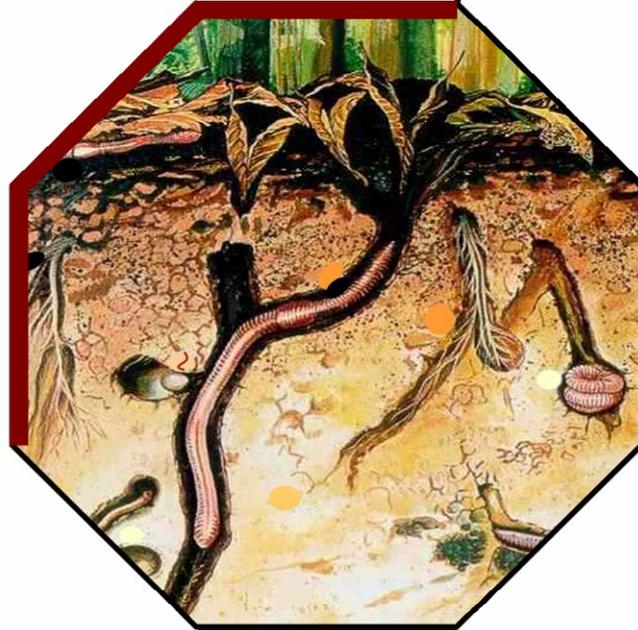


CHAPTER

3



ASSESSMENT OF ANECIC BEHAVIOR
IN SELECTED EARTHWORM SPECIES:
EFFECTS ON WHEAT SEED BURIAL,
SEEDLING ESTABLISHMENT,
WHEAT GROWTH,
AND LITTER INCORPORATION

3.1 ABSTRACT

Anecic earthworm species function as ecosystem engineers by structuring the soil environment, incorporating large amounts of litter and seeds into soil and, thereby, drive the composition of plant communities. The aim of the present greenhouse experiment was to investigate the effects of three anecic earthworm species on wheat seed burial, seedling establishment, wheat growth and litter incorporation. Anecic earthworms differed substantially in their behavior and effect on plant establishment. *Aporrectodea longa* did not incorporate litter into the soil, on the contrary, *L. terrestris* (-69%) and *L. rubellus friendoides* (-75%) reduced the litter layer considerably during 9 weeks of incubation. Moreover, *L. terrestris* and *L. rubellus friendoides* buried more wheat seeds into the soil than *A. longa*. Less seeds germinated when buried by *A. longa* compared to *L. terrestris*. The results show that anecic earthworm species differentially affect wheat seed burial, litter incorporation and wheat establishment. The effects of *L. terrestris* and *L. rubellus friendoides* are conform to the characteristics of anecic earthworm species whereas those of *A. longa* rather resemble endogeic species. The present study is the first proof of the anecic behavior of *L. rubellus friendoides*.

3.2 INTRODUCTION

Earthworms are a major component of many terrestrial ecosystems (Edwards and Bohlen 1996). In non-acidic soils they usually dominate the biomass of soil invertebrates and function as ecosystem engineers by structuring the environment of the soil community (Jones et al. 1994, Lavelle et al. 1998, Scheu and Setälä 2002). Through burrowing, casting and mixing of litter and soil (bioturbation) they influence aggregate stability, soil structure, infiltration of water, aeration of deeper soil layers, nutrient cycling and mineralization, microbial biomass, and other soil invertebrates (Edwards and Bohlen 1996, Eisenhauer et al. 2007). These changes have important consequences for plant communities and potentially the herbivore system (Scheu 2003, Brown et al. 2004). The degree of mixing soil layers varies with earthworm species which are categorized into three main ecological groups: epigeic, endogeic and anecic species (Bouché 1977, Edwards and Bohlen 1996). Anecic species are intermediate between litter-dwelling epigeics and soil-dwelling endogeics in that they feed, at least partly, on litter but live in the soil in burrows. These moderate to large earthworms form vertical permanent burrows up to 2 m deep and incorporate litter from the soil surface into deeper soil layers but also transport mineral soil materials to the surface by casting (Bouché 1977, Sims and Gerard 1999). *Lumbricus terrestris* L. has been the subject of several studies and functions as a model earthworm species (Shumway and Koide 1994, Edwards and Bohlen 1996, Maraun et al. 1999, Milcu et al. 2006a). *Aporrectodea longa* Ude is another common earthworm species grouped as anecic (Pearce 1978, Schmidt et al. 1997, Lowe and Butt 2002). *Lumbricus rubellus friendoides* Bouché resembles *L. terrestris* in body size and shape but there is no proof on its anecic behavior.

The aim of the present study was to investigate the effects of these three earthworm species on wheat seed burial, seedling establishment, wheat growth and litter incorporation, and their classification into the anecic species group.

3.3 MATERIALS AND METHODS

We set up mesocosms consisting of PVC tubes (inner diameter 26 cm, height 20 cm) which were sealed at the bottom with a 1 mm mesh. The mesocosms were filled with 10 kg of sieved (1 cm) and homogenized soil (height of soil column 18 cm) and placed in a temperature controlled greenhouse at a day/night regime of 16/8 h and $20/16 \pm 2^\circ\text{C}$ (Figure 3.1A). The soil (Gleyic Cambisol: 9% sand, 69% silt, 22% clay, pH 6.6, carbon

content 1.01%, water content 17%) was taken from plot 20 of the Heidfeldhof experimental field station (University of Hohenheim, Baden-Württemberg, Germany; 48° 42' 58" N, 9° 10' 53" E, altitude 402 m). A layer of mixed litter consisting mainly of grass leaves (5 g, 2.53% N, C-to-N ratio 17.3) was placed on the soil surface to simulate natural conditions and to investigate litter incorporation. The litter had been collected at the Jena Biodiversity Experiment field site (Thuringia, Germany; Roscher et al. 2004), dried at 60°C for three days, and cut into pieces about 5 cm in length. The mesocosms were watered every second day (200-ml portions of deionized water) and germinating weeds were removed for 14 days. Subsequently, one adult *L. terrestris* (average fresh weight with gut content 2.53 ± 0.26 g), *L. rubellus friendoides* (2.76 ± 0.19 g) or *A. longa* (2.20 ± 0.75 g) was introduced in each bucket creating four treatments (Control [without earthworms], with one individual of *L. terrestris*, *L. rubellus friendoides* or *A. longa*; 7 replicates each). *Lumbricus terrestris* was collected at the Jena Biodiversity Experiment field site and *L. rubellus friendoides* and *A. longa* at the Heidfeldhof experimental field station (University of Hohenheim) by electro shocking in November 2006. Furthermore, we applied 48 wheat seeds (*Triticum aestivum*, var. Triso) on the soil surface to investigate seed burial and seedling establishment. The number of remaining seeds at the soil surface and the number of seedlings were counted weekly. After 9 weeks the wheat shoot biomass was harvested and remaining litter material on the soil surface sampled, dried (60°C, 3 days), and weighed. We broke up the soil core, inspected it by eye for spatial distribution of earthworm burrows, and weighed the earthworms (fresh weight with gut content).

One-way ANOVAs (analysis of variance; STATISTICA 6.0; StatSoft, Tulsa, USA) were used to analyze the effects of “earthworm treatment” on the dependent factors “seeds incorporated into soil”, “number of seedlings”, “number of ears”, “total shoot biomass per plant”, “litter remaining on soil surface” and “earthworm weight” after 9 weeks. Additionally, the numbers of seeds incorporated into soil and the number of seedlings after 1, 2, 3, 4, 5 and 9 weeks were analyzed using repeated measures ANOVA with “time” as repeated factor and “earthworm treatment” as categorical factor using the statistical software system R 2.4.0. If necessary, normal distribution and homogeneity of variance were improved by log-transformation ($\log[x+1]$). Means presented in text and figures were calculated using non-transformed data (\pm SD). Comparisons of means (Tukey’s HSD test $\alpha=0.05$) were performed using STATISTICA 6.0 (Statsoft, Tulsa, USA).

3.4 RESULTS

Earthworms did not affect wheat growth parameters (total shoot biomass per plant and number of ears; Table 1). Furthermore, there was no significant effect on seed incorporation and seedling establishment over time ($F_{15,119}=0.51$, $P=0.93$ and $F_{6,59}=0.28$, $P=0.95$, respectively). However, *L. terrestris* and *L. rubellus friendoides* created only few large, mainly vertical orientated burrows, whereas, *A. longa* formed large numbers of vertical and horizontal burrows dispersed throughout the entire soil core (optical inspection; Fig. 3.1B, C, D). Moreover, *A. longa* lost weight (-12%), whereas *L. terrestris* and *L. rubellus friendoides* gained weight considerably during the experiment (+67% and +70%, respectively; $F_{2,16}=30.86$, $P<0.0001$; Fig. 3.2A). *Aporrectodea longa* did not incorporate litter into the soil, on the contrary, *L. terrestris* and *L. rubellus friendoides* reduced the litter layer substantially by -69% and -75%, respectively (Table 3.1; Fig. 3.2B). *Lumbricus terrestris* (-76%) and *L. rubellus friendoides* (-77%) buried more of the provided wheat seeds into the soil than *A. longa* (-57%). However, the removal of seeds from the soil surface in the control treatment (-23%; incorporation due to watering; Table 3.1; Fig. 3.2C) was still significantly lower than in the treatment with *A. longa*. The number of seedlings did not vary significantly between the control, *L. terrestris* and *L. rubellus friendoides* treatments. Interestingly, less seeds germinated when buried by *A. longa* compared to *L. terrestris* (-50%; Table 3.1; Fig. 3.2D).

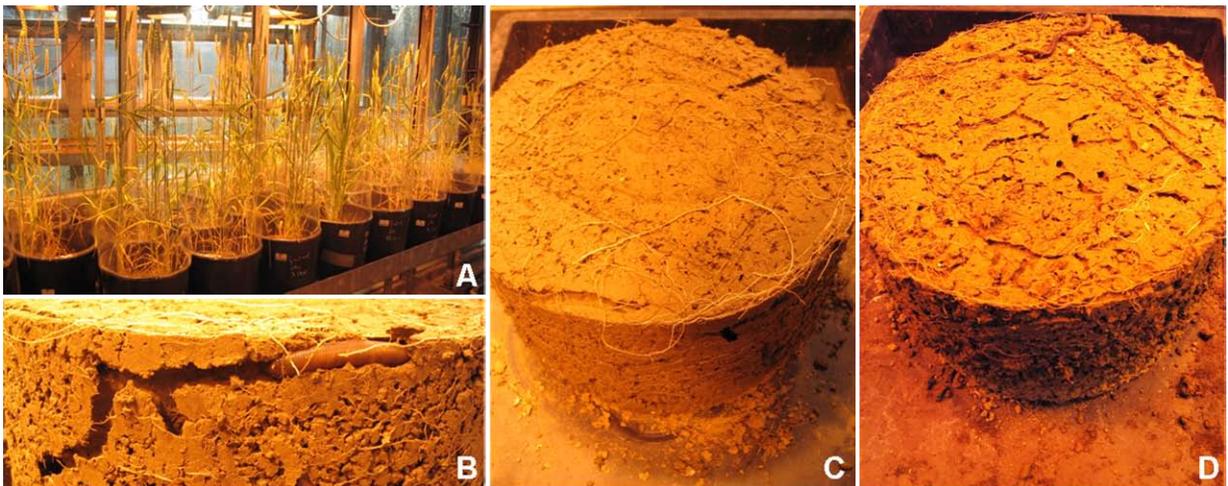


Figure 3.1 | Photos taken at the end of the experiment (after nine weeks). (A) Part of the 28 mesocosms in the greenhouse. (B) Soil core (section) of the treatment with *Lumbricus terrestris* showing an animal with large burrows. (C) Soil core of the treatment with *Lumbricus rubellus friendoides* showing only few but large burrows (bottom view). (D) Soil core of the treatment with *Aporrectodea longa* showing numerous small burrows (bottom view). Photos by S. Marhan.

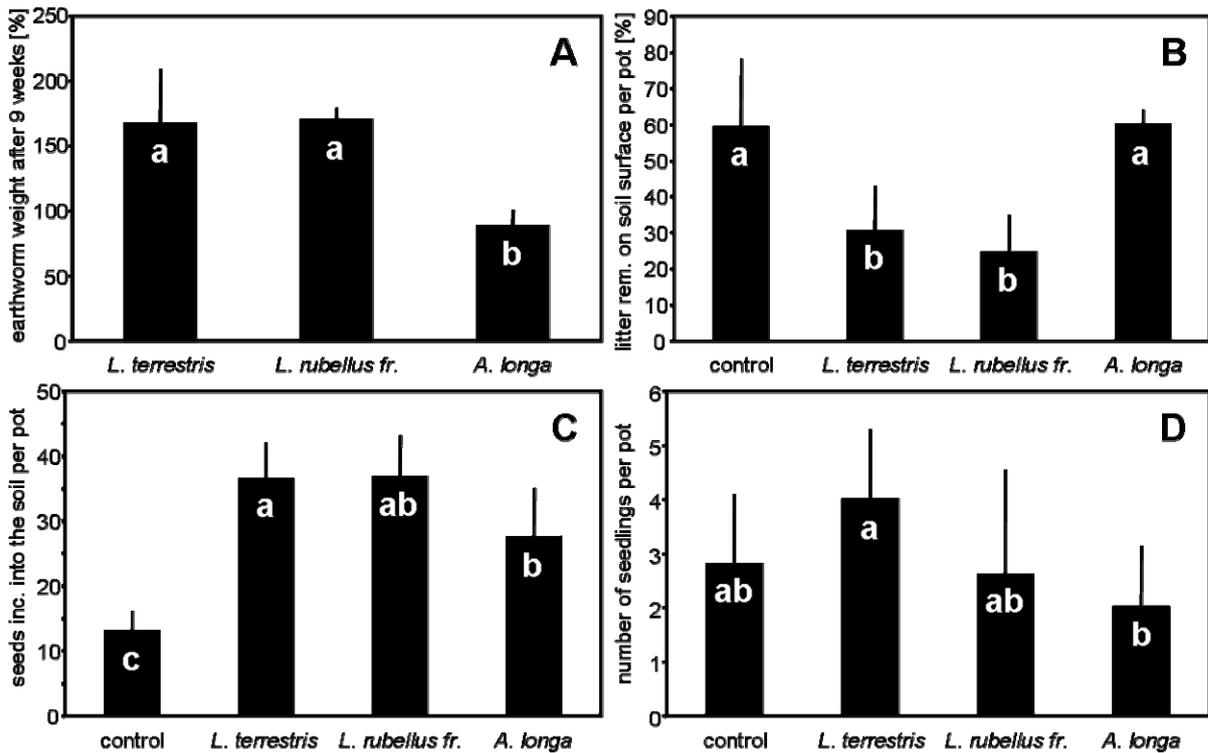


Figure 3.2 | (A) Changes in body fresh weight of *Lumbricus terrestris*, *Lumbricus rubellus friendoides* and *Aporrectodea longa* during the experiment [% of initial], effects of earthworms (Control, *Lumbricus terrestris*, *Lumbricus rubellus friendoides* and *Aporrectodea longa*) on (B) litter remaining on the soil surface per pot [%], (C) number of seeds incorporated into the soil per pot, and (D) number of established seedlings per pot. Bars with different letters vary significantly (Tukey's HSD test, $\alpha < 0.05$).

Table 3.1 | One-way ANOVA table of F-values for the effect of earthworm species (control without earthworms, with one individual of *Lumbricus terrestris*, *Lumbricus rubellus friendoides* or *Aporrectodea longa*) on wheat growth parameters and on seed and litter incorporation.

	Df	Df error	F-value	P-value
Seeds incorporated	3	20	17.62	<0.0001
Number of seedlings	3	20	3.01	0.054
Number of ears	3	20	1.85	0.17
Total shoot biomass per plant	3	20	1.69	0.20
Remaining litter	3	20	11.61	0.00013

Df, degrees of freedom.

3.5 DISCUSSION

The investigated earthworm species differed significantly in their burial behavior and effect on wheat plant establishment. Earthworms did not affect wheat growth parameters but this presumably was due to the large variation in plant biomass and the low earthworm density used (one individual per mesocosm is equivalent to only 19 ind. m⁻²). However, the burrowing behaviors of the three earthworm species differed considerably. *Lumbricus terrestris* and *L. rubellus friendoides* created only few large, mainly vertical orientated burrows, whereas, *A. longa* formed large numbers of vertical and horizontal burrows dispersed throughout the entire soil core. Furthermore, soil surface activity differed significantly. *Aporrectodea longa* did not incorporate litter into the soil, on the contrary, *L. terrestris* and *L. rubellus friendoides* reduced the litter layer substantially, thereby, providing resources for soil microflora and microarthropods and nutrients for plants. *Aporrectodea longa* only fed on mineral soil which was poor in organic matter. *Aporrectodea longa* presumably depends on other macrodecomposers which are able to provide more decayed organic material. Lowe and Butt (2002) stated that *A. longa* performed well feeding on organic matter in the soil profile and Satchell (1980) classified *A. longa* as an intermediate soil-litter feeder. Moreover, Curry and Schmidt (2007) assumed *Aporrectodea* species to be primary geophageous with natural abundances of ¹⁵N and ¹³C between litter-feeders and endogeics (Schmidt et al. 1997). Data on ¹⁴C assimilation indicate that *A. longa* feeds on older and more decayed carbon sources than epigeic and epi-anecic species (Briones et al. 2005). Consequently, *A. longa* lost weight, whereas *L. terrestris* and *L. rubellus friendoides* gained weight considerably during the experiment feeding on the distinct litter layer. On the contrary, other studies documented a rather anecic behavior of *A. longa* (Pearce 1978, Lowe and Butt 2002, Chan et al. 2004). Thus, further studies are required to investigate the inconsistent behavior of *A. longa*. *Lumbricus terrestris* and *L. rubellus friendoides* buried more of the provided wheat seeds into the soil than *A. longa* but, surprisingly, the number of seedlings did not vary significantly between the control, *L. terrestris* and *L. rubellus friendoides* treatments. Interestingly, less seeds germinated when buried by *A. longa* compared to *L. terrestris*. Obviously, *A. longa* buries seeds in a way that is unfavorable for germination, whereas, *L. terrestris* provides a more favorable environment. Milcu et al. (2006a) observed that *L. terrestris* buries seeds irrespective of size and shape. Although in general recruitment of seedlings was lower in presence of *L. terrestris*, those seedlings that managed to establish benefited from lower intra- and interspecific competition and beneficial

nutrient and water conditions in earthworm burrows. This is the first proof of *L. rubellus friendoides* incorporating litter and seeds into the soil and, thereby, functioning as an ecosystem engineer.

3.6 CONCLUSIONS

The present study shows that anecic earthworms function as ecosystem engineers and, thereby, drive the establishment of plant seedlings. However, anecic earthworm species vary considerably in their characteristics in plant seed burial, litter incorporation and influence on seedling establishment. *Lumbricus terrestris* and *L. rubellus friendoides* had similar effects on the soil system and resemble characteristics of the anecic functional group. In contrast, the behavior of *A. longa* was hardly conform to anecic earthworm species, rather, its burying and feeding behavior matched that of endogeic species. Further studies investigating the burial of seeds of different size and the differences in seed burial characteristics between earthworm species are needed for understanding the direct effects of earthworms on seedling recruitment and plant community structure in natural ecosystems.

CHAPTER

4



INVASIBILITY OF EXPERIMENTAL
GRASSLAND COMMUNITIES:
THE ROLE OF EARTHWORMS,
PLANT FUNCTIONAL GROUP
IDENTITY, AND SEED SIZE

4.1 ABSTRACT

Invasions of natural communities by non-indigenous species threaten native biodiversity and are currently rated as one of the most important global-scale environmental problems. The mechanisms that make communities resistant to invasions and drive the establishment success of seedlings are essential both for management and for understanding community assembly and structure. Especially in grasslands, anecic earthworms are known to function as ecosystem engineers, however, their direct effects on plant community composition and on the invasibility of plant communities via plant seed burial, ingestion and digestion are poorly understood.

In a greenhouse experiment we investigated the impact of *Lumbricus terrestris* L., plant functional group identity and seed size of plant invader species and plant functional group of the established plant community on the number and biomass of plant invaders. We set up 120 microcosms comprising four plant community treatments, two earthworm treatments and three plant invader treatments containing three seed size classes.

Earthworm performance was influenced by an interaction between plant functional group identity of the established plant community and that of invader species. The established plant community and invader seed size affected the number of invader plants significantly, while invader biomass was only affected by the established community. Since earthworm effects on the number and biomass of invader plants varied with seed size and plant functional group identity they probably play a key role in seedling establishment and plant community composition.

Seeds and germinating seedlings in earthworm burrows may significantly contribute to earthworm nutrition, but this deserves further attention. *Lumbricus terrestris* likely behaves like a “farmer” by collecting plant seeds which cannot directly be swallowed or digested. Presumably, these seeds are left in middens and become eatable after partial microbial decay. Increased earthworm numbers in more diverse plant communities likely contribute to the positive relationship between plant species diversity and resistance against invaders.

4.2 INTRODUCTION

What determines the success or failure of an invading plant species? Numerous studies have focussed on this topic since invasions of natural communities by non-indigenous species are a threat to native biodiversity and are currently rated as one of the most important global-scale environmental problems (Vitousek et al. 1996). The properties and mechanisms that make communities resistant to invasions and drive the establishment success of seedlings are essential both for management (D'Antonio and Vitousek 1992, Pimentel et al. 2000) and for understanding community assembly and structure (Fargione et al. 2003).

Biodiversity is one feature of communities that has long been hypothesized to reduce invasions by using resources more completely than simple communities (Elton 1958, Levine and D'Antonio 1999, Tilman 1999, Fargione and Tilman 2005). Thereby, complementarity is thought to be an important factor since it may result in species having trade-offs in their efficiency of using different resources, in colonization and competitive abilities, or in their success under different environmental conditions (Fargione and Tilman 2005). However, an ecosystem's susceptibility to invasion is influenced by many factors (Crawley et al. 1999, Levine and D'Antonio 1999). Physical hazards and pathogens may control seedling establishment to a greater extent than competition by neighbouring plants (Ryser 1993). Moreover, some species are hardly able to establish without shelter of vegetation (Ryser 1993). Further, the number and size of plant seeds and plant traits affecting seed dispersal are major factors driving seedling establishment.

Compared to plant seeds in the soil seed bank, those on the soil surface are more vulnerable to predation by birds, rodents and insects and to germination in unfavorable conditions (Roberts 1970). Thus, seed burial is a key factor in prolonging the survival of seeds (Harper 1957). Seeds may enter the soil seed bank through a variety of agents, and some of these (e.g. cultivation and the activity of animals) are also capable of returning buried seeds to the surface (Thompson et al. 1994). Several studies indicated that after the displacement of seeds from the parent plant to the soil surface, earthworms play an important role in the subsequent displacement of seeds on the soil surface or burial into the soil (Grant 1983, Willems and Huijsmans 1994, Decaens et al. 2003, Milcu et al. 2006a).

Earthworms are a major component of many terrestrial ecosystems (Edwards and Bohlen 1996). In non-acidic soils of temperate grasslands they usually dominate the biomass of soil invertebrates and, especially anecic species, function as ecosystem engineers by structuring the environment of the soil community (Jones et al. 1994, Lavelle et al. 1998,

Scheu and Setälä 2002). Through burrowing, casting and mixing of litter and soil (bioturbation) they influence aggregate stability, soil structure, infiltration of water, aeration of deeper soil layers, microbial biomass and nutrient mineralization (Edwards and Bohlen 1996, Maraun et al. 1999, Tiunov and Scheu 1999, Eisenhauer et al. 2007).

Modification of the physical structure of soil by creating and modifying microhabitats functions as a small-scale disturbance which likely affects plant recruitment and therefore potentially plant community structure (Connell 1978, Fox 1979). Furthermore, earthworm casts and burrows might be important regeneration niches for plant seedlings (Crawley 1992). Surface-foraging species such as *Lumbricus terrestris* are effective in burying seeds, while the surface casts produced by many species often contain seeds (McRill and Sagar 1973, Grant 1983, Thompson et al. 1994, Milcu et al. 2006a). Thereby, earthworms may affect seedling establishment by a variety of mechanisms, through selective ingestion and digestion of seeds (McRill and Sagar 1973, Shumway and Koide 1994, CHAPTER 6), downward or upward seed transport (Grant 1983, CHAPTER 3) and acceleration (Ayanlaja et al. 2001, CHAPTER 6) or delaying of seed germination (Grant 1983, Decaens et al. 2001). A grassland field study by Thompson et al. (1994) indicated that the composition of seeds in bulk soil and earthworm casts differ; seeds in earthworm casts were substantially smaller (<0.3 mg) than the majority of plant seeds of the soil seed bank (0.3 – 1 mg). Therefore, seed selection by earthworms may help to explain the frequently reported differences between the species composition of the seed bank and the standing vegetation (Thompson et al. 1994, Grant 1983), and earthworm activity may be an important factor in plant population dynamics, floristic composition and weed control (Grant 1983). In grasslands about 70% of all seedlings emerged out of earthworm casts (Grant 1983).

Moreover, earthworms may influence seedling establishment and survival through litter removal, which was shown to result in a 5-fold increase in the density of herb seedlings (Wilby and Brown 2001). However, there is little evidence for the effect of earthworms on plant performance starting with changes in seed germination and seedling recruitment. A recent microcosm study revealed that *L. terrestris* strongly affects seed dispersal, seed burial, seedling recruitment, and the spatial distribution of seedlings of plant species of different functional groups which probably affects plant community composition (Milcu et al. 2006a). However, Milcu and colleagues worked with microcosms without an established plant community which gives little evidence for natural conditions in grassland communities.

Building on the study of Milcu et al. (2006a), we set up a microcosm greenhouse experiment to test the following hypotheses:

- (1) Plant invaders perform better in bare grounds than in established plant communities;
- (2) Plant invaders perform better in established plant communities that lack the plant functional group of the invaders;
- (3) Herb invaders perform better in legume than in grass communities due to better nitrogen availability (Dromph et al. 2006);
- (4) Large seeded invaders perform better than intermediate and small ones;
- (5) Earthworms reduce the number but increase the biomass of the established plant invader individuals;
- (6) Earthworms change the structure of plant invader communities by promoting large seeded invaders.

