

EARTHWORMS IN A PLANT DIVERSITY GRADIENT: DIRECT AND INDIRECT EFFECTS ON PLANT COMPETITION AND ESTABLISHMENT

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Für meine Familie

Die vorliegende Arbeit wurde unter der Leitung von **Prof. Dr. Stefan Scheu** am Institut für Zoologie der Technischen Universität Darmstadt durchgeführt und von der **Deutschen Forschungsgemeinschaft** (DFG) im Rahmen des Jena Experimentes (FOR 456: *The role of biodiversity for element cycling and trophic interactions: An experimental approach in a grassland community*) gefördert.

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Ich erkläre hiermit an Eides statt, dass ich die vorliegende Dissertation selbständig und nur mit den angegebenen Hilfsmitteln angefertigt habe. Ich habe noch keinen Promotionsversuch unternommen.

Darmstadt, den 28.03.2008

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„Ich habe keine besondere Begabung, sondern bin nur leidenschaftlich neugierig.“

Albert Einstein 1879-1955

*„Gehe nicht, wohin der Weg führen mag, sondern dorthin,
wo kein Weg ist und hinterlasse eine Spur.“*

Jean Paul 1763-1825

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“EARTHWORMS IN A PLANT DIVERSITY GRADIENT: DIRECT AND INDIRECT EFFECTS ON PLANT COMPETITION AND ESTABLISHMENT”

as a part of

"The Jena Experiment - The role of biodiversity for element cycling and trophic interactions: an experimental approach in a grassland community"

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Eisenhauer N, Partsch S, Parkinson D and Scheu S (2007) Invasion of a deciduous forest by earthworms: Changes in soil chemistry, microflora, microarthropods and vegetation. *Soil Biology and Biochemistry* 39: 1099-1110.

CHAPTER 3

Eisenhauer N, Marhan S and Scheu S (2008) Assessment of anecic behavior in selected earthworm species: Effects on wheat seed burial, seedling establishment, wheat growth and litter incorporation. *Applied Soil Ecology* 38: 79-82.

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Eisenhauer N, Straube D and Scheu S (2008) Efficiency of two widespread non-destructive extraction methods under dry soil conditions for different ecological earthworm groups. *European Journal of Soil Biology* 44: 141-145.

CHAPTER 4

Eisenhauer N and Scheu S (2008) Invasibility of experimental grassland communities: The role of earthworms, plant functional group identity, and seed size. *Oikos*, in press.

Habekost M, **Eisenhauer N**, Scheu S and Gleixner G (2008) Seasonal and plant diversity induced changes of a microbial community in a grassland with different species mixtures four years after establishment. *Soil Biology and Biochemistry*, accepted.

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CHAPTER 5

Eisenhauer N, Milcu A, Bessler H, Sabais A, Engels C and Scheu S (in preparation) Invasibility and stability of temperate grasslands: Biodiversity and ecosystem engineers (anecic earthworms) as determinants.

CHAPTER 6

Eisenhauer N, Schuy M, Butenschoen O and Scheu S (in preparation) The soil seed bank: A safe place to endure?

Eisenhauer N, Habekost M, Scherber C, Milcu A, Partsch S, Gleixner G, Weisser WW and Scheu S (in preparation) Microbial parameters and nutrient limitations in grassland communities varying in plant species and functional group diversity.

Eisenhauer N, König S, Sabais A, Renker K, Buscot F, and Scheu S (in preparation) Interacting effects of earthworms and mycorrhiza fungi on plant performance.

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Eisenhauer N and Scheu S (2007) Earthworms as drivers of the competition between grasses and legumes. ESA/SER Joint Meeting, San José, California, USA.

Eisenhauer N and Scheu S (2007) Earthworms as drivers of the competition between grasses and legumes. 37th Annual Conference Ecological Society of Germany, Austria and Switzerland, Marburg, Germany.

Eisenhauer N and Scheu S (2008) Direct effects of earthworms on plant communities. Seminar: Probleme der Freilandökologie, Gießen, Germany.

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Martin Schuy (2007) Direkte und indirekte Wirkung von Regenwürmern auf die Keimung von Pflanzensamen. Examensarbeit, Darmstadt University of Technology.

Daniela Straube (2007) Effizienz zweier Extraktionsmethoden für Regenwürmer bei trockenen Bodenbedingungen. Forschungspraktikum, Darmstadt University of Technology.

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Felix Schonert (2008) Der Einfluss von Pflanzenartendiversität und funktionellen Pflanzengruppen auf die Bodenmakrofauna in einer Grünlandgesellschaft. Examensarbeit, Darmstadt University of Technology.

Svenja Gass (2008) Einfluss von Pflanzendiversität auf funktionelle Nematodengruppen in einer Grünlandgesellschaft. Examensarbeit, Darmstadt University of Technology.

Daniela Straube (2008) Invasion of exotic earthworms into a deciduous forest: Effects on soil chemistry, microflora, microarthropods, plant seed bank and vegetation. Diploma thesis, Darmstadt University of Technology.

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OVERVIEW OF MANUSCRIPTS

The present thesis comprises the following manuscripts:

CHAPTER 2 | EARTHWORMS AS DRIVERS OF THE COMPETITION BETWEEN GRASSES AND LEGUMES

by Nico Eisenhauer and Stefan Scheu. Submitted to Soil Biology and Biochemistry.

This manuscript investigates the role of earthworms for the competition between grasses and legumes for soil nutrients and highlights the intimate interrelationship between the above- and belowground systems. It shows that:

- Earthworms modulate the competition between grasses and legumes by mobilizing soil N and thereby increasing the competitive strength of grasses.
- Earthworms function as essential driving agents of grass-legume associations by (a) increasing grass yield, (b) increasing the amount of N in grass hay, (c) increasing the infestation rate of grasses with aphids, and (d) potentially reducing the attractiveness of grass-legume associations to pollinators.

Nico Eisenhauer is the overall author of this manuscript. He developed the main ideas and experimental setup. He personally collected and analyzed the data, created the graphs and tables, wrote the whole manuscript, and communicated with referees and editors.

Stefan Scheu was the supervisor of the experiment presented in this manuscript. He was involved in the development of the experimental setup and critically reviewed previous versions of the present manuscript.

CHAPTER 3 | ASSESSMENT OF ANECIC BEHAVIOR IN SELECTED EARTHWORM SPECIES: EFFECTS ON WHEAT SEED BURIAL, SEEDLING ESTABLISHMENT, WHEAT GROWTH, AND LITTER INCORPORATION

by Nico Eisenhauer, Sven Marhan, and Stefan Scheu. Applied Soil Ecology (2008) 38: 79-82, doi:10.1016/j.apsoil.2007.07.002.

This manuscript investigates the effects of three apparently anecic earthworm species on wheat seed burial, seedling establishment, wheat growth, and litter incorporation. It shows that:

- The three investigated anecic earthworm species differ substantially in their behavior and in their effect on plant establishment.

- The behavior of *L. terrestris* and *L. rubellus friendoides* is characteristic for anecic earthworm species whereas that of *A. longa* rather resemble that of endogeic species.
- The present study is the first experimental evidence for anecic behavior in *L. rubellus friendoides*.

Nico Eisenhauer is the overall author of this manuscript. He developed the main ideas and experimental setup. He personally collected and analyzed the data, created the graphs and tables, wrote the whole manuscript, communicated with referees, editors and typesetters, and accomplished the whole publication process from submission in March 2007 until print publication in January 2008.

Sven Marhan was involved in the development of the experimental setup, helped harvesting the greenhouse experiment, and commented on earlier versions of this manuscript.

Stefan Scheu was the supervisor of the experiment presented in this manuscript. He was involved in the development of the experimental setup and critically reviewed previous versions of the present manuscript.

CHAPTER 4 | INVASIBILITY OF EXPERIMENTAL GRASSLAND COMMUNITIES: THE ROLE OF EARTHWORMS, PLANT FUNCTIONAL GROUP IDENTITY, AND SEED SIZE

by Nico Eisenhauer and Stefan Scheu. *Oikos* (2008), in press.

This manuscript investigates the impacts of *Lumbricus terrestris*, 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. It shows that:

- Earthworm effects on the number and biomass of invader plants vary with seed size and plant functional group identity.
- Earthworms 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.

Nico Eisenhauer is the overall author of this manuscript. He developed the main ideas and experimental setup. He personally collected and analyzed the data, created the graphs and tables, wrote the whole manuscript, communicated with referees, editors and typesetters.

Stefan Scheu was the supervisor of the experiment presented in this manuscript. He was involved in the development of the experimental setup and critically reviewed previous versions of the present manuscript.

CHAPTER 5 | INVASIBILITY AND STABILITY OF TEMPERATE GRASSLANDS: BIODIVERSITY AND ECOSYSTEM ENGINEERS (ANECIC EARTHWORMS) AS DETERMINANTS

by Nico Eisenhauer, Alexandru Milcu, Holger Bessler, Alexander Sabais, Christof Engels, and Stefan Scheu. In preparation for *Acta Oecologica*.

This manuscript investigates modifications of the invasibility and stability of grassland communities varying in plant species and functional group diversity by *Lumbricus terrestris*. It shows that:

- Increasing diversity enhances the stability of the plant community which is primarily due to the higher probability of grass presence in the resident community.
- Plant species richness is more important than number of plant functional groups for the invasion resistance of grassland communities.
- 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.
- Plant species invasion and community stability are driven by a complex interaction between the diversity, functional identity, the structural complexity of plant communities, and by belowground ecosystem engineers such as anecic earthworms.

Nico Eisenhauer is the overall author and developed the main ideas of this manuscript. He personally collected (2006) and analyzed the data on plant invaders, created the graphs and tables, and wrote the whole manuscript.

Alexandru Milcu collected the data on plant invaders in 2004 and 2005. He commented on earlier versions of the manuscript.

Holger Bessler provided data on fine root biomass.

Alexander Sabais helped collecting data on plant invaders (2006) and commented on earlier versions of the manuscript.

Christof Engels was involved in the setup of continuous fine root samplings.

Stefan Scheu was the supervisor of the experiments presented in this manuscript. He was involved in the development of the experimental setup and critically reviewed previous versions of the present manuscript.

CHAPTER 6 | THE SOIL SEED BANK: A SAFE PLACE TO ENDURE?

by Nico Eisenhauer, Martin Schuy, Olaf Butenschoen, and Stefan Scheu. In preparation for *Oikos*.

This manuscript tested whether endogeic earthworms ingest and digest seeds from grassland plant species, the passage of seeds through the gut of endogeic earthworms modifies plant seed germination, and whether excreta (mucus and casts) of an endogeic earthworm species (*Aporrectodea caliginosa*) modify plant seed germination. It shows that:

- Selective ingestion and digestion of plant seeds by endogeic earthworm species presumably alter the composition of the soil seed bank and, consequently, plant community assembly.
- Ingestion of seeds by earthworms likely strongly impacts plant seed survival and germination by stimulating germination of several species while digesting seeds from others.
- Effects of earthworm excreta on plant seeds are earthworm and plant species-specific and therefore likely contribute to earthworm-mediated changes in vegetation structure.

Nico Eisenhauer is the overall author of this manuscript. He developed the main ideas and experimental setup. He helped collecting the data, analyzed the data, created the graphs and tables, and wrote the whole manuscript.

Martin Schuy collected the data and commented on earlier versions of the manuscript.

Olaf Butenschoen was involved in the development of the experimental setup and commented on earlier versions of the manuscript.

Stefan Scheu was the supervisor of the experiments presented in this manuscript. He was involved in the development of the experimental setup and reviewed previous versions of the present manuscript.

CHAPTER 7 | EFFICIENCY OF TWO WIDESPREAD NON-DESTRUCTIVE EXTRACTION METHODS UNDER DRY SOIL CONDITIONS FOR DIFFERENT ECOLOGICAL EARTHWORM GROUPS

by Nico Eisenhauer, Daniela Straube, and Stefan Scheu. *European Journal of Soil Biology* (2008) 44: 141-145, doi:10.1016/j.ejsobi.2007.10.002.

This manuscript investigates 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. It shows that:

- The mustard method is more efficient for the extraction of anecic earthworms, whereas the octet method is inappropriate in reflecting the actual earthworm community structure under dry soil conditions.
- The efficiency of both methods can not be 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

Nico Eisenhauer is the overall author of this manuscript. He developed the main ideas and experimental setup. He helped collecting the data, analyzed the data, created the graphs and tables, wrote the whole manuscript, communicated with referees, editors and typesetters and accomplished the whole publication process from submission in August 2007 until print publication in February 2008.

Daniela Straube collected the data and commented on earlier versions of the manuscript.

Stefan Scheu was the supervisor of the experiments presented in this manuscript. He was involved in the development of the experimental setup and reviewed previous versions of the present manuscript.

SUMMARY

The human-caused 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, however, the majority of biodiversity experiments in temperate grasslands focussed on a limited number of ecosystem processes, e.g. aboveground plant productivity. 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 in understanding of biodiversity loss. Although the decomposer subsystem drives essential ecosystem processes, it has received only limited consideration in previous biodiversity-experiments. 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. In many terrestrial ecosystems earthworms dominate the invertebrate biomass and are the most important decomposer group by structuring the whole belowground system and by directly and indirectly affecting the aboveground subsystem.

The design of The Jena Experiment offers the unique opportunity to investigate the relationship between biodiversity and ecosystem processes while simultaneously manipulating trophic interactions. Thereby, it is possible for the first time to explore the consequences of human-induced diversity loss while considering interrelationships between plant communities and important animal ecosystem engineers. In the present thesis I performed two field experiments and four greenhouse experiments in order to extract the main direct and indirect interacting mechanisms between earthworms and grassland plant communities varying in diversity.

The objectives of the first greenhouse experiment were to quantify the effects of earthworms on grass-legume competition in model grassland systems in order to improve the understanding of ecological mechanisms structuring grass-legume associations. We established model grassland systems in microcosms that were harvested twice to simulate the widespread biannual mowing regime in Central European grasslands. The presence of *Lumbricus terrestris* L. increased the productivity of grasses and legumes after 6 weeks but only that of grasses after another 10 weeks. Analyses of $^{15}\text{N}/^{14}\text{N}$ ratios indicate that, compared to legumes, grasses more efficiently exploit soil mineral N and benefit from legume presence through reduced “intra-functional group” competition. Earthworms appeared to modulate the

competition between grasses and legumes by mobilizing soil N and thereby fostering the competitive strength of grasses. Moreover, earthworms were shown to affect the aboveground system and to function as essential driving agents of grass-legume associations by increasing grass yield, the amount of N in grass hay, the infestation rate of grasses with aphids, and potentially by reducing the attractiveness (number of flowerheads) of grass-legume associations to pollinators.

The second greenhouse experiment investigated the effects of three apparently anecic earthworm species on wheat seed burial, seedling establishment, wheat growth, and litter incorporation. In contrast to *Aporrectodea longa* Ude, *L. terrestris* and *Lumbricus rubellus friendoides* Bouché reduced the litter layer considerably and buried more wheat seeds. 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* were conform to the characteristics of anecic earthworm species whereas those of *A. longa* rather resemble endogeic species.

The aim of the third greenhouse experiment was to investigate the impact of *L. terrestris*, 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. Earthworm performance was influenced by an interaction between plant functional group identity of the established plant community and that of invader species. 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.

The first field survey aimed to explore modifications of the invasibility and stability of grassland communities varying in plant species and functional group diversity by *L. terrestris*. We weeded experimental subplots (differing in *L. terrestris* densities) by removing, counting and weighing non-target plant species. The results show that increasing diversity enhances the stability of the plant community which was primarily due to the higher probability of grass presence in the resident community. Earthworm performance likely is not affected by plant diversity per se but by the presence of certain plant functional groups (legumes and grasses). 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. Moreover, plant species invasion and community stability are driven by a complex interaction

between the diversity, functional identity, and structural complexity of plant communities and by belowground ecosystem engineers such as anecic earthworms.

The fourth greenhouse experiment investigated direct and indirect impacts of endogeic earthworms on grassland plant seeds. Seed ingestion and digestion and germination in presence of earthworm excreta appeared to be plant and earthworm species-specific. Ingestion of seeds by earthworms likely strongly impacts plant seed survival and germination by stimulating germination of some species while digesting seeds from others. Selective ingestion and digestion of plant seeds by endogeic earthworm species presumably alter the composition of the soil seed bank and, consequently, plant community assembly.

A second field survey investigated 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. The mustard method was shown to be more efficient for the extraction of anecic earthworms, whereas the octet method was inappropriate in reflecting the actual earthworm community structure under dry soil conditions. The efficiency of both methods could not be improved by beforehand water addition. Moreover, the present study highlights the differing ecology of earthworm groups by showing that anecic earthworms, in contrast to endogeics, remain active during dry periods.

Overall, the present thesis indicates that earthworm performance is unresponsive to manipulations of plant community diversity. Rather earthworms are affected by the presence of nutrient rich resources provided by legumes. Earthworm effects on the aboveground system appeared to be manifold playing a decisive role via four different fundamental ecosystem processes. First, (anecic) earthworms act as decomposers by increasing nutrient availability for plants and driving the competition between plants. Second, (anecic) earthworms are important ecosystem engineers by creating structures of increased nutrient availability (middens) functioning as small scale disturbances and regeneration niches for plant seedlings. Thereby, earthworms were shown to promote plant diversity. Third, (anecic) earthworms function as important seed dispersers by seed burial and ingestion and egestion of plant seeds. 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. Fourth, earthworms directly affect plant community assembly by functioning as seed predators whereas seed predation is earthworm and plant species-specific.

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.

ZUSAMMENFASSUNG

Der anthropogen bedingte rasche Biodiversitätsverlust ist einer der dramatischsten Aspekte des globalen Wandels, der Bedenken über die Konsequenzen für Ökosystemprozesse ausgelöst hat. Während der letzten zwei Jahrzehnte ist die Erforschung der Zusammenhänge zwischen Biodiversität und Ökosystemprozessen zunehmend in den wissenschaftlichen Fokus gerückt. Die Mehrzahl an Biodiversitätsexperimenten wurde in temperierten Grasländern durchgeführt, untersuchte dabei allerdings eine begrenzte Anzahl an Ökosystemprozessen, wie zum Beispiel oberirdische Produktivität. Ober- und unterirdische Ökosystemkomponenten wurden bisher meist unabhängig voneinander untersucht. Dabei ignorierte man die fundamentale Rolle von ober- und unterirdischen Rückkopplungsprozessen zum Verständnis der Folgen von Biodiversitätsverlust. Obwohl das Zersetzersystem elementare Ökosystemprozesse steuert, hat es in bisherigen Biodiversitätsexperimenten wenig Beachtung gefunden. Die Bodenfauna lenkt Nährstoffkreisläufe, den Umsatz von organischem Material und die Charakteristik der Bodenstruktur, welches ausnahmslos Schlüsselprozesse für die Produktivität und den Kohlenstoffspeicher darstellen. Regenwürmer dominieren die Invertebratenbiomasse in zahlreichen terrestrischen Ökosystemen und stellen dabei die wichtigste Zersetzergruppe dar, indem sie das gesamte Bodensystem strukturieren und das oberirdische System direkt und indirekt beeinflussen.

Das Design des Jena-Experimentes bietet die einzigartige Gelegenheit, den Zusammenhang zwischen Biodiversität und Ökosystemprozessen bei simultaner Manipulation von trophischen Interaktionen zu untersuchen. Dabei ist zum ersten Mal die Betrachtung der Konsequenzen von anthropogen bedingtem Biodiversitätsverlust unter Einbeziehung der Zusammenhänge zwischen Pflanzengemeinschaften und tierischen Ökosystem-Ingenieuren möglich. Im Rahmen meiner Promotion führte ich zwei Feld- und vier Gewächshausexperimente durch, um die wichtigsten direkten und indirekten mechanistischen Zusammenhänge zwischen Regenwürmern und verschiedenen diversen Pflanzengemeinschaften zu erforschen.

Ziel des ersten Gewächshausexperimentes war es, den Einfluss von Regenwürmern auf die Konkurrenz zwischen Gräsern und Leguminosen zu quantifizieren, um die ökologischen Mechanismen zu verstehen, welche die in der Landwirtschaft weit verbreiteten Kleegrasmischungen strukturieren. Dafür wurden Pflanzengemeinschaften in Mikrokosmen etabliert, welche an zwei Terminen geerntet wurden, um ein gebräuchliches Mahdregime in europäischen Grasländern zu simulieren. Nach sechs Wochen war die oberirdische Biomasse

von Gräsern und Leguminosen in Anwesenheit von *Lumbricus terrestris* L. erhöht, wobei nach zehn Wochen nur eine erhöhte Grasbiomasse registriert wurde. Die Analyse der Stickstoffisotope zeigte, dass im Gegensatz zu Leguminosen Gräser mineralischen Stickstoff im Boden effektiv aufnehmen. Gräser profitieren von der Anwesenheit von Leguminosen durch eine reduzierte „intra-funktionelle“ Konkurrenz. Regenwürmer verändern die Konkurrenzsituation zwischen Gräsern und Leguminosen, indem sie Stickstoff im Boden mobilisieren und dadurch die Konkurrenzkraft der Gräser stärken. Darüber hinaus konnte gezeigt werden, dass Regenwürmer als fundamentale Steuergrößen der oberirdischen Gemeinschaft fungieren, indem sie den Ertrag und die Güte von Grasgemeinschaften erhöhen, die Befallsrate von Gräsern durch Blattläuse erhöhen und wahrscheinlich die Attraktivität von Kleegrasmischungen für Bestäuber durch eine geringere Anzahl an Blüten reduzieren.

Das zweite Gewächshausexperiment untersuchte die Einflüsse von drei scheinbar anözischen Regenwurmart auf das Vergraben von Weizensamen, die Etablierung von Keimlingen, das Weizenwachstum und die Einarbeitung von Streu in den Boden. Im Gegensatz zu *Aporrectodea longa* Ude, reduzierten *L. terrestris* und *Lumbricus rubellus friendoides* Bouché die Streuschicht und vergruben mehr Weizensamen. Die Ergebnisse verdeutlichen, dass sich anözische Regenwurmart wesentlich in ihrem Einfluss auf die Einarbeitung von Streu und Samen in den Boden und auf die Etablierung von Keimlingen unterscheiden. Die Effekte von *L. terrestris* und *L. rubellus friendoides* entsprechen denen anözischer Regenwürmer, wohingegen diejenigen von *A. longa* eher endogäischen Eigenschaften entsprechen.

Das dritte Gewächshausexperiment untersuchte die Effekte von *L. terrestris*, der Zugehörigkeit zu bestimmten funktionellen Pflanzengruppen und der Samengröße von Pflanzeneinwanderern und funktioneller Identität der etablierten Pflanzengemeinschaft auf die Anzahl und die Biomasse etablierter Einwandererpflanzen. Die Regenwurmbiomasse wurde von einer Interaktion zwischen der funktionellen Identität der etablierten Pflanzengemeinschaft und derjenigen der Pflanzeneinwanderer beeinflusst. Da der Effekt von Regenwürmern auf die Anzahl und Biomasse der etablierten Pflanzeneinwanderern von der Samengröße und der funktionellen Identität der Pflanzensamen abhängt, spielen sie wahrscheinlich eine entscheidende Rolle während der Etablierung von Keimlingen und steuern die Zusammensetzung der Pflanzengemeinschaft. Samen und Keimlinge sind vermutlich ein bedeutender Bestandteil der Ernährung von Regenwürmern.

Die erste Feldstudie untersuchte, ob Regenwürmer die Stabilität und Einwanderungsanfälligkeit von Pflanzengemeinschaften unterschiedlicher Diversität verändern. Dafür

wurden experimentelle Teilflächen, die sich in ihrer Regenwurmdichte unterschieden, gejätet und Einwandererpflanzen identifiziert, gezählt und gewogen. Die Ergebnisse zeigen, dass die Stabilität von Pflanzengemeinschaften mit steigender Diversität zunimmt. Das lag primär an der erhöhten Wahrscheinlichkeit der Präsenz von Gräsern in der Pflanzengemeinschaft. Die Anzahl und Biomasse von *L. terrestris* wurde hauptsächlich von der Anwesenheit bestimmter funktionellen Pflanzengruppen (Gräser und Leguminosen) beeinflusst, nicht aber von der Diversität der Pflanzengemeinschaft an sich. Indem Regenwurmdichten erfolgreich im Feld manipuliert wurden, konnte zum ersten Mal gezeigt werden, dass Regenwürmer die Ausbreitung von Samen und die Keimlingsetablierung beeinflussen. Darüber hinaus konnte gezeigt werden, dass die Stabilität und Einwanderungsanfälligkeit von Pflanzengemeinschaften von der komplexen Interaktion zwischen Diversität, funktioneller Identität, struktureller Komplexität der Pflanzengemeinschaft und Ökosystemingenieuren, wie z.B. anözischen Regenwürmern, abhängen.

Das vierte Gewächshausexperiment untersuchte die direkten und indirekten Auswirkungen von endogäischen Regenwurmarten auf Pflanzensamen von Graslandarten. Die Ergebnisse zeigten, dass das Verschlucken und Verdauen von Samen und die Keimungsrate in Anwesenheit von Regenwurmexkreten von der Regenwurm- und der Pflanzenart abhängen. Das Verschlucken durch Regenwürmer hat vermutlich einen starken Einfluss auf das Überleben und die Keimungsrate von Pflanzensamen, da manche Samen während der Darmpassage verdaut wurden, während andere danach eine erhöhte Keimungsrate zeigten. Die Ergebnisse dieses Experimentes deuten darauf hin, dass der selektive Samenfraß und die artspezifische Verdauung von Pflanzensamen durch endogäische Regenwürmer die Zusammensetzung der Samenbank und damit die Beschaffenheit der Pflanzengemeinschaft fundamental beeinflussen können.

Eine zweite Feldstudie untersuchte die Effizienz der elektrischen Oktettmethode und der Senfmethode zur Extraktion von Regenwürmern unterschiedlicher ökologischer Gruppen bei trockenen Bodenverhältnissen. Es wurde gezeigt, dass die Senfmethode effizienter anözische Regenwürmer extrahiert, während die Oktettmethode ungeeignet ist, um unter trockenen Bedingungen die tatsächliche Struktur der Regenwurmgemeinschaft darzustellen. Die Effizienz beider Methoden kann nicht durch vorherige Wasserzugabe verbessert werden. Darüber hinaus betont diese Studie, dass sich Regenwürmer aus verschiedenen ökologischen Gruppen in ihrem Verhalten drastisch unterscheiden. Im Gegensatz zu endogäischen Arten bleiben anözische Regenwürmer auch während trockener Perioden aktiv.

Zusammenfassend hat die vorliegende Arbeit aufgezeigt, dass Regenwürmer nicht von der Diversität der Pflanzengemeinschaft abhängen. Sie werden eher von der Anwesenheit nährstoffreicher Ressourcen beeinflusst, welche vor allem von Leguminosen bereitgestellt werden. Es wurde gezeigt, dass Effekte von Regenwürmern auf das oberirdische System sehr facettenreich sind. Dabei konnten vier fundamentale Mechanismen identifiziert werden. Erstens fungieren (anözische) Regenwürmer als wichtige Zersetzer, indem sie die Nährstoffverfügbarkeit und damit die Konkurrenz zwischen Pflanzen steuern. Zweitens sind (anözische) Regenwürmer entscheidende Ökosystemingenieure, indem sie Strukturen (Auswürfe) schaffen, die als kleinräumige Störungen und Regenerationsnischen für Keimlinge fungieren. Dadurch können Regenwürmer die Diversität von Pflanzengemeinschaften erhöhen. Drittens wirken Regenwürmer als wichtige Samenvektoren, indem sie Samen vergraben, verschlucken und teilweise wieder ausscheiden. Das Vergraben von Samen stellt wahrscheinlich einen essentiellen Mechanismus dar, der das Überleben von bestimmten Pflanzenarten erhöht. Das ist in trockenen Perioden von besonderer Bedeutung, in denen die Samenreifung und –ausbreitung stattfindet und *L. terrestris* ebenfalls aktiv ist. Viertens beeinflussen Regenwürmer die Zusammensetzung der Pflanzengemeinschaft direkt, indem sie als selektive Granivore auftreten.

Die vorliegende Arbeit betont durch ihren kombinierten Ansatz der Untersuchung ober- und unterirdischer Systeme deren enge Verknüpfung und unterstreicht die Notwendigkeit der Berücksichtigung beider Systeme bei der Interpretation, Abschätzung und Modellierung von anthropogen bedingten weltweiten Umweltveränderungen.

CHAPTER

1



GENERAL INTRODUCTION

1.1 BIODIVERSITY AND ECOSYSTEM PROCESSES

One of the most striking features of the earth's biota is its extraordinary diversity, estimated to include about 10-100 million species. However, until today only about 1.8 million species are described (Table 1.1; Soulé 1991, Naeem et al. 1999, Loreau et al. 2002). One of the most dramatic aspects of contemporary **global change** is the rapid decline of species diversity in many ecosystems. On a global scale, even at the lowest estimated current extinction rate, about half of all species could be extinct within 100 years due to human activities which is similar to the magnitude of the five mass extinctions in the 3.5 billion year history of life on earth (Naeem et al. 1999). The major **drivers of the high current extinction rate** are habitat modifications and destruction, increased rate of invasions of accidentally introduced non-native species, over-exploitation, climate changes and nitrogen and CO₂ depositions (Naeem et al. 1999, Sala et al. 2000). Indeed, human population size is further on growing increasing the demand for resources and deteriorating the perspective for global biodiversity. Thereby, **human impacts** are affecting the whole globe (Fig. 1.1A) and causing species extinction rates up to one thousand times higher than that of fossil records (Fig. 1.1B, Pimm et al. 1995). However, modelling of future conditions predict the extinction rate to be even ten times higher than the current rate (Millenium Ecosystem Assessment 2005).

| Group | Number of described species |
|------------------------------------|-----------------------------|
| Bacteria and blue-green algae | 4,800 |
| Fungi | 47,000 |
| Algae | 26,900 |
| Bryophytes | 24,000 |
| Gymnosperms | 750 |
| Angiosperms | 250,000 |
| Pteridophytes | 10,000 |
| Protozoans | 30,800 |
| Sponges | 5,000 |
| Cnidaria | 9,000 |
| Roundworms and earthworms | 24,000 |
| Crustaceans | 38,000 |
| Insects | 1 100,000 |
| Other Arthropods and invertebrates | 132,500 |
| Molluscs | 50,000 |
| Starfish | 6,100 |
| Fishes (Teleosts) | 19,000 |
| Amphibians | 4,200 |
| Reptiles | 6,300 |
| Birds | 9,200 |
| Mammals | 4,170 |
| Total | 1,801,720 |

Table 1.1 | Number of described species that are currently existing per taxonomic group of organisms (modified after <http://www.globalchange.umich.edu/globalchange2/current/lectures/biodiversity/biodiversity.html>).

