

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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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.

CHAPTER 7

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.

CHAPTER 2

Eisenhauer N and Scheu S (2008) Earthworms as drivers of the competition between grasses and legumes. *Soil Biology and Biochemistry*, submitted.

IN PREPARATION

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.

Eisenhauer N, Migunova VD and Scheu S (in preparation) Structure of nematode communities in an experimental grassland plant diversity gradient.

Eisenhauer N, Scherber C, Sabais A, Partsch S, Weisser WW and Scheu S (in preparation) Above- and belowground insecticides in a plant diversity gradient: Effects on soil basal respiration and microbial biomass.

Habekost M, **Eisenhauer N**, Scheu S and Gleixner G (in preparation) Linking microbial community and plant diversity in an experimental grassland approach.

Oelmann Y, Bender F, Rosenkranz S, Scherber C, **Eisenhauer N**, Temperton V, Scheu S, Weisser WW, and Wilcke W (in preparation) Insect herbivory contributes to N and P fluxes in throughfall and soil solution in experimental grassland mixtures.

Pérès G, Cozic Y, Cluzeau D, Menasseri S, Sousana JF, Scheu S, **Eisenhauer N**, Gleixner G, Habekost M and Weigelt A (in preparation) Effects of plant species diversity and plant functional group on soil stability.

Sabais A, Partsch S, **Eisenhauer N** and Scheu S (in preparation) Influence of plant species diversity, plant functional group diversity and an insecticide on the occurrence of soil mesofauna (Collembola) in a grassland community.

Sabais A, **Eisenhauer N** and Scheu S (in preparation) Influence of plant species diversity, plant functional group diversity and an insecticide on the occurrence of soil macrofauna in a grassland community.

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Eisenhauer N and Scheu S (2005) Invasion of European earthworms in a North American aspen forest: Effects on soil chemistry, microflora, fauna, and vegetation. Workshop above ground and below ground: Decomposer and primary producer interactions (Joint meeting of the GfÖ and DBG, Witzenhausen, Germany).

Eisenhauer N, Parkinson D and Scheu S (2006) Invasion of European earthworms into a North American aspen forest: Effects on soil chemistry, microflora, fauna and vegetation. The 8th International Symposium on Earthworm Ecology (Kraków, Poland).

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.

Eisenhauer N, Milcu A, Sabais A, and Scheu S (2008) Invasibility of experimental grassland communities: The role of plant diversity, plant functional group identity and earthworms. Multitrophic Interactions Workshop, Göttingen, Germany.

SUPERVISIONS

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.

Volker Hörsch (2008) Wechselwirkungen zwischen Zersettern (Lumbricidae: *Aporrectodea caliginosa* und *Lumbricus terrestris*) und phytopathogenen Pilzen (*Fusarium graminearum*): Einfluss auf das Wachstum und den Befall von Weizen (*Triticum aestivum*) durch Pflanzenfresser (*Rhopalosiphum padi*). Examensarbeit, Darmstadt University of Technology.

Matthias Klier (2008) Nematoden in einem Pflanzendiversitätsgradienten: Einfluss auf Mikroorganismen und Pflanzenwachstum. Examensarbeit, Darmstadt University of Technology.

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 Regenwurmarten 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 Regenwurmarten 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 Regenwurmartenspezies 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 Samenreife 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>).

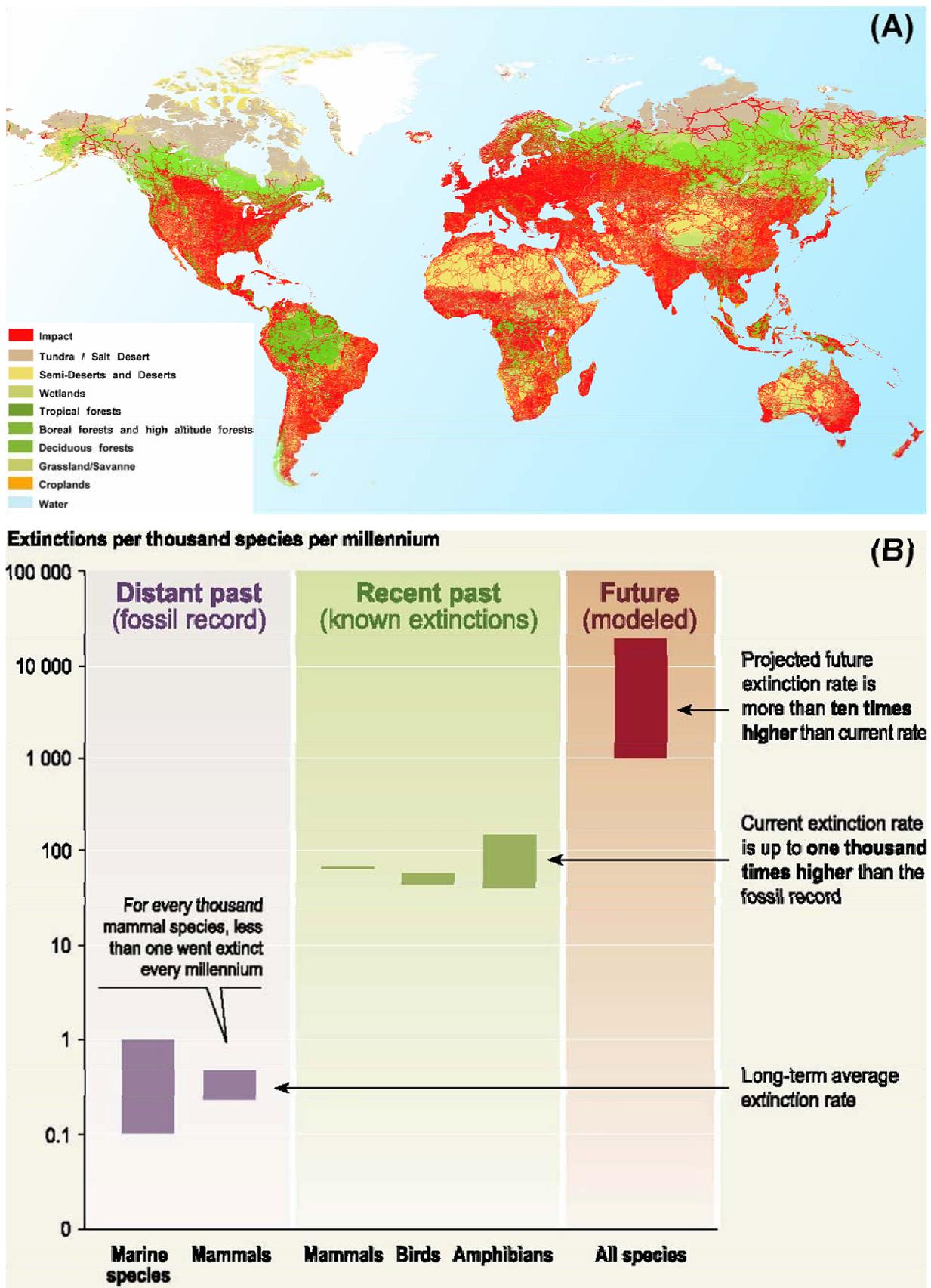


Figure 1.1 | (A) Map of human impacts on the ecosystems of the world (Globio 2007). (B) Species extinction rates of the distant past, recent past and the future based on habitat changes from 1970 to 2050 (Millenium Ecosystem Assessment 2005).