4.3 MATERIALS AND METHODS

Experimental setup

We set up microcosms consisting of PVC tubes (diameter 16 cm, height 38 cm) covered by a 1 mm mesh at the bottom to prevent earthworms (*L. terrestris*) from escaping but allow drainage of water. Furthermore, a plastic barrier (10 cm height, open at the top) prevented earthworm escape from experimental containers. The soil (pH 8.1, carbon concentration 4.6%, nitrogen concentration 0.3%, C-to-N ratio 15.7, water content 14%) was taken from the southeastern edge of the field site of The Jena Experiment (Jena, Thuringia, Germany, Roscher et al. 2004). The Jena Experiment is a long-term grassland study investigating the interactions between plant diversity and ecosystem processes, focusing on element cycling and trophic interactions (Roscher et al. 2004). A total of 120 microcosms each filled with 6 kg (fresh weight; height of soil core 30 cm) of sieved (1 cm), defaunated (heating for four days at 50°C) and homogenized soil were placed in a temperature controlled greenhouse at a day/night regime of 16/8 h and 20/16 ± 2°C (Fig. 4.1A). Before starting the experiment the microcosms were watered regularly for a month (100 ml of deionized water every second day) to leach nutrients released as a result of the defaunation procedure and to remove germinating weeds (unwanted plants from the seedbank). Twelve pre-germinated plant individuals (height 3–6 cm) consisting of two functional groups (grasses and legumes, selected from the species pool of The Jena Experiment, Central European Arrhenatherion grassland, Roscher et al. 2004), were transplanted into $\frac{3}{4}$ of the microcosms creating four plant community treatments (bare ground, grass community, legume community and mixed community). Grass communities contained four individuals of each *Phleum pratense* L.,

Dactylis glomerata L. and *Lolium perenne* L., legume communities contained four individuals of each *Trifolium pratense* L., *T. repens* L. and *Medicago varia* Martyn, mixed communities contained two individuals of each of the six plant species and bare grounds contained no plants (Fig. 4.1B, C). Dried litter (3 g at experimental start and 2 g per microcosm after the first harvest, respectively; carbon concentration 41.2%, nitrogen concentration 2.7%, C-to-N ratio 15.4, dried at 60°C for three days and cut into pieces about 3 cm in length) collected at The Jena Experiment field site and consisting predominantly of grass leaves, was placed on top of the soil prior to the addition of earthworms to simulate field soil surface conditions (Fig. 4.1D). Two adult *L. terrestris* (average fresh weight with gut content 4.25 ± 0.69 g, weighed individually; ~ 100 ind./m²) were introduced to half of the microcosms creating two treatments (with and without earthworms).

After the first harvest 30 seeds (three invader species, ten seeds each) were added with the litter to the soil surface. To each plant community treatment we added seeds out of three plant functional groups separately (grass invaders, legume invaders and herb invaders). To account for different seed size classes (small, intermediate and large seeds) we used *Poa trivialis* L. (small, 2.1 x 0.7 mm), *Alopecurus pratensis* L. (intermediate, 5.0 x 1.8 mm) and *Arrhenatherum elatius* L. (large, 8.0 x 1.8 mm) as grass invader species, *Bellis perennis* L. (small, 1.6 x 0.9 mm), *Taraxacum officinale* Web. (intermediate, 4.0 x 1.0 mm) and *Tragopogon pratensis* L. (large, 12.0 x 1.3 mm) as herb invader species, and *Trifolium campestre* Schreb. (small, 1.0 x 0.8 mm), *Lotus corniculatus* L. (intermediate, 1.5 x 1.3 mm) and *Onobrychis viciifolia* Scop. (large, 6.1 x 4.9 mm) as legume invader species. These species are among successful indigenous invader plant species on the field site of The Jena Experiment (C. Roscher, pers. comm.) and, therefore, are predestinated species to explore the mechanisms of seedling invasion and establishment. We set up five replicates of each of the 24 treatments (Plant Community [4] x Earthworms [2] x Invader Functional Group [3]).

The experiment lasted for four months, six weeks to the first harvest followed by invader seed addition and another ten weeks to the second harvest. Light intensity varied between 450 and 650 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ depending on weather conditions. The water regime was successively increased from irrigating four times a week with 100 ml (weeks 1-3) to irrigating daily with 100 ml (weeks 4-9) and 150 ml (weeks 10-16) deionized water. Microcosms were randomized every two weeks.

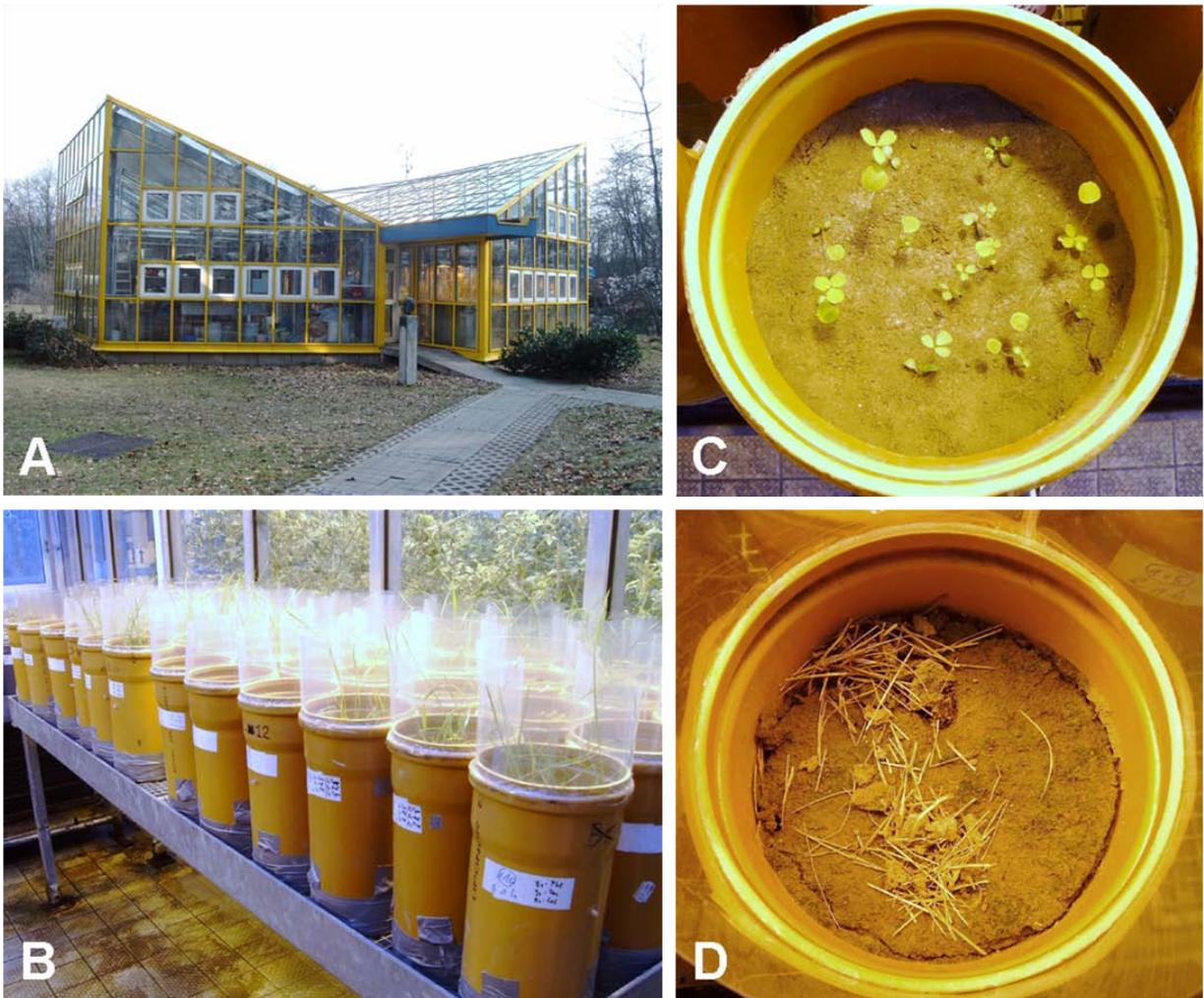


Figure 4.1 | (A) Photograph of the experimental greenhouse. (B) Photograph taken about two weeks after experimental start showing a bench with some of the 120 numbered and randomized microcosms under controlled greenhouse conditions. (C) Photograph of one microcosm (legume community) directly after transplanting the pre-germinated plants. (D) Photograph of one microcosm (bare ground) one week after experimental start showing the formation of middens by two *Lumbricus terrestris* individuals. Photos by N. Eisenhauer.

Sampling

The experiment was divided into two parts, with the first part of the experiment lasting for six weeks to establish the plant communities. Shoot biomass was harvested cutting shoots 3 cm above soil surface level (first harvest). Thereafter, we added 30 invader seeds to each microcosm to simulate the anthropogenic plant seed dispersal accompanying the mowing of grasslands. The second part of the experiment lasted for ten weeks and plant individuals (including invader species) were harvested separately cutting shoots at soil surface level (second harvest). Legume and herb invaders were separated to species level, whereas grasses were only recorded as grass invaders. Roots were sieved from the soil using a 1 mm mesh. Invader roots could not be separated from the roots of the established plant community. Shoot and root material were dried at 60°C for three days.

Earthworms were collected by hand, weighed individually (fresh weight with gut content) and earthworm cocoons were counted. Moreover, we calculated the difference between earthworm weight at the start and the end of the experiment.

Statistical analysis

Two factorial ANOVAs (analyses of variance) implemented in STATISTICA 7.1 (Statsoft) were used to analyze the effects of plant community (bare ground, grass community, legume community and mixed community) and invader functional group (grass invaders, legume invaders and herb invaders) on earthworm weight and cocoon numbers. Only microcosms containing both earthworm individuals at the end of the experiment were included. When necessary, normal distribution and homogeneity of variance were improved by log-transformation.

We performed Friedman ANOVAs as a nonparametric alternative to one-way analysis of variance to analyze the effects of invader seed size on the number (% established invader plants) and biomass (shoot biomass) of invader plants per microcosm. Therefore, we predefined three invader seed size classes (small seeds [*Bellis perennis* and *Trifolium campestre*], intermediate seeds [*Taraxacum officinale* and *Lotus corniculatus*] and large seeds [*Tragopogon pratensis* and *Onobrychis viciifolia*]) and summed up the number and biomass of the corresponding plant species. Data on invader grass species were not included since grass seedlings could not be identified to species level.

Two factorial ANOVAs were used to analyze the effect of plant community and earthworm presence on the relative number and biomass of established invaders belonging to three seed size classes (small, intermediate and large). Therefore, we only used microcosms with three or more established invader plants; the legume and mixed community had to be excluded due to low germination rates.

Four factorial ANOVAs were used to analyze the effects of grass presence, legume presence, earthworm presence and identity of invader plant functional group on the number and biomass of invader plants per microcosm. Therefore, the number and biomass of plant species belonging to the corresponding plant functional groups were added up. Because of low numbers of germinating plants, data on individual invader plant species were not analyzed separately. Further, correlations were carried out to identify associations between the shoot biomass, root biomass and total biomass of the established community and the number and biomass of established invader plants. Therefore, bare ground microcosms were not

considered to evaluate the main processes driving invader establishment in established communities.

Comparisons of means (Tukey's HSD test, $\alpha = 0.05$) were performed using STATISTICA 7.1 (Statsoft). Means presented in text and figures were calculated using non-transformed data (\pm SE).

4.4 RESULTS

Established plant communities

Generally, the establishment of plant communities was successful since 99% of the plant individuals survived the transplantation into experimental microcosms. Total shoot biomass of the grass community (11.19 ± 0.57 g) and the mixed community (10.36 ± 0.58 g) exceeded that of the legume community (7.29 ± 0.66 g) at the first harvest. However, at the second harvest legume communities (20.85 ± 0.38 g) and mixed communities (18.13 ± 0.23 g) produced considerably more shoot biomass than grass communities (13.09 ± 0.49 g). Root biomass was higher in grass (37.20 ± 1.98 g) and mixed communities (33.63 ± 1.48 g) than in legume communities (18.01 ± 1.19 g). Further results on the productivity of the plant communities and the competition between plant individuals are discussed elsewhere (CHAPTER 2).

Effects on earthworms

A total of 110 of 120 earthworms (92%) survived the four months of the experiment. On average 8.4 ± 4.8 cocoons were produced per microcosm. Plant community ($F_{3,37} = 0.53$, $P = 0.67$) and invader functional group ($F_{2,37} = 0.23$, $P = 0.80$) had no effect on earthworm weight, whereas the interaction of these factors affected earthworm weight significantly ($F_{6,37} = 3.41$, $P = 0.009$; Fig. 4.2A). Earthworms gained weight when legume seeds were added to the grass community and lost weight when legume seeds were added to the legume and mixed community. On the contrary, earthworms lost weight when grass seeds were added to the grass community but gained weight when grass seeds were added to the legume and mixed community. The addition of herb seeds increased the earthworm weight slightly only in the legume community.

In contrast, earthworm cocoon numbers were only affected by the plant community with higher numbers in bare grounds (21.08 ± 0.86) than in the grass (7.58 ± 1.41), legume (9.23 ± 1.18) and mixed community (8.36 ± 1.74 ; $F_{3,37} = 23.32$, $P < 0.0001$; Fig. 4.2B).

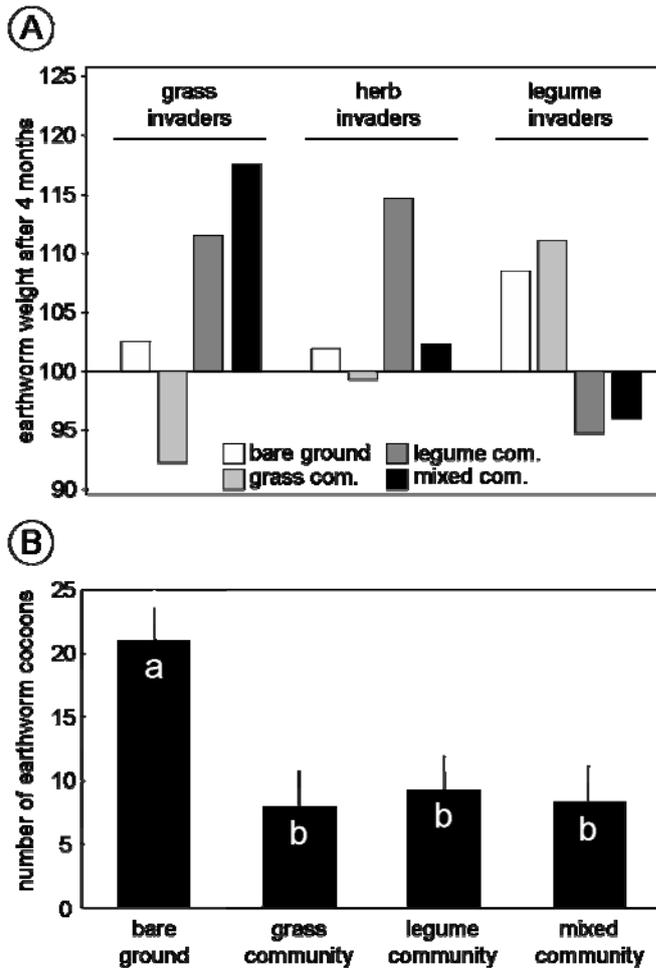


Figure 4.2 | (A) Changes in body fresh weight of *Lumbricus terrestris* during the experiment [% of initial] as affected by the established plant community (bare ground, grass community, legume community and mixed community) and the functional group of the invading plant species (grass, legume and herb invaders); (B) variations in the number of *Lumbricus terrestris* cocoons in different established plant communities. Means with standard errors. Bars with different letters vary significantly (Tukey's HSD test, $\alpha < 0.05$).

Seed size and earthworms

On average, 3.18 ± 0.37 ($10.60 \pm 1.23\%$ of applied seeds) invader plants with a biomass of 0.53 ± 0.12 g per microcosm established in the ten weeks of the experiment (second period).

Seed size influenced the number of established invader plants significantly; more large seeded invaders ($4.35 \pm 1.40\%$) established than small ($2.37 \pm 0.78\%$) and intermediate seeded invaders ($2.03 \pm 0.71\%$; Table 4.1, Fig. 4.3A). On the contrary, there was no effect of seed size on the biomass of invader plants (Table 4.1, Fig. 4.3B).

Generally, more invader plants established in the bare ground treatment ($22.41 \pm 2.82\%$, 2.04 ± 0.36 g) and the grass community ($17.11 \pm 2.11\%$, 0.103 ± 0.014 g) than in the legume ($0.33 \pm 0.19\%$, 0.003 ± 0.002 g) and mixed community ($2.89 \pm 1.18\%$, 0.013 ± 0.006 g; $F_{3,115} = 84.11$, $P < 0.0001$ and $F_{3,115} = 85.12$, $P < 0.0001$ for the number and biomass of invader plants, respectively). However, while small seeded invaders had higher numbers in the bare ground treatment compared to the treatments with established grass community, large seeded invaders showed the opposite pattern with higher numbers in the grass community (Fig. 4.3A). Remarkably, small seeded invaders did not establish in the

legume community at all. However, invaders produced relatively little biomass in already established plant communities, irrespective of the seed size (Fig. 4.3B).

The presence of earthworms reduced the number of established plants of small (-66%) and intermediate (-66%) seeded invaders significantly, and the number of large seeded invaders in trend (-29%; Table 4.1, Fig. 4.3C). However, while earthworm presence did not affect the biomass of small and large seeded invaders, intermediate seeded invaders produced more biomass in presence of earthworms (+158%; Table 4.1, Fig. 4.3D).

While the relative number and biomass of small seeded invaders were significantly higher in the bare ground treatment without earthworms ($56 \pm 9\%$, $60 \pm 13\%$) than in grass communities without ($13 \pm 7\%$, $10 \pm 6\%$) and with earthworms ($12 \pm 6\%$, $10 \pm 5\%$), the number and biomass of large seeded invaders were lower in the bare ground treatment without earthworms ($18 \pm 7\%$, $3 \pm 1\%$) as compared to the bare ground treatment with earthworms ($66 \pm 8\%$, $58 \pm 13\%$) and the grass communities without ($62 \pm 10\%$, $60 \pm 11\%$) and with earthworms ($62 \pm 16\%$ in trend, $64 \pm 15\%$; Table 4.2, Fig. 4.4A, B).

Table 4.1 | Friedman ANOVA table of χ^2 -values for the effect of (A) plant invader seed size (small, intermediate and large) on the on the number (% established invader plants) and shoot biomass of invader plants and (B) plant community (bare ground, grass community, legume community and mixed community) and earthworms (with and without) on the number and biomass of invader plants of different seed size (small, intermediate and large).

	Df	N	established plants		invader biomass	
			χ^2 -value	P-value	χ^2 -value	P-value
(A)						
Seed size	2	119	10.32	0.0057	4.39	0.1116
(B)						
Plant community						
small	3	29	32.66	<.0001	37.62	<.0001
intermediate	3	29	22.87	<.0001	24.90	<.0001
large	3	29	26.52	<.0001	22.42	<.0001
Earthworms						
small	1	59	6.23	0.0126	0.00	1.0000
intermediate	1	59	11.84	0.0006	3.86	0.0493
large	1	59	2.91	0.0881	0.73	0.3948

Df, degrees of freedom; N, number of observations.
Significant effects ($P < 0.05$) are given in bold.

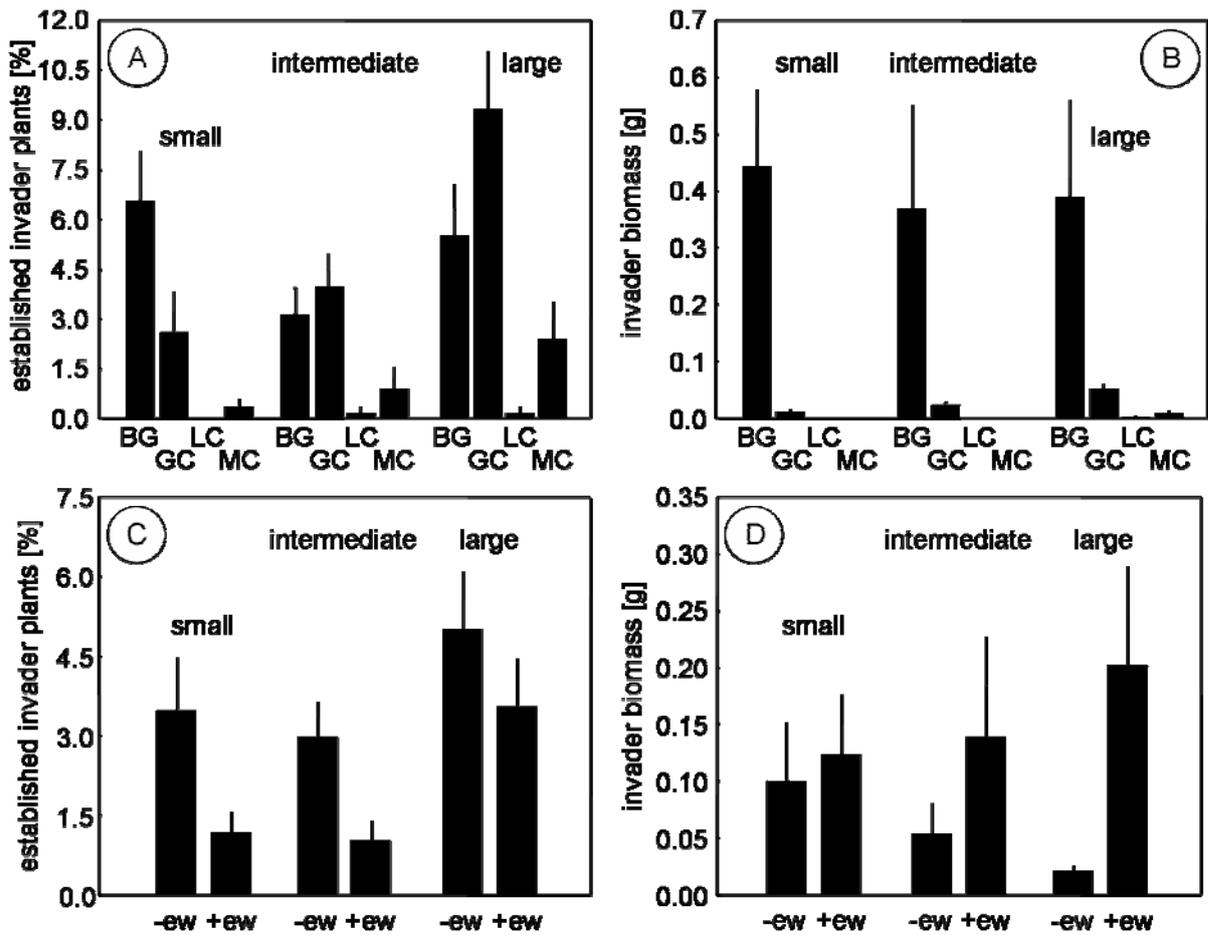


Figure 4.3 | Variations in (A) the number [% of applied seeds] and (B) the biomass [g] of established invader plants as affected by the seed size of the invader plants (small, intermediate and large) and the established plant community (bare ground [BG], grass community [GC], legume community [LC] and mixed community [MC]); variations in (C) the number [% of applied seeds] and (D) the biomass [g] of established invader plants as affected by the seed size of the invader plants and the presence of earthworms (*Lumbricus terrestris*; -ew: without and +ew: with). Means with standard errors.

Table 4.2 | ANOVA table of F-values for the effect of plant community (PC; bare ground, grass community, legume community and mixed community) and earthworms (E; with and without) on the relative number (% established invader plants) and shoot biomass of invader plants of different seed size (small, intermediate and large).

	Df	Df error	established plants		invader biomass	
			F-value	P-value	F-value	P-value
Small seeded invaders						
PC	1	95	8.96	0.0060	8.79	0.0064
E	1	95	1.85	0.1861	1.43	0.2433
PC x E	1	95	3.17	0.0869	2.50	0.1262
Intermediate seeded invaders						
PC	1	95	0.10	0.7499	0.00	0.9813
E	1	95	1.01	0.3252	0.89	0.3534
PC x E	1	95	0.06	0.8014	0.34	0.5627
Large seeded invaders						
PC	1	95	2.38	0.1352	12.80	0.0014
E	1	95	3.58	0.0698	13.45	0.0011
PC x E	1	95	7.42	0.0114	19.15	0.0002

Df, degrees of freedom.

Significant effects ($P < 0.05$) are given in bold.

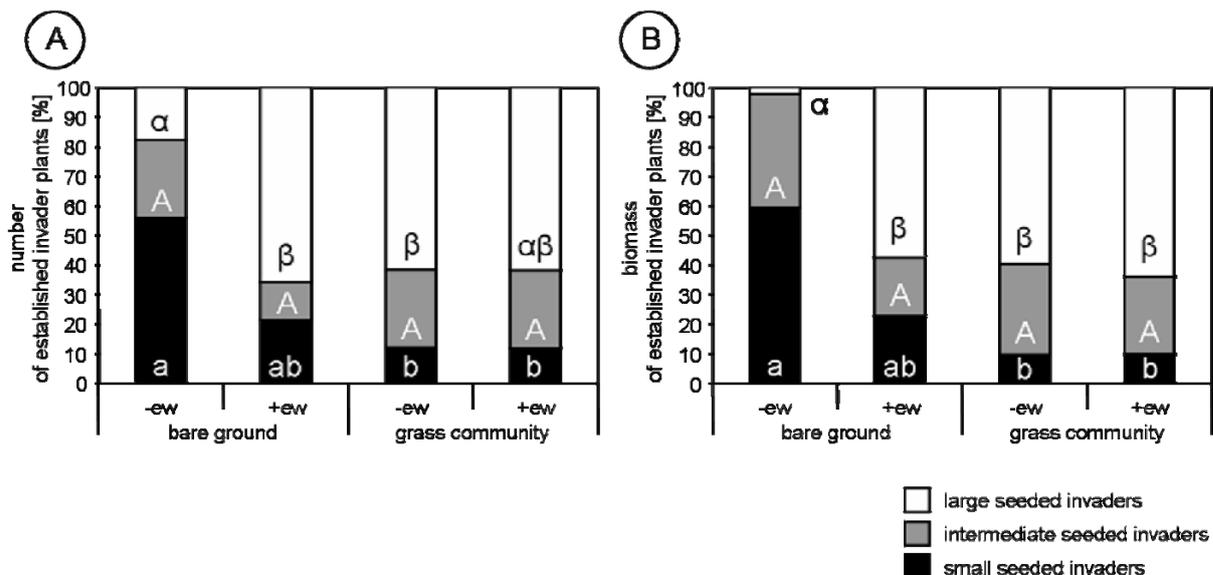


Figure 4.4 | Variations in the proportion of (A) the number and (B) the biomass of different seed size classes (small, intermediate and large seeded invaders) as affected by the plant community (bare ground and grass community) and the presence of earthworms (*Lumbricus terrestris*; -ew: without and +ew: with). Respective bars with different letters vary significantly (Tukey's HSD test, $\alpha < 0.05$).