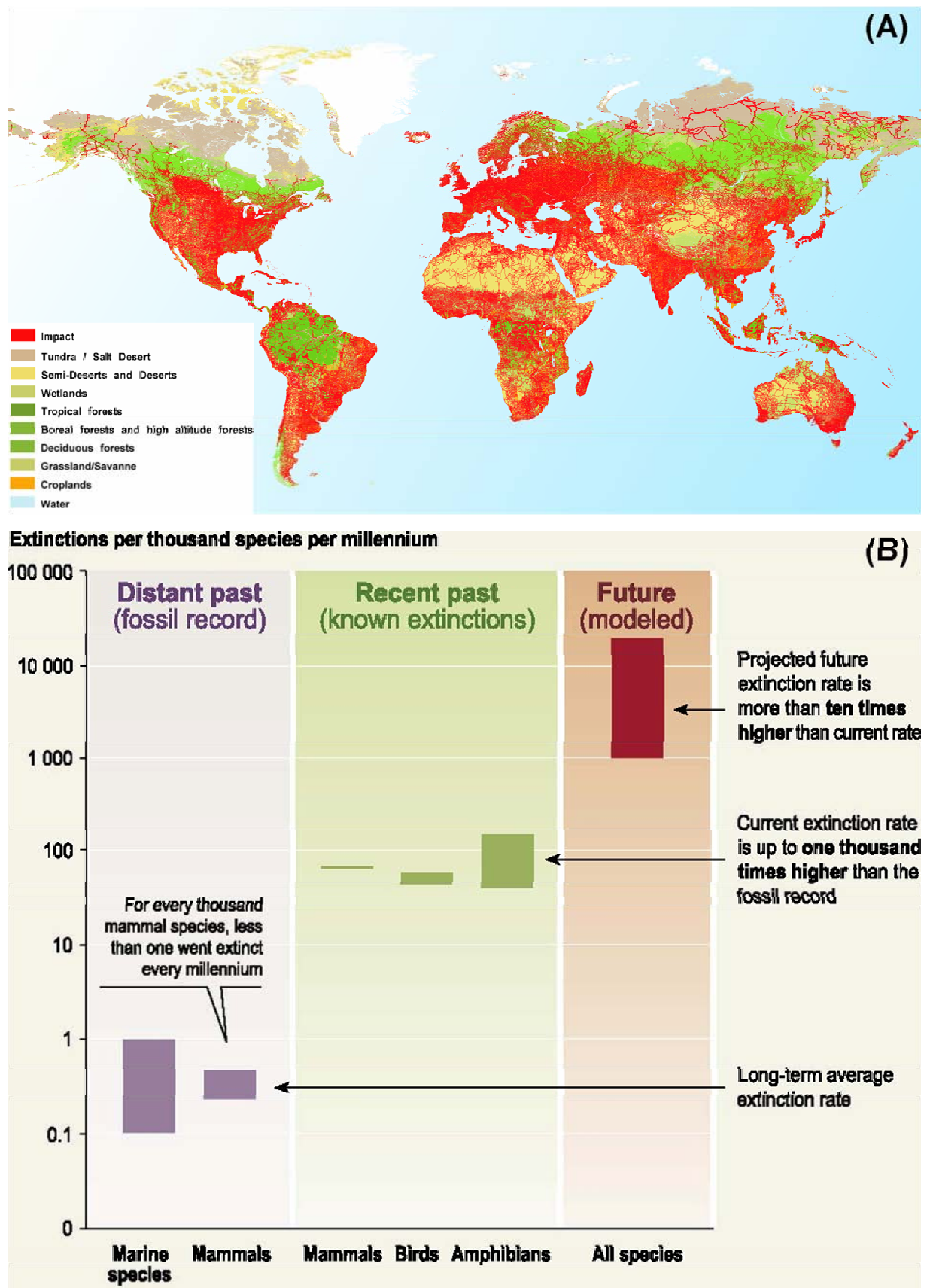
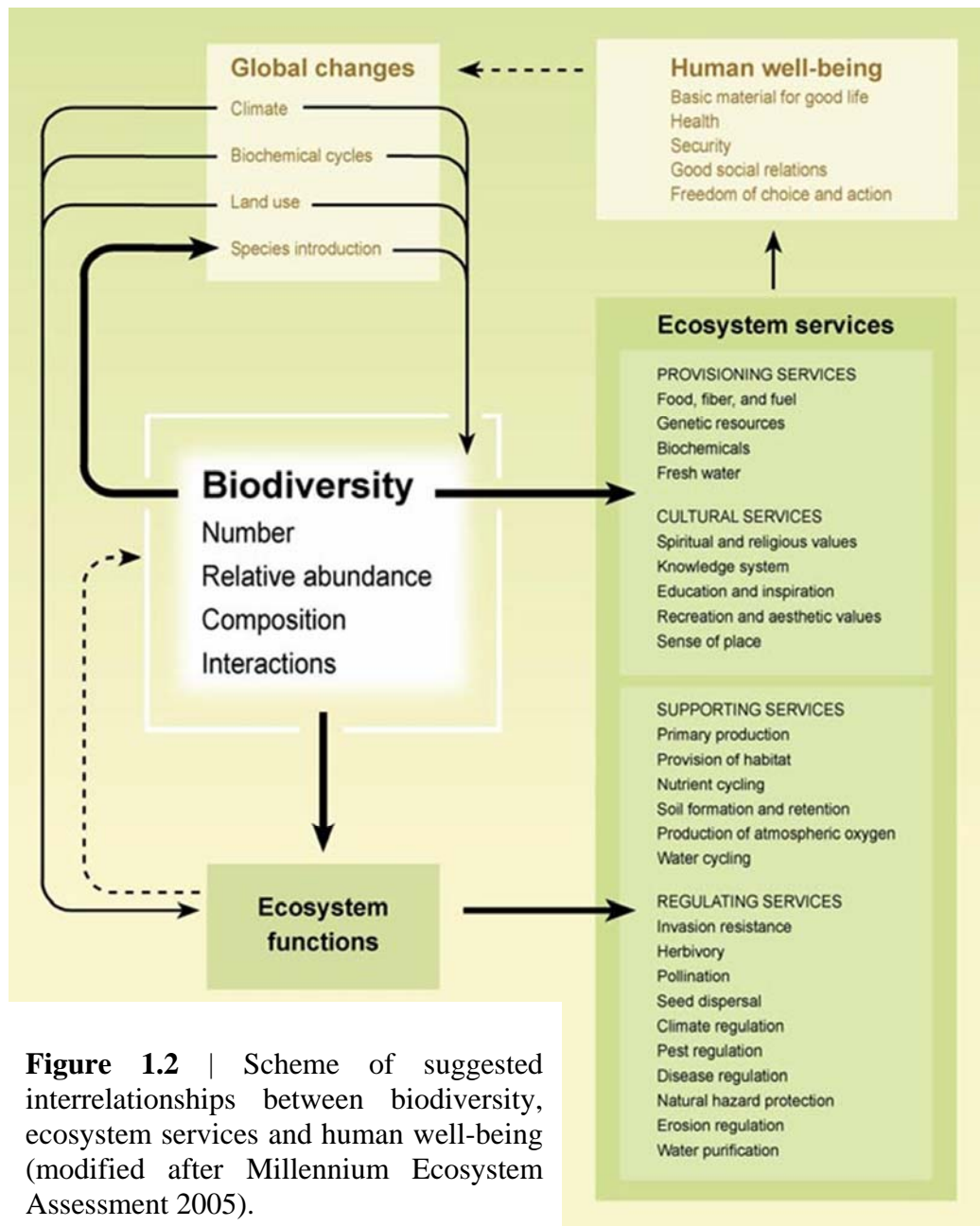


Figure 1.1 | (A) Map of human impacts on the ecosystems of the world (Globio 2007). (B) Species extinction rates of the distant past, recent past and the future based on habitat changes from 1970 to 2050 (Millenium Ecosystem Assessment 2005).

What are the **consequences of such declines in biodiversity** and how might they affect human well-being? Numerous ecosystem processes affect human well-being, such as primary production, nutrient cycling, soil formation and retention, production of atmospheric oxygen, water cycling, invasion resistance, herbivory, pollination, seed dispersal, climate regulation, pest regulation, and decomposition (Fig. 1.2). Therefore, the concern over the consequences of biodiversity loss for ecosystem processes and the uncertainty of 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). It is widely accepted that biodiversity plays an important role for ecosystem processes and stability, however, there are few studies that link biodiversity to changes in human well-being.



Until today more than 50 different hypotheses have been proposed to describe the consequences of biodiversity loss which can be grouped into three classes of biodiversity-functioning hypotheses (Fig. 1.3; Naeem et al. 2002):

1. *Species are primarily redundant*

Redundancy hypotheses imply that loss of species is compensated for by other species or the addition of such species adds nothing new to the system. Related to this class of hypotheses is the “**rivet hypothesis**” by Ehrlich and Ehrlich (1992) comparing the role of species with rivets holding together a machine: some rivets or species are redundant in their function, increasing the reliability of the system. However, after the number of rivets drops below a certain threshold, the system fails.

2. *Species are primarily singular*

Singular hypotheses imply that species contribute to ecosystem functioning in ways that are unique, thus their loss or addition causes detectable changes in functioning. **Keystone species** or **ecosystem engineers** are often cited as examples of singular species. Thereby, two mechanisms are distinguished. “**Sampling effects**” or “**selection effects**” are due to the increased probability of presence of highly competitive species at high diversity levels (Huston 1997) whereas “**complementarity**” of species is due to positive interactions between species or due to tradeoffs in species’ efficiency in using different resources, in colonization and competitive abilities or in their success under different environmental conditions (Loreau 2000, Loreau and Hector 2001).

3. *Species impacts are context-dependent and therefore idiosyncratic or unpredictable*

This class of hypotheses imply that the impact of loss or addition of a species depends on conditions, such as community composition, site fertility, and disturbance regime, under which the local extinction or addition occurs. One important related hypothesis suggests that biodiversity provides an insurance or a buffer (“**insurance hypothesis**”; Yachi and Loreau 1999) against environmental fluctuations, because different species respond differently to these fluctuations. This leads to a more predictable aggregate community or ecosystem properties. Consequently, species that are functionally redundant for an ecosystem process at a given time are no longer redundant through time.

However, it is crucial to note that the term “biodiversity” does not only refer to the number of species; it also includes genetic and functional diversity across population, community, habitat, ecosystem, landscape, and global scales. The present thesis focuses on biodiversity in terms of species richness and number of functional groups.

Although there has been an impressive development of research inquiry on the role of biodiversity in the functioning of ecosystems, there is still an ongoing debate on the reliability and interpretation of results (Mooney 2002). Taking weaknesses of previous biodiversity experiments into account (e.g. sampling effects) **a new generation of experiments are required** to investigate the mechanisms and the underlying biology of biodiversity-ecosystem process relationships (Spehn et al. 2005, CHAPTER 1.2). Further, the cooperation of scientists from different disciplines might enable a more holistic view of interrelationships between biodiversity and several ecosystem processes like element cycling and trophic interactions.

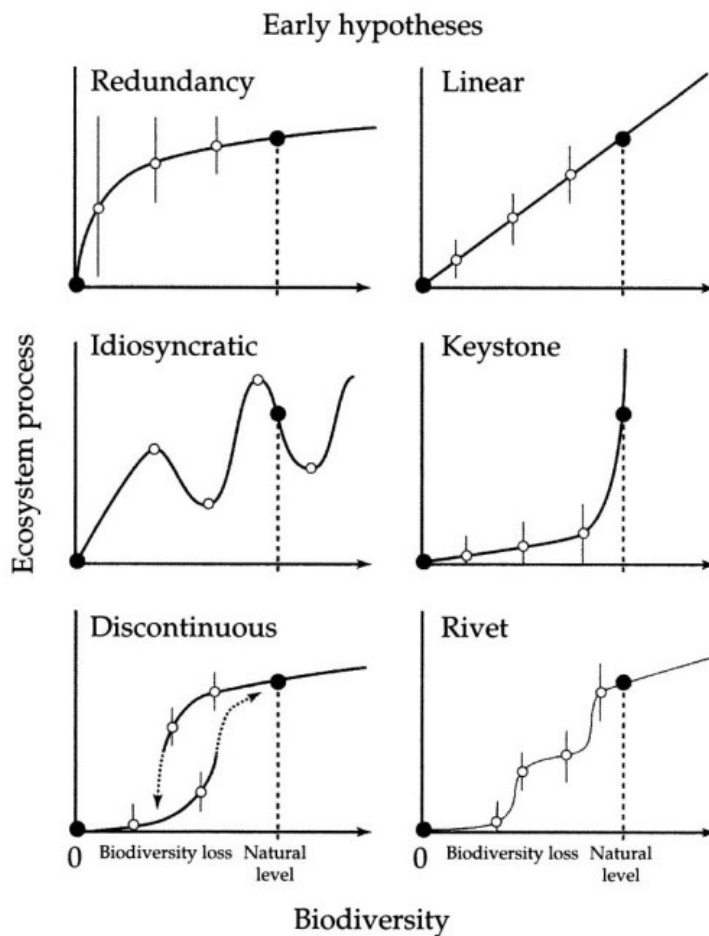


Figure 1.3 | Graphs of early hypotheses on the relationship between biodiversity and ecosystem functioning (Naeem et al. 2002).

1.2 BIODIVERSITY EXPERIMENTS

Until today **more than 150 biodiversity-function experiments** have been performed (Cardinale et al. 2007). The most famous experiments were performed in temperate grasslands or used species assemblages of temperate grasslands and are described in the following. However, effects of biodiversity on biomass production has been shown to be

consistent across studies of bacterial, fungal, plant, and animal assemblages inhabiting terrestrial, freshwater, and marine ecosystems (Cardinale et al. 2007).

Previous Biodiversity Experiments

The **Cedar Creek Biodiversity Experiment** was set up in 1994 by establishing a diversity gradient from 1-16 (-32) grassland plant species on plots with 9 x 9 m (Fig. 1.4A, B); Tilman 1997, 2001). Several ecosystem variables were measured (e.g. plant productivity, arthropod abundances), however, the design did not allow for distinguishing effects of plant species richness from plant functional group richness. Moreover, some findings have been criticized for using nutrient additions to create diversity gradients. Thereby, fertilization effects could not be separated from diversity effects.

The **Ecotron Biodiversity Experiment** manipulated biodiversity in a system of controlled-environment chambers (Fig. 1.4C) by establishing model communities with different numbers of species and complexity, whilst keeping trophic structure intact (Naeem et al. 1994). Primary producers were self-pollinating herbaceous annual plants, primary consumers were herbivorous insects and snails, secondary consumers (predators) were insect parasitoids and decomposers were Collembola and earthworms (Fig. 1.4D). Results indicated that plant species richness is positively correlated with several ecosystem processes, including productivity and CO₂ fluxes. However, the Ecotron Biodiversity Experiments have been criticized since species richness was not replicated and the selection of species was non-random, i.e. that species identity effects could not be separated from species richness effects (Hodgson et al. 1998).

The European **BIODEPTH** experiment (Biodiversity and Ecological Processes in Terrestrial Herbaceous Ecosystems: experimental manipulations of plant communities) was designed to investigate the effects of declining biodiversity on ecosystem processes and to elucidate the underlying population dynamic and ecophysiological processes (Hector et al. 1999). Therefore, the same core experiment in grassland communities was carried out at eight European sites (Switzerland, Germany, Great Britain, Ireland, Sweden, Portugal and Greece) forming two orthogonal transects across Europe (NW-SE and NE-SW), thus embracing a wide range of climates and soil types (Fig. 1.4E). The plot size was 2 x 2 m containing plant communities with 1-32 herbaceous plant species (Fig. 1.4F). Results of the BIODEPTH experiment were already manifold since eleven ecosystem variables were measured (e.g. above- and belowground productivity, decomposition, soil animals). However, findings of the BIODEPTH experiments have been criticized for containing sampling effects (Wardle 1999).

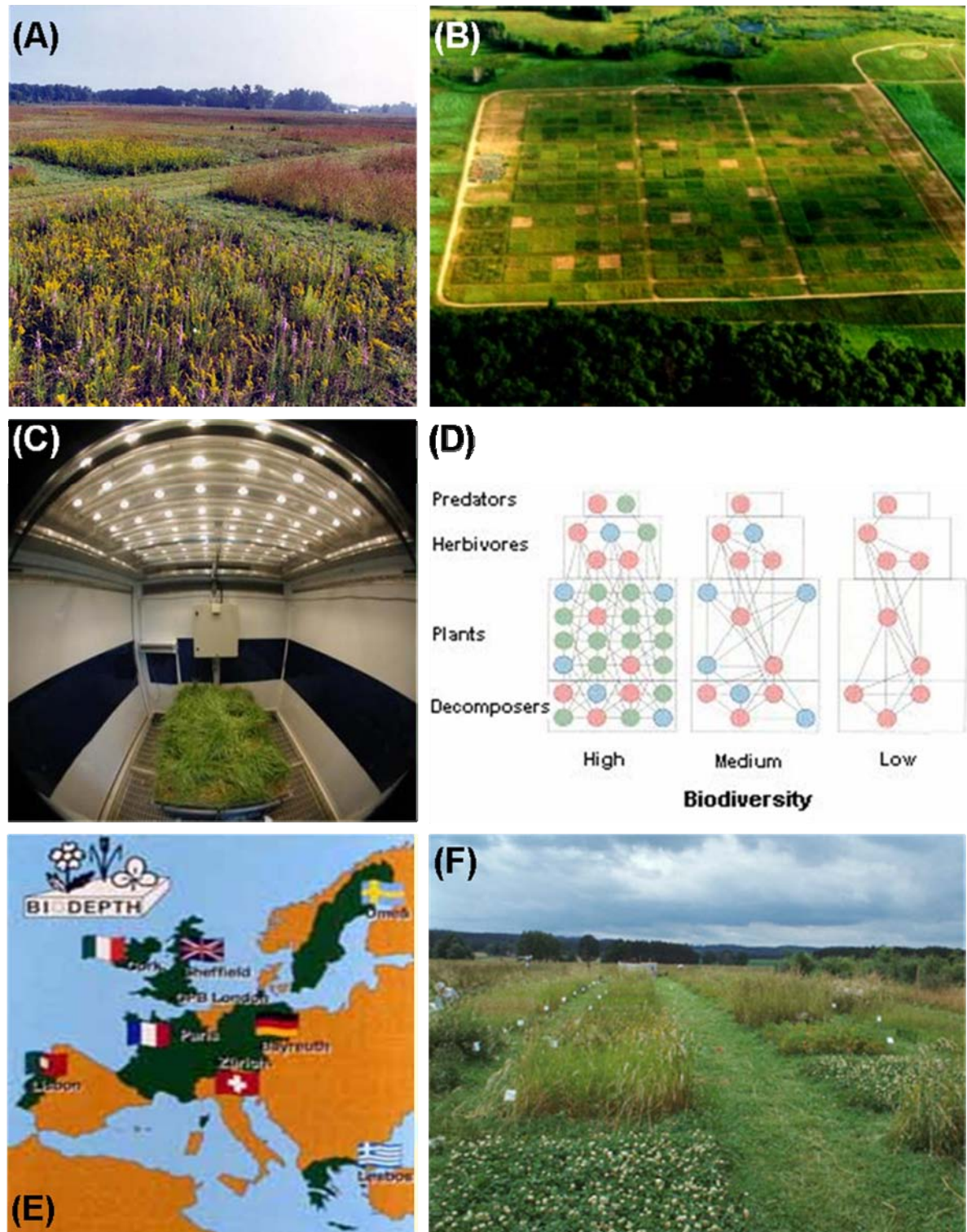


Figure 1.4 | (A) Photograph of single experimental plots containing different plant species combinations and (B) photograph of the experimental field site of the Cedar Creek Experiment (<http://www.cedarcreek.umn.edu/wicc/>). (C) Photograph of one experimental chamber and (D) scheme of the experimental design of the Ecotron Biodiversity experiment (<http://www3.imperial.ac.uk/portal/page/portallive/>). (E) Map of Europe; countries with experimental field sites of the BIODDEPTH experiment are indicated by green colour (http://www.naturlink.pt/.../not2032_lang1_part5847.jpg). (F) Experimental plots of the BIODDEPTH experiment in Bayreuth, Germany (<http://www.biotree.bgc-jena.mpg.de/background/index.html>).

The Jena Experiment – Exceeding current knowledge...

Although there is agreement that biodiversity affects ecosystems in general terms, it remains unclear to which extent it is simply the number of species, the number of functional attributes of the species in a community (number of functional groups), the particular mixture of species (community composition), or the presence of single species that are responsible for such effects (Schmid et al. 2002, Cardinale et al. 2006). Moreover, plant diversity is only one component of the diversity of an ecosystem and the effects of other groups of organisms are only beginning to be explored. Consequently, there is a lack of studies on **trophic interactions** although there is evidence that trophic interactions between plants, herbivores, predators, microorganisms and decomposers greatly affect plant performance and ecosystem processes (Wardle 2002, Scheu 2003, Cardinale et al. 2006). Thus, The Jena Experiment was established to offer a platform for cooperation between ecologists from different disciplines to investigate the whole **complex of compartments** and associated organisms that have largely been neglected in past discussions on the role of biodiversity for ecosystem processes.

Furthermore, in the design of The Jena Experiment the results and critique of previous experiments have been considered. An important example is the decoupling of species richness and functional group richness and having a balanced representation of plant functional groups in experimental mixtures to combine the study of both possible effects (Roscher et al. 2004). Further, the statistical separation of “sampling” from “complementarity effects” has been considered.

One of the essential differences between The Jena Experiment and previous biodiversity experiments is that experiments focussing on trophic interactions have been included in the **experimental design**. The large experimental plot size of 20 x 20 m has been selected to allow for the establishment of specific invertebrate communities in the respective plant community and, therefore, allow for the first time the detailed investigation of ecosystem processes despite plant biomass productivity. In the framework of The Jena Experiment “**Subproject 5 – Soil Fauna**” set out to manipulate different target soil animal groups (Collembola, Lumbricidae and Nematoda) to investigate their interacting impact with plant diversity on ecosystem processes.

The Jena Experiment – Experimental setup

The field site of The Jena Experiment is located in the floodplain of the Saale river at the northern edge of the city of Jena (Thuringia, Germany; 50°55'N, 11°35'E, 130 m NN; Fig. 1.5A). Mean annual air temperature is 9.3°C (measured at a meteorological

station 3 km south of the field site; Roscher et al. 2004) and mean annual precipitation is about 587 mm (Kluge and Müller-Westermeier 2000). The soil of the experimental site is an Eutric Fluvisol (FAO-Unesco 1997) developed from up to 2 m-thick loamy fluvial sediments (Roscher et al. 2004). Before the establishment of the experiment the site was used as an arable field for the last 40 years and highly fertilized over the last decades for the growing of vegetables and wheat (Roscher et al. 2004). 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.

After the last harvest in autumn 2000 the field was ploughed and kept fallow throughout 2001. In order to reduce the weed pressure the field was harrowed three times and treated with Glyphosate (N-(Phosphonomethyl)-glycine, Roundup) in July 2001 (Roscher et al. 2004). In spring 2002, the experimental area was harrowed twice before the plots were established. Seeds were obtained from commercial suppliers and the desired seedling density was 1000 seedlings per m² divided equally among the species of each mixture (Roscher et al. 2004). The species mixtures were sown from 11–16 May 2002 (Fig. 1.5B).

Table 1.2 | Plant species pool of The Jena Experiment. Four plant functional groups (grasses, small herbs, tall herbs, and legumes) had been defined a priori according to a cluster analysis of 17 functional traits (Roscher et al. 2004).

| Grasses | Small herbs | Tall herbs | Legumes |
|------------------------------|-----------------------------|--------------------------------|------------------------------|
| <i>Alopecurus pratensis</i> | <i>Ajuga reptans</i> | <i>Achillea millefolium</i> | <i>Lathyrus pratensis</i> |
| <i>Anthoxanthum odoratum</i> | <i>Bellis perennis</i> | <i>Anthriscus sylvestris</i> | <i>Lotus corniculatus</i> |
| <i>Arrhenatherum elatius</i> | <i>Glechoma hederacea</i> | <i>Campanula patula</i> | <i>Medicago lupulina</i> |
| <i>Avenula pubescens</i> | <i>Leontodon autumnalis</i> | <i>Cardamine pratensis</i> | <i>Medicago x varia</i> |
| <i>Bromus erectus</i> | <i>Leontodon hispidus</i> | <i>Carum carvi</i> | <i>Onobrychis viciifolia</i> |
| <i>Bromus hordeaceus</i> | <i>Plantago lanceolata</i> | <i>Centaurea jacea</i> | <i>Trifolium campestre</i> |
| <i>Cynosurus cristatus</i> | <i>Plantago media</i> | <i>Cirsium oleraceum</i> | <i>Trifolium dubium</i> |
| <i>Dactylis glomerata</i> | <i>Primula veris</i> | <i>Crepis biennis</i> | <i>Trifolium fragiferum</i> |
| <i>Festuca pratensis</i> | <i>Prunella vulgaris</i> | <i>Daucus carota</i> | <i>Trifolium hybridum</i> |
| <i>Festuca rubra</i> | <i>Ranunculus repens</i> | <i>Galium album</i> | <i>Trifolium pratense</i> |
| <i>Holcus lanatus</i> | <i>Taraxacum officinale</i> | <i>Geranium pratense</i> | <i>Trifolium repens</i> |
| <i>Luzula campestris</i> | <i>Veronica chamaedrys</i> | <i>Heracleum sphondylium</i> | <i>Vicia cracca</i> |
| <i>Phleum pratense</i> | | <i>Knautia arvensis</i> | |
| <i>Poa pratensis</i> | | <i>Leucanthemum vulgare</i> | |
| <i>Poa trivialis</i> | | <i>Pastinaca sativa</i> | |
| <i>Trisetum flavescens</i> | | <i>Pimpinella major</i> | |
| | | <i>Ranunculus acris</i> | |
| | | <i>Rumex acetosa</i> | |
| | | <i>Sanguisorba officinalis</i> | |
| | | <i>Tragopogon pratensis</i> | |

The target plant community of the experiment is semi-natural species-rich mesophilic grassland (Molinio-Arrhenatheretea meadows, **Arrhenatherion community**, Ellenberg 1996). A pool of 60 native grassland 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 84 large plots of 20 x 20 m (Fig. 1.5C, D; 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 (Table 1.2; 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 (Fig. 1.5B). Further information on the design and setup of The Jena Experiment is given in Roscher et al. (2004).

Experimental plots were divided into subplots to allow for the establishment of nested **project-specific treatments** and destructive measurements (Fig. 1.5E, F). Experiments shown in the present thesis were performed on large plots containing 1, 4, and 16 plant species (earthworm and control subplots; CHAPTER 5) or at the edge of the experimental field site (CHAPTER 7).

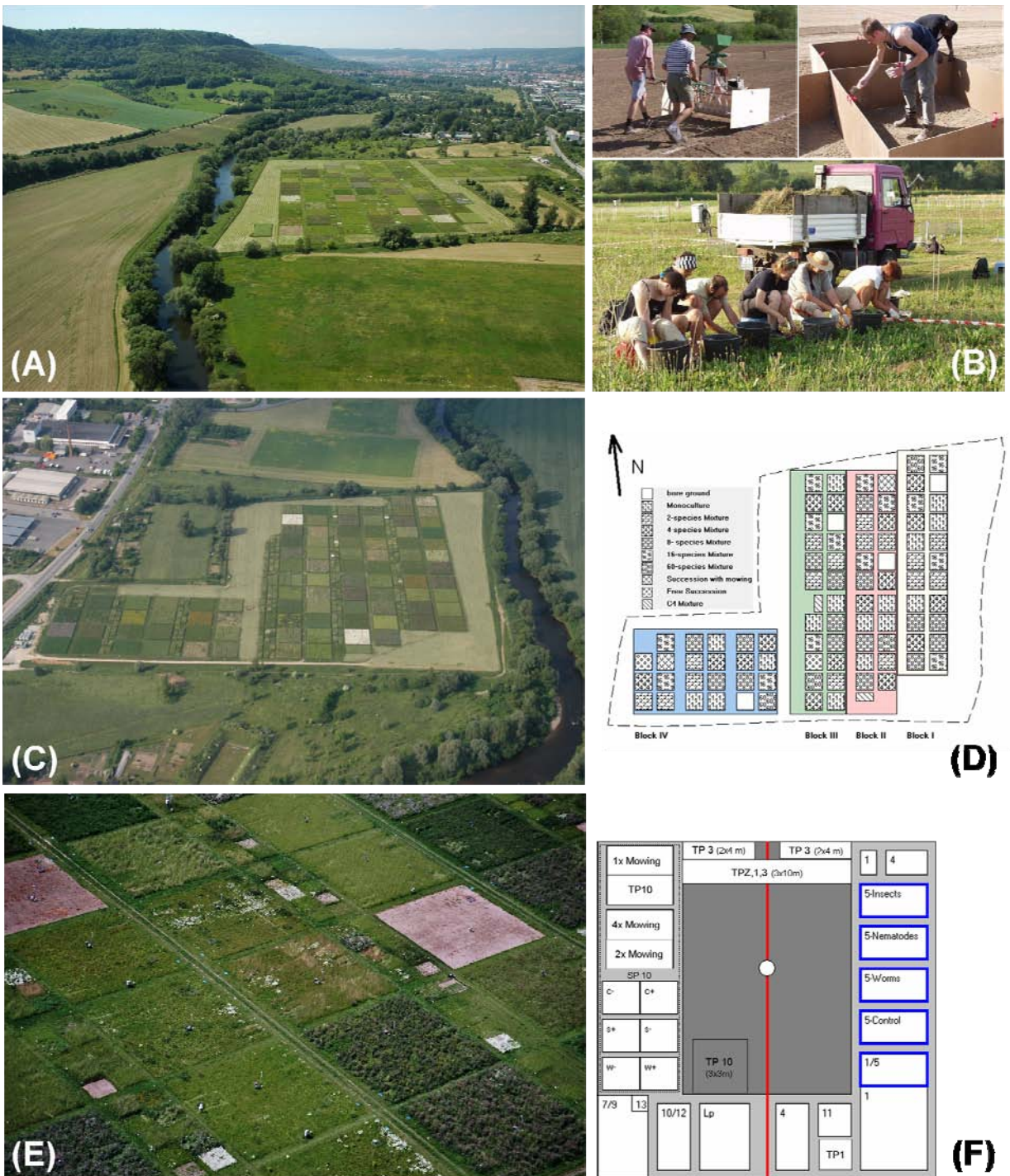
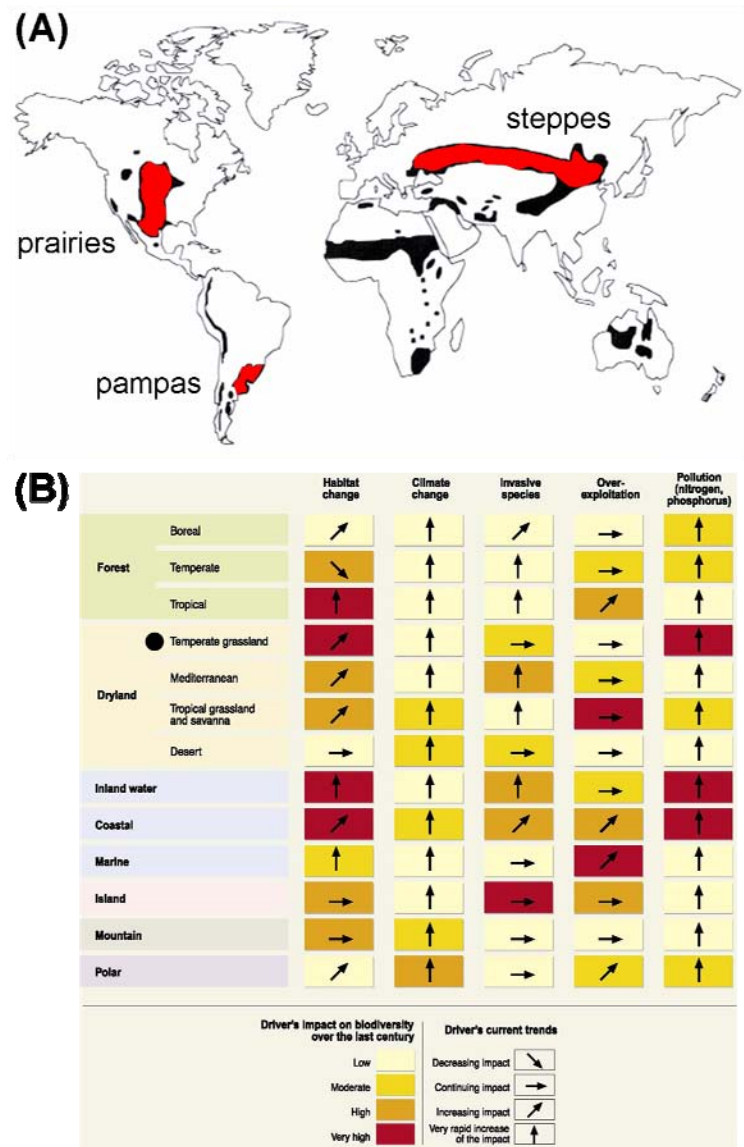


Figure 1.5 | (A) Photograph of the location of The Jena Experiment field site in the floodplain of the Saale river at the northern edge of Jena (Thuringia, Germany; Photo by A. Weigelt). (B) Photographs of the establishment (sowing of target species) and maintenance (weeding of non-target plant species) of experimental plots (Photos by Subproject Z – Coordination and A. Weigelt). (C) Photograph of the experimental field site of The Jena Experiment (Photo by J. Baade). (D) Design of The Jena Experiment indicating plant species diversity levels of the large plots and the four blocks. (E) Photograph of some single large and small plots containing different plant species combinations (Photo by A. Weigelt). (F) Layout of a large plot with overview over subplots of different subprojects (Subplots of Subproject 5 – Soil Fauna are indicated by blue frames).

