Justus-Liebig-Universität Gießen
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Effects of Ground Cover on Seedling Emergence and Establishment

Habilitationsschrift
zur Erlangung der Lehrbefähigung für die Fächer
„Vegetationsökologie und Landschaftsökologie“
im Fachbereich Agrarwissenschaften, Ökotrophologie und Umweltmanagement der
Justus-Liebig-Universität Gießen
vorgelegt von

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Gießen 2011

Justus Liebig University Gießen

Faculty of Agricultural Sciences, Nutritional Sciences and Environmental Management

Institute of Landscape Ecology and Resources Management

Division of Landscape Ecology and Landscape Planning

Effects of Ground Cover on Seedling Emergence and Establishment

Habilitation Thesis
for the Acquirement of the Facultas Docendi for the Subjects
“Vegetation Ecology and Landscape Ecology”
at the
Faculty of Agricultural Sciences, Nutritional Sciences and Environmental Management
Justus-Liebig-Universität Gießen

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Gießen 2011

Contents

1 Introduction

Species selection in a plant community can be described as an adaptive assembly process that determines the invasibility of a system by species that (i) reach the local site, (ii) tolerate the local environmental conditions, and (iii) successfully co-exist in interactions with other organisms of the same or other trophic levels (Weiher and Keddy 1995, Lortie et al. 2004, Temperton et al. 2004). These interactions include the antagonistic processes of competition and facilitation (Holmgren et al. 1997, Brooker & Callaghan 1998, Bruno et al. 2003), which will be greatly governed by the effects of the most abundant species within a plant community on abiotic and biotic conditions. Thus, for co-occurring species, dominant ground cover, dead or alive, will have unavoidable impact through interferences at different life stages of plants (During & Van Tooren 1990, Facelli & Pickett 1991a, Reader 1993, Foster & Gross 1997, Eckstein 2005, Grime 1998, Ladd & Facelli 2008, Fayolle et al. 2009) and also on ecosystem processes (Wardle et al. 1998).

Once viable diaspores reach a site, their success depends on the availability of safe sites for seed germination (Chambers & Macmahon 1994, Hulme 1994, Leimu et al. 2002, Grubb 1977, Eriksson & Ehrlén 1992, Weiher & Keddy 1999, Donath et al. 2006). Therefore, differences of microsites in quality and quantity, in time and space have a great impact on community composition, diversity and succession through their effects on seed germination and seedling emergence (Davis et al. 2000, Münzbergová 2004, Fenner & Thompson 2005). These changes may be initiated by natural and anthropogenic processes altering the type and amount of ground cover present. Ground cover can either consist of living plant tissue, such as vascular plants or bryophytes, which would lead to direct plant–plant interactions, or dead plant material, i. e. litter, which leads to "after-death" interactions (sensu Facelli & Facelli 1993).

Several studies on plant-plant interactions emphasize that the direction and net effect of these interactions depends largely on the vital rate considered, on environmental conditions, such as climate and soil depth, as well as on the age and life stage of benefactor and beneficiary (Rousset & Lepart 2000, Hastwell & Facelli 2003, Eckstein 2005, Donath et al. 2006, Lortie & Callaway 2006, Maestre et al. 2005, Michalet 2006). A general rule drawn from these studies is that the strength of facilitative effects in a plant-plant interaction is related to the degree of abiotic stress (Bertness & Callaway 1994, Callaway & Walker 1997). In case of litter, moderate cover seems to exert positive effects on seedling emergence under dry conditions (e. g. Becerra et al. 2004, Rotundo & Aguiar 2005, Violle et al. 2006, Quested & Eriksson 2006), while under more benign conditions the effect tends to be neutral or even negative (Xiong et al. 1999).

A meta-analysis by Xiong & Nilsson (1999) highlights that across studies litter effects on seed germination, seedling emergence and establishment, and on biomass and species richness were predominantly negative, despite several studies revealing positive effects. Interestingly, the patterns altered in case of grasslands: both the effects of grass-litter (versus other litter types) and the effects of litter in grasslands (versus other ecosystem types) could be positive or negative (Xiong & Nilsson 1999).