Plant functional groups and earthworms

Generally, significantly more grass invaders ($14.97 \pm 2.87\%$) established than legume ($8.50 \pm 1.67\%$) and herb invaders ($8.42 \pm 1.67\%$; Table 4.3, Fig. 4.5A). Remarkably, the biomass of grass (0.64 ± 0.24 g) and herb invaders (0.70 ± 0.23 g) did not vary but were significantly higher than that of legume invaders (0.24 ± 0.10 g; Table 4.3, Fig. 4.5B).

The number of grass invaders and the biomass of grass and herb invaders were significantly lower in presence of grasses in the established community (Fig. 4.5A, B). However, in presence of legumes in the established community the number and biomass of invader plants were decreased substantially, irrespective of plant functional group identity (Fig. 4.5A, B).

The presence of earthworms decreased the total number of invader plants, irrespective of grass and legume presence in the established plant community (-48%; Fig. 4.5C, D). However, while the presence of grasses had no significant effect on the total number of invader plants, the presence of legumes decreased the number of total invader plants considerably (-92%; Fig. 4.5C, D). Furthermore, in presence of grasses and legumes the total biomass of invader plants decreased substantially (-94% and -99% respectively; Fig. 4.5E). However, the total biomass of invader plants was only increased in the presence of earthworms in bare ground treatments (+158%; Fig. 4.5E). Remarkably, there was no effect of earthworm presence on total invader biomass in already established plant communities (Fig. 4.5E). However, generally, the number of grass and legume invader plants was decreased in presence of earthworms (-45% and -67%, respectively), while the number of herb invader plants remained unaffected (Fig. 4.5F). Furthermore, there were significant negative correlations between the number ($R^2 = 0.48$, $P < 0.0001$; Fig. 4.6) and biomass ($R^2 = 0.32$, $P < 0.0001$; data not shown) of total invader plants and the shoot biomass of the established plant community. In contrast, there were weak positive correlations between the number ($R^2 = 0.04$, $P = 0.0644$) and biomass ($R^2 = 0.06$, $P = 0.02$) of total invader plants and the root biomass of the established plant community but low R^2 values indicate that root biomass was of minor importance. Moreover, there was no correlation between the number ($R^2 < 0.01$, $P = 0.8015$) and biomass ($R^2 = 0.01$, $P = 0.5066$) of total invader plants and the total biomass of the established plant community.

Table 4.3 | ANOVA table of F-values for the effect of the presence of grasses (G; with and without), legumes (L; with and without), earthworms (E; with and without) and invader functional group (IF; grass invaders, legume invaders and herb invaders) on the number (% established invader plants) and shoot biomass of invader plants.

	Df	Df error	established plants		invader biomass	
			F-value	P-value	F-value	P-value
G	1	95	0.42	0.5211	94.80	<.0001
L	1	95	443.64	<.0001	142.00	<.0001
E	1	95	30.33	<.0001	9.72	0.0024
IF	2	95	6.58	0.0021	6.84	0.0017
G x L	1	95	10.90	0.0014	99.19	<.0001
G x E	1	95	3.45	0.0663	10.73	0.0015
L x E	1	95	2.44	0.1216	10.76	0.0015
G x IF	2	95	13.97	<.0001	11.95	<.0001
L x IF	2	95	18.85	<.0001	8.39	0.0004
E x IF	2	95	5.93	0.0038	0.02	0.9837
G x L x E	1	95	0.04	0.8458	10.56	0.0016
G x L x IF	2	95	2.93	0.0581	10.68	<.0001
G x E x IF	2	95	1.28	0.2829	0.01	0.9930
L x E x IF	2	95	0.18	0.8329	0.02	0.9811
G x L x E x IF	2	95	2.13	0.1237	0.00	0.9986

Df, degrees of freedom.

Significant effects ($P < 0.05$) are given in bold.

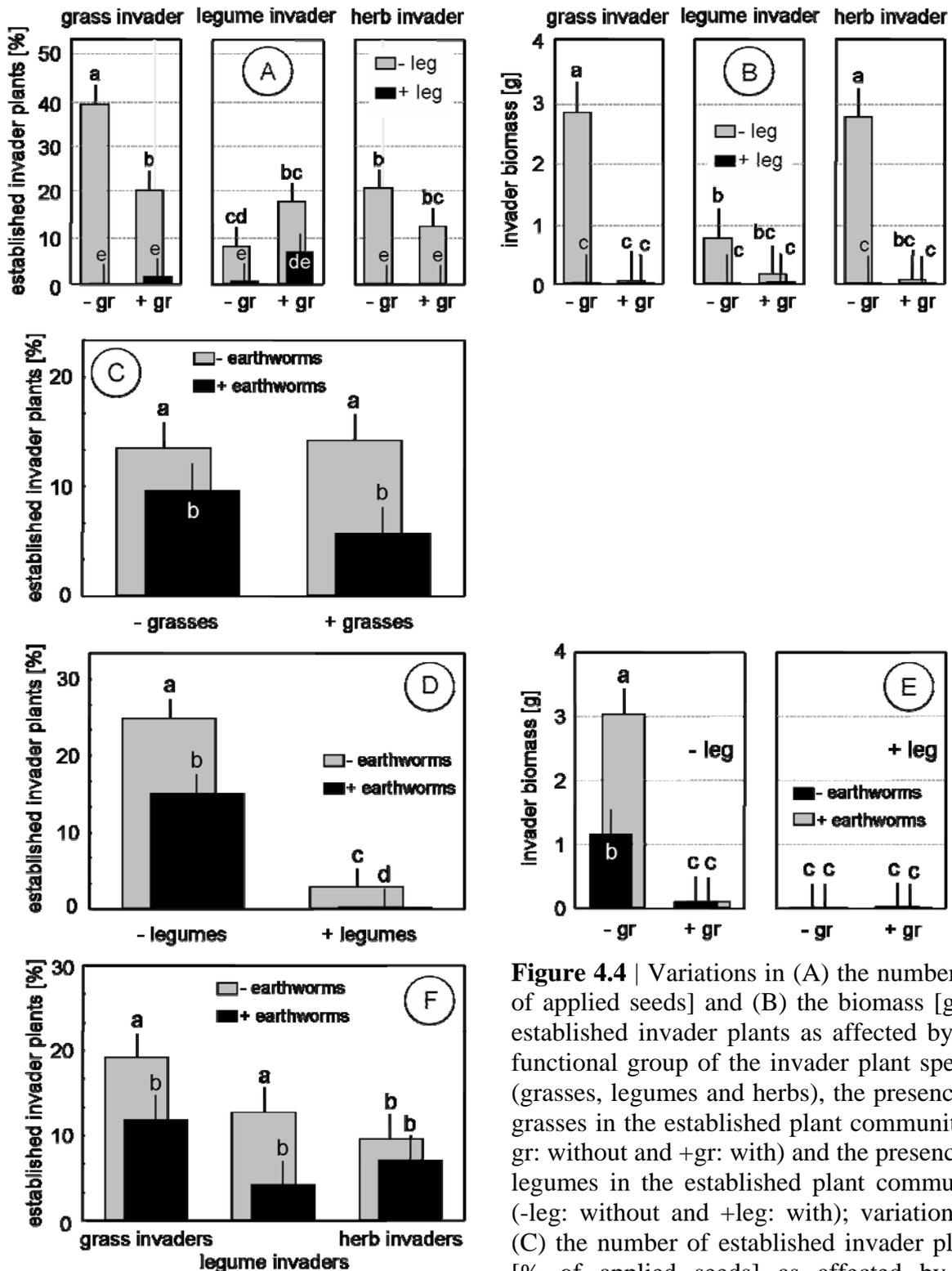


Figure 4.4 | Variations in (A) the number [% of applied seeds] and (B) the biomass [g] of established invader plants as affected by the functional group of the invader plant species (grasses, legumes and herbs), the presence of grasses in the established plant community (-gr: without and +gr: with) and the presence of legumes in the established plant community (-leg: without and +leg: with); variations in (C) the number of established invader plants [% of applied seeds] as affected by the presence of grasses in the established

plant community and the presence of earthworms (*Lumbricus terrestris*; -ew: without and +ew: with); variations in (D) the number and (E) the biomass of established invader plants as affected by the presence of grasses (only in [E]) and legumes in the established plant community and the presence of earthworms; variations in (F) the number of established invader plants as affected by the functional group of the invader plant species (grass invaders, legume invaders and herb invaders) and the presence of earthworms. Means with standard errors. Bars with different letters vary significantly (Tukey’s HSD test, $\alpha < 0.05$).

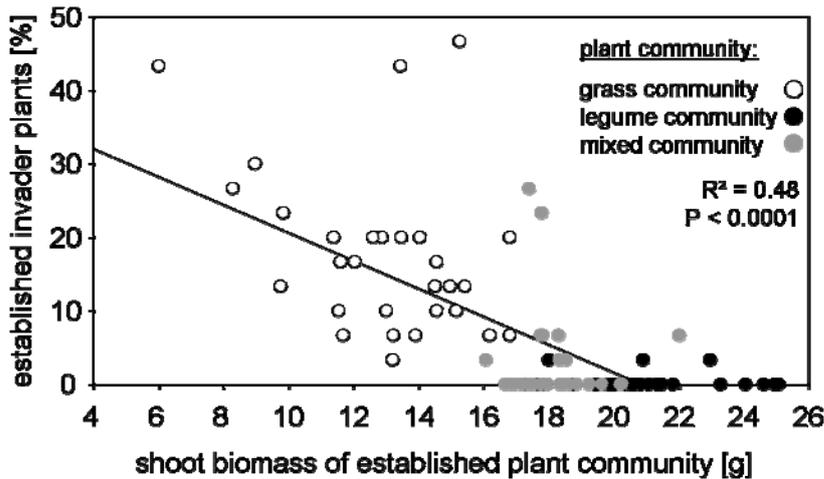


Figure 4.5 | Regression between the shoot biomass of the established plant community ([g]; grass community, legume community and mixed community) and the number of established invader plants [% of applied seeds].

4.5 DISCUSSION

Mechanisms that make communities resistant to invasions and drive the establishment success of plant seedlings are essential for understanding community assembly and structure (Fargione et al. 2003). Seed burial is a key factor in prolonging the survival of seeds (Harper 1957) and in the establishment of plant invaders. Thereby, anecic earthworms may affect seedling establishment by a variety of mechanisms, e.g. through selective ingestion and digestion of seeds (McRill and Sagar 1973, Shumway and Koide 1994, CHAPTER 6) and downward or upward seed transport (Grant 1983, CHAPTER 3). However, the question if *L. terrestris* functions as a driving agent of plant community composition in semi-natural grasslands has been unanswered.

Generally, *L. terrestris* buried or consumed the whole amount of litter and all seeds irrespective of seed size and plant functional group identity during the first week of the second experimental period. Although we used relatively high numbers of anecic earthworms (~100 ind./m²; to compensate for dying individuals) compared to semi-natural conditions at the field site of The Jena Experiment (~30 ind./m²; CHAPTER 5), we are convinced that observed earthworm effects resemble those under field conditions as anecic earthworms bury plant seeds very fast (Milcu et al. 2006a). However, we could not distinguish between buried and ingested plant seeds but large seeds of invaders presumably could not be swallowed by *L. terrestris* since it is unable to feed on particles with a diameter larger than 2 mm (Shumway and Koide 1994, CHAPTER 6).

Effects on earthworms

Earthworms lost weight when grass invaders were added to grass communities and legume invaders to legume communities, but gained weight when grass invaders were added to legume communities and legume invaders to grass communities. Since germination and growth of species of a specific plant functional group may be inhibited by the presence of plants of the same plant functional group in the direct vicinity (intra-functional group competition), grass invaders probably germinated better in legume communities and legume invaders in grass communities (Fargione and Tilman 2005). Assuming that earthworms benefit from germinating seeds, *L. terrestris* likely performed better in treatments with the invader species and the established plant community differing in plant functional group identity due to increased food supply. The higher earthworm weight in presence of grass invaders than in the presence of legume invaders in mixed communities presumably was due to the dominance of legumes in the established plant community in the second part of the experiment. Again, grass invaders may have germinated better than legume invaders due to reduced intra-functional group competition resulting in increased food supply for earthworms.

Further, the number of earthworm cocoons was significantly higher in the bare ground treatment than in each of the plant treatments. There are two explanations for this observation which may have acted in combination. First, the water content was higher in the bare ground treatment due to the lack of transpiration by plants (data not shown); indeed, the soil in planted microcosms was temporarily dry which may have detrimentally affected earthworm performance (Berry and Jordan 2001). Second, earthworms presumably fed on invader plant seedlings previously pulled into their burrows (Lee 1985) which germinated better in bare grounds than in established plant communities (Maron 1997). These findings are in strong contrast to the view that digestion of plant seeds by earthworms is of minor importance (Curry and Schmidt 2007), although it is well documented that *L. terrestris* buries, ingests and digests plant seeds (McRill and Sagar 1973, Grant 1983, Milcu et al. 2006a, CHAPTER 6). However, the role of plant seeds, in particular that of germinating seeds, for earthworm nutrition remains unclear, but recent work (CHAPTER 6) and the present study indicate that at least certain plant seeds are effectively digested by anecic earthworms.

Seed size

Seed size is a key trait of plant species, determining both competitive and colonizing ability (Turnbull et al. 2004). Generally, seed mass of plant species is thought to result from a trade-off between producing few, large seeds, each with high probability of successful

establishment, versus producing many small seeds, each with a low probability of establishment (Moles and Westoby 2006). Thereby, larger and better provisioned seedlings associated with large seeds have the ability to survive periods of resource shortage imposed by drought, shade and herbivory (Westoby et al. 1996). Indeed, in the present study large seeded invaders established better than intermediate and small seeded invaders, confirming our hypothesis (4). Large seeded invaders established even in already established plant communities which supports observations of Leishman and Westoby (1994) and Burke and Grime (1996) that large seeded invaders have higher survival rates of harsh environmental conditions, e.g. due to shading and competition with established plants. Interestingly, however, the biomass of established invader plants at the end of the experiment did not vary with seed size suggesting that once established, plant fitness is independent of seed size. Not surprisingly and confirming hypothesis (1), plant invaders performed best in the bare ground treatment reflecting the significance of small scale disturbances causing open gaps in the vegetation for invader establishment.

Plant functional group

Conforming to our hypotheses (2) and (3) the establishment of invaders depend on the plant functional group present in the established plant community. In contrast to these hypotheses, invader establishment was not driven by open “functional gaps” in the established community (i.e. absence of the plant functional group of the invading plant species in the established plant community). In fact, presence of legumes in the established plant community had a stronger negative effect on invader establishment than the presence of grasses; generally, the number of invader plants was very low in presence of legumes irrespective of grass and earthworm presence, and of plant functional group identity of the invader plants. The results suggest that invader establishment in fact is not facilitated by legumes and the associated nitrogen fixation. Rather, the strong negative correlation between the shoot biomass of the established plant community and the number of invader plants indicates that shading by legumes was the major factor driving invader plant establishment. Complementarity is thought to be an important factor affecting plant community invasibility since there are trade-offs in the efficiency of using different resources and in colonization and competitive abilities (Fargione and Tilman 2005). In contrast to this view and our hypothesis (2) the results suggest that complementarity is of little importance during the first stages of invader establishment. Competition with established plants presumably is driven by light as indicated by the significant correlation between the shoot biomass of the established plant

community and the number of invader plants. Weak or missing correlations between root biomass of the established plant community and the number and biomass of invader plants indicate that belowground competition for soil nutrients is of minor importance during the first stages of invader establishment, however, very likely it becomes important in later stages of invader plant development.

Effects of earthworms

Moles and Westoby (2006) proposed an equation for the survival of the most important life stages of plants. As indicated in Fig. 4.7, earthworms affect four of seven life stages directly [survival of post-dispersal seed predation (Grant 1983; Thompson et al. 1994), storage in soil (Thompson et al. 1994), germination (Ayanlaja et al. 2001) and seedling survival (Lee 1985)] reflecting the capacity of earthworms to drive plant recruitment and plant community composition. Surprisingly, the majority of the previous studies concentrated on indirect effects of earthworms on plants (reviewed by Scheu 2003, Brown et al. 2004). However, a recent study indicated that earthworms indeed drive seedling establishment and the invasibility of established plant communities directly (Milcu et al. 2006a). Building on this, the present study for the first time investigated the significance of earthworms for the establishment of plant invaders in semi-natural plant communities in context of characteristics of the established plant community (plant functional group identity, above- and below-ground biomass), seed size and plant functional group identity of invading plant species.

We had hypothesized (5) that earthworms reduce the number but increase the biomass of plant invader individuals. Indeed, the number of established invader plants was decreased in small, intermediate and, in trend, large seeded invaders in presence of earthworms, but in contrast to our hypothesis the biomass of invader plants was only increased in intermediate sized species in presence of earthworms. Milcu et al. (2006a) proposed that especially larger seeds, which are known to have a higher risk of granivory than smaller seeds (Heske and Brown 1990), benefit from being buried by earthworms by preventing above-ground seed predation and causing favourable environmental conditions for germination, recruitment and growth in earthworm middens (Blanchart et al. 1999). According to our results, however, this may only in part apply to established plant communities. *Lumbricus terrestris* is known to ingest and digest plant seeds (McRill and Sagar 1973, Shumway and Koide 1994) with digestion rates of ingested seeds between 34 and 83% depending on plant species identity (CHAPTER 6). Smaller seeds (< 2 mm) are preferentially ingested and this may explain the detrimental effect of earthworms on the number of small and intermediate seeded invader

plants in the present experiment. Since earthworm casts are known to be essential regeneration niches in grassland communities (Grubb 1977, Grant 1983), earthworms probably drive plant community composition directly due to selective seed predation. Moreover, *L. terrestris* likely behaves like a “farmer” by collecting plant seeds which cannot directly be swallowed or digested but are stocked in middens and become eatable after partial microbial decay.

Overall, *L. terrestris* likely governs plant community composition and decreases the invasibility of grassland systems for weed plant species. Since more diverse plant communities support higher numbers of earthworms (Milcu et al. 2008, CHAPTER 5), earthworm activity, granivory and herbivory on small invader seedlings in its burrows might contribute to the increased resistance of diverse plant communities against invasions (Elton 1958, Tilman 1999, Fargione and Tilman 2005). Further, the present study indicates that the effect of earthworms on invader plant species varies with plant functional group identity in that numbers of grass and legume invaders were decreased by earthworm presence while herb invaders remained unaffected. Consequently, as we hypothesized (6) earthworms presumably not only change the number of invader plants but also plant invader composition by affecting plant functional groups differently and by promoting large seeded species.

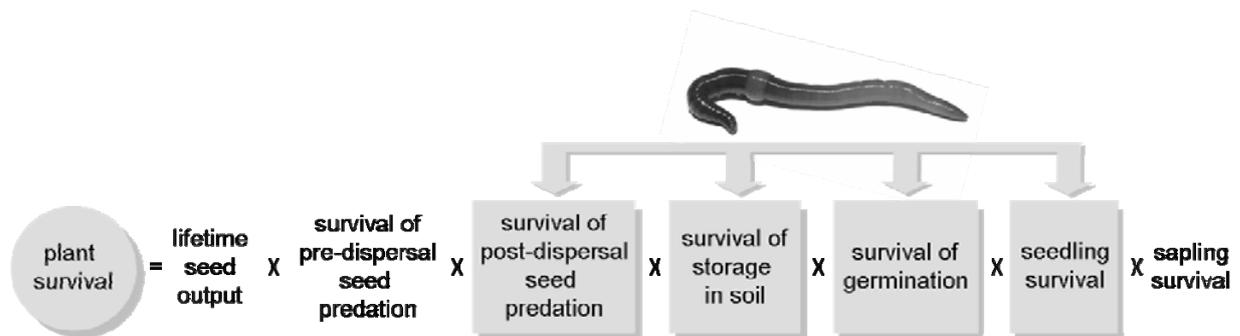


Figure 4.7 | Plant survival as a function of the survival of different plant life stages with emphasis on life stages directly influenced by earthworms. Modified after Moles and Westoby (2006).

4.6 CONCLUSIONS

Small scale disturbances and open gaps represented by the bare ground treatments were shown to be essential regeneration niches but also patches of increased probability for plant invasion in grassland communities. *Lumbricus terrestris* functions as a driving agent of plant recruitment and plant community composition by burying, ingesting and digesting plant seeds. Since earthworm numbers increase in more diverse plant communities they likely contribute to the positive relationship between plant species diversity and resistance against invaders. However, in regions devoid an indigenous earthworm fauna invasive species, such as *L. terrestris*, may have different impacts on the plant community, e.g. by decreasing the fitness of endemic plant species and possibly favouring the establishment of invading plant species whose seeds may be adjusted to the burial and ingestion by earthworms. Taking the significant impact of earthworms on plant seeds and seedlings and the potential contribution of seeds and seedlings in earthworm nutrition into account, we hypothesize that plants and earthworms might have co-evolved in temperate regions of Central Europe. This topic deserves further attention with regard to the proceeding anthropogenic dispersal of European earthworms worldwide and the homogenization of habitats.

CHAPTER

5



INVASIBILITY AND STABILITY
OF TEMPERATE GRASSLANDS:
BIODIVERSITY AND ECOSYSTEM
ENGINEERS (ANECIC EARTHWORMS)
AS DETERMINANTS

5.1 ABSTRACT

Invasions of natural communities by non-indigenous species are currently rated as one of the most important global-scale environmental problems. Biodiversity is one feature of communities that has long been hypothesized to reduce invasions and increase stability by using resources more completely than simple communities. Disturbances by ecosystem engineers affect the distribution, establishment, and abundance of species but this has been widely ignored in studies on diversity-invasibility relationships.

We determined natural plant invasion into plots varying in the number of plant species (1, 4, and 16) and plant functional groups (1, 2, 3, and 4) for three vegetation periods two years after establishment of The Jena Experiment. We sampled subplots with earthworm addition and earthworm reduction to investigate effects of important ecosystem engineers in temperate grasslands. Additionally, we performed a seed-dummy experiment to investigate the role of earthworms in secondary seed dispersal in a plant diversity gradient.

Transposition of seeds decreased considerably in earthworm reduction subplots indicating successful density manipulations. Seed dispersal by earthworms decreased with increasing plant species diversity and presence of grasses but increased in presence of small herbs suggesting that dense vegetation inhibits surface activity of earthworms.

Invasibility decreased and stability increased with increasing plant species diversity. Thereby, coverage of the resident plant community (light availability) and fine root biomass (belowground nutrient competition) presumably govern community invasibility. However, the present study highlights the intimate relationship between earthworms as ecosystem engineers and plant species diversity, functional group identity and structural complexity for the invasibility and stability of grassland communities. Earthworms modulated the diversity-invasibility relationship by increasing plant invader numbers and diversity, and by decreasing stability of grassland communities. Overall the results document that fundamental processes in plant communities are modulated by soil fauna calling for closer cooperation between soil animal and plant ecologists.

5.2 INTRODUCTION

Anthropogenic activity has played an important role in facilitating biological invasions and is reducing biodiversity worldwide (Pimm et al. 1995, Vitousek et al. 1996). Invasions of natural communities by non-indigenous species are currently rated as one of the most important global-scale environmental problems (Vitousek et al. 1996). The increasing loss of biodiversity has generated concern over the consequences for ecosystem functioning and thus understanding the relationship between both has become a major focus in ecological research during the last two decades (Schulze and Mooney 1994, Kinzig et al. 2002, Loreau et al. 2002, Fargione and Tilman 2005). Until today more than 150 biodiversity-function experiments have been performed (Cardinale et al. 2007), however, the mechanisms responsible for biodiversity-function relationships have been hotly debated without reaching consensus on the relative importance of sampling of species and complementarity of niches until today (Huston 1997, Loreau 2000, Cardinale et al. 2007).

Understanding the mechanisms that make communities resistant to invasions and drive the establishment success of seedlings is essential both for management (D'Antonio and Vitousek 1992, Pimentel et al. 2000) and for community assembly and structure (Fargione et al. 2003). Biodiversity is one feature of communities that has long been hypothesized to reduce invasions and increase stability by using resources more completely than simple communities (Elton 1958, Levine and D'Antonio 1999, Tilman 1999, Fargione and Tilman 2005, Weigelt et al. 2008). Thereby, complementarity is thought to be an important factor since it may result in species having trade-offs in their efficiency of using different resources, in colonization and competitive abilities, or in their success at different environmental conditions (Fargione and Tilman 2005). However, an ecosystem's susceptibility to invasion is influenced by many factors (Crawley et al. 1999, Levine and D'Antonio 1999). Species diversity of plant communities result from dynamics in plant mortality and seedling establishment. Thereby, local processes like small scale disturbances and formation of regeneration niches are essential factors driving the establishment of seedlings (Grub 1977, Milcu et al. 2006a). In this context, ecosystem engineering (the modification, maintenance, creation or destruction of habitats) clearly has the potential to affect the distribution, establishment and abundance of species (Jones et al. 1997, Wright and Jones 2004), e.g. ants were reported to have considerable effects on the vegetation structure in grassland by creating gaps and translocating plant seeds (King 2007). Surprisingly, however, ecosystem engineers have widely been ignored in studies investigating diversity-invasibility relationships. For a

more holistic view on the factors driving invasion resistance and stability of plant communities, considering keystone fauna groups is essential. Among these, earthworms should without doubt be ranked first (Huhta 2007).

