Efficiency of two Widespread Non-Destructive Extraction Methods under Dry Soil Conditions for Different Ecological Earthworm Groups
7.1 **Abstract**

Reliable non-destructive extraction methods are required for the assessment of the size and composition of earthworm communities where physical disturbances are not acceptable. The aim of the present study was to investigate the efficiency of the electrical octet method and the mustard extraction method for sampling of different ecological groups of earthworms (anecics, endogeics and epigeics) under dry soil conditions. We hypothesized that (1) the extraction efficiency of the mustard method and the octet method will vary with ecological earthworm group and (2) beforehand water addition to dry soil will increase the extraction efficiency of the octet method but not that of the mustard method.

Endogeic earthworm species were extracted in low numbers irrespective of the extraction method indicating their inactivity during dry periods. The mustard method was more efficient for the extraction of anecic earthworms even under dry soil conditions, whereas the octet method was inappropriate in reflecting the actual earthworm community structure. Surprisingly, the efficiency of both methods was not improved by beforehand water addition. These findings are essential to be considered when working under dry soil conditions e.g. in the context of environmental monitoring.
7.2 INTRODUCTION

Earthworms may be sampled in a variety of ways, depending on behavioral traits and habitat preferences (Coleman et al. 2004). Reliable extraction methods are required for the assessment of the size and composition of earthworm communities. Sieving and hand-sorting soil have been considered the most accurate earthworm sampling techniques (Lee 1985). Further, there is a consensus that hand-sorting soil of the upper 20 cm with proximate formalin extraction is the preferred and standardized earthworm extraction method (Römbke et al. 2006). However, these methods cause strong physical disturbance of the soil, are labor intensive, and in the case of hand-sorting of little efficiency for anecic earthworm species (Callaham and Hendrix 1997, Schmidt 2001a, Lawrence and Bowers 2002). Furthermore, chemical repellants such as potassium permanganate (Evans and Guild 1947), formalin (Raw 1959, Callaham and Hendrix 1997, Schmidt 2001b) and household detergents (East and Knight 1998) have been used but they are toxic to earthworms and other soil organisms (Lee 1985, Gunn 1992). For a detailed survey of the common earthworm extraction methods listing advantages and disadvantages see Coleman et al. (2004). However, physical disturbances accompanying most extraction methods may be unacceptable at some sites like the study site of The Jena Experiment (Roscher et al. 2004) where experimental sites have to be retained. Non-destructive or “environmental friendly” alternative extraction methods are the mustard method (Gunn 1992, Chan and Munro 2001) and the electrical octet method (Thielemann 1986, Schmidt 2001a). The application of mustard solution or allyl isothiocyanate which is a component imparting the sharp taste to mustard (Zaborski 2003), causes earthworms to move to the soil surface due to chemical irritation (Gunn 1992). Advantages of the mustard method include high efficiency for deep-burrowing anecic species and the simple application. However, it may be less effective for other earthworm ecological groups and depend on soil type and soil moisture (Chan and Munro 2001, Bartlett et al. 2006). The octet method has been proposed for comparative surveys but its efficiency also varies with soil conditions in particular with soil moisture (Zaller and Arnone III 1999b, Schmidt 2001b).

In general, choosing the appropriate method for earthworm extraction depends on the purpose of the study (e.g. quantitative sampling versus qualitative biodiversity surveys) and on soil conditions (Coleman et al. 2004). However, the knowledge on earthworm extraction methods under varying soil conditions is scarce. The aim of the present study was to investigate the efficiency of two non-destructive earthworm extraction methods for different ecological earthworm groups (anecics, endogeics and epigeics) and that of beforehand water
addition under dry soil conditions. Thereby, extraction efficiency might vary with different ecological earthworm groups; while epigeic species reside mainly in the upper organic layers and endogeic species typically live in the upper mineral soil in horizontal burrows, anecic earthworms live in permanent vertical burrows up to 2 m deep populating the entire soil profile (Bouché 1977). We hypothesized that (1) the extraction efficiency of the mustard method and the octet method will vary with differing ecological earthworm groups and (2) beforehand water addition to dry soil will increase the extraction efficiency of the octet method but not that of the mustard method.

7.3 MATERIALS AND METHODS

The study site

The study was carried out at the field site of The Jena Experiment (Roscher et al. 2004) which is located near the Saale River in the vicinity of Jena (altitude 130 m NN, Thüringen, Germany). The site was formerly used as typical Central European mesophilic grassland. The soil is a Eutric Fluvisol (FAO-UNESCO 1997) developed from up to 2 m-thick loamy fluvial sediments. Mean annual air temperature is 9.3°C and annual precipitation is 587 mm. The plant community of the experiment is semi-natural grassland at varying diversity levels (Arrhenatherion community; Ellenberg 1996, Roscher et al. 2004). The sampling for the present study was carried out at the edge of the field site which is dominated by grasses of the species pool of The Jena Experiment (Roscher et al. 2004) and has been mown twice a year since the establishment in 2002.