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.

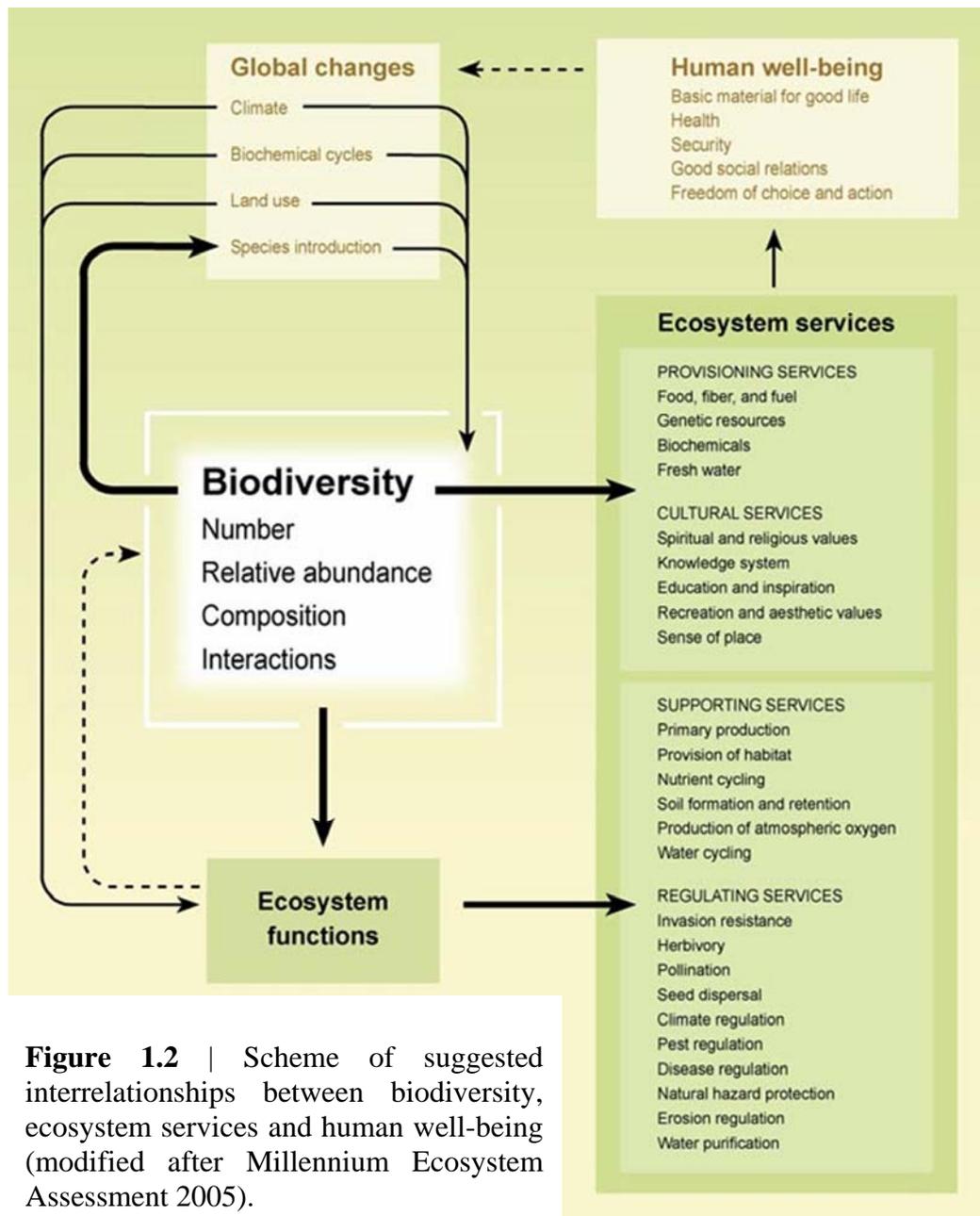


Figure 1.2 | Scheme of suggested interrelationships between biodiversity, ecosystem services and human well-being (modified after Millennium Ecosystem Assessment 2005).

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.

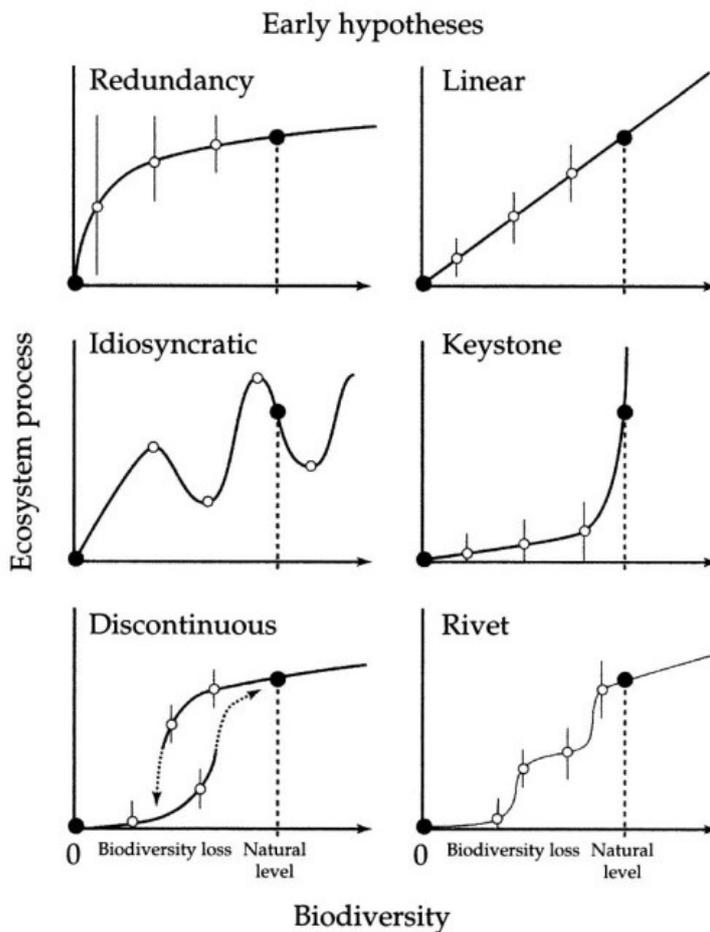


Figure 1.3 | Graphs of early hypotheses on the relationship between biodiversity and ecosystem functioning (Naeem et al. 2002).

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).