It is increasingly recognized that after phase I dispersal of seeds, i.e. the displacement of seeds from the parent to the soil surface, earthworms play an important role in phase II dispersal, i.e. the subsequent displacement of seeds on the soil surface and burial into the soil (Grant 1983, Willems and Huijsmans 1994, Decaens et al. 2003, Milcu et al. 2006a, CHAPTER 4). Selective ingestion and digestion of seeds (McRill and Sagar 1973, Shumway and Koide 1994, CHAPTER 6), downward or upward seed transport (Grant 1983), acceleration (Ayanlaja et al. 2001; CHAPTER 6) or delaying of seed germination (Grant 1983, Decaens et al. 2001) are the main mechanisms by which earthworms affect seedling establishment, and these processes likely are important for seedling mortality and establishment under natural conditions (Zaller and Saxler 2007, CHAPTER 4). However, this has not been proven.

Large surface feeding anecic earthworms, such as *Lumbricus terrestris* L. (Lumbricidae), are a dominant component of decomposer communities in virtually all non-acidic pastures and meadows of temperate regions. Furthermore, *L. terrestris* is a peregrine species which has been spread worldwide with European agricultural practices including ecosystems previously devoid of earthworms (Bohlen et al. 2004, Eisenhauer et al. 2007). Anecic earthworms function as ecosystem engineers modifying the physical structure of soils by changing soil aggregation, soil porosity and the distribution and abundance of microorganisms and other soil invertebrates (Wickenbrock and Heisler 1997, Maraun et al. 1999, Tiunov and Scheu 1999, Eisenhauer et al. 2007). Modification of the soil physical structure by creating and modifying microhabitat functions acts as small-scale disturbances which likely affect plant recruitment and, therefore, plant community structure (Connell 1978, Fox 1979). Consequently, following the “intermediate disturbance hypothesis” (Connell 1978), earthworm middens might increase the spatial heterogeneity of habitats and plant diversity locally. Thereby, earthworm casts and middens might function as important regeneration niches for plant seedlings (Crawley 1992, Milcu et al. 2006a).

Here we report results from three years of natural plant invasion into experimental grassland communities of The Jena Experiment, a large grassland experiment focussing on the role of biodiversity for element cycling and trophic interactions (Roscher et al. 2004). The present study is, to our knowledge, the first focussing on the mechanisms of invasion resistance and stability in a plant diversity gradient as modulated by ecosystem engineers. The main objectives of the study were:

1. Why is biodiversity a barrier for species invasion and what are the driving mechanisms making a diverse community resistant to the establishment of invader plants?
2. What is more important, plant species diversity or plant functional group diversity?
3. Are there keystone plant functional groups affecting invasion resistance?
4. Are manipulations of earthworm densities efficient in the field and are they able to modulate ecosystem functions?
5. Are ecosystem engineers important drivers of plant invader establishment and do they affect plant community diversity?
6. Are earthworms modifying the stability of grassland communities?

5.3 MATERIALS AND METHODS

Experimental setup

The present study was part of The Jena Experiment, a large field experiment investigating the role of biodiversity for element cycling and trophic interactions in grassland communities (Roscher et al. 2004). The study site is located on the floodplain of the Saale river (altitude 130 m NN) at the northern edge of Jena (Thuringia, Germany). Mean annual air temperature 3 km south of the field site is 9.3°C and annual precipitation is 587 mm (Kluge and Müller-Westermeier 2000). The site had been used as an arable field for the last 40 years and the soil is an Eutric Fluvisol (FOA-Unesco 1997).

The experiment was established in May 2002. The studied system represents Central European mesophilic grasslands traditionally used for haymaking (Arrhenatherion community; Ellenberg 1988). A pool of 60 native plant species was used to establish (by independent random draws with replacement) a gradient of plant species (1, 2, 4, 8, 16, and 60) and functional group diversity (1, 2, 3, and 4) in a total of 90 plots of 20 x 20 m (Fig. 5.1A; Roscher et al. 2004). Plant species were aggregated into four plant functional groups: grasses (16 species), small herbs (12 species), tall herbs (20 species), and legumes (12 species) by using (1) above- and belowground morphological traits, (2) phenological traits, and (3) the ability for N₂ fixation as attribute classes (Roscher et al. 2004). Experimental plots were mown twice a year (June and September), as is typical for hay meadows and weeded twice a year (April and July) to maintain the target species composition.

Plots were assembled into four blocks following a gradient in soil characteristics, such as stone surface cover (0-23%), sand content (45-628 g kg⁻¹), and CaCO₃ concentration (40-

391 g kg⁻¹). Each block contains an equal number of plots and plant species and functional group diversity levels and was divided into subplots to allow for the establishment of nested project-specific treatments and destructive measurements. Further information on the design and setup of The Jena Experiment is given in Roscher et al. (2004).

Manipulation of earthworm densities

Subplots for manipulating earthworm density were established on the 1, 4, and 16 plant species diversity levels in September 2003. On each plot two randomly selected subplots of 2 x 4 m were used to establish the following treatments: control and earthworm. Earthworm subplots were further divided into two earthworm density treatments (subplots with earthworm addition and earthworm reduction). Subplots (1 x 1 m) were enclosed with PVC shields aboveground (20 cm) and belowground (15 cm) to prevent the escape or colonization of *L. terrestris* which is the only large surface active (anecic) earthworm species occurring at the field site of The Jena Experiment. Earthworm addition subplots received 25 adult individuals of *L. terrestris* (average fresh weight with gut content 4.10 ± 0.61 g) per year (15 individuals in spring and 10 in autumn). The earthworm addition treatment was established since earthworm density was low after establishment of The Jena Experiment which involved repeated disk cultivation to reduce weed density, a practice which is known to detrimentally affect earthworms (Edwards and Bohlen 1996). Further, two earthworm extraction campaigns were performed per year (spring and autumn) on the adjacent earthworm reduction subplots by electro-shocking (Fig. 5.1B). This non-destructive method has been shown to provide comparable estimates of earthworm biomass (Vetter et al. 1996) and community composition (Zaller and Arnone 1999b). A combination of four octet devices (DEKA 4000, Deka Gerätebau, Marsberg, Germany; Thielemann 1986) was used. On each subplot earthworm extraction was performed for 35 minutes, increasing the voltage from 250 V (10 min) to 300 V (5 min), 400 V (5 min), 500 V (5 min), and 600 V (10 min). Extracted earthworms were identified, counted and weighed (fresh weight with gut content) to investigate the effects of plant diversity and plant functional groups on earthworm performance (number and biomass). Additionally, control subplots were established in autumn 2005 by installing aboveground PVC shields to investigate the efficiency of earthworm density manipulations (seed dummy experiment).

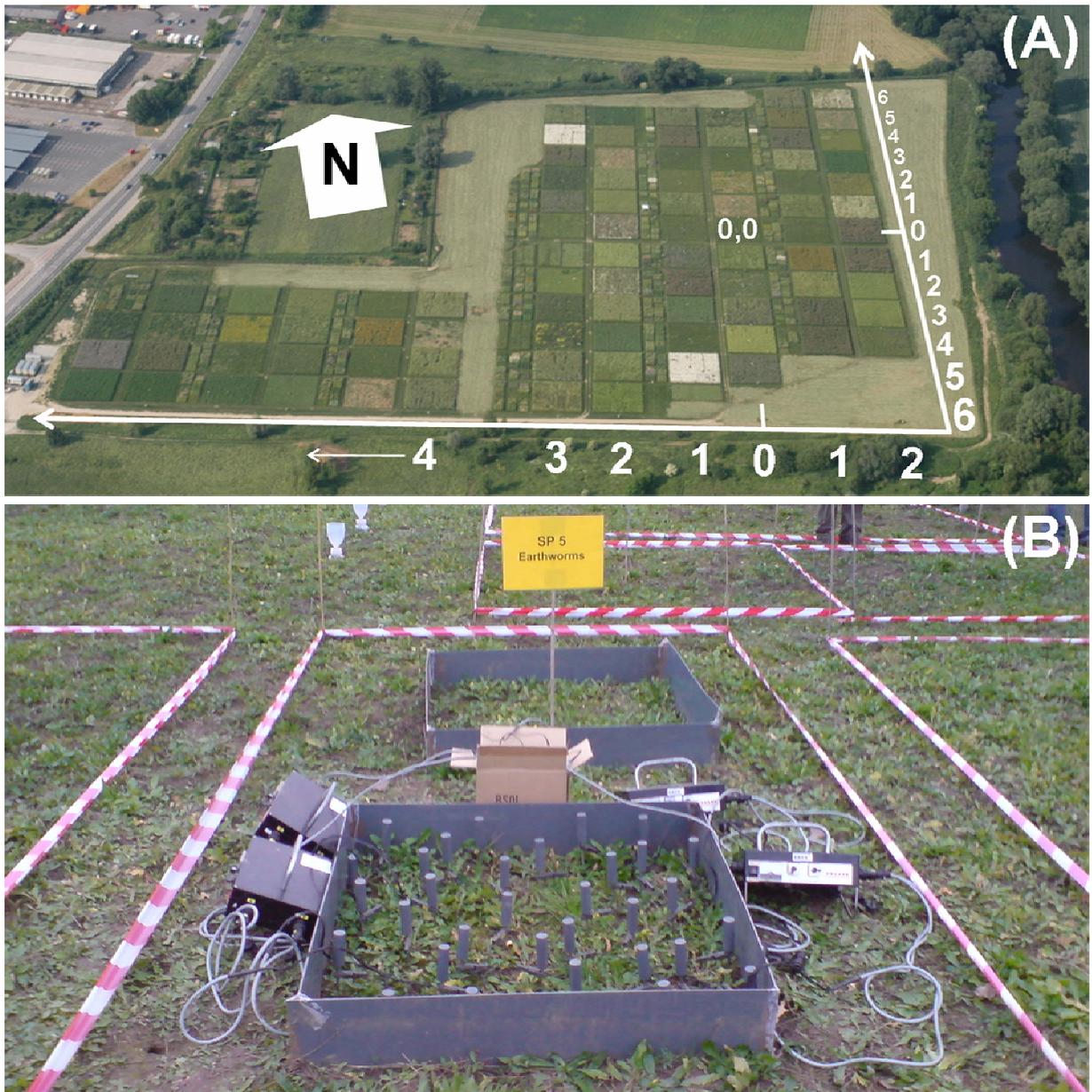


Figure 5.1 | (A) Photograph of the field site of The Jena Experiment taken in 2004 showing the main experimental plots (20 x 20 m) varying in sown plant species diversity (1, 2, 4, 8, 16, and 60) and plant functional group diversity (1, 2, 3, and 4) and the X- (horizontal axis) and Y-coordinates (vertical axis). Photo by J. Baade. (B) Photograph of one exemplary earthworm subplot, the enclosures for earthworm density manipulations (earthworm addition and earthworm reduction), and four octet devices used for earthworm extraction by electroshocking. Photo by N. Eisenhauer.

Above- and belowground plant community parameters

Coverage of the plant community and fine root biomass were determined to provide explanatory variables (above- and belowground plant community parameters) for the establishment success of plant invaders. Therefore, the coverage of the plant community [% coverage of soil surface; 1 m²] was determined on the control subplots in May 2006. To determine fine root biomass (root diameter ≤ 2 mm) three soil samples were taken per plot from June to July 2006 (diameter 4.8 cm; to a depth of 30 cm). Pooled soil samples per plot were homogenized and cut into pieces using scissors (maximal length of root fragments 1 cm). Roots were washed out of one subsample per plot (50 g soil) using a 0.5 mm mesh, placed on Petri dishes to remove mineral soil particles, dried (70°C; 24 h), and weighed.

Seed dummy experiment

Aboveground PVC shields were also installed at control subplots (in autumn 2005) to account for possible edge effects of the enclosures and, consequently, differing earthworm activity under unequal microclimatic conditions. The seed dummy experiment was performed in May 2006, five weeks after the last earthworm density manipulation to investigate the efficiency of density manipulations for *L. terrestris* (via earthworm soil surface activity). Since *L. terrestris* is known to bury seeds irrespective of size and shape (Milcu et al. 2006a, CHAPTER 6), nine seed dummies (little glass beads; diameter 2 mm) spaced 25 cm were deployed in each earthworm treatment (control, earthworm addition, and earthworm reduction). Each seed dummy was marked with a flag to allow detecting movement and burying of the dummies. The number of moved and buried dummies was determined one week after application. There was no heavy rain and wind during the experiment which could have moved the dummies. To evaluate potential influence of voles the number of vole holes was determined per subplot, however, it was not correlated with the number of moved and buried seed dummies (data not shown). Thus, any movement of seed dummies was ascribed to earthworms.

Plant invaders

To investigate the effects of earthworms, sown plant species diversity, plant functional group diversity, and identity of certain plant functional groups on the invasibility of grassland communities, earthworm subplots (earthworm addition and earthworm reduction) were weeded in April 2004, 2005, and 2006. All plant individuals which did not belong to the respective initial target plant community were removed. Focusing on the main mechanisms of

plant invader establishment, we did not distinguish between experimental and non-experimental invader species. Weeded plants were identified, counted, separated into plant functional groups (grasses, herbs and legumes), dried (60°C, 72 h) and weighed. Moreover, invader diversity was determined by counting herb and legume invader species per subplot. Thereby, grass invaders were not considered since they were not identified to species level.

Further, we determined the stability of the plant communities by calculating the variability in plant invasion resistance. The coefficient of variation (CV; [%]) of the number and biomass of grass and herb invaders at the three weeding dates was used as measure of variability:

$$CV = \text{standard deviation}_{(\text{dependent variable; 2004-2006})} / \text{mean}_{(\text{dependent variable; 2004-2006})} * 100\%$$

Statistical analysis

Numbers and biomass of *L. terrestris* extracted during six earthworm extraction campaigns (autumn 2003, 2004, and 2005 and spring 2004, 2005, and 2006) were summed up per subplot and log-transformed to improve normal distribution and homogeneity of variance. ANOVA as part of the general linear models (GLM, type I sum of squares) was used to analyze the effects of block (B), sown plant species diversity (S), plant functional group diversity (Fg), and presence/absence of grasses (Gr), small herbs (Sh), tall herbs (Th), and legumes (Leg) on the number and biomass of *L. terrestris* individuals, on the coverage of the plant community and on fine root biomass in a hierarchical order.

Further, split plot ANOVA (GLM, type I sum of squares) was used to analyze the effects of block, sown plant species diversity, plant functional group diversity, presence/absence of grasses, small herbs, tall herbs, legumes, and earthworms (Ew; control, earthworm addition, earthworm reduction) on the number of moved and buried seed dummies.

Data on the number and biomass of total plant invaders, grass invaders, herb invaders and plant invader diversity in April 2004, 2005, and 2006 were log-transformed to improve normal distribution and homogeneity of variance. Data on legume invaders were not analyzed separately and will not be considered due to very low numbers. Split plot ANCOVA (GLM, type I sum of squares) was used to analyze the effects of x- and y-coordinates, sown plant species diversity, plant functional group diversity, presence/absence of grasses, small herbs, tall herbs, legumes, and earthworms (earthworm addition and earthworm reduction) on the number and biomass of total plant invaders, grass invaders, herb invaders, and plant invader diversity in April 2004, 2005, and 2006. Therefore, x- and y-coordinates were fitted as

covariates to account for possible edge effects of seed import to experimental plots (x for east-west direction and y for north-south direction; Fig. 5.1A). As mentioned above, for earthworm performance, plant community coverage, and seed dummy movement and burial the factor ‘block’ was used instead of x- and y-coordinates since variations in abiotic soil parameters were more important than distance to the edge of the field site.

Moreover, split plot ANCOVA (GLM, type I sum of squares) was used to analyze the effects of x- and y-coordinate, sown plant species diversity, plant functional group diversity, presence/absence of grasses, small herbs, tall herbs, legumes, and earthworms on the CV of the number and biomass of grass and herb invaders.

Repeated measures split plot ANCOVA (GLM, type I sum of squares) was used to analyze the effects of x- and y-coordinates, sown plant species diversity, plant functional group diversity, presence/absence of grasses, small herbs, tall herbs, legumes, earthworms, and time (2004, 2005, and 2006) on the number and biomass of total plant invaders.

The experimental design does not allow to fully separate the effects of S and FG which are partially confounded; consequently, no interaction term between S and FG was calculated. F-values given in text and tables refer to those where the respective factor (and interaction) was fitted first (Schmid et al. 2002). ‘Block’ or ‘X’- and ‘Y’-coordinates (covariates) were always fitted first, followed by ‘S’ and ‘Fg’. Then the effects of presence/absence of certain plant functional groups were calculated followed by ‘Plot’, ‘Ew’, and interactions between ‘Ew’ and ‘S’ and ‘Fg’, respectively.

Treatments analyzed at the plot scale (Block, S, Fg, Gr, Sh, Th, and Leg) were tested against the variance between plots to avoid pseudoreplication whereas treatments analyzed on the subplot scale (Ew, Ew×S, and Ew×Fg) were tested against the variance between subplots.

After fitting the maximal model, it was simplified by sequential deletion of factors with minor explanatory value to define the minimal adequate model. Therefore, the Akaike Information Criterion (AIC, Burnham and Anderson 1998) was used. Smaller values of AIC indicate higher predictive power of the respective statistical model.

Analyses of variance and comparisons of means (Tukey HSD test $\alpha = 0.05$) were performed using SAS V9.1 (SAS Institute Inc., Cary, USA). Means (\pm SD) presented in text and figures were calculated using non-transformed data.

To identify associations between the number and biomass of total plant invaders and coverage of the plant community and fine root biomass, respectively, correlations were carried out using STATISTICA 7.1 (Statsoft, Tulsa, USA).

5.4 RESULTS

Earthworm performance

In total 65.78 ± 32.68 ind./m² (53.34 ± 30.73 g/m² fresh weight) of *L. terrestris* were extracted during the six sampling campaigns between autumn 2003 and spring 2006. Abiotic soil conditions affected *L. terrestris* density significantly and biomass in trend with higher values at block 1 (92.17 ± 41.66 ind./m²; 63.00 ± 37.34 g/m²) than at block 3 (50.00 ± 27.72 ind./m²; 36.17 ± 21.74 g/m²; Table 5.1). Moreover, earthworm densities depended on plant species diversity with 76.00 ± 35.41 ind./m² and 68.21 ± 30.19 ind./m² in 4- and 16-species mixtures, respectively, but only 53.44 ± 30.27 ind./m² in monocultures, whereas plant functional group diversity had no significant effect. The presence of grasses reduced the density (-19%) and biomass (-33%) of *L. terrestris*, whereas the presence of legumes increased earthworm density (+46%) and in particular earthworm biomass considerably (+82%; Table 1). Fitting the effect of legumes before plant species diversity eliminated the significant diversity effect ($F_{2,34} = 1.88$, $P = 0.17$ for earthworm density and $F_{2,34} = 0.11$, $P = 0.90$ for earthworm biomass) suggesting that the diversity effect was due to the presence of legumes. Effects on endogeic earthworms and earthworm community structure are discussed elsewhere (Milcu et al. 2008).

Above- and belowground parameters of the plant community

The coverage of the plant community was strongly affected by plant species diversity with higher coverage in 16- ($84.40 \pm 8.90\%$) and 4-species mixtures ($65.63 \pm 19.07\%$) than in monocultures ($45.31 \pm 26.74\%$; Table 5.1). Moreover, plots containing two ($79.79 \pm 14.79\%$), three ($77.71 \pm 17.46\%$), and four ($76.88 \pm 12.86\%$) plant functional groups had higher coverage of the respective plant community than plant functional group monocultures ($49.17 \pm 25.99\%$). The presence of legumes increased the coverage of the plant community considerably (+59%). Fitting the effect of legumes before plant species diversity and plant functional group diversity attenuated the effect of plant diversity but did not eliminate it ($F_{2,34} = 8.11$, $P = 0.0013$ for plant species diversity and $F_{3,34} = 3.36$, $P = 0.03$ for plant functional group diversity; Table 5.1), suggesting that diversity effects were not only due to the presence of legumes. On the contrary, the presence of small herbs decreased plant community coverage (-13%; Table 5.1).

Fine root biomass was strongly affected by the presence of certain plant functional groups (Table 5.1). While fine root biomass was increased considerably in presence of grasses

(+52%; $631.04 \pm 233.75 \text{ g/m}^2$) compared to plots without grasses ($415.53 \pm 183.17 \text{ g/m}^2$), it was decreased in presence of legumes (-22%). However, plant species and functional group diversity did not affect fine root biomass. Further effects on belowground productivity are discussed elsewhere (H. Bessler et al., unpubl.).

Seed dummy experiment

On average 6.32 ± 1.61 seed dummies were moved or buried one week after the start of the experiment of which 4.04 ± 1.85 seed dummies were buried. The number of moved and buried seed dummies differed strongly between earthworm treatments. They were similar in the control (6.76 ± 1.37) and earthworm addition treatment (7.00 ± 1.40), but considerably lower in the earthworm reduction treatment (5.20 ± 1.44 ; Table 5.1). The number of buried seed dummies was even more reduced in earthworm reduction treatments (2.80 ± 1.41) compared to control (-38%; 4.57 ± 1.75) and earthworm addition treatments (-41%; 4.74 ± 1.76 ; Table 5.1, Fig. 5.2A) indicating a reduction in earthworm soil surface activity by -38%. Moreover, the number of buried seed dummies was higher in mixtures with three and four than in those with one and two plant functional groups in the control (significant interaction between earthworm treatment and plant functional group diversity; Table 5.1). On the contrary, in earthworm addition and reduction treatments the highest numbers of seed dummies were buried in plant functional group monocultures (Table 5.1).

Significantly more seed dummies were moved and buried in monocultures (6.79 ± 1.47) than in 4-species (6.15 ± 1.57) and 16-species mixtures (5.98 ± 1.70 , Fig. 5.2B), however, the number of buried seed dummies was not affected by plant diversity (Table 5.1). Further, the presence of grasses reduced the number of moved and buried (-10%) and the number of buried seed dummies (-17%; Table 5.1). On the contrary, the presence of small herbs increased the number of moved and buried seed dummies slightly but significantly (+3%) and the number of buried seed dummies in trend (+11%).

Table 5.1 ANOVA table of F-values for the effect of block (B), sown plant species diversity (S), plant functional group diversity (Fg), grasses (Gr), small herbs (Sh), tall herbs (Th), legumes (Leg), Plot, and earthworm treatment (Ew) on the number and biomass of *Lumbricus terrestris*, the coverage of the plant community, the fine root biomass per m², the number of moved and buried seed dummies, and the number of buried seed dummies. Explanatory variables are ordered by the sequence in which they were tested. Significant effects and distinct tendencies are given in bold.

Variable	Df	Test variance	<i>L. terrestris</i> number	<i>L. terrestris</i> biomass	coverage plant community	fine root biomass	seed dummies moved and buried	seed dummies buried
B	3	Plot	3.69*	2.87(*)	1.70 ns	excluded	2.23 ns	2.33(*)
S	2	Plot	3.86* ↑	1.52 ns	15.54*** ↑	excluded	3.26* ↓	2.04 ns
Fg	3	Plot	1.90 ns	1.23 ns	8.92*** ↑	1.19 ns	excluded	0.95 ns
Gr	1	Plot	5.24* ↓	12.17** ↓	0.92 ns	10.57** ↑	4.11* ↓	5.01* ↓
Sh	1	Plot	excluded	excluded	24.26*** ↓	excluded	5.30* ↑	3.04(*) ↑
Th	1	Plot	excluded	excluded	excluded	0.71 ns	excluded	excluded
Leg	1	Plot	4.19* ↑	10.44** ↑	10.56** ↑	15.84*** ↓	excluded	excluded
Plot	34	Subplot	-	-	-	-	1.86**	2.78***
Ew	2	Subplot	-	-	-	-	33.05*** ↑	36.94*** ↑
Ew×S	4	Subplot	-	-	-	-	excluded	1.24 ns
Ew×Fg	6	Subplot	-	-	-	-	excluded	3.68**
Number of Df max model			12	12	12	12	24	24
Number of Df min model			10	10	11	6	8	22
AIC max model			-139.00	-133.70	-150.40	-198.86	34.67	- 4.19
AIC min model			-142.56	-137.67	-152.40	-201.27	34.54	-10.19

Annotations: Df, degrees of freedom; max, maximal; min, minimal; ns, not significant; AIC, Akaike Information Criterion.
 ↑, increase; ↓, decrease; P>0.1, ns; P<0.1, (*); P<0.05, *; P<0.01, **; P<0.001 ***

Total plant invaders

Generally, the number of plant invaders increased during the three years of experimental weeding from 51.10 ± 65.14 ind./m² in 2004 to 63.08 ± 107.20 ind./m² in 2005 and 92.40 ± 99.13 ind./m² in 2006 ($F_{2,170} = 88.62$, $P < 0.001$). Effects of the main factors studied did not change significantly with time (not shown). The most successful plant invader species were *Poa trivialis* L. (grass) and *Taraxacum officinale* Web. (herb), however, species identity was not considered in the present study.

Generally, x-coordinate fitted as covariate did not significantly affect the number and biomass of plant invaders, however, the y-coordinate significantly affected the number of herb invaders in 2006 with lower numbers in the center than at the edge of the field site (Tables 5.2, 5.3, and 5.4).

Plant species diversity strongly affected the number and biomass of plant invaders at each of the three weeding dates (Tables 5.2, 5.3, and 5.4). In 2004, total number and biomass of invaders decreased in trend with increasing plant species diversity with 58.16 ± 71.37 ind./m² (37.13 ± 57.00 g/m²) and 57.41 ± 66.54 ind./m² (19.73 ± 26.36 g/m²) in monocultures and 4-species mixtures, respectively, but only 35.82 ± 54.96 ind./m² (5.7 ± 10.53 g/m²) in 16-species mixtures (Table 5.2, Fig. 5.2C, D). Though total invader biomass was decreased substantially in presence of grasses (-80%), it was increased in presence of legumes (+17%; Table 5.2).

