Acta Oecologica 64 (2015) 10–20

Contents lists available at ScienceDirect

Acta Oecologica

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

Original article

Non-native earthworms promote plant invasion by ingesting seeds and modifying soil properties

Julia Clause [a], [*], Estelle Forey [a], Christopher J. Lortie [b], Adam M. Lambert [c], Sébastien Barot [d]

[a] ECODIV Laboratory, University of Rouen, 1 Place Emile Blondel, 76821 Mont Saint Aignan, France
[b] Department of Biology, York University, Toronto, ON M3J 1P3, Canada
[c] Marine Science Institute, University of California, Santa Barbara, CA 93106, USA
[d] IRD-IEES-P, ENS-Ulm, 46 rue d'Ulm, 75230 Paris Cedex 5, France

ARTICLE INFO

Article history:
Received 30 May 2014
Received in revised form
5 February 2015
Accepted 13 February 2015
Available online

Keywords:
Invasive species
Californian grassland
Earthworm casts
Feedback
Interactions
Seed bank

ABSTRACT

Earthworms can have strong direct effects on plant communities through consumption and digestion of seeds, however it is unclear how earthworms may influence the relative abundance and composition of plant communities invaded by non-native species. In this study, earthworms, seed banks, and the standing vegetation were sampled in a grassland of central California. Our objectives were i) to examine whether the abundances of non-native, invasive earthworm species and non-native grassland plant species are correlated, and ii) to test whether seed ingestion by these worms alters the soil seed bank by evaluating the composition of seeds in casts relative to uningested soil. Sampling locations were selected based on historical land-use practices, including presence or absence of tilling, and revegetation by seed using *Phalaris aquatica*. Only non-native earthworm species were found, dominated by the invasive European species *Aporrectodea trapezoides*. Earthworm abundance was significantly higher in the grassland blocks dominated by non-native plant species, and these sites had higher carbon and moisture contents. Earthworm abundance was also positively related to increased emergence of non-native seedlings, but had no effect on that of native seedlings. Plant species richness and total seedling emergence were higher in casts than in uningested soils. This study suggests that there is a potential effect of non-native earthworms in promoting non-native and likely invasive plant species within grasslands, due to seed-plant-earthworm interactions via soil modification or to seed ingestion by earthworms and subsequent cast effects on grassland dynamics. This study supports a growing body of literature for earthworms as ecosystem engineers but highlights the relative importance of considering non-native-native interactions with the associated plant community.

1. Introduction

Recent studies have focused on direct impacts of earthworms on plant communities via their ingestion and sometimes digestion of seeds. Earthworms aggregate seeds in their casts, which contain more viable seeds than in the surrounding soil (Decaëns et al., 2003). This ingestion is often species-specific with earthworms selecting seeds according to traits such as size or oil content (Clause et al., 2011; Grant, 1983) or by plant functional groups (Milcu et al., 2006; Zaller and Saxler, 2007). Seedlings that emerge from casts are likely to benefit from their higher nutrient content and physical protection (Bityutskii et al., 2012; Decaëns et al., 2003; Zhang and Schrader, 1993). The outcome in terms of seedling growth and survival should depend on the combination of plant and earthworm species (Eisenhauer et al., 2009a; Milcu et al., 2006). Thus, direct impacts of seed-earthworm interactions through seed ingestion are important for the composition and structure of plant communities (Forey et al., 2011).

Many grasslands are invaded by non-native plant species globally (Seastedt and Pyšek, 2011). Invasion by non-native earthworms can be an important factor of plant invasion because earthworms can negatively affect both soil and plant biodiversity patterns (Hale et al., 2008; Hendrix et al., 2008; Hendrix and Bohlen, 2002; Holdsworth et al., 2007). Non-native (=exotic) species are non-

---

[*] Corresponding author.
E-mail address: juliamclause@gmail.com (J. Clause).

http://dx.doi.org/10.1016/j.actao.2015.02.004
1146-609X/© 2015 Published by Elsevier Masson SAS.

native to a region and have been introduced into a region through human activities. Through subsequent dispersal and population expansion, they can cause significant economic or environmental damages to incipient communities, thereby becoming invasive species (IUCN, 2000). One of the most documented ecosystem alterations by non-native earthworms is the modification of the northern American hardwood forests following the colonization by European earthworms (Frelich et al., 2006; Hale et al., 2005). Indirect impacts of earthworms on plant community via soil modifications have also often been reported (Eisenhauer et al., 2009b, 2007; Holdsworth et al., 2007; Nuzzo et al., 2009), but only two studies have focused on the direct impact of non-native, invasive earthworms on plant communities through seed ingestion (Drouin et al., 2014; Eisenhauer et al., 2009b). In the first study, the effects of invasive earthworms, *Lumbricus terrestris* and *Octoaedra tyrtaeum* on seedling emergence in American northern hardwood forests were examined (Eisenhauer et al., 2009b). The presence of the endogeic *O. tyrtaeum* significantly increased the emergence of all seedlings while the presence of the anecic *L. terrestris* increased the emergence of herb seedlings only. The second study showed that invasive earthworms reduce seed germination of seven species and survival of three species of trees in southern Québec (Drouin et al., 2014). Both studies showed an impact of non-native, invasive earthworms on the seed bank and standing vegetation.

Several mechanisms have been identified to explain a positive interaction between non-native earthworms and non-native plants. In disturbed systems, mutualisms and synergisms between non-native plant and non-native animal species impact both plant and animal communities (Catford et al., 2012; Mitchell et al., 2006; Richardson et al., 2000). These facilitative interactions include the predation of native species by generalist non-native predators (Mitchell et al., 2006; Parker et al., 2006) or the modification of the environment physico-chemical properties (Didham et al., 2007; see Mitchell et al., 2006). The interactions between non-native species, and with their physico-chemical environment can lead to positive feedback loops, leading to drastic and irreversible changes in ecosystem functioning and the composition of communities that characterize an ‘invasional meltdown’ (Simberloff and Holle, 1999). Similarly, interactions of invasive earthworms with invaded plant communities favors a decline in native plant species and appears to facilitate plant invasions, at least in American northern hardwood forests (Eisenhauer et al., 2009b; Frelich et al., 2006; Hale et al., 2005). Nuzzo et al. (2009) also suggested that earthworm invasion, rather than non-native plant invasion, was the driving force behind changes in forest plant communities in Northeastern North America.

The general hypothesis of this study is that the abundance of non-native earthworm species is correlated with non-native grassland plant species and that selective ingestion of non-native seeds influences the species composition of seed in casts relative to that in the soil. The following predictions were tested: i) the abundance of non-native earthworms is positively correlated with the abundance of non-native plants, ii) non-native earthworms influence non-native seedling emergence from the seed bank via a facilitative non-native-non-native interaction and, iii) non-native earthworms favor the seedling emergence of herbs and grasses compared to leguminous species due to their preferential selection of seeds.

2. Materials and methods

2.1. Study system

This study was conducted at the Kenneth S. Norris Rancho Marino Reserve (KNRMR) in San Luis Obispo County, California (N 35°32.36–N 35°31.36 and W 121° 05.70–W 121° 04.8). Annual average temperatures range from 2 °C in January to 37 °C in July. Average annual rainfall is 460 mm. Our sampling was done on 26.7 ha of coastal grassland located on Concepcion loam (NCRS Soil Survey USDA, 2013). Concepcion loams are very deep loamy sands with moderate drainage and moderate available water capacity. Soil pH is slightly acidic and increases with depth.

This coastal grassland site was formerly used for agricultural purposes and was tilled until the 1950s (D. Canestro, reserve manager, personal communication). Soil disturbance and tillage are known to promote plant invasion worldwide (MacDougall and Turkington, 2005; Seastedt and Pyšek, 2011). Non-native species such as *Bromus* spp., *Plantago lanceolata*, *Festuca perennis*, and *Erodyum botrys* are categorized as invasive in California grasslands (Calflora, 2012), and are abundant throughout much of the grasslands at KNRMR. However, the area of grassland located along the coastal cliff had never been tilled and harbors native plant species including *Agoseris apargioides*, *Armeria maritima, Calystegia macrostegia*, *Distichlis spicata* and *Isocoma menziesii*. In addition to tillage, half of the study area, including a portion of the tilled and untilled areas, was sown with the grass *Phalaris aquatica* before the land was taken out of agricultural production (D. Canestro, personal communication). *P. aquatica* is recognized as an invasive species in San Luis Obispo County, although the California Invasive Plant Council classifies its potential impact on native ecosystems as moderate (see Calflora, 2012). We believed that its presence might have affected soil properties and belowground communities, although the relative frequency of the species remained low. Therefore, sown and unsown areas were both sampled.