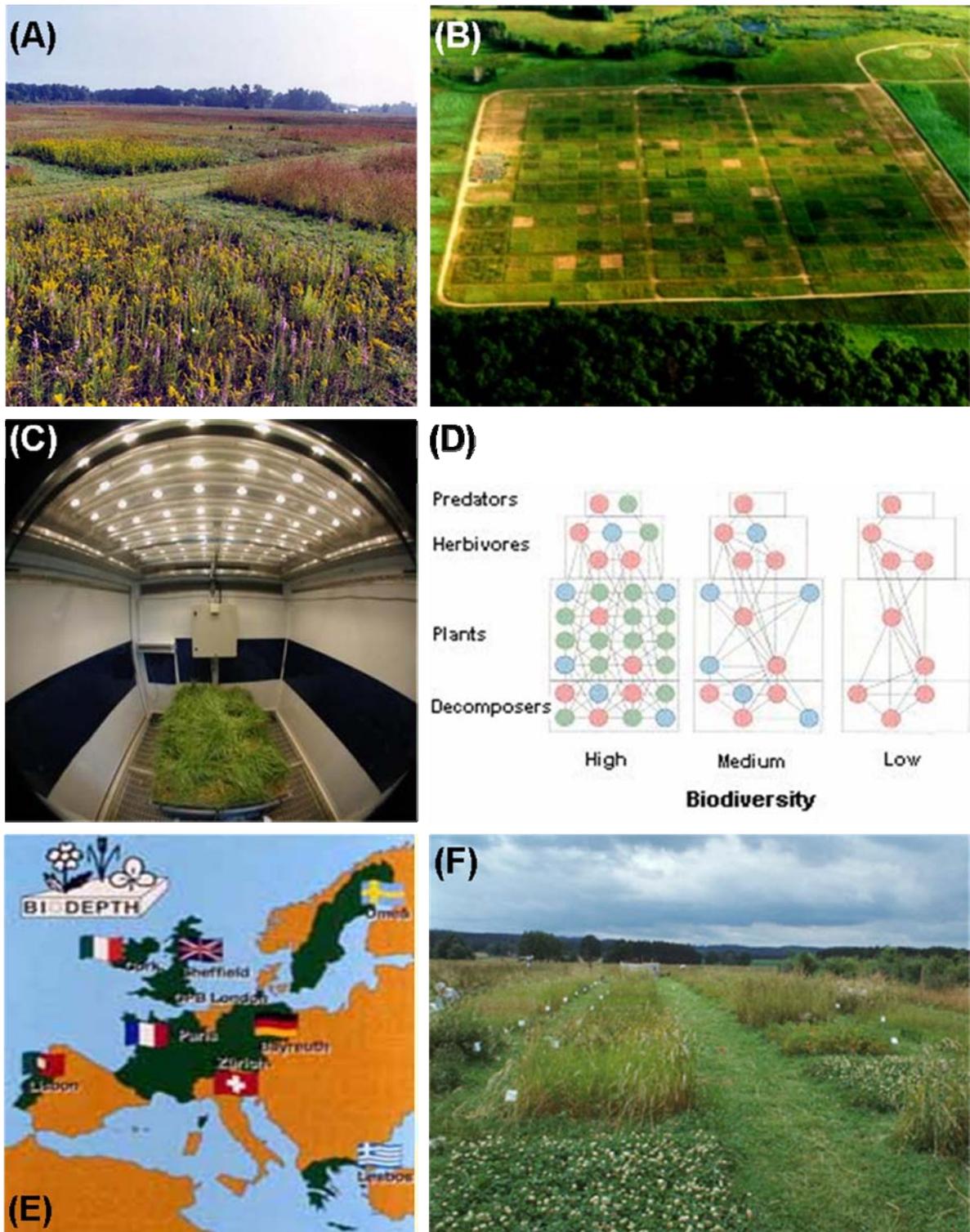


Figure 1.4 | (A) Photograph of single experimental plots containing different plant species combinations and (B) photograph of the experimental field site of the Cedar Creek Experiment (<http://www.cedarcreek.umn.edu/wicc/>). (C) Photograph of one experimental chamber and (D) scheme of the experimental 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 BIODEPTH experiment are indicated by green colour (http://www.naturlink.pt/.../not2032_lang1_part5847.jpg). (F) Experimental plots of the BIODEPTH 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).

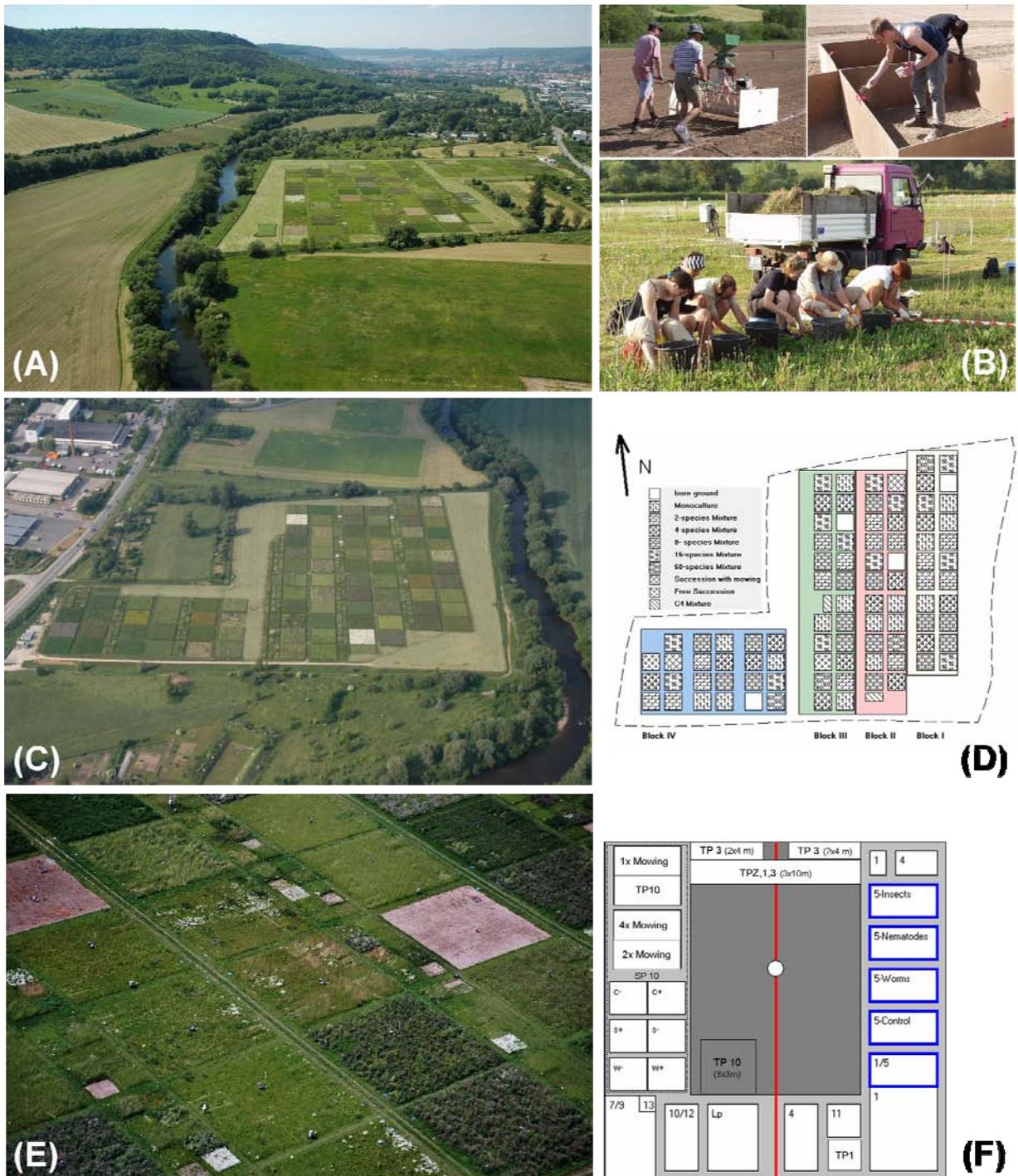


Figure 1.5 | (A) Photograph of the location of The Jena Experiment field site in the floodplain of the Saale river at the northern edge of Jena (Thuringia, Germany; Photo by A. Weigelt). (B) Photographs of the establishment (sowing of target species) and maintenance (weeding of non-target plant species) 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.

