection via the ingestion and digestion of 23 grassland plant species spanning a range of 14 morphological and chemical traits by two common earthworm species: the anecic *Lumbricus terrestris* and the endogean *Allolobophora chlorotica*.

Results Both earthworm species ingested seeds from all plant species. *A. chlorotica* digested almost all ingested

seeds (out of the 15 % ingested), whereas *L. terrestris* excreted them in varying quantities (out of the 86 % ingested), depending on plant species identity. Seed ingestion rate by *L. terrestris* was driven by seed oil content and earthworm initial weight. The apparent effect of seed length was explained via seed oil content. Seed digestion rate by *L. terrestris* was negatively impacted by seed size. Seed ingestion rate by *A. chlorotica* tended to be impacted by seed protein content and seed length.

Conclusion Earthworms–seed interactions depend on a variety of seed traits and earthworm identity. Thus, earthworms, via their specific feeding behavior, might facilitate or impede the regeneration of certain plant species and drive plant communities.

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Introduction

The impact of earthworms on plants through the modification of soil properties has been shown extensively (van Groenigen et al. 2014). Earthworm impacts on plant communities through the ingestion of seeds and consequences for seed survival are, however, less well studied, although they have been shown to be significant (Forey et al. 2011). Earthworms transport seeds upward or downward through the soil (Willems and Huijsmans 1994; Zaller and Saxler 2007). Seed burial might be an essential mechanism to escape aboveground seed predation or harsh environmental conditions (Traba et al. 2006), but might also deprive access to light for germination (Donath and Eckstein 2012). On the other hand, transporting seeds upwards might facilitate the emergence of buried seeds (Decaëns et al. 2003).

The ingestion of seeds by earthworms alters seed germination and seedling establishment (Aira and Pearce 2009; Eisenhauer et al. 2009). These alterations are likely due to seed damage in the earthworm gut (Curry and Schmidt 2007). In general, a percentage of ingested seeds (ranging between 0 and 100 %) that is both seed-specific and earthworm-specific was not recovered after ingestion because seeds were too damaged or digested (McRill and Sagar 1973). While some seeds are not viable anymore after gut passage, some viable seeds are excreted in nutrient-rich earthworm casts and burrows (Zhang and Schrader 1993), which may positively or negatively impact the plant development of viable seeds (Laossi et al. 2010). Animal feces are commonly recognized as regeneration niches, especially in cattle dung (Gardener et al. 1993). Therefore, seed selection by earthworms represents a first step in modifying plant community composition. These direct seed-earthworm interactions are known to be seed- and earthworm-specific (Eisenhauer et al. 2009; Clause et al. 2011). Significantly different plant compositions were found between seed banks in cast and in soil along a chalk grassland ecotone, which suggests that seed ingestion by earthworms plays a significant role in plant composition dynamics (Clause et al. 2016).

Seed palatability, which is the intrinsic property of the food that is assessed without considering post-

ingestive consequences (Yeomans 1998), is likely determined by seed chemical and morphological properties for earthworms. Most studies show that earthworms mostly ingest seeds according to seed size (see Forey et al. 2011). Additionally, the effects of morphological seed traits, such as shape, volume, and mass, have rarely been tested (Grant 1983; Clause et al. 2011), and chemical traits of seeds have only been studied in one case (Clause et al. 2011). This last study indicated that the anecic earthworm *Lumbricus terrestris* and the epigeic *Satchellius mammalis* ingest seeds of chalk grasslands according to seed oil content as well as their size. This suggests that seed ingestion might be an ‘active’ ingestion process for nutritive purposes, i.e. granivory. Other types of chemical compounds might attract earthworms, such as protein content (Harrison et al. 2003) and volatile compounds (Paczkowski et al. 2013).

Seed chemical and morphological traits are also likely to impact seed digestion by earthworms. Small seeds are generally digested or destroyed in higher proportions than large seeds because of contraction of the earthworm gizzard, grinding and enzymatic activity in the earthworm gut (Marhan and Scheu 2005; Curry and Schmidt 2007), and a longer retention time (Levey and Grajal 1991; Stanley and Lill 2002). Seed protein content and lipid content probably also influence seed digestion and nutrient uptake by earthworms (Clause et al. 2011). However, chemical and morphological seed traits may be correlated, and there has been no study on the relative importance of a broad spectrum of different seed traits for ingestion and digestion by earthworms thus far.

Anecic and endogeic earthworm species are known to have different feeding behaviors. Anecic species mostly feed on plant litter, whereas endogeic species mostly feed on soil organic matter (Curry and Schmidt 2007). Both ingest seeds, although anecic *L. terrestris* ingests more seeds than endogeic species (Eisenhauer et al. 2009). Their feeding behaviors are associated with their anatomical external and internal characteristics, such as the size of their prostomium or the development of certain organs such as the typhlosolis, a mid-dorsal invagination in the midgut that may be involved in nutrient uptake efficiency (Makeschin 1997). Those species-specific differences might have an impact on seed ingestion or digestion.

The objective of this study was to disentangle the influence of chemical traits (e.g. oil content) and morphological characteristics (e.g. seed size) on ingestion and digestion by earthworms. Therefore, we ran an

experiment similar to that of Clause et al. (2011) with two earthworm species (one anecic and one endogeic species) and 23 seed species with very diverse chemical and morphological traits, including seed size and oil, protein, and fatty acid contents. We hypothesized that (i) the species compositions of ingested and digested seeds differ between *L. terrestris* and *A. chlorotica*, and (ii) their ingestion preferences depend on specific seed traits, most likely driven by seed size as well as oil and protein content.

Materials and methods

Earthworm and seed species

Ingestion of seeds by adult *Lumbricus terrestris* (4.28 ± 1.03 g, $n = 230$) and *Allolobophora chlorotica* (0.24 ± 0.05 g, $n = 230$) was tested. *L. terrestris* was commercially purchased and *A. chlorotica* was sampled from a wood border on chalk substrate in Hénouville (Upper-Normandy, France). All earthworms were kept in boxes containing soil and grass litter (*Brachypodium perenne* L., *Lolium perenne* L.) collected from a chalk grassland at least two weeks prior to the experiment (Fründ et al. 2010). Time between sampling and the experiment was between two and four weeks. All live earthworms were weighed with guts that were voided before the experimentation to test for an effect of earthworm weight on ingestion and digestion patterns.

Seeds of 23 chalk grassland species were chosen for their wide range of size, mass, shape, texture, oil and protein contents across many plant families: *Achillea millefolium* L.; *Agrostis capillaris* L., *Brachypodium pinnatum* L., *Carex flacca* L., *Centaurea nigra* L., *Daucus carota* L., *Deschampsia cespitosa* L., *Festuca lemanii* L., *Galium mollugo* L., *Genista tinctoria* L., *Holcus lanatus* L., *Lolium perenne* L., *Lotus corniculatus* L., *Medicago lupulina* L., *Ononis spinosa* L., *Origanum vulgare* L., *Poa pratensis* L., *Ranunculus acris* L., *Sanguisorba minor* L., *Seseli libanotis* L., *Teucrium scorodonia* L., *Trifolium repens* L., and *Urtica dioica* L. (see Online Resource 1 for details on seed traits). Seeds were commercially purchased from Emorgate Seeds (King's Lynn, UK) or provided by the Caen and Bailleul Botanical gardens (France). 'Seeds' refers both to seeds and to fruits with not easily detached structures. For example, grass caryopses (except for *B. pinnatum*) and the fruit of *S. minor* were measured

as seeds. Microscopic control showed that seed processing prior to the experiment did not damage seeds. Germination rates of seeds prior to the experiment ranged from 0 % for *C. nigra* and *G. tinctoria* to 100 % for *Origanum vulgare*.