The KNRMR had never been sampled for earthworms (S. James, personal communication). In Santa Barbara County, Wood and James (1993) identified eight introduced earthworm species (seven European and one South American species) and two native species that were never recorded before (*Ocnerodrilus* sp. and *Argilophilus* sp.). Preliminary sampling at KNRMR (J. Clause measurements) showed 0 to 120 individuals m^{-2} with variation across grasslands and forests. In the study area, only non-native endogeic earthworm species were found and identified: *Aporrectodea trapezoides* (25%), *Aporrectodea caliginosa* (12%), *Allolobophora chlorotica* (0.9%) and *A. rosea* (0.1%). *A. trapezoides* is recognized as an invasive species in Californian grasslands (Hendrix and Bohlen, 2002; Winsome et al., 2006).

2.2. Experimental design

All sampling was done in December 2011 along 20 transects parallel to the coastline running North to South. Five 12 m transects were sampled in each of the following four factor combinations (FC) – invaded/unsown, invaded/sown, uninvaded/unsown, uninvaded/sown. These factor combinations were the result of previous land management and were not the result of manipulation from our part. The presence/absence of the native plant species listed above was the basis of our invaded vs. uninvaded factor (see Section 2.1). The sowing of *P. aquatica* 40 years ago in some areas, but not others, was the basis of our sown vs. unsown factor. Each transect was subdivided into three 4-m plots equaling a total of 60 plots. Standing vegetation, earthworms, casts, and soil were sampled in each plot (Fig. 1).

2.3. Earthworm sampling

The December early rain (not measured) facilitated earthworm sampling. In each plot, a single 50 × 50 × 25 cm^3 hole was dug (Fig. 1). The soil was excavated and earthworms were manually sampled and hand-sorted. This method of sampling has been

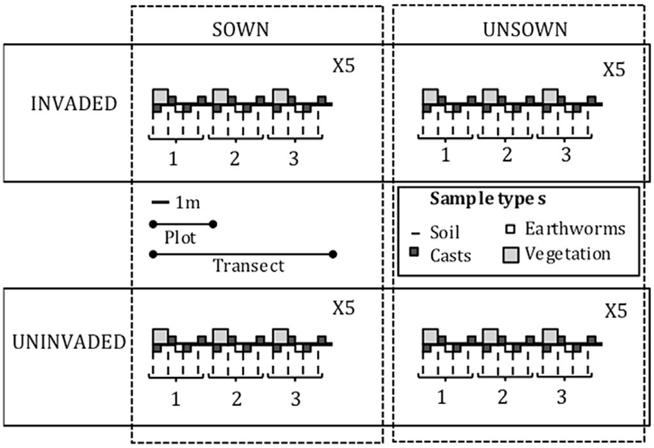

Fig. 1. Schema of the sampling protocol in each of the four sowing/invasion combinations. Standing vegetation, earthworms, casts and soil cores were sampled in three plots within each transect. Four soil cores and four cast samples were pooled within each plot. Each soil core was divided into three depths (0–2 cm, 2–5 cm, 5–10 cm) before soil analyses.

proven to be the most efficient since a higher diversity of earthworm species can be collected (Lawrence and Bowers, 2002). Earthworms were then killed and fixed in denatured alcohol. Earthworms were counted and identified with the Fender and McKey-Fender identification key (Fender and McKey-Fender, 1990).

2.4. Soil analyses

In each plot, a soil core was sampled for soil analyses (ø 2 cm; Fig. 1). Soil samples were sieved (2 mm-mesh sieve). Soil moisture content was measured after oven-drying 10 g of soil (100 °C, 24 h). NH_4^+ and NO_3^- contents were measured after extraction from fresh soil with a 2 M KCl solution (QuikChem 8500, Lachat Instruments, Colorado, USA). All samples were then air-dried and total carbon and total nitrogen contents were measured with a CN Elemental Analyzer (Carlo Erba Instrumentazione, Milan, Italy). pH and conductivity were measured with a glass electrode (soil:water at 1:5, ISO 10390).

2.5. Sampling of standing vegetation

In each plot, absolute cover abundance for each species was estimated within a 1 × 1 m^2 quadrat (Fig. 1). Species or genera were identified with the nomenclature of Carter et al. (2003). The invasiveness of species (native, non-native and non-native, invasive) was determined with the Calflora database (Calflora, 2012) (Table 1). In addition to invasiveness, species were also categorized according to their plant functional group: herbs (non-leguminous), grasses and leguminous species (Table 1). The aim was to determine what plant communities were more or less associated with the presence of earthworms.

2.6. Seedling emergence from soil and cast seed banks

In each plot, earthworm surface casts were manually collected in four 50 × 50 cm^2 equally spaced quadrats (one person, 5 min/quadrat). Casts from these four quadrats were then pooled to obtain a single sample per plot (Fig. 1).

In each plot, four soil seed banks were collected with a soil core (ø 2 cm, every 50 cm, circa 31 cm^3). Each soil core was divided into three depths (a = 0–2 cm, b = 2–5 cm, c = 5–10 cm) to allow for a comparison of patterns of seedling emergence between soil and cast samples. Samples from the four soil cores were pooled to obtain one sample per plot and per depth.

All soil/cast samples were dried (36 °C, 36 h), weighed and spread onto trays to allow seeds to germinate. Trays were previously filled with vermiculite, covered with wet cheesecloth and placed into a greenhouse under daylight. Samples were watered daily and control trays were set up to check for external aerial seed contamination. No contamination of the trays occurred. Seed germination was tracked weekly for six months and seedlings were identified and counted. Identified seedlings were eliminated and seedlings that could not be identified were grown further until identification was possible. Some seedlings could not be identified to species or to the genus. Thus, *Bromus* sp. and *Briza* sp. were pooled into a general category "annual exotic species" (Table 1). As with the standing vegetation, the invasiveness of species was determined with the Calflora database (Calflora, 2012), and seedlings were grouped into grass, herb, or legume functional groups (Table 1). Seedlings that died during the experimentation before identification were only taken into account in the calculation of the total seedling density (0.2% of the total density).

2.7. Statistical analyses

A Principal Component Analysis (PCA) was conducted to reveal relationships between soil properties and the earthworm community. PCA is a multivariate analysis that reduces the original number of dimensions, i.e. variables considered, of a dataset into fewer dimensions (*principal components*) that represent linear combinations of the original variables. PCA is represented by i) a two-dimensional graph (plane) where each axis corresponds to a principal component, and ii) by a circle of correlations that shows how much each variable explains the data variability as well as correlations between variables and the first two axes. After checking for colinearity, the PCA was performed with a matrix of 60 individuals (= plots) and seven variables (six soil parameters + earthworm total abundance). Abundances of each earthworm species and proportions of native, non-native and non-native, invasive species in the standing vegetation were used as illustrative variables, i.e. they did not contribute to the total dispersion of the data, but improved the interpretation of variability.

Non-parametric permutation tests were used to compare species frequencies of native, non-native and non-native, invasive species or each functional group between all sample types (n. permutations = 10,000). Appropriate p-values for non-parametric tests for multiple comparisons were performed with selected using a Bonferroni correction.

A generalized linear mixed modeling (GLMM) analysis was used to examine the effect of the factor combinations (FC; invaded/unsown, invaded/sown, uninvaded/unsown, uninvaded/sown), of sample type (S; cast and soil layers a, b and c) and earthworm total abundance (EW) on the species richness and abundance of germinating seedlings (n = 240). We considered the following response variables of germinating seedlings: total species richness and abundance, grouped into "total", "natives", "non-natives", "invasives", "herbs", "grasses" and "legumes". Species that could not clearly be considered as native, non-native or non-native, invasive species were called "undetermined" and were not considered in the grouped analyses (see Tables 1 and 2). GLMMs are used to account for dependence of replicates. In our case, all response variables were best modeled by a Poisson distribution and, because plots were nested into transects, the transect identity was used as random factor (Bolker et al., 2009). Models of best fit were selected using Akaike Information Criterion (AIC) from the full model (with Full model = response

Table 1
List of plant species grouped by native vs. non-native species for Californian grasslands and into plant functional groups. The presence within sample types for each factor combination (sown/uninvaded, sown/invaded, unsown/uninvaded, insown/invaded) is also listed. The following are the abbreviations listed. Veg: standing vegetation, Cast: casts, a,b,c: soil layers a,b,c (0–2 cm, 2–5 cm, 5–10 cm). Non-native species identified as invasive are marked with (i). Plant abbreviations are indicated.