Temperate Grasslands

One of the most important biomes for mankind is temperate grassland. It is present at all continents except the Antarctic (Fig. 1.6A). Naturally, grasslands are present in rather dry geographical regions of temperate zones and characterized by cold winters (Fig. 1.6A; grasslands are given in black). Temperate grasslands are the prairies in central North America, the pampas in Argentina and Uruguay, and the steppes in Asia (Fig. 1.6A; temperate grasslands given in red). Important ecological factors of these areas are dry periods, fires and grazing by large mammals. All these factors prevent the establishment of shrubs and trees (Campbell 2000). However, anthropogenic deforestation in the course of the establishment of agricultural monocultures and pastures artificially created the grasslands of central Europe. Since grasslands have traditionally been used and transformed for agriculture, human impacts had and have fundamental effects on grassland biodiversity (MA 2005; Fig. 1.6B); under the MA scenarios, a further 10-20% of grassland and forestland is projected to be converted by 2050 (primarily to agriculture). Further, impacts like invasive species and pollution are threatening grassland biodiversity with unknown consequences for human well-being.



1.3 ABOVE- AND BELOWGROUND LINKAGES

Ecologists are becoming increasingly aware of the role of aboveground–belowground relationships in controlling ecosystem processes and properties (Wardle et al. 2004, Bardgett et al. 2005). Traditionally, above- and belowground systems were regarded as separate entities, however, this view is surprising since **plants** live in both spheres simultaneously (Schröter et al. 2004). Thereby, a plant may function as an integrator of these subsystems, because above- and belowground consumers are largely spatially separated with the plant as a connector (Wardle et al. 2004). Since studies considering both subsystems are scarce, aboveground consequences of belowground interactions and vice versa are widely unknown. However, scenarios and modelling of effects of global change on ecosystem processes should adequately consider above- and belowground processes and the interactions between them (Schröter et al. 2004).

Additionally, **generalist predators** like carabids, staphilinids and spiders were shown to be important connectors of the above- and belowground subsystems since they occur and feed in both subsystems (von Berg et al. 2008). Further, several invertebrates inhabit both subsystems in different life-stages (e.g. many Coleoptera and Diptera species) or live and feed in both as adults (e.g. many ant and termite species). Moreover, earthworms, particularly anecic species, are increasingly recognized as ecosystem engineers by affecting the chemical and physical characteristics of the soil (Lavelle et al. 1998). Some recent studies indicated that earthworms, thereby, drive plant competition (Kreuzer et al. 2004, Wurst et al. 2005) and community composition (Grant 1983, Milcu et al. 2006a, Zaller and Saxler 2007).

In summary, plants and generalist predators are considered most important links and two main pathways are distinguished connecting the above- and belowground system (Scheu 2001): (A) Soil animal-mediated effects on plant performance affecting herbivores and the aboveground community which may be considered as **bottom-up control** of the aboveground community by belowground animals; (B) Generalist predators benefit from belowground energy supply, i.e. strengthening **top-down forces** aboveground when generalist predators switch their attacks from decomposers to herbivores.

Plants as drivers of the soil animal community

The soil animal community relies on carbon sources like plant residues and root exudates entering the soil system (Albers et al., 2006; Ostle et al., 2007). Consequently, the quality and quantity of plant residues and exudates should drive the soil animal community. Since plant productivity is known to increase with increasing plant diversity, above- and

belowground diversity is supposed to be linked (Tilman et al. 2001, Coleman et al. 2004, Spehn et al. 2005). Other potential mechanisms by which plant diversity might affect decomposer performance are the release of more diverse carbon compounds and increased litter diversity in more diverse plant communities which might increase the diversity of the decomposer community (Hooper et al. 2000). Hooper and colleagues (2000) defined a step-by-step process for the main mechanism by which heterogeneity of carbon substrates will positively influence belowground diversity: (A) diversity of primary producers leads to diversity of carbon inputs belowground, (B) carbon resource heterogeneity leads to diversity of herbivores and detritivores, and (C) diversity of detritivores and belowground herbivores leads to diversity of organisms at higher trophic levels in belowground food webs. However, in contrast to the aboveground herbivore system, the decomposer community appears to be less affected by plant community composition (Salamon et al. 2004, Wardle 2004, Milcu et al. 2008). Since dead organic matter is the basal resource of the decomposer food web and, therefore, the soil animal community, co-evolutionary processes between plants and decomposers are unlikely to have shaped plant-decomposer-relationships. Rather the concentration of nitrogen in litter materials appears to be a key effect of plants on the soil animal community (Spehn et al. 2000, Milcu et al. 2008). Therefore, legumes, as a keystone plant functional group, were shown to be of particular importance for decomposer systems due to the high quality of litter entering the soil system (Spehn et al. 2000, Milcu et al. 2008). However, this topic deserves further attention since only few studies have investigated the relationship between plant diversity and the composition of the soil animal community which likely plays a fundamental role in essential ecosystem processes like decomposition and nutrient cycling.

Soil fauna and decomposer effects on plants

The majority of animals in terrestrial habitats are invertebrate members of the decomposer community, however, the soil system is still one of the most poorly investigated habitats of the planet (Wolters 2001, Coleman et al. 2004). Though, soil decomposer animals and microorganisms are essential for nutrient mineralization (Bradford et al. 2002). Moreover, it is well documented that the enhanced nutrient turnover in soil in presence of decomposer animals leads to a higher plant nutrient acquisition and therefore stimulates plant growth (Scheu et al. 1999, Kreuzer et al. 2004, Partsch et al. 2006). Thereby, interactions between soil decomposer animals and microorganisms not only affect decomposition processes and nutrient cycling but also modify the growth and competition between plant species and,

thereby, the composition of plant communities (Kreuzer et al. 2004, Wurst et al. 2005). Decomposer effects were shown to propagate even into the aboveground food web (Wurst et al. 2003, Poveda et al. 2005, Schütz et al. 2008). Moreover, decomposers were shown to alter the flowering of plants and presumably the visitation of flowers by pollinators (Poveda et al. 2005).

In addition to these indirect effects, macro-decomposers like earthworms affect plant communities directly via burial, ingestion and digestion of plant seeds (Grant 1983, Milcu et al. 2006a, Zaller and Saxler 2007). However, this has not been proven under natural conditions.

1.4 EARTHWORMS

Earthworms are a major component of many terrestrial ecosystems (Lee 1985, 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 (Lavelle et al. 1998). The importance of earthworms for the whole ecosystem was already recognized by Aristotle (about 330 BC) denoting earthworms the “**intestines of the soil**”. The scientific literature on earthworms began with Linnaeus’ taxonomic description of *Lumbricus terrestris* L. more than 200 years ago. Later, Darwin (1881) outlined the beneficial effects of earthworms in his book “**The formation of vegetable mould through the actions of worms, with observations of their habitats**” by stating “It may be doubted whether there are many other animals which have played so important a part in the history of the world, as have these lowly organized creatures.” Since then, a large number of studies investigated the role of earthworms for soil formation, decomposition, nutrient cycling, distribution of soil microorganisms and animals, and plant growth (Lee 1985, Edwards and Bohlen 1996, Scheu 2003, Brown et al. 2004). Through burrowing, casting and mixing of litter and soil (bioturbation) earthworms influence aggregate stability, soil structure, infiltration of water, aeration of deeper soil layers, nutrient cycling and mineralization, microbial biomass, and other soil invertebrates (Lee 1985, Edwards and Bohlen 1996, McLean and Parkinson 2000, Eisenhauer et al. 2007). These changes have important consequences for plant communities and the herbivore system and possibly for the whole aboveground food web (Scheu 2001, Scheu 2003, Wurst et al. 2003, Poveda et al. 2005).

The term “earthworms” comprises a diverse group of the taxon Oligochaeta (Annelida) of more than 3500 species (Coleman et al. 2004). The majority of European

earthworms belong to the taxon Lumbricidae pooling approximately 600 species. In Germany about 38 earthworm species are found (Schaefer 2006).

Earthworm ecology

Earthworms are grouped into three functional categories based on their morphology, their behavior and feeding ecology, and their microhabitats (Fig. 1.7; Bouché 1977).

Epigeic species reside mainly in the upper organic layers and cause limited mixing of mineral and organic layers. **Endogeic** species live in horizontal burrows in the upper mineral soil layers mainly consuming mineral soil materials. **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).

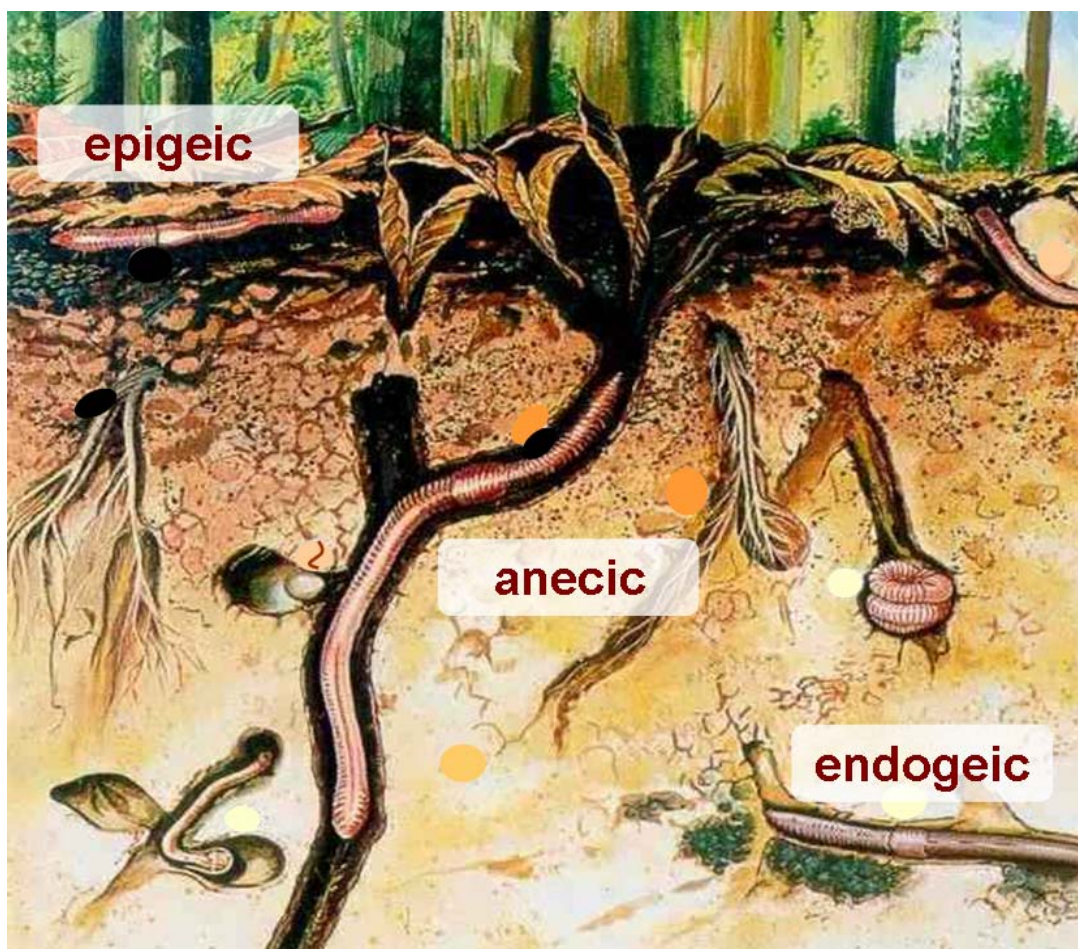


Figure 1.7 | Pictorial representation of the characteristics of the three ecological groups of earthworms as proposed by Bouché (1977; modified after http://www.umweltbundesamt.de/..hinweise_9.jpg).

Earthworm reproduction

Earthworms are **hermaphrodites** with both male and female reproductive organs and they usually cross-fertilize (Edwards and Bohlen 1996). When two individuals copulate they exchange sperm and store it in spermathecae (Fig. 1.8). The sperm is later released, along with eggs, into cocoons secreted by the glandular clitellum where they get fertilized (Coleman et al. 2004). **Cocoons** are deposited into the soil, the embryo worms develop and young worms emerge when temperature and moisture conditions are suitable. Earthworms, particularly larger species, may reach an age of up to 10 to 12 years, but in nature earthworms generally survive only about 2 to 4 years (Lee, 1985). In addition, some earthworm species are parthenogenetic, such as *Octolasion tyrtaeum* Sav., reproducing without mating (Sims and Gerard, 1999). Parthenogenesis provides an effective means by which certain species can establish populations in new habitats. Interestingly, *O. tyrtaeum* is known to be a successful peregrine species, e.g. in North America (Eisenhauer et al. 2007).

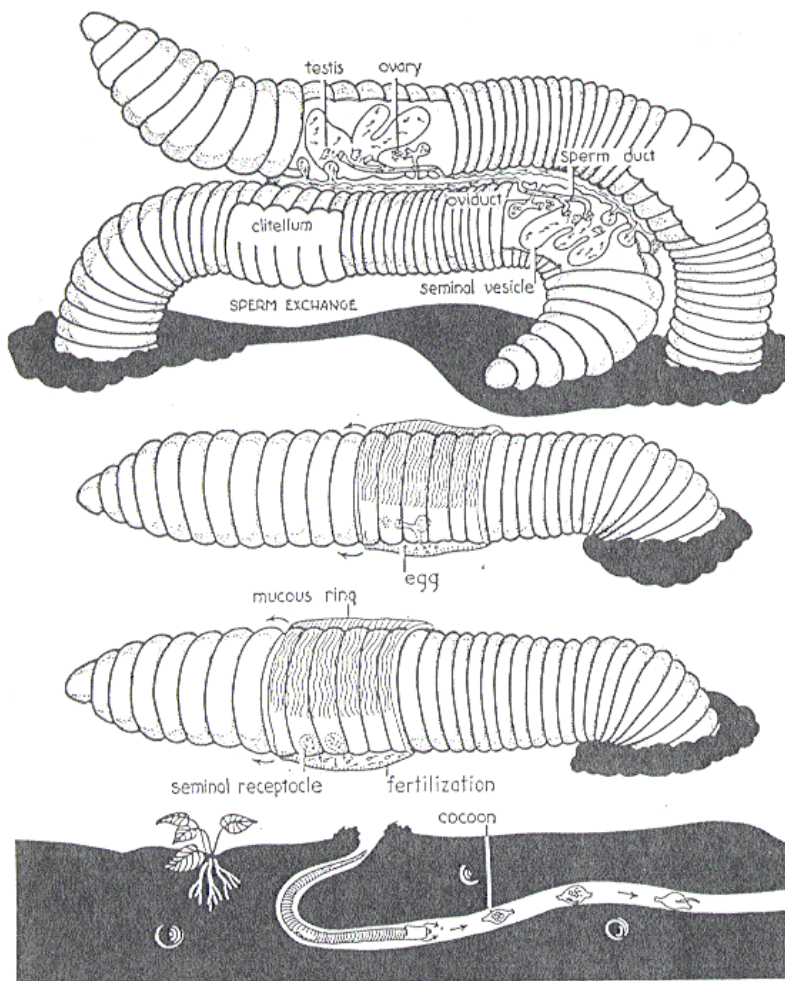


Figure 1.8 | (A) Scheme of earthworm copulation, egg and cocoon formation and cocoon deposition in soil (<http://www.sciencefun4all.net/.../Worms/reproduction.gif>).

Earthworm distribution and abundance

Earthworms occur worldwide in habitats where soil water content and temperature are favourable. Since the suitable **pH** for the most lumbricid species ranges from slightly acid to slightly alkaline, earthworms are rare in soils with pH lower than 4 (Satchell 1955, Lee 1985). However, epigeic species are usually more tolerable to low pH conditions than species living in the soil (Edwards and Bohlen 1996). Moreover, some epigeic species of cold regions where the soil is frozen in winter were shown to be freeze-tolerant, i.e. they endure ice formation in extracellular body fluids and accumulate high concentrations of glucose as a response to freezing (Holmstrup 2003). Generally, the **temperature** tolerance of earthworms is narrow, ranging from 0-30°C with the optimum for temperate species typically being in the range of 10-20°C. However, some tropical and subtropical species are adapted to temperatures above 30°C (Edwards and Bohlen 1996). Earthworm respiration depends upon diffusion of gases through the body wall, therefore, this has to be kept **moist**. However, earthworms are able to tolerate desiccation to some extent, to enter a temporary dormant state (diapause) and to produce resistant cocoons during unfavourable periods (Edwards and Bohlen 1996). Earthworm distribution is further limited by **soil texture**, i.e. they are absent in soils with coarse texture presumably due to the physical abrasion of their body wall and the high susceptibility of drought under these conditions.

As already stated above, earthworms usually dominate the biomass of soil invertebrates with up to 2-3 t per hectare (Blakemore 2002). However, earthworm density and biomass vary with various habitats (Table 1.3). In temperate grasslands, where the present thesis was conducted, earthworm densities range from 50 to 200 ind./m² and 10 to 50 g fresh weight/m² (Edwards and Bohlen 1996) and annual turnover rates of soil through earthworms castings were reported to be about 40-70 t/ha (Bouché 1983).

Table 1.3 | Typical ranges of earthworm density and biomass in various habitats (summarized from Lee (1985) and Edwards and Bohlen (1996) in Coleman et al. (2004)).

| Habitat | Earthworms per m ² | Earthworm biomass (g fw per m ²) |
|-----------------------------|-------------------------------|--|
| Temperate hardwood forest | 100-200 | 20-100 |
| Temperate coniferous forest | 10-100 | 30-35 |
| Temperate pastures | 300-1000 | 50-100 |
| Temperate grassland | 50-200 | 10-50 |
| Sclerophyll forest | <10-50 | <10-30 |
| Taiga | <10-25 | ≤10 |
| Tropical rainforest | 50-200 | <10-50 |
| Arable soil | <10-200 | <10-50 |

fw, fresh weight

Earthworm nutrition and co-occurrence

While there is a considerable volume of published information on the feeding ecology of earthworms (reviewed in Curry and Schmidt 2007), there are still many aspects which are not fully understood. One essential open question is the rather **enigmatic co-occurrence** of superficially very similar species. The primary food source for earthworms is dead plant material, particularly plant leaf litter. These **saprophagous** animals feed preferentially on dead and decaying plant residues that have a broad range in their physical and chemical composition (Curry and Schmidt 2007). Analysis of earthworm gut contents revealed the presence of a wide range of organic materials. Pearce (1978) found fragments of grass and other plant leaves, roots, algal cells, earthworm setae, plant seeds, fungi, protozoa, fragments of arthropod cuticle, and amorphous humus in a range of species from a permanent pasture in Wales. Although different earthworm species are supposed to overlap considerably in their diet, Pearce (1978) concluded that the six species co-occurring at the investigated location fell into five separate **dietary groups** distinguishable on the basis of their ecological grouping, particle size and quantities of organic and mineral materials ingested. Typically, within a particular soil, less than six earthworm species are found and the species often effectively partition the soil volume according to their functional categories. Milcu et al. (2008) supported these observations for the field site of The Jena Experiment by showing that five earthworm species co-occur there (*Allolobophora chlorotica* Sav. (Fig. 1.9A), *Aporrectodea caliginosa* Sav. (Fig. 1.9C), *Aporrectodea rosea* Sav. (Fig. 1.9B), *L. terrestris* (Fig. 1.9E), and *O. tyrtaeum* (Fig. 1.9D).

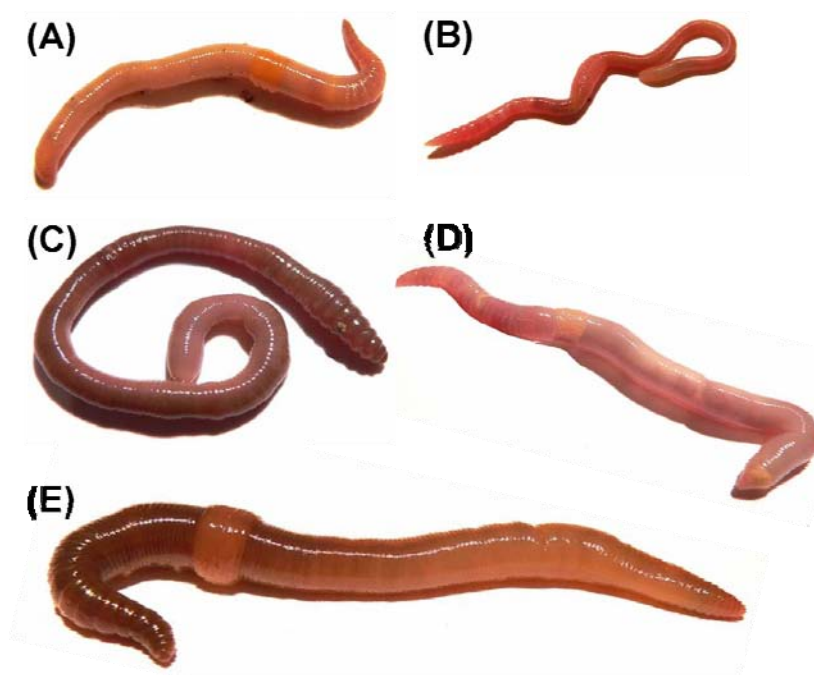


Figure 1.9 | Photographs of the earthworm species occurring at the field site of The Jena Experiment. (A) *Allolobophora chlorotica*, (B) *Aporrectodea rosea*, (C) *Aporrectodea caliginosa*, (D) *Octolasion tyrtaeum*, and (E) *Lumbricus terrestris*. Photos by H. Schuy.

However, recent extractions revealed that two further earthworm species are currently invading the field site of The Jena Experiment (*Aporrectodea longa* Ude (anecic) and *Lumbricus castaneus* Sav. (epigeic)).

Earthworms and plants

As already described above, a large number of studies focussed on earthworm effects on plant performance. Earthworms generally are assumed to be beneficial soil animals which is mainly based on the belief that they **promote plant growth** (Lee 1985, Edwards and Bohlen 1996). However, most studies concentrated on the effect of earthworms on single plant species and on arable systems while only few have investigated effects on plant communities (Scheu 2003, Brown et al. 2004). Scheu (2003) identified seven main mechanisms by which earthworms affect plant performance and thereby herbivores (Fig. 1.10). Though, he distinguished direct (root feeding and interactions with seeds) and indirect interactions (changing root structure, mineralization of nutrients, hormone-like effects, and dispersal of beneficial and detrimental microorganisms).

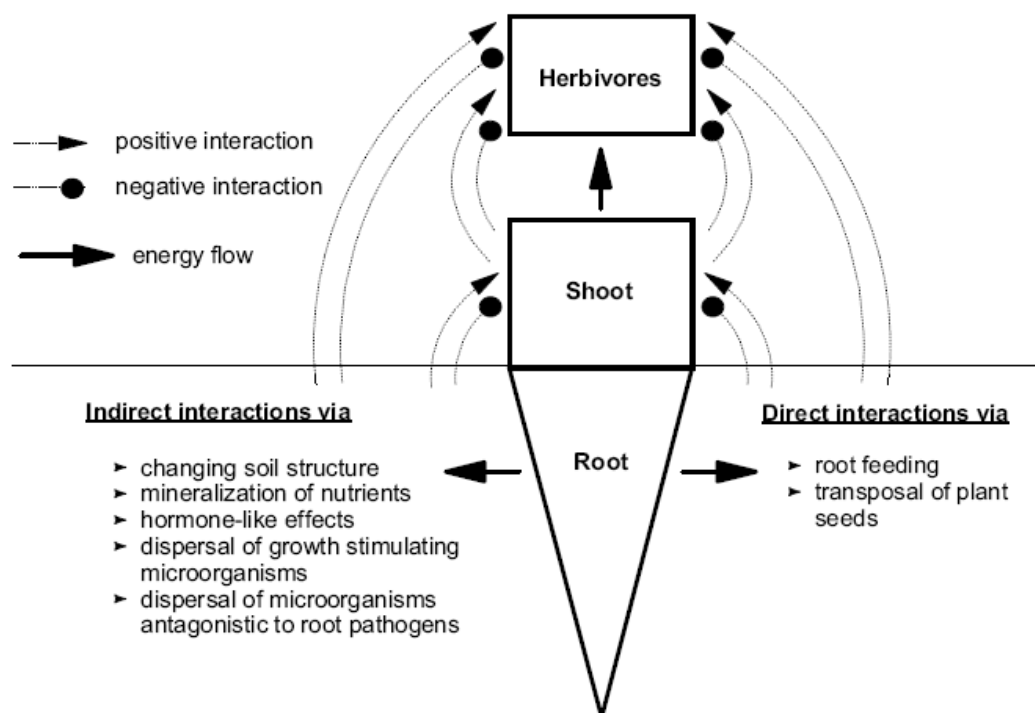


Figure 1.10 | Mechanisms by which earthworms affect plant growth and the herbivore community above the ground (Scheu 2003).

1.5 OBJECTIVES

The design of The Jena Experiment offers the unique opportunity to investigate the relationship between biodiversity and ecosystem processes while simultaneously manipulating trophic interactions. Thereby, it is possible for the first time to explore the consequences of anthropogenic induced diversity loss while considering interrelationships between plant communities and important animal ecosystem engineers. In the framework of The Jena Experiment, the precedent dissertation of Alexandru Milcu (2005) primarily investigated the effects of plant diversity on the performance of the decomposer community (microorganisms and earthworms) and litter decomposition which is an essential ecosystem process. Building on the outcomes of his experiments, **the present thesis aimed to explore the main mechanisms by which earthworms affect plant communities varying in plant species and functional group diversity**. Besides the understanding of **indirect** earthworm effects on grassland plant communities, I concentrated on **direct interactions** between earthworms and plants via plant seeds. In addition to the field experiments, five greenhouse experiments were performed to extract the main interacting mechanisms between earthworms and grassland plant communities. It was not possible to integrate all experiments performed in the present thesis since measurements and samplings will be continued in the next two years. Beside a further greenhouse experiment investigating the role of earthworm-mycorrhiza interactions in different grassland plant communities, I performed field surveys on the effects of earthworms on grassland plant communities varying in species richness and number of functional groups and on single model plant species (phytometers). In addition, earthworm samplings were performed to investigate the effects of the plant community on earthworm performance and earthworm nutrition via ^{15}N analysis.

In **CHAPTER 2**, the role of earthworms for the competition between grasses and legumes for soil nutrients were investigated. Studies of earthworm effects on plant communities are scarce but indicated that earthworms likely affect plant competition (Kreuzer et al. 2004, Wurst et al. 2005). However, the mechanisms behind the modification of plant competition are not fully understood. Thus, the objectives of this greenhouse experiment were to quantify the effects of earthworms on grass-legume competition in model grassland systems. In order to improve the understanding of ecological mechanisms structuring grass-legume associations the following questions were investigated:

(A) What are the driving factors for the competition between grasses and legumes – are grass and legume species competing for resources and is N availability driving this competition?

- (B) Is the competition between grasses and legumes modified by earthworms and, if yes, which mechanisms are responsible for these modifications?
- (C) Is increased soil N availability to grasses propagating into the herbivore system – connecting the above- and below-ground system?
- (D) What are the mechanisms behind the phenomenon that grasses benefit from legume presence – do grasses indeed benefit from legume fixed N?

In **CHAPTER 3**, the effects of three apparently anecic earthworm species on plant seed burial (wheat seeds), seedling establishment, plant growth, and litter incorporation were investigated. Particularly anecic earthworm species are supposed to function as ecosystem engineers in temperate grasslands. However, it is unclear if and how anecic earthworms differ in essential ecosystem processes like plant seed burial and litter incorporation. Therefore, this greenhouse experiment intended to assess the behavior of two common earthworm species grouped as anecic and occurring at the field site of The Jena Experiment (*A. longa* and *L. terrestris*) and an additional earthworm species with unknown autecology (*Lumbricus rubellus friendoides* Bouché).

In **CHAPTER 4**, the impacts of *L. terrestris*, 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 were investigated. 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 (Milcu et al. 2006a) probably affecting plant community composition. 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), this greenhouse experiment was conducted to test the following hypotheses:

- (A) Plant invaders perform better in bare grounds than in established plant communities;
- (B) Plant invaders perform better in established plant communities that lack the plant functional group of the invaders;
- (C) Herb invaders perform better in legume than in grass communities due to better nitrogen availability;
- (D) Large seeded invaders perform better than intermediate and small ones;
- (E) Earthworms reduce the number but increase the biomass of the established plant invader individuals;

(F) Earthworms change the structure of plant invader communities by promoting large seeded invaders.