Ingestion and digestion of seeds

We followed the method of Eisenhauer et al. (2009) to study the ingestion, digestion, and excretion of seeds. After voiding their gut by making them fast for 48 h on moist filter paper (15 °C, darkness), earthworms were placed on moist filter paper in petri dishes (15 °C, 24 h, darkness) with 1 g of sieved soil (5 mm; collected from chalk grassland, Upper Normandy, France) with 20 seeds of a single plant species, placed at the soil surface. Thereafter, earthworms were removed and transferred into a different set of petri dishes with moist filter paper for 48 h (15 °C, darkness) to recover as many seeds as possible. Adding soil simulates natural conditions at the soil surface and provides sand particles that improve grinding and nutrient assimilation from organic matter in the earthworm gut (Marhan and Scheu 2005; Curry and Schmidt 2007). Each treatment combination (two earthworm treatments and 23 seed treatments) was replicated ten times (460 petri dishes in total). At the end of the experiment, casts were gently manually broken apart with water, and the number of non-ingested and egested seeds was counted. Seeds that were not recovered in casts were considered as non-ingested seeds, and egested seeds in casts were considered as ingested. The difference between the total number of seeds used (20) and the sum of the non-ingested and egested seeds was considered as the number of digested seeds. Digestion of seeds was only calculated for treatments where at least three earthworm individuals per species ingested at least three plant seeds, for statistical purposes.

Seed traits selection and measurements

Seed mass, size, and shape

Fourteen traits were selected to test the impact of seed traits on ingestion and digestion by earthworms. For each plant species, seed mass was calculated as the average of 20 seeds, individually weighed with a precision balance (precision: 10^{-7} g). Seed length, width, and thickness were measured on 10 seeds with a Zeiss

AxioCam HR camera. Length ranged from 1.81 mm (*O. vulgare*) to 8.41 mm (*S. minor*) (Online Resource 1). Seed shape was estimated using Thompson et al. (1993) shape index on the 10 seeds, where the shape (V_s) is determined by dividing the seed length, width, and thickness by the length and by calculating the variance of these three values with $V_s = [\sum (x - \bar{x})^2/n]^{1/2}$ (Thompson et al. 1993). As such, values for seed shape vary between 0 (perfectly spherical) and 0.2 (elongated or disc-shaped seeds). Values ranged from 0.002 (*T. repens*) to 0.168 (*F. lemanii*) (Online Resource 1).

Seed carbon and protein contents

Seeds of each species were ground to obtain between 2.2 mg (*O. vulgare*) and 3.0 mg (*L. corniculatus*) of seed powder that were weighed and analyzed for C and N contents. The total carbon and nitrogen contents were measured by combustion on two replicates per seed species (CHN Analyzer, Fisher Scientific, Germany). Protein content for each sample was determined with the Kjehldal method ($N \times 6.25$). Values for the total carbon content ranged from 42.4 % (*L. perenne*) to 54.2 % (*O. vulgare*) (Online Resource 1). Values for protein content varied from 14.3 % (*L. perenne*) to 41.8 % (*O. spinosa*) (Online Resource 1).

Seed water content, oil content, and fatty acid composition

Seed water content was calculated by subtracting the fresh weight of 10 seeds from the dry weight (107 °C, 17 h) and by dividing the result by the fresh weight. The mean value was calculated from five replicates. Values for the seed water content varied from 4.3 % (*A. millefolium*) to 19.4 % (*C. flacca*) (Online Resource 1).

Oil content was extracted and quantified by supercritical fluid extraction with carbon dioxide according to Seal et al. (2008). For this, 0.5 g (± 0.001 g) of seeds were ground and mixed with 1.5 (± 0.001 g) of Wetsupport™ before further analysis with a ISCO SFX 3560 fat analyser (6000 psi, 80 °C). Oil content was quantified by weighing the final vacuum-dried (70 °C, 1 h, $n = 3$) extract and by dividing it by the initial seed dry weight in the mix. Controls were performed with 5 drops of sunflower oil. Values for oil content varied from 0.9 % (*L. perenne*) to 34.9 % (*U. dioica*) (Online Resource 1).

Fatty acid composition of seeds was analyzed with a Gas Chromatography-Mass Spectrometry (GC-MS) according to Colville et al. (2012) after oil extraction. The compounds were detected using a Mass Spectrometer (Thermo Finnigan Trace DSQ; ionization energy 70 eV, scan frequency range m/z 10–500 per 0.3 s) and identified through comparison with the NIST mass spectral database and analytical standards (F.A.M.E. Mix C4-C24, Supelco). Excalibur® software (Fisher Scientific) was used to facilitate the identification of the most abundant chemicals components. Quantification of fatty acid methyl esters was performed using standard curves of quantitative standard mixtures (F.A.M.E. Mix GLC-10, -30 and -50, Supelco). Many fatty acids were detected in each seed species (Online Resource 2). In further analyses, we only kept the five fatty acids that were identified in all species and in the largest amounts: palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1 ω 9), linoleic acid (18:2 ω 6), and α -linolenic acid (18:3 ω 3) (Online Resource 1).

Data analysis

To test the effects of seed species, earthworm species, and their interaction on ingestion and digestion, we ran analyses of variance using generalized linear models (GLM), with binomial or quasibinomial distribution to overcome overdispersion (see Zuur et al. 2007). Post hoc comparisons among seed species were performed within each earthworm species with a Tukey HSD test ($\alpha = 0.05$). Means of ingested and digested seeds (%) were compared for each plant species between both earthworm species, with a 2-sample non-parametric Wilcoxon-Mann-Whitney test ($\alpha = 0.05$). Due to the low ingestion by *A. chlorotica*, 13 plant species could not be considered in the comparison of digestion patterns between both earthworm species.

The variability of seed morphological characteristics was described using a Principal Component Analysis (PCA) on a 12 traits \times 23 seed species matrix. Variables were log-transformed to improve normality. Variables were then centered and standardized by standard deviation. Seed ingestion and digestion by *L. terrestris* and *A. chlorotica*, as well as earthworm weight at the start of the experiment, were added to the analysis as illustrative variables. Illustrative variables do not contribute to the correlation circle and to axes, but their correlation with PCA axes can be tested. Illustrative variables were projected on the PCA axes to see how they were

associated with the different seed traits. Additional correlation tests were run between ingestion as well as digestion and each illustrative variable.

To disentangle which seed traits determined ingestion and digestion by earthworms, while accounting for correlations among traits, we performed a structural equation modeling (SEM). The first step in SEM requires establishing an a priori model based on known and expected relationships among variables. Based on results of the PCA and regression analyses, we decided to only keep variables that showed significant or marginally significant correlations with response variables. Data followed the same transformations as for the PCA analysis, with an additional arcsine-transformation of continuous percentages of ingestion and digestion. We fitted the model by testing for the overall goodness of fit using the maximum likelihood (ML) estimation procedure. Relationships among the remaining variables were chosen based on our knowledge of seeds, and on the model fitting procedure to reach the best model as possible. Adequate model fits are indicated by a non-significant χ^2 test ($P > 0.05$), low 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.

PCA analysis was carried out using the *ade4* (Dray and Dufour 2007) and *FactoMineR* (Husson et al. 2013) modules within the R environment (R Core Team 2013). SEM was performed using *Amos 5* (Amos Development Corporation, Crawfordville, FL, USA).

Results

Ingestion of seeds

Both earthworm species ingested seeds of all plant species. Seed ingestion rates depended on the interaction between the seed species and the earthworm species (GLM, $\chi^2_{(45,412)} = 6612$, $P < 0.001$). Overall, the total ingestion rate was higher for *L. terrestris* (86 ± 1 %) than for *A. chlorotica* (15 ± 1 %; GLM, $\chi^2_{(1456)} = 5180$, $P < 0.001$; Fig. 1). Higher seed ingestion for *L. terrestris* than for *A. chlorotica* was also found within each seed species, except for seeds of *T. repens* (Fig. 1 and Table 1).