In 2005, both plant species and plant functional group diversity significantly affected the total number and biomass of plant invaders (Table 5.3). While the total number of plant invaders was similar in monocultures (78.44 ± 130.44 ind./m²) and 4-species mixtures (79.10 ± 113.36 ind./m²), it was considerably lower in 16-species mixtures (27.21 ± 50.30 ind./m²). Total biomass of plant invaders decreased more linearly from monocultures (56.12 ± 54.00 g/m²) to 4-species mixtures (27.21 ± 42.16 g/m²) and 16-species mixtures (12.26 ± 38.04 ; Table 5.3, Fig. 5.2C, D). Further, the number and biomass of grass invaders decreased from treatments with a single plant functional group (72.45 ± 72.02 ind./m², 54.46 ± 55.10 g/m²) to mixtures with two (48.42 ± 106.75 ind./m², 27.83 ± 49.84 g/m²), four (48.51 ± 121.41 ind./m², 7.09 ± 7.65 g/m²) and three plant functional groups (30.42 ± 108.03 ind./m², 3.43 ± 6.93 g/m²; Fig. 5.2E, F). Like in 2004, total invader biomass was decreased considerably in presence of grasses in 2005 (-85%).

In 2006, total number and biomass of plant invaders varied with plant species diversity but not with plant functional group diversity (Table 5.4; Fig. 5.2C, D). Remarkably, the total number of plant invaders was similar in monocultures (79.94 ± 50.51 ind./m²) and in 16-

species mixtures (61.36 ± 92.46 ind./m²), but considerably higher in 4-species mixtures (132.03 ± 127.27 ind./m²). In contrast, total biomass of plant invaders was similar in monocultures (86.50 ± 85.46 g/m²) and 4-species mixtures (78.90 ± 139.10 g/m²), but considerably lower in 16-species mixtures (14.06 ± 24.93 g/m²). Further, the total number of plant invaders was increased in presence of tall herbs (+111%). Moreover, in tendency the total number of invader plants varied with earthworm treatments; it was higher in earthworm addition treatments (+10%) than in reduction treatments.

While the coverage of the plant community correlated negatively with the total number and biomass of plant invaders, fine root biomass was not correlated with the number but correlated negatively with the biomass of total plant invaders (Fig. 5.3).

Invader grasses

In 2004, the significant effects of plant species diversity on total number and biomass of invader plants were primarily due to the distinct effects on the number and biomass of grass invaders decreasing from 18.59 ± 27.64 ind./m² (20.55 ± 42.10 g/m²) in monocultures and 24.34 ± 59.50 ind./m² (9.29 ± 16.93 g/m²) in 4-species mixtures to 3.61 ± 12.04 ind./m² (1.08 ± 4.14 g/m²) in 16-species mixtures (Table 5.2). Further, the number of grass invaders was higher in single plant functional group treatments (22.32 ± 46.28 ind./m²) and mixtures with three plant functional groups (27.63 ± 51.60 ind./m²) than in mixtures with two (2.94 ± 9.44 ind./m²) and four plant functional groups (0.25 ± 0.77 ind./m²). Biomass of grass invaders followed a similar pattern and was lowest in single plant functional group treatments (19.24 ± 37.69 g/m²) as compared to mixtures containing two (1.36 ± 5.18 g/m²), three (5.51 ± 10.33 g/m²) and four plant functional groups (1.79 ± 6.73 g/m²; Table 5.2). Further, the number and biomass of grass invaders decreased substantially in presence of grasses (-73% and -86%, respectively) but increased in presence of legumes (+15% and +48%, respectively; Table 5.2). Moreover, the number of grass invaders decreased in trend in earthworm addition compared to reduction treatments (-19%; Table 5.2).

In 2005, the number and biomass of grass invaders decreased significantly with increasing plant species diversity from monocultures (57.84 ± 52.71 ind./m² and 24.05 ± 37.78 g/m²) to 4-species (45.69 ± 61.28 ind./m² and 18.45 ± 37.13 g/m²) and 16-species mixtures (15.93 ± 38.98 ind./m² and 8.61 ± 30.33 g/m²; Table 5.3). Moreover, the number and biomass of invader grasses was significantly affected by plant functional group diversity, decreasing from single functional group treatments (60.81 ± 52.95 ind./m² and 27.43 ± 42.00 g/m²) to mixtures containing two (31.01 ± 51.05 ind./m² and 20.01 ± 41.93 g/m²),

four (20.04 ± 54.73 ind./m² and 2.46 ± 6.71 g/m²), and three plant functional groups (16.79 ± 46.02 ind./m² and 2.17 ± 6.73 g/m²; Table 5.3).

In 2006, the number and biomass of grass invaders was affected by plant species diversity but not by plant functional group diversity, being significantly higher in 4-species mixtures (129.06 ± 140.82 ind./m² and 71.96 ± 131.51 g/m²) than in monocultures (40.69 ± 48.92 ind./m² and 36.87 ± 57.51 g/m²), and in 16-species mixtures (39.39 ± 77.42 ind./m² and 4.31 ± 7.92 g/m²; Table 5.4, Fig. 5.2C, D). Moreover, the number of grass invaders was decreased in presence of grasses (-20%) and the biomass of grass invaders increased considerably in presence of legumes (+132%; Table 5.4). Further, the number of grass invaders was increased in earthworm addition treatments compared to reduction treatments (+18%; Table 5.4).

Invader herbs

Number and biomass of herb invaders were not affected by plant species and functional group diversity in 2004 (mean number 35.02 ± 46.03 ind./m² and mean biomass 10.80 ± 19.90 g/m²; Table 5.2). However, the biomass of herb invaders was decreased in trend in presence of grasses (-73%).

In contrast to 2004, the number and biomass of herb invaders was significantly affected by plant species and functional group diversity in 2005 (Table 5.3). While the number and biomass of herb invaders decreased from monocultures (28.63 ± 29.71 ind./m² and 31.05 ± 32.17 g/m²) to 4-species (20.50 ± 20.52 ind./m² and 8.76 ± 11.35 g/m²) and 16-species mixtures (16.43 ± 30.30 ind./m² and 3.65 ± 8.69 g/m²), they decreased from single functional group treatments (28.16 ± 26.65 ind./m² and 26.29 ± 29.52 g/m²) to mixtures containing four (22.69 ± 38.56 ind./m² and 4.63 ± 6.14 g/m²), two (17.06 ± 21.85 ind./m² and 7.82 ± 11.31 g/m²), and three plant functional groups (9.81 ± 14.36 ind./m² and 1.26 ± 2.38 g/m²).

Like in 2005, the number and biomass of herb invaders decreased with increasing plant species diversity in 2006 from monocultures (38.72 ± 25.66 ind./m² and 48.68 ± 50.77 g/m²) to 4-species (25.91 ± 21.62 ind./m² and 18.28 ± 23.54 g/m²) and 16-species mixtures (21.71 ± 30.93 ind./m² and 9.03 ± 19.78 g/m²; Table 5.4). Further, herb invader biomass decreased with increasing plant functional group diversity from functional group monocultures (40.69 ± 46.67 g/m²) to mixtures containing two (19.05 ± 30.55 g/m²), three (9.98 ± 15.97 g/m²), and four plant functional groups (8.80 ± 15.25 g/m²). Moreover, the number of herb invaders was decreased in trend in presence of legumes (-32%; Table 5.4).

Invader diversity

Generally, plant invader diversity decreased significantly from monocultures (4.22 ± 2.49 , 2.38 ± 1.41 , and 3.63 ± 1.29 invader species in 2004, 2005 and 2006, respectively) to 4-species mixtures (3.44 ± 2.42 , 1.47 ± 0.98 , and 2.97 ± 1.12 invader species) and 16-species mixtures (1.61 ± 1.89 , 0.68 ± 0.72 , and 1.75 ± 0.70 invader species; Tables 5.2, 5.3, and 5.4). Moreover, increasing plant functional group diversity decreased plant invader diversity significantly in 2005 from 2.23 ± 1.27 invader species in single plant functional group treatments to 1.31 ± 1.25 , 0.63 ± 0.72 , and 0.81 ± 0.54 invader species in mixtures with two, three and four plant functional groups, respectively (Table 5.3). Similarly, plant invader diversity also decreased with increasing plant functional group diversity in 2006 from 3.41 ± 1.30 invader species in single plant functional group treatments to 2.56 ± 1.09 , 2.19 ± 1.22 , and 2.12 ± 0.96 invader species in mixtures with two, three and four plant functional groups, respectively (Table 5.4)

Presence/absence of certain plant functional groups affected plant invader diversity only in 2006 with a decrease in invader diversity in presence of tall herbs (-5%) and legumes (-34%; Table 5.4). Further, plant invader diversity was increased significantly in earthworm addition treatments compared to reduction treatments (+12%; Table 5.4).

Table 5.2 ANOVA table of F-values for the effect of field edge (X and Y), sown plant species diversity (S), plant functional group diversity (Fg), grasses (Gr), small herbs (Sh), tall herbs (Th), legumes (Leg), Plot, and earthworm treatment (Ew) on the number and biomass of invader plants (total), invader grasses, invader herbs, and plant invader diversity in April 2004. Explanatory variables are ordered by the sequence in which they were tested. Significant effects and distinct tendencies are given in bold.

Variable	Df	Test variance		Invader total		Invader grasses		Invader herbs		Invader diversity	
		number	biomass	number	biomass	number	biomass	number	biomass	number	diversity
X	1	Plot	excluded	excluded	2.11 ns	excluded	excluded	excluded	excluded	excluded	excluded
Y	1	Plot	excluded	0.63 ns	excluded	excluded	excluded	0.71 ns	excluded	excluded	excluded
S	2	Plot	2.05 ns	3.18 (*) ↓	4.68 * ↓	4.61 * ↓	1.07 ns	1.62 ns	5.76 ** ↓	2.13 ns	2.13 ns
Fg	3	Plot	1.78 ns	1.81 ns	4.28 * ↓	3.63 * ↓	0.70 ns	0.91 ns	0.91 ns	0.91 ns	0.91 ns
Gr	1	Plot	1.22 ns	4.63 * ↓	11.83 ** ↓	4.83 * ↓	excluded	3.51 (*) ↓	2.44 ns	2.44 ns	2.44 ns
Sh	1	Plot	excluded	excluded	excluded	1.41 ns	excluded	excluded	excluded	excluded	excluded
Th	1	Plot	excluded	excluded	2.53 ns	excluded	excluded	excluded	excluded	excluded	excluded
Leg	1	Plot	0.73 ns	4.48 * ↑	6.29 * ↑	7.23 * ↑	0.14 ns	2.24 ns	2.30 ns	2.30 ns	2.30 ns
Plot	34	Subplot	8.78 **	9.12 **	7.68 **	5.08 **	6.22 **	8.50 **	9.61 **	9.61 **	9.61 **
Ew	1	Subplot	0.59 ns	excluded	3.47 (*) ↓	0.30 ns	0.39 ns	0.66 ns	0.19 ns	0.19 ns	0.19 ns
Ew×S	2	Subplot	excluded	excluded	excluded	excluded	excluded	excluded	excluded	excluded	excluded
Ew×Fg	3	Subplot	0.95 ns	excluded	1.47 ns	1.25 ns	0.67 ns	excluded	1.09 ns	1.09 ns	1.09 ns
Number of Df max model			17	17	17	17	17	17	17	17	17
Number of Df min model			11	8	12	15	10	9	11	11	11
AIC max model			-249.41	-271.02	-278.84	-253.92	-223.28	-291.36	-433.50	-433.50	-433.50
AIC min model			-259.41	-281.84	-286.69	-258.61	-235.17	-297.74	-443.06	-443.06	-443.06

Annotations: Df, degrees of freedom; max, maximal; min, minimal; ns, not significant; AIC, Akaike Information Criterion.
 ↑, increase; ↓, decrease; P>0.1, ns; P<0.1, (*); P<0.05, *; P<0.01, **; P<0.001, ***

Table 5.3 ANOVA table of F-values for the effect of field edge (X and Y), sown plant species diversity (S), plant functional group diversity (Fg), grasses (Gr), small herbs (Sh), tall herbs (Th), legumes (Leg), Plot, and earthworm treatment (Ew) on the number and biomass of invader plants (total), invader grasses, invader herbs, and plant invader diversity in April 2005. Explanatory variables are ordered by the sequence in which they were tested. Significant effects and distinct tendencies are given in bold.

Variable	Df	Test variance		Invader total		Invader grasses		Invader herbs		Invader diversity	
		number	biomass	number	biomass	number	biomass	number	biomass	number	biomass
X	1	Plot	excluded	excluded	excluded	excluded	excluded	excluded	excluded	excluded	excluded
Y	1	Plot	excluded	excluded	excluded	excluded	excluded	excluded	2.30 ns	excluded	excluded
S	2	Plot	5.33** ↓	14.76*** ↓	3.34* ↓	4.04* ↓	4.14* ↓	12.57** ↓	12.55*** ↓	4.14* ↓	12.57** ↓
Fg	3	Plot	3.92* ↓	10.32*** ↓	2.88* ↓	4.67** ↓	3.17* ↓	8.67*** ↓	8.70*** ↓	3.17* ↓	8.67*** ↓
Gr	1	Plot	excluded	5.05* ↓	12.72*** ↓	17.79*** ↓	0.95 ns	excluded	excluded	0.95 ns	excluded
Sh	1	Plot	excluded	0.42 ns	1.42 ns	excluded	excluded	excluded	excluded	excluded	1.05 ns
Th	1	Plot	0.35 ns	excluded	3.34(*) ↑	5.65* ↓	excluded	excluded	excluded	excluded	excluded
Leg	1	Plot	0.57 ns	excluded	excluded	2.01 ns	excluded	excluded	excluded	2.01 ns	excluded
Plot	34	Subplot	19.62***	9.90***	21.62***	14.14***	15.48***	9.46***	7.24***	15.48***	7.24***
Ew	1	Subplot	0.81 ns	1.62 ns	excluded	excluded	excluded	2.19 ns	0.81 ns	excluded	0.81 ns
Ew×S	2	Subplot	0.89 ns	excluded	excluded	excluded	excluded	excluded	0.47 ns	excluded	0.47 ns
Ew×Fg	3	Subplot	1.22 ns	excluded	excluded	excluded	excluded	excluded	1.51 ns	excluded	1.51 ns
Number of Df max model			17	17	17	17	17	17	17	17	17
Number of Df min model			13	8	8	8	7	7	13	7	13
AIC max model			-325.12	-305.56	-319.13	-320.31	-320.52	-313.01	-501.86	-320.52	-313.01
AIC min model			-333.12	-313.58	-326.74	-328.59	-327.52	-325.08	-509.86	-327.52	-325.08

Annotations: Df, degrees of freedom; max, maximal; min, minimal; ns, not significant; AIC, Akaike Information Criterion.
 ↑, increase; ↓, decrease; P>0.1, ns; P<0.1, (*); P<0.05, *; P<0.01, **; P<0.001, ***

Table 5.4 ANOVA table of F-values for the effect of field edge (X and Y), sown plant species diversity (S), plant functional group diversity (Fg), grasses (Gr), small herbs (Sh), tall herbs (Th), legumes (Leg), Plot, and earthworm treatment (Ew) on the number and biomass of invader plants (total), invader grasses, invader herbs, and plant invader diversity in April 2006. Explanatory variables are ordered by the sequence in which they were tested. Significant effects and distinct tendencies are given in bold.

Variable	Df	Test variance	Invader total		Invader grasses		Invader herbs		Invader	
			number	biomass	number	biomass	number	biomass	number	diversity
X	1	Plot	excluded	excluded	2.58 ns	excluded	excluded	excluded	excluded	excluded
Y	1	Plot	excluded	excluded	excluded	excluded	5.14*	2.25 ns	1.37 ns	
S	2	Plot	3.62* ↓	8.01** ↓	6.90** ↓	6.03** ↓	3.89* ↓	8.37*** ↓	12.88*** ↓	
Fg	3	Plot	1.39 ns	excluded	0.62 ns	excluded	excluded	4.33* ↓	4.94** ↓	
Gr	1	Plot	1.53 ns	excluded	7.08* ↓	0.77 ns	excluded	excluded	excluded	excluded
Sh	1	Plot	0.81 ns	excluded	excluded	excluded	excluded	excluded	excluded	excluded
Th	1	Plot	4.36* ↑	excluded	1.96 ns	excluded	excluded	excluded	excluded	5.35* ↓
Leg	1	Plot	excluded	0.91 ns	1.15 ns	4.19* ↑	2.84(*) ↓	excluded	excluded	6.73* ↓
Plot	34	Subplot	12.20***	9.43***	28.24***	10.73***	7.56***	8.58***	2.76***	
Ew	1	Subplot	3.04(*) ↑	0.38 ns	3.60(*) ↑	excluded	excluded	excluded	4.49* ↑	
Ew×S	2	Subplot	excluded	1.10 ns	0.30 ns	excluded	excluded	1.05 ns	excluded	excluded
Ew×Fg	3	Subplot	1.07 ns	0.49 ns	0.18 ns	0.43 ns	1.22 ns	0.48 ns	excluded	excluded
Number of Df max model			17	17	17	17	17	17	17	17
Number of Df min model			12	9	15	7	7	11	9	9
AIC max model			-334.91	-294.37	-354.70	-276.41	-301.31	-289.05	-474.26	
AIC min model			-341.44	-306.37	-358.70	-289.03	-315.17	-301.05	-476.91	

Annotations: Df, degrees of freedom; max, maximal; min, minimal; ns, not significant; AIC, Akaike Information Criterion.
 ↑, increase; ↓, decrease; P>0.1, ns; P<0.1, (*); P<0.05, *; P<0.01, **; P<0.001, ***

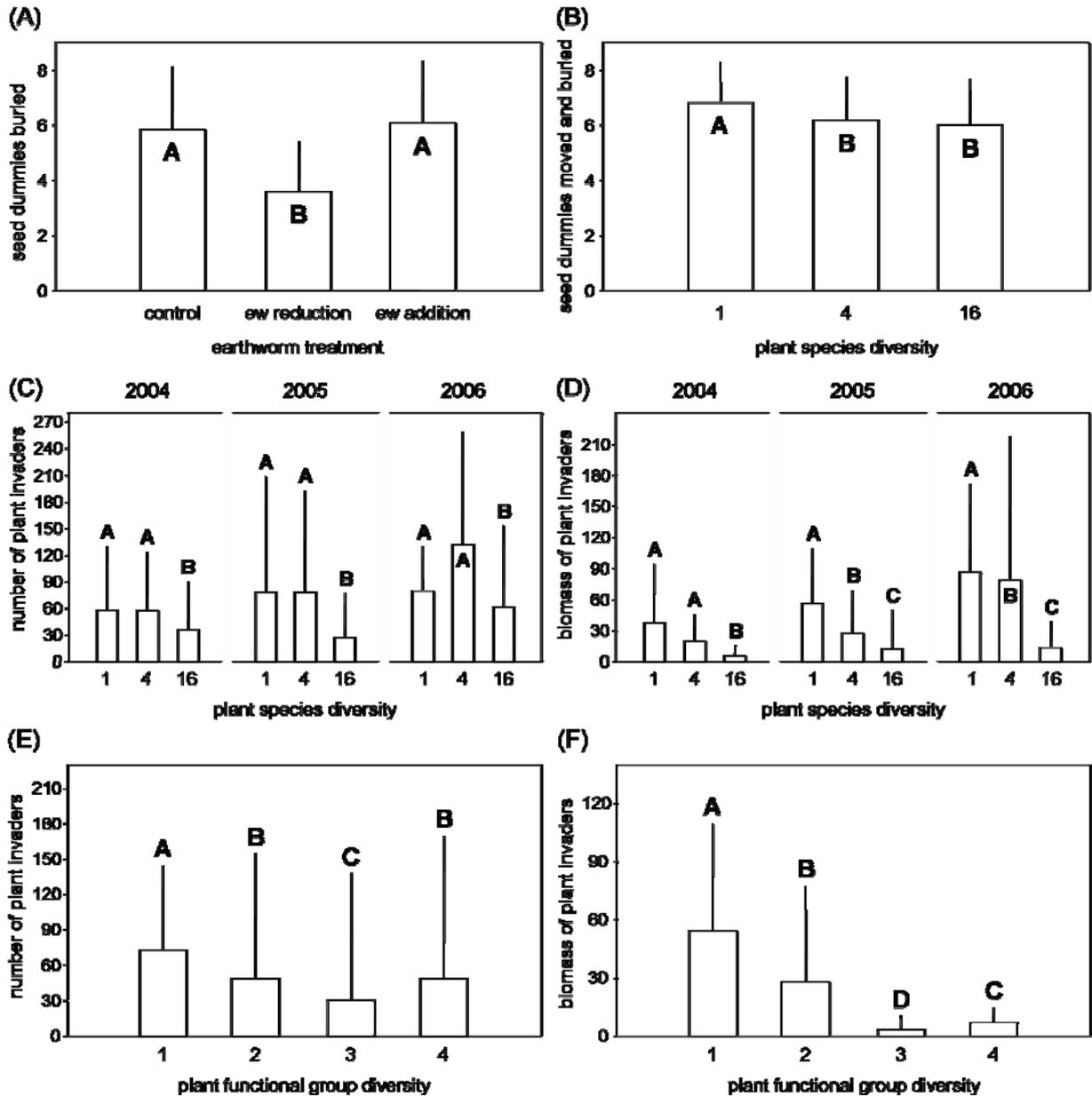


Figure 5.2 | (A) Effects of earthworm treatment (control, earthworm [ew] reduction, and earthworm addition) on the number of buried seed dummies after seven days (May 2006); (B) Effects of sown plant species diversity (1, 4, and 16-species mixtures) on the number of moved and buried seed dummies after seven days (May 2006); Effects of sown plant species diversity (1, 4, and 16-species mixtures) on (C) the number of plant invaders per m² and (D) the biomass of plant invaders per m² (g dry weight; April 2004, 2005, and 2006); Effects of plant functional group diversity (1, 2, 3, and 4 plant functional groups) on (E) the number of plant invaders per m² and (D) the biomass of plant invaders per m² (g dry weight; April 2005). Means with standard deviations. Bars with different letters vary significantly (Tukey's HSD test, P < 0.05).

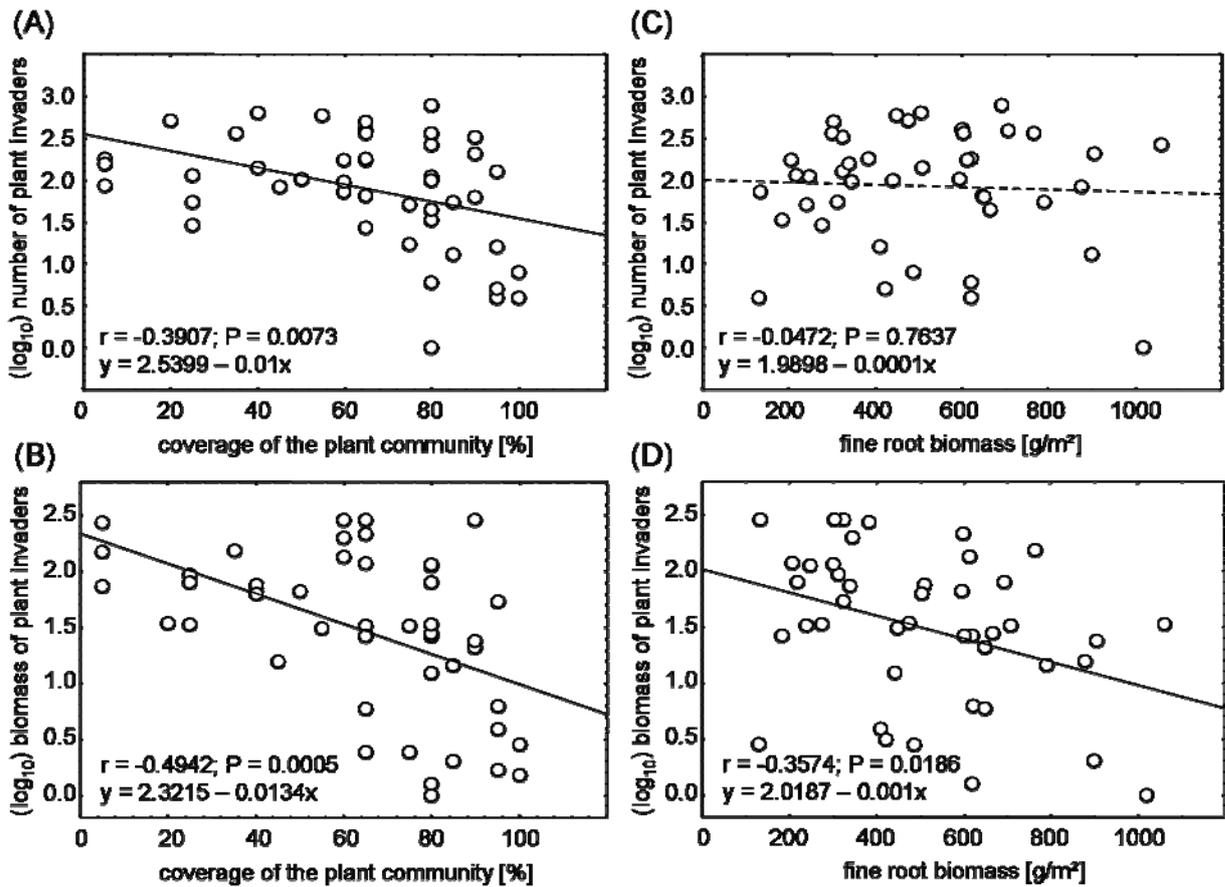


Figure 5.3 | Regressions of the coverage of the plant community [%] and (A) the number of total plant invaders ($\text{ind.}/\text{m}^2$; \log_{10} -transformed data) and (B) the biomass of total plant invaders (g/m^2 ; \log_{10} -transformed data) and regressions of the fine root biomass [g/m^2] and (C) the number of total plant invaders ($\text{ind.}/\text{m}^2$; \log_{10} -transformed data) and (D) the biomass of total plant invaders (g/m^2 ; \log_{10} -transformed data).