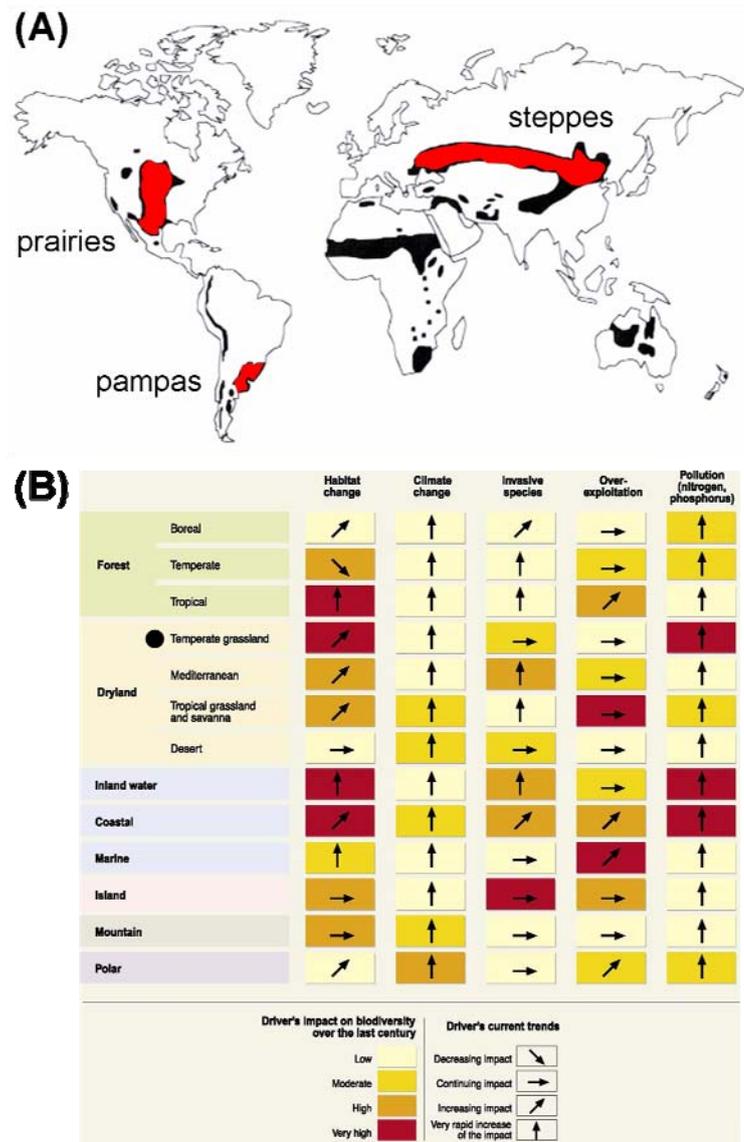


Figure 1.6 | (A) Map of the temperate grasslands of the world (modified after Coupland 1992). (B) Main direct drivers of biodiversity loss distinguishing between impacts over the last century (colour) and current trends (arrows) in different biomes (modified after Millennium Ecosystem Assessment 2005).

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).

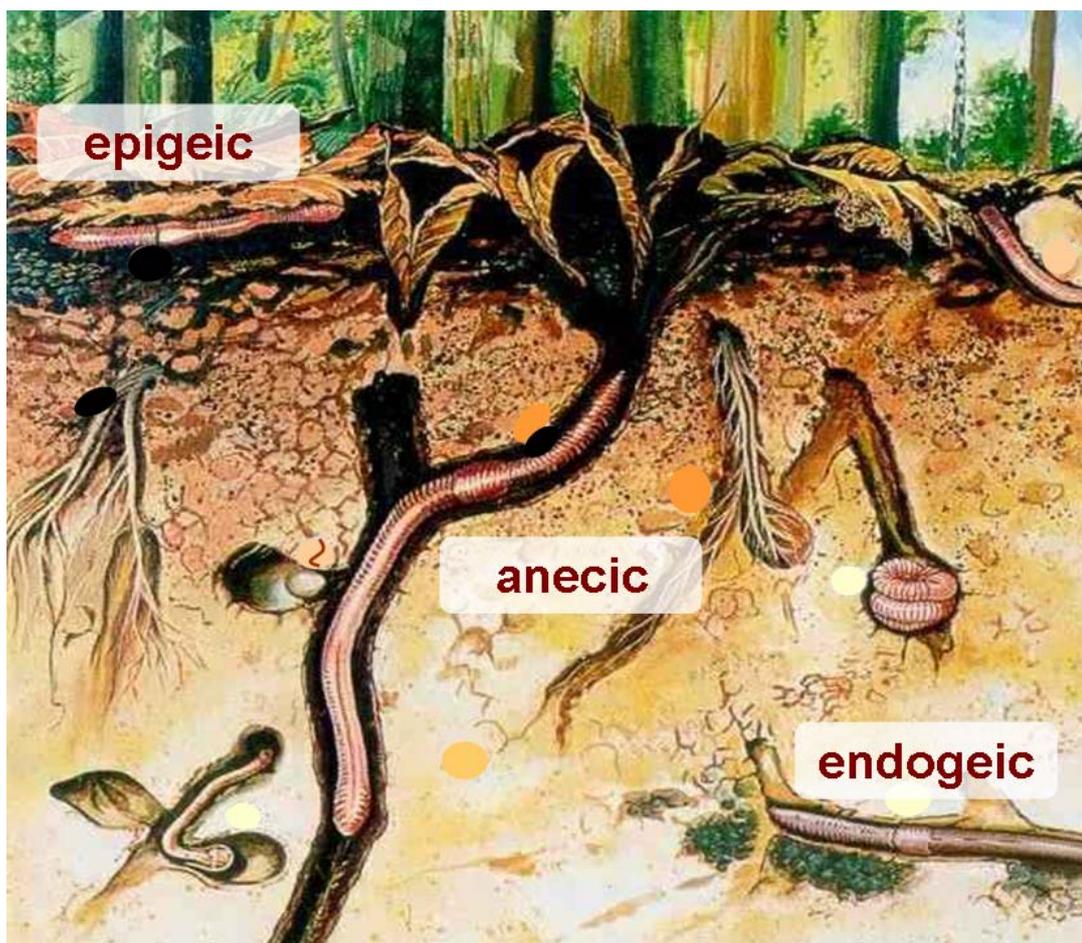


Figure 1.7 | Pictorial representation of the characteristics of the three ecological groups of earthworms as proposed by Bouché (1977; modified after http://www.umweltbundesamt.de/..hinweise_9.jpg).

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).