Seed ingestion by *L. terrestris* varied among species (GLM, $\chi^2_{(22,205)} = 901$, $P < 0.001$). *L. terrestris* ingested 100 % of seeds of *O. vulgare*, *Urtica dioica*, *T. scorodonia*, *S. libanotis*, and *O. spinosa* (Fig. 1). Ingestion rates were high (>75 %) for all other seed species except for seeds of *F. lemanii* (67 ± 6 %), *B. pinnatum* (52 ± 8 %), and *T. repens* (38 ± 9 %; Fig. 1).

Seed ingestion by *A. chlorotica* also varied among species (GLM, $\chi^2_{(22,207)} = 530$, $p < 0.001$). *A. chlorotica* ingested more seeds of *O. spinosa* (56 ± 3 %) and *T. repens* (49 ± 9 %) than of any other seed species (Fig. 1 and Table 1). The other seed species with similar ingestion rates were *A. millefolium* (28 ± 3 %), *U. dioica* (23 ± 5 %), *T. scorodonia*, *L. perenne* (18 ± 3 %), *L. corniculatus* (16 ± 2 %), *D. carota* (15 ± 4 %), and *A. capillaris* (13 ± 4 %).

Digestion of seeds

Seed digestion depended on the interaction between the seed species and the earthworm species (GLM, $\chi^2_{(32, 288)} = 1028$, $P < 0.001$). Overall, *A. chlorotica* digested more of the ingested seeds (99 ± 1 %) than *L. terrestris* (45 ± 2 %; GLM, $\chi^2_{(1319)} = 683$, $P < 0.001$; Fig. 2 and Table 1).

Seed digestion by *L. terrestris* varied among plant species (GLM, $\chi^2_{(22,203)} = 318$, $P < 0.001$). The seeds with the highest digestion rates were that of *T. repens* (83 ± 7 %) and *A. capillaris* (73 ± 5 %), followed by *D. carota* (60 ± 5 %), *A. millefolium* (59 ± 5 %), *G. mollugo* (59 ± 7 %), *B. pinnatum* (57 ± 10 %), *O. vulgare* (54 ± 6 %), *L. corniculatus* (52 ± 9 %), and *U. dioica* (47 ± 6 %; Fig. 2 and Table 1).

Seed digestion by *A. chlorotica* reached 100 % for all ingested seeds, except for *A. capillaris* that was less digested (GLM, $\chi^2_{(9,85)} = 28$, $P = 0.001$; Fig. 2 and Table 1). Digestion of 13 seed species could not be determined due to low ingestion rates by *A. chlorotica*: grasses *H. lanatus*, *D. cespitosa*, *C. flacca*, *P. pratensis*, *F. lemanii*, *B. pinnatum*, herbs *G. mollugo*, *C. nigra*, *R. acris*, *S. libanotis*, and *S. minor*, and legumes *M. lupulina* and *G. tinctoria*.

Correlative analyses of seed traits

The first PCA axis explained ca. 28 % of the total variability between seed traits and was mostly associated with seed chemical traits: seed content in oil, carbon, and stearic, oleic, palmitic, and linoleic acids (Table 2 and

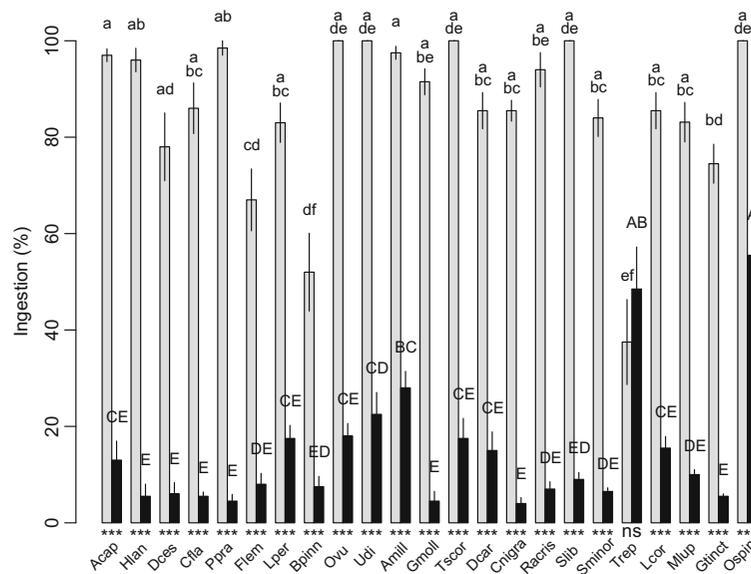


Fig. 1 Number of seeds (% \pm SEM) of 23 different seed species ingested by *L. terrestris* (grey) and *A. chlorotica* (black). Bars with different letters vary significantly within *L. terrestris* (lowercase) or within *A. chlorotica* (uppercase). Asterisks below bars indicate significant differences between ingestion patterns by both earthworms for each plant species (Wilcoxon-Mann-Whitney; *** $P < 0.001$, ns non-significant). Seed species were arranged according to plant functional identity (grasses, herbs, and legumes) and seed size (small-seeded species and large-seeded species):

A. capillaris (Acap), *H. lanatus* (Hlan), *D. cespitosa* (Dces), *C. flacca* (Cfla), *P. pratensis* (Ppra), *F. lemanii* (Flem), *L. perenne* (Lper), *B. pinnatum* (Bpinn), *O. vulgare* (Ovu), *U. dioica* (Udi), *A. millefolium* (Amill), *G. mollugo* (Gmoll), *T. scorodonia* (Tscor), *D. carota* (Dcar), *C. nigra* (Cnigra), *R. acris* (Racris), *S. libanotis* (Slib), *S. minor* (Sminor), *T. repens* (Trep), *L. corniculatus* (Lcor), *M. lupulina* (Mlup), *G. tinctoria* (Gtinct), *O. spinosa* (Ospin)

Fig. 3b). Six out of eight traits explaining axis 1 were chemical traits (Table 2). However, length and shape also significantly explained axis 1 (Table 2 and Fig. 3b). Long seeds that were enriched in lineoleic, oleic, stearic, and palmitic acids, and that were represented by grasses *L. perenne*, *P. pratensis*, *D. cespitosa* and *B. pinnatum*, were opposed to small, round, oil- and carbon-rich seeds, represented by *U. dioica*, *O. vulgare*, *A. millefolium*, and *T. scorodonia* (Figs 3a and b). Variability of ingestion and digestion rates, represented as illustrative variables, were not explained by this axis (Table 2). However, a high mean ingestion was associated with high values of protein, carbon, and oil content with $\alpha = 0.01$.

PCA axis 2 accounted for additional ca. 23 % of the total variability among seed traits, and appeared to be a size-related axis (Table 2 and Fig. 3b). It opposed wide, thick, and heavy seeds represented by *O. spinosa*, *S. libanotis*, *R. acris*, and *G. tinctoria*, to narrow, shallow, or light seeds, such as *A. capillaris*, *D. cespitosa*, *O. vulgare*, *P. pratensis*, *A. millefolium*, and *F. lemanii* (Figs 3a and b). Digestion of seeds by *L. terrestris* was significantly represented by this axis (Table 2).

L. terrestris digested preferentially small, round, light seeds in contrast to wide, thick, and heavy ones.

Axis 3 accounted for ca. 14 % of the total variability among seed traits. As for axis 1, it was strongly associated with seed fatty acid content, here alpha-linolenic, palmitic, and stearic acids (Table 2). It was also strongly associated with seed protein content (Table 2), and opposed *G. tinctoria* and *O. vulgare* to *D. carota*, *F. lemanii*, *C. flacca*, *B. pinnatum*, and *S. libanotis* (data not shown).