Sampling

The sampling took place during a period with low precipitation in April 2007 (4.1 mm; measured at The Jena Experiment field site by the Max Planck Institute for Biogeochemistry, Jena). Normally, precipitation in April is about 27.5 mm at the field site (mean of 2003-2006). Thus, the mean soil water content of the upper 15 cm was only 12% (mean field capacity of Ap-horizon 18% [Baade 2001]; Table 7.1). Four adjoined blocks were established parallel to the river to account for changes in soil abiotic conditions (Table 7.1) as a function of distance from the river (Roscher et al. 2004). At each block (ca. 60 by 280 m) we established 20 plots of 0.25 m², spaced at 1 m intervals, by removing carefully the upper 2-3 cm of the soil with a rake (80 plots in total). The removed topsoil was hand-sorted for earthworms and detected individuals (primarily epigeics, see below) from each plot were
preserved alive in separate plastic bags filled with Jena soil. To test the influence of beforehand water addition on the efficiency of the octet and the mustard method we added 5 l water to half of the pits 45 and 30 min before extraction started (10 l of water in total) which increased soil water content of the upper 15 cm to 18% (+50%; Table 7.1). Five replicates per extraction method and water treatment were taken at each block. Moreover, we took soil samples from the upper 15 cm to determine soil water contents.

Table 7.1 | Variations in soil parameters (lime, clay, silt and sand content) of the upper 40 cm (Baade 2001, Kreutziger 2006), field capacity of the upper 75 cm (Baade 2001), gravimetric soil water content of the upper 15 cm (with [+] and without [-] water addition) and number [ind./0.25 m²] and biomass [g/0.25 m²] of anecic and endogeic earthworms at the four blocks of The Jena Experiment field site. Data on earthworms were derived from an extraction using the octet method in October 2006 over a period of appropriate weather conditions (high precipitation and mild temperatures).

<table>
<thead>
<tr>
<th>Block 1</th>
<th>Block 2</th>
<th>Block 3</th>
<th>Block 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lime content</td>
<td>6 %</td>
<td>10 %</td>
<td>13 %</td>
</tr>
<tr>
<td>Clay content</td>
<td>14 %</td>
<td>21 %</td>
<td>24 %</td>
</tr>
<tr>
<td>Silt content</td>
<td>41 %</td>
<td>54 %</td>
<td>61 %</td>
</tr>
<tr>
<td>Sand content</td>
<td>45 %</td>
<td>25 %</td>
<td>15 %</td>
</tr>
<tr>
<td>Field capacity</td>
<td>18 %</td>
<td>17 %</td>
<td>17 %</td>
</tr>
<tr>
<td>Water content (-)</td>
<td>13 %</td>
<td>12 %</td>
<td>13 %</td>
</tr>
<tr>
<td>Water content (+)</td>
<td>20 %</td>
<td>16 %</td>
<td>17 %</td>
</tr>
<tr>
<td>Number of anecics</td>
<td>8</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Biomass of anecics</td>
<td>14</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Number of endogeics</td>
<td>20</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>Biomass of endogeics</td>
<td>5</td>
<td>4</td>
<td>3</td>
</tr>
</tbody>
</table>

To test the efficiency of the octet method earthworms were sampled in subplots of 0.25 m² by electroshocking (DEKA 4000, Deka Gerätebau, Marsberg, Germany; Thielemann 1986). On each plot we extracted earthworms 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).

Mustard solutions were prepared by shaking 100 g of dry mustard powder (Intermarkt GmbH, Koblenz, Germany) with 5 l of water 24 h before extraction (Chan and Munro 2001). Additional 5 l of water were added to each bucket and the solution was mixed intensively just before application. We applied 5 l of mustard solution to each pit and another 5 l after 15 min and collected earthworms for 35 min in total. We recovered only few earthworms moving out
of the soil after this period. Extracted earthworms from each plot were preserved alive in separate plastic bags filled with Jena soil at 5°C (see above). Afterwards, we determined all earthworms alive in the laboratory to species level (including juveniles) and counted and weighed them (fresh weight with gut content).