These effect patterns are also interesting to look at from the perspective of species and vegetation conservation and restoration. European semi-natural grasslands, which carry an exceptionally high diversity of plants (Ellenberg 1996, Korneck et al. 1998) are continuously subject to great changes. Although intensification of management is certainly the key driver for species loss in agricultural landscapes, also disruption of a continuous management will induce severe changes in plant communities (Korneck et al. 1998, Luoto et al. 2003, Wellstein et al. 2007, Enyedi et al. 2008). In case of grassland communities there is evidence that the accumulation of dead plant material, i. e. litter, is a very important driver for changes in the plant community after the cessation of regular management (Schiefer 1986, Ellenberg 1996) since it interferes with seed germination and seedling establishment (Virágh and Bartha, 1996, Bakker and Berendse, 1999, Kahmen et al., 2002). Where management ceased there is typically an increasing cover of litter, which goes in line with species loss (Schiefer 1986, Ellenberg 1996). Consequently, in several cases it was shown that species loss was stopped by the removal of the litter cover which creates safe sites for species to re-emerge from the seeds either freshly spread from remnant plants or from the seeds which survived in the seed bank (Spačková et al. 1998, Lepš 1999, Ruprecht et al. 2010a). While in these cases species are still present at the sites, in other cases species may be completely lost from the sites as well as in the close surroundings and show only limited re-colonisation ability (Bischoff 2002, Donath et al. 2003). Under such circumstances different methods for the re-introduction of grassland species are available (Kiehl et al. 2010); basically one can re-introduce pre-grown plants at the restoration sites or spread seeds of the target species at the restoration sites. In the latter case a very successful method is the application of seed-containing plant material from remnant stands of species rich grasslands (cf. Hölzel & Otte 2003, Donath et al. 2007, Kiehl et al. 2010). Despite its application in ample restoration projects, there is little empirical evidence on the optimal amount of plant material to balance positive and negative effects of litter on seedling emergence.

The overall effects of ground cover, e. g. moss or litter, on seeds are mediated through physical, mechanical and chemical processes (During & Van Tooren 1990, Facelli & Pickett 1991a). Allelopathy, i. e. the leaching or volatilization of phytotoxins from plant tissues, is considered as one possible mechanism acting on seed germination (Baskin & Baskin 2001), but also on seedling establishment (Schlatterer & Tisdale 1969, Chang-Hung & Chiu-Chung 1975, Werner 1975, Bosy & Reader 1995, Hovstad & Ohlson 2008), and plant growth and distribution (del Moral & Cates 1971, Rice 1972, Newman & Rovira 1975). Chemicals involved are generally secondary metabolites such as coumarins, terpenoids, phenolics or tannins (Rice 1984, Harborne 1993). However, since there are close links between plant secondary compounds, palatability, litter quality and decomposition (Grime et al. 1996), allelochemicals may also affect other components and functions of the ecosystem and thus play an important role in ecosystem regulation (Wardle et al. 1998, Mazzoleni et al. 2007).

Changes of the physical conditions may result in increased seedling emergence through the alleviation of limited water availability due to reduced evaporation and runoff, especially in water-limited habitats (Rotundo & Aguiar 2005). Additionally, the insulating effect of ground cover possibly will reduce temperature extremes and reduce soil temperature amplitudes that seeds are confronted with (van Cleve et al. 1983). This effect may, however, lead to reduced seed germination when temperature amplitudes are below certain thresholds that are needed to induce germination (Thompson & Grime 1983). In addition, beneath a ground cover light quantity and quality, i. e. red/red-far ratio, may be lowered to levels unfavourable for seed germination (Keizer *et al*. 1985, Bosy & Reader 1995, Holmgren et al. 1997, Jensen & Gutekunst 2003).

Mechanical effects of both litter and bryophytes may be positive when seeds are guarded against herbivores through a protective cover (During & Van Tooren 1990, Reader 1993, Facelli 1994). This protective effect of litter seems to increase with the amount of cover (Facelli 1994, Reader 1993, Shaw 1968, Sydes & Grime 1981). There is, however, a direct and important trade-off between shelter from predation and seedling establishment because the relative position of seeds in a litter cover or soil cover will also directly affect germination and seedling emergence (Burmeier et al. 2010, Facelli & Pickett 1991, Rotundo & Aguiar 2005). With increasing thickness of cover on top of seedlings, growth is retarded, since the young plants are forced to pierce through a cover of soil (Burmeier et al. 2010, Zhang & Maun 1990) and/or litter (Chambers 2000, Fowler 1986) to reach the light. On the other hand, with increasing thickness of litter beneath seedlings, the ability of the radicle to establish soil contact is diminished (Mallik et al. 1984, Rotundo & Aguiar 2005). Thus, differences in the relative position of seeds at the time of germination further accentuate how ground cover may affect seedling emergence (Facelli & Pickett 1991a, Rotundo & Aguiar 2005). In addition, a seed's position will also change during time: while newly shed seeds will be situated in the top layers of litter or moss carpets, they will gradually descend through decomposition at the base of the cover and input of new plant litter on top or through apical moss growth, respectively.