Plant species	Abb	Invasiveness	Plant functional group	Sown, uninvaded	Sown, invaded	Unsown, uninvaded	Unsown, invaded
Agoseris apargioides (Less.) Greene	Aap	Native	Herb	Veg		Veg	
Armeria maritima (Mill.) Willd.	Ama	Native	Herb	Veg		Veg	
Anagallis arvensis L.	Aar	Non-native	Herb	Veg,Cast,a,b,c	Veg,Cast,a,b,c	Veg,Cast,a,b,c	Veg,Cast,a,b,c
Brachypodium distachyon Beauv	Bdi	Non-native (i)	Grass			b,c	Cast, a,b,c
Calandrinia sp.	Casp	Native	Herb	Cast,b		a,b,c	
Calystegia macrostegia (Greene) Brummitt	Cma	Native	Herb	Veg		Veg	
Cerastium glomeratum Thuill.	Cgl	Non-native	Herb	Veg		Veg	
Clarkia davyi (Jeps.) F.H.Lewis & M.R.Lewis	Cda	Native	Herb			Cast,c	
Crassula sp.	Crsp	Native or non-native	Herb			b	
Cirsium vulgare (Savi) Ten.	Cvu	Non-native (i)	Herb		Veg		
Danthonia californica Bol.	Dca	Native	Grass			Veg	
Distichlis spicata (L.) Greene	Dsp	Native	Grass	Veg		Veg	
Erigeron glaucus	Egl	Native	Herb			Veg	
Erodium botrys (Cav.) Bertol.	Ebo	Non-native (i)	Herb	Veg	Veg	Veg	Veg
Festuca perennis Lam.	Fpe	Non-native (i)	Grass	Veg,b	Veg	Veg	Veg,a
Festuca sp.	Fsp	Native to invasive	Grass	Veg	Veg	Veg	Veg
Geranium dissectum L.	Gdi	Non-native (i)	Herb				a
Geranium sp.	Gsp	Native to invasive	Herb		Veg		Veg
Gnaphalium palustre Nutt.	Gpa	Native	Herb	c		Cast,a,c	
Grindelia stricta DC	Gst	Native	Herb	Veg			
Hemizonia congesta DC.	Hco	Native	Herb	a,b,c		a,b,c	Cast,a,b,c
Hirschfeldia incana (L.) Lagréze-Fossat	Bge	Non-native (i)	Herb		Veg		
Hypochaeris radicata L.	Hra	Non-native (i)	Herb	Veg		Veg	Veg
Isocoma menziesii (Hook. & Arn.) G. Nesom	Ime	Native	Herb	Veg		Veg	
Juncus sp.	Jsp	Native	Grass	Cast,a,b,c	Cast,a,b,c	Cast,a,b,c	Cast,a,b,c
Lotus humistratus Greene	Lhu	Native	Legume			c	
Lythrum hyssopifolia L.	Lhy	Non-native (i)	Herb				Cast,a,b,c
Medicago polymorpha L.	Mpo	Non-native (i)	Legume	c			Cast
Nassella pulchra (Hitchc.) Barkworth	Npu	Native	Grass				c
Oxalis pes-caprae L.	Ope	Non-native (i)	Herb			Veg	
Phalaris aquatica L.	Paq	Non-native (i)	Grass	Veg	Veg		
Plantago lanceolata L.	Pla	Non-native (i)	Herb	Veg	Veg	Veg	Veg
Plantago sp.	Psp	Native to invasive	Herb	Cast,a,b,c	Cast,c	Cast,b,c	
Rumex acetosella L.	Rac	Non-native (i)	Herb			Veg	Veg
Silene gallica L.	Sga	Non-native	Herb	Cast,a,b,c	Cast,a,b,c	Cast,a,b,c	Cast
Sonchus sp.	Ssp	Non-native to invasive	Herb		Cast	Cast	
Spergularia macrotheca (Hornem.) Heynh.	Sma	Native	Herb	c		a,c	a
Stellaria media (L.) Vill.	Sme	Non-native	Herb	a,b	c	Cast,a,b,c	Cast,a,b,c
Trifolium sp.	Tsp	Native or non-native	Legume	Veg		Veg,c	Veg
Trifolium subterraneum L.	Tsu	Non-native	Legume	Veg		a,b	Veg,b
Vulpia microstachys (Nutt.) Munro	Vmi	Native	Grass	Cast,a,b,c	a	a,b	a
Vulpia myuros (L.) C.C.Gmel.	Vmy	Non-native	Grass	Cast,a,b,c	Cast,a,b,c	Cast,a,b,c	Cast,a,b,c
Annual exotic grasses	AEG	Non-native (i)	Grass	Cast,a,b,c	Cast,a,b,c		Cast,a

variable ~ FC + S + FC*S + EW + 1|transect identity). Three-way interactions were not significant. Tests for multiple comparisons were performed with a Bonferroni correction to determine which samples were significantly different from each other within each factor interaction (FC*S; See Online Resources 1 and 2). Mann–Whitney U tests were performed to compare the germination patterns of seedlings of non-native vs. native species in casts.

To test the contribution of qualitative (factor combination and sample type) and quantitative (earthworm abundance) variables on plant composition, we performed a distance-based redundancy analysis (db-RDA) according to Legendre and Anderson (1999). db-RDA is similar to PCA, but is adapted to non-Euclidean distances such as data of species frequency. We used the Bray–Curtis distance measure on a matrix of species frequencies with 300 samples (rows) and 40 plant species (columns). For each seed bank sample, species frequencies were calculated as the number of seedlings of each species divided by the total number of seedlings in that sample. Singletons (i.e. species that were represented by only one individual in a single sample) were excluded from our analysis. The effects of each variable were tested with a Monte-Carlo permutation test (n. permutations = 9999).

All analyses were performed with the 'R' statistical and programming environment (R Core Team, 2013) including the following packages: 'ade4' (Dray and Dufour, 2007) and 'FactoMineR' (Husson et al., 2013) for the PCA, 'lme4' (Bates et al., 2013) and 'multcomp' (Hothorn et al., 2013) for the GLMM, and 'vegan' (Oksanen et al., 2013) for the db-RDA.

3. Results

3.1. Earthworm abundance and soil properties

Earthworm abundance and composition, and plant composition were correlated with factor combinations. The first PCA axis explained 40% of the total variance and showed a gradient of invasion where the invaded/sown combination was isolated from other combinations (Fig. 2a). This invaded/sown combination was characterized by high levels of C/N, C, moisture and NO_3^-, as well as a high abundance of earthworms, as indicated by the length of the arrows in the positive values of Axis 1 of the correlation circle (r = 0.8, r = 0.8, r = 0.4, r = 0.8 and r = 0.6 respectively; $p < 0.001$; Fig. 2b; Online Resource 1). The uninvaded areas were

Table 2

Mean species richness and seedling emergence (%) in each sample types in all factor combinations tested in greenhouse trials. The responses are grouped into native, non-native and non-native, invasive species and into grass, herb and legume species. Total proportions of grass, herb and legume species within each degree of invasiveness are included. A mean comparison was tested with a non-parametric permutation test (perm.anova, n.perm = 10000) to compare differences in richness and germination. Standard errors are indicated within parentheses and different letters denote significant differences between sample types at $p < 0.05$.

	Vegetation	Cast	Soil layer a	Soil layer b	Soil layer c	Statistical significance
Species richness	5.4 (0.3)^{a}	2.5 (0.2)^{b}	2.8 (0.1)^{b}	3.2 (0.2)^{b}	3.2 (0.1)^{b}	$F_{(4,300)} = 2.56^{****}$
Species frequencies (%)						
Natives	8.5 (2.0)^{b}	16.3 (3.5)^{ab}	11.8 (2.4)^{ab}	15.7 (2.8)^{ab}	19.8 (2.7)^{a}	$F_{(4,300)} = 2.56^{****}$
Grasses	37.3	78.1	71.8	75.6	91.0	
Herbs	62.7	21.9	28.2	24.4	7.8	
Legumes	0.0	0.0	0.0	0.0	1.2	
Non-natives	45.0 (4.4)^{b}	56.1 (4.8)^{ab}	73.2 (3.9)^{a}	73.9 (3.2)^{a}	73.5 (2.8)^{a}	$F_{(4,300)} = 11.68^{****}$
Grasses	64.9	33.5	24.5	13.6	14.7	
Herbs	33.8	66.0	75.2	85.2	84.9	
Legumes	1.2	0.5	0.2	1.2	0.4	
Undetermined[a]	46.5 (4.2)^{a}	20.9 (4.1)^{b}	12.8 (3.1)^{b}	10.4 (2.3)^{b}	6.6 (1.7)^{b}	$F_{(4,300)} = 24.75^{****}$
Grasses	85.5	80.0	93.7	86.8	75.6	
Herbs	1.5	20.0	6.3	13.2	22.0	
Legumes	13.0	0.0	0.0	0.0	2.4	
Invasives[b]	31.7 (4.1)^{a}	0.8 (0.6)^{b}	0.2 (0.2)^{b}	0.5 (0.4)^{b}	1.1 (0.2)^{b}	$F_{(4,300)} = 55.60^{****}$
Grasses	62.4	33.3	100.0	50.0	55.6	
Herbs	37.6	33.3	0.0	50.0	22.2	
Legumes	0.0	33.3	0.0	0.0	22.2	
Grasses	71.3 (2.5)^{a}	46.2 (4.4)^{b}	39.5 (3.3)^{b}	30.0 (3.2)^{b}	32.9 (3.2)^{b}	$F_{(4,300)} = 24.10^{****}$
Herbs	21.8 (2.5)^{c}	46.6 (4.4)^{b}	58.7 (3.3)^{ab}	69.4 (3.1)^{a}	66.2 (3.1)^{a}	$F_{(4,300)} = 33.10^{****}$
Legumes	6.9 (1.6)^{a}	0.6 (0.6)^{b}	0.1 (0.1)^{b}	0.6 (0.5)^{b}	0.9 (0.6)^{b}	$F_{(4,300)} = 11.87^{****}$

$^{*}p < 0.05$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

[a] Species that could not be categorized as native or non-native (see Materials and methods).