![Figure 7.1](image)

**Figure 7.1** | (A) Scheme of the field site of The Jena Experiment. Blocks are indicated by different colors. Experimental plots are indicated by different numbers. (B) Edge of the field site of The Jena Experiment where the samplings took place indicated by the red bar. (C) Scheme of extracted plots per block.
**Statistical analysis**

ANOVA as part of the general linear models (GLMs) was used to analyze the effects of varying soil type of the four blocks (Soil), earthworm extraction method (Method), water treatment (Water) and the resultant interactions on the number and biomass of extracted anecic earthworms, using SAS 8 statistical package (SAS Inst., Cary, Florida, USA). Normal distribution and homogeneity of variance were improved by log-transformation (log[x+1]). Moreover, Friedman ANOVA was used as a nonparametric alternative to one-way repeated measures analysis of variance to analyze the effects of varying soil type of the four blocks (Soil), earthworm extraction method (Method), and water treatment (Water) on the number and biomass of extracted endogeic and epigeic earthworms using STATISTICA 6.0 (StatSoft, Tulsa, USA). Means presented in text and figures were calculated using non-transformed data (± SD). Comparisons of means (Tukey’s HSD test \( \alpha = 0.05 \)) were performed using SAS 8 statistical package.

**7.4 RESULTS**

We extracted six earthworm species belonging to three functional groups (Bouché 1977), anecic (*Lumbricus terrestris* L.), endogeic (*Aporrectodea caliginosa* Savigny, *A. rosea* Savigny, *Alolobophora chlorotica* Savigny and *Oeclolasion tyrtaeum* Savigny), and epigeic (*L. castaneus* Savigny). On average 4 ± 4 individuals (16 ind. m\(^{-2}\)) and ca. 2 ± 3 g fresh weight (10 g m\(^{-2}\)) of earthworms were extracted using the octet method. In contrast, 13 ± 10 individuals (51 ind. m\(^{-2}\)) and 17 ± 12 g (65 g m\(^{-2}\)) of earthworms were extracted using the mustard method.

The number and biomass of extracted anecic earthworms were significantly higher using the mustard method (12 ± 9 ind. 0.25 m\(^{-2}\), 16 ± 12 g 0.25 m\(^{-2}\)) than using the octet method (3 ± 4 ind. 0.25 m\(^{-2}\), 2 ± 3 g 0.25 m\(^{-2}\); Table 7.2; Fig. 7.1A). The number and biomass of extracted epigeic and endogeic earthworms were very low irrespective of extraction method. While there was no difference in the efficiency of extraction methods on the number and biomass of extracted epigeic earthworms (Table 7.3; Fig. 7.1A), the biomass of extracted endogeic earthworms was significantly higher using the octet method (0.28 ± 0.52 g 0.25 m\(^{-2}\)) than using the mustard method (0.08 ± 0.18 g 0.25 m\(^{-2}\)) and the number of endogeic individuals showed a similar trend (0.55 ± 0.93 and 0.23 ± 0.42 g 0.25 m\(^{-2}\), respectively; Table 7.3; Fig. 7.1A). Significantly more endogeic earthworms were extracted in block 3 than in blocks 1 and 2 (Table 7.3).
Generally, anecic earthworms were extracted most successfully, adding up to 96% of all extracted individuals and 99% of the earthworm biomass using the mustard method, but only 81% and 87% using the octet method, respectively. Further, 2% (number) and 0.5% (biomass) of the extracted earthworms were endogeics using the mustard method, however, endogeic earthworms added up to 15% (number) and 12% (biomass) of all extracted individuals using the octet method. Epigeic earthworms contributed only a marginal proportion to the total number and biomass of extracted earthworms irrespective of the extraction method ranging between 0.5% and 5%.

In general, there was no effect of beforehand water application on the efficiency of both earthworm extraction methods (Tables 7.2 and 7.3). Interestingly, the biomass of extracted anecic earthworms did not vary at different blocks using the octet method, significantly more earthworm biomass was extracted at block 1 than at block 3 and 4 using the mustard method (Table 7.2; Figure 7.1B).

<table>
<thead>
<tr>
<th>Soil</th>
<th>11.39</th>
<th>&lt;.0001</th>
<th>4.52</th>
<th>0.0062</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method</td>
<td>58.18</td>
<td>&lt;.0001</td>
<td>92.70</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Water</td>
<td>2.46</td>
<td>0.1216</td>
<td>0.37</td>
<td>0.5475</td>
</tr>
<tr>
<td>Soil x Method</td>
<td>1.75</td>
<td>0.1667</td>
<td>2.50</td>
<td>0.0677</td>
</tr>
<tr>
<td>Soil x Water</td>
<td>0.17</td>
<td>0.9189</td>
<td>0.62</td>
<td>0.6047</td>
</tr>
<tr>
<td>Method x Water</td>
<td>1.34</td>
<td>0.2509</td>
<td>0.66</td>
<td>0.4198</td>
</tr>
<tr>
<td>Soil x Method x Water</td>
<td>0.38</td>
<td>0.7685</td>
<td>0.04</td>
<td>0.9910</td>
</tr>
</tbody>
</table>

Table 7.2 | ANOVA table of F-values for the effect of soil type of the four blocks (Soil), earthworm extraction method (Method) and water treatment (Water) on the number and biomass of extracted anecic earthworms. Significant effects and distinct tendencies are given in bold.
Table 7.3 | Friedman ANOVA table of Chi Square-values for the effect of soil type (Soil), earthworm extraction method (Method) and water treatment (Water) on the number and biomass of extracted epigeic and endogeic earthworms. Significant effects and distinct tendencies are given in bold.