This effect will also depend on seed size, which directly influences seed mobility, and leads to different starting conditions even before the onset of germination (Bekker et al. 1998, Burmeier et al. 2010). Studies on the formation of soil seed banks showed that smaller seeds are more likely to be incorporated into the soil seed bank than larger seeds (Bekker et al. 1998, Burmeier et al. 2010, Schmiede et al. 2009). Three mechanisms are related to seed size and may have a strong influence on the performance of seeds after germination (Leishman et al. 2000, Moles & Westoby 2004b): the seedling size effect, the reserve effect and the metabolic effect (Westoby et al. 1996). Through these mechanisms, differences in seed size seem to have a distinct influence on seed and seedling fate (Leishman et al. 2000). Due to their larger reserves of resources (Baskin & Baskin 2001), seedlings of large-seeded species show higher survival rates under harsh conditions (Bosy & Reader 1995, Turnbull et al. 1999).

These effects may be further altered by the activity of soil-dwelling invertebrates such as earthworms, which not only incorporate organic matter into the mineral soil (Eisenhauer et al. 2008) but may also change the speed of seed movement in both a litter cover and the soil. Seeds may either be merely co-carried with the litter into the soil, or may be selectively targeted as food by earthworms (Zaller & Saxler 2007). The final depth at which seeds come to rest in the soil will greatly determine their chance for successful emergence; in general emergence will decrease with increasing burial depth (e. g. Burmeier et al. 2010). For small seeded species, which are more likely to be persistent (Thompson et al. 1993), burial deep into the soil will speed up the formation of a persistent seed bank (Schmiede et al. 2009), while it will most likely remove large seeds from the regeneration pool (Burmeier et al. 2010). Thus, depending on seed size, earthworms may play a decisive, yet ambivalent, role in the early phase of plant establishment.

Despite a number of studies on the effects of ground cover on seed germination and species composition there were surprisingly few experimental studies trying to untangle the influence of different components of these processes. Against this background, the general aim of this work was to experimentally address effects of different qualities and quantities of ground cover on seedling emergence and seedling establishment under different marginal conditions. Specifically the studies addressed the following main questions:

- Does the effect of ground cover change from positive under dry conditions to negative under more favourable water supply (chapters 2, 4)?
- Are the positive effects of ground cover overridden by the negative effects on seedling emergence and establishment at higher cover amounts (chapters 2, 4, 5, 6)?
- Are there ecosystem-specific effects of litter types, i. e. is the emergence of woodland species differently affected by grassland litter than by woodland litter and vice versa (chapter 4)?
- Do allelopathic secondary compounds, e. g. of a dominant grass species, affect seed germination and seedling performance (chapters 3, 4)?
- How does the relative seed position influence seedling emergence and how does this response change depending on different cover types and amounts (chapters 5, 6)?

- To what extent do the effects of ground cover depend on species specific traits, e. g. seed size, of the study species (chapters 2, 4, 5, 6)?
- Does seed transport by earthworms change the effect of litter cover and relative seed position on seedling emergence (chapter 6)?

Overview

Chapter 2 describes an experimental study on the effects of different quantities of grass litter on familial pairs of flood plain species under contrasting water availability. **Chapter 3** focuses on the allelopathic impacts of secondary compounds of a dominant grass species on co-occurring grasses and herbs in dry grassland. **Chapter 4** addresses habitat-specific litter effects of woodlands and grasslands on familial pairs of grassland and woodland species, respectively. **Chapter 5** also addresses the effects of different type ground cover, i. e. grass litter and moss, on seedling emergence. In addition to the before mentioned studies, this experimental study investigates also differential effects of relative seed position on seedling emergence. Based on these findings, **chapter 6** contains an experimental set-up that aims to assess how seed position effects are changed by soil dwelling invertebrates, i. e. earthworms. **Chapter 7** contains a closing discussion of the **chapters 2-6**. **Chapter 8** and **chapter 9** present an English and German summary, respectively. The reference list in **chapter 10** includes all references used throughout this work.