[b] Percentage of the total seedling emergence that were non-native, invasive only.

characterized by a high pH, as indicated by the length of the pH arrow pointing towards negative values of Axis 1 (Fig. 2b). The second axis explained 19% of the total variance and discriminated the uninvaded/sown combination. It was characterized by high levels of C/N and NH_4^+ and by a low abundance of earthworms (r = 0.5, r = 0.4, r = −0.6 respectively; $p < 0.001$, Fig. 2b; Online Resource 1). Adding supplementary variables confirmed that the invaded/sown combination was dominated by non-native and non-native, invasive plants, and showed that this standing vegetation was associated with a high abundance of earthworms (r = 0.58; $p < 0.001$). This earthworm population was mostly composed of juveniles, *A. caliginosa* and *A. trapezoides* (r = 0.91, r = 0.65 and r = 0.59 respectively; $p < 0.001$). Uninvaded areas contained a larger population of native plant species and were characterized by a low abundance of earthworms (r = −0.4; $p < 0.01$; Online Resources 1 and 2).

3.2. Seed bank composition across samples

Within the whole study, a total of 2361 seedlings germinated from casts and soil seed bank samples (casts:388, a:549, b:700, c:724). A total of 23 and 24 species or genera were identified from

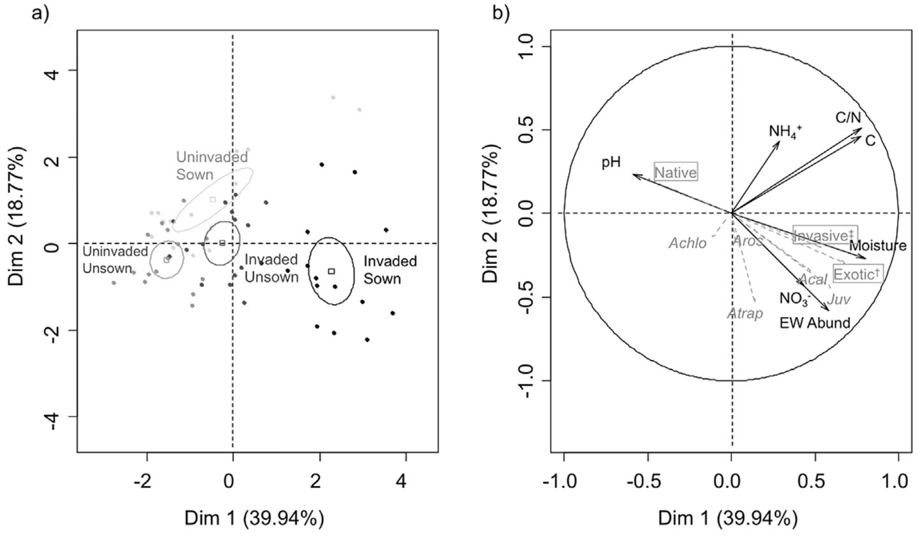

Fig. 2. Principal Component Analysis (PCA) with eight variables (seven soil characteristics and earthworm abundance) within the four factor combinations. Plot of individual samples grouped by treatment (a) and correlation circle of variables (b). Earthworm species and invasiveness of plant species (framed) were used as illustrative variables in the correlation circle: *Atrap: Ap. trapezoides*, *Acal*: *Ap. caliginosa*, *Aros*: *Ap. rosea*, *Achlo*: *All. chlorotica*, *Juv*: juveniles. Dim 1 and Dim 2 indicate the percentage of variance explained by axes 1 and 2. Ellipses indicate the center of gravity of samples with 67% of samples within the ellipse.

the standing vegetation and from the seed banks, respectively. Overall, significantly more species were sampled in the standing vegetation than in the other sample types (permanova; $F_{(4,300)} = 2.56$; $p < 0.0001$; Table 2). The proportion of native species was higher in all soil and cast samples than in the standing vegetation, although a significant difference was only found between the standing vegetation and soil layer c ($p = 0.01$). All samples were dominated by non-native species (not tested) and only the standing vegetation showed a high proportion of non-native, invasive species ($F_{(4,300)} = 55.60$; $p < 0.0001$; Table 2). Grasses dominated the standing vegetation, while herbs dominated soil samples (not tested). The proportion of grasses and legumes was significantly higher in the standing vegetation than in the other sample types, but that of herbs was significantly lower ($p < 0.0001$; Table 2). Native species were most represented by grasses in the soil and cast samples, but by herbs in the standing vegetation (Table 2). On the other hand, non-native species were most represented by grasses in the standing vegetation and by herbs in the cast and soil samples. Invasive species were mostly grasses in the standing vegetation and soil layers a and c (Table 2). A very low percentage of invasive species and legumes emerged from all samples.

3.3. *Effects of earthworms and factor combinations on seedling emergence*

Earthworm abundance significantly affected only the seedling emergence, and increased that of non-native and non-native, invasive species (GLMM; $p = 0.05$ and $P = 0.04$ within model; Table 3).

The interaction between factor combination and sample type influenced species richness ($p = 0.02$; Table 3). Species richness was higher in casts than in the soil samples except in the sown/invaded combination ($p = 0.002$; Fig. 3a; Online Resource 1). This sown/invaded combination had a significantly lower species richness and seedling emergence in casts than in the surface soil layer (Online Resource 2). Both the factor combination and the sample type – but not their interaction – affected the total seedling emergence ($p = 0.02$ and $p < 0.0001$ respectively; Table 3). For all samples types, it was the highest in the unsown/invaded combination (Fig. 3b; Online Resource 2). Total seedling emergence was also higher in casts than in soil samples in all combinations, except in the sown/invaded combination ($p = 0.03$; Fig. 3b; Online Resource 2).

The interaction between factor combination and sample type influenced the emergence of native and non-native seedlings ($p = 0.001$ and $p = 0.02$; Table 3). More native seedlings emerged from the unsown/invaded combination than from the other factor combinations ($p < 0.0001$; Fig. 3c; Online Resource 2). Only in that unsown/invaded combination did more native seedlings emerge from casts than from soil samples ($p = 0.003$, Fig. 3c; Online Resource 2). Non-native seedling emergence was significantly higher in the unsown/invaded combination than in all the other ones ($p < 0.001$; Fig. 3d; Online Resource 2). Contrary to native species, more non-native seeds emerged from casts than from the soil samples in the unsown/uninvaded combination only ($p = 0.04$; Fig. 3d; Online Resource 2). Both the factor combination and earthworm abundance influenced the seedling emergence of invasive species ($p = 0.005$ and $p = 0.04$; Table 3). More invasive seeds emerged from casts than from soil samples in the unsown/invaded combination (statistics not shown) but no difference of emergence was found between combinations. Significantly more non-native seeds than native seeds germinated in casts in all combinations (Mann–Whitney U test; $V = 1297$; $p < 0.0001$), except in the invaded/unsown combination (Mann–Whitney U test; $V = 70$; $p = 0.3$).

The factor combination and the sample type individually affected the emergence of herbs (GLMM, $p = 0.0001$ and $p < 0.0001$ respectively; Table 3). The emergence of grasses was influenced by their interaction ($p = 0.01$; Table 3) and the emergence of legumes was significantly affected by the factor combination only ($p = 0.04$; Table 3). More grasses emerged in the invaded sampled areas than in the uninvaded sampled areas ($p < 0.0001$; Fig. 3e; Online Resource 2), with more grass seedlings emerging from casts than from other samples ($p = 0.02$; Fig. 3e; Online Resource 2). More herbs emerged in the unsown/invaded combination than in the other combinations ($p = 0.0002$; Fig. 3f; Online Resource 2), with a similar pattern for seedling emergence in casts. The seedling emergence of herbs was the lowest in casts in the sown/invaded combination. Results showed no significant difference of legume emergence between factor combinations or between sample types (statistics not shown). The species richness and densities of emerging seedlings decreased with sample depth.

3.4. *Earthworm and treatment effect on plant community structure*

The distance-based redundancy analysis (db-RDA) showed that the factor combination, sample type (standing vegetation, cast or soil layers), their interaction and the earthworm abundance

Table 3

The impacts of the sowing/invasion factor combination (FC), sample type (S), their interactions and earthworm abundance on seedling emergence. A Generalized Linear Mixed Models (GLMM) with the Akaike Information Criterion (AIC) is presented for all seeds (Total), seeds from native (Natives), non-native species (Non-natives) and non-native, invasive (Invasives) and belonging to the three functional groups (Grasses, Herbs and Legumes).