<table>
<thead>
<tr>
<th></th>
<th>Endogeic earthworms</th>
<th></th>
<th>Epigeic earthworms</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>number</td>
<td>biomass</td>
<td>number</td>
<td>biomass</td>
</tr>
<tr>
<td></td>
<td>Chi Squ.  P-value</td>
<td>Chi Squ. P-value</td>
<td>Chi Squ.  P-value</td>
<td>Chi Squ. P-value</td>
</tr>
<tr>
<td>Soil</td>
<td>9.63    0.0220</td>
<td>6.35     0.0959</td>
<td>4.62     0.2020</td>
<td>4.67     0.1976</td>
</tr>
<tr>
<td>Method</td>
<td>3.56    0.0594</td>
<td>4.26     0.0389</td>
<td>0.69     0.4054</td>
<td>0.29     0.5930</td>
</tr>
<tr>
<td>Water</td>
<td>0.09    0.8919</td>
<td>0.23     0.6481</td>
<td>1.74     0.1790</td>
<td>0.72     0.3945</td>
</tr>
</tbody>
</table>

Figure 7.2 | Effects of the earthworm extraction method (mustard, octet) (A) on the number and biomass of extracted earthworms belonging to different ecological groups (anecic, endogeic, epigeic) and (B) on the biomass of extracted anecic earthworms at the four blocks of The Jena Experiment.

*** P<0.0001; * P<0.05; (*) P<0.1; ns: not significant.
Bars with different letters vary significantly (Tukey’s HSD test, α<0.05).
CHAPTER 7 | Earthworm extraction methods

7.5 Discussion

Reliable non-destructive extraction methods are required for the assessment of the size and composition of earthworm communities where physical disturbances are not acceptable. The present study expands the knowledge about the mode of functioning and the efficiency of the octet and the mustard method under dry soil conditions. Although, the results support hypothesis (1), hypothesis (2) has to be rejected in part. Results on the mustard method showed an extremely biased pattern towards anecic species which presumably was due to the inactivity of endogeic species during dry periods and the inefficiency of extracting endogeic earthworms using this method (Bartlett et al. 2006, Chan and Munro 2001). Our findings on anecic earthworm densities resembled results of earlier extractions at the study site showing higher earthworm density and biomass in block 1 than in blocks 2-4 (Table 7.1). However, although the mustard method turned out to be very efficient in extracting anecic earthworms even under dry soil conditions, potential nutrient effects have to be taken into consideration in long term field experiments. The missing differences between the two extraction methods in epigeic earthworms were expected since L. castaneus is just invading the field site and occurs in low numbers (N. Eisenhauer, unpubl.).

Surprisingly, the octet method was not only ineffective in extracting anecic species, also it did not reflect the distribution pattern of anecic earthworms under varying soil conditions. These results disagree with previous studies indicating that the octet method is a reliable and useful method for estimating earthworm populations (Schmidt 2001b, Zaller and Arnone III 1999b). Moreover, the actual earthworm community composition was not reflected by the octet method (Table 7.1, Fig. 7.1). Nevertheless, more endogeic earthworms were extracted using the octet method than using the mustard method. This might have been due to the fact that the mustard solution primary percolates through the vertical burrows of anecic earthworms while affecting endogeic earthworms only marginally.

Unexpectedly, there was no effect of beforehand water addition on the efficiency of the octet and the mustard method, although, the soil water content was increased by +50%. While the missing effect on endogeic earthworms can be explained by the fact that they were inactive due to the dry soil conditions and the short period of time between water application and sampling, the missing effect on anecic earthworms remains rather unclear.
7.6 CONCLUSIONS

The mustard method appeared to be an efficient method for the extraction of anecic earthworms even under dry soil conditions. The octet method was inappropriate in reflecting the actual community structure under dry conditions and the efficiency was not improved by beforehand water addition. The present study highlights the differing ecology of earthworm groups by showing that anecic earthworms, in contrast to endogeics, remain active during dry periods. These findings are essential to be considered when working under dry soil conditions, e.g. for environmental monitoring.
General Discussion
8.1 BACKGROUND AND OBJECTIVES OF THIS THESIS