In **CHAPTER 5**, the modulation of invasion resistance and stability in a plant diversity gradient by earthworms was investigated. Although 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), surprisingly, however, ecosystem engineers have widely been ignored in studies investigating diversity-invasibility relationships. Therefore, the main questions of this field study were:

- (A) 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?
- (B) What is more important, plant species diversity or plant functional group diversity?
- (C) Are there keystone plant functional groups affecting invasion resistance?
- (D) Are manipulations of earthworm densities efficient in the field and are they able to modulate ecosystem functions?
- (E) Are ecosystem engineers important drivers of plant invader establishment and do they affect plant community diversity?
- (F) Are earthworms modifying the stability of grassland communities?

In **CHAPTER 6**, direct and indirect effects of endogeic earthworms on grassland plant seeds were investigated. The soil seed bank is considered a basic way to escape unfavourable environmental conditions and seed predation (Thompson et al. 2001, Azcárate and Peco 2003). However, in soil seeds may be ingested by endogeic earthworms which consume large amounts of mineral soil. Thus, we tested whether:

- (A) Endogeic earthworms ingest and digest grassland plant seeds;
- (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.

In **CHAPTER 7**, the efficiency of two widespread non-destructive earthworm extraction methods (electrical octet method and mustard extraction) for sampling of different ecological groups of earthworms were investigated under dry soil conditions. Reliable extraction methods are required for the assessment of the size and composition of earthworm communities and for the manipulation of earthworm densities in the field. Further, the activity of different ecological earthworm groups was unclear during dry periods which is an essential ecological factor of temperate grasslands (CHAPTER 1.2). Thus, we tested whether:

- (A) Extraction efficiency of the mustard method and the octet method varies with ecological earthworm group;
- (B) Beforehand water addition to dry soil increases the extraction efficiency of the octet method but not that of the mustard method;
- (C) Earthworms belonging to different ecological groups vary in their activity during dry periods.

The results of all experiments are discussed in **CHAPTER 8** in a holistic way. Thus, direct and indirect earthworm effects on grassland plant communities as observed by the single experiments serve as elements for the integral discussion of the role of earthworms in temperate grasslands (Fig. 1.11).

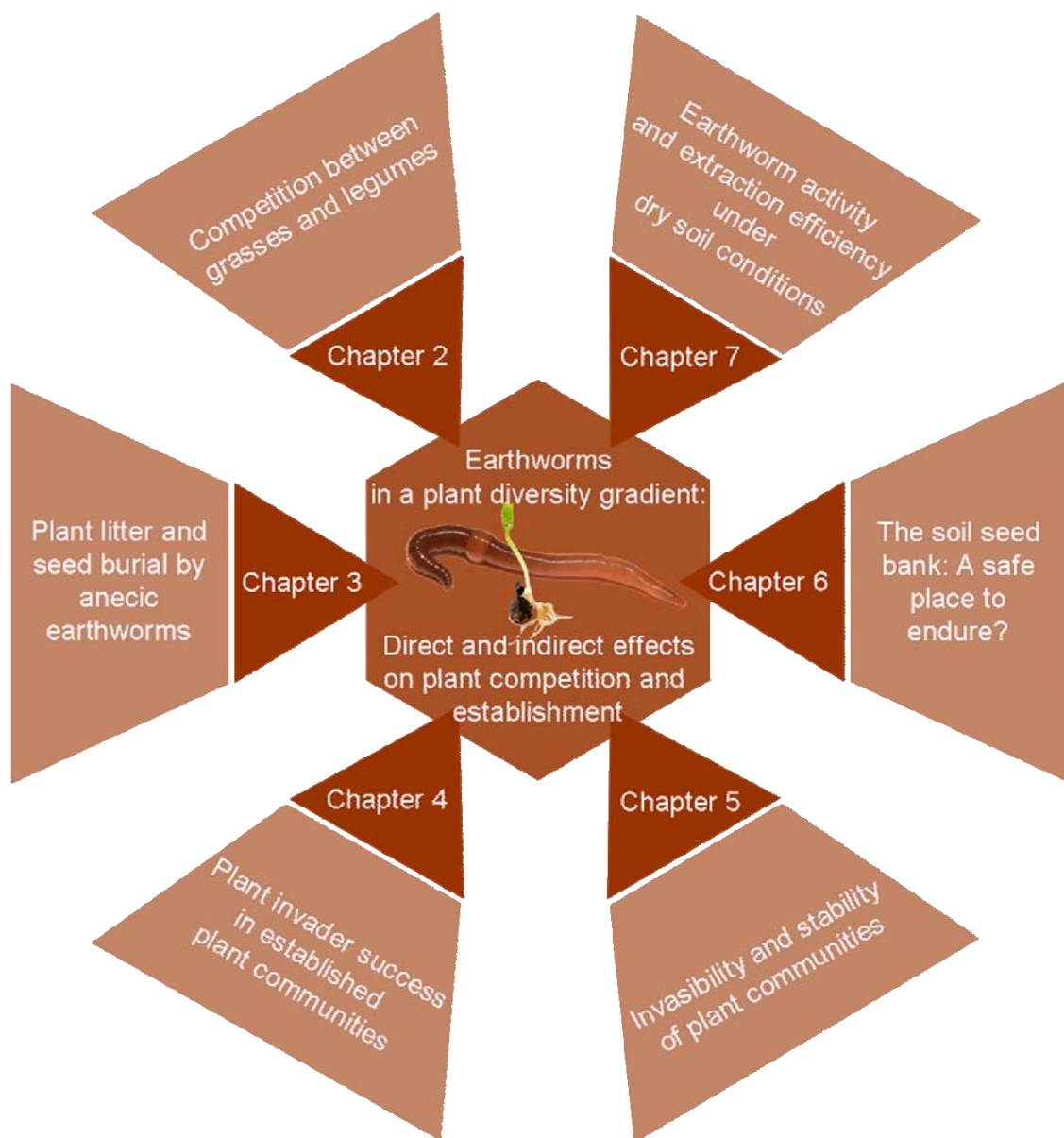
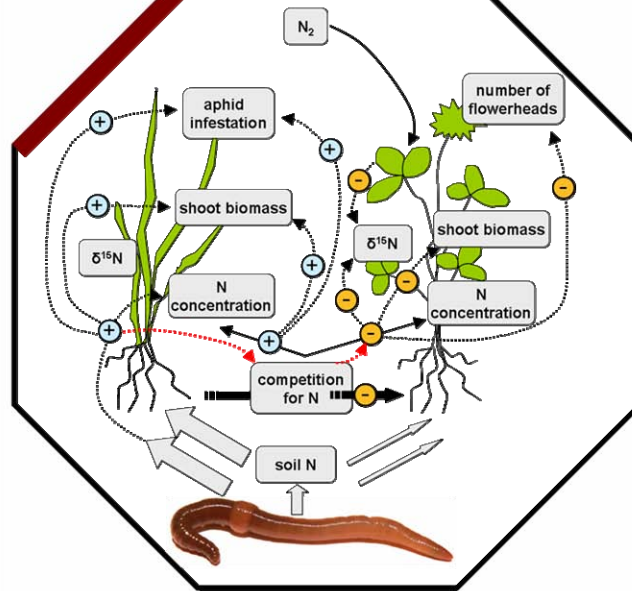


Figure 1.11 | Structure of the present thesis. Further explanations can be found in the respective chapter.

CHAPTER

2



EARTHWORMS
DRIVE THE COMPETITION BETWEEN
GRASSES AND LEGUMES

2.1 ABSTRACT

Grasses and legumes are grown together worldwide to improve total herbage yield and the quality of forage, however, the causes of population oscillations of grasses and legumes are poorly understood. Especially in grasslands, earthworms are among the most important detritivore animals functioning as ecosystem engineers, playing a key role in nutrient cycling and affecting plant nutrition and growth. The objectives of the present greenhouse experiment were to quantify the effects of earthworms on grass-legume competition in model grassland systems at two harvesting dates - simulating the widespread biannual mowing regime in Central European grasslands in order to address the following questions: (A) What are the driving factors for the competition between grasses and legumes? (B) Is the competition between grasses and legumes modified by earthworms? (C) Is increased soil nitrogen (N) availability to grasses propagating into the herbivore system? (D) Which mechanisms cause grasses to benefit from legume presence?

The presence of earthworms increased the productivity of grasses and legumes after 6 weeks but only that of grasses after another 10 weeks. In functional group mixture, the presence of grasses and earthworms decreased legume shoot biomass, the amount of N in shoot tissue and the number of legume flowerheads while the presence of legumes and earthworms increased the amount of N in grass shoots and the infestation of grasses with aphids. Analyses of $^{15}\text{N}/^{14}\text{N}$ ratios indicate that, compared to legumes, grasses more efficiently exploit soil mineral N and benefit from legume presence through reduced “intra-functional group” competition. In contrast to previous experiments, we found no evidence for N transfer from legumes to grasses. However, legume presence improved total herbage and N yield.

Earthworms modulate the competition between grasses and legumes by mobilizing soil N and thereby increasing the competitive strength of grasses. Earthworms function as essential driving agents of grass-legume associations by (a) increasing grass yield, (b) increasing the amount of N in grass hay, (c) increasing the infestation rate of grasses with aphids, and (d) potentially reducing the attractiveness of grass-legume associations to pollinators.

2.2 INTRODUCTION

Grasses and legumes are grown together worldwide to improve herbage yield and the quality of forage, especially when soil nitrogen (N) limits yield. Consequently, grass-legume associations have been intensively studied (Munoz and Weaver 1999, Hu and Jones 2001, Ngulube et al. 2004). Legumes acquire a large portion of their N from symbiotic N₂ fixation, whereas grasses depend on N mineralized from soil organic matter (or mineral fertilizers). Low-input pasture and meadow systems based on mixtures of grasses and legumes have long been proposed as a sustainable alternative to intensive N-fertilizer based grasslands, but causes of population oscillations of these two plant functional groups are poorly understood (Schwinning and Parsons 1996). If soil fertility is high, grasses and legumes compete predominantly for light and little for soil nutrients. If N is limiting, grasses may benefit from N fixed by legumes which may reduce the competitive strength of legumes (Schwinning and Parsons 1996). Although grasses may benefit from capturing legume-fixed N (Mulder et al. 2002, Temperton et al. 2006), this may not always be the case (Munoz and Weaver 1999).

Generally, nutrients in soil are mineralized by the decomposer community and decomposers depend on plants for their carbon (C) supply. Thus decomposer-plant interactions affect plant growth and intra- and inter-specific competition (Scheu 2003, Wurst et al. 2005, Endlweber and Scheu 2006). Decomposer animals benefit from carbon resources entering the soil via plant roots, e.g. as root exudates or via mycorrhizal fungi (Albers et al. 2006, Ostle et al. 2007). In parallel to this, by changing the distribution and availability of nutrients, and the activity and composition of the microbial community, decomposers indirectly affect plant growth and plant community composition (Edwards and Bohlen 1996, Scheu and Setälä 2002, Partsch et al. 2006).

Earthworms are a major component the decomposer fauna of many terrestrial ecosystems (Lee 1985, Edwards and Bohlen 1996). In non-acidic soils 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. 1988, 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, Wickenbrock and Heisler 1997, Maraun et al. 1999, Tiunov and Scheu 1999, Eisenhauer et al. 2007) with important consequences for plant growth and competitive interactions between plant species (Scheu 2003, Wurst et al. 2005, Partsch et al. 2006).

Especially in grasslands earthworms are known to play a key role in nutrient cycling and physical soil improvement (Spehn et al. 2000), and therefore in plant growth (Scheu 2003). However, most studies concentrated on the effect of earthworms on single plant species, only few have investigated effects on plant communities (Scheu 2003, Brown et al. 2004). Kreuzer et al. (2004) showed that the effect of earthworms is more pronounced in grasses than in legumes suggesting that earthworm effects vary with plant functional groups. Further, Wurst et al. (2005) suggested that earthworms enhance the competitive ability of *Lolium perenne* (grass) against *Trifolium repens* (legume) by increasing the supply of N for grasses. Although earthworm activity did not affect total above-ground biomass production in calcareous grassland (Zaller and Arnone 1999b), different plant species varied in their degree of association with earthworm casts (Zaller and Arnone 1999a). Graminoid species were closely associated with casts and, moreover, nutrient-rich earthworm casts stimulated the ramet production of grassland plant species. However, the mechanisms behind the modification of plant competition are not fully understood.

Thus, the objectives of the present study were to quantify the effects of earthworms on grass-legume competition in model grassland systems at two harvesting dates - simulating the widespread biannual mowing regime in Central European grasslands. In order to improve the understanding of ecological mechanisms structuring grass-legume associations the following questions were investigated:

- (A) What are the driving factors for the competition between grasses and legumes – are grass and legume species competing for resources and is N availability driving this competition?
- (B) Is the competition between grasses and legumes modified by earthworms and, if yes, which mechanisms are responsible for these modifications?
- (C) Is increased soil N availability to grasses propagating into the herbivore system – connecting the above- and below-ground system?
- (D) What are the mechanisms behind the phenomenon that grasses benefit from legume presence – do grasses indeed benefit from legume fixed N?

2.3 MATERIALS AND METHODS

Experimental setup

We set up microcosms consisting of PVC tubes (inner diameter 16 cm, height 38 cm) covered by a 1 mm mesh at the bottom to prevent earthworms (*Lumbricus terrestris*) from escaping but allow drainage of water. Furthermore, a plastic barrier (10 cm height) prevented

earthworms from escaping 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 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, focussing on element cycling and trophic interactions (Roscher et al. 2004). A total of 90 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 $20/16 \pm 2^\circ\text{C}$. 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 (4 weeks old, 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 each microcosm creating three plant community treatments (Grasses, Legumes and Mixtures). Grasses only treatments contained four individuals of each *Phleum pratense* L., *Dactylis glomerata* L., and *Lolium perenne* L., legumes only treatments contained four individuals of *Trifolium pratense* L., *T. repens* L., and *Medicago varia* Martyn and mixtures contained two individuals of each of the six plant species. 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 of all microcosms prior to the addition of earthworms to simulate field surface soil conditions. Two adult *Lumbricus terrestris* L. (average fresh weight with gut content 4.25 ± 0.69 g, weighed individually) were introduced in half of the microcosms creating two treatments (with and without earthworms). We set up 15 replicates of each of the six treatments (plant community [3] x earthworms [2]).

The experiment lasted for four months, with a first harvest at week 6 and a second harvest at week 16. 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. Thereby, all microcosms received the same amount of water to avoid effects of different water availability. Microcosms were randomized every two weeks.

Aphids (*Rhopalosiphum padi* L.) occurred accidentally in all microcosms starting at week 1 of the experiment and infested only grass species. We added four larvae of *Chrysoperla carnea* Steph. to each microcosm after week 2 and 4, respectively, to reduce aphid infestation rates. After the first harvest, we allowed the movement and interchange of aphids (via hanging over vegetation) by placing microcosms in close vicinity to investigate the effects of earthworm and legume presence on aphid infestation rates of grasses.

Sampling

At the first harvest shoot biomass from different plant individuals was harvested separately cutting shoots 3 cm above soil surface level. At the second harvest plant individuals were harvested separately cutting shoots at soil surface level. Roots were washed out of the soil using a 1 mm mesh; it was not possible to separate roots from different individuals and different plant functional groups. Shoot and root material was dried at 60°C for three days. We performed two harvests to simulate the widespread biannual mowing regime in Central European grasslands and to investigate short- and long-term treatment effects.

To detect the main N sources driving the competition between grasses and legumes we ground the shoot material of grasses and legumes (individual shoots pooled per plant functional group; second harvest) harvested from each microcosm separately.

Prior to the second harvest the number of legume flowerheads and the number of aphids (*R. padi*) were counted to investigate if treatment effects propagate into the above-ground system.

Earthworms were collected by hand, weighed individually (fresh weight with gut content) and earthworm cocoons were counted to investigate the effect of the plant community on earthworm performance (second harvest).

¹³C and ¹⁵N analysis

We measured ¹⁵N/¹⁴N isotope ratio (δ¹⁵N) in plant shoot material to quantify treatment effects on the relative contribution of biological N₂-fixation by legumes, on the transfer of legume-derived N to grasses, and on the competition of grasses and legumes for soil N. Moreover, we measured ¹³C/¹²C isotope ratio (δ¹³C) to investigate treatment effects on the competition of grasses and legumes for water since water stress is known to alter plant physiology which is reflected in changes in ¹³C fractionation (Brugnoli et al. 1998, Anderson et al. 2000). Approximately 3 mg of the powdered plant shoot material (individuals of one

plant functional group pooled per microcosm; second harvest) were weighed into tin capsules. Total C concentration, $\delta^{13}\text{C}$, total N concentration and $\delta^{15}\text{N}$ were determined by a coupled system consisting of an elemental analyzer (NA 1500, Carlo Erba, Milan) and a gas isotope mass spectrometer (MAT 251, Finnigan; Reineking et al. 1993). Isotope natural abundance was expressed using the delta notation with $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ [‰] = $(R_{\text{sam}} - R_{\text{std}})/(R_{\text{std}} \times 1000)$. R_{sam} and R_{std} refer to $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in samples and standard, respectively. Pee Dee River belemnite marine limestone (PDB) and atmospheric N_2 were used as standard for ^{13}C and ^{15}N determination, respectively. Acetanilide ($\text{C}_8\text{H}_9\text{NO}$; Merck, Darmstadt, Germany) was used for internal calibration.

Further, we determined the amount of N per plant individual (per plant functional group) and per microcosm by multiplying shoot biomass with the N concentration of the corresponding plant functional group of each microcosm.

Calculations

We calculated the difference between the individual earthworm weights at the start and the end of the experiment (second harvest). For statistical analyses of earthworm weight and cocoon number only microcosms were used that contained all earthworm individuals at the end of the experiment.

Data on shoot and root biomass were summed up per microcosm. The weight of legume flowerheads was included in aboveground biomass data, but the number of flowerheads was also analyzed separately. Total shoot biomass, number of flowerheads and number of aphids were divided by the number of plant individuals occurring in the specific microcosm (shoot biomass per grass or legume individual, number of flowerheads per legume individual, number of aphids per grass individual) to account for the fact that there were twelve grass and legume individuals, respectively, in plant functional group “monocultures” but only six grass and legume individuals in mixtures. Normal distribution and homogeneity of variance were improved by log-transformation, if necessary. Means presented in text and figures are based on non-transformed data ($\pm\text{SD}$).

Statistical analyses

Analysis of variance (ANOVA; type III SS) implemented in SAS 9.1 (SAS Inst., Cary, North Carolina, USA) was used to analyze the effects of Plant community (grasses, legumes and mixtures) and Earthworms (with and without *L. terrestris*) on plant biomass productivity (shoot biomass per microcosm, root biomass per microcosm, total plant biomass per

microcosm, shoot-to-root ratio and amount of N in plant shoots per microcosm). In addition, ANOVA was used to investigate the effects of Plant functional group (with grasses or legumes, and without grasses or legumes, respectively) and Earthworms on shoot biomass per plant individual, on aphid infestation rates (number of aphids per grass individual), on the number of flowerheads per legume individual, on N concentration, on $\delta^{15}\text{N}$, on carbon concentration, on $\delta^{13}\text{C}$, on the amount of N, and on the C-to-N ratio of grass and legume shoots. Further, single factor ANOVA was used to analyze the effect of earthworms on plant productivity (shoot biomass per microcosm, root biomass per microcosm, total plant biomass per microcosm, and shoot-to-root ratio) for each plant community treatment and to analyze the effect of Plant community on earthworm performance (fresh weight and cocoon production). Comparisons of means (Tukey's HSD test; $\alpha = 0.05$) were performed using SAS 9.1 (SAS Inst., Cary, North Carolina, USA).

2.4 RESULTS

Earthworms

A total of 82 of 90 earthworms (91%) survived the 4 months of the experiment whereas earthworm disappearance occurred evenly across all treatments. On average 8.4 ± 4.8 cocoons were produced per microcosm. Neither earthworm weight nor the number of cocoons produced were affected by Plant community ($F_{2,34} = 0.71$, $P = 0.50$ and $F_{2,34} = 0.55$, $P = 0.58$, respectively). Generally, *L. terrestris* buried the whole amount of litter during the first week after its application (at experimental start and after the first harvest).

Plant productivity

Total shoot biomass of the treatment with grasses only and the mixture exceeded that of the treatment with legumes only after 6 weeks (+54% and +42%, respectively; first harvest; Table 2.1, Fig. 2.1A). However, 10 weeks after the first harvest the opposite was true since the legumes only treatment exceeded that of the grasses only treatment (+36%) and the mixture (+12%; second harvest; Fig. 2.1B). Contrary to shoot biomass, root biomass in the treatment with grasses only and the mixture exceeded that in the legumes only treatment at the second harvest (Fig. 2.1C). Therefore, total biomass per microcosm (shoot and root biomass) was significantly higher in the treatment with grasses only and the mixture than it was in the legumes only treatment (Fig. 2.1D). Consequently, shoot-to-root ratio of the legumes only treatment exceeded that of the grasses only treatment and the mixture (Fig. 2.1E).

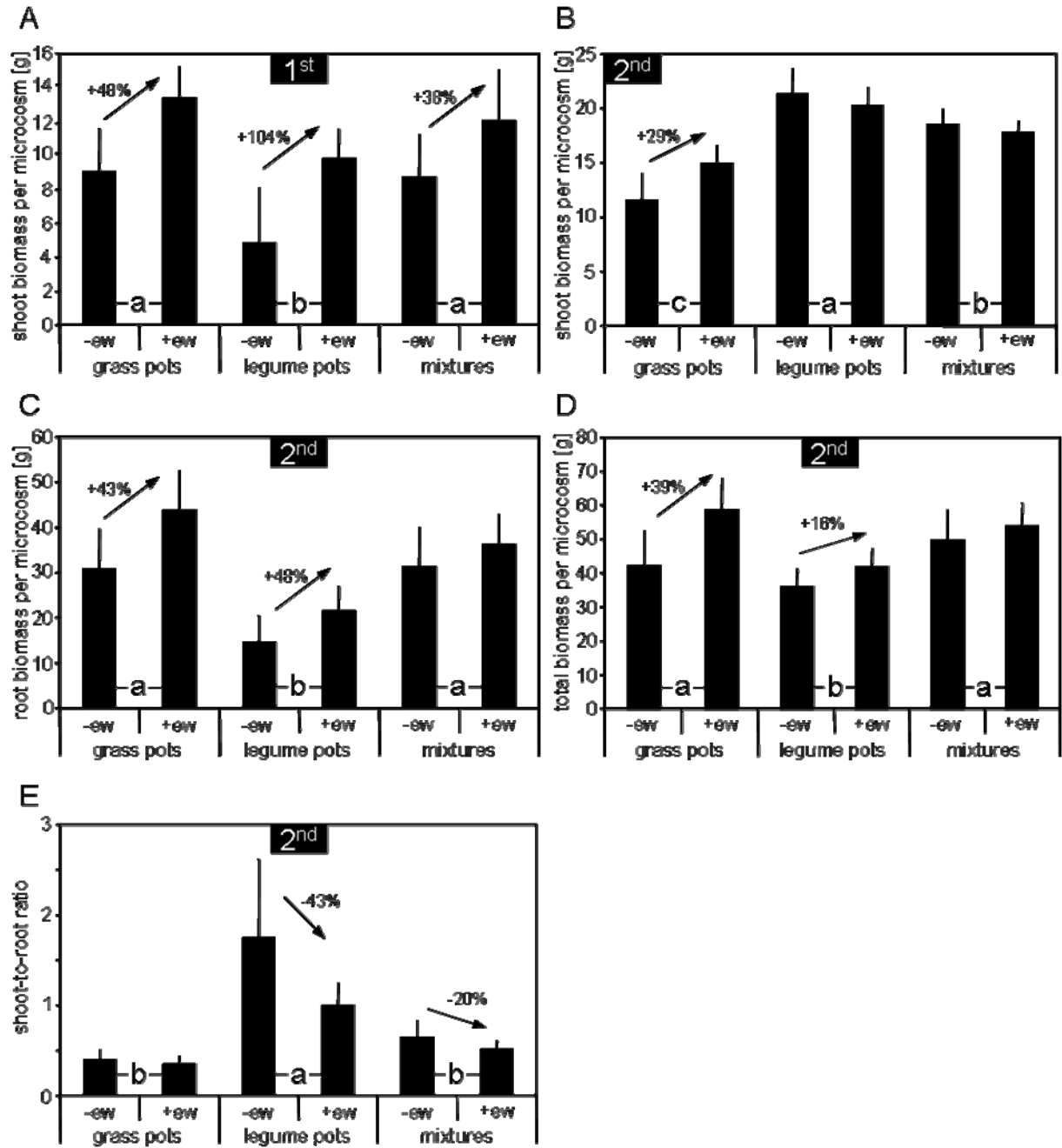


Figure 2.1 | Effects of Plant community (grasses only treatment, legumes only treatment and mixture) and Earthworms (with [+ew] and without [-ew] *Lumbricus terrestris*) on (A) shoot biomass per microcosm (first [1st] harvest), (B) shoot biomass per microcosm (second [2nd] harvest), (C) root biomass per microcosm (2nd harvest), (D) total biomass per microcosm (shoots and roots; 2nd harvest) and (E) shoot-to-root ratio (2nd harvest). Means with standard deviations. Pairs of bars (Plant community treatments) with different letters vary significantly (Tukey's HSD test, $P < 0.05$).

Table 2.1 | ANOVA table of F-values on the effects of Plant community (grasses, legumes and mixtures) and Earthworms (with and without *Lumbricus terrestris*) on shoot biomass (first [1st] harvest after 6 weeks, second [2nd] harvest after 16 weeks), root biomass, total biomass per microcosm (2nd harvest), shoot-to-root ratio (2nd harvest), and amount of nitrogen per microcosm (2nd harvest). Significant effects are given in bold.

| Dependent variable | Independent variable | Df | F-value | P-value |
|-------------------------------------|----------------------|-------|---------------|------------------|
| Shoot biomass 1 st | Plant community (PC) | 2, 84 | 14.13 | <.0001 |
| | Earthworms | 1, 84 | 34.22 | <.0001 |
| | PC X Earthworms | 2, 84 | 4.39 | 0.0154 |
| Shoot biomass 2 nd | Plant community | 2, 84 | 110.75 | <.0001 |
| | Earthworms | 1, 84 | 5.03 | 0.0276 |
| | PC X Earthworms | 2, 84 | 15.77 | <.0001 |
| Root biomass 2 nd | Plant community | 2, 84 | 67.59 | <.0001 |
| | Earthworms | 1, 84 | 32.01 | <.0001 |
| | PC X Earthworms | 2, 84 | 2.03 | 0.1382 |
| Total biomass 2 nd | Plant community | 2, 84 | 26.34 | <.0001 |
| | Earthworms | 1, 84 | 31.79 | <.0001 |
| | PC X Earthworms | 2, 84 | 5.11 | 0.0081 |
| Shoot-to-root ratio 2 nd | Plant community | 2, 84 | 130.53 | <.0001 |
| | Earthworms | 1, 84 | 17.86 | <.0001 |
| | PC X Earthworms | 2, 84 | 3.35 | 0.0399 |
| Amount of nitrogen | Plant community | 2, 84 | 660.90 | <.0001 |
| | Earthworms | 1, 84 | 9.63 | 0.0026 |
| | PC X Earthworms | 2, 84 | 13.82 | 0.0081 |

Df: degrees of freedom.

All legume individuals had root nodules, however, there were no differences in nodulation rates among treatments (data not shown).

The presence of earthworms increased total shoot biomass of the grasses only treatment (+48%), the legumes only treatment (+104%), and the mixture (+38%) at the first harvest (Tables 2.1, 2.2, Fig. 2.1A), but only the shoot biomass of grasses at the second harvest (+29%; Tables 2.1, 2.2, Figs. 2.1B, 2.2A). Furthermore, earthworms increased total root biomass of the treatment with grasses only (+43%) and legumes only (+48%), whereas, root biomass of the mixture did not vary significantly (second harvest; Fig. 2.1C). Overall,

earthworms only increased total plant biomass in the grasses (+39%) and legumes only treatments (+16%; Fig. 2.1D). Generally, legumes had higher shoot-to-root ratios than grasses, however, earthworms decreased the shoot-to-root ratio in the legumes only treatment (-43%) and the mixture (-20%; Fig. 2.1E) considerably.

The shoot biomass of grass individuals was increased at the first (+42% and +70%) and second harvest (+24% and +37%) in presence of earthworms and legumes, respectively (Table 2.3, Figs. 2.2A, 2.2B). In presence of grasses the shoot biomass of legume individuals was decreased at the first harvest (-77%), whereas earthworms increased the shoot biomass of legume individuals irrespective of the presence of grasses (+42% and +104% with and without grasses, respectively; Table 2.4, Fig. 2.2D). However, at the second harvest shoot biomass of legume individuals was only decreased when both grasses and earthworms were present (Fig. 2.2E).

Table 2.2 | ANOVA table of F-values on the effects of earthworms (with and without *Lumbricus terrestris*) on shoot biomass (SB; first [1st] harvest after 6 weeks, second [2nd] harvest after 16 weeks), root biomass (RB), total biomass per microcosm (BM; 2nd harvest), shoot-to-root ratio (SR; 2nd harvest), and the amount of nitrogen per microcosm (AN; 2nd harvest). Significant effects are given in bold.

| | <i>Grasses only</i> | <i>Legumes only</i> | <i>Mixtures</i> |
|--------------------|---|--|---|
| SB 1 st | F_{1,28} = 18.56 P = 0.0002 | F_{1,28} = 14.89 P = 0.0006 | F_{1,28} = 8.07 P = 0.0083 |
| SB 2 nd | F_{1,28} = 16.00 P = 0.0004 | F _{1,28} = 2.10 P = 0.1582 | F _{1,28} = 3.13 P = 0.0878 |
| RB 2 nd | F_{1,28} = 17.52 P = 0.0003 | F_{1,28} = 13.15 P = 0.0011 | F _{1,28} = 3.75 P = 0.0693 |
| BM 2 nd | F_{1,28} = 21.98 P = <.0001 | F_{1,28} = 9.88 P = 0.0039 | F _{1,28} = 2.52 P = 0.1233 |
| SR 2 nd | F _{1,28} = 1.27 P = 0.2698 | F_{1,28} = 12.58 P = 0.0014 | F_{1,28} = 5.08 P = 0.0322 |
| AN 2 nd | F_{1,28} = 17.21 P = 0.0003 | F _{1,27} = 0.26 P = 0.6173 | F _{1,28} = 1.43 P = 0.2421 |

Aphid infestation

All grass species were similarly infested with aphids (data not shown). On average there were 1612 ± 1176 aphids per grass individual. The number of aphids was increased in presence of earthworms (+95%) and legumes (+84%; Table 2.3, Fig. 2.2C). Further, the number of aphids per grass individual was positively correlated with the N concentration [%] of grass shoot tissue ($R^2 = 0.35$; $P < 0.0001$; Fig. 2.4).