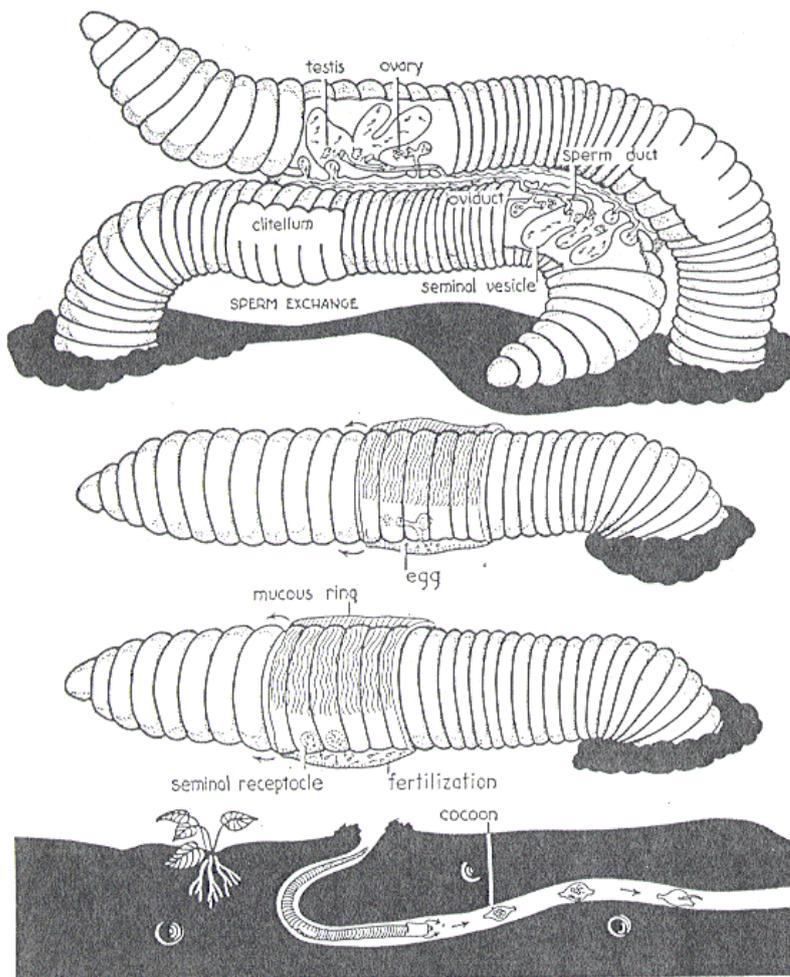


Figure 1.8 | (A) Scheme of earthworm copulation, egg and cocoon formation and cocoon deposition in soil (<http://www.sciencefun4all.net/.../Worms/reproduction.gif>).

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).

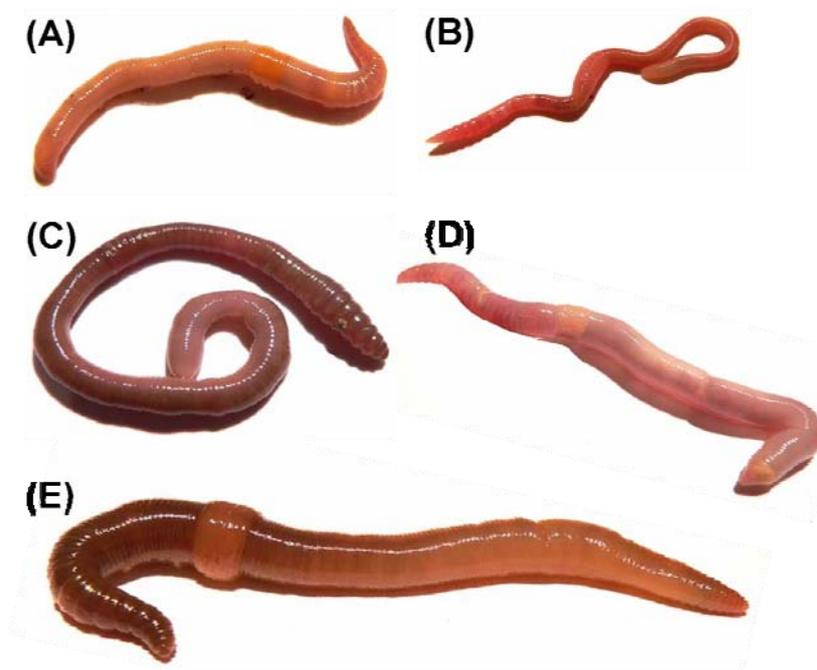


Figure 1.9 | Photographs of the earthworm species occurring at the field site of The Jena Experiment. (A) *Allolobophora chlorotica*, (B) *Aporrectodea rosea*, (C) *Aporrectodea caliginosa*, (D) *Octolasion tyrtaeum*, and (E) *Lumbricus terrestris*. Photos by H. Schuy.

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).

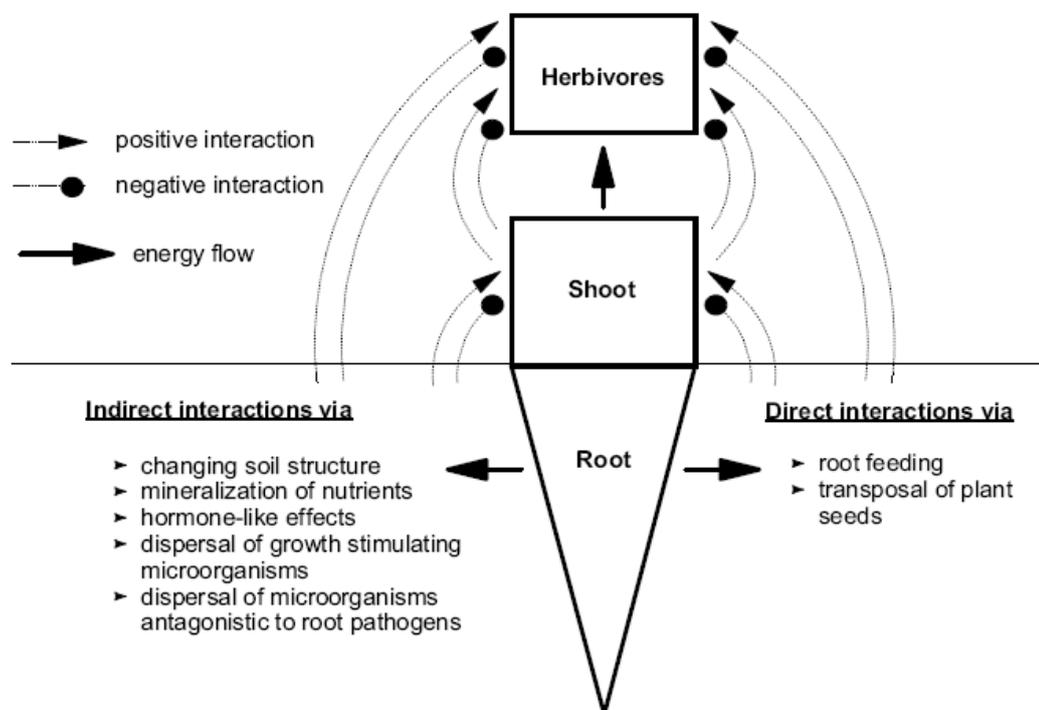


Figure 1.10 | Mechanisms by which earthworms affect plant growth and the herbivore community above the ground (Scheu 2003).

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).