Anthropogenic activities are responsible for contemporary global change phenomena. The rapid **loss of biodiversity** is one of the most dramatic aspects which has generated concern over the **consequences for ecosystem functioning**. During the last two decades understanding biodiversity-ecosystem process relationships have become a major focus in ecological research (Schulze and Mooney 1994, Kinzig et al. 2002, Loreau et al. 2002, Naeem 2002, Fargione and Tilman 2005). The majority of biodiversity experiments in temperate grasslands focussed on a limited number of ecosystem processes, e.g. aboveground plant productivity. However, terrestrial ecosystems consist of **above- and belowground components** that interact in their influence on ecosystem-processes and properties (Fig. 8.1; Wardle et al. 2004). Surprisingly, above- and belowground components of ecosystems have traditionally been considered in isolation from one another ignoring the fundamental role of aboveground-belowground feedbacks in controlling ecosystem processes (van Dam et al. 2003, Wardle et al. 2004, Bardgett et al. 2005). The increasing recognition of this **lack of more holistic studies** points at the demand for surveys considering linkages between above- and belowground biota which are crucial for understanding the consequences of biodiversity loss (Bardgett et al. 2005, Spehn et al. 2005). This understanding will be gained by evaluating how **plants function as integrators of these subsystems** connecting above- and belowground food webs (Wardle et al. 2004). Moreover, there is the need for a deeper understanding of the role of aboveground–belowground feedback mechanisms in plant community dynamics and to consider this knowledge in the **modelling of global change** effects (Schröter et al. 2004).

Although the **decomposer subsystem** drives essential ecosystem processes, it has received only limited consideration in previous biodiversity-experiments (but see Bradford et al. 2002, Hedlund et al. 2003, Spehn et al. 2005, Milcu et al. 2008). The **soil fauna** is known to govern nutrient cycling, organic matter turnover, and maintenance of soil physical structure, processes that are key determinants of primary production and ecosystem carbon storage (Lavelle et al. 1998, Scheu et al. 1999, Bradford et al. 2002, Wardle et al. 2004, Bardgett et al. 2005). In many terrestrial ecosystems **earthworms** dominate the invertebrate biomass and are the most important decomposer group by structuring the soil system (Lee 1985, Edwards and Bohlen 1996). Besides several indirect mechanisms by which earthworms affect the aboveground system (Scheu 2003, Brown et al. 2004), they are supposed to have also direct impacts (Milcu et al. 2006a, Zaller and Saxler 2007).
The present thesis aimed to evaluate the main direct and indirect mechanisms by which earthworms affect grassland plant communities varying in plant species richness, number of plant functional groups and plant functional group identity. In order to improve the understanding of aboveground-belowground interactions I present the outcomes of two field surveys (CHAPTER 5, CHAPTER 7) and four greenhouse experiments (CHAPTER 2, CHAPTER 3, CHAPTER 4, CHAPTER 6) conducted in the framework of The Jena Experiment. Greenhouse experiments were performed since they have the advantage to ensure constant environmental conditions and allow for exact manipulation of the soil fauna and plant community composition. Thereby, it is possible to extract single mechanisms from complex interrelationships. First, I discuss how the present thesis enlarged the knowledge on the effects of plant communities on earthworm performance. Second, the main mechanisms are illustrated by which earthworms affect plant communities investigated in this thesis. Third, I discuss the outcomes of this thesis particularly with regard to anthropogenic activity causing fundamental factors of current global change. Finally, I close the thesis by listing the implications in a comprehensive way.
8.2 EFFECTS OF PLANTS ON EARTHWORMS

Generally, soil decomposer communities strongly depend on plant-derived carbon sources entering the soil system via dead plant materials and root exudates. Since plant species differ in the quality and quantity of resources that they return to soil, individual plant species were proposed to have important effects on specific components of the soil decomposer community and the processes that they regulate (Wardle et al. 2004). While effects of the plant community were shown to be inconsistent for microorganisms (Zak et al. 2003, Spehn et al. 2005, Milcu et al. 2006b, Milcu et al. 2008, N. Eisenhauer et al., unpubl.), nematodes (Hedlund et al. 2003, Bezemer et al. 2004, Brinkmann et al. 2005, N. Eisenhauer et al., unpubl.), and soil mesofauna (Salamon et al. 2004, Partsch et al. 2006, A. Sabais et al., unpubl.), earthworms appeared to depend rather on the quantity and quality of litter than on plant community composition per se (Zaller and Arnone 1999b, Spehn et al. 2000, Milcu et al. 2008, N. Eisenhauer et al., unpubl.). However, other studies argued that earthworms presumably are unresponsive to floristic changes (Wardle et al. 1999, Hedlund et al. 2003). In the BIODEPTH experiment, the performance of anecic earthworms was primarily affected by the presence of legumes (Spehn et al. 2000). Similarly, earthworm extractions performed in the framework of The Jena Experiment showed a positive effect of plant species richness which was also due to the presence of legumes (Milcu et al. 2008, Chapter 5). On the contrary, earthworm performance decreased in presence of grasses presumably mainly due to the low quality (C-to-N ratio) of grass litter. Anecic earthworms were affected most by the presence of legumes likely due to their predominant foraging on fresh organic matter (Edwards and Bohlen 1996). Indeed, previous studies on the feeding ecology of L. terrestris showed its preference for litter materials with high C-to-N ratio and for litter inoculated with microorganisms (reviewed by Curry and Schmidt 2007). Therefore, a decrease in biomass production and quality, respectively, should affect anecic earthworms faster and more strongly than endogeic earthworms, which feed on humified organic matter (Spehn et al. 2000). Legumes function as key plant functional group by fixing N and increasing N availability in the soil (Temperton et al. 2006, Roscher et al. 2008). Besides the positive effect of legume leaf litter entering the soil, however, Milcu et al. (2006b) found earthworms also to benefit from legumes without legume leaves entering the soil. They suggested that earthworms exploit belowground resources of legumes, potentially dead roots with associated rhizobia rich in N. In contrast to the suggestions of Milcu et al. (2006b), the outcomes of a greenhouse experiment indicate that root exudates of the plant community had
no impact on earthworm performance (Chapter 2). Performance of *L. terrestris* was rather influenced by the availability and germination success of plant seeds (Chapter 4). Moreover, results presented in Chapter 6 show that anecic and endogeic earthworm species ingest and digest considerable amounts of plant seeds. Both ingestion and digestion strongly depends on seed size and surface attributes (Chapter 6). 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 3, Chapter 4, Chapter 6), the role of plant seeds and germinating seedlings for earthworm nutrition remains unclear. However, results of the present thesis indicate that plant seeds are an important component of earthworm nutrition and that plant communities might affect earthworm performance via characteristics in seed output.