Legume flowerheads

On average there were 1.89 ± 1.01 flowerheads per legume individual. The number of flowerheads was decreased to less than half in presence of grasses. Moreover, in the mixture the number of flowerheads was decreased in presence of earthworms (-36%; second harvest; Table 2.4, Fig. 2.2F).

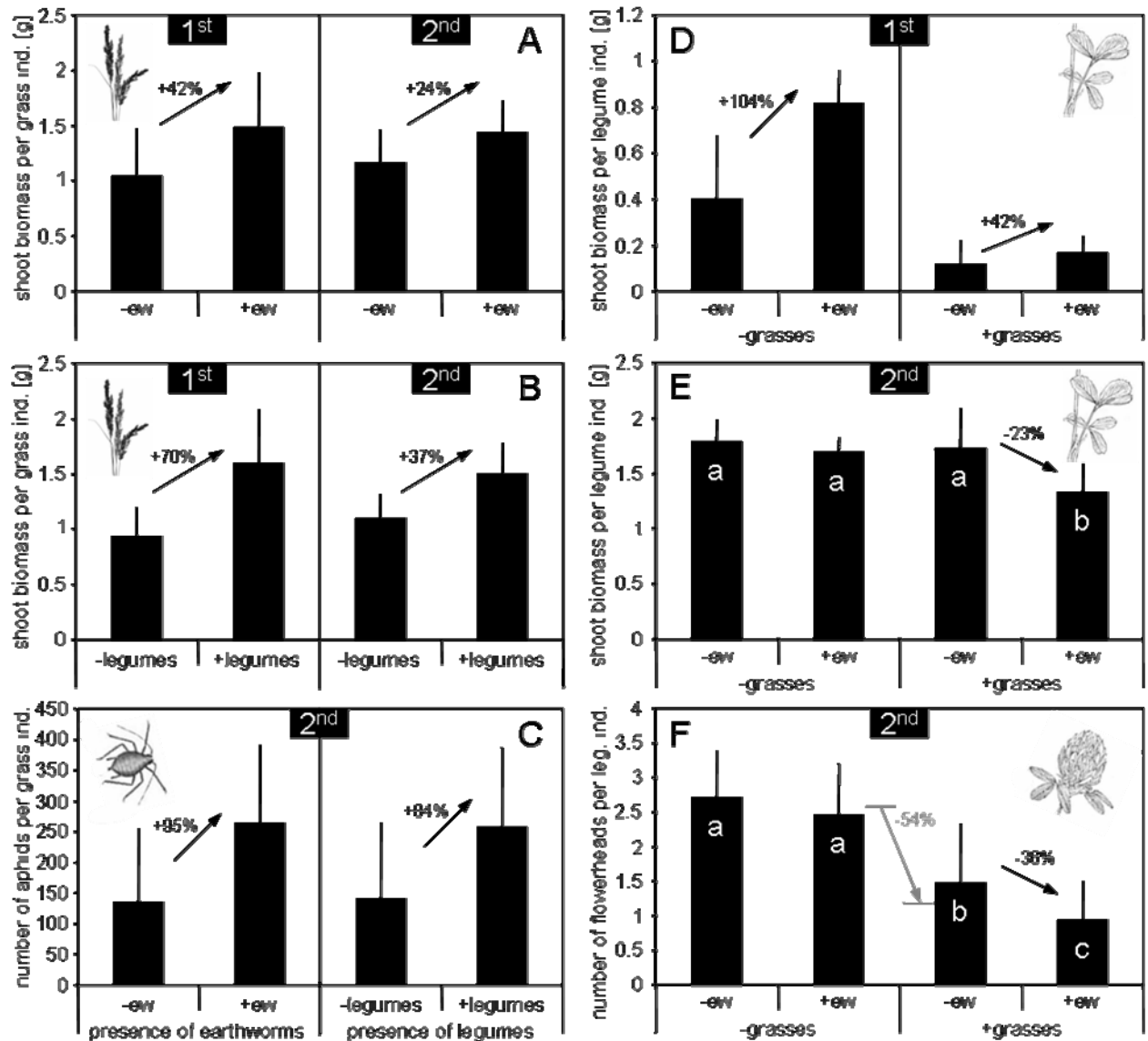


Figure 2.2 | (A) Effects of Earthworms (with [+ew] and without [-ew] *Lumbricus terrestris*) on shoot biomass per grass individual (first [1st] and second [2nd] harvest); (B) Effects of legumes (with [+legumes] and without [-legumes] legumes) on shoot biomass per grass individual (1st and 2nd harvest); (C) Effects of earthworms and legumes on the number of aphids per grass individual (2nd harvest); Effects of grasses (with [+grasses] and without [-grasses] grasses) and earthworms on (D) shoot biomass per legume individual (1st harvest), (E) shoot biomass per legume individual (2nd harvest), and (F) number of flowerheads per legume individual (2nd harvest). Means with standard deviations. Bars with different letters vary significantly (Tukey's HSD test, $P < 0.05$).

Table 2.3 | ANOVA table of F-values on the effects of legumes (with and without legumes) and earthworms (with and without *Lumbricus terrestris*) on shoot biomass of grasses (dry weight per individual; first [1st] harvest after 6 weeks, second [2nd] harvest after 16 weeks), and number of aphids (*Rhopalosiphum padi*) per grass individual (2nd harvest) in the grasses only treatment and the mixture with grasses and legumes. Significant effects are given in bold.

| Dependent variable | Independent variable | Df | F-value | P-value |
|-------------------------------|----------------------|-------|--------------|------------------|
| Grass biomass 1 st | Legumes | 1, 56 | 46.70 | <.0001 |
| | Earthworms | 1, 56 | 24.54 | <.0001 |
| | Legumes X Earthworms | 1, 56 | 0.51 | 0.4760 |
| Grass biomass 2 nd | Legumes | 1, 56 | 47.66 | <.0001 |
| | Earthworms | 1, 56 | 24.41 | <.0001 |
| | Legumes X Earthworms | 1, 56 | 0.99 | 0.3248 |
| Aphids 2 nd | Legumes | 1, 56 | 25.00 | <.0001 |
| | Earthworms | 1, 56 | 32.13 | <.0001 |
| | Legumes X Earthworms | 1, 56 | 1.73 | 0.1940 |

Df: degrees of freedom.

Table 2.4 | ANOVA table of F-values on the effects of grasses (with and without grasses) and earthworms (with and without *Lumbricus terrestris*) on shoot biomass of legumes (dry weight per individual; first [1st] harvest after 6 weeks, second [2nd] harvest after 16 weeks), and number of flowerheads per individual (2nd harvest) in the legumes only treatment and the mixture with grasses and legumes. Significant effects are given in bold.

| Dependent variable | Independent variable | Df | F-value | P-value |
|--------------------------------|----------------------|-------|--------------|------------------|
| Legume biomass 1 st | Grasses | 1, 56 | 44.03 | <.0001 |
| | Earthworms | 1, 56 | 14.85 | 0.0003 |
| | Grasses X Earthworms | 1, 56 | 0.08 | 0.7720 |
| Legume biomass 2 nd | Grasses | 1, 56 | 14.29 | 0.0004 |
| | Earthworms | 1, 56 | 15.30 | 0.0003 |
| | Grasses X Earthworms | 1, 56 | 7.06 | 0.0102 |
| Flowerheads 2 nd | Grasses | 1, 56 | 48.37 | <.0001 |
| | Earthworms | 1, 56 | 6.25 | 0.0154 |
| | Grasses X Earthworms | 1, 56 | 2.92 | 0.0929 |

Df: degrees of freedom.

Nitrogen and carbon concentration, C-to-N ratio, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$

The concentration and the amount of N in grass shoot tissue were increased in presence of legumes (+23% and +68%, respectively) and in presence of earthworms (+12% and +36%, respectively; second harvest; Table 2.5, Fig. 2.3A). The C-to-N ratio of grass shoots was decreased in presence of legumes (-18%) and earthworms (-11%; Table 2.5). $\delta^{15}\text{N}$ values of grass shoots did not vary significantly (6.01 ± 0.70).

The concentration of N in legume shoots was decreased in presence of grasses (-4%) but increased in presence of earthworms (+8%; Table 2.6, Fig. 2.3B) whereas the C-to-N ratio was increased in presence of grasses (+4%) and decreased in presence of earthworms (-8%; Table 2.6). However, earthworm presence did not affect the amount of N in legume shoot tissue in absence of grasses but in presence of grasses and earthworms the amount of N in legume shoot tissue was decreased significantly (-16%; Table 2.6). $\Delta^{15}\text{N}$ of legume shoots was decreased in presence of grasses but increased in presence of earthworms (Table 2.6, Fig. 2.3C). If both earthworms and grasses were present $\delta^{13}\text{C}$ of legume shoots was decreased (Table 2.6, Fig. 2.3D).

In total, the amount of N per microcosm (shoot material) was highest in the legumes only treatment, whereas the grasses only treatment contained the lowest amount of N (Table 2.1, Fig. 2.3E). In presence of earthworms the amount of N was only increased in the grasses only treatment (+44%; Table 2.2).

Table 2.5 | ANOVA table of F-values on the effects of legumes (with and without legumes) and earthworms (with and without *Lumbricus terrestris*) on nitrogen concentration, $\delta^{15}\text{N}$, carbon concentration, $\delta^{13}\text{C}$, C-to-N ratio, and the amount of nitrogen of grass shoots (second harvest) in the grasses only treatment and the mixture with grasses and legumes. Significant effects are given in bold.

| Dependent variable | Independent variable | Df | F-value | P-value |
|------------------------|----------------------|-------|--------------|------------------|
| Nitrogen concentration | Legumes | 1, 56 | 30.58 | <.0001 |
| | Earthworms | 1, 56 | 9.34 | 0.0034 |
| | Legumes X Earthworms | 1, 56 | 0.05 | 0.8200 |
| $\Delta^{15}\text{N}$ | Legumes | 1, 56 | 0.62 | 0.4336 |
| | Earthworms | 1, 56 | 0.42 | 0.5216 |
| | Legumes X Earthworms | 1, 56 | 0.36 | 0.5500 |
| Carbon concentration | Legumes | 1, 56 | 2.66 | 0.1084 |
| | Earthworms | 1, 56 | 0.01 | 0.9115 |
| | Legumes X Earthworms | 1, 56 | 0.21 | 0.6496 |
| $\Delta^{13}\text{C}$ | Legumes | 1, 56 | 0.78 | 0.3817 |
| | Earthworms | 1, 56 | 0.24 | 0.6252 |
| | Legumes X Earthworms | 1, 56 | 0.00 | 0.9942 |
| C-to-N ratio | Legumes | 1, 56 | 28.30 | <.0001 |
| | Earthworms | 1, 56 | 9.42 | 0.0033 |
| | Legumes X Earthworms | 1, 56 | 0.09 | 0.7608 |
| Amount of nitrogen | Legumes | 1, 56 | 76.93 | <.0001 |
| | Earthworms | 1, 56 | 27.93 | <.0001 |
| | Legumes X Earthworms | 1, 56 | 0.34 | 0.5625 |

Df: degrees of freedom.

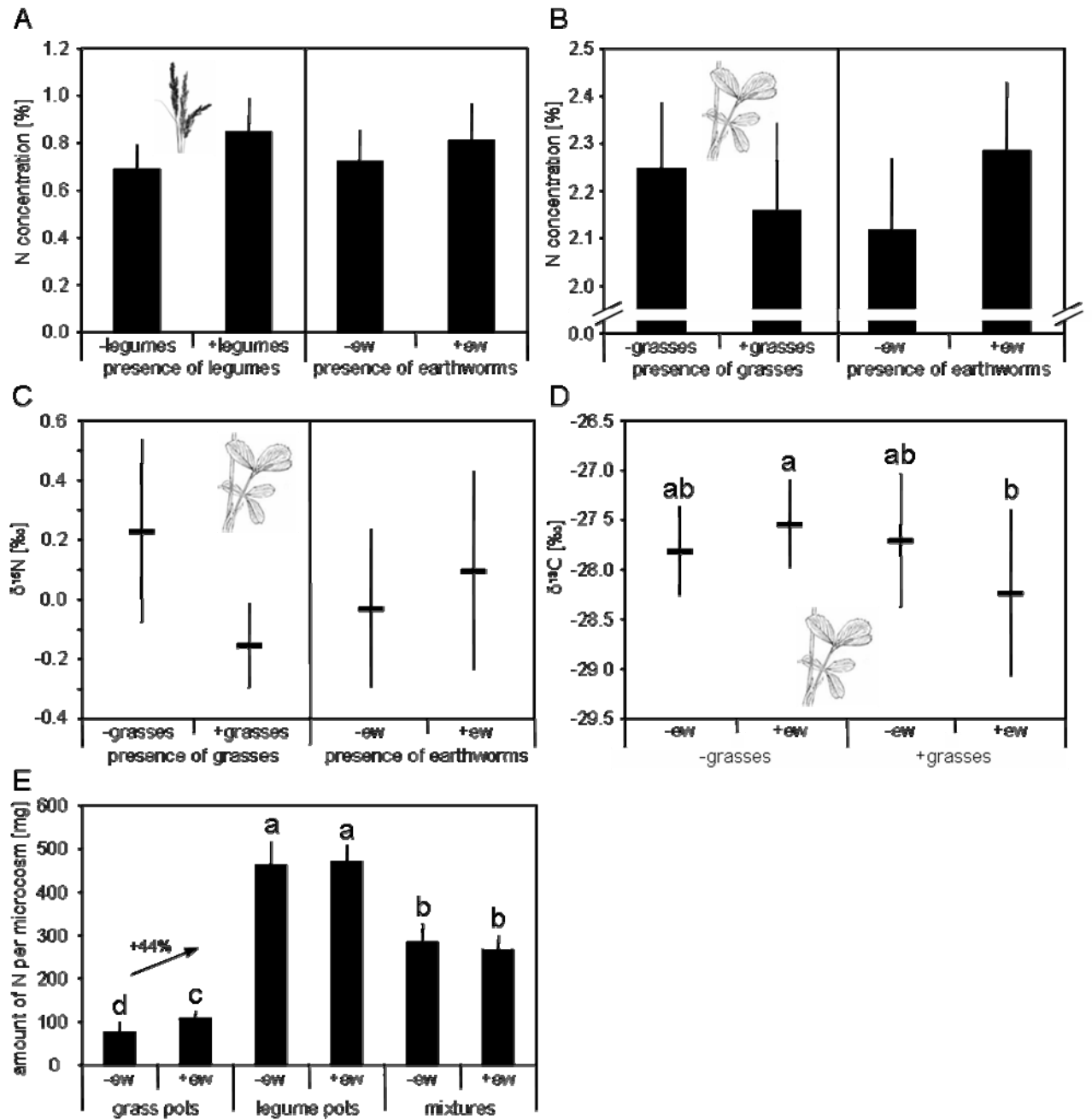


Figure 2.3 | (A) Effects of legumes (with [+legumes] and without [-legumes] legumes) and earthworms (with [+ew] and without [-ew] *Lumbricus terrestris*) on nitrogen concentration of grass shoots (second [2nd] harvest); (B) Effects of grasses (with [+grasses] and without [-grasses] grasses) and earthworms on nitrogen concentration of legume shoots (2nd harvest); (C) Effects of grasses and earthworms on $\delta^{15}\text{N}$ of legume shoots (2nd harvest); (D) Effects of grasses and earthworms on $\delta^{13}\text{C}$ of legume shoots (2nd harvest); (E) Effects of plant community (grasses only treatment, legume only treatment and mixture) and earthworms (with [+ew] and without [-ew] *Lumbricus terrestris*) on the amount of nitrogen per microcosm [mg]. Means with standard deviations. Bars with different letters vary significantly (Tukey's HSD test, $P < 0.05$).

Table 2.6 | ANOVA table of F-values on the effects of grasses (with and without grasses) and earthworms (with and without *Lumbricus terrestris*) on nitrogen concentration, $\delta^{15}\text{N}$, carbon concentration, $\delta^{13}\text{C}$, C-to-N ratio, and the amount of nitrogen of legume shoots (second harvest) in the legumes only treatment and the mixture with grasses and legumes. Significant effects are given in bold.

| Dependent variable | Independent variable | Df | F-value | P-value |
|------------------------|----------------------|-------|--------------|------------------|
| Nitrogen concentration | Grasses | 1, 55 | 5.96 | 0.0179 |
| | Earthworms | 1, 55 | 20.07 | <.0001 |
| | Grasses X Earthworms | 1, 55 | 0.59 | 0.4449 |
| $\Delta^{15}\text{N}$ | Grasses | 1, 55 | 52.64 | <.0001 |
| | Earthworms | 1, 55 | 4.80 | 0.0327 |
| | Grasses X Earthworms | 1, 55 | 0.10 | 0.7573 |
| Carbon concentration | Grasses | 1, 55 | 0.31 | 0.5790 |
| | Earthworms | 1, 55 | 1.27 | 0.2642 |
| | Grasses X Earthworms | 1, 55 | 0.09 | 0.7639 |
| $\Delta^{13}\text{C}$ | Grasses | 1, 55 | 3.18 | 0.0800 |
| | Earthworms | 1, 55 | 0.60 | 0.4402 |
| | Grasses X Earthworms | 1, 55 | 6.19 | 0.0159 |
| C-to-N ratio | Grasses | 1, 55 | 4.91 | 0.0309 |
| | Earthworms | 1, 55 | 22.06 | <.0001 |
| | Grasses X Earthworms | 1, 55 | 0.44 | 0.5113 |
| Amount of nitrogen | Grasses | 1, 55 | 19.82 | <.0001 |
| | Earthworms | 1, 55 | 3.65 | 0.0614 |
| | Grasses X Earthworms | 1, 55 | 4.34 | 0.0419 |

Df: degrees of freedom.

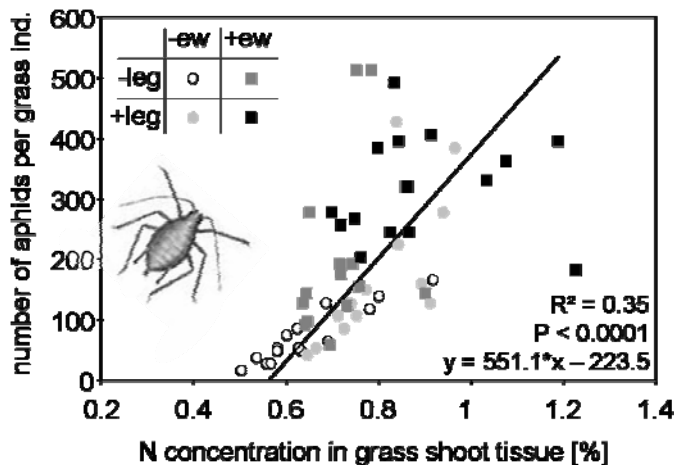


Figure 2.4 | Regression of the number of aphids per grass individual and the N concentration [%] in grass shoot tissue (-ew: without earthworms; +ew: with earthworms; -leg: without legumes; +leg: with legumes).

2.5 DISCUSSION

Plant competition for light and nitrogen

The presence of grasses decreased legume shoot biomass at the first harvest through competition, presumably for light. Grasses established and grew fast, thereby suppressing legumes. Similar findings by Munoz and Weaver (1999) also have been explained by shading of legumes by grasses. On the contrary, at the second harvest grasses decreased legume shoot biomass only when earthworms were also present. Presumably, grasses were only able to suppress legumes when earthworms increased the supply of mineral N thereby fostering the competitive strength of grasses against legumes.

Earthworms modulate plant competition through nitrogen allocation

The present study indicates that earthworms are important driving agents of the competition between grasses and legumes, with their effect varying with time. Short-term effects of earthworms stimulated plant growth irrespective of plant functional group (first harvest). This is in line with the majority (79%) of the previous studies investigating earthworm effects on plant shoot biomass (Scheu 2003). However, the effect of earthworms on shoot biomass of already established plant communities was less consistent (second harvest). Results of the second harvest indicate that, in contrast to grasses, once established legumes are able to satisfy their N supply through N₂ fixation of associated root-nodule bacteria but, still, also depend on mineralized N in soil.

Earthworms and legumes affected the performance of grasses in a similar and predominantly beneficial way. Both increased biomass of individual shoots, shoot N concentration and the amount of N in grass shoot tissue. The responses suggest that grasses benefited from increased N mineralization in presence of earthworms and possibly from the leakage/transfer of N fixed by legumes. In fact, shoot N concentration of grasses and legumes was increased in presence of earthworms suggesting that earthworms indeed increased N supply to plants. However, earthworms only increased the amount of N in grasses not in legumes reflecting that in presence of earthworms grasses flourished at the expense of legumes. Legume presence did not increase the supply of N to grasses via N transfer of fixed N₂. $\Delta^{15}\text{N}$ values of grass shoots neither were affected by legumes nor by earthworms. This suggests that grasses exclusively relied on soil derived N. In the mixture, grass individuals had only to compete with five other grass individuals and six legume individuals (low “intra-functional group” competition), whereas in the grasses only treatment, grass individuals

competed with eleven other grass individuals for soil N (high “intra-functional group” competition). Rather than benefiting from legume fixed N, the presence of legumes increased the biomass of individual grass shoots, N concentration and the amount of N in grass shoot tissue through decreased “intra-functional group” competition. Munoz and Weaver (1999) also observed that grasses did not receive N from clover but there is also evidence for uptake of legume-fixed N by grasses and, consequently, increased productivity (Mulder et al. 2002, Temperton et al. 2006, Ayres et al. 2007). One explanation for the missing transfer of legume derived N to grasses might have been the defaunation procedure of soil prior to the start of the experiment since Dromph et al. (2006) showed that N transfer between legumes and non-legumes depends on the density of root infestation by parasitic nematodes – probably causing N leakage from infested roots. Presumably, depending on rhizosphere interactions and the types of competitors, grasses may benefit from both reduced “intra-functional group” competition and N transfer from legumes.

Increased $\delta^{15}\text{N}$ values of legume shoots in presence of earthworms suggest that legumes increased the uptake of N from soil mineralized by earthworms. Lower $\delta^{15}\text{N}$ values of legume shoots in presence of grasses indicate that when competing with grasses legumes rely more on N_2 fixed by rhizobia. Consequently, compared to legumes grasses more efficiently exploit mineral N in soil. This is consistent to the findings of Munoz and Weaver (1999) who reported that fertilization with N fostered the competitive strength of ryegrass compared to clover. Recent studies indicated that earthworms are also able to enhance the competitive ability of grasses against legumes (Kreuzer et al. 2004, Wurst et al. 2005) but the present study is the first to uncover the responsible mechanisms at the level of plant functional groups by using three common plant species per functional group and ^{15}N analysis. The modulation of grass-legume competition might also play a significant role in natural grasslands since Zaller and Arnone (1999a) reported graminoid species to be more highly associated with earthworm casts than other plant species.

In addition to $\delta^{15}\text{N}$, changes in $\delta^{13}\text{C}$ values suggest that the decline in legumes in presence of earthworms was not only due to increased capture of N by grasses but also by increased uptake of water thereby increasing water stress in legumes. It is known that ^{13}C discrimination in plants correlates negatively with water availability (Brugnoli et al. 1998, Anderson et al. 2000). In presence of earthworms total plant biomass in mixtures was higher and the soil dried out earlier than in legume only treatments (second harvest), suggesting that earthworms also fostered the build-up of a more extended root system of grasses, thereby increasing the competitive strength for water against legumes.

Root biomass of grasses and legumes were generally increased in presence of earthworms resulting in a decreased shoot-to-root ratio in legumes. In previous studies the response of root biomass to earthworm presence was inconsistent with an increase in 50% but a decrease in 38% of the studies reviewed by Scheu (2003). Since the plant root system functions as a foraging system capturing resources in soil (Hutchings et al. 2000) earthworms may stimulate root growth by casting, i.e. the formation of nutrient rich patches. Indeed, burrows of *L. terrestris* are known to be “hotspots” of microbial activity and nutrient availability (Maraun et al. 1999, Tiunov and Scheu 1999, Tiunov and Scheu 2000). Further, Zaller and Arnone (1999a) reported that especially graminoid plant species were associated with earthworm casts in calcareous grassland. Thus, foraging and growth of roots in the vicinity of earthworm burrows may stimulate resource allocation to roots resulting in a more pronounced root system.

Earthworm effects on the above-ground food web

Increased infestation of grasses by aphids was due to increased plant tissue N concentrations in presence of earthworms and by decreased “intra-functional group” competition in presence of legumes. Increased susceptibility of grasses to aphid infestation in presence of decomposers has been reported previously (Scheu et al. 1999), although in other studies aphid reproduction remained unaffected (Bonkowski et al. 2001) or was even reduced (Wurst et al. 2003, Schütz et al. 2008, X. Ke and S. Scheu, unpubl.). Increased aphid infestation has been related to decomposer-mediated increase in N concentration in plant tissue and this was also responsible for increased aphid numbers in our experiment. Herbivore performance is known to strongly depend on plant tissue N concentrations and therefore, earthworm-mediated increase in plant tissue N concentrations likely propagate into the herbivore system. Thus, the activity of the below-ground decomposer community may strongly impact the above-ground system by altering the infestation by herbivores and, thereby, the above-ground food web.

Earthworms not only affected yield related parameters of legumes and grasses but also the flowering of legumes. Presumably, due to fostering the competitive strength of grasses, earthworms influence the plant community composition, thereby, decreasing the proportion of legume biomass and the number of legume flowerheads. Consequently, even though earthworms likely increase plant productivity, they potentially influence pollinators and the rate of pollination of legumes negatively as Poveda et al. (2005) showed that the number of flower visits is strongly correlated with the number of flowers per plant.

The quality of forage

In total, the presence of earthworms only increased the amount of shoot N in the grasses only treatment. The legumes only treatment and the mixture contained significantly higher amounts of shoot N than the grasses only treatment but were not affected by earthworm presence. These results suggest that earthworms are able to increase the amount of N in grass hay but they play an inferior role in grass-legume associations where the presence of legumes may be more important and probably increases the quality of forage and herbage yield under field conditions.

2.6 Conclusions

Competition for nutrients is one of the main processes structuring plant communities and closely links plants to the decomposer community. The present study emphasizes the importance of earthworms as regulatory forces of nutrient mineralization and driving agents of plant competition (Fig. 2.5). Increased availability of mineral N in soil due to earthworm presence enhanced plant growth, in particular that of grasses, thereby fostering the competitive strength of grasses against legumes. Similarly, legumes also beneficially affected grasses. Rather than due to transfer/leakage of N fixed by legumes, this presumably was caused by legumes decreasing the “intra-functional group” competition among grasses. Earthworms increased the yield of grass “monocultures”, the amount of N in grass hay and potentially reduce the attractiveness of grass-legume associations to pollinators and the rate of pollination of legumes by reducing the amount of flowerheads. Moreover, earthworms potentially affect the above-ground food web by increasing the susceptibility of grasses for being infested by aphids. Our findings highlight the intimate interrelationship between the above- and below-ground systems and accentuate the particular significance of earthworms linking these two systems.

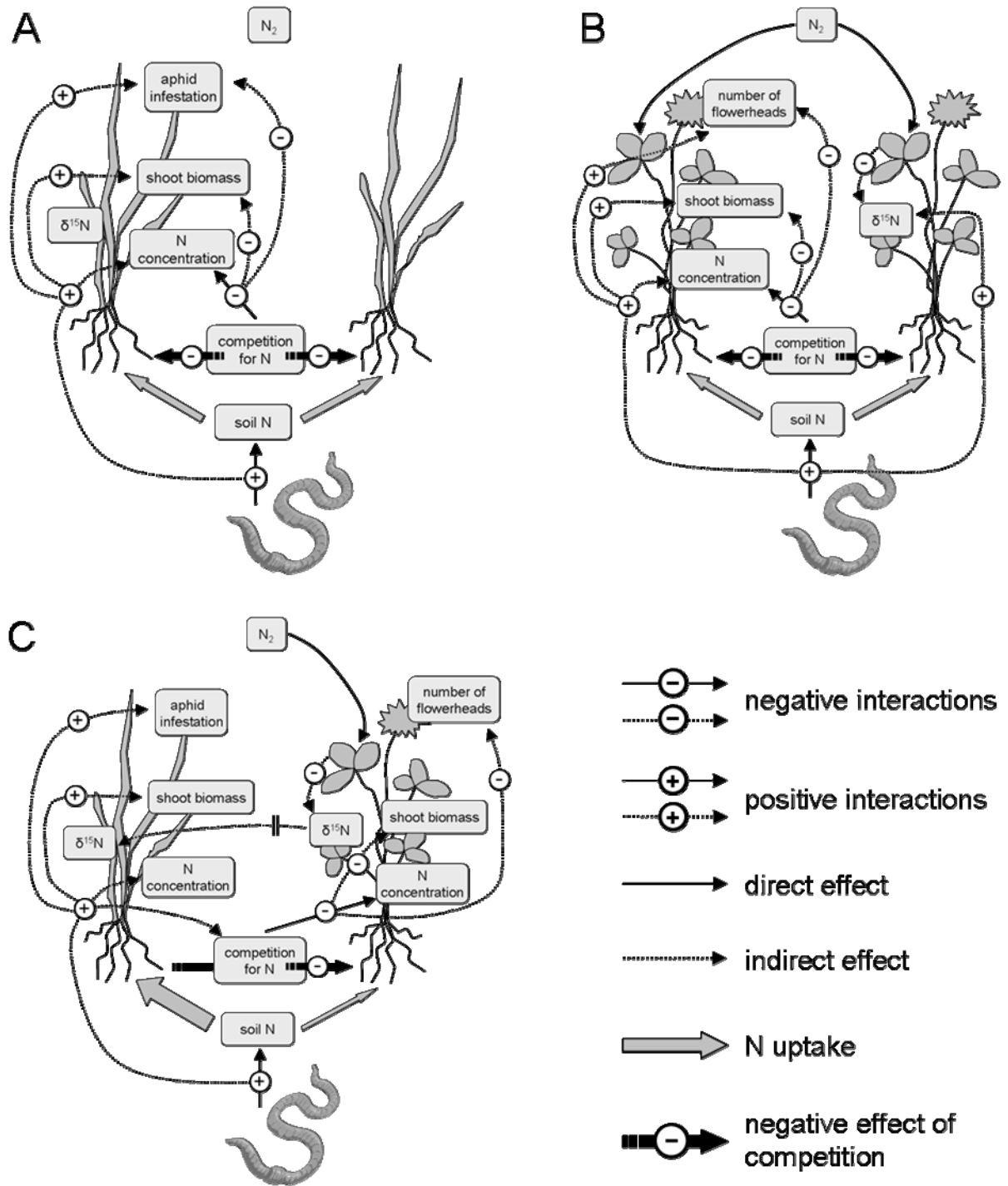
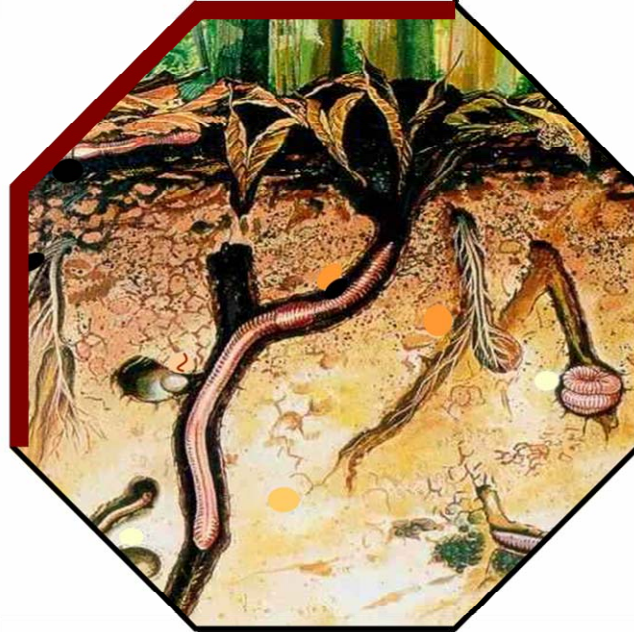


Figure 2.5 | Summary of probable and hypothetical mechanisms by which earthworms may affect the competition between grasses and legumes for N based on experimental data on shoot N concentration, shoot biomass of grasses and legumes, aphid infestation of grasses, number of legume flowerheads, and $\delta^{15}\text{N}$ of grass and legume shoots: “Intra- and inter-functional group” competition between (A) grasses, (B) legumes and (C) grasses and legumes.