Publications included in this thesis

- **Chapter 2:** Eckstein RL & Donath TW 2005 Interactions between litter and water availability affect seedling emergence in four familial pairs of floodplain species. Journal of Ecology 93: 807-81
- **Chapter 3:** Ruprecht E, Donath TW, Otte A & Eckstein RL 2008 Chemical effects of dominant grass on seed germination of four familial pairs of dry grassland species. Seed Science Research 18: 239-248
- **Chapter 4:** Donath TW & Eckstein RL 2008 Grass and oak litter exert different effects on seedling emergence of herbaceous perennials from grasslands and woodlands. Journal of Ecology 96: 272-280

- **Chapter 5:** Donath TW & Eckstein RL 2010 Effects of bryophytes and grass litter on seedling emergence vary by vertical seed position and seed size. Plant Ecology 207: 257-268
- **Chapter 6:** Donath TW & Eckstein RL: Litter effects on seedling establishment interact with seed position and earthworm activity. Plant Biology: accepted
  doi: 10.1111/j.1438-8677.2011.00490.

2 Interactions between litter and water availability affect seedling emergence in four familial pairs of floodplain species

Eckstein RL & Donath TW 2005 Journal of Ecology 93: 807-816

Summary

1 We studied seedling emergence in four familial pairs of floodplain herbs in response to the experimental manipulation of soil moisture and litter cover to analyse (i) whether the effect of litter changes from negative under humid to positive under dry conditions, and (ii) whether the response to changing water- and light conditions with increasing litter cover varies among species and plant families.

2 We carried out a controlled pot experiment using four levels of litter cover (0, 2, 4 and 8 g litter per pot, corresponding to 0, 0.2, 0.4 and 0.8 kg m^{-2}) and two levels of water-addition, leading to constantly humid substrate or intermittently dry topsoil.

3 Across water-additions, percentage emergence reached a peak at low levels of litter cover (0.2 and 0.4 kg m^{-2}). There was a significant litter*water-addition interaction in six species, with positive effects of litter under intermittently dry conditions and negative or neutral effects under constantly humid conditions. Litter lowered maximum temperature as well as amplitude, and alleviated soil humidity under low water-supply, while imposing increasingly shaded conditions. Analysis of species- and family-specific responses suggested that germination under a litter cover of 0.8 kg m^{-2} was significantly reduced in small-seeded species (i. e. those that tend to have higher light demands for germination).

4 Our results suggest that transfer of seed-containing plant litter can aid restoration projects if applied at between 0.2 and 0.4 kg m^{-2}. Below these levels, establishment of most species may be inhibited by drought, while higher amounts will increasingly suppress seedling emergence, especially of small-seeded species.

5 In addition to facilitation effects observed between living plants, dead plant remains may also exert positive effects of on establishment. The sign of the litter effect on seedling emergence depends on soil humidity, with negative effects seen above a threshold amount, which is species- and family-specific and is closely related to seed size. Whether positive litter effects in grasslands are a consequence of coevolution remains to be examined.

Introduction

Dead plant remains, i. e. litter, are an important component of biogeochemical nutrient cycles. The rate of litter degradation via decomposition has large impacts on ecosystem productivity and community composition (e. g. Aerts & Chapin 2000, Quested et al. 2005). However, apart from these long-term effects on ecosystem processes, the accumulation of litter may also exert various short-term effects on plant communities (Facelli & Pickett 1991a). These may be direct, e. g. when litter acts as a physical barrier for the establishment of seedlings, or indirect through changes in abiotic conditions (e. g., soil temperature, light quantity and quality; cf. Bosy & Reader 1995). Litter accumulation may have similar consequences to those of increased fertilisation (Foster & Gross 1997, 1998), which suggests that such 'after-death-interactions' may be an important functional link between the higher productivity which results from atmospheric nutrient inputs, and reduced species richness (Facelli & Facelli 1993, Berendse 1999).

In a thorough meta-analysis Xiong & Nilsson (1999) showed that, in accordance with many empirical studies, litter effects on seed germination, seedling emergence and establishment (e. g. Bosy & Reader 1995, Špačková et al. 1998, Jensen & Meyer 2001, Jensen & Gutekunst 2003), and on biomass and species richness (e. g. Foster & Gross 1998, Jensen & Meyer 2001) were predominantly negative. This analysis additionally revealed, however, that small amounts of litter may facilitate germination (Xiong & Nilsson 1999). Interestingly, both the effects of grass-litter (in contrast to other litter types) and the effects of litter in grasslands (in contrast to other ecosystem types) could be positive or negative (Xiong & Nilsson 1999). Facilitative litter effects under certain conditions have been observed in some field experiments (Fowler 1986, Nakamura 1996, Suding & Goldberg 1999, Xiong et al. 2003). In parallel with theoretical conceptual models on interspecific interactions between living plants (Callaway & Walker 1997, Holmgren et al. 1997), we also suggest that dead plant litter may exert facilitative effects on seedling emergence in situations where litter cover will alleviate stressful abiotic conditions (e. g. drought). It is, however, likely to hamper germination and emergence under more favourable conditions or beyond a certain threshold level of litter cover, when other factors (e. g. light) will deteriorate.