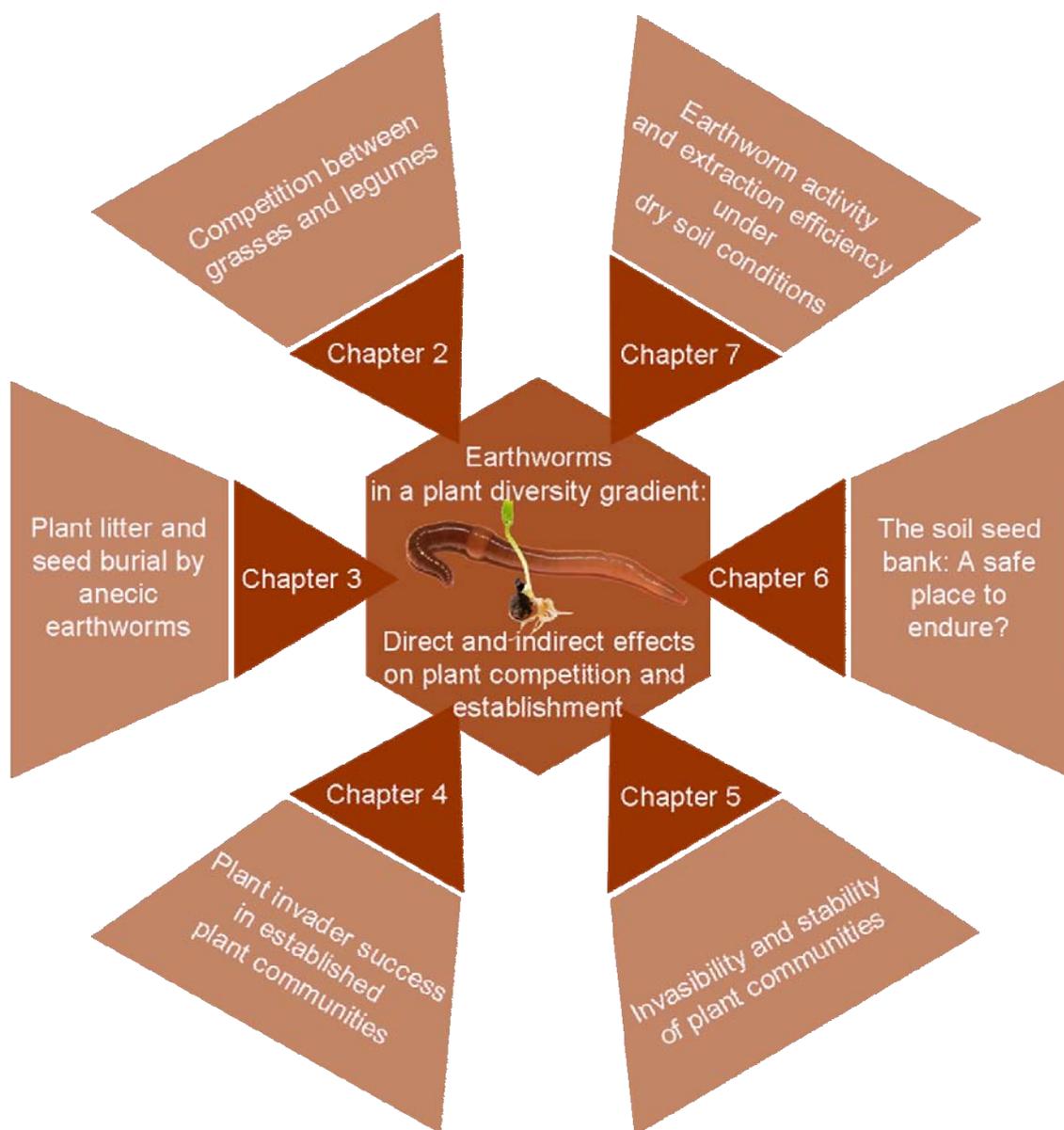


Figure 1.11 | Structure of the present thesis. Further explanations can be found in the respective chapter.

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 ($\delta^{15}\text{N}$) 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 ($\delta^{13}\text{C}$) 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).

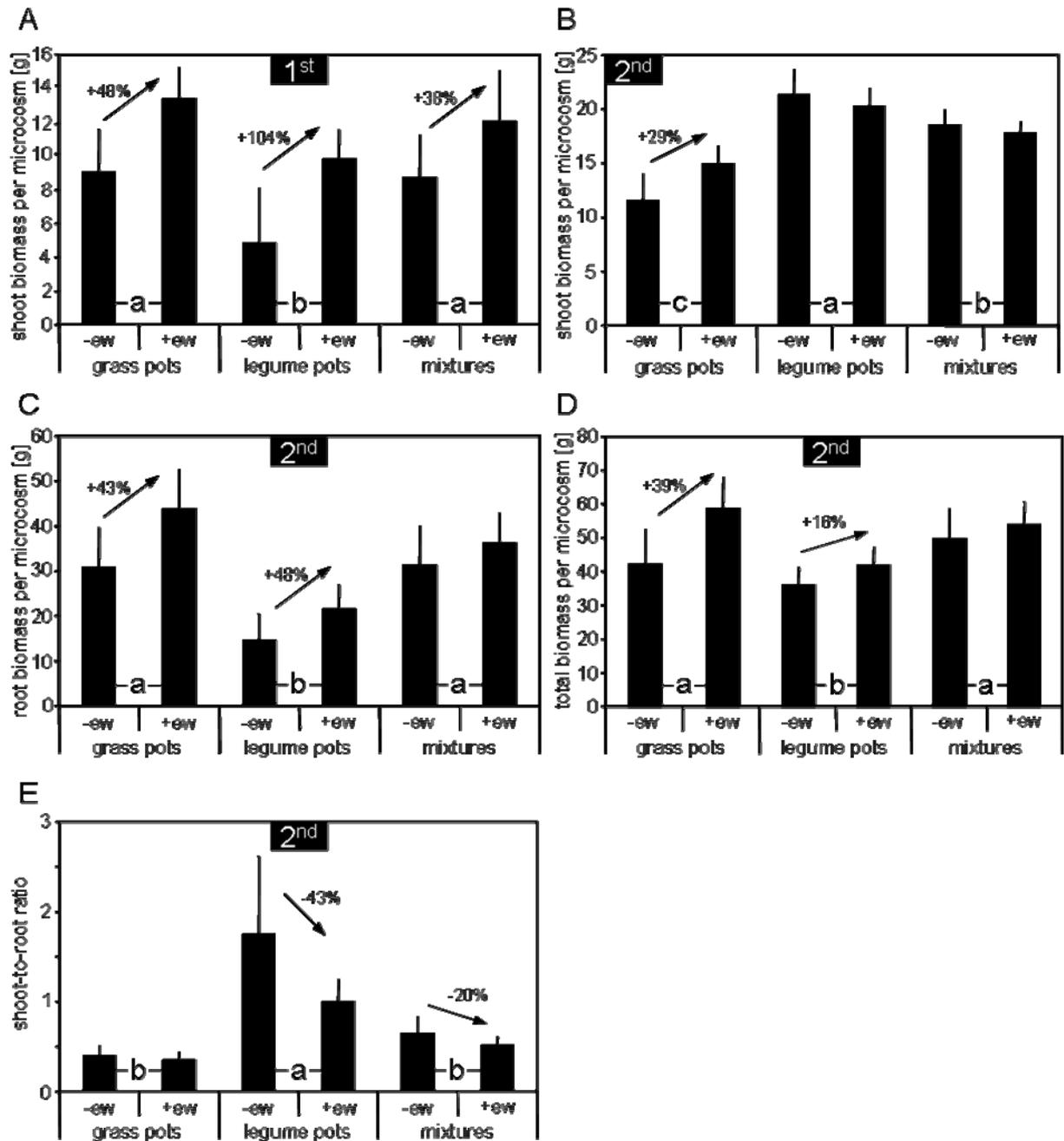


Figure 2.1 | Effects of Plant community (grasses only treatment, legumes only treatment and mixture) and Earthworms (with [+ew] and without [-ew] *Lumbricus terrestris*) on (A) shoot biomass per microcosm (first [1st] harvest), (B) shoot biomass per microcosm (second [2nd] harvest), (C) root biomass 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).