Stability of plant communities

Generally, the variability of the number and biomass of grass (98% and 106%, respectively) and herb invaders (83% and 97%, respectively) was high. Fitting the x- and y-coordinates as covariates suggest that the CV of grass and herb invaders did not depend on distance from the edge of the experimental field site (number and biomass; Table 5.5). Further, plant functional group diversity, and the presence of small herbs, tall herbs, and legumes did not affect the CV of the number and biomass of grass invaders. However, the CV was lower in 16-species mixtures (64 and 61% for invader number and biomass, respectively) than in monocultures (97 and 117%) and in 4-species mixtures (128 and 135%; Table 5.5). Presence of grasses did not affect the CV of the number of invader grasses but decreased the CV of the biomass of invader grasses considerably (-35%). Moreover, the CV of the number of grass invaders was increased in trend in the earthworm addition treatment (+11%; Table 5.5).

Although the CV of the number and biomass of herb invaders was not affected by plant diversity and the presence of certain plant functional groups, the interactions between earthworm treatment and plant species and functional group diversity had significant effects (Table 5.5; Fig. 5.4). While the CV of the number and biomass of herb invaders did not differ in monocultures, the respective CVs were increased in earthworm addition treatments in 4-species mixtures (+25 and -13% for invader number and biomass, respectively) but decreased in 16-species mixtures (-11% and -13%; Fig. 5.4A, B). Similarly, the CV of the number and biomass of herb invaders did not differ in single plant functional group treatments, however, they were increased in earthworm addition treatments in mixtures with two (+37 and +42% for invader number and biomass, respectively) and three plant functional groups (+37% and +32%) but decreased in mixtures with four plant functional groups (-33% and -26%; Fig. 5.4C, D).

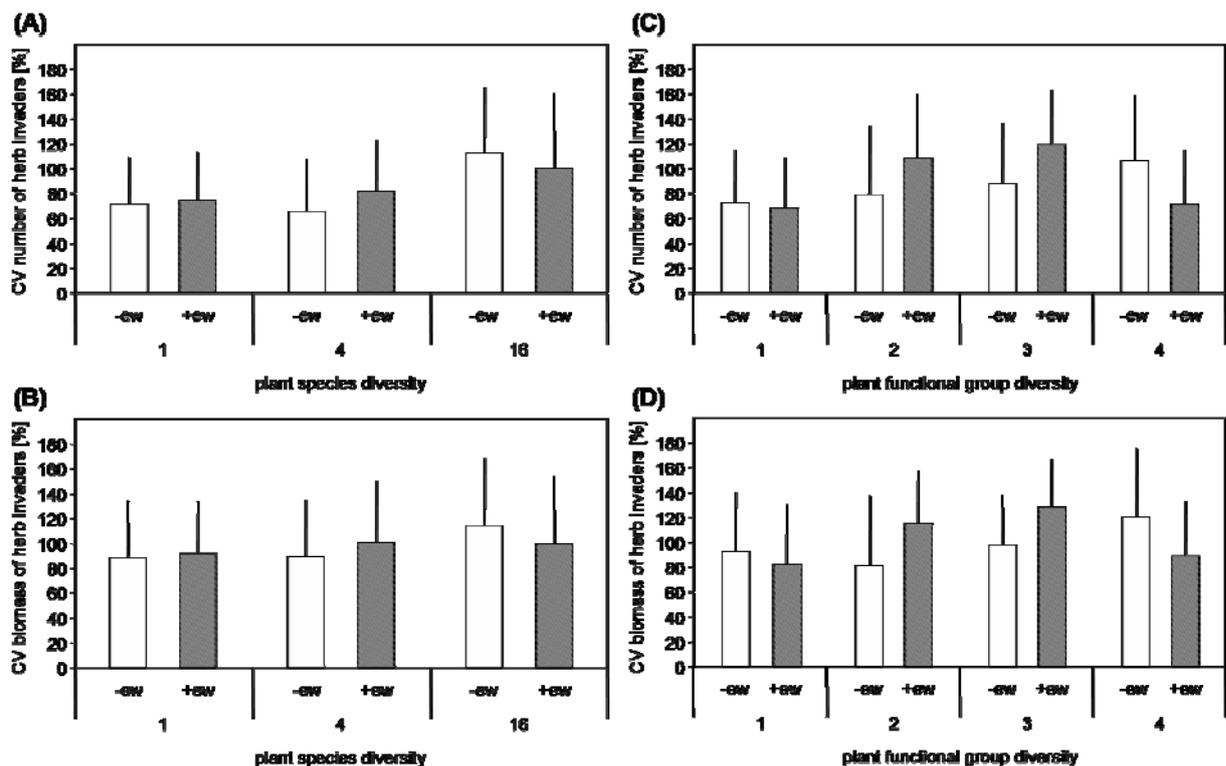


Figure 5.4 | Effects of sown plant species diversity (1, 4, and 16-species mixtures) and earthworm treatment (earthworm reduction [-ew] and earthworm addition [+ew]) on the coefficient of variance (CV; [%]) of (A) the number and (B) biomass of herb invaders in the years 2004 to 2006 and effects of plant functional group diversity (1, 2, 3, and 4 plant functional groups) and earthworm treatment on the CV [%] on (C) the number and (B) biomass of herb invaders in the years 2004 to 2006. Means with standard deviations

Table 5.5 | ANOVA table of F-values for the effect of field edge (X and Y), sown plant species diversity (S), plant functional group diversity (Fg), grasses (Gr), small herbs (Sh), tall herbs (Th), legumes (Leg), Plot, and earthworm treatment (Ew) on the coefficient of variance (CV) of the number and biomass of invader grasses and invader herbs in April 2004, 2005, and 2006. Explanatory variables are ordered by the sequence in which they were tested. Significant effects and distinct tendencies are given in bold.

Variable	Df	Test variance	CV invader grasses		CV invader herbs	
			number	biomass	number	biomass
X	1	Plot	excluded	excluded	excluded	excluded
Y	1	Plot	excluded	excluded	excluded	excluded
S	2	Plot	5.50** ↓	15.15*** ↓	0.37 ns	excluded
Fg	3	Plot	1.68 ns	1.19 ns	0.98 ns	0.85 ns
Gr	1	Plot	1.56 ns	13.68*** ↓	excluded	1.86 ns
Sh	1	Plot	0.85 ns	1.65 ns	excluded	1.47 ns
Th	1	Plot	excluded	excluded	excluded	excluded
Leg	1	Plot	excluded	excluded	0.45 ns	excluded
Plot	34	Subplot	8.86***	10.04***	1.04 ns	1.09 ns
Ew	1	Subplot	3.52^(*) ↑	excluded	excluded	excluded
Ew×S	2	Subplot	0.96 ns	excluded	3.50* ↓	4.66* ↓
Ew×Fg	3	Subplot	excluded	excluded	3.18* ↓	4.33*** ↓
Number of Df max model			17	17	17	17
Number of Df min model			11	7	11	10
AIC max model			545.17	-280.88	-220.26	-221.09
AIC min model			540.42	-290.61	-230.26	-229.09

Annotations: Df, degrees of freedom; max, maximal; min, minimal; ns, not significant; AIC, Akaike Information Criterion.

↑, increase; ↓, decrease; ↓, nonlinear effect; P>0.1, ns; P<0.1, ^(*); P<0.05, *; P<0.01, **; P<0.001***

5.5 DISCUSSION

Biological invasion is a widespread but poorly understood phenomenon (Collins et al. 2007). The “biodiversity-invasibility hypothesis” by Elton (1958) postulates that high diversity increases the competitive environment of communities and makes them more difficult to invade. Numerous biodiversity experiments have been conducted since that time and several mechanisms have been proposed to explain the often observed negative relationship between diversity and invasibility. Beside the decreased chance of empty ecological niches but the increased probability of competitors that preclude invasion success, diverse communities are assumed to use resources more completely and therefore limit the ability of invaders to establish (Elton 1958, Tilman 1999, Dukes 2002). However, interacting effects of the plant community and essential ecosystem engineers have not been considered so far.

Why is biodiversity increasing invasion resistance?

Results of the present experiment show that competition for resources is one key factor driving the successful recruitment of plant invaders. The coverage of the plant community increased significantly with increasing plant species and plant functional group diversity underlining the often observed positive relationship between plant species diversity and aboveground productivity (Loreau et al. 2002, Hooper et al. 2005, Roscher et al. 2005, Cardinale et al. 2007). The coverage of the resident plant community was negatively correlated with both the number and the biomass of plant invaders. Presumably, increased competition for light in more diverse plant communities is one mechanism reducing successful recruitment of invader plants. Results of a recent greenhouse experiment support this assumption (CHAPTER 2). Belowground competition for nutrients presumably was of minor importance for invader establishment since fine root biomass (reflecting belowground competition) was not correlated with the number of plant invaders. However, invader biomass correlated negatively with fine root biomass suggesting that belowground competition shaped the performance of invader plants during later stages of plant development. The results underline that high diversity increases the competitive environment of plant communities by increasing the use of plant resources (light, belowground nutrients) as proposed by Tilman (1999) and Fargione and Tilman (2005). Functional dissimilarity of the resident plant community and plant invaders presumably was of minor importance. In fact, the presence of grasses decreased the number and biomass of plant invaders irrespective of the identity of the

functional group of the invaders in 2004; the presence of herbs in the resident community did not significantly affect the performance of herb invaders. Rather, the more pronounced fine root system in presence of grasses likely decreased the successful establishment of invaders. On the contrary, in presence of legumes more plant invaders established, presumably due to significantly lower amount of fine roots in the upper soil layer (upper 30 cm) and, thereby, decreased competition for nutrients.

Consequently, we confirm that competition for light is of capital importance in the first stages of invader establishment (Cascorbi 2007). Light availability likely is driven by the productivity of the resident plant community which increases with diversity due to “complementarity effects” (Roscher et al. 2005). If once established, however, during later stages of invader establishment, belowground competition might also be important. As indicated by Hooper et al. (2005) keystone functional groups (like grasses and legumes) may be essential determinants of invader establishment. Hence, the present results indicate that both, “complementarity effects” and “sampling effects” play significant roles during invader establishment. Moreover, increasing diversity enhanced the stability of the plant community which was primarily due to the higher probability of grass presence in the resident community.

What is more important, plant species diversity or plant functional group diversity?

The design of The Jena Experiment for the first time allows to separate plant species and plant functional group diversity effects in a rigorous way (Roscher et al. 2004). In each of the three years of the experiment plant species diversity was more important than plant functional group diversity. These results are in contrast to the “redundancy hypothesis” (functional compensation) of Walker (1992) which assumes that the roles of species in ecosystems overlap. Rather, the results indicate that individual species contribute to the resistance of plant communities against species invasions as proposed by the “rivet hypothesis” (Ehrlich and Ehrlich 1992, Lawton 1994). This hypothesis assumes that species are relatively specialized and their ability to compensate the loss of ecosystem functioning is less pronounced than in the redundancy hypothesis. Loss of biodiversity therefore is likely to increase the invasion of exotic plant species into European grasslands.

Are there key plant functional groups affecting invasion resistance?

Previous studies have shown that functional dissimilarity between constituent species is the main driving force in the biodiversity-process relationship (Fargione and Tilman 2005,

Wardle and Zackrisson 2005). More precisely, the presence of a certain plant functional groups might represent an invasion barrier for species that have the same plant functional group affiliation because the ecological niche is already occupied (Elton 1958). However, as already discussed above, certain plant functional groups may govern plant invader development after initial establishment irrespective of plant functional group affiliation of the respective plant invader.

Results of the present study suggest that grasses reduce the number and biomass of total plant invaders, presumably due to a pronounced root system in the upper soil layers and, thereby, an increased competition for nutrients. On the contrary, legumes produced low fine root biomass and often increased the number and biomass of plant invaders. Legumes are keystone plants driving essential ecosystem processes, such as nitrogen-fixation (Mosier 2002), aboveground biomass production (Spehn et al. 2005) and decomposition (Milcu et al. 2008). Therefore, in addition to decreased root competition (CHAPTER 2), legumes probably enhance invader success by providing nitrogen-rich root exudates and litter.

Are earthworms modulating ecosystem functioning?

Long-term density manipulations of soil invertebrates in the field are very labor-intensive and may not be perfectly successful. By adding earthworms to our field site we intended to increase the densities of *L. terrestris* since they were low after establishment of The Jena Experiment (A. Milcu, unpubl.). However, four years after establishment earthworm densities were saturated as indicated by similar earthworm soil surface activity (seed-dummy experiment) in earthworm addition and control treatments. On the contrary, earthworm soil surface activity was decreased significantly by -38% in earthworm reduction plots. Considering that our manipulations only reduced the impact of earthworms on seed dispersal and burial, the experiment only reflects part of the full effects of earthworms on plant communities and invader success. Indeed greenhouse experiments suggest that earthworms strongly affect the fate of plant seeds and seedling recruitment (Milcu et al. 2006a, CHAPTER 4). By successfully manipulating earthworm densities in the field the present study for the first time documents that earthworms in fact modulate seed dispersal and invader establishment.

Do animal ecosystem engineers drive invasibility and stability of grassland communities?

Ecosystem engineering – the modification, maintenance, creation or destruction of habitats – affects the distribution, establishment and abundance of species (Jones et al. 1997,

Wright and Jones 2004). Surprisingly, animal ecosystem engineers have widely been ignored in studies focussing on diversity-invasibility relationships. Earthworms, especially anecic species (CHAPTER 3), are among the most prominent belowground ecosystem engineers with distinct effects on aboveground plant communities (Lavelle et al. 1998, Scheu 2003, Huhta 2007).

During the first two years of the experiment the effects of earthworms on plant invader establishment were non-significant. However, in the third year after establishment there was a distinct trend of increased numbers of total and grass invaders and a significant increase in plant invader diversity in earthworm addition plots. Results of the seed-dummy experiment indicate that earthworms likely modulated plant invasion and invader establishment success by dispersal and burial of plant seeds. Indeed, greenhouse experiments showed that earthworms translocate, bury, swallow and digest plant seeds and thereby alter plant community assembly (Milcu et al. 2006a, CHAPTER 3, CHAPTER 4). Interestingly, earthworm-plant seed interactions vary with plant species and are driven by seed size, shape and surface structure. Moreover, there is field evidence that seed predation and transport are important mechanisms by which earthworms can alter the diversity of grassland ecosystems (Zaller and Saxler 2007). Grant (1983) found 70% of the seedlings in temperate grasslands to germinate from earthworm casts, although casts only covered about 25% of the soil surface. This indicates that earthworm middens and casts function as important regeneration niches in grassland communities (Crawley 1992). Further, earthworm middens and casts represent nutrient-rich patches with comparatively low competition with the resident plant community (Milcu et al. 2006a) which likely facilitates seedling establishment thereby compensating seed loss due to digestion. Generally, the establishment of seedlings depends strongly on local processes like small scale disturbances (Grubb 1977) and earthworm middens represent small scale disturbances of intermediate strength known to increase diversity (Connell 1978). Moreover, Zaller and Arnone (1999a) reported distinct associations between earthworm casts and certain plant species in calcareous grassland.

Milcu et al. (2006a) suggested that by seed translocation from the seed bank to soil surface earthworms increase the resilience of grassland communities. In contrast to this assumption, our results indicate that at least in grassland communities of intermediate diversity rather the opposite is true. As described above, earthworm middens formed by anecic species represent small-scale disturbances increasing invasibility and, thereby, decreasing stability of grassland communities. Interestingly, this phenomenon depends on the diversity of the resident plant community with missing effects in low (monocultures) and

relatively high diverse (16-species mixtures and mixtures with four plant functional groups, respectively) plant communities.

As indicated by our earthworm extractions, monocultures maintain only low numbers and biomass of anecic earthworms. Consequently, earthworm effects on the plant community are of minor importance. Moreover, single plant species and functional group treatments provide ample gaps for invader establishment. But why were earthworm effects missing in high diverse plant communities where earthworm biomass was high? The seed-dummy experiment showed that despite the high biomass and density soil surface activity decreased with increasing plant species diversity suggesting that the more dense vegetation (Roscher et al. 2005, Lorenzen et al. 2008) hampered finding and translocating of plant seeds by *L. terrestris*. Consequently, in addition to reducing the number of open gaps, light and nutrient availability, diverse plant communities might be more stable against plant invasion due reduced soil surface activity of anecic earthworms. This conclusion is supported by the fact that earthworm soil surface activity was decreased and invasion resistance was increased in presence of grasses. Grasses produce large numbers of shoots thereby increasing structural complexity. The associated reduction in earthworm surface activity likely contributed to the reduced numbers and biomass of *L. terrestris* in presence of grasses, and this further reduced invasibility. On the contrary, earthworm performance was increased in presence of legumes and this likely contributed to the high invasibility of legume plant communities. Also, high earthworm surface activity in communities with small herbs, i.e. with low structural complexity, likely contributed to the sensitivity of these communities to invaders. Thus, the present study indicates that plant species invasion and community stability is driven by a complex interaction between the diversity, functional identity and structural complexity of plant communities, but also by belowground ecosystem engineers such as anecic earthworms.

5.6 CONCLUSIONS

Proceeding biodiversity loss due to invasion by exotic species and other global change phenomena facilitated by man threatens important ecosystem functions. Generally, competition for resources is one key factor driving invasibility and stability of grassland communities. However, recognizing the importance of non-trophic interactions such as ecosystem engineering in controlling patterns of species richness and ecosystem functioning is an important step in ecology (Wright and Jones 2004). The present study highlights the intimate relationship between earthworms and plant species diversity, functional group

identity and structural complexity in the invasibility and stability of grassland communities. Principal mechanisms of plant communities are modulated by faunal components calling for future cooperation between plant and animal ecologists and for studies aiming a holistic view of processes.

CHAPTER

6



THE SOIL SEED BANK:
A SAFE PLACE TO ENDURE?

6.1 ABSTRACT

The soil seed bank is considered a basic way to escape unfavourable environmental conditions and seed predation. Anecic earthworms are increasingly recognized as important dispersers and predators of plant seeds. However, the role of endogeic earthworms which live and feed in the soil on the soil seed bank is unknown. We tested whether (A) endogeic earthworms (*Aporrectodea rosea*, *Allolobophora chlorotica*, *Octolasion tyrtaeum*, *Aporrectodea caliginosa*) ingest and digest grassland plant seeds (*Phleum pratense*, *Bellis perennis*, *Trifolium repens*, *Poa trivialis*, *Plantago lanceolata*, *Medicago varia*), (B) the passage of seeds through the gut of endogeic earthworm modifies plant seed germination, (C) excreta (mucus and casts) of endogeic earthworm modify plant seed germination. As a reference effects of the well-studied anecic species *Lumbricus terrestris* were determined. Endogeic earthworms ingested and digested all of the studied plant seeds, however, both ingestion and digestion were earthworm and plant species specific. Moreover, passage through the gut of endogeic earthworms and their excreta modified plant seed germination (gut passage: *Po. trivialis*; excreta: *Ph. pratense* and *Pl. lanceolata*). The results indicate that endogeic earthworms may strongly impact the composition of the soil seed bank and, consequently, plant community assembly via direct and indirect interactions with plant seeds. Since post-dispersal seed predation is a key factor for the structure of plant communities with the effect on seed survival potentially exceeding that of pre-dispersal predation, seed predation and changes in germination of seeds by endogeic earthworm species deserves further attention.

6.2 INTRODUCTION

Seeds play a key role in the survival of plant species (Adams et al. 2005). Soil seed banks have been investigated focussing on the maintenance and restoration of species rich plant communities since they provide a source for re-establishment of species which are lost from the above-ground vegetation (Wellstein et al. 2007). The composition of seed banks depend on the contribution of present and former plant communities, seed rain from adjacent areas and on seed longevity (Rice 1989, Hutchings and Booth 1996). However, seed survival prior to the germination phase is primarily driven by processes during secondary seed dispersal, including both horizontal and vertical movements (burial) and post-dispersal seed predation (Chambers and MacMahon 1994). These processes determine the final composition of the soil seed bank (Juan et al. 2006). One of the most important and widely studied influences on seed banks is seed predation, altering the number and distribution of seeds (Anderson and MacMahon 2001). In some perennial communities, aboveground seed predation may destroy more than 95% of the seeds produced (Thompson 1992). The soil seed bank was therefore usually seen as a way to escape unfavourable environmental conditions such as severe drought or frost (Cohen 1966) and, in addition, offering significant protection from predation by vertebrates, birds and ants (Thompson et al. 2001, Azcárate and Peco 2003).

Although seed burial is an essential secondary dispersal process that reduces the risk of desiccation and predation (Hulme 1993), it may also reduce successful germination, emergence, and establishment (Traba et al. 2004). Burial in particular results in translocation of small seeds which enter deeper soil layers where they may remain viable long time (Thompson et al. 1993, 2001). It is increasingly recognized that after phase I dispersal of seeds, i.e. the displacement of seeds from the parent to the soil surface, anecic earthworms play an important role in phase II dispersal, i.e. the subsequent displacement of seeds on the soil surface and burial into the soil (Grant 1983, Willems and Huijsmans 1994, Decaens et al. 2003, Milcu et al. 2006a, CHAPTER 3, CHAPTER 4). By burying seeds anecic earthworms may influence seed and seedling fates in both positive and negative ways. On the one hand they may enhance seed survival by reducing exposure of seeds to vertebrate seed predators (Heithaus 1981). On the other hand seeds buried below some critical depth may fail to emerge (Traba et al. 2004). Thus, dispersal may give rise to a conflict between the dispersal needs of a plant and its requirements for successful establishment. However, generally, seed burial by anecic earthworms is thought to be primarily beneficial, by reducing seed predation and, in

addition, by creating gaps for seed germination and nutrient rich regeneration niches in earthworm middens (Grant 1983, Milcu et al. 2006a, CHAPTER 5).

Post-dispersal seed predation has been discovered as a key factor for explaining demographic changes in plant communities (Hulme 1998), and may affect seed survival more than pre-dispersal predation (Moles et al. 2003). While seed predation by small mammals, birds, and ants has been studied intensively (Hulme 1998, López et al. 2000, Anderson and MacMahon 2001), surveys on earthworms as important seed predators in post-dispersal stages are scarce (CHAPTER 4). Studies on earthworm nutrition questioned the role of plant seeds as food source (Curry and Schmidt 2007) and focussed only on anecic earthworm species (McRill and Sagar 1973, Grant 1983, Shumway and Koide 1994). Thereby, the soil seed bank was treated like a “black box” by considering it as a mainly predation-free spatial niche for plant seeds. Seed predation by endogeic earthworm species, which consume large amounts of mineral soil and organic matter, has not been investigated (Fig. 6.1A). Especially in temperate grasslands, earthworms contribute most to invertebrate biomass in soils with densities of up to 400 ind./m² (Edwards and Bohlen 1996). In temperate climates, the upper 15 cm of soil, containing an essential component of the soil seed bank, may be turned over completely every 10 to 20 years (Edwards and Bohlen 1996). Consequently, ingestion, digestion and damaging of plant seeds during gut passage through endogeic earthworms may strongly affect seed bank composition, plant recruitment and plant community structure. Thereby, selective feeding of earthworms on plant seeds which has been shown for *Lumbricus terrestris* L. to depend on seed size (Shumway and Koide 1994), shape (McRill and Sagar 1973) and surface structure (Shumway and Koide 1994), is likely to favour certain plant species.

Beside the direct effects of earthworms on plant seeds, there is evidence that via excreta earthworms alter plant seed germination and seedling establishment indirectly. However, results have been inconsistent showing that earthworm casts may break seed dormancy and increase germination and root initiation of plant seeds (Tomati et al. 1988, Ayanlaja et al. 2001) but also decrease germination of several grassland plant species (Grant 1983, Decaens et al. 2001).

We used the earthworm species pool of typical European grassland communities to investigate both, direct and indirect effects of anecic (*L. terrestris*) and, for the first time, endogeic earthworms (*Aporrectodea rosea* Sav., *Allolobophora chlorotica* Sav., *Octolasion tyrtaeum* Sav., and *Aporrectodea caliginosa* Sav.) on seeds and germination of six common grassland plant species. The main objectives of the present study were to investigate (1) if endogeic earthworms ingest and digest plant seeds, (2) if plant germination is modified by the

gut passage through earthworms, (3) if plant seed germination is modified by earthworm excreta, and (4) if the soil seed bank is a safe place to endure?

6.3 MATERIALS AND METHODS

Experimental setup (A)

Ingestion of plant seeds: To investigate the ingestion of plant seeds, adult individuals of *Ap. rosea* Sav. (0.15 ± 0.05 g; fresh weight with gut content), *Al. chlorotica* Sav. (0.23 ± 0.11 g), *O. tyrtaeum* Sav. (0.66 ± 0.39 g), *Ap. caliginosa* Sav. (0.50 ± 0.14 g), and *L. terrestris* L. (4.45 ± 1.06 g) extracted from the grassland field site of The Jena Experiment were used. Further, seeds from six plant species consisting of three plant functional groups (grasses: *Phleum pratense* L. [1.6 x 0.7 x 0.7 mm] and *Poa trivialis* L. [2.1 x 0.7 x 0.3 mm], herbs: *Bellis perennis* L. [1.6 x 0.7 x 0.1 mm] and *Plantago lanceolata* L. [2.7 x 1.1 x 0.8 mm], and legumes: *Trifolium repens* L. [1.0 x 1.0 x 0.6 mm] and *Medicago varia* Mart. [2.0 x 1.4 x 0.9 mm]) selected from the species pool of The Jena Experiment (Roscher et al. 2004) were used. The Jena Experiment is a large grassland experiment investigating the role of biodiversity for element cycling and trophic interactions. Earthworms were kept on moist filter paper for 48 h for voiding their guts (15°C, darkness). Thereafter, individual earthworms were placed on filter paper (three sheets) moistened with 3 ml of deionized water in a Petri dish (diameter 8 cm). To each Petri dish 1 g sieved soil (2 mm; from the field site of The Jena Experiment) and 20 seeds of one plant species were deployed. Soil was added to simulate natural conditions since small stones and sand particles are known to grind ingested organic material in the earthworm gut (Schulmann and Tiunov 1999, Marhan and Scheu 2005, Curry and Schmidt 2007). Each treatment (five earthworm treatments and six plant treatments) was replicated ten times. During the experiment Petri dishes were incubated in the dark for 24 h at 15°C. Thereafter, earthworms were removed and the number of remaining seeds per Petri dish was counted. Disappeared seeds were assumed to have been ingested (Fig. 6.2; Experiment A [I] Ingestion).