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).

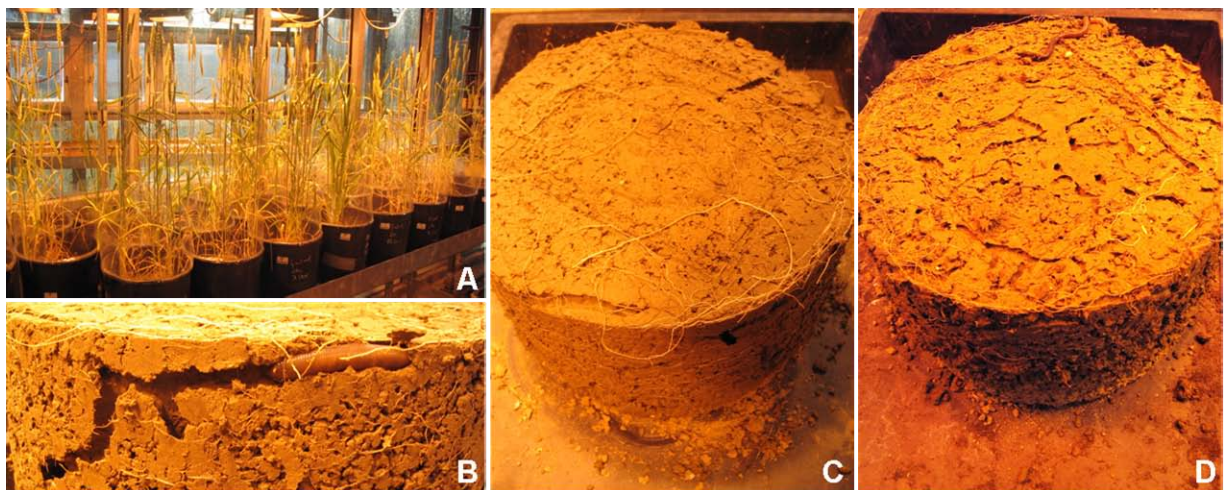


Figure 3.1 | Photos taken at the end of the experiment (after nine weeks). (A) Part of the 28 mesocosms in the greenhouse. (B) Soil core (section) of the treatment with *Lumbricus terrestris* showing an animal with large burrows. (C) Soil core of the treatment with *Lumbricus rubellus friendoides* showing 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.

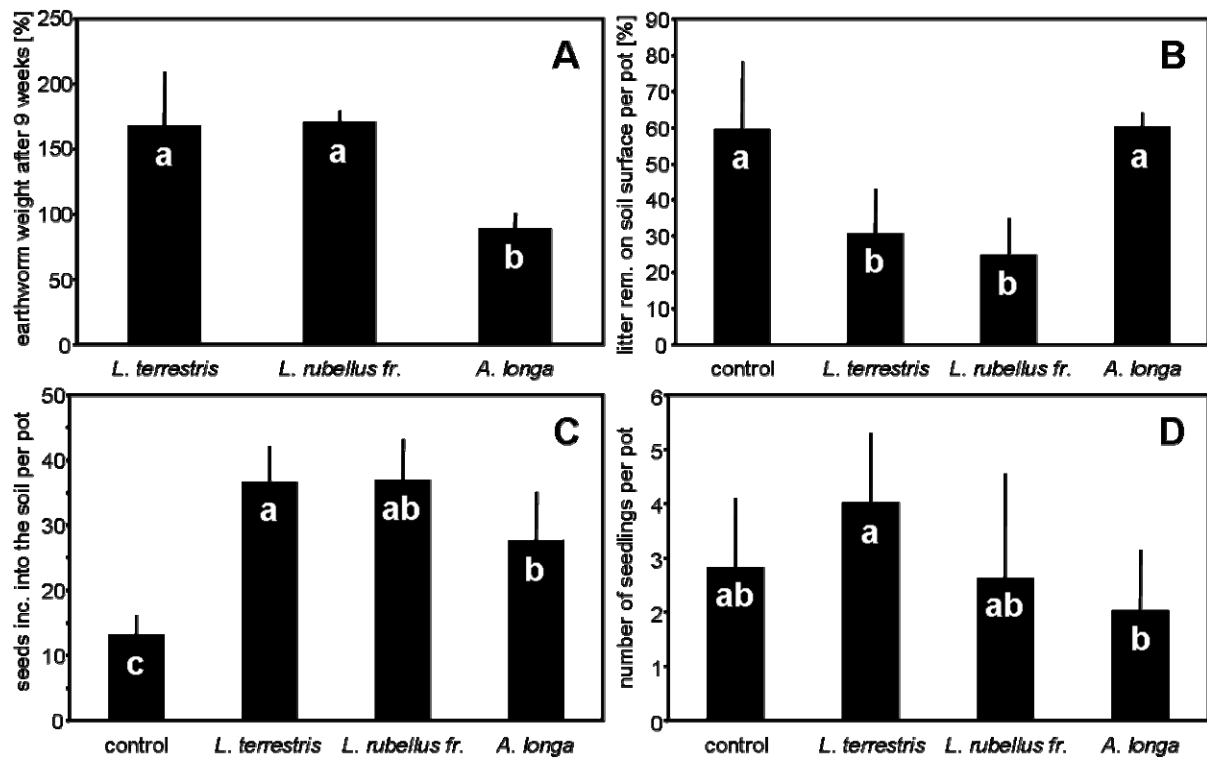


Figure 3.2 | (A) Changes in body fresh weight of *Lumbricus terrestris*, *Lumbricus rubellus friendoides* and *Aporrectodea longa* during the experiment [% of initial], effects of earthworms (Control, *Lumbricus terrestris*, *Lumbricus rubellus friendoides* and *Aporrectodea longa*) on (B) litter remaining 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 $20/16 \pm 2^\circ\text{C}$ (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.

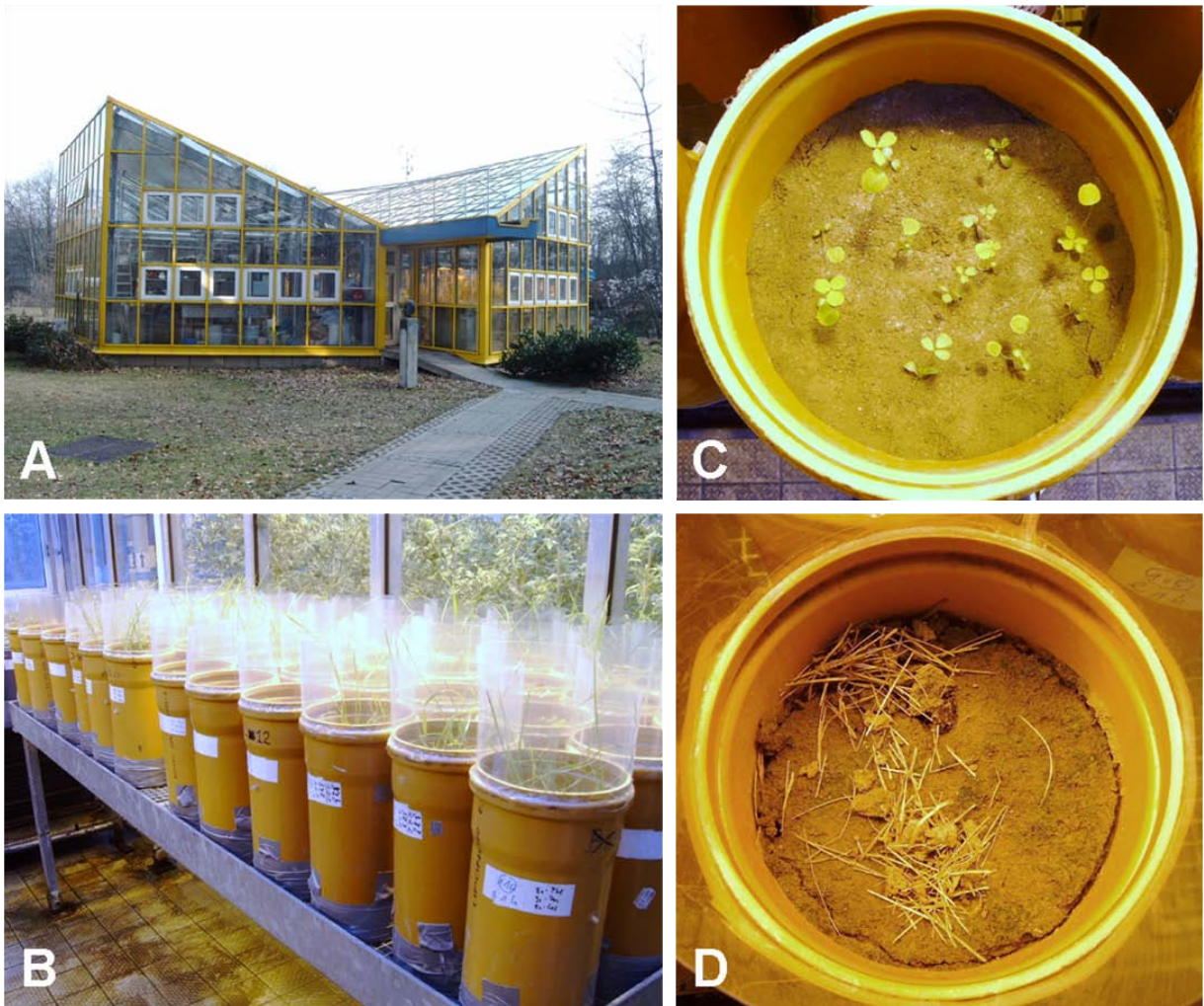


Figure 4.1 | (A) Photograph of the experimental greenhouse. (B) Photograph taken about two weeks after experimental start showing a bench with some of the 120 numbered and randomized microcosms under controlled greenhouse conditions. (C) Photograph of one microcosm (legume community) directly after transplanting 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).

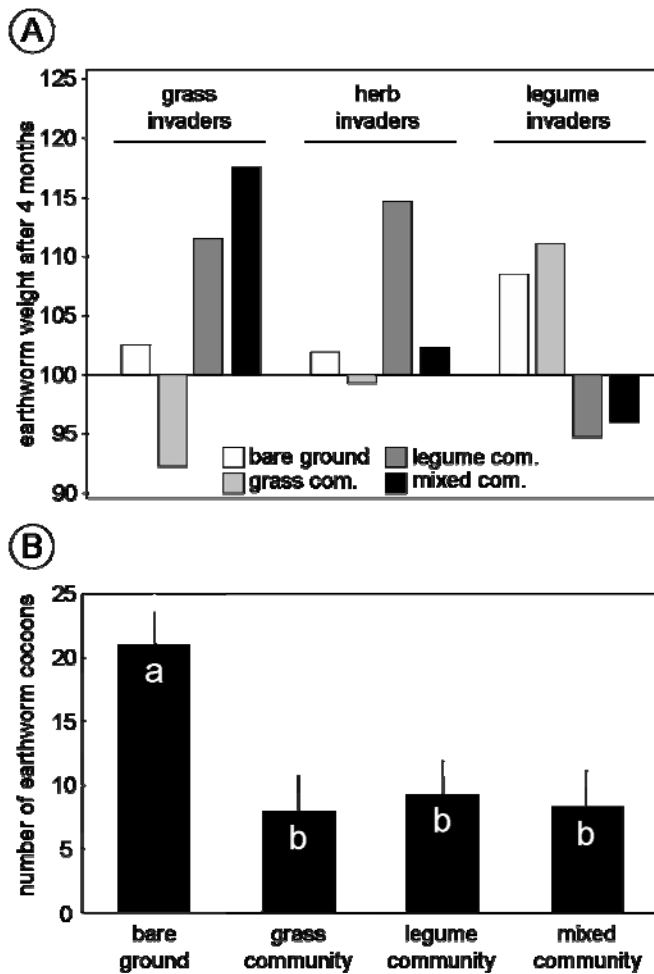


Figure 4.2 | (A) Changes in body fresh weight of *Lumbricus terrestris* during the experiment [% of initial] as affected by the established plant community (bare ground, grass community, legume community and mixed community) and the functional group of the invading plant species (grass, legume and herb 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).

| | | | established plants | | invader biomass | |
|-----------------|----|-----|--------------------|------------------|-----------------|------------------|
| | Df | N | 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.

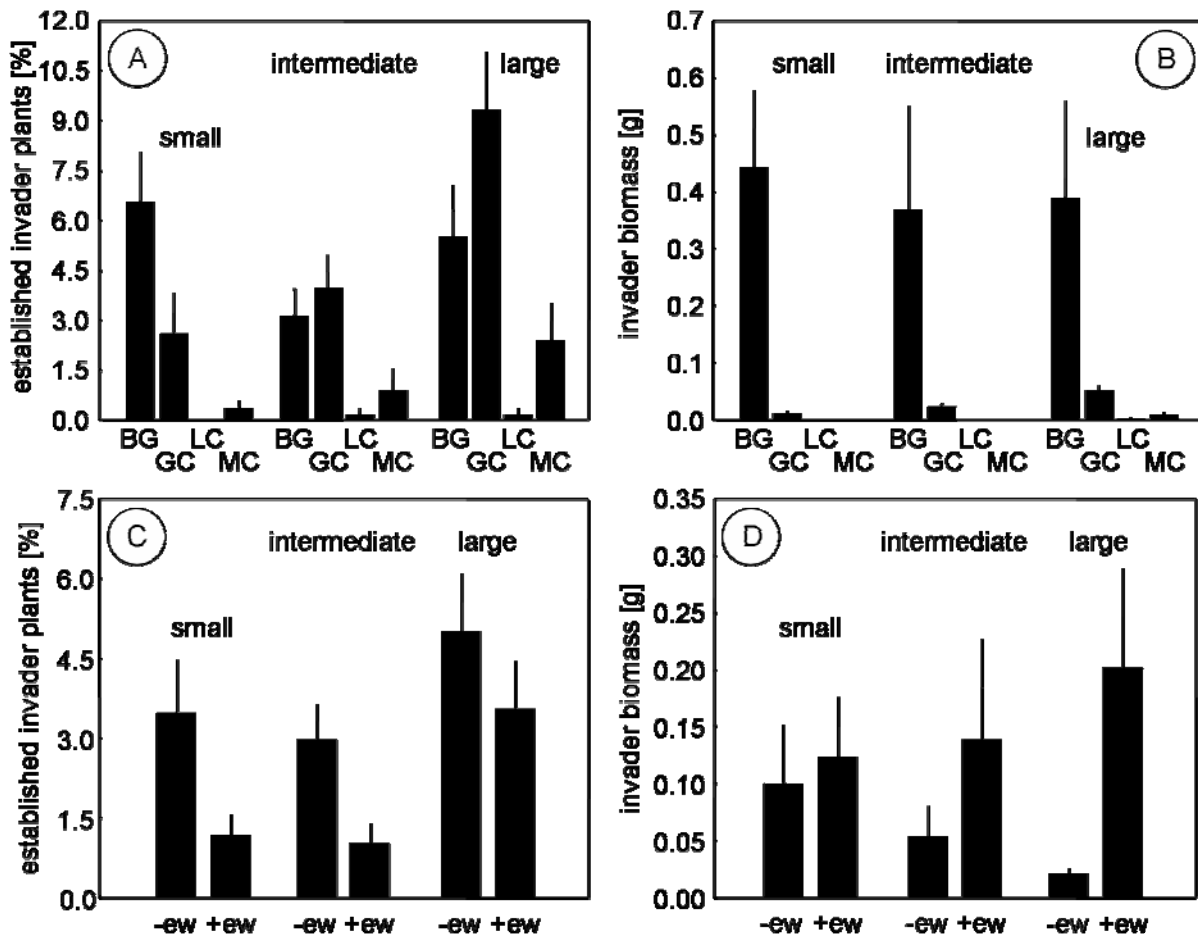


Figure 4.3 | Variations in (A) the number [% of applied seeds] and (B) the biomass [g] of established invader plants as affected by the seed size of the invader plants (small, intermediate and large) and the established plant community (bare ground [BG], grass community [GC], legume community [LC] and 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.

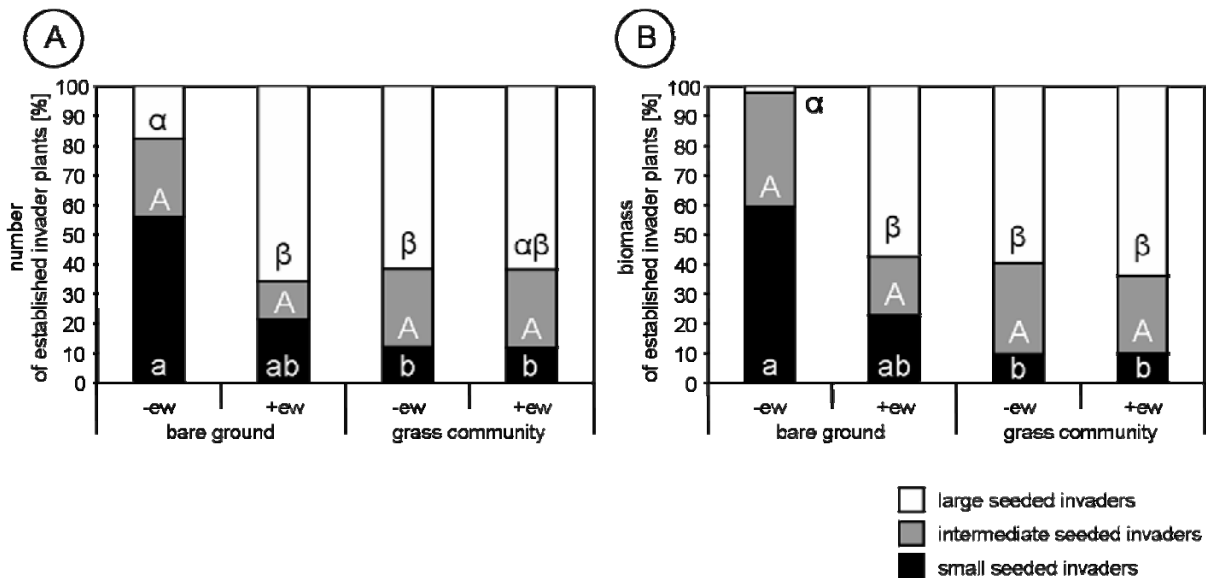


Figure 4.4 | Variations in the proportion of (A) the number and (B) the biomass of different seed size classes (small, intermediate and large seeded invaders) as affected by the plant community (bare ground and grass community) and the presence of earthworms (*Lumbricus terrestris*; -ew: without and +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.

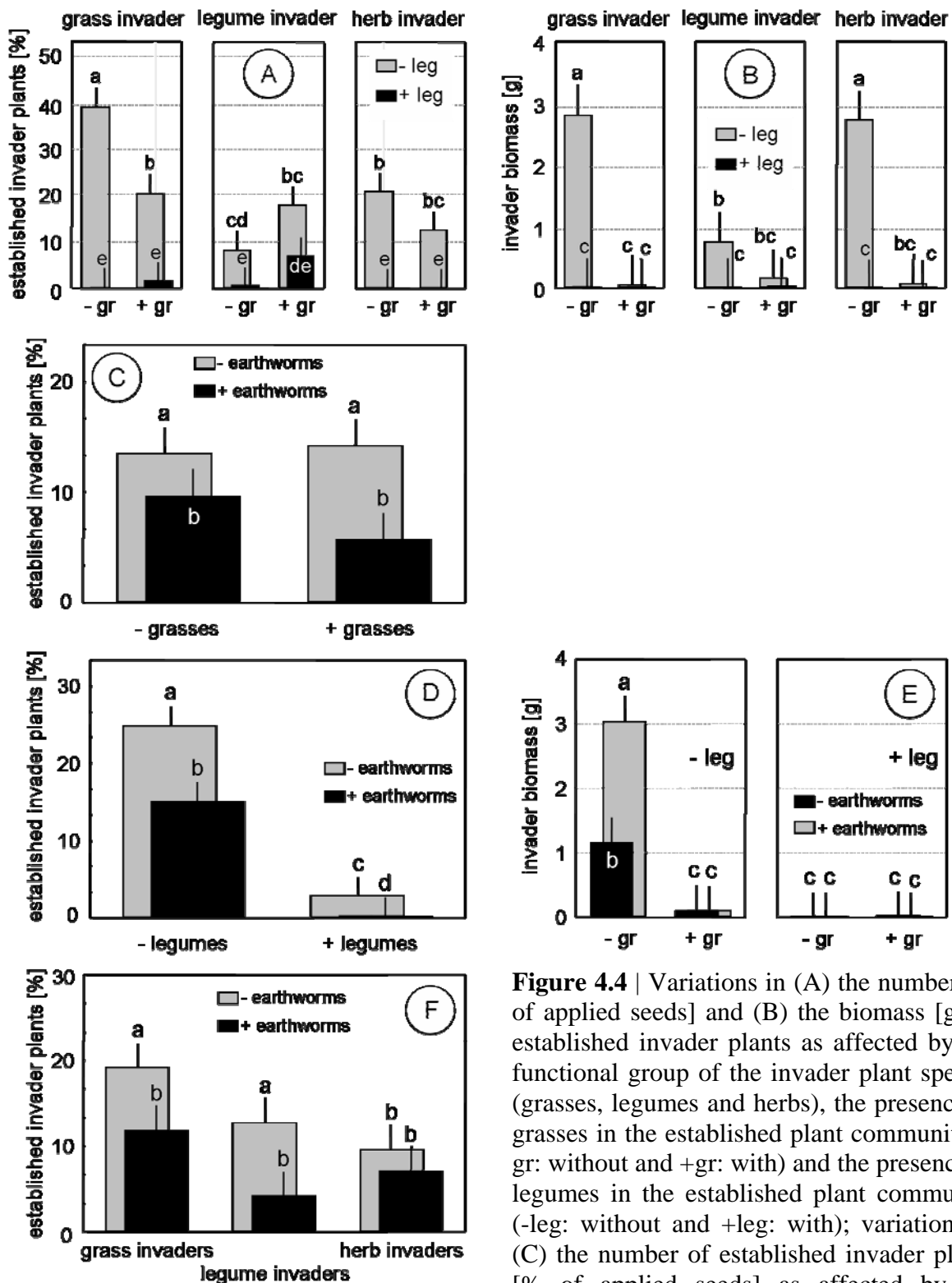
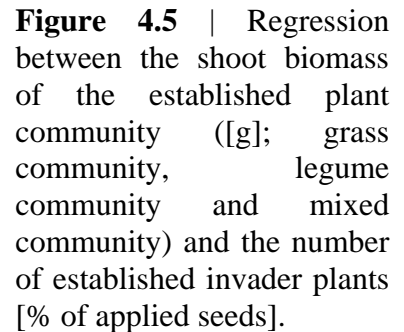


Figure 4.4 | Variations in (A) the number [% of applied seeds] and (B) the biomass [g] of established invader plants as affected by the functional group of the invader plant species (grasses, legumes and herbs), the presence of grasses in the established plant community (-gr: without and +gr: with) and 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, $\alpha < 0.05$).



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.

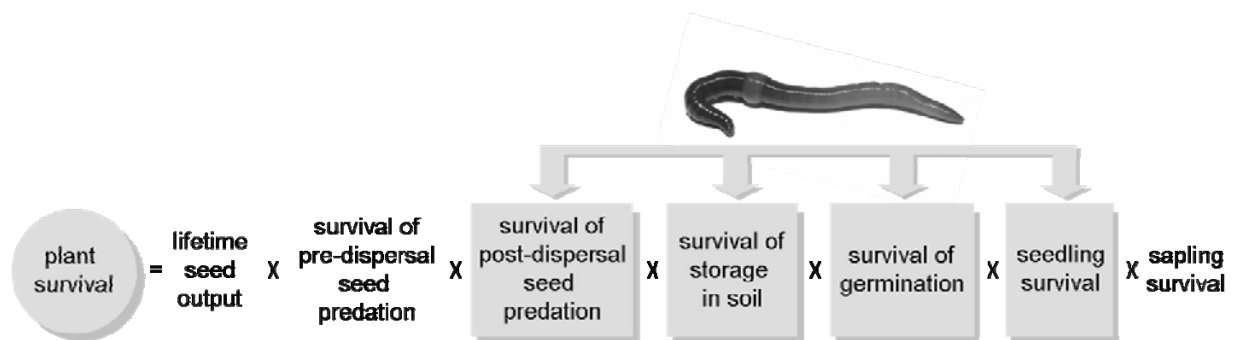


Figure 4.7 | Plant survival as a function of the survival of different plant life stages with emphasis on life stages directly influenced by earthworms. Modified after Moles and Westoby (2006).

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).

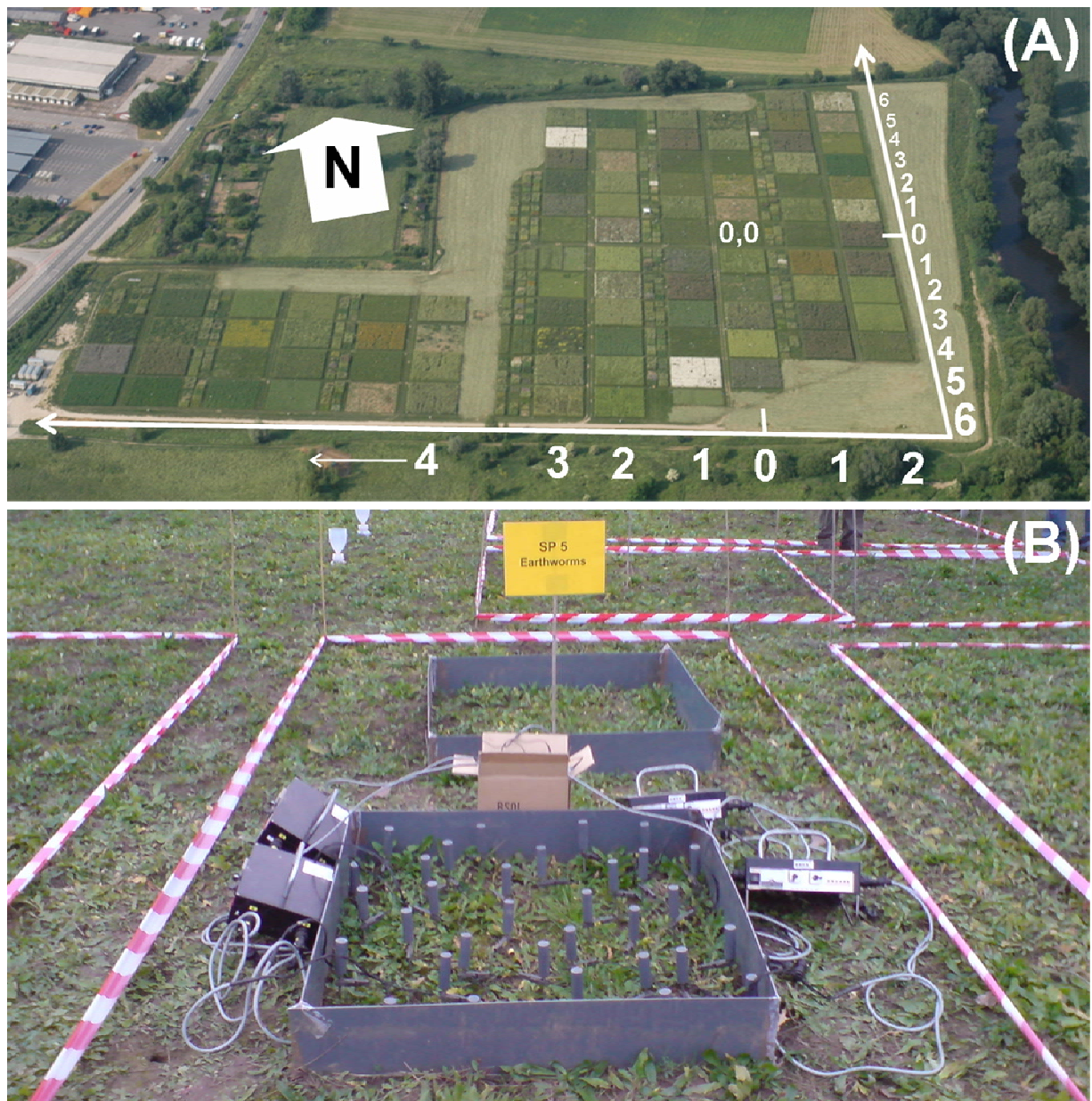


Figure 5.1 | (A) Photograph of the field site of The Jena Experiment taken in 2004 showing the main experimental plots (20 x 20 m) varying in sown plant species diversity (1, 2, 4, 8, 16, and 60) and plant functional group diversity (1, 2, 3, and 4) and the X- (horizontal axis) and Y-coordinates (vertical 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 electro-shocking. 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 | |
|------------------------|----|---------------|----------------|------------------|------------------|----------------|----------------|------------------|-----------------|-----------|
| | | | number | biomass | number | biomass | number | biomass | diversity | diversity |
| X | 1 | Plot | excluded | excluded | excluded | 2.11 ns | excluded | excluded | excluded | excluded |
| Y | 1 | Plot | excluded | 0.63 ns | excluded | excluded | excluded | 0.71 ns | 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 |
| Fg | 3 | Plot | 1.78 ns | 1.81 ns | 4.28* ↓ | 3.63* ↓ | 0.70 ns | 0.91 ns | | |
| Gr | 1 | Plot | 1.22 ns | 4.63* ↓ | 11.83** ↓ | 4.83* ↓ | excluded | 3.51(*) ↓ | 2.44 ns | excluded |
| Sh | 1 | Plot | excluded | excluded | excluded | 1.41 ns | excluded | excluded | excluded | excluded |
| Th | 1 | Plot | excluded | excluded | 2.53 ns | 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 | |
| Plot | 34 | Subplot | 8.78*** | 9.12*** | 7.68*** | 5.08*** | 6.22*** | 8.50*** | 9.61*** | |
| Ew | 1 | Subplot | 0.59 ns | excluded | 3.47(*) ↓ | 0.30 ns | 0.39 ns | 0.66 ns | 0.19 ns | |
| Ew×S | 2 | Subplot | 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 | |
| Number of Df max model | | | 17 | 17 | 17 | 17 | 17 | 17 | 17 | |
| Number of Df min model | | | 11 | 8 | 12 | 15 | 10 | 9 | 11 | |
| AIC max model | | | -249.41 | -271.02 | -278.84 | -253.92 | -223.28 | -291.36 | -433.50 | |
| AIC min model | | | -259.41 | -281.84 | -286.69 | -258.61 | -235.17 | -297.74 | -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 | |
|------------------------|----|---------------|-----------------|-------------------|-------------------|-------------------|-----------------|------------------|-------------------|-------------------|
| | | | number | biomass | number | biomass | number | biomass | diversity | diversity |
| X | 1 | Plot | excluded | excluded | excluded | excluded | excluded | excluded | excluded | excluded |
| Y | 1 | Plot | excluded | excluded | excluded | excluded | excluded | excluded | excluded | excluded |
| S | 2 | Plot | 5.33** ↓ | 14.76*** ↓ | 3.34* ↓ | 4.04* ↓ | 4.14* ↓ | 12.57** ↓ | 12.55*** ↓ | 12.55*** ↓ |
| Fg | 3 | Plot | 3.92* ↓ | 10.32*** ↓ | 2.88* ↓ | 4.67** ↓ | 3.17* ↓ | 8.67*** ↓ | 8.70*** ↓ | 8.70*** ↓ |
| Gr | 1 | Plot | excluded | 5.05* ↓ | 12.72*** ↓ | 17.79*** ↓ | 0.95 ns | excluded | excluded | 1.65 ns |
| Sh | 1 | Plot | excluded | 0.42 ns | 1.42 ns | excluded | excluded | excluded | excluded | 1.05 ns |
| Th | 1 | Plot | 0.35 ns | excluded | 3.34(*) ↑ | 5.65* ↓ | excluded | excluded | excluded | excluded |
| Leg | 1 | Plot | 0.57 ns | excluded | excluded | 2.01 ns | 2.01 ns | excluded | excluded | excluded |
| Plot | 34 | Subplot | 19.62*** | 9.90*** | 21.62*** | 14.14*** | 15.48*** | 9.46*** | 7.24*** | 7.24*** |
| Ew | 1 | Subplot | 0.81 ns | 1.62 ns | excluded | excluded | excluded | 2.19 ns | excluded | 0.81 ns |
| Ew×S | 2 | Subplot | 0.89 ns | excluded | excluded | excluded | excluded | excluded | excluded | 0.47 ns |
| Ew×Fg | 3 | Subplot | 1.22 ns | excluded | excluded | excluded | excluded | excluded | excluded | 1.51 ns |
| Number of Df max model | | | 17 | 17 | 17 | 17 | 17 | 17 | 17 | 17 |
| Number of Df min model | | | 13 | 8 | 8 | 8 | 7 | 7 | 13 | 13 |
| AIC max model | | | -325.12 | -305.56 | -319.13 | -320.31 | -320.52 | -313.01 | -501.86 | -501.86 |
| AIC min model | | | -333.12 | -313.58 | -326.74 | -328.59 | -327.52 | -325.08 | -509.86 | -509.86 |