	Full model[a]	Factor combination (FC)	Sample (S)	Earthworm abundance (EW)	FC* S	Selected model			
						Formula	d.f.	LL	AIC
Species richness	854	1074	861***	1075	853*	FC* S	17	−409.7	853
Seedling abundance									
Total	1454	1543***	1467***	1558	1454	FC + S	9	−717.0	1452
Natives	795	802***	831	830	793**	FC* S	18	−378.3	793
Non-natives	1360	1455***	1375***	1465.	1361*	FC* S + EW	18	−660.8	1360[b]
Invasives[c]	114	114**	123	119*	126	FC + EW	6	−50.1	112
Grasses	922	940***	937*	940.	921*	FC* S	18	−442.3	921
Herbs	1250	1304***	1262***	1319	1250	FC + S	9	−614.7	1247
Legumes	116	103*	109	105	115	FC	5	−46.7	103

d.f. degree of freedom; LL: log-likelihood.

*,**,***, '.' Levels of significance with $\alpha < 0.05, 0.01, 0.001, 0.1$ of models compared to a null model with no factor.

[a] Full model = FC + S + FC*S + EW + 1|transect identity.

[b] The full model was selected due to its lower AIC and significant effect of earthworm abundance with $P = 0.05$.

[c] Percentage of the total seedling emergence that were non-native, invasive only.

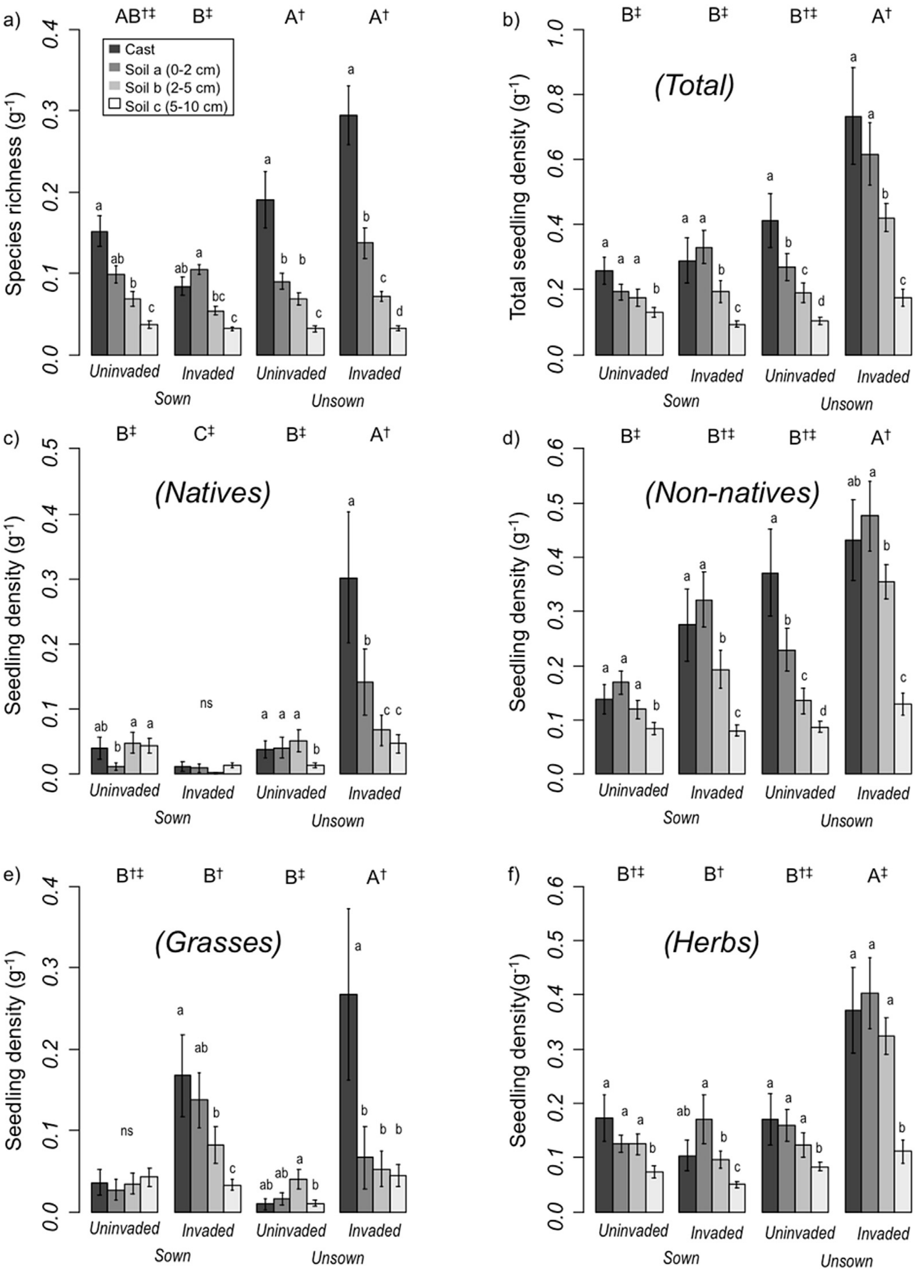

Fig. 3. Species richness (a) and seed densities of total germinated seeds (b), germinated seeds of native species (c), non-native species (d), herb species (e) and grass species (f). Capital letters indicate a significant different effect of the sample compartment between treatments. Small letter indicates significant different effects of the sample compartment within each sowing/invasion factor combinations ($p < 0.05$). Symbols (†,‡) indicate a significant different effect of treatment within casts. ns: not significant.

explained approximately 20% of the total variance of the species composition for all analyses except that of legumes (43%; Table 4). Sample type was the most important explanatory factor for variations in total species composition (43%) and was superior to the interaction (33%) and the factor combination alone (22%). The variation in the composition of native species was most explained by the interaction between sample type and factor combination (47%) than by each factor alone (app. 25% each) (Table 4). Finally, the variation in the composition of non-native, grass, herb and legume species was explained more by the sample type than by the other factors. The abundance of earthworms had no effect on the species composition for any plant categories.

The graphic representation of the db-RDA showed that the influence of sample type on species composition was associated with an effect of the standing vegetation (Fig. 4). Axis 1 explained 8.5% of the total variance and discriminated the standing vegetation from

Table 4
Contribution of the sowing/invasion factor combination (FC) and sample type (S), their interaction, and earthworm abundance (EW) on the structure and composition of the seedling and plant community. A Monte-Carlo permutation test was used (n = 9999) on the db-RDA. % c.i.: Percentage of constrained inertia.

	Total			Natives			Non-natives			Invasives[a]			Grasses			Herbs			Legumes		
	Inertia	p	% c. i.	Inertia	p	% c. i.	Inertia	p	% c. i.	Inertia	p	% c. i.	Inertia	p	% c. i.	Inertia	p	% c. i.	Inertia	p	% c. i.
Total	964.23			964.23			913.15			387.47			818.39			618.95			108.88		
Treatment (T)	49.56	<0.0001	22.12	19.37	<0.0001	25.57	49.88	<0.0001	24.63	24.48	<0.0001	29.66	41.75	<0.0001	22.17	31.62	<0.0001	24.08	5.91	<0.0001	12.68
Sample Tyoe (S)	97.27	<0.0001	43.41	19.69	<0.0001	25.99	79.88	<0.0001	39.45	28.31	<0.0001	34.29	82.28	<0.0001	43.70	54.44	<0.0001	41.46	23.26	<0.0001	49.89
EW Abundance (EW)	3.00	0.2092	1.34	1.00	0.55	1.32	2.68	0.32	1.32	1.13	0.34	1.37	2.53	0.27	1.34	1.93	0.27	1.47	0.15	0.63	0.31
T * C	74.24	<0.0001	33.13	35.70	<0.0001	47.12	70.06	<0.0001	34.60	28.63	<0.0001	34.69	61.74	<0.0001	32.79	43.34	<0.0001	33.00	17.04	<0.0001	36.54
Residual	740.15			346.65			710.66			304.90			630.09			487.63			62.26		

[a] Percentage of the total seedling emergence that were non-native, invasive only.

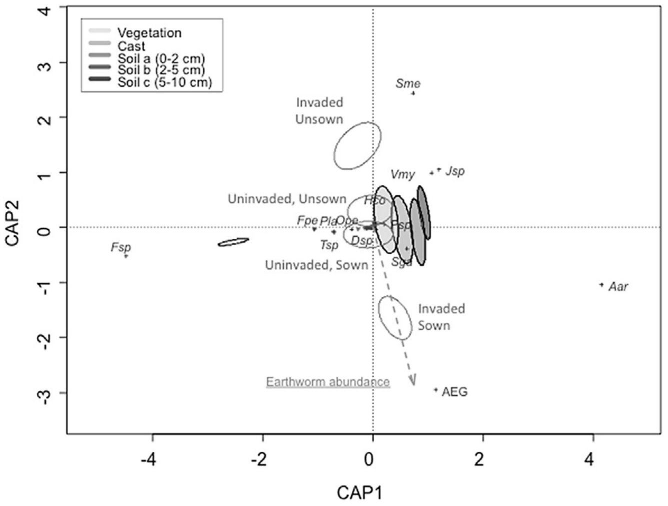

Fig. 4. Seedling species composition within the sowing/invasion factor combination and samples (Standing vegetation, Cast, soil layers a, b and c) using a distance-based redundancy analysis (db-RDA) with the Bray–Curtis measure of dissimilarity. Ellipses indicate gravity center of factors with a 0.95% confidence interval.

the other sample types. This was due to the abundance of *Festuca* sp. and of some invasive, grass and legume species that were only present in the standing vegetation. Axis 2 explained 3.4% of the total variance and strongly opposed the unsown/invaded to the sown/invaded combination. This was due to the abundance of *Stellaria media* and of other herb species in the unsown treatments, and to the abundance of annual exotic grasses and the absence of native species in the sown/invaded combination. Patterns for the plant composition of non-native species showed that species composition was more similar between standing vegetation and seedlings from casts than between standing vegetation and seedlings from soil layers. Species composition of native plant seedlings was more similar between the soil surface layer and standing vegetation than between the cast seed bank and standing vegetation.