Taking the significant impact of the presence of specific plant functional groups (grasses and legumes), plant seeds and seedlings into account, earthworms likely primarily depend on the quality of resources. Therefore, the present thesis supports the assumption that positive effects of plant diversity on earthworm performance are rather due to sampling effects than due to complementarity effects.

### 8.3 Effects of Earthworms on Plant Communities

Since the first scientific recognition of earthworm effects being essential factors for plant performance (Darwin 1881) a large number of studies focused on this topic. Earthworms were assumed to be beneficial soil animals promoting plant growth (Lee 1985, Edwards and Bohlen 1996). However, the majority of studies performed in this context concentrated on the performance of single arable plant species, i.e. on the yield of crop plants (Scheu 2003). Thus, the role of earthworms for plant performance in natural habitats and for more complex plant communities was neglected. Recent studies primarily conducted in our working group indicate that, indeed, earthworms may affect the competition between plant species and thereby plant community assembly (Kreuzer et al. 2004, Wurst et al. 2005, Milcu et al. 2006a). Building on these findings, the present thesis aimed to uncover the main mechanisms by which earthworms affect plant community assembly, both indirectly (Chapter 2) and directly (Chapter 3, Chapter 4, Chapter 5, Chapter 6).

The results of the study presented in Chapter 2 supported the assumption that competition for soil nutrients is one of the main processes structuring plant communities and
closely links plants to the decomposer community. **Earthworms function as regulatory forces of nutrient mineralization and driving agents of plant competition.** Increased availability of mineral N in soil due to earthworm presence enhanced plant growth, particularly that of grasses, thereby fostering the competitive strength of grasses against legumes. Moreover, earthworms fundamentally affect grass-legume associations by increasing grass yield, the amount of N in grass hay (quality of forage), the infestation rate of grasses with aphids, and potentially by reducing the attractiveness of grass-legume associations to pollinators. These essential indirect impacts of earthworms on plant communities could not have been confirmed in the field so far (Zaller and Arnone 1999b, N. Eisenhauer et al., unpubl.). However, findings by Zaller and Arnone (1999a) in calcareous grassland indicate that primarily grass species show a close association with earthworm surface casts. Moreover, the number of established grass seedlings was increased significantly in presence of earthworms (CHAPTER 5). Consequently, both greenhouse (Kreuzer et al. 2004, Wurst et al. 2005, CHAPTER 2) and field studies (Zaller and Arnone 1999a, CHAPTER 5) indicate that particularly grasses benefit from earthworm presence with essential consequences for plant community assembly and thereby presumably for the aboveground food web.