Individual correlations showed that mean seed ingestion and ingestion of seeds by *L. terrestris* were significantly positively correlated with seed oil and carbon contents, and marginally negatively correlated with seed length (Table 3). Seed digestion by *L. terrestris* was significantly negatively correlated to seed size, i.e. length, width, thickness, volume and mass, and marginally negatively correlated with stearic acid content. Seed ingestion by *A. chlorotica* was not significantly correlated to any seed trait, but tended to be positively correlated with seed protein content, and to be negatively correlated with seed length (Table 3).

Table 1 Summary of the generalized linear model (GLM; binomial) to test the impact of earthworm species on seed ingestion and digestion ($n = 10$ microcosms). Df: degree of freedom, residual degree of freedom

Seed species	Ingested seeds			Digested seeds		
	df	χ^2	P	df	χ^2	P
Total	1456	5180	<0.001	1319	682.5	<0.001
<i>A. capillaris</i>	1,18	342.1	<0.001	1,14	0.72	0.59
<i>H. lanatus</i>	1,18	402.1	<0.001	-	-	-
<i>D. cespitosa</i>	1,18	242.7	<0.001	-	-	-
<i>C. flacca</i>	1,18	304.5	<0.001	-	-	-
<i>P. pratensis</i>	1,18	449.6	<0.001	-	-	-
<i>F. lemanii</i>	1,18	164.1	<0.001	-	-	-
<i>L. perenne</i>	1,18	186.7	<0.001	1,18	61.76	<0.001
<i>B. pinnatum</i>	1,18	104.5	<0.001	-	-	-
<i>O. vulgare</i>	1,18	352.9	<0.001	1,18	39.64	<0.001
<i>U. dioica</i>	1,18	320.8	<0.001	1,18	59.43	<0.001
<i>A. millefolium</i>	1,18	244.3	<0.001	1,18	50.99	<0.001
<i>G. mollugo</i>	1,18	364.1	<0.001	-	-	-
<i>T. scorodonia</i>	1,18	356.7	<0.001	1,17	51.57	<0.001
<i>D. carota</i>	1,18	219.89	<0.001	1,18	27.91	<0.001
<i>C. nigra</i>	1,18	317.4	<0.001	-	-	-
<i>R. acris</i>	1,18	362.2	<0.001	-	-	-
<i>S. libanotis</i>	1,18	430.23	<0.001	-	-	-
<i>S. minor</i>	1,18	278.8	<0.001	-	-	-
<i>T. repens</i>	1,18	5.0	0.38	1,18	24.86	<0.001
<i>L. corniculatus</i>	1,18	216.4	<0.001	1,18	27.98	0.003
<i>M. lupulina</i>	1,18	215.67	<0.001	-	-	-
<i>G. tinctoria</i>	1,18	226.1	<0.001	-	-	-
<i>O. spinosa</i>	1,18	149.2	<0.001	1,18	174.75	<0.001

SEM on seed trait selection

The final structural equation model showed that seed ingestion by *L. terrestris* was significantly determined by seed oil content, and by the earthworm weight (Chi-square SEM, $\chi^2_4 = 7$, $P = 0.12$, RMSEA = 0.19, $P_{RMSEA} = 0.14$, AIC = 29.30; Fig. 4). Seed ingestion rate increased when seeds were richer in oil content (SPC = 0.44, $P = 0.04$) and when earthworms were heavier (i.e. bigger) (SPC = 0.37, $P = 0.02$; Fig. 4a). Seed length did not directly impact seed ingestion rate (SPC = 0.04; $P = 0.83$), but was negatively correlated to seed oil content (SPC = -0.28). Seed oil and carbon contents were strongly positively correlated (SPC = 0.59; Fig. 4). Other seed traits had no significant effect on seed ingestion and were not considered in the SEM (see Table 3).

No suitable SEM was found to explain the relative impact of seed traits on seed digestion by *L. terrestris* and seed ingestion by *A. chlorotica*, and no significant pathway was found (data not shown). Results for seed digestion by *A. chlorotica* could not be calculated due to low replication.

Discussion

Seed ingestion and digestion vary with earthworm and seed species

As expected, different seed-feeding behaviors were found between the anecic and endogeic earthworms used in our experiment. As in other studies, we report

Fig. 2 Number of seeds (% ± SEM) of 23 different seed species digested by *L. terrestris* (grey) and *A. chlorotica* (black). Symbols and abbreviations as in Fig. 1

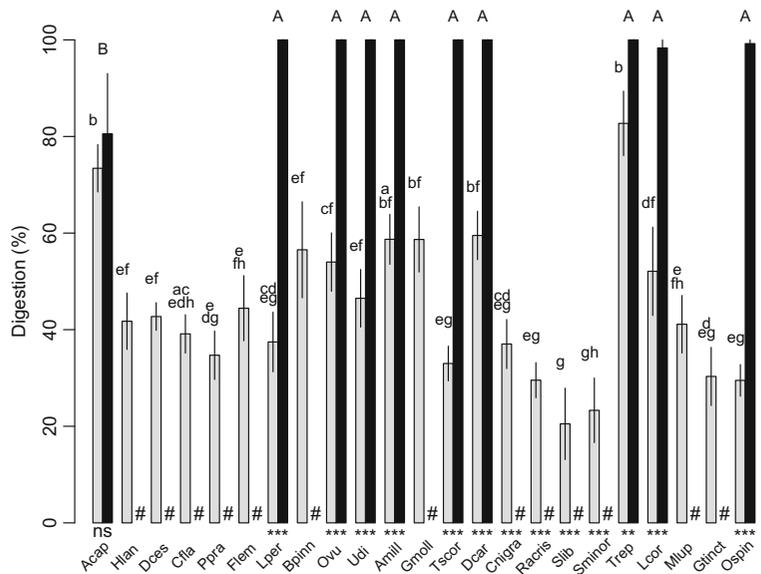


Table 2 Eigenvector scores of plant traits in three main PCA axes, obtained from a matrix of 14 traits x 23 species. Values are ranked in order of absolute magnitude along PCA1. Eigenscores significantly correlated to each PCA axis ($\alpha = 0.05$) are indicated in bold.

Values in parentheses indicate variance accounted for by each axis. Ingestion and digestion responses were used as illustrative variables were projected onto PCA biplot

	PCA1 (27.83 %)	PCA2 (22.88 %)	PCA3 (14.57 %)
Active variables			
Oil content ^a	-0.766	0.214	0.077
Length ^a	0.756	0.129	-0.408
Stearic acid ^a	0.724	0.184	0.461
Oleic acid ^a	0.685	0.095	-0.141
Palmitic acid ^a	0.613	-0.102	0.598
Carbon content ^a	-0.545	0.412	-0.181
Linoleic acid ^a	0.506	-0.320	-0.069
Shape	0.482	-0.633	-0.258
Water content	0.392	-0.115	-0.184
Protein content ^a	-0.375	0.082	0.677
Mass [†]	0.345	0.843	-0.194
α -Linolenic acid ^a	0.288	0.071	0.796
Width	0.217	0.923	0.032
Thickness ^a	0.199	0.910	-0.099
Illustrative variables			
mean % seed ingested by earthworms	-0.364	0.083	0.326
% seed ingested by <i>L. terrestris</i> ^b	-0.290	-0.029	0.141
% seed digested by <i>L. terrestris</i>	-0.259	-0.542	0.087
% seed ingested by <i>A. chlorotica</i> ^a	-0.273	0.014	0.287
<i>L. terrestris</i> initial weight	-0.027	-0.131	0.179
<i>A. chlorotica</i> initial weight	-0.091	0.254	-0.256