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 | diversity | 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 | 5.35* ↓ | |
| Leg | 1 | Plot | excluded | 0.91 ns | 1.15 ns | 4.19* ↑ | 2.84(*) ↓ | 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 | |
| Number of Df min model | | | 12 | 9 | 15 | 7 | 7 | 11 | 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, ***

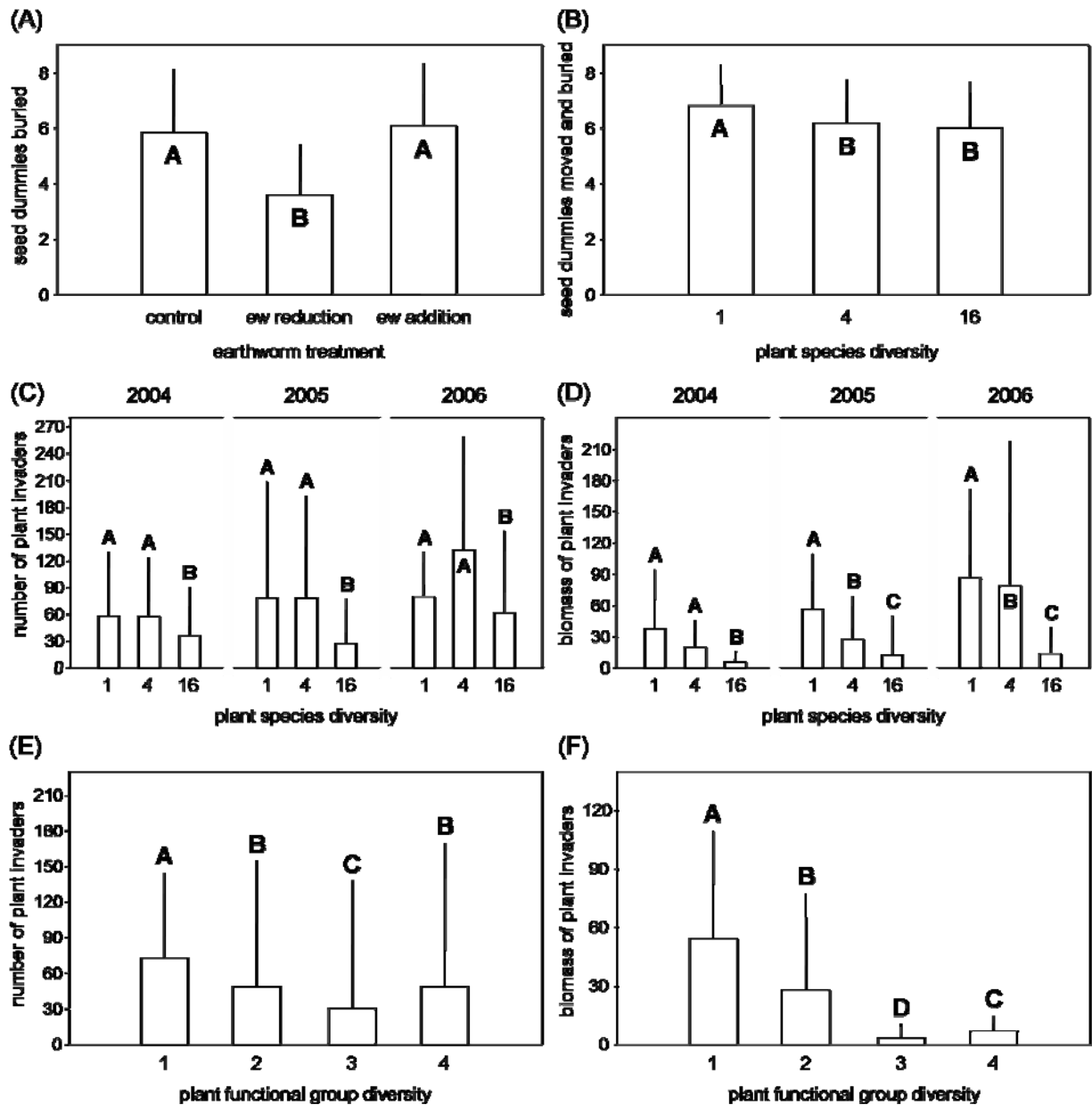


Figure 5.2 | (A) Effects of earthworm treatment (control, earthworm [ew] reduction, and earthworm addition) on the number of buried seed dummies after seven days (May 2006); (B) Effects of sown plant species diversity (1, 4, and 16-species mixtures) on the number of moved and buried seed dummies after 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, $P < 0.05$).

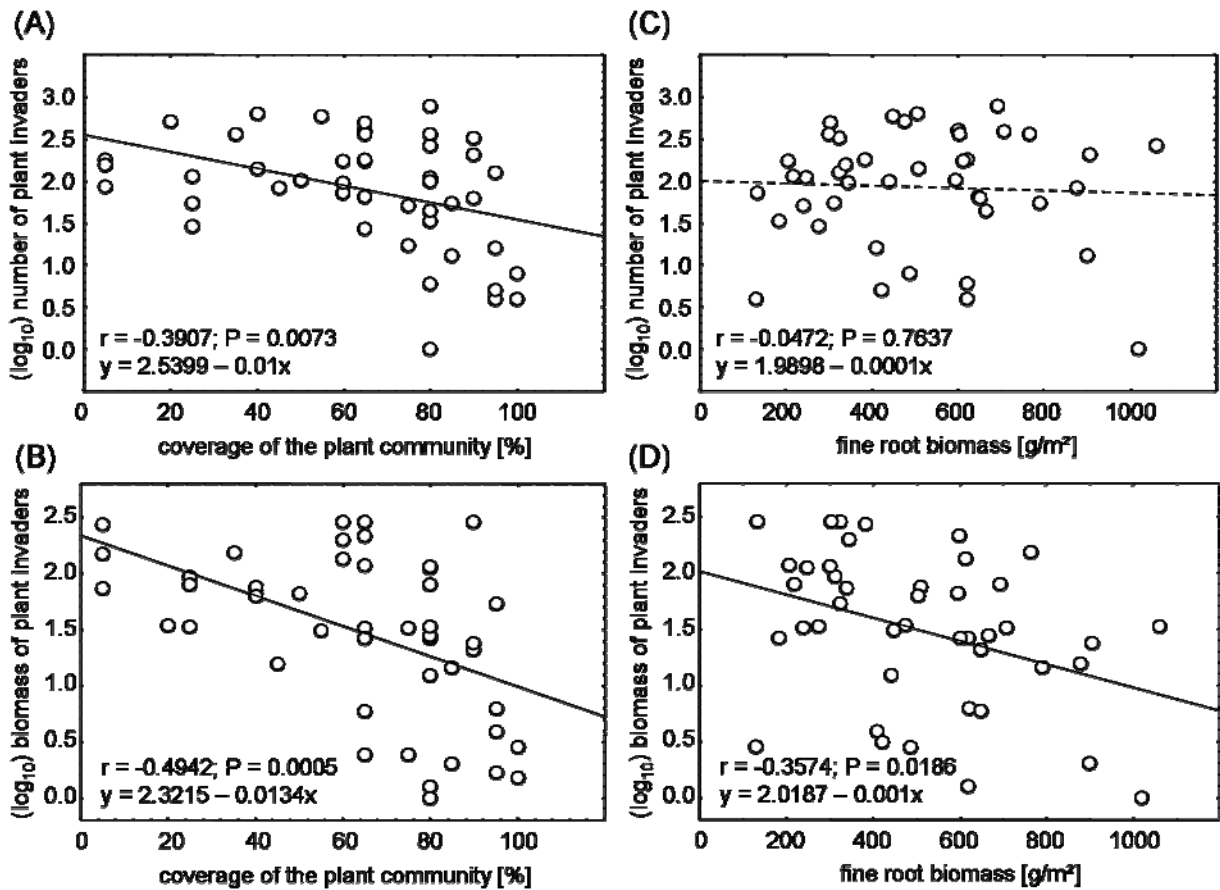


Figure 5.3 | Regressions of the coverage of the plant community [%] and (A) the number of total plant invaders ($\text{ind.}/\text{m}^2$; \log_{10} -transformed data) and (B) the biomass of total plant invaders (g/m^2 ; \log_{10} -transformed data) and regressions of the fine root biomass [g/m^2] and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

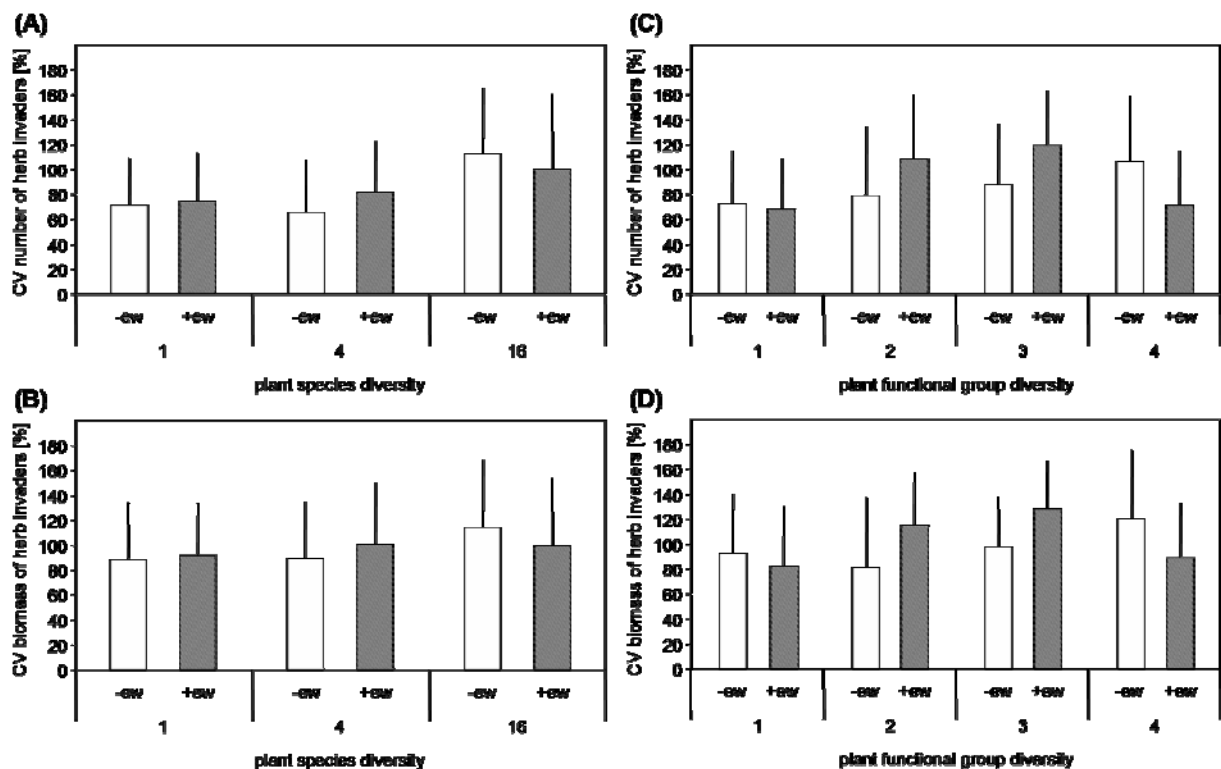


Figure 5.4 | Effects of sown plant species diversity (1, 4, and 16-species mixtures) and earthworm treatment (earthworm reduction [-ew] and earthworm addition [+ew]) on the coefficient of variance (CV; [%]) of (A) the number and (B) biomass of herb invaders in the years 2004 to 2006 and effects of plant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

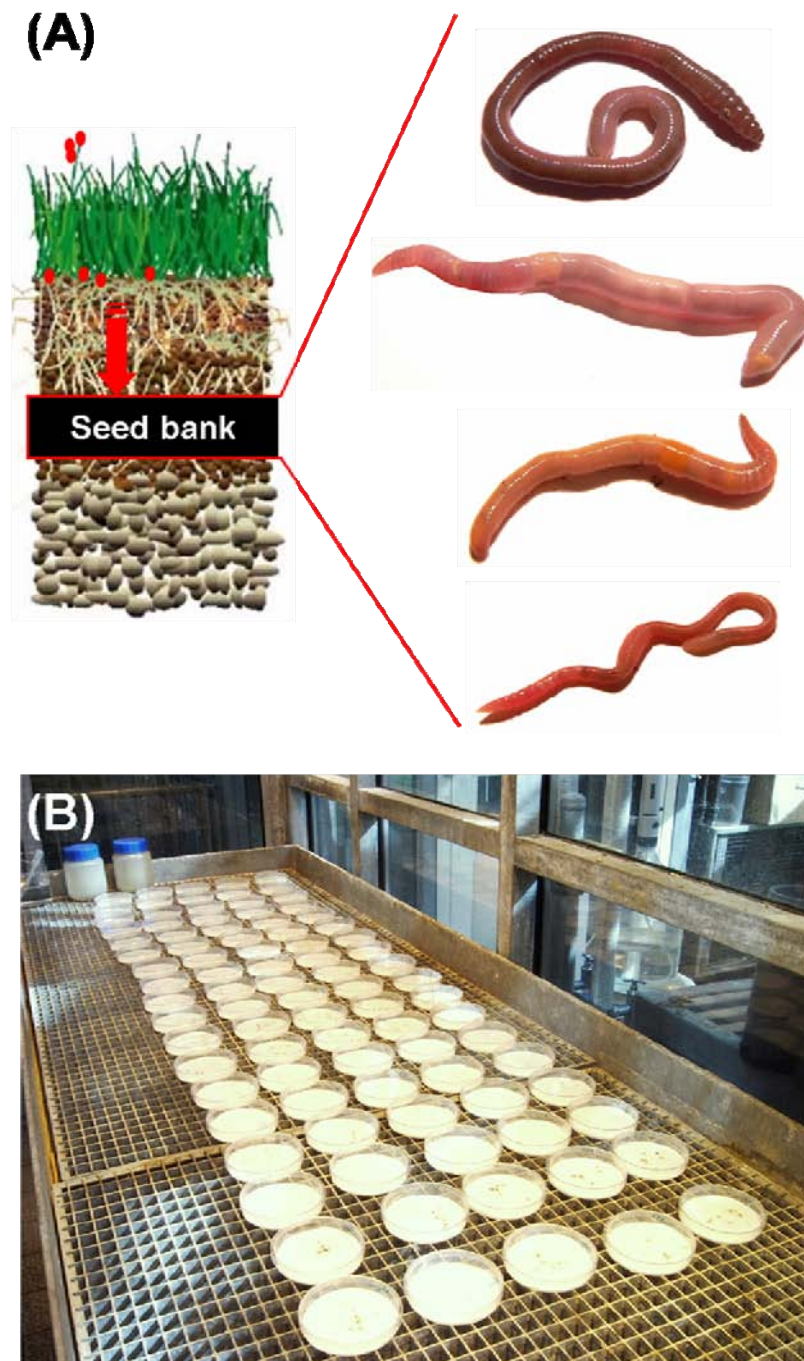


Figure 6.1 | (A) Scheme of the translocation of plant seeds (red ovals) to the soil seed bank. Endogeic earthworm species (at the field site of The Jena Experiment: *Aporrectodea caliginosa*, *Octolasion tyrtaeum*, *Allolobophora chlorotica*, and *Aporrectodea rosea*, from top to bottom) live and feed 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).

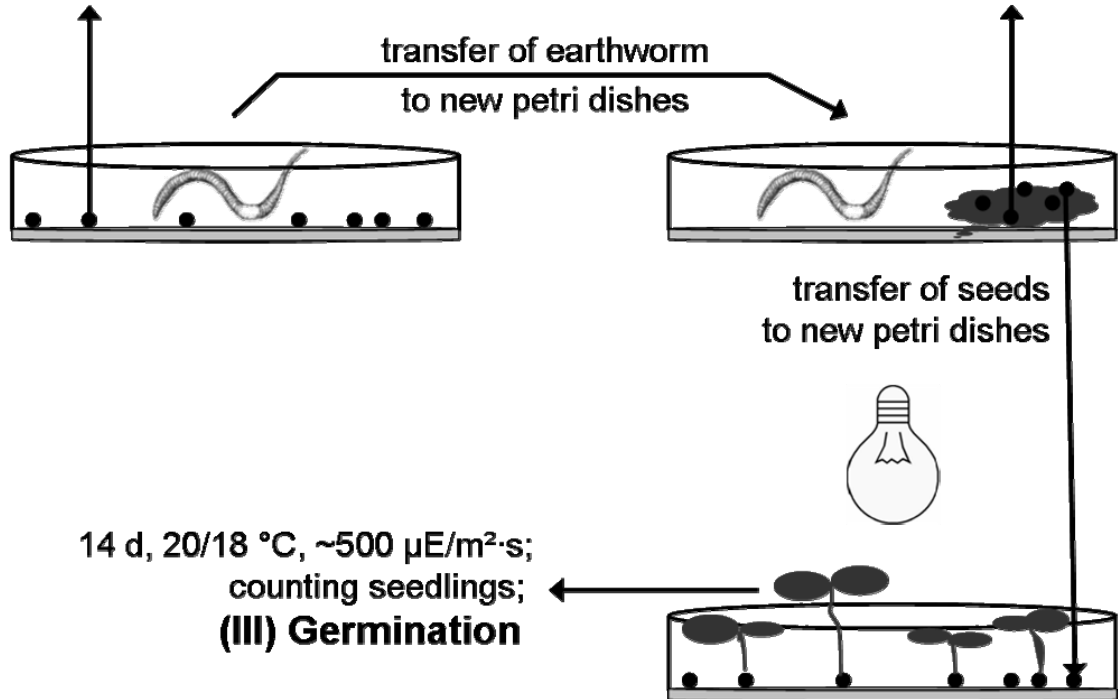
Experiment (A)

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

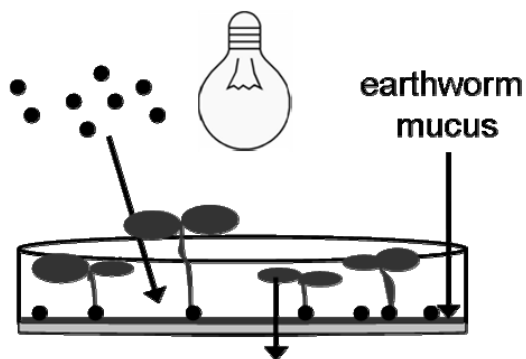
(I) Ingestion

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

(II) Digestion

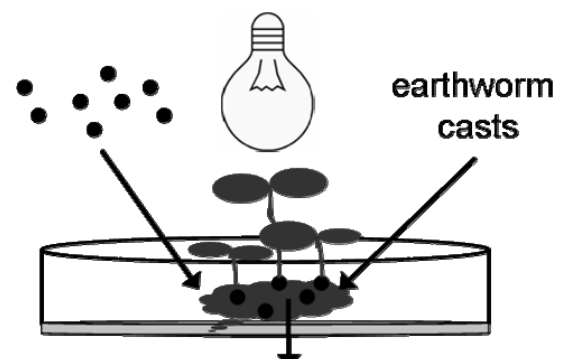


Experiment (B)



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, ***

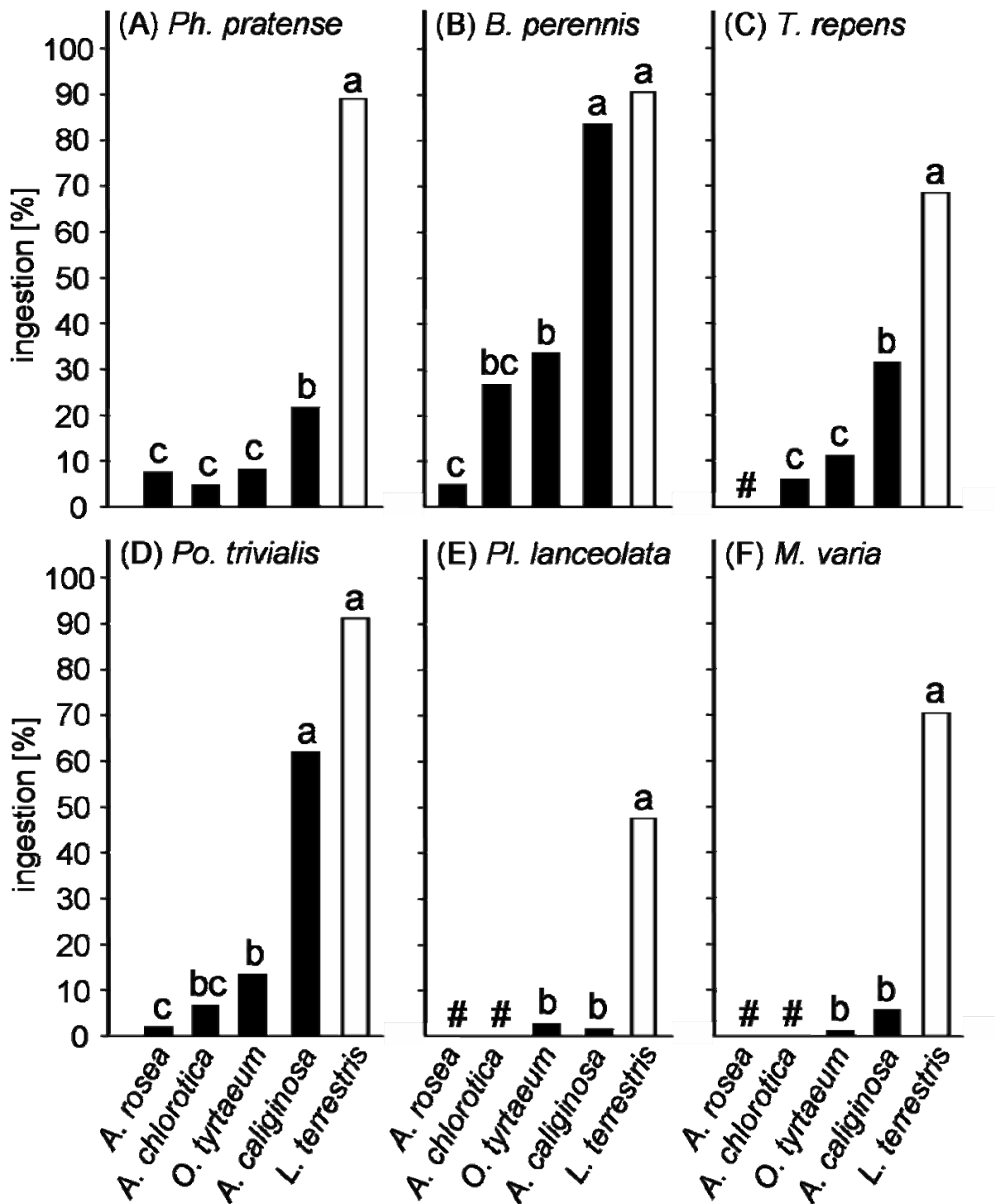


Figure 6.3 | Number of seeds [%] from different plant species [(A) *Phleum pratense*, (B) *Bellis perennis*, (C) *Trifolium repens*, (D) *Poa trivialis*, (E) *Plantago lanceolata*, and (F) *Medicago varia*] ingested by different earthworm species (*Aporrectodea rosea*, *Allolobophora chlorotica*, *Octolasion 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).

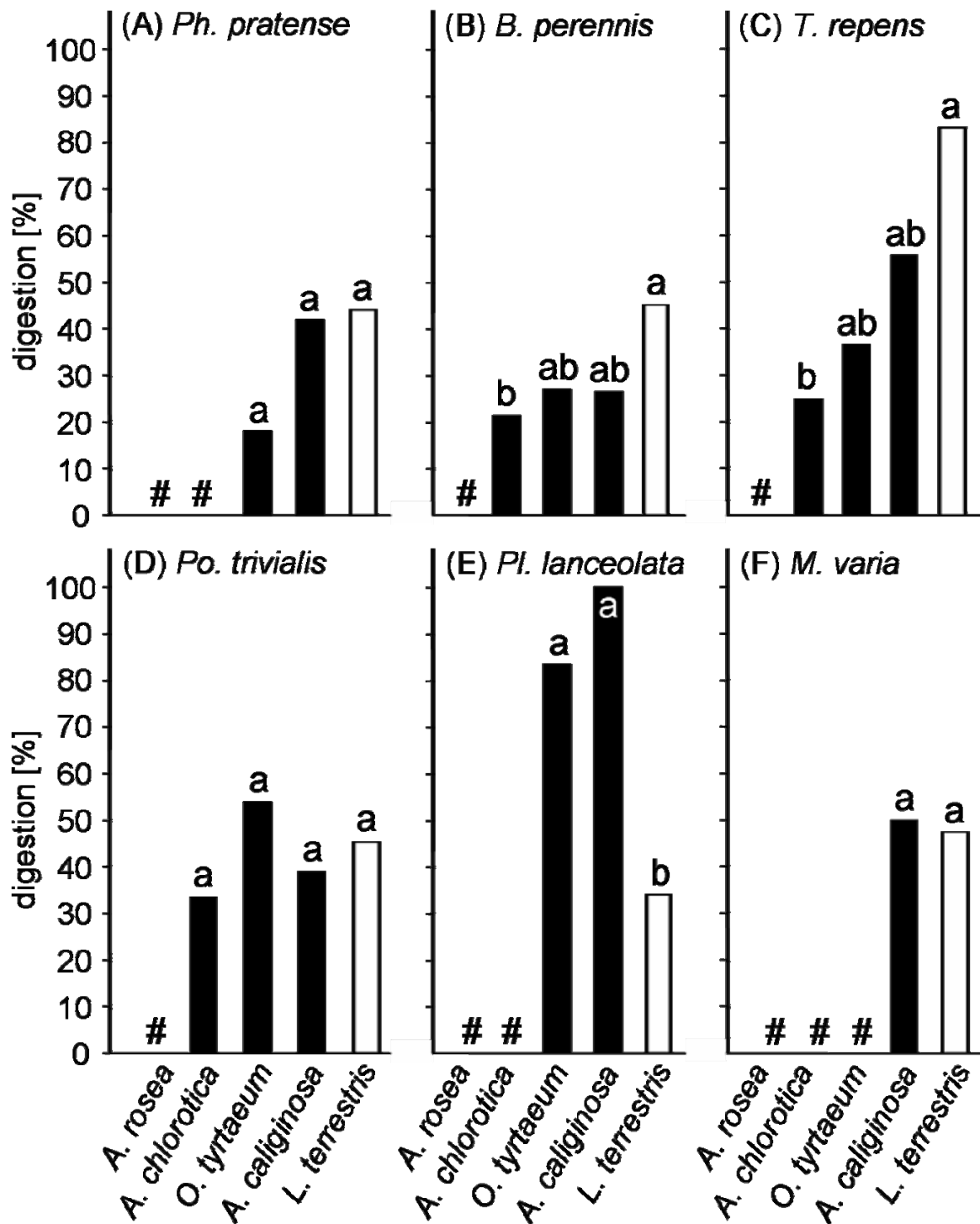


Figure 6.4 | Number of ingested seeds [%] from different plant species [(A) *Phleum pratense*, (B) *Bellis perennis*, (C) *Trifolium repens*, (D) *Poa trivialis*, (E) *Plantago lanceolata*, and (F) *Medicago varia*] digested by different earthworm species (*Aporrectodea rosea*, *Allolobophora chlorotica*, *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).