4. Discussion

The impact of non-native species in many ecosystems is a major global threat and better understanding the dynamics is an important ecological contribution to these issues. In this study, the relative co-occurrence of non-native worms and plants was investigated, as well as the potential impacts of earthworms on the invasion of semi-natural grasslands by non-native plant species. The abundances of non-native (to invasive) earthworms and non-native plants were positively correlated suggesting that both benefit from the same set of environmental conditions, e.g. a disturbance that promotes the success of all invasive species. Alternatively, there may be a positive feedback between non-native plants and non-native earthworms. Overall, earthworms favored the emergence of non-native over native species through the ingestion of seeds.

4.1. The co-occurrence of non-native worms and plants: a positive-feedback loop

The historical land use practices of plowing and sowing of *P. aquatica* 40 years ago favored the establishment of non-native plant communities that provided a suitable habitat for the development of non-native earthworm species such as *A. trapezoides* (see Catford et al., 2012; D'Antonio and Vitousek, 1992; MacDougall and Turkington, 2005; Stohlgren et al., 2003). In disturbed systems, the empty niche hypothesis proposes that non-native plant species often colonize canopy gaps (Levine and D'Antonio, 1999). These species are often better competitors and can sometimes capitalize on nutrient more than native species (Huenneke et al., 1990). Non-native seedlings can also establish better than native species in these systems as well (Hernandez and Sandquist, 2011; Moles et al., 2008). The colonization of ecosystems by non-native, invasive plant species can lead to significant changes in soil chemical properties such as pH, nitrogen or water content (Asner and Vitousek, 2005; Ehrenfeld et al., 2001). Litter of non-native, invasive plants is often of higher quality (low C:N ratio) than that of native plants and can show a higher decomposition rate (Allison and Vitousek, 2004; Ehrenfeld et al., 2001). This favors the uptake of nitrate by the non-native plants and its invasion. Non-native plants thus often modify nutrient cycling and create a positive feedback loop that favors their establishment. Hence, introduction of non-native plants can lead to dramatically altered conditions in grasslands independent of other engineering of other species such as earthworms. However, these is every indication that many animals can continue to promote these non-native cycles (Lake and Leishman, 2004; Maron and Vilà, 2001; Parker et al., 2006).

The positive earthworm-non-native plant relationship detected here supports previous research on earthworms and invasion but these studies were from forest not grasslands (Kourtev et al., 1999; Nuzzo et al., 2009). Earthworms prefer moist soils (Lee, 1985) with a high quality of organic matter determined by a vegetation litter of high quality, i.e. C/N close to or less than 20:1 like that of grass and herbaceous plants in non-acidic soils (Curry, 2004). The high abundance of non-native earthworms in the invaded/sown areas dominated by non-native grass suggests that non-native plant presence provides a suitable habitat for the development of non-native earthworm species both via appropriate soil conditions from the plants and from the habitat disturbance resulting from tillage. Winsome et al. (2006) also found that the non-native *A. trapezoides* dominated in grazed and fertilized grasslands, whereas native *Argilophilus marmoratus* maintained its dominance in undisturbed grasslands in Northern California.

Earthworms in turn also significantly impact soil physical, chemical, and microbiological properties (Bityutskii et al., 2012; Huang et al., 2010; Lavelle et al., 1998; Lee, 1985). We found that the increased abundance of earthworms was not only correlated with higher NO_3^- and soil moisture but also with a higher germination of non-native seeds in the casts tested. This suggests that earthworms could play a critical role in furthering a positive feedback loop established by non-native plants by accelerating the decomposition of the high-quality plant litter under non-native plant communities, and by promoting the germination of their seeds post ingestion. However, field or laboratory independent manipulations of earthworms and plants would be needed to test whether the invasion is driven by plants or by earthworms in a causative capacity, but this evidence indicates that these feedbacks could exist in Californian grasslands – particularly those subject to agricultural uses.

4.2. Earthworm effects on non-native seedling emergence

The promotion of non-native plant species by earthworm ingestion supports previous research in forest by Eisenhauer et al. (2007). Both plant community structure and composition density emerging from casts was greater than from soil samples in that study similar to our findings using density and plant species richness. Increased seedling emergence in casts could be caused by the passage of seeds through the earthworm gut, leading to a seed coat damage and the breaking of the seed dormancy (Ayanlaja et al., 2001; Traveset et al., 2008). Seedling survival and growth can also be promoted by higher nutrient contents and by molecules analogous to phytohormones already detected in casts (Chaoui et al., 2003; Clause et al., 2014; Tomati et al., 1988). Therefore, earthworm casts can represent an important part of the regeneration niche of some non-native plant species by both aggregating seeds and influencing important soil characteristics for seeds and plants.

The sensitivity of native versus non-native plant species to these potential drivers of community change is an important issue in invasion biology in general (Berg and Ellers, 2010; Funk et al., 2008). The implication of this survey is that earthworms can be agents of change that influence important soil characteristics in favor of non-native species. We found that the emergence of non-native seedlings from casts was higher than that of native seedlings and that these increases were particularly important within the uninvaded/unsown blocks where plant invasion may be actively occurring due to ingestion and cast dynamics. Interestingly, this suggests that the ingestion and egestion of seeds by non-native earthworms had a larger positive ecological effect on non-native seedling emergence in undisturbed habitats than in other habitats.

4.3. Earthworm effect and species composition

The composition of seeds within casts correlated more strongly with the general soil seed bank than with the standing vegetation. Such a difference between the standing vegetation and the seed banks can be explained by the dominance of *Festuca* sp. and *P. aquatica* that were present in the standing vegetation but not within casts. These dominant grasses can reproduce vegetatively and this likely reduced their presence within the seed bank in general and in the casts. The growth of grasses could also have particularly benefited from earthworm activities because of a competitive advantage against slower other species (Eisenhauer et al., 2009a; Eisenhauer and Scheu, 2008). The very low emergence of legume seedlings from our cast and soil samples prevented us from making assumption on the impact of non-native earthworms on legumes although Eisenhauer et al. (2007) found a decreased emergence of legume species in casts in forests. Plant species richness is thus also sensitive to earthworm effects.

4.4. On the lack of true replication

As mentioned before, we did not implement our ‘sowing’ and ‘invasion’ treatments. These were historical land practices within the reserve. The study site was unique in that it presented native, non-native and non-native, invasive plant species, as well as non-native and non-native, invasive earthworms. Other sites in central California appeared to be devoid of earthworms (personal observation). Though the use of GLMMs takes into account some of the pseudoreplication issues inherent to our sampling design (see Schank and Koehnle, 2009), we acknowledge that our study is exploratory because we could sample only one reserve with different blocks subject to land-use practices. This was also a correlative study in that we did not actively introduce earthworms. More *in situ* work involving sampling of earthworms, soil, cast and standing vegetation should be conducted in ecosystems where both native and non-native, invasive plant and earthworm species are present to conclude on the possibility of facilitation of non-native plants by non-native earthworms, via seed ingestion and/or soil modification. Alternatively, manipulative experiments implementing treatments with and without non-native earthworm species, e.g. in mesocosms, would be necessary to obtain a clear-cut conclusion. Although the historical land use via tillage and sowing led to more complexity in the results, it also revealed that these management practices can have a significant impact on plant–earthworm interactions and should be taken into account in further studies.

5. Conclusions

We suggest that disturbances in Californian grasslands have a significant impact on plant-seed-earthworm interactions and that these interactions can be an important consideration in better understanding plant invasions in grasslands in general. We propose that non-native plants and non-native earthworms could form a positive feedback loop via a modification of soil physico-chemical properties that mutually benefited them. Additionally, non-native earthworms favored non-native species via the ingestion of their seeds and their seedling emergence in casts. This could be a real concern in native or relatively uninvaded grasslands. When two or more species together have a higher invasive impact on a habitat than if each had invaded it alone (Simberloff and Holle, 1999), a positive feedback loop can in theory become an 'invasional meltdown' phenomenon that cannot be controlled (Simberloff, 2006). Our invaded/sown sampled area also showed signs of an 'invasional meltdown' as very few seeds of native species emerged from the casts and from the soil seed bank, and no native plant was found in the standing vegetation. Biological control of the invasion might thus be limited due to a dramatic loss of the native seed bank (Simberloff, 2006; Simberloff and Holle, 1999). Control of non-native earthworms may also be another important mitigation strategy.