Digestion of plant seeds: After removal, earthworms were left for 48 h on moist filter paper in fresh Petri dishes for voiding their guts (15°C, darkness). Thereafter, the number of plant seeds in earthworm casts was determined. The difference between the number of ingested and the number of egested seeds was taken as the number of digested seeds by the respective earthworm individual (Fig. 6.2; Experiment A [II] Digestion). Digestion of plant

seeds was only calculated for treatments where at least three earthworm individuals per species ingested at least three plant seeds.

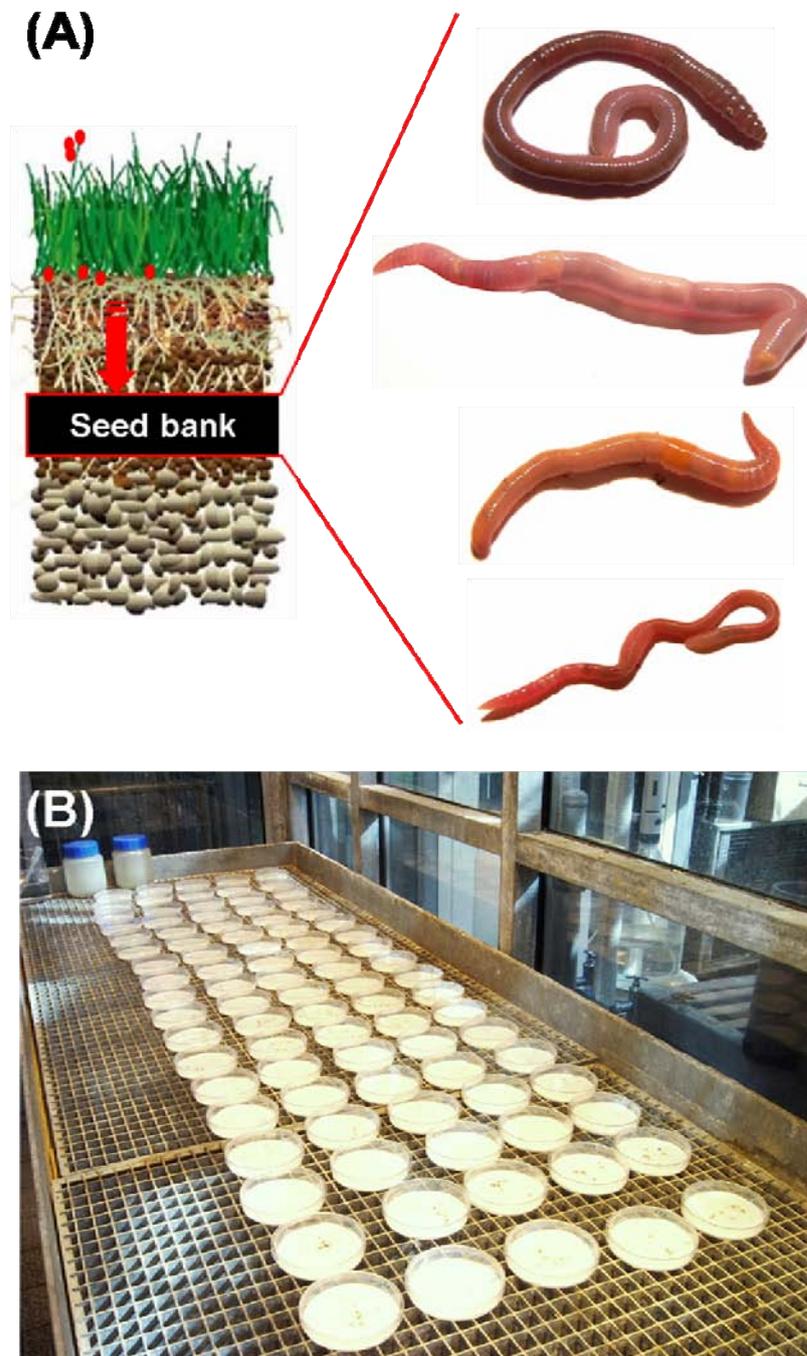


Figure 6.1 | (A) Scheme of the translocation of plant seeds (red ovals) to the soil seed bank. Endogeic earthworm species (at the field site of The Jena Experiment: *Aporrectodea caliginosa*, *Octolasion tyrtaeum*, *Allolobophora chlorotica*, and *Aporrectodea rosea*, from top to bottom) live and feed in the upper mineral soil. (B) Photograph showing some of the Petri dishes containing plant seeds on moist filter paper under greenhouse conditions. Photo by N. Eisenhauer.

Germination after earthworm gut passage: Germination of plant seeds out of earthworm casts was determined in a temperature controlled greenhouse (day/night 16/8 h and $20/18 \pm 2^\circ\text{C}$, $\sim 500 \mu\text{E}/\text{m}^2\cdot\text{s}$). Control treatments were used as a reference by adding 2 g (fresh weight) of sieved Jena soil (2 mm) on moist filter paper per Petri dish. Twenty plant seeds were added to the soil which had not been offered to earthworms. Germination was recorded for 14 days as describes above (Fig. 6.1B, 2; Experiment A [III] Germination). Germination of plant seeds was only calculated for treatments where at least three earthworm individuals per species egested at least three plant seeds.

Experimental setup (B)

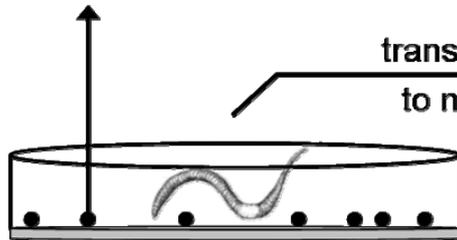
Germination in presence of earthworm mucus: Twenty plant seeds per Petri dish from one plant species were placed on filter paper. Petri dishes were either irrigated with 3 ml of deionized water (control treatment) or earthworm mucus solution (*Ap. caliginosa* and *L. terrestris*) in intervals of 48 h. Therefore, beakers were filled with 100 ml of deionized water and 30 cleaned earthworm individuals were added for 15 min. Earthworms were stimulated to produce mucus via tactile skin irritation. Earthworm mucus solution was sieved (1 mm) and prepared freshly at all times. Each treatment (three irrigation treatments times six plant treatments) was replicated five times. Germination of plant seeds was recorded for 14 days in the greenhouse as described above (Fig. 6.2; Experiment B [I] Mucus).

Germination in presence of earthworm casts: Thirty individuals of *Ap. caliginosa* or *L. terrestris*, were kept for 48 h on moist filter paper for voiding their guts (15°C , darkness). Thereafter, casts per earthworm species were homogenized and distributed evenly on fresh filter paper in Petri dishes (ca. 2 g fresh weight). Control treatments received 2 g (fresh weight) of sieved Jena soil (1 mm). Twenty plant seeds from one plant species were added to each Petri dish and sprinkled with 3 ml deionized water in intervals of 48 h. Each treatment (three cast treatments and six plant treatments) was replicated ten times. Germination of plant seeds was determined for 14 days in the greenhouse as described above (Fig. 6.2: Experiment B [II] Casts).

Experiment (A)

24 h, 15 °C, darkness;
counting remaining seeds;

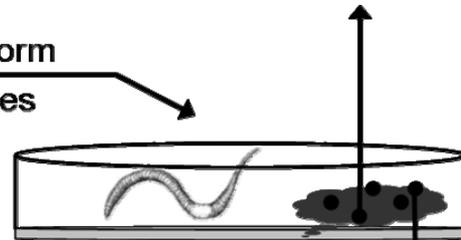
(I) Ingestion



transfer of earthworm
to new petri dishes

48 h, 15 °C, darkness;
counting seeds in casts;

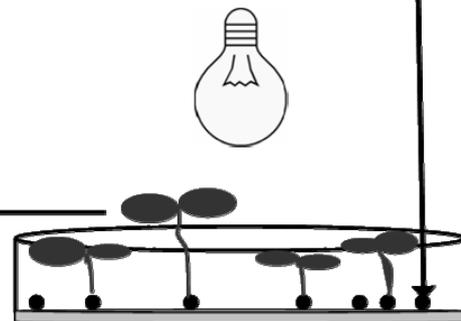
(II) Digestion



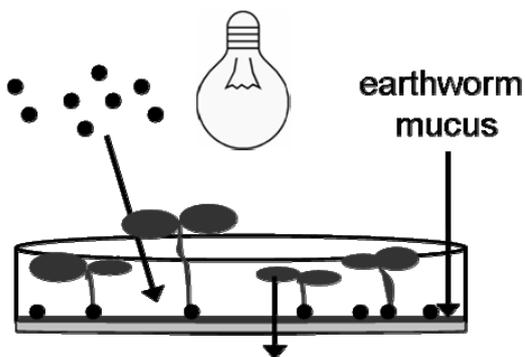
transfer of seeds
to new petri dishes

14 d, 20/18 °C, ~500 $\mu\text{E}/\text{m}^2\cdot\text{s}$;
counting seedlings;

(III) Germination

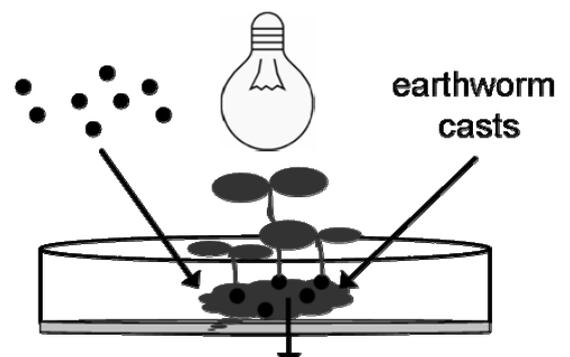


Experiment (B)



14 d, 20/18 °C, ~500 $\mu\text{E}/\text{m}^2\cdot\text{s}$;
counting seedlings;

(I) Mucus



14 d, 20/18 °C, ~500 $\mu\text{E}/\text{m}^2\cdot\text{s}$;
counting seedlings;

(II) Casts

Figure 6.2 | Experimental setup (scheme). Experiment (A) investigated direct effects of different earthworm species on plant seeds, i.e. ingestion of plant seeds (I), digestion of ingested plant seeds (II), and germination of plant seeds after earthworm gut passage. Experiment (B) investigated indirect effects of earthworm mucus (I) and casts (II) on the germination of plant seeds.

Statistical analysis

Data on seed ingestion, digestion, germination after earthworm gut passage, and germination in presence of earthworm mucus and casts were tested for normal distribution and homogeneity of variance and log-transformed, if necessary. Means (\pm SD) presented in text and figures were calculated using non-transformed data.

Analysis of variance (ANOVA) was used to analyze the effects of earthworm species identity (*Ap. rosea*, *Al. chlorotica*, *O. tyrtaeum*, *Ap. caliginosa*, and *L. terrestris*) on the number of ingested plant seeds [%], digested plant seeds [%], and germinated plant seeds after gut earthworm gut passage [%] for each plant species separately (*P. pratense*, *B. perennis*, *T. repens*, *Po. trivialis*, *Pl. lanceolata*, and *M. varia*). Additionally, correlations were carried out to identify associations between earthworm species-specific ingestion of plant seeds and seed size (length, surface, and volume). Further, ANOVAs were performed to analyze the effects of earthworm mucus and casts (control, *Ap. caliginosa*, and *L. terrestris*) on the germination of plant seeds for each plant species separately.

Analyses of variance, correlations, and comparisons of means (Tukey's HSD test, $\alpha = 0.05$) were performed using STATISTICA 7.1 (Statsoft).

6.4 RESULTS

Ingestion of plant seeds

Generally, seed ingestion depended strongly on seed size, i.e. the ingestion of seeds was negatively correlated with seed size. Thereby, seed volume had higher explanatory values than seed length and seed surface, respectively (Table 6.1).

Ingestion of plant seeds varied considerably among earthworm species with higher numbers in large than in small earthworms (Fig. 6.3). Thus, earthworm species were arranged from the smallest (*Ap. rosea*) to the biggest species (*L. terrestris*) in all tables and figures. As an exception, *O. tyrtaeum* was arranged before *Ap. caliginosa* due to the considerable higher seed ingestion by the latter.

Lumbricus terrestris ($89 \pm 14\%$) ingested more *Ph. pratense* seeds than endogeic species, however, *Ap. caliginosa* ($22 \pm 13\%$) ingested significantly more seeds than *Ap. rosea* ($8 \pm 15\%$), *Al. chlorotica* ($5 \pm 7\%$), and *O. tyrtaeum* ($8 \pm 14\%$; Table 6.2, Fig. 6.3A). On the contrary, *L. terrestris* ($91 \pm 8\%$) and *Ap. caliginosa* ($84 \pm 21\%$) ingested similar numbers of *B. perennis* seeds but significantly more than *Ap. rosea* ($5 \pm 6\%$), *Al. chlorotica* ($27 \pm 31\%$), and *O. tyrtaeum* ($33 \pm 37\%$; Table 6.2, Fig. 6.3B). Moreover, *O. tyrtaeum* ingested more

B. perennis seeds than *Ap. rosea*. *Lumbricus terrestris* ($69 \pm 17\%$) ingested more *T. repens* seeds than *Ap. caliginosa* ($32 \pm 25\%$) and this species more than *Al. chlorotica* ($6 \pm 13\%$) and *O. tyrtaeum* ($11 \pm 24\%$). *Aporrectodea rosea* ingested no *T. repens* seeds at all (Table 6.2, Fig. 6.3C). Furthermore, *L. terrestris* ($91 \pm 7\%$) and *Ap. caliginosa* ($62 \pm 26\%$) ingested more *Po. trivialis* seeds than *Ap. rosea* ($2 \pm 4\%$), *Al. chlorotica* ($7 \pm 9\%$) and *O. tyrtaeum* ($14 \pm 9\%$; Table 6.2, Fig. 6.3D). Though, *O. tyrtaeum* ingested significantly more *Po. trivialis* seeds than *Ap. rosea* (Table 6.2, Fig. 6.3D). Generally, endogeic earthworm species ingested only a small proportion of *Pl. lanceolata* and *M. varia* seeds. While *Ap. rosea* and *Al. chlorotica* ingested no seeds at all, *O. tyrtaeum* and *Ap. caliginosa* ingested only $3 \pm 4\%$ and $2 \pm 2\%$ of *Pl. lanceolata* seeds, respectively, and $1 \pm 2\%$ and $6 \pm 10\%$ of *M. varia* seeds, respectively. However, *L. terrestris* ingested significantly more *Pl. lanceolata* ($48 \pm 36\%$) and *M. varia* seeds ($71 \pm 24\%$) than endogeic species (Table 6.2, Fig. 6.3E, F).

Table 6.1 | Coefficients of correlation (r) and coefficients of determination (R^2) of regressions between seed size (length [mm], surface [mm^2], and volume [mm^3]) and the ingestion of seeds by different earthworm species (*Aporrectodea rosea*, *Allolobophora chlorotica*, *Octolasion tyrtaeum*, *Aporrectodea caliginosa*, and *Lumbricus terrestris*).

Earthworm species	Length		Surface		Volume	
	r	R^2	r	R^2	r	R^2
<i>Aporrectodea rosea</i>	-0.29	0.08**	-0.36	0.13***	-0.37	0.14***
<i>Allolobophora chlorotica</i>	-0.34	0.12**	-0.43	0.18***	-0.48	0.23***
<i>Octolasion tyrtaeum</i>	-0.38	0.14***	-0.42	0.18***	-0.42	0.18***
<i>Aporrectodea caliginosa</i>	-0.42	0.18***	-0.62	0.38***	-0.70	0.48***
<i>Lumbricus terrestris</i>	-0.33	0.11**	-0.51	0.26***	-0.53	0.28***

P < 0.01, **; P < 0.001, ***

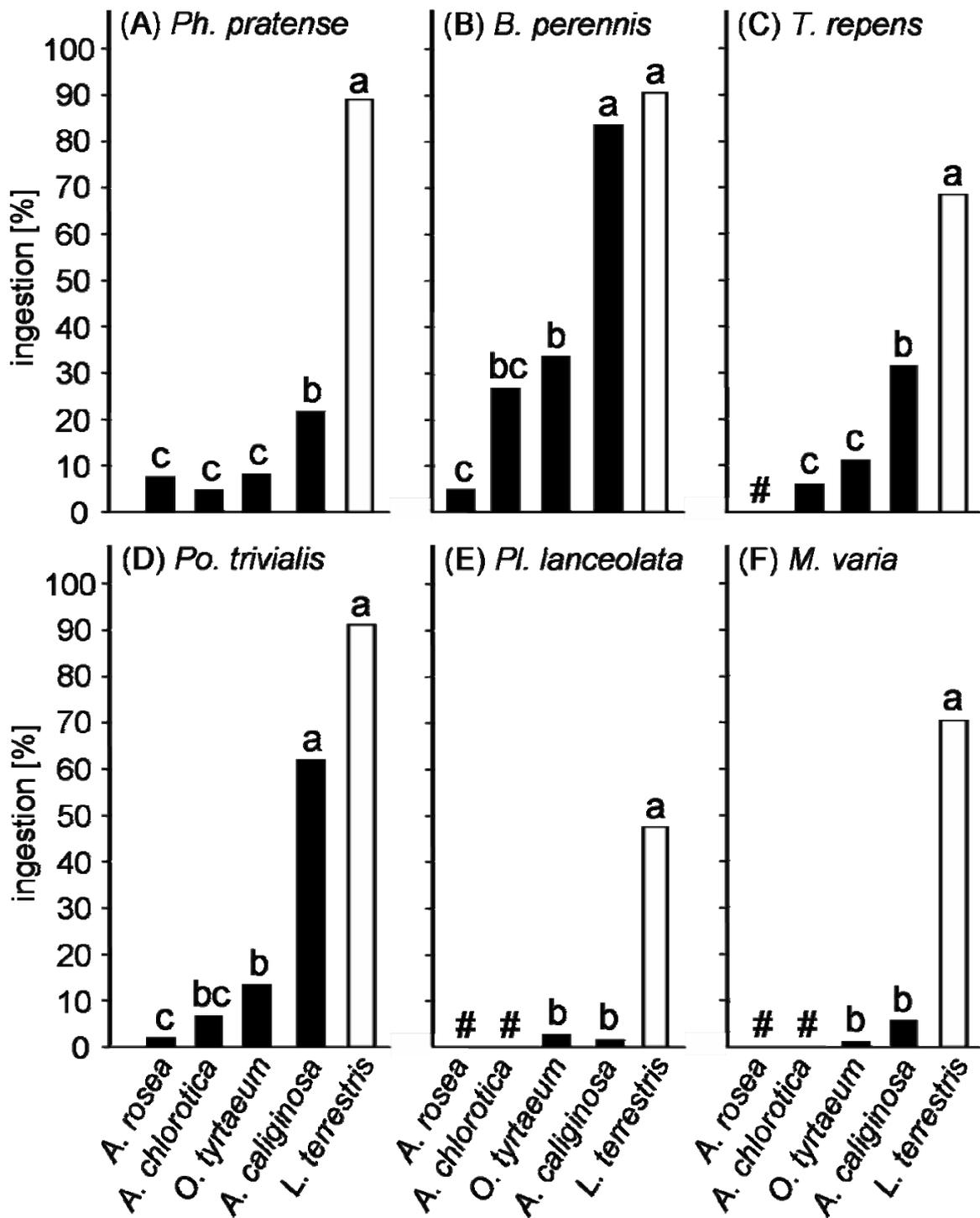


Figure 6.3 | Number of seeds [%] from different plant species [(A) *Phleum pratense*, (B) *Bellis perennis*, (C) *Trifolium repens*, (D) *Poa trivialis*, (E) *Plantago lanceolata*, and (F) *Medicago varia*] ingested by different earthworm species (*Aporrectodea rosea*, *Allolobophora chlorotica*, *Octolasion tyrtaeum*, *Aporrectodea caliginosa*, and *Lumbricus terrestris*). Black bars indicate endogeic and white bars anecic earthworm species, respectively. Bars with different letters vary significantly (Tukey's HSD test, $\alpha < 0.05$). #: not ingested.

Table 6.2 | ANOVA table of F- and P-values for the effect of earthworm species (*Aporrectodea rosea*, *Allolobophora chlorotica*, *Octolasion tyrtaeum*, *Aporrectodea caliginosa*, and *Lumbricus terrestris*) on the (A) number of ingested plant seeds [%]; *Phleum pratense*, *Bellis perennis*, *Trifolium repens*, *Poa trivialis*, *Plantago lanceolata*, and *Medicago varia*), (B) the number of digested plant seeds [%], and (C) the number of germinated plant seeds after earthworm gut passage [%]; control, *Ap. rosea*, *Al. chlorotica*, *O. tyrtaeum*, *Ap. caliginosa*, and *L. terrestris*).

Plant species	Ingestion			Digestion			Germination		
	Df	F-value	P-value	Df	F-value	P-value	Df	F-value	P-value
<i>Ph. pratense</i>	4,45	18.85	<0.001	2,16	0.86	0.501	2,22	3.02	0.069
<i>B. perennis</i>	4,45	14.72	<0.001	3,25	3.32	0.036	4,31	2.53	0.060
<i>T. repens</i>	3,36	19.57	<0.001	3,18	5.12	0.010	2,15	1.02	0.383
<i>Po. trivialis</i>	4,45	30.96	<0.001	3,23	2.59	0.078	2,26	8.63	<0.001
<i>Pl. lanceolata</i>	2,27	15.50	<0.001	2,13	8.27	0.005	1,13	8.89	0.011
<i>M. varia</i>	2,27	18.70	<0.001	1,11	0.08	0.784	1,17	3.30	0.087

Significant effects ($P < 0.05$) are given in bold.

Df, degrees of freedom.

Digestion of plant seeds

Digestion of plant seeds depended on both, earthworm species and plant species identity (Table 6.2, Fig. 6.4). Digestion of *Ph. pratense* seeds could not be determined for *Ap. rosea* and *Al. chlorotica*, and did not differ significantly between *O. tyrtaeum* ($18 \pm 10\%$), *Ap. caliginosa* ($42 \pm 33\%$), and *L. terrestris* ($44 \pm 17\%$; Table 6.2, Fig. 6.4A). Also, digestion of *B. perennis* and *T. repens* seeds could not be determined for *Ap. rosea* and *Al. chlorotica* ($21 \pm 31\%$ and $25 \pm 35\%$), but *O. tyrtaeum* ($27 \pm 41\%$ and $37 \pm 52\%$) and *Ap. caliginosa* ($26 \pm 23\%$ and $56 \pm 22\%$) digested a considerable fraction of the ingested seeds. However, *L. terrestris* ($45 \pm 19\%$ and $83 \pm 17\%$) digested significantly more *B. perennis* and *T. repens* seeds than *Al. chlorotica* (Fig. 6.4B, C). Further, *Al. chlorotica* ($33 \pm 47\%$), *O. tyrtaeum* ($54 \pm 31\%$), *Ap. caliginosa* ($39 \pm 27\%$), and *L. terrestris* ($45 \pm 14\%$) did not differ in the digestion of ingested *Po. trivialis* seeds, whereas digestion by *Ap. rosea* could not be determined (Fig. 6.4D). While digestion of *Pl. lanceolata* seeds could not be determined for *Ap. rosea* and *Al. chlorotica*, *O. tyrtaeum* ($83 \pm 29\%$) and *Ap. caliginosa* ($100 \pm 0\%$) digested significantly more seeds than *L. terrestris* ($34 \pm 31\%$; Fig. 6.4E). Furthermore, *Ap. caliginosa* ($47 \pm 13\%$) and *L. terrestris* ($50 \pm 17\%$) did not differ in the digestion of *M. varia* seeds, while digestion could not be determined for *Ap. rosea*, *Al. chlorotica*, and *O. tyrtaeum* (Fig. 6.4F).

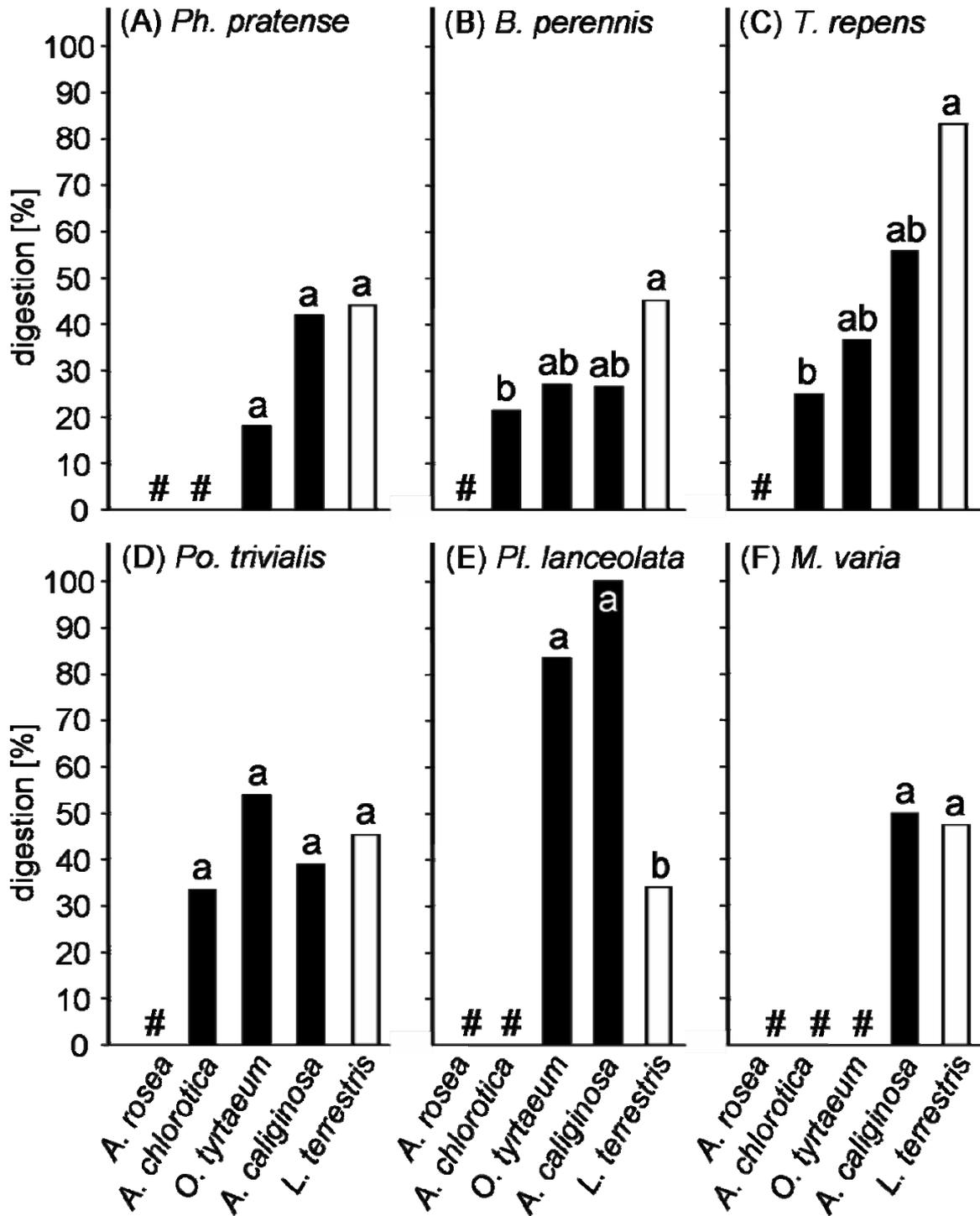


Figure 6.4 | Number of ingested seeds [%] from different plant species [(A) *Phleum pratense*, (B) *Bellis perennis*, (C) *Trifolium repens*, (D) *Poa trivialis*, (E) *Plantago lanceolata*, and (F) *Medicago varia*] digested by different earthworm species (*Aporrectodea rosea*, *Allolobophora chlorotica*, *Octolasion tyrtaeum*, *Aporrectodea caliginosa*, and *Lumbricus terrestris*). Black bars indicate endogeic and white bars anecic earthworm species, respectively. Bars with different letters vary significantly (Tukey's HSD test, $\alpha < 0.05$). #: not calculated.