^{a,b} Variables that were respectively log- or arcsin-transformed prior to PCA analysis

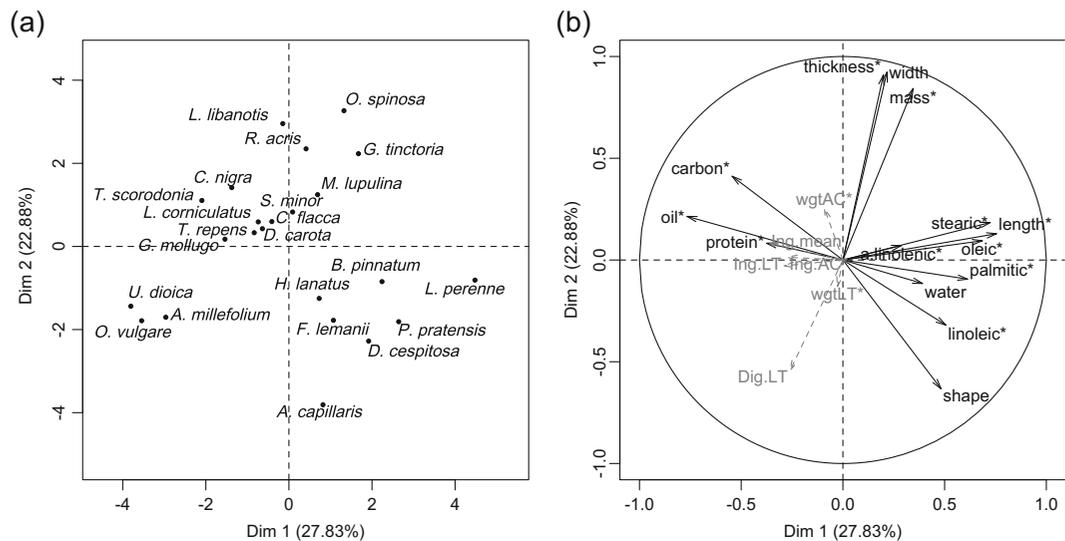


Fig. 3 PCA ordination of 23 seed species, on the basis of 14 seed morphological and chemical traits. **a** Location of individual species on the ordination plane represented by axis 1 (27.85 % of variance) and axis 2 (22.80 % of variance). **b** Correlation circle of active variables (seed traits; solid arrows) that contribute to axes 1 and 2, and display of illustrative variables that do not contribute to

the circle (dashed arrows). Illustrative variables are seed ingestion (%) by both earthworms (Ing.mean), *Lumbricus terrestris* alone (Ing.LT), *Allolobophora chlorotica* alone (Ing.AC), seed digestion (%) by *L. terrestris* alone (Dig.LT), and earthworm weights (wgtLT and wgtAC). Variables marked with an asterisk (*) were log-transformed prior to analysis

higher seed ingestion by the large anecic *L. terrestris* for all seed species than by the smaller earthworm species, here the endogeic *A. chlorotica*. In contrast to Eisenhauer et al. (2009), we found that *A. chlorotica* could ingest all seed species.

L. terrestris digested only part of the seeds that it ingested, and the seed species with the highest digestion rates tended to match the ones with the highest ingestion rates. This suggests that *L. terrestris* prefers ingesting seeds that it can digest, supporting the results of previous studies (McRill and Sagar 1973; Eisenhauer et al. 2009). Notably, *A. chlorotica* fully digested all the seeds that it ingested, with the exception of *A. capillaris*. This is surprising, since Eisenhauer et al. (2009) found no or little seed digestion by *A. chlorotica*. Our results on seed digestion by *A. chlorotica* must be treated with caution though, considering the relatively low percentage of seed ingestion and the fact that we could not consider 13 out of 23 seed species due to low ingestion and digestion rates. However, these results suggest that *A. chlorotica* is particularly efficient at either physically or chemically degrading the seeds that it ingests. We suggest that due to the low assimilation of soil organic matter by endogeic species, the digestion of seeds may significantly contribute to their nutrition. This might be

facilitated by their well-developed typhlosolis, a mid-dorsal invagination in the midgut that may be involved in nutrient uptake efficiency, compared to that in anecic species (Makeschin 1997).

Enzymatic activity occurs in the earthworm gut, either through gut microfloral activity or from the earthworm own digestive processes (see Curry and Schmidt 2007 for a review). However, although earthworms can process carbohydrates and proteins, they digest little cellulose or phenolic material, and no lignin (Curry and Schmidt 2007). Seed coats are often lignified to maintain the impermeability of mature seeds, and to protect them from morphological damage (Souza and Marcos-Filho 2001). Therefore, earthworms must first overcome a physical barrier of lignin before they can digest seeds. As litter and soil material that are ground in the earthworm gizzard (Marhan and Scheu 2005), seeds and seed coats are probably physically damaged before reaching the earthworm's intestines. Damage of seeds must also be linked with the seed retention time in the earthworm gut. The longer the seed stays in the gut, the more likely it is to be damaged or destroyed (Tewksbury et al. 2008). Thus, future studies should investigate the interplay between physical and chemical processes in seed digestion in earthworm guts.

Table 3 Summary of simple linear regression analysis and correlations (r) between seed ingestion and digestion rates by earthworms (*L. terrestris*, *A. chlorotica*) and seed andearthworm traits (n = 23 ; $\alpha = 0.05$). df: degree of freedom, F: Fisher index, r: coefficient of correlation

Seed trait	mean % seed ingested by earthworms			% seed ingested by <i>L. terrestris</i> ^b			% seed digested by <i>L. terrestris</i>			% seed ingested by <i>A. chlorotica</i> ^a		
	df	F	r	df	F	r	df	F	r	df	F	r
Length ^a	1,21	5.37*	-0.45	1,21	3.33	-0.26	1,21	5.12*	-0.44*	1,21	3.51	-0.38
Width	1,21	<0.01	<0.01	1,21	0.36	-0.06	1,21	8.87**	-0.54**	1,21	0.01	-0.03
Thickness ^a	1,21	0.23	-0.1	1,21	1.24	-0.2	1,21	6.81*	-0.49*	1,21	0.13	0.08
Mass ^a	1,21	1.21	-0.23	1,21	2.46	-0.28	1,21	9.63**	-0.56**	1,21	0.54	-0.16
Shape	1,21	2.02	-0.29	1,21	0.03	-0.04	1,21	<0.01	0.01	1,21	1.94	-0.29
Volume ^a	1,21	0.81	-0.19	1,21	1.58	-0.26	1,21	13.46**	-0.63**	1,21	0.61	-0.17
Water content	1,21	0.19	-0.09	1,21	0.60	-0.12	1,21	0.21	0.10	1,21	<0.01	<0.01
Oil content ^a	1,21	7.17*	0.50*	1,21	12.29**	0.59**	1,21	0.51	-0.15	1,21	0.03	0.04
Protein content ^a	1,21	1.18	0.23	1,21	0.02	-0.04	1,21	2.08	0.30	1,21	3.99	0.40
Carbon content ^a	1,21	10.62**	0.58**	1,21	8.07**	0.48*	1,21	1.19	-0.23	1,21	1.09	0.22
Linoleic acid ^a	1,21	0.62	-0.17	1,21	1.53	-0.22	1,21	0.31	0.12	1,21	0.21	-0.10
Oleic acid ^a	1,21	0.17	-0.09	1,21	0.26	0.01	1,21	0.77	-0.19	1,21	1.78	-0.28
Stearic acid ^a	1,21	0.01	0.02	1,21	0.03	-0.01	1,21	3.18	-0.36	1,21	0.04	-0.04
Palmitic acid ^a	1,21	0.35	0.13	1,21	0.08	0.11	1,21	0.19	-0.09	1,21	<0.01	-0.02
α -Linolenic acid ^a	1,21	0.58	0.16	1,21	0.85	0.09	1,21	0.77	-0.19	1,21	0.16	0.09
LT weight [†]	1,21	6.31*	0.48*	1,21	3.48	0.38	1,21	0.89	0.20	-	-	-
AC weight [†]	1,21	0.12	-0.08	-	-	-	-	-	-	1,21	0.66	0.17