Acknowledgments

The authors would like to thank the Carla D'Antonio and Viviane Vincent from the D'Antonio lab (UCSB) for material and professional support, particularly through the Schuyler Endowment Funds to Carla D'Antonio. They would also like to thank Don Canestro, manager of the reserve, who enabled an easy access to the KNRM Reserve Station. Special thanks must be addressed to Samuel James for his advice and expertise regarding earthworm identification, Anya Reid, and Maris and Lorna McCarthy for their technical help in sampling, Stephanie Ma for soil analyses and Kate E. Forrest for seedling identification. Finally, the authors thank the two anonymous reviewers for their useful comments and advice. CJL and a proportion of this research were funded by an NSERC DG. Experiments performed for this study comply with US law.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.actao.2015.02.004.

Author contributions

JC, EF & CJL originally formulated the idea. JC, CJL, AL, EF & SB developed methodology. JC conducted fieldwork and performed statistical analyses. JC wrote the manuscript that was edited by CJL, AL, EF & SB. All authors have approved the final article.

References

Allison, S.D., Vitousek, P.M., 2004. Rapid nutrient cycling in leaf litter from invasive plants in Hawai'i. Oecologia 141, 612–619. http://dx.doi.org/10.1007/s00442-004-1679-z.

Asner, G.P., Vitousek, P.M., 2005. Remote analysis of biological invasion and biogeochemical change. Proc. Natl. Acad. Sci. U. S. A. 102, 4383–4386. http://dx.doi.org/10.1073/pnas.0500823102.

Ayanlaja, S.A., Owa, S.O., Adigun, M.O., Senjobi, B.A., Olaleye, A.O., 2001. Leachate from earthworm castings breaks seed dormancy and preferentially promotes radicle growth in jute. HortScience 36, 143–144.

Bates, D., Maechler, M., Bolker, B., Walker, S., 2013. lme4: Linear Mixed-effects Models Using Eigen and S4.

Berg, M.P., Ellers, J., 2010. Trait plasticity in species interactions: a driving force of community dynamics. Evol. Ecol. 24, 617–629. http://dx.doi.org/10.1007/s10682-009-9347-8.

Bityutskii, N.P., Kaidun, P.I., Yakkonen, K.L., 2012. The earthworm (Aporrectodea caliginosa) primes the release of mobile and available micronutrients in soil. Pedobiologia 55, 93–99. http://dx.doi.org/10.1016/j.pedobi.2011.11.003.

Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H., White, J.-S.S., 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol. Evol. 24, 127–135. http://dx.doi.org/10.1016/j.tree.2008.10.008.

Calflora, 2012. Search for Plants [WWW Document]. Calflora Inf. Calif. Plants Educ. Res. Conserv. Search Plants 2012 Berkeley CA Calflora Database (first). URL. http://www.calflora.org/ (accessed 06.05.12.).

Carter, J.L., Carter, M.A., Stevens, D.J., 2003. Common Southwestern Native Plants: an Identification Guide. Mimbres Pub.

Catford, J.A., Daehler, C.C., Murphy, H.T., Sheppard, A.W., Hardesty, B.D., Westcott, D.A., Rejmánek, M., Bellingham, P.J., Pergl, J., Horvitz, C.C., Hulme, P.E., 2012. The intermediate disturbance hypothesis and plant invasions: implications for species richness and management. Perspect. Plant Ecol. Evol. Syst. 14, 231–241. http://dx.doi.org/10.1016/j.ppees.2011.12.002.

Chaoui, H.I., Zibilske, L.M., Ohno, T., 2003. Effects of earthworm casts and compost on soil microbial activity and plant nutrient availability. Soil Biol. Biochem. 35, 295–302. http://dx.doi.org/10.1016/S0038-0717(02)00279-1.

Clause, J., Barot, S., Richard, B., Decaëns, T., Forey, E., 2014. The interactions between soil type and earthworm species determine the properties of earthworm casts. In: Appl. Soil Ecol., XVI International Colloquium on Soil Zoology & XIII International Colloquium on Apterygota, Coimbra, 2012 – Selected Papers, vol. 83, pp. 149–158. http://dx.doi.org/10.1016/j.apsoil.2013.12.006.

Clause, J., Margerie, P., Langlois, E., Decaëns, T., Forey, E., 2011. Fat but slim: criteria of seed attractiveness for earthworms. Pedobiologia 54. http://dx.doi.org/10.1016/j.pedobi.2011.08.007. Supplement, S159–S165.

Curry, J.P., 2004. Factors affecting the abundance of earthworms in soils. In: Edwards, C.A. (Ed.), Earthworm Ecology. CRC Press, Boca Raton, FL, pp. 91–113.

D'Antonio, C.M., Vitousek, P.M., 1992. Biological invasions by exotic grasses, the grass/fire cycle, and global change. Annu. Rev. Ecol. Syst. 23, 63–87. http://dx.doi.org/10.1146/annurev.es.23.110192.000431.

Decaëns, T., Mariani, L., Betancourt, N., Jiménez, J.J., 2003. Seed dispersion by surface casting activities of earthworms in Colombian grasslands. Acta Oecol. 24, 175–185. http://dx.doi.org/10.1016/S1146-609X(03)00083-3.

Didham, R.K., Tylianakis, J.M., Gemmell, N.J., Rand, T.A., Ewers, R.M., 2007. Interactive effects of habitat modification and species invasion on native species decline. Trends Ecol. Evol. 22, 489–496. http://dx.doi.org/10.1016/j.tree.2007.07.001.

Dray, S., Dufour, A., 2007. The ade4 package: implementing the duality diagram for ecologists. J. Stat. Softw. 22, 1–20.

Drouin, M., Bradley, R., Lapointe, L., Whalen, J., 2014. Non-native anecic earthworms (Lumbricus terrestris L.) reduce seed germination and seedling survival of temperate and boreal trees species. Appl. Soil Ecol. 75, 145–149. http://dx.doi.org/10.1016/j.apsoil.2013.11.006.

Ehrenfeld, J.G., Kourtev, P., Huang, W., 2001. Changes in soil functions following invasions of exotic understory plants in deciduous forests. Ecol. Appl. 11, 1287–1300. http://dx.doi.org/10.1890/1051-0761(2001)011[1287:CISFFI]2.0.CO;2.

Eisenhauer, N., Milcu, A., Sabais, A.C.W., Scheu, S., 2009a. Earthworms enhance plant regrowth in a grassland plant diversity gradient. Eur. J. Soil Biol. 45, 455–458. http://dx.doi.org/10.1016/j.ejsobi.2009.06.001.

Eisenhauer, N., Partsch, S., Parkinson, D., Scheu, S., 2007. Invasion of a deciduous forest by earthworms: changes in soil chemistry, microflora, microarthropods and vegetation. Soil Biol. Biochem. 39, 1099–1110. http://dx.doi.org/10.1016/j.soilbio.2006.12.019.

Eisenhauer, N., Scheu, S., 2008. Earthworms as drivers of the competition between grasses and legumes. Soil Biol. Biochem. 40, 2650–2659. http://dx.doi.org/10.1016/j.soilbio.2008.07.010.

Eisenhauer, N., Straube, D., Johnson, E.A., Parkinson, D., Scheu, S., 2009b. Exotic ecosystem engineers change the emergence of plants from the seed bank of a deciduous forest. Ecosystems 12, 1008–1016. http://dx.doi.org/10.1007/s10021-009-9275-z.

Fender, W.M., McKey-Fender, D., 1990. Oligochaeta: Megascolecidae and other earthworms from western North America. In: Soil Biology Guide. Wiley and Sons, New York, NY, pp. 357–378.

Forey, E., Barot, S., Decaëns, 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. http://dx.doi.org/10.1016/j.actao.2011.03.001.

Frelich, L.E., Hale, C.M., Scheu, S., Holdsworth, A.R., Heneghan, L., Bohlen, P.J., Reich, P.B., 2006. Earthworm invasion into previously earthworm-free temperate and boreal forests. Biol. Invasions 8, 1235–1245. http://dx.doi.org/10.1007/s10530-006-9019-3.

Funk, J.L., Cleland, E.E., Suding, K.N., Zavaleta, E.S., 2008. Restoration through reassembly: plant traits and invasion resistance. Trends Ecol. Evol. 23, 695–703. http://dx.doi.org/10.1016/j.tree.2008.07.013.

Grant, J.D., 1983. The activities of earthworms and the fates of seeds. In: Satchell, J.E. (Ed.), Earthworm Ecology. Springer, Netherlands, pp. 107–122.

Hale, C.M., Frelich, L.E., Reich, P.B., 2005. Exotic European earthworm invasion dynamics in northern hardwood forests of Minnesota, USA. Ecol. Appl. 15, 848–860. http://dx.doi.org/10.1890/03-5345.

Hale, C.M., Frelich, L.E., Reich, P.B., Pastor, J., 2008. Exotic earthworm effects on hardwood forest floor, nutrient availability and native plants: a mesocosm study. Oecologia 155, 509–518. http://dx.doi.org/10.1007/s00442-007-0925-6.