Surface-foraging species such as *L. terrestris* are to known to effectively bury seeds, while 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, CHAPTER 4, CHAPTER 5) and acceleration (Ayanlaja et al. 2001, CHAPTER 6) or delaying of seed germination (Grant 1983, Decaens et al. 2001, CHAPTER 6). A grassland field study by Thompson et al. (1994) indicated that the compositions 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 was proposed to help explaining the frequently reported differences between the species composition of the seed bank and the standing vegetation (Grant 1983, Thompson et al. 1994, Zaller and Saxler 2007). Further, in grasslands about 70% of all seedlings emerged out of earthworm casts (Grant 1983). Results of this thesis fundamentally expand the knowledge on interactions between earthworms and plant seeds by showing that effects vary with earthworm species identity, seed size and plant functional group affiliation (CHAPTER 4, CHAPTER 5, CHAPTER 6).
The first greenhouse experiment showed that anecic earthworms occurring at the field site of The Jena Experiment (A. longa and L. terrestris) differ substantially in their direct (seed burial) and indirect effects (litter incorporation) on plant establishment and performance (CHAPTER 3). Since L. terrestris is the dominating anecic earthworm species at the field site of The Jena Experiment, I assume that its soil surface activity has fundamental consequences for plant community assembly. A further greenhouse experiment indicated that earthworm middens function as small scale disturbances, regeneration niches and patches of increased probability for plant invasion of particularly large seeded plants in grassland communities (CHAPTER 4). Earthworm effects on small and intermediate sized seeds were rather detrimental. Consequently, L. terrestris was suggested to govern plant community composition by decreasing 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 was suggested to contribute to the increased resistance of diverse plant communities against invasions (Elton 1958, Tilman 1999, Fargione and Tilman 2005, CHAPTER 4). However, results from the field survey indicate that beneficial effects of earthworm soil surface activity on seedling establishment might overbalance detrimental mechanisms (CHAPTER 5). Earthworms modulated the diversity-invasibility relationship by increasing plant invader numbers, particularly that of grasses, and diversity, and by decreasing the stability of grassland communities. This is primarily due to the soil surface activity of L. terrestris which significantly reduced the number of seed dummies on the soil surface (CHAPTER 5). Seeds might benefit from burial by escaping aboveground seed predation (Cohen 1966, Thompson et al. 2001, Azcárate and Peco 2003). Moreover, seeds likely find favourable environmental conditions for germination and growth in L. terrestris middens due to increased water-holding capacity and nutrient availability (James 1991, Blanchard et al. 1999). These might be essential mechanisms increasing the survival of seeds from certain plant species since L. terrestris was shown to stay active even during dry periods, e.g. in late summer during seed set (CHAPTER 7). However, the present thesis highlights that earthworm effects on the invasibility and stability of grassland communities depend on plant diversity, plant functional group identity and structural complexity of the established plant community (CHAPTER 5).

A further greenhouse experiment revealed that besides interactions between anecic earthworms and plant seeds, impacts of endogeic earthworms on the soil seed bank likely are also significant for plant community assembly (CHAPTER 6). In contrast to the widespread
assumption that entering the soil seed bank is a basic way to escape unfavourable environmental conditions and seed predation (Cohen 1966, Thompson et al. 2001, Azcárate and Peco 2003), the present thesis indicate that endogeic earthworms, which consume large amounts of the upper mineral soil layers, ingest and digest plant seeds. However, both, ingestion and digestion likely are earthworm and plant species specific (Chapter 6). On the contrary, seeds that survived the passage through the earthworm gut primarily benefited from gut passage by showing increased germination rates. In addition, seed germination of some plant species is also modified by earthworm excreta (mucus and casts; Chapter 6). Taking the significant impact of earthworms on plant seeds and seedlings and the potential contribution of seeds and seedlings in earthworm nutrition into account, I hypothesize that certain plant species and earthworms might have co-evolved in temperate regions of Central Europe.

8.4 CONSEQUENCES OF HUMAN-CAUSED GLOBAL CHANGE

Biodiversity loss

Anthropogenic activities have caused a dramatic decline in global biodiversity via numerous mechanisms raising the question about consequences for ecosystem functioning (Chapter 1). The decline in plant diversity in general is assumed to affect soil heterotrophic organisms in two ways: (1) by decreasing plant biomass production (decreasing resource availability), and (2) less diverse mixtures probably provided a less balanced diet in terms of food quality and a less constant supply in time (Spehn et al. 2000).

Since earthworms are known to be important components of terrestrial decomposer communities and drive several fundamental ecosystem processes like litter incorporation and decomposition (Chapter 2, Chapter 3), nutrient cycling (Chapter 2), seed survival (Chapter 6), and seedling establishment (Chapter 3, Chapter 4, Chapter 5) consequences of biodiversity loss for earthworm performance are essential to be considered. Results of the present thesis indicate, however, that earthworms are rather unresponsive to changes in plant diversity, both, at the level of plant species and plant functional groups. Earthworm performance likely depends on the presence of legumes (Chapter 5) being a key plant functional group by providing litter and root exudates rich in N. Moreover, legumes were shown to increase the productivity of the whole plant community (Aarssen 1997, Loreau et al 2002, Roscher et al. 2005), suggesting a sampling effect type of response. So far, there is very little evidence of effects of species richness of grassland plant communities on
earthworm performance and ecosystem processes driven by earthworms from biodiversity experiments in the field. Rather, earthworm performance likely is mainly driven by abiotic factors of the soil, e.g. pH and sand content (Milcu et al. 2008).