†: significant with $P < 0.1$; * $P < 0.05$; ** $P < 0.01$ ^{a,b} Variables that were respectively log- or arcsin-transformed prior to analysis

Seed oil content drives seed ingestion by *L. terrestris*

Structural equation modeling revealed that ingestion was primarily driven by high seed oil content and not seed size. This result supports the idea that seed size alone does not explain seed selection by earthworms, despite morphological limitations of earthworms to ingest seeds larger than the size of their mouth (Shumway and Koide 1994). Interestingly, the SEM analysis showed that the effect of seed length, which was the most significant proxy measure for seed size in correlation analyses, was explained by seed oil content, which was negatively related to seed length. Similarly, Clause et al. (2011) found a strong negative correlation between seed oil content and seed size with a smaller dataset, but the present study is the first one being able to disentangle the effects of those two seed traits on seed selection by earthworms.

Seed oil content, which was highly correlated with carbon content, was the main seed trait determining seed

palatability for *L. terrestris*. The fact that earthworms preferentially ingest seeds with high oil content indeed suggests that they select seeds as high-quality food material, i.e. with high energy content. In fact, earthworms preferentially ingest litter based on its contents in nitrogen, cellulose, lignin or the presence of particular fungi (Moody et al. 1995; Curry and Schmidt 2007). A recent study showed that *L. terrestris* consumed legume seedlings at the radicle stage more than seeds or seedlings at the cotyledon stage, and more than grass seeds and seedlings with lower N concentrations (Eisenhauer et al. 2010). We found that, unlike oil content, seed protein content was not an important criterion of seed selection by earthworms. Lipids are a more readily assimilated energy source than protein (see Levey and Grajal 1991), and the handling cost of degrading proteins might not benefit earthworms. Although *L. terrestris* ingests small seeds more easily than larger seeds, it overcomes a size morphological barrier to ingest large oil-rich seeds, such as those of *C. nigra*.

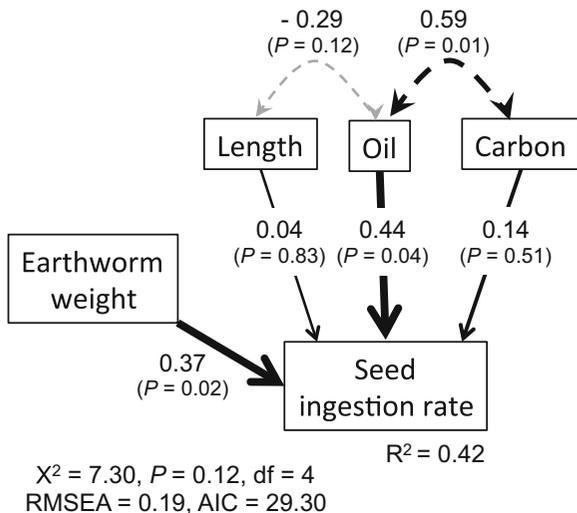


Fig. 4 Effects of seed traits (length, oil, carbon content) of 23 seed species and earthworm weight on seed ingestion rate by earthworm *Lumbricus terrestris*. Numbers adjacent to arrows are indicative of the effect size (standardized path coefficients) and significance (P -value in brackets) of the relationship. Continuous arrows indicate causal relationships between variables. Dashed double-headed arrows indicate undirected correlations between exogenous variables. *Black* and *grey solid arrows*, respectively, show positive and negative relationships between variables. The width of arrows is proportional to the strength of path coefficients. R^2 denotes the proportion of variance explained. RMSEA: Root Mean Square Error of Approximation

An optimum size/oil content ratio may exist, beyond which seeds become less attractive to earthworms. Also, earthworms might only ingest some seeds once they have germinated and become more attractive (Eisenhauer et al. 2010).

Volatile compounds, tannins, and other secondary chemicals might also impact seed palatability, and attract or repel earthworms. Earthworms are equipped with chemoreceptors that help them locate food sources. For example, volatile compounds produced by microorganisms attract earthworms (Zirbes et al. 2011). Also, compounds produced by imbibed seeds and spread through mucilage attract or repel other invertebrates, such as beetles (Law and Gallagher 2015) or ants (Yang et al. 2013). A negative relation was recently observed between seed oil content and mucilage production by the seed coat of *Arabidopsis thaliana* (Shi et al. 2012). The production of mucilage in our seed species and the production of attracting or repelling volatile compounds, and their relation to seed oil content that could alter feeding behavior of earthworms should be investigated in the future. They might explain a

chemical recognition of oil-rich seeds by earthworms. The perception of volatile compounds by earthworms is also likely to depend on the hardness of the seed coat. Paulsen et al. (2013) showed that volatile compounds emitted by soft seeds attracted desert hamsters, whereas non-emitting hard seeds escaped seed predators. Dimorphic seed species benefit from the attraction of dispersers by a pool of physiologically non-dormant seeds that emit volatile compounds, because dispersers subsequently also disperse physically dormant seeds that will survive the predator gut (Paulsen et al. 2013). In our case, the dimorphic species *T. repens* that produces both hard and soft seeds (Baskin and Baskin 2004), might also have been less ingested by earthworms due to a reduced attraction of hard seeds from the dry chalk grasslands habitat (see Paulsen et al. 2013). Scarification of seeds prior to the experiment might increase the consumption of those seeds by earthworms. The comparison of the seed coat thickness and hardness of wild seed species could better explain seed preferences by earthworms.

Seed palatability may depend on other fatty acids than the tested one, which are present in lower amounts and/or that are seed-specific. Saturated margaric (17:0) and arachidic (20:0) acids were also commonly found, but in lower amounts, except in *O. vulgare*, where arachidic acid was the fatty acid with highest concentration (Online Resource 2). No fatty acid stood out for *B. pinnatum*, but myristic (14:0) and 7-isopropyl-10-methyl-1-oxo-1,5-dithia-spiro[5,5]undecane-2-carboxylic acid were found as seventh most important fatty acid in *L. perenne* and *T. repens*, respectively, which were ingested in lower amounts. A repellent role of these fatty acids should be explored. Brassylic and sebacic acids were only found in *S. libanotis*, and arachidonic acid (20:4 ω 6c) was only found in *T. scorodonia*, which may be associated with an attractive effect.

Earthworm weight was also found to have an impact on seed ingestion. Earthworm weight is linked to earthworm overall size and thus to mouth size: larger and heavier earthworms likely have a larger mouth and ingest more seeds. Earthworm feeding behavior changes with adulthood (Curry and Schmidt 2007), and some of the adult earthworms that we used might not have fully developed adult feeding preferences. The fact that ingestion of seeds by *A. chlorotica* did not depend on seed traits or earthworm weight is probably due to different feeding behaviors between ecological groups. Endogeic species primarily ingest soil particles, and seed

palatability may be less important than ‘soil palatability’ and soil-related compounds (Curry and Schmidt 2007). However, some of our results suggest that they might be attracted to seed protein content rather than oil content.