Hendrix, P.F., Bohlen, P.J., 2002. Exotic earthworm invasions in North America: ecological and policy implications. BioScience 52, 801–811. http://dx.doi.org/10.1641/0006-3568(2002)052[0801:EEIINA]2.0.CO;2.

Hendrix, P.F., Callaham, M.A., Drake, J.M., Huang, C.-Y., James, S.W., Snyder, B.A., Zhang, W., 2008. Pandora's box contained bait: the global problem of introduced earthworms*. Annu. Rev. Ecol. Evol. Syst. 39, 593–613. http://dx.doi.org/10.1146/annurev.ecolsys.39.110707.173426.

Hernandez, R.R., Sandquist, D.R., 2011. Disturbance of biological soil crust increases emergence of exotic vascular plants in California sage scrub. Plant Ecol. 212, 1709–1721. http://dx.doi.org/10.1007/s11258-011-9943-x.

Holdsworth, A.R., Frelich, L.E., Reich, P.B., 2007. Effects of earthworm invasion on plant species richness in northern hardwood forests. Conserv. Biol. 21, 997–1008. http://dx.doi.org/10.1111/j.1523-1739.2007.00740.x.

Hothorn, T., Bretz, F., Westfall, P., Heiberger, R.M., Schuetzenmeister, A., 2013. Multcomp: Simultaneous Inference in General Parametric Models.

Huang, C.-Y., Hendrix, P.F., Fahey, T.J., Bohlen, P.J., Groffman, P.M., 2010. A simulation model to evaluate the impacts of invasive earthworms on soil carbon dynamics. Ecol. Model. 221, 2447–2457. http://dx.doi.org/10.1016/j.ecolmodel.2010.06.023.

Huenneke, L.F., Hamburg, S.P., Koide, R., Mooney, H.A., Vitousek, P.M., 1990. Effects of soil resources on plant invasion and community structure in Californian serpentine grassland. Ecology 71, 478–491. http://dx.doi.org/10.2307/1940302.

Husson, F., Josse, J., Lê, S., Mazet, J., 2013. FactoMineR: Multivariate Exploratory Data Analysis and Data Mining with R.

IUCN, 2000. IUCN Guidelines for the Prevention of Biodiversity Loss Caused by Alien Invasive Species, p. 24. Presented at the 51st Meeting of the IUCN Council, Gland, Switzerland.

Kourtev, P.S., Huang, W.Z., Ehrenfeld, J.G., 1999. Differences in earthworm densities and nitrogen dynamics in soils under exotic and native plant species. Biol. Invasions 1, 237–245. http://dx.doi.org/10.1023/A:1010048909563.

Lake, J.C., Leishman, M.R., 2004. Invasion success of exotic plants in natural ecosystems: the role of disturbance, plant attributes and freedom from herbivores. Biol. Conserv. 117, 215–226. http://dx.doi.org/10.1016/S0006-3207(03)00294-5.

Lavelle, P., Pashanasi, B., Charpentier, F., Gilot Villenave, C., Rossi, J.-P., Derouard, L., Andre, J., Ponge, J.-F., Bernier, N., 1998. Effects of earthworms on soil organic matter and nutrient dynamics at a landscape scale over decades. In: Edwards, C. (Ed.), Earthworm Ecology. CRC Press, pp. 103–122.

Lawrence, A.P., Bowers, M.A., 2002. A test of the “hot” mustard extraction method of sampling earthworms. Soil Biol. Biochem. 34, 549–552. http://dx.doi.org/10.1016/S0038-0717(01)00211-5.

Lee, K.E., 1985. Earthworms: Their Ecology and Relationships with Soils and Land Use. Academic Press, New York.

Legendre, P., Anderson, M.J., 1999. Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. Ecol. Monogr. 69, 1–24. http://dx.doi.org/10.1890/0012-9615(1999)069[0001:DBRATM]2.0.CO;2.

Levine, J.M., D'Antonio, C.M., 1999. Elton revisited: a review of evidence linking diversity and invasibility. Oikos 87, 15. http://dx.doi.org/10.2307/3546992.

MacDougall, A.S., Turkington, R., 2005. Are invasive species the drivers or passengers of change in degraded ecosystems? Ecology 86, 42–55. http://dx.doi.org/10.1890/04-0669.

Maron, J.L., Vilà, M., 2001. When do herbivores affect plant invasion? Evidence for the natural enemies and biotic resistance hypotheses. Oikos 95, 361–373. http://dx.doi.org/10.1034/j.1600-0706.2001.950301.x.

Milcu, A., Schumacher, J., Scheu, S., 2006. Earthworms (Lumbricus terrestris) affect plant seedling recruitment and microhabitat heterogeneity. Funct. Ecol. 20, 261–268. http://dx.doi.org/10.1111/j.1365-2435.2006.01098.x.

Mitchell, C.E., Agrawal, A.A., Bever, J.D., Gilbert, G.S., Hufbauer, R.A., Klironomos, J.N., Maron, J.L., Morris, W.F., Parker, I.M., Power, A.G., Seabloom, E.W., Torchin, M.E., Vázquez, D.P., 2006. Biotic interactions and plant invasions. Ecol. Lett. 9, 726–740. http://dx.doi.org/10.1111/j.1461-0248.2006.00908.x.

Moles, A.T., Gruber, M.A.M., Bonser, S.P., 2008. A new framework for predicting invasive plant species. J. Ecol. 96, 13–17. http://dx.doi.org/10.1111/j.1365-2745.2007.01332.x.

Nuzzo, V.A., Maerz, J.C., Blossey, B., 2009. Earthworm invasion as the driving force behind plant invasion and community change in northeastern North American forests. Conserv. Biol. 23, 966–974. http://dx.doi.org/10.1111/j.1523-1739.2009.01168.x.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2013. Vegan: Community Ecology Package.

Parker, J.D., Burkepile, D.E., Hay, M.E., 2006. Opposing effects of native and exotic herbivores on plant invasions. Science 311, 1459–1461. http://dx.doi.org/10.1126/science.1121407.

R Core Team, 2013. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Richardson, D.M., Allsopp, N., D'antonio, C.M., Milton, S.J., Rejmánek, M., 2000. Plant invasions — the role of mutualisms. Biol. Rev. 75, 65–93. http://dx.doi.org/10.1111/j.1469-185X.1999.tb00041.x.

Schank, J.C., Koehnle, T.J., 2009. Pseudoreplication is a pseudoproblem. J. Comp. Psychol. Wash. D.C. 1983 (123), 421–433. http://dx.doi.org/10.1037/a0013579.

Seastedt, T.R., Pyšek, P., 2011. Mechanisms of plant invasions of North American and European grasslands. Annu. Rev. Ecol. Evol. Syst. 42, 133–153. http://dx.doi.org/10.1146/annurev-ecolsys-102710-145057.

Simberloff, D., 2006. Invasional meltdown 6 years later: important phenomenon, unfortunate metaphor, or both? Ecol. Lett. 9, 912–919. http://dx.doi.org/10.1111/j.1461-0248.2006.00939.x.

Simberloff, D., Holle, B.V., 1999. Positive interactions of nonindigenous species: invasional meltdown? Biol. Invasions 1, 21–32. http://dx.doi.org/10.1023/A:1010086329619.

Stohlgren, T.J., Barnett, D.T., Kartesz, J.T., 2003. The rich get richer: patterns of plant invasions in the United States. Front. Ecol. Environ. 1, 11–14. http://dx.doi.org/10.1890/1540-9295(2003)001[0011:TRGRPO]2.0.CO;2.

Tomati, U., Grappelli, A., Galli, E., 1988. The hormone-like effect of earthworm casts on plant growth. Biol. Fertil. Soils 5, 288–294. http://dx.doi.org/10.1007/BF00262133.

Traveset, A., Rodríguez-Pérez, J., Pías, B., 2008. Seed trait changes in disperser's guts and consequences for germination and seedling growth. Ecology 89, 95–106. http://dx.doi.org/10.1890/07-0094.1.

USDA, S.S.S., Natural Resources Conservation Service, 2013. NRCS Web Soil Survey [WWW Document]. http://websoilsurvey.nrcs.usda.gov/. URL (accessed 25.09.13.).

Winsome, T., Epstein, L., Hendrix, P.F., Horwath, W.R., 2006. Competitive interactions between native and exotic earthworm species as influenced by habitat quality in a California grassland. Appl. Soil Ecol. 32, 38–53. http://dx.doi.org/10.1016/j.apsoil.2005.01.008.

Wood, H.B., James, S.W., 1993. Native and Introduced Earthworms from Selected Chaparral, Woodland, and Riparian Zones in Southern California. Department of Agriculture, Forest Service, Pacific Southwest Research Station, Albany, CA: U.S. Gen. Tech. Rep. PSW-142. ed.

Zaller, J.G., Saxler, N., 2007. Selective vertical seed transport by earthworms: implications for the diversity of grassland ecosystems. Eur. J. Soil Biol. 43 (Suppl. 1), S86–S91. http://dx.doi.org/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. http://dx.doi.org/10.1007/BF00361617.