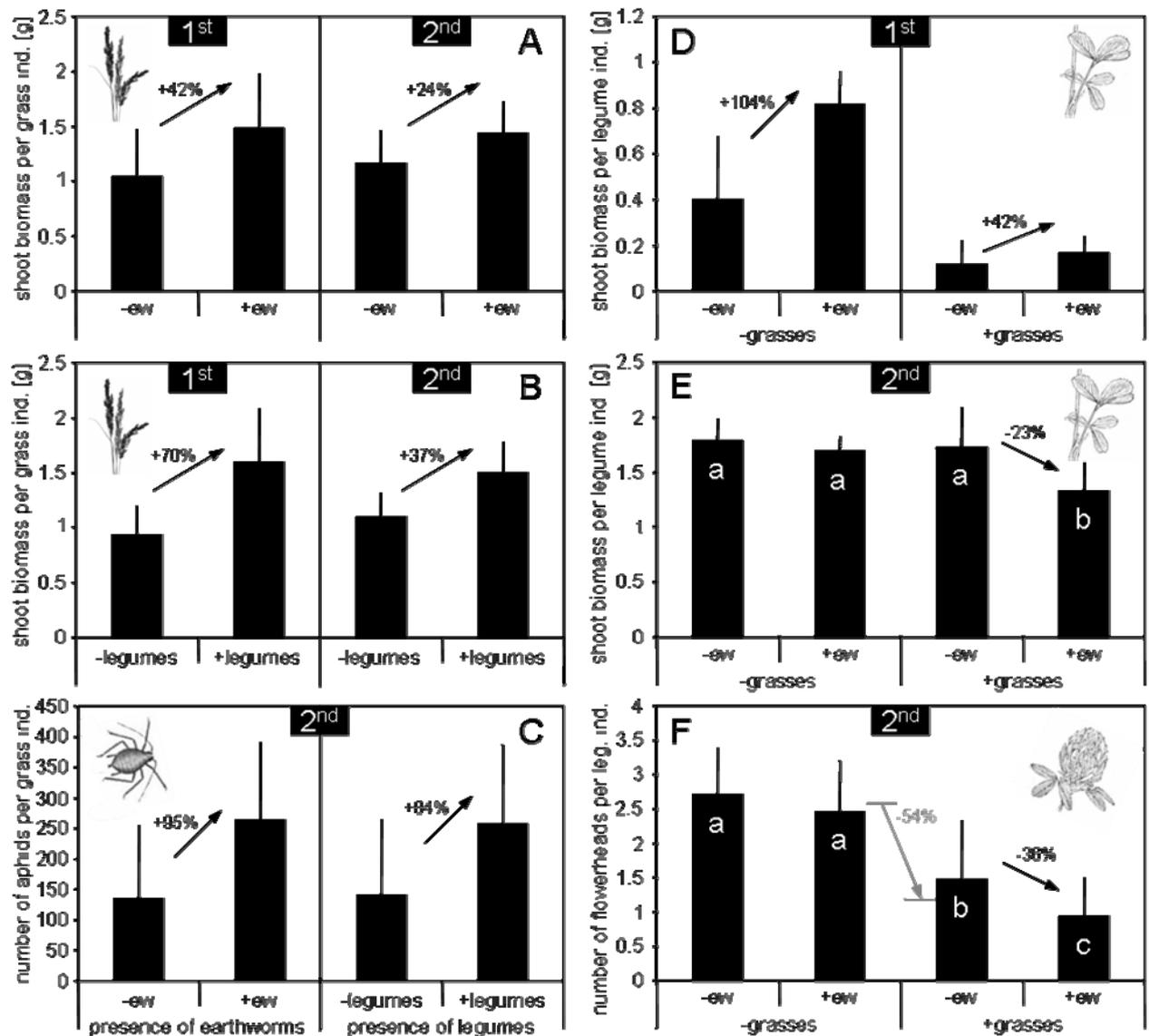


Figure 2.2 | (A) Effects of Earthworms (with [+ew] and without [-ew] *Lumbricus terrestris*) on shoot biomass per grass individual (first [1st] and second [2nd] harvest); (B) Effects of legumes (with [+legumes] and without [-legumes] legumes) on shoot biomass per grass individual (1st and 2nd harvest); (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.