Seed size impacts seed digestion

Seed digestion by *L. terrestris* was strongly impacted by seed size. However, we were unable to identify which specific component of the seed size (i.e. length, width or thickness) impacted seed digestion. Using more seeds, more seed species, and more replicates might be future steps to refine this analysis. No significant correlation between oil and seed digestion was found, unlike previous studies that showed a positive correlation between oil content and seed digestion by earthworms (Clause et al. 2011), and their assimilation of N and fatty acids in body tissue through the action of specific gut microbiota (Sampedro et al. 2006). However, protein and stearic content, respectively, tended to correlate positively and negatively with seed digestion, although this was not significant.

Small and light seeds were more digestible than large/heavy seeds. In birds, the retention time of seeds in the gut depends on seed size: small seeds take longer to pass through the animal gut than large seeds (Stanley and Lill 2002). This was explained by a cost of handling large seeds that displace gut volume and prevent the ingestion of other material, and an increased energy demand for locomotion (Levey and Grajal 1991). In earthworm gut, small seeds are likely to take longer to be egested, and to be more physically damaged in the gizzard or digested by enzymatic activity than large seeds. This mechanism could explain our results of high digestion rates of small seeds compared to large seeds by *L. terrestris*, but not for *A. chlorotica*. From a methodological perspective, this implies that particular attention should be paid that earthworms do not re-ingest large seeds in laboratory experiments, e.g. by removing seeds from the experiment within 24 h. No experiment has measured at the same time seed selection and gut transit time of individual seeds by earthworms. Considering the estimated 8 h of food-retention time by *L. terrestris* in culture (Hartenstein and Amico 1983), we believe that all our seeds were effectively egested after 48 h of fasting. However, differences of retention time for different seed species remain to be investigated to better evaluate seed damage to seed and check for a potential re-ingestion of seeds.

Consequences for the ecological and evolutionary relations between plants and earthworms

The influence of seed traits on species-specific impact of earthworms on seed partial or full digestion might impact plant evolution strategies via impacts on plant performances and dispersal. Seed chemical and morphological properties determine functional traits, such as dormancy, seed defense, or germination (Milberg and Lamont 1997; Traveset et al. 2007; Dalling et al. 2011), and might determine mutualistic relations between earthworms and plant species. A weight gain was observed in earthworms after seed and seedling consumption (Eisenhauer et al. 2010), and depending on seed and earthworm species, earthworms can be either seed predators or dispersal agents. Large-seeded species have lower digestion rates and are more rapidly excreted than undigested small-seeded species. While the influence of large animals on the evolution on seed and fruit characteristics has been thoroughly exemplified (Galetti et al. 2013), the impact of smaller animals on the evolution of these characteristics has hardly been studied. Our results suggest that in ecosystems with high earthworm densities, earthworms could represent an important selection pressure for plants, by selecting for plants with large oil-poor seeds, such as grasses. This could be tested by comparing seed traits of the same plant species in earthworm-free and earthworm-invaded areas in Northern America (see Fisichelli et al. 2012). Grasses were also shown to benefit from lower seedling consumption by earthworms and from increased soil fertility associated with their activity (Laossi et al. 2009), which increases their competitive behavior. On the other hand, longer seed retention time of small seeds and their viability upon egestion might favor dispersal into the soil, and storage in the soil seed bank (Willems and Huijsmans 1994).

Patterns of seed preferences vary between laboratory and in situ experiments, due to laboratory conditions that do not follow earthworm natural living conditions (Willems and Huijsmans 1994). Although the impacts of earthworms on seed preferences and plant development observed in laboratory experiments and in situ experiments may be consistent (Eisenhauer et al. 2010), the generalization of our results to in situ situations should only be derived with caution. In particular, laboratory experiments might overestimate the influence of seed-earthworm interactions.

To conclude, the impact of seed ingestion and digestion by earthworms on seed bank dynamics and above-ground seed communities is seed-specific (Forey et al. 2011). The combined species-specific effects of earthworms on plants at different stages of the plant life cycle via seed selection, burial, partial or full digestion, germination, seedling establishment, and plant growth confirm that earthworms have the capacity to influence the structure and composition of plant communities (Laossi et al. 2009; Laossi et al. 2010). To comprehensively assess the impact of one or several earthworm species on plant species and communities, one must consider direct and indirect earthworm effects on the entire plant life cycle, i.e. through seed ingestion, digestion, germination, plant growth (root, shoot), and reproduction.

Data accessibility

The datasets analyzed during the current study are available from the corresponding author on reasonable request. Summarized data on seed traits are present in the manuscript and its supporting information.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest

References

- Aira M, Pearce TG (2009) The earthworm *Lumbricus terrestris* favours the establishment of *Lolium perenne* over *Agrostis capillaris* seedlings through seed consumption and burial. *Appl Soil Ecol* 41:360–363. doi:10.1016/j.apsoil.2008.11.007
- Baskin JM, Baskin CC (2004) A classification system for seed dormancy. *Seed Sci Res* 14:1–16. doi:10.1079/SSR2003150
- Clause J, Margerie P, Langlois E et al (2011) Fat but slim: Criteria of seed attractiveness for earthworms. *Pedobiologia* 54(Supplement):S159–S165. doi:10.1016/j.pedobi.2011.08.007
- Clause J, Barot S, Forey E (2016) Earthworms promote greater richness and abundance in the emergence of plant species across a grassland-forest ecotone. *J Plant Ecol*. doi:10.1093/jpe/rtw008rtw008
- Colville L, Bradley EL, Lloyd AS et al (2012) Volatile fingerprints of seeds of four species indicate the involvement of alcoholic fermentation, lipid peroxidation, and Maillard reactions in seed deterioration during ageing and desiccation stress. *J Exp Bot* 63:6519–6530. doi:10.1093/jxb/ers307
- Curry JP, Schmidt O (2007) The feeding ecology of earthworms – a review. *Pedobiologia* 50:463–477. doi:10.1016/j.pedobi.2006.09.001
- Dalling JW, Davis AS, Schutte BJ, Elizabeth Arnold A (2011) Seed survival in soil: interacting effects of predation, dormancy and the soil microbial community. *J Ecol* 99:89–95. doi:10.1111/j.1365-2745.2010.01739.x
- Decaëns T, Mariani L, Betancourt N, Jiménez JJ (2003) Seed dispersion by surface casting activities of earthworms in Colombian grasslands. *Acta Oecol* 24:175–185. doi:10.1016/S1146-609X(03)00083-3
- Donath TW, Eckstein RL (2012) Litter effects on seedling establishment interact with seed position and earthworm activity. *Plant Biol* 14:163–170. doi:10.1111/j.1438-8677.2011.00490.x
- Dray S, Dufour A (2007) The ade4 package: implementing the duality diagram for ecologists. *J Stat Softw* 22:1–20
- Eisenhauer N, Schuy M, Butenschoen O, Scheu S (2009) Direct and indirect effects of endogeic earthworms on plant seeds. *Pedobiologia* 52:151–162. doi:10.1016/j.pedobi.2008.07.002
- Eisenhauer N, Butenschoen O, Radsick S, Scheu S (2010) Earthworms as seedling predators: importance of seeds and seedlings for earthworm nutrition. *Soil Biol Biochem* 42:1245–1252. doi:10.1016/j.soilbio.2010.04.012
- Fisichelli NA, Frelich LE, Reich PB, Eisenhauer N (2012) Linking direct and indirect pathways mediating earthworms, deer, and understory composition in Great Lakes forests. *Biol Invasions* 15:1057–1066. doi:10.1007/s10530-012-0350-6
- Forey E, Barot S, Decaëns T et al (2011) Importance of earthworm–seed interactions for the composition and structure of plant communities: a review. *Acta Oecol* 37:594–603. doi:10.1016/j.actao.2011.03.001
- Fründ H-C, Butt K, Capowiez Y, Eisenhauer N, Emmerling C, Ernst G, Potthoff M, Schädler M, Schrader S (2010) Using earthworms as model organisms in the laboratory: recommendations for experimental implementations. *Pedobiologia* 53:119–125. doi:10.1016/j.pedobi.2009.07.002
- Galetti M, Guevara R, Côrtes MC et al (2013) Functional extinction of birds drives rapid evolutionary changes in seed size. *Science* 340:1086–1090. doi:10.1126/science.1233774
- Gardener CJ, McIvor JG, Jansen A (1993) Survival of seeds of tropical grassland species subjected to bovine digestion. *J Appl Ecol* 30:75. doi:10.2307/2404272
- Grace JB (2006) Structural equation modeling and natural systems. Cambridge University Press, Cambridge
- Grant JD (1983) The activities of earthworms and the fates of seeds. In: Satchell JE (ed) *Earthworm Ecology* from Darwin to Vermiculture. Chapman and Hall Ltd, London, pp. 107–122