Moreover, impacts of earthworms on seedling establishment and plant community assembly likely are intimately interrelated with plant community diversity with the most pronounced effects in grassland communities with four plant species and two or three plant functional groups, respectively. There, earthworms were shown to increase the diversity of plant communities by creating small scale disturbances (Connell 1978, CHAPTER 5). Since grassland species mixtures containing four plant species are extremely low in diversity, earthworm-plant seed interactions might also not be threatened by the decline in biodiversity. However, earthworms perform rather poor in agricultural monocultures where their impact on ecosystem processes likely is decreased strongly (Edwards and Bohlen 1996, CHAPTER 5).

Dispersal of peregrine earthworm species

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). Invasions by belowground organisms have received less attention than invasions by aboveground organisms, in part due to the cryptic nature of the soil environment and the less apparent consequences of such invasions (Bohlen 2006). One of the most apparent and dramatic examples of belowground invaders is the invasion of regions previously devoid of earthworms by non-native earthworm species. As described above, much of earthworm effects occurs because of their role as ecosystem engineers capable of substantially changing the physical and chemical characteristics of the soil environment, with consequences for the entire soil food web, nutrient distribution, invertebrate and plant communities, and thereby ecosystem structure and functioning (Fig. 8.2; Bohlen et al. 2004, Hale et al. 2006, Eisenhauer et al. 2007). The present thesis indicate that beside indirect facilitation processes by changing soil conditions, earthworms possibly favour invasive plants that are adapted to earthworm ingestion and gut passage (CHAPTER 4, CHAPTER 6). As described above, taking the significant impact of earthworms on plant seeds and seedlings and the potential contribution of seeds and seedlings in earthworm nutrition into account, it is likely that plants and earthworms have co-evolved in temperate regions of Central Europe (CHAPTER 4, CHAPTER 6). Moreover, invasive earthworms probably change soil seed bank composition and plant community assembly. Indeed, preliminary results of a study investigating the effects of invasive earthworm species on the soil seed bank of a deciduous
aspen forest (Alberta, Canada) indicate that earthworms modify plant germination (D. Straube, unpubl.). The presence of _O. tyrtaeum_ increased the germination of native herbaceous plant species considerably. However, this topic deserves further attention with regard to the proceeding **anthropogenic dispersal of European earthworms** worldwide and the **homogenization of habitats**.

**Figure 8.2** | Scheme illustrating mechanisms by which above- and belowground invaders influence ecosystem structure and functioning (Bohlen 2006).
8.5 CLOSING THE LOOP: CONCLUSIONS AND IMPLICATIONS

There is great demand for new insights from studies on aboveground-belowground interactions that should be used to improve our predictions of the effects of human-induced environmental changes in biodiversity and ecosystem properties and to enhance the efficiency of human interventions in restoration and conservation efforts (Wardle et al. 2004). In this context, the present thesis expands the knowledge on the role of earthworms as important belowground ecosystem engineers in respect of human-caused global change processes like biodiversity loss and dispersal of exotic species.

Earthworm effects on the aboveground system appeared to be manifold playing a decisive role via four different fundamental ecosystem processes (Fig. 8.3):

A | First, (anecic) earthworms act as decomposers by incorporating litter into the soil and increasing nutrient availability for plants. Thereby, earthworms drive the competition between plants and plant community assembly (Chapter 2).

B | Second, (anecic) earthworms are important ecosystem engineers by removing the litter layer and creating structures of increased nutrient availability (middens). These structures function as small scale disturbances and regeneration niches for plant seedlings increasing the heterogeneity of the habitat and affecting plant community assembly and diversity (Chapter 3, Chapter 4, Chapter 5).

C | Third, (anecic) earthworms function as important seed dispersers by seed burial and ingestion and egestion of plant seeds (Chapter 5, Chapter 6). Further, earthworm gut passage and earthworm excreta affect seed germination and thereby plant community assembly (Chapter 6). Seed burial might be an essential mechanism increasing the survival of seeds from certain plant species since L. terrestris was shown to stay active even during dry periods, e.g. in late summer during seed set (Chapter 7).

D | Fourth, earthworms function as seed predators whereas seed predation is earthworm and plant species specific directly affecting plant community assembly (Chapter 4, Chapter 6).

The present combined approach of above- and belowground systems emphasizes their intimate interrelationships demanding for the consideration of both systems when interpreting, estimating and modelling human-induced global change phenomena.
Figure 8.3 | Scheme of the direct, indirect and supposed effects of earthworms on the plant community and the aboveground food web as indicated by the present thesis. Index numbers refer to the respective chapter and capital letters refer to the roles of earthworms in temperate grasslands (A, Decomposer; B, Ecosystem engineer; C, Seed disperser; D, Seed predator).
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