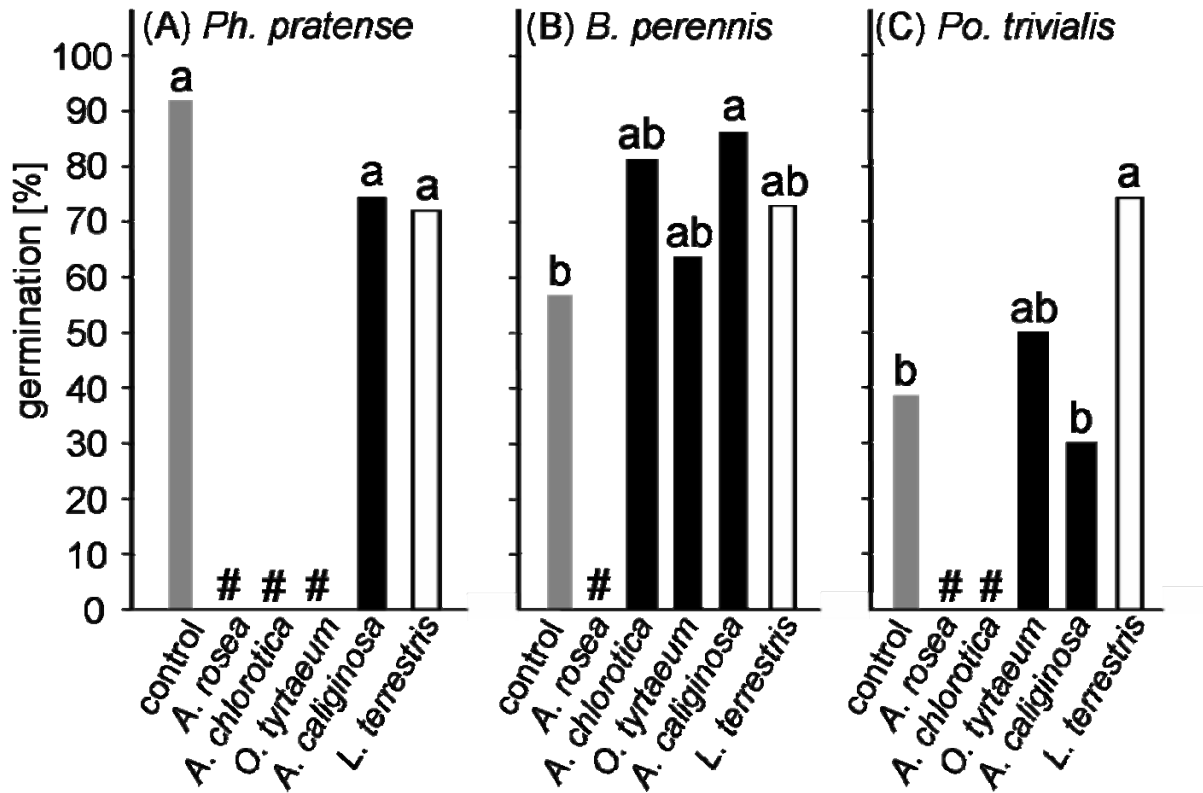


Figure 6.5 | Number of germinated seeds [%] from different plant species [(A) *Phleum pratense*, (B) *Bellis perennis*, and (C) *Poa trivialis*] after gut passage of different earthworm species (*Aporrectodea rosea*, *Allolobophora chlorotica*, *Octolasion tyrtæum*, *Aporrectodea caliginosa*, and *Lumbricus 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.

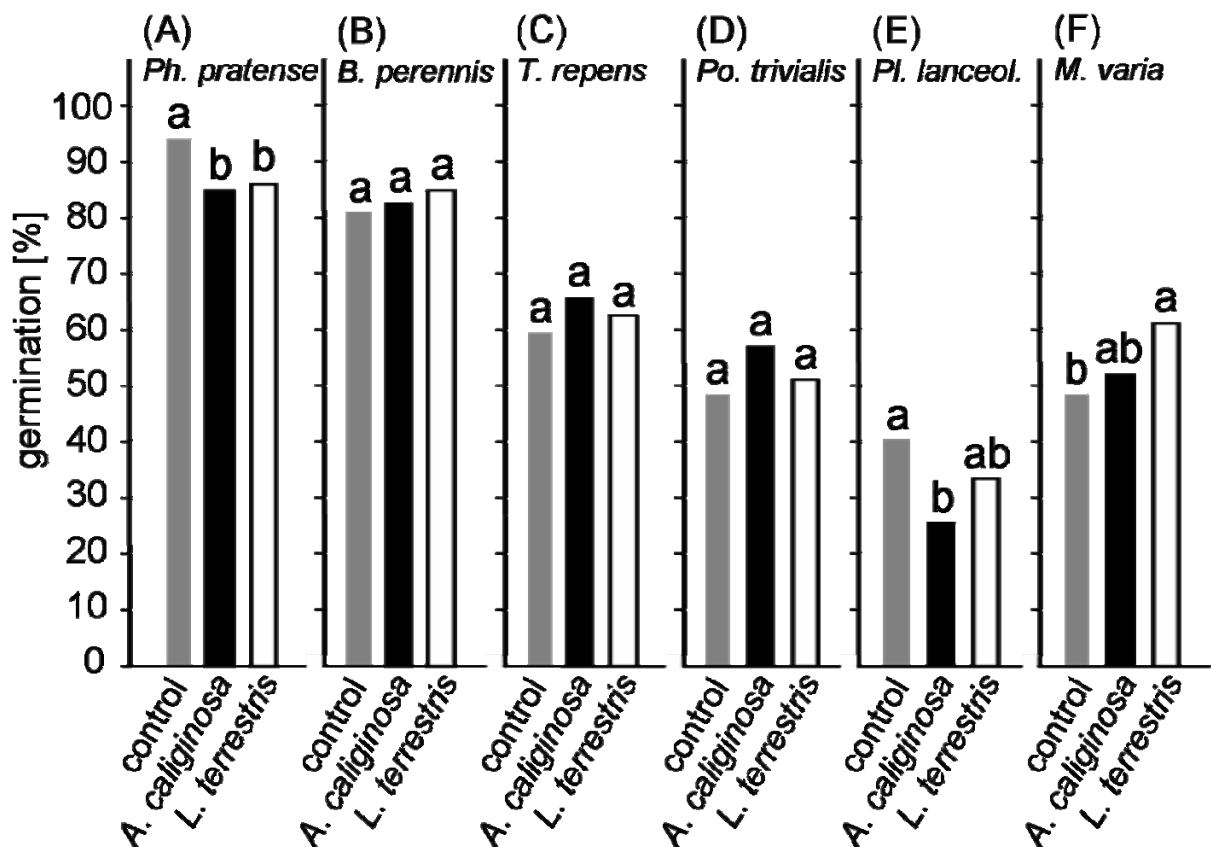


Figure 6.6 | Number of germinated seeds [%] from different plant species [(A) *Phleum pratense*, (B) *Bellis perennis*, (C) *Trifolium repens*, (D) *Poa trivialis*, (E) *Plantago lanceolata*, and (F) *Medicago varia*] as affected by earthworm mucus (control, *Aporrectodea caliginosa*, and *Lumbricus 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).

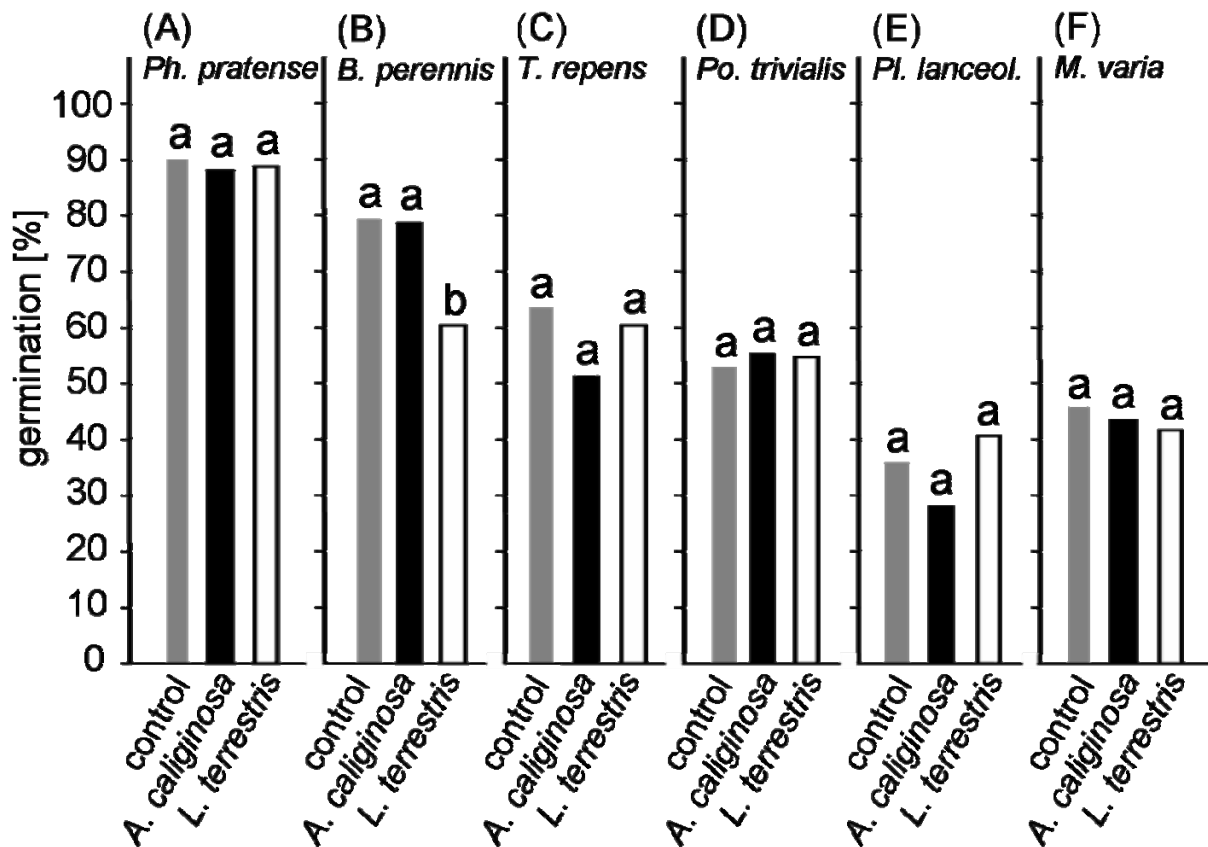


Figure 6.7 | Number of germinated seeds [%] from different plant species [(A) *Phleum pratense*, (B) *Bellis perennis*, (C) *Trifolium repens*, (D) *Poa trivialis*, (E) *Plantago lanceolata*, and (F) *Medicago varia*] as affected by earthworm casts (control, *Aporrectodea caliginosa*, and *Lumbricus 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.

CHAPTER

7



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).

| | Block 1 | Block 2 | Block 3 | Block 4 |
|----------------------|---------|---------|---------|---------|
| Lime content | 6 % | 10 % | 13 % | 28 % |
| Clay content | 14 % | 21 % | 24 % | 22 % |
| Silt content | 41 % | 54 % | 61 % | 69 % |
| Sand content | 45 % | 25 % | 15 % | 9 % |
| Field capacity | 18 % | 17 % | 17 % | 20 % |
| Water content (-) | 13 % | 12 % | 13 % | 10 % |
| Water content (+) | 20 % | 16 % | 17 % | 17 % |
| Number of anecics | 8 | 4 | 2 | 5 |
| Biomass of anecics | 14 | 7 | 4 | 9 |
| Number of endogeics | 20 | 12 | 6 | 13 |
| Biomass of endogeics | 5 | 4 | 3 | 6 |

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).

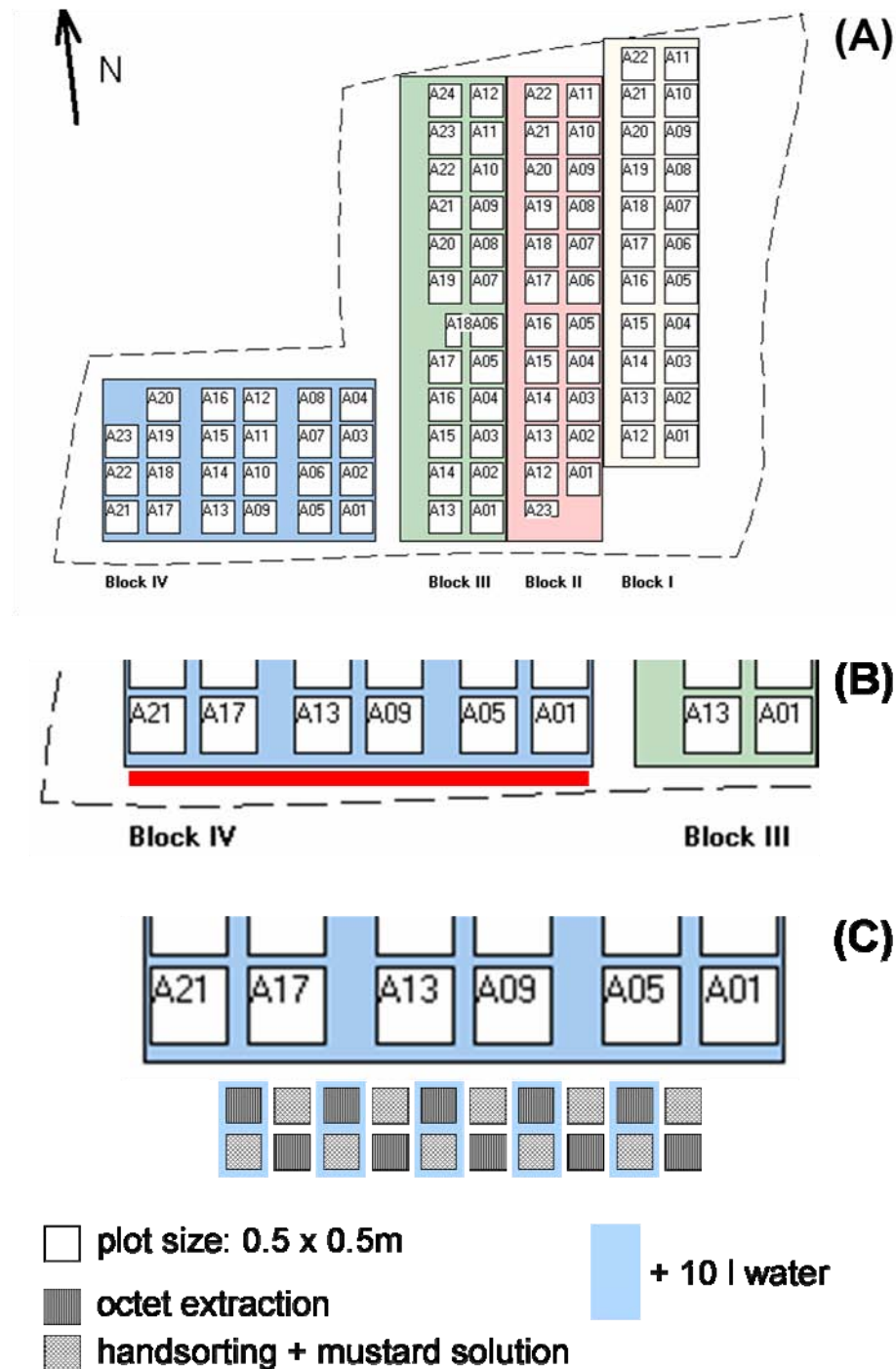


Figure 7.1 | (A) Scheme of the field site of The Jena Experiment. Blocks are indicated by different colors. Experimental plots are indicated by different numbers. (B) Edge of the field site of The Jena Experiment where the samplings took place indicated by the red bar. (C) Scheme of extracted plots per 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 (\pm 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, *Allolobophora chlorotica* Savigny and *Octolasion tyrtaeum* Savigny), and epigeic (*L. castaneus* Savigny). On average 4 ± 4 individuals (16 ind. m^{-2}) and ca. $2 \pm 3 \text{ g}$ fresh weight (10 g m^{-2}) of earthworms were extracted using the octet method. In contrast, 13 ± 10 individuals (51 ind. m^{-2}) and $17 \pm 12 \text{ g}$ (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 ($12 \pm 9 \text{ ind. } 0.25 \text{ m}^2$, $16 \pm 12 \text{ g } 0.25 \text{ m}^2$) than using the octet method ($3 \pm 4 \text{ ind. } 0.25 \text{ m}^2$, $2 \pm 3 \text{ g } 0.25 \text{ m}^2$; Table 7.2; Fig. 7.1A). The number and biomass of extracted epi- and endogeic earthworms were very low irrespective of extraction method (Fig. 7.1A). 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 ($0.28 \pm 0.52 \text{ g } 0.25 \text{ m}^2$) than using the mustard method ($0.08 \pm 0.18 \text{ g } 0.25 \text{ m}^2$) and the number of endogeic individuals showed a similar trend (0.55 ± 0.93 and $0.23 \pm 0.42 \text{ g } 0.25 \text{ m}^2$, 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 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.

| | Anecic earthworms | | | |
|-----------------------|-------------------|------------------|--------------|------------------|
| | number | | biomass | |
| | F-value | P-value | F-value | P-value |
| Soil | 11.39 | <.0001 | 4.52 | 0.0062 |
| Method | 58.18 | <.0001 | 92.70 | <.0001 |
| Water | 2.46 | 0.1216 | 0.37 | 0.5475 |
| Soil x Method | 1.75 | 0.1667 | 2.50 | 0.0677 |
| Soil x Water | 0.17 | 0.9189 | 0.62 | 0.6047 |
| Method x Water | 1.34 | 0.2509 | 0.66 | 0.4198 |
| Soil x Method x Water | 0.38 | 0.7685 | 0.04 | 0.9910 |

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.

| | Endogeic earthworms | | | | Epigeic earthworms | | | |
|--------|---------------------|---------------|-------------|---------------|--------------------|---------|----------|---------|
| | number | | biomass | | number | | biomass | |
| | Chi Squ. | P-value | Chi Squ. | P-value | Chi Squ. | P-value | Chi Squ. | P-value |
| Soil | 9.63 | 0.0220 | 6.35 | 0.0959 | 4.62 | 0.2020 | 4.67 | 0.1976 |
| Method | 3.56 | 0.0594 | 4.26 | 0.0389 | 0.69 | 0.4054 | 0.29 | 0.5930 |
| Water | 0.09 | 0.8919 | 0.23 | 0.6481 | 1.74 | 0.1790 | 0.72 | 0.3945 |

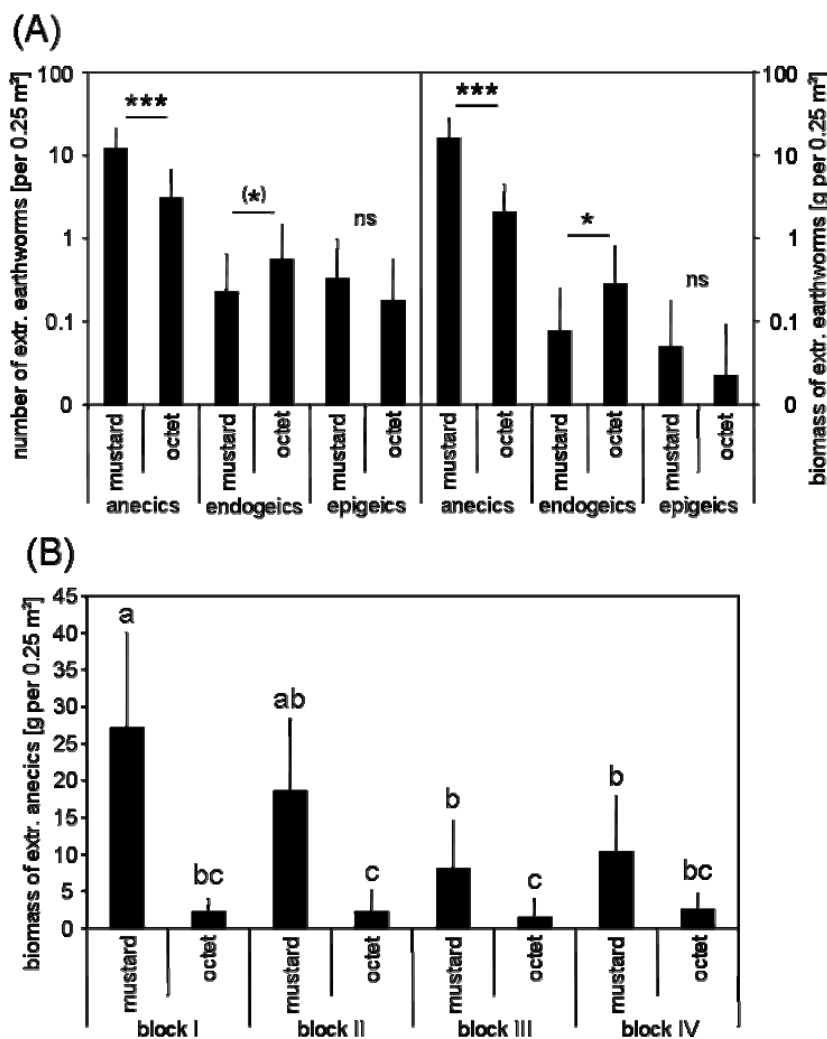


Figure 7.2 | Effects of the earthworm extraction method (mustard, octet) (A) on the number and biomass of extracted earthworms belonging to different ecological groups (anecic, endogeic, epigeic) and (B) on the biomass of extracted anecic earthworms at the four blocks of The Jena Experiment. *** $P < 0.0001$; * $P < 0.05$; (*) $P < 0.1$; ns: not significant. Bars with different letters vary significantly (Tukey's HSD test, $\alpha < 0.05$).

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 primarily 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.

CHAPTER

8



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).

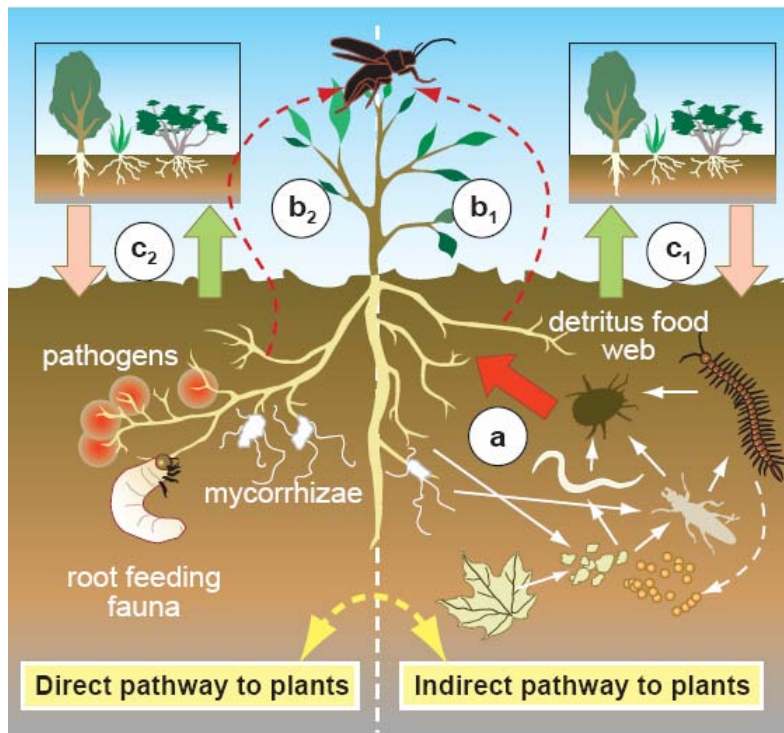


Figure 8.1 | Scheme of direct (left) and indirect (right) consequences for aboveground communities by the soil food web (Wardle et al. 2004) illustrating that feeding activities in the detritus food web stimulate **indirectly** nutrient turnover, plant nutrient acquisition, plant performance, and thereby influence aboveground herbivores. Soil biota affect plants **directly** by feeding on roots and forming antagonistic and mutualistic interactions with plants. Further, the soil food web controls the development of plant communities which in turn influence soil biota.

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 BIODDEPTH 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 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

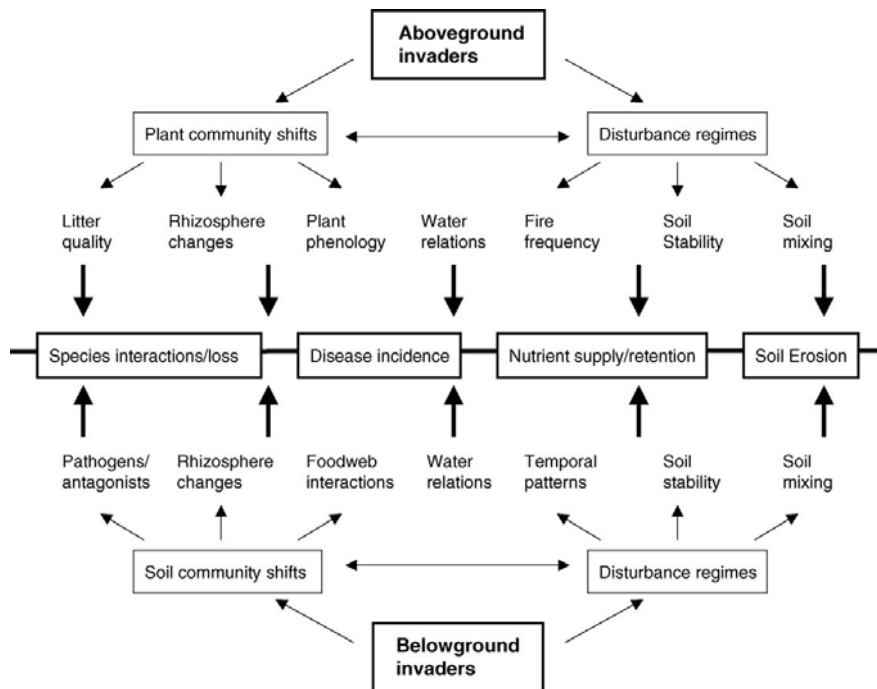


Figure 8.2 | Scheme illustrating mechanisms by which above- and belowground invaders influence ecosystem structure and functioning (Bohlen 2006).

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.

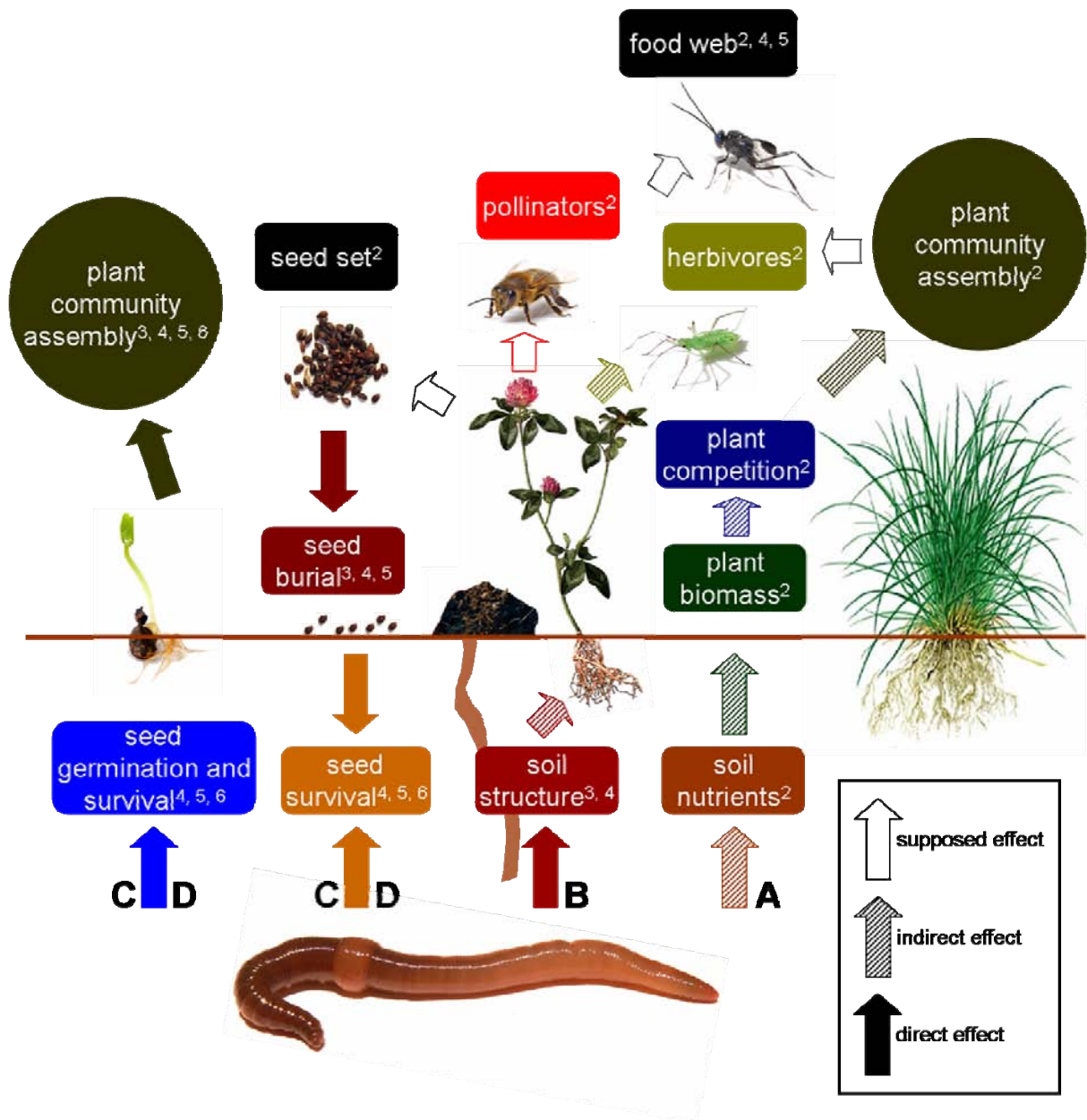


Figure 8.3 | Scheme of the direct, indirect and supposed effects of earthworms on the plant community and the aboveground food web as indicated by the present thesis. Index numbers refer to the respective chapter and capital letters refer to the roles of earthworms in temperate grasslands (A, **Decomposer**; B, **Ecosystem engineer**; C, **Seed disperser**; D, **Seed predator**).

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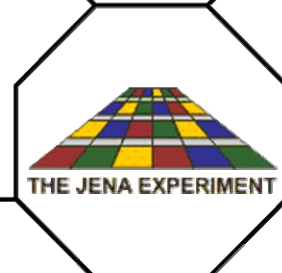
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*„Gibt es intelligentes Leben?
Vielleicht sollten wir den Begriff nicht allzu hoch hängen. Ist es nicht schon eine Form von
Intelligenz, wenn ein Regenwurm sich nach dem Regen ausgräbt, weil er sonst ersäuft.
Sicher wäre es intelligenter, sich gar nicht erst einzugraben.
Menschen haben da einen guten Kompromiss gefunden.
Sie graben sich erst ein, wenn sie tot sind –
also zu einem Zeitpunkt, an dem Ersaufen keine ernsthafte Gefahr mehr darstellt.“*

Dieter Nuhr in *Gibt es intelligentes Leben?* (2006)

ALMOST THE END.

„Selbst Schimmel trocknet bei 80°C!“



THE END.