- Harrison SK, Regnier EE, Schmoll JT (2003) Postdispersal predation of Giant ragweed (*Ambrosia trifida*) seed in no-tillage corn. *Weed Sci* 51:955–964
- Hartenstein R, Amico L (1983) Production and carrying capacity for the earthworm *Lumbricus terrestris* in culture. *Soil Biol Biochem* 15:51–54. doi:10.1016/0038-0717(83)90118-9
- Husson F, Josse J, Lê S, Mazet J (2013) FactoMineR: Multivariate exploratory data analysis and data mining with R
- Laossi K-R, Noguera DC, Bartolomé-Lasa A et al (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. doi:10.1016/j.soilbio.2009.05.009
- Laossi K-R, Noguera D-C, Barot S (2010) Earthworm-mediated maternal effects on seed germination and seedling growth in three annual plants. *Soil Biol Biochem* 42:319–323. doi:10.1016/j.soilbio.2009.11.010
- Law JJ, Gallagher RS (2015) The role of imbibition on seed selection by *Harpalus pensylvanicus*. *Appl Soil Ecol* 87:118–124. doi:10.1016/j.apsoil.2014.11.015
- Levey DJ, Grajal A (1991) Evolutionary implications of fruit-processing limitations in cedar waxwings. *Am Nat* 138:171–189
- Makeschin F (1997) Earthworms (Lumbricidae: Oligochaeta): important promoters of soil development and soil fertility. In: Fauna in soil ecosystems: recycling processes, nutrient fluxes, and agricultural production. Marcel Dekker Inc. G. Benckiser, New York, pp. 173–223
- Marhan S, Scheu S (2005) Effects of sand and litter availability on organic matter decomposition in soil and in casts of *Lumbricus terrestris* L. *Geoderma* 128:155–166. doi:10.1016/j.geoderma.2004.07.001
- McRill M, Sagar GR (1973) Earthworms and seeds. *Nature* 243:482–482. doi:10.1038/243482a0
- Milberg P, Lamont BB (1997) Seed/cotyledon size and nutrient content play a major role in early performance of species on nutrient-poor soils. *New Phytol* 137:665–672. doi:10.1046/j.1469-8137.1997.00870.x
- Moody SA, Briones MJI, Pearce TG, Dighton J (1995) Selective consumption of decomposing wheat straw by earthworms. *Soil Biol Biochem* 27:1209–1213. doi:10.1016/0038-0717(95)00024-9
- Paczkowski S, Paczkowska M, Dippel S et al (2013) Volatile combustion products of wood attract *Acanthocnemus nigricans* (Coleoptera: Acanthocnemidae). *J Insect Behav* 27:228–238. doi:10.1007/s10905-013-9430-4
- Paulsen TR, Colville L, Kranner I, Daws MI, Högstedt G, Vandvik V, Thompson K (2013) Physical dormancy in seeds: a game of hide and seek? *New Phytol* 198:496–503. doi:10.1111/nph.12191
- Sampedro L, Jeannotte R, Whalen JK (2006) Trophic transfer of fatty acids from gut microbiota to the earthworm *Lumbricus terrestris* L. *Soil Biol Biochem* 38:2188–2198. doi:10.1016/j.soilbio.2006.02.001
- Seal CE, Kranner I, Pritchard HW (2008) Quantification of seed oil from species with varying oil content using supercritical fluid extraction. *Phytochem Anal* 19:493–498. doi:10.1002/pca.1072
- Shi L, Katavic V, Yu Y et al (2012) Arabidopsis *glabra2* mutant seeds deficient in mucilage biosynthesis produce more oil. *Plant J* 69:37–46. doi:10.1111/j.1365-313X.2011.04768.x
- Shumway DL, Koide RT (1994) Seed preferences of *Lumbricus terrestris* L. *Appl Soil Ecol* 1:11–15. doi:10.1016/0929-1393(94)90019-1
- Souza FHDD, Marcos-Filho J (2001) The seed coat as a modulator of seed-environment relationships in Fabaceae. *Bras J Bot* 24:365–375. doi:10.1590/S0100-84042001000400002
- Stanley MC, Lill A (2002) Does seed packaging influence fruit consumption and seed passage in an avian frugivore? *Condor* 104:136–145. doi:10.1650/0010-5422(2002)104[0136:DSPIFC]2.0.CO;2
- Tewksbury JJ, Reagan KM, Machnicki NJ et al (2008) Evolutionary ecology of pungency in wild chilies. *Proc Natl Acad Sci* 105:11808–11811. doi:10.1073/pnas.0802691105
- Thompson K, Band SR, Hodgson JG (1993) Seed size and shape predict persistence in soil. *Funct Ecol* 7:236–241. doi:10.2307/2389893
- Traba J, Azcárate FM, Peco B (2006) The fate of seeds in Mediterranean soil seed banks in relation to their traits. *J Veg Sci* 17:5–10. doi:10.1111/j.1654-1103.2006.tb02417.x
- Traveset A, Robertson AW, Rodríguez-Pérez J (2007) A review on the role of endozoochory in seed germination. In: Seed dispersal: theory and its application in a changing world., CABI Publishing, Wallingford, UK. Dennis AJ, Schupp EW, Green RJ, Westcott DA, pp 78–103
- van Groenigen JW, Lubbers IM, Vos HMJ et al (2014) Earthworms increase plant production: a meta-analysis. *Sci Rep* 4:6365
- Willems JH, Huijsmans KGA (1994) Vertical seed dispersal by earthworms: a quantitative approach. *Ecography* 17:124–130. doi:10.1111/j.1600-0587.1994.tb00084.x
- Yang X, Baskin CC, Baskin JM et al (2013) Hydrated mucilage reduces post-dispersal seed removal of a sand desert shrub by ants in a semiarid ecosystem. *Oecologia* 173:1451–1458. doi:10.1007/s00442-013-2735-3
- Yeomans MR (1998) Taste, palatability and the control of appetite. *Proc Nutr Soc* 57:609–615. doi:10.1079/PNS19980089
- Zaller JG, Saxler N (2007) Selective vertical seed transport by earthworms: implications for the diversity of grassland ecosystems. *Eur J soil Biol* 43(Supplement 1):S86–S91. doi:10.1016/j.ejsobi.2007.08.010
- Zhang H, Schrader S (1993) Earthworm effects on selected physical and chemical properties of soil aggregates. *Biol Fertil Soils* 15:229–234. doi:10.1007/BF00361617
- Zirbes L, Mescher M, Vrancken V et al (2011) Earthworms use odor cues to locate and feed on microorganisms in soil. *PLoS One* 6:e21927. doi:10.1371/journal.pone.0021927
- Zuur AF, Ieno EN, Smith GM (2007) Analysing ecological data. Springer, New York