Germination after earthworm gut passage

Generally, germination of small plant seeds (*Ph. pratense*, *B. perennis*, and *T. repens*) was higher than that of large seeds (+62%; $F_{1,162} = 96.26$, $P < 0.001$; *Po. trivialis*, *Pl. lanceolata*, and *M. varia*).

Germination of *Ph. pratense* seeds after earthworm gut passage could only be determined for *Ap. caliginosa* ($78 \pm 15\%$) and *L. terrestris* ($74 \pm 23\%$) but did not differ significantly from germination in control treatments ($92 \pm 9\%$), although the decline after gut passage through *L. terrestris* was marginally significant (Tukey's HSD test, $P = 0.067$; Table 6.2, Fig. 6.5A). Germination of *B. perennis* seeds was increased considerably after gut passage through *Ap. caliginosa* ($86 \pm 11\%$) as compared to control treatments ($57 \pm 28\%$), however, the gut passage through *Al. chlorotica* ($81 \pm 20\%$), *O. tyrtaeum* ($63 \pm 28\%$) and *L. terrestris* ($73 \pm 22\%$) did not modify germination significantly (Fig. 6.5B). Further, gut passage through *Ap. caliginosa* ($40 \pm 41\%$) and *L. terrestris* ($39 \pm 28\%$) did not affect germination of *T. repens* seeds ($44 \pm 11\%$; Table 6.2). Germination of *Po. trivialis* seeds was increased considerably after gut passage through *L. terrestris* ($74 \pm 13\%$) compared to control treatments ($40 \pm 14\%$), whereas the gut passage through *O. tyrtaeum* ($33 \pm 29\%$) and *Ap. caliginosa* ($43 \pm 23\%$) did not affect seed germination (Table 6.2, Fig. 6.5C). Moreover, germination of *Pl. lanceolata* seeds was increased significantly after passage through the gut of *L. terrestris* ($52 \pm 19\%$) compared to control treatments ($29 \pm 12\%$; Table 6.2). Similarly, germination of *M. varia* seeds was increased in trend after gut passage through *L. terrestris* ($58 \pm 33\%$) as compared to control treatments ($37 \pm 17\%$; Table 6.2).

Germination in presence of earthworm mucus

Germination of plant seeds in presence of *Ap. caliginosa* and *L. terrestris* mucus was earthworm and plant species specific.

Germination of *Ph. pratense* seeds was decreased significantly in presence of *Ap. caliginosa* ($85 \pm 7\%$) and *L. terrestris* mucus ($86 \pm 5\%$) as compared to control treatments ($94 \pm 7\%$; Table 6.3, Fig. 6.6A). On the contrary, mucus of *Ap. caliginosa* and *L. terrestris* had no effect on the germination of *B. perennis* ($83 \pm 9\%$ and $85 \pm 11\%$), *T. repens* ($66 \pm 14\%$ and $63 \pm 15\%$), and *Po. trivialis* seeds ($57 \pm 17\%$ and $51 \pm 17\%$) as compared to control treatments ($81 \pm 10\%$, $60 \pm 16\%$, and $49 \pm 13\%$, respectively; Fig. 6.6B, C, D).

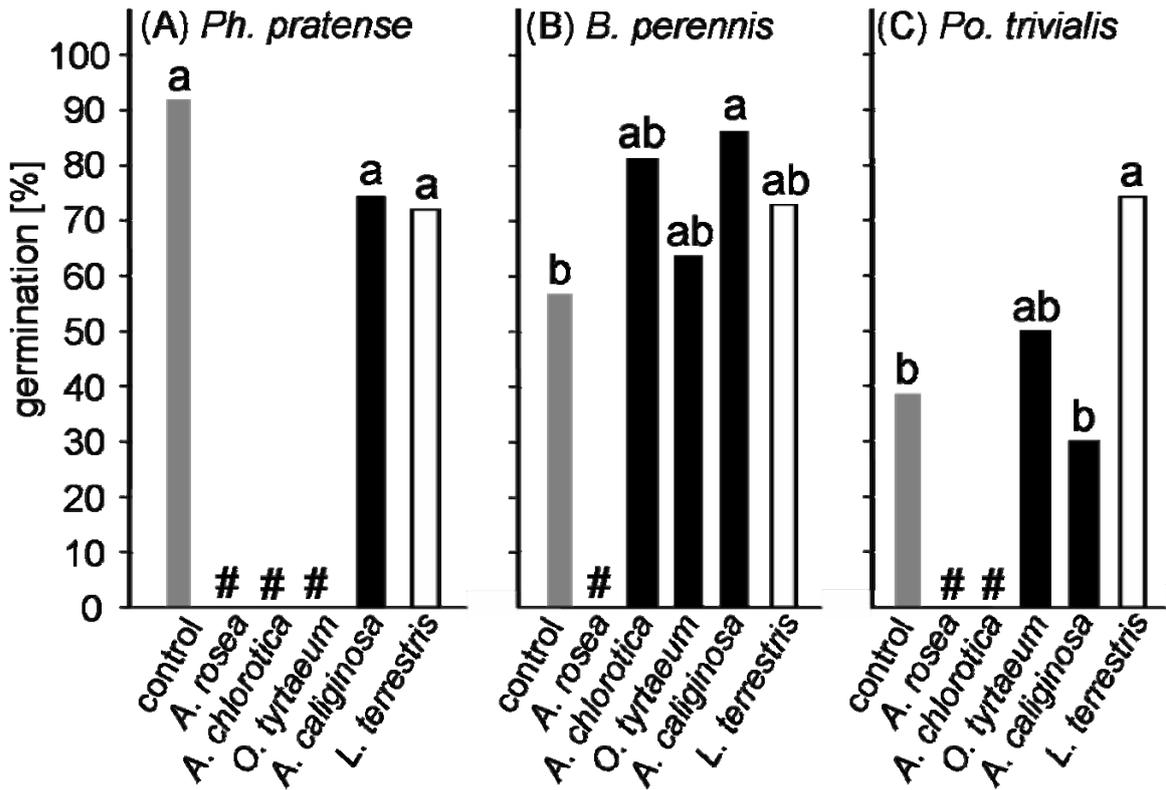


Figure 6.5 | Number of germinated seeds [%] from different plant species [(A) *Phleum pratense*, (B) *Bellis perennis*, and (C) *Poa trivialis*] after gut passage of different earthworm species (*Aporrectodea rosea*, *Allolobophora chlorotica*, *Octolasion tyrtæum*, *Aporrectodea caliginosa*, and *Lumbricus terrestris*). Grey bars indicate control treatments, black bars endogeic earthworm species, and white bars anecic earthworm species, respectively. Bars with different letters vary significantly (Tukey's HSD test, $\alpha < 0.05$). #: not calculated.

However, while mucus of *L. terrestris* ($34 \pm 10\%$) did not modify germination of *Pl. lanceolata* seeds compared to control treatments ($41 \pm 12\%$), mucus of *Ap. caliginosa* decreased germination significantly ($26 \pm 10\%$; Fig. 6.6E). Further, compared to control treatments ($49 \pm 10\%$) mucus of *L. terrestris* ($61 \pm 11\%$) increased germination of *M. varia* seeds while mucus of *Ap. caliginosa* had no effect ($52 \pm 11\%$; Fig. 6.6F).

Table 6.3 | ANOVA table of F- and P-values for the effect of earthworm mucus and casts (control, *Aporrectodea caliginosa*, and *Lumbricus terrestris*) on the number of germinated plant seeds ([%]; *Phleum pratense*, *Bellis perennis*, *Trifolium repens*, *Poa trivialis*, *Plantago lanceolata*, and *Medicago varia*).

Plant species	Earthworm mucus			Earthworm casts		
	Df	F-value	P-value	Df	F-value	P-value
<i>Phleum pratense</i>	2,27	6.38	0.005	2,42	0.08	0.917
<i>Bellis perennis</i>	2,27	0.40	0.668	2,42	4.68	0.015
<i>Trifolium repens</i>	2,27	0.40	0.675	2,42	1.65	0.202
<i>Poa trivialis</i>	2,27	0.77	0.472	2,42	0.06	0.954
<i>Plantago lanceolata</i>	2,27	4.38	0.016	2,42	2.51	0.093
<i>Medicago varia</i>	2,27	3.80	0.035	2,42	0.39	0.678

Significant effects ($P < 0.05$) are given in bold.

Df, degrees of freedom.

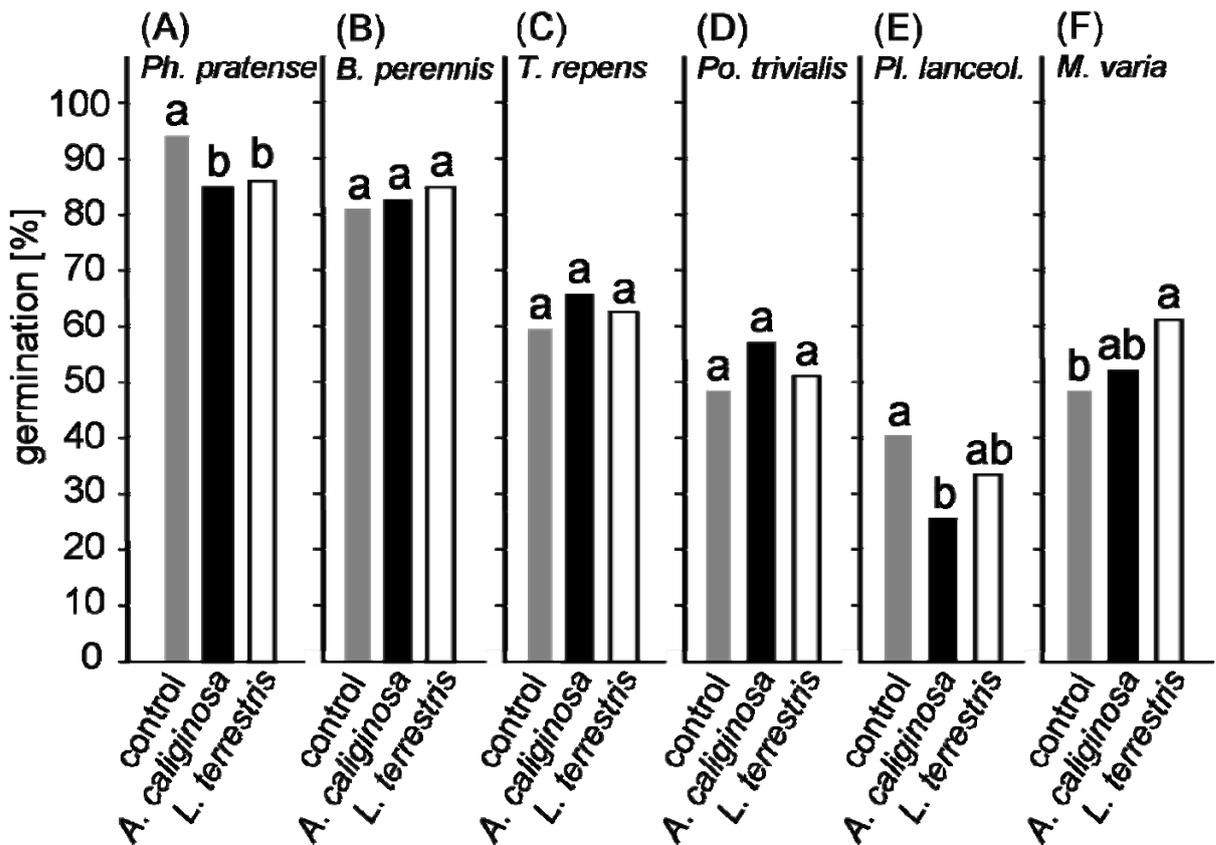


Figure 6.6 | Number of germinated seeds [%] from different plant species [(A) *Phleum pratense*, (B) *Bellis perennis*, (C) *Trifolium repens*, (D) *Poa trivialis*, (E) *Plantago lanceolata*, and (F) *Medicago varia*] as affected by earthworm mucus (control, *Aporrectodea caliginosa*, and *Lumbricus terrestris*). Grey bars indicate control treatments, black bars endogeic earthworm species, and white bars anecic earthworm species, respectively. Bars with different letters vary significantly (Tukey's HSD test, $\alpha < 0.05$).

Germination in presence of earthworm casts

No weeds (non-target plant species) germinated out of earthworm casts and Jena soil. Earthworm casts did not significantly modify germination of *Ph. pratense* ($90 \pm 10\%$), *T. repens* ($64 \pm 15\%$), *Po. trivialis* ($53 \pm 14\%$), *Pl. lanceolata* ($36 \pm 14\%$) and *M. varia* ($46 \pm 12\%$) seeds (Table 6.4, Fig. 6.7A, C, D, E, F). However, germination of *B. perennis* seeds was decreased in presence of *L. terrestris* casts ($60 \pm 15\%$) compared to control treatments ($79 \pm 16\%$; Fig. 6.7B).

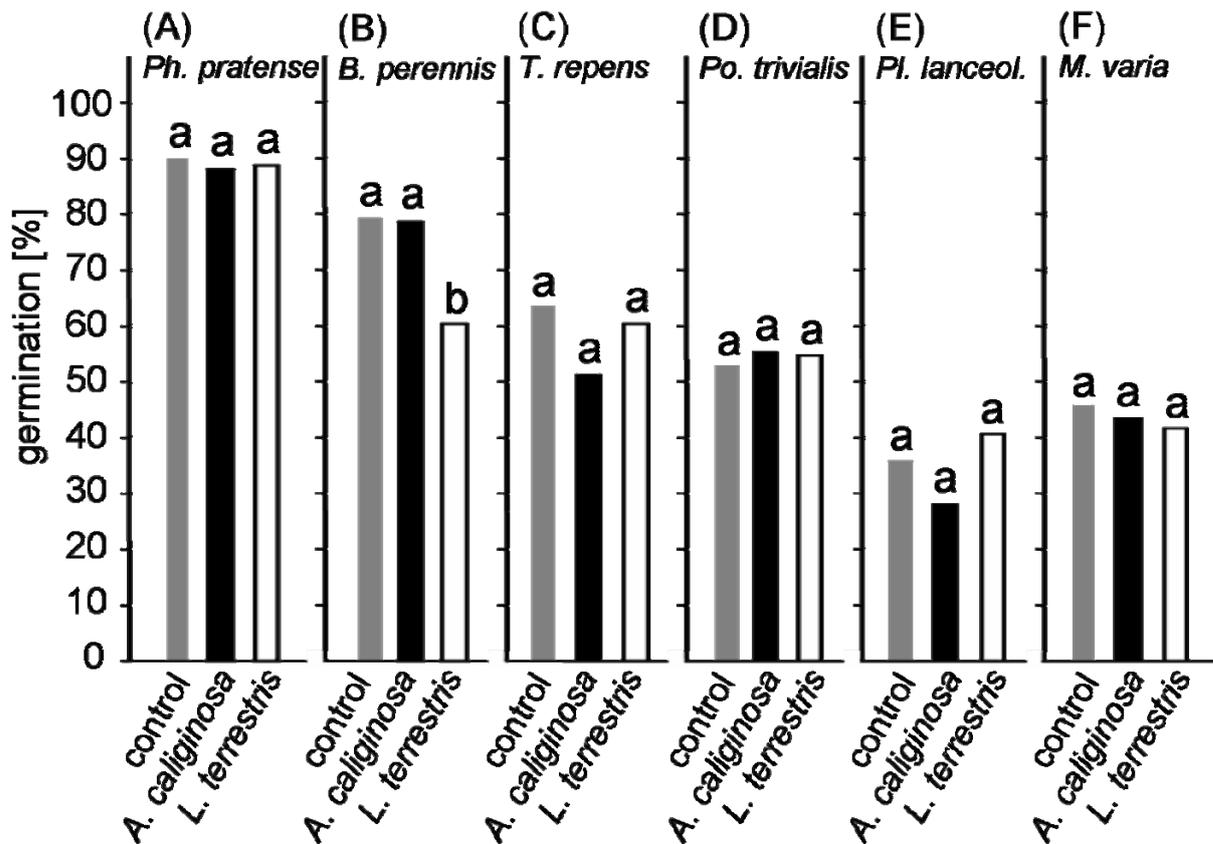


Figure 6.7 | Number of germinated seeds [%] from different plant species [(A) *Phleum pratense*, (B) *Bellis perennis*, (C) *Trifolium repens*, (D) *Poa trivialis*, (E) *Plantago lanceolata*, and (F) *Medicago varia*] as affected by earthworm casts (control, *Aporrectodea caliginosa*, and *Lumbricus terrestris*). Grey bars indicate control treatments, black bars endogeic earthworm species, and white bars anecic earthworm species, respectively. Bars with different letters vary significantly (Tukey's HSD test, $\alpha < 0.05$).

6.5 DISCUSSION

Until today the soil seed bank was treated almost uniformly as “black box” by considering it a mainly predation-free spatial niche for plant seeds. However, endogeic earthworm species live and feed primarily in the upper mineral soil. Taking into account previous studies on anecic earthworm species (McRill and Sagar 1973, Grant 1983, Shumway and Koide 1994), we hypothesized that also endogeic species ingest and digest plant seeds and, thereby, affect seed germination and plant community assembly. To our knowledge this has not been investigated before.

Do endogeic earthworms ingest and digest plant seeds?

Generally, ingestion of plant seeds depended strongly on seed and earthworm size. More small seeds (*Ph. pratense*, *B. perennis*, and *T. repens*) were ingested than large seeds (*Po. trivialis*, *Pl. lanceolata*, and *M. varia*) supporting previous studies on *L. terrestris* (Grant 1983, Shumway and Koide 1994). Moreover, small endogeic earthworms like *Ap. rosea* and *Al. chlorotica* ingested even small seeds in low numbers. On the contrary, larger endogeic species like *O. tyrtaeum* and *Ap. caliginosa* ingested seeds of all plant species investigated. Although the mean fresh weight of *O. tyrtaeum* exceeded that of *Ap. caliginosa*, seed ingestion was considerably higher in *Ap. caliginosa*. Consequently, seed ingestion is not only determined by earthworm size. Rather, species specific feeding habits are likely responsible for the distinct differences between these two species. Moreover, *Ap. caliginosa* appeared to favour slender (*Po. trivialis*) over round seeds (*T. repens*). Overall, the results suggest that ingestion of plant seeds by endogeic earthworms, particularly larger species, is widespread.

In contrast to seed ingestion, seed digestion did not depend on earthworm size. Although seed digestion could not be determined for *Ap. rosea*, the other endogeic earthworm species digested 18-100% of the ingested seeds irrespective of seed size. High seed digestion rates are in contrast to the view that earthworms consume poor-quality food material and compensate low assimilation by high consumption rates (Curry and Schmidt 2007). Although we did not determine assimilation efficiency, earthworms likely use seeds as a high quality food source as indicated by CHAPTER 4. Grant (1983) assumed that “lost” seeds during the gut passage may be destroyed by earthworm gizzard contraction and enzyme activity. Small stones and sand presumably enforce grinding of seeds in the earthworm gut as has been shown for litter material (Schulmann and Tiunov 1999, Marhan and Scheu 2005, Curry and Schmidt 2007). Moreover, enzyme activity (cellulases) provided by the ingested microflora

(Lattaud et al. 1998) likely contributes to seed digestion. Since assimilation efficiencies of endogeic species for soil organic matter is particularly low (<2.5%; Bolton and Phillipson 1976), digestion of seeds may significantly contribute to earthworm nutrition. This needs to be investigated in future experiments. Differences in earthworm seed predation may contribute to the rather enigmatic co-occurrence of superficially very similar species of endogeic earthworms. Moreover, the results indicate that selective feeding and digestion of plant seeds by endogeic earthworm species is affecting plant community assembly.

Is plant germination modified by earthworm gut passage?

Studies on germination of plant seeds after earthworm gut passage are scarce. Grant (1983) reported decreased and delayed germination of numerous grassland plant species after the gut passage through *L. terrestris* and *Aporrectodea longa*. However, certain plant species might benefit from gut passage since slight damage of seeds may break seed dormancy. Results of the present study suggest that seed germination of numerous plant species are influenced by earthworm gut passage implying that plants might have adapted to the ingestion, gut passage, and egestion by earthworms. Particularly the gut passage through *L. terrestris* altered germination of several plant species; it increased the germination of *Po. trivialis* (+34%), *Pl. lanceolata* (+23%) and *M. varia* (+21%) but decreased that of *Ph. pratense* (-18%). Moreover, the gut passage through *Ap. caliginosa* increased germination of *B. perennis* seeds (+29%). The primarily stimulating effect of earthworm gut passage on germination of grassland plant species likely was due to mechanical forces, such as scratching the seed coat (Marhan and Scheu 2005, Curry and Schmidt 2007), but also to chemical stimuli, such as increased nutrient concentrations. Moreover, phytohormone-like substances and enzymes produced by microorganisms associated with earthworm guts and casts may have contributed to breaking seed dormancy (Ayanlaja et al. 2001, El Harti et al. 2001). Ingestion of seeds by earthworms therefore likely strongly impacts plant seed survival and germination by stimulating germination of several species while digesting seeds from others. These two mechanisms likely contribute to the discrepancy between species composition of standing vegetation and the soil seed bank (Grant 1983).

Is plant seed germination modified by earthworm excreta?

Earthworm excreta clearly have the potential to alter plant seed germination. Beside increased nutrient availability in presence of earthworm excreta, earthworm casts were shown to accelerate seed germination by increasing water permeability of the seed surface (Tomati et

al. 1988) and by breaking seed dormancy (Ayanlaja et al. 2001). Moreover, earthworm excreta were shown to contain rhizogenic substances similar to indolacetic acid (El Harti et al. 2001) and are able to alter protein synthesis in seedlings (Tomati et al. 1990). Indeed, germination of *M. varia* seeds was increased by +12% in presence of *L. terrestris* mucus. Surprisingly, however, results of the present study showed that plant seed germination was primarily decreased in presence of earthworm casts and mucus reducing germination of seeds of *Ph. pratense* by -9 and -8% (in presence of *Ap. caliginosa* and *L. terrestris* mucus, respectively), that of *Pl. lanceolata* by -15% (*Ap. caliginosa* mucus), and that of *B. perennis* by -19% (*L. terrestris* casts). Previous studies also documented decreased seed germination in presence of *L. terrestris* casts (Grant 1983, Decaens et al. 2001). Potentially, seeds get damaged by enzymes like cellulases in earthworm casts produced by associated microorganisms (Urbasek 1990, Lattaud et al. 1998). Furthermore, high concentrations of ammonium as present in earthworm excreta are known to induce seed dormancy and delay germination (Crocker and Barton 1953, Satchell 1967). Again, as already described for effects of earthworm gut passage, effects of earthworm excreta on seeds were earthworm and plant species-specific and therefore likely contribute to earthworm-mediated changes in vegetation structure.

6.6 CONCLUSIONS: *Is the soil seed bank a safe place to endure?*

Delayed germination and the formation of soil seed banks is a strategy to escape unfavourable environmental conditions (Cohen 1966) and to protect seeds from predation by vertebrates, birds and ants (Thompson et al. 2001, Azcárate et al. 2003). Previous studies have shown that species with small, rounded seeds accumulate in soil seed banks, while larger seeds do not (Thompson et al. 2001). Small seeds have been suggested to experience less predation and therefore are more likely to be buried. The formed soil seed bank was considered to function as predator free (or reduced) space. However, in soil seeds may be ingested by endogeic earthworms which dominate the biomass of soil invertebrates in temperate grasslands and consume large amounts of mineral soil. Results of the present experiment indicate that endogeic earthworms indeed ingest and digest a considerable amount of plant seeds, especially small seeds. Moreover, the gut passage through endogeic earthworms and excreta modify the germination of a number of plant species. Since the effects were earthworm and plant species specific, endogeic earthworms likely strongly impact the composition of the soil seed bank and, consequently, plant community assembly.

Since post-dispersal seed predation is a key factor driving plant community composition (Hulme 1998) and affects seed survival more than pre-dispersal predation (Moles et al. 2003) the role of endogeic earthworm species deserves further attention.