A major trait closely related to germination success under environmental stress (e. g. shade) is seed size (e. g. Baskin & Baskin 2001). Small seeds run the risk of being deeply incorporated into the soil (Milberg et al. 2000, Baskin & Baskin 2001), where consequent germination would be detrimental, and there has therefore been strong selection against dark germination in small seeded species (Hodkinson et al. 1998, Milberg et al. 2000, Baskin & Baskin 2001). In contrast, seedlings of large-seeded species show higher survival rates under unfavourable conditions (Bosy & Reader 1995, Turnbull et al. 1999), due to their larger reserves of resources (Baskin & Baskin 2001). However, penetration of a thick litter cover may lead to the depletion of seed resources and smaller seedling sizes as compared to siblings that germinated on bare ground. Initially small size differences may have large fitness consequences during later stages (Cook 1980).

Despite a wealth of studies on the effects of litter and vegetation on seed germination and species composition (see reviews by Facelli & Pickett 1991a and Xiong & Nilsson 1999 and references therein) there have been relatively few empirical studies simultaneously manipulating the amount of litter and the availability of abiotic resources (but see Gross 1984, Fowler 1986, Hamrick & Lee 1987, Xiong et al. 2003). Therefore, the main objective of the present paper was to evaluate the effects of litter for seedling emergence of species from floodplain grasslands at two contrasting water-availabilities. We selected two species from each of four plant families and included *family* and *species* (nested within *family*) in our statistical analysis to account for possible phylogenetic effects on seed size and germination requirements since, at least in European ecosystems, seed size and the degree of dark dormancy are closely related (Leishman et al. 2000).

Since current conservation projects are using the transfer of litter from species-rich source stands to old-fields and ex-arable fields to restore floodplain meadows (Donath et al. 2003, Hölzel & Otte 2003), another objective was to evaluate experimentally the balance between positive and negative effects of litter on seedling emergence.

We specifically addressed the following hypotheses:

1a The effects of litter on seedling emergence are predominantly positive under dry conditions and negative or neutral under favourable water supply.

1b Possible facilitative effects of litter cover on seedling emergence decrease with the amount of litter, since positive effects on soil moisture will become outweighed by negative effects on light interception.

2 If seed size is related to a species' degree of dark dormancy, there should be species and family specific effects of litter on seedling emergence.

3 If emergence through a thick litter cover depletes the seedling's reserves, there should be significant effects of litter treatments on final seedling biomass.

Material and Methods

Study species

We selected pairs of typical flood meadow species that belonged to the same plant family to be able to account for phylogenetic effects on seed size and germination requirements. In three of the four families (Asteraceae, Rubiaceae and Violaceae), the two species were congeneric (Table 1), while for the Apiaceae we used species from different genera. The ranking of species in terms of seed mass was consistent with their family identity, i. e. the smallest seeds were found in the Asteraceae while the largest seeds were found in the two Apiaceae. Three of the study species, i. e. *Viola elatior*, *V. pumila* and *Peucedanum officinale*, are considered to be threatened by extinction in Germany (Jedicke 1997) and the *Viola* species are listed among species deserving special conservation efforts in Central Europe (Schnittler & Günther 1999).

Seed sources

Seeds of the species were collected from several natural populations at different sites along the northern Upper Rhine in autumn 2003. These seeds were subsequently dry stored in darkness at room temperature until sowing on December 17, 2003. This sowing date left enough time for cold stratification in the field, which is crucial for successful germination of, for instance, Apiaceae (Baskin & Baskin, 2001). An initial viability test with a 1% Tetrazolium-chloride solution (Bennett & Loomis 1949) on additional seed batches showed that almost all seeds (>90% for all species except *Peucedanum officinale*) used in the experiment were viable (Table 1).

Table 1. Families, seed mass (mg) and percentage of seed viability (Tetrazolium-test) of the study species. All species are iteroparous, perennial hemicryptophytes. Data on seed mass are from Hölzel & Otte (2004).

Species	**Family**	**Seed mass**	**Viability (%)**
Galium boreale L.	Rubiaceae	0.68	95
Galium wirtgenii F