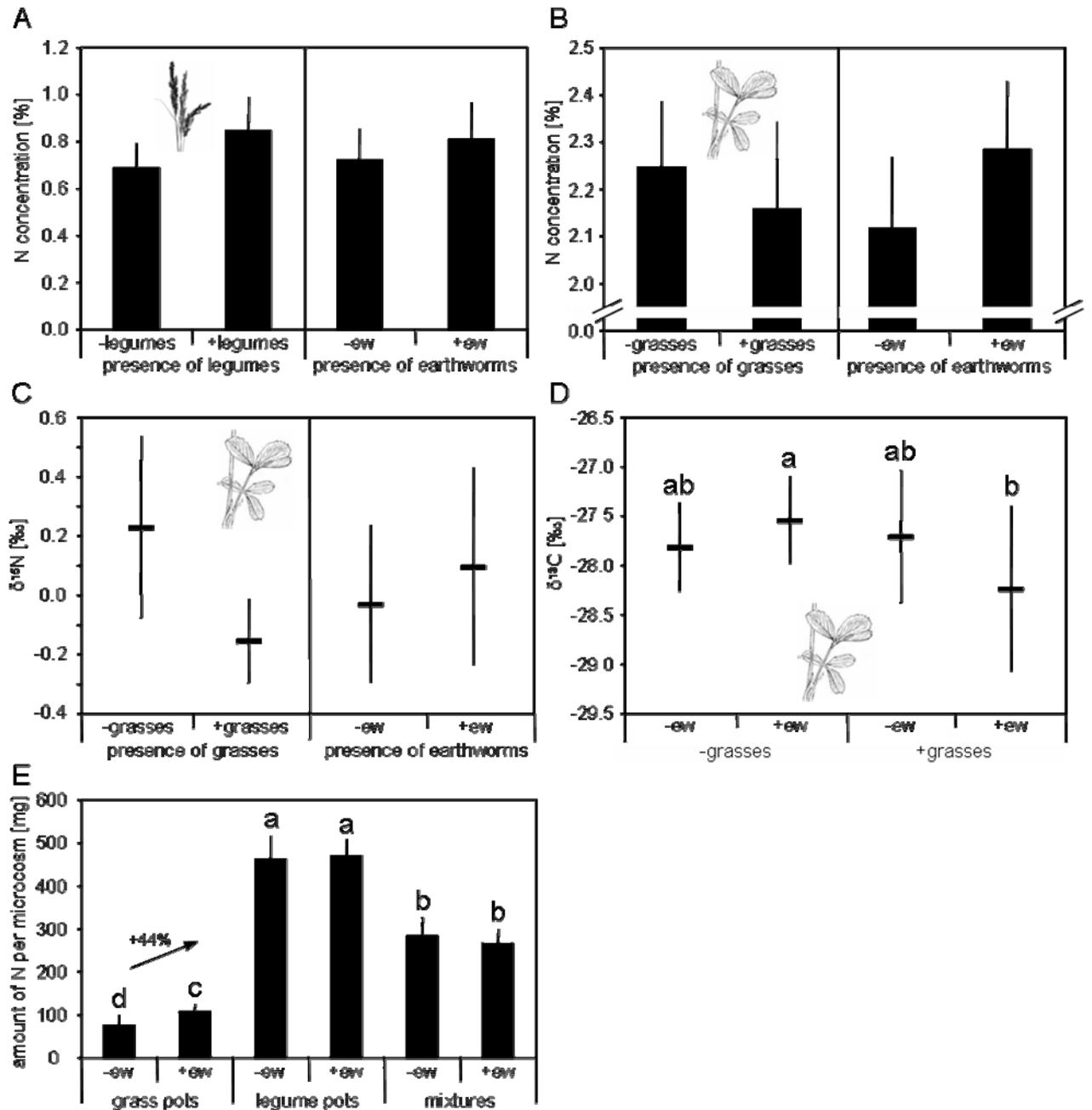


Figure 2.3 | (A) Effects of legumes (with [+legumes] and without [-legumes] legumes) and earthworms (with [+ew] and without [-ew] *Lumbricus terrestris*) on nitrogen concentration of grass shoots (second [2nd] harvest); (B) Effects of grasses (with [+grasses] and without [-grasses] grasses) and earthworms 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.

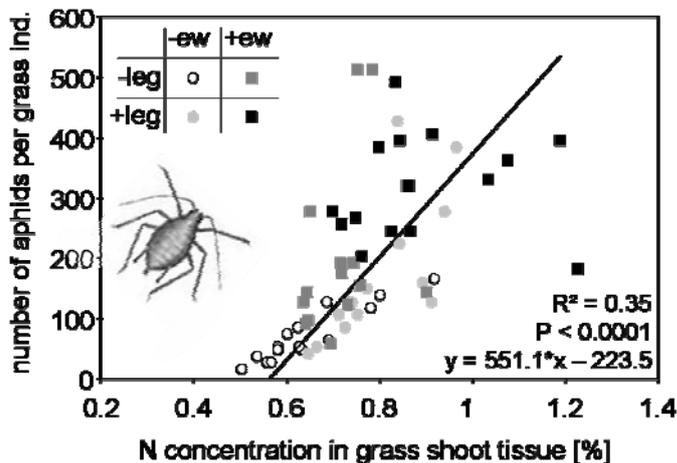


Figure 2.4 | Regression of the number of aphids per grass individual and the N concentration [%] in grass shoot tissue (-ew: without earthworms; +ew: with earthworms; -leg: without legumes; +leg: with legumes).

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.

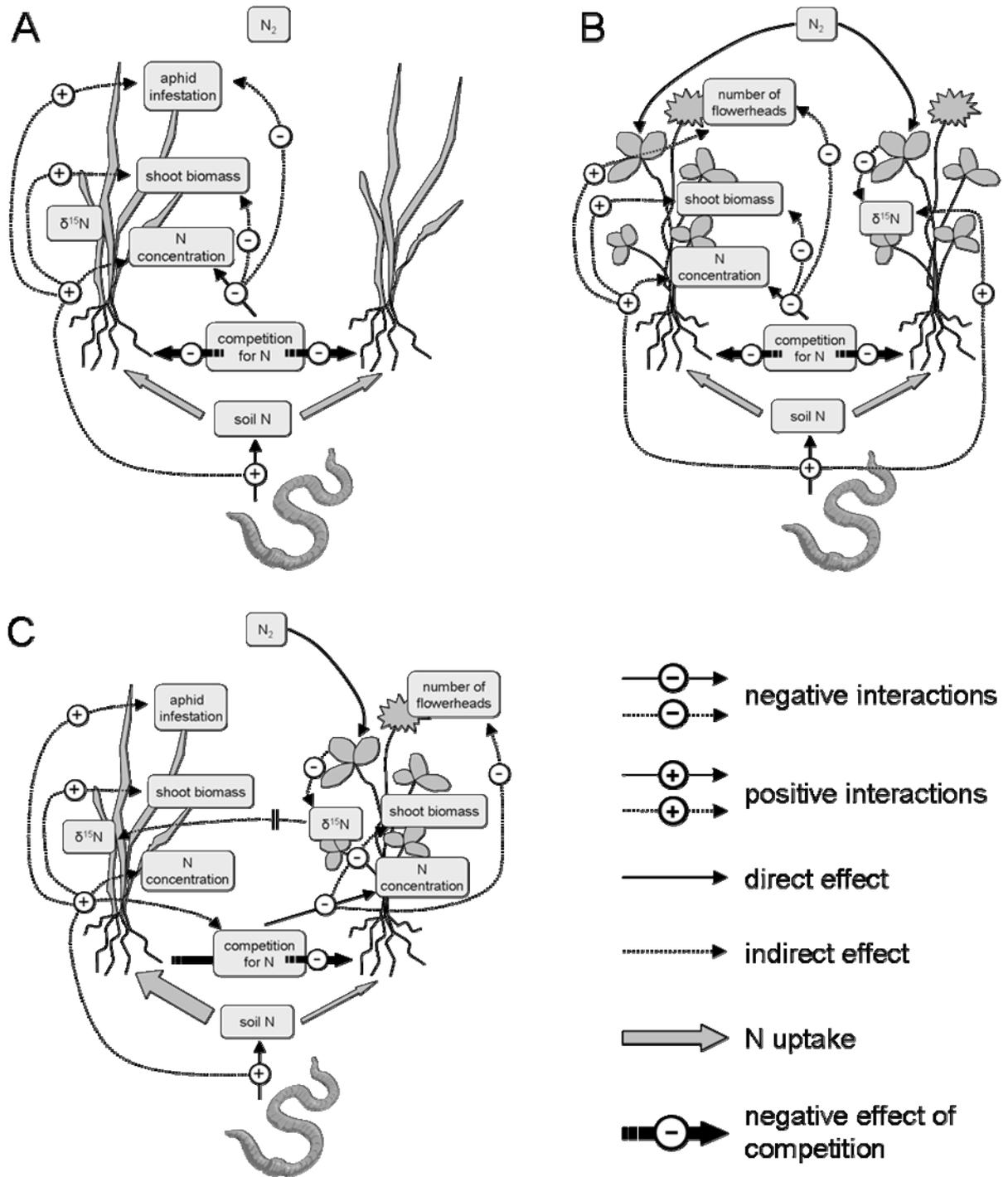


Figure 2.5 | Summary of probable and hypothetical mechanisms by which earthworms may affect the competition between grasses and legumes for N based on experimental data on shoot N concentration, shoot biomass of grasses and legumes, aphid infestation of grasses, number of legume flowerheads, and $\delta^{15}\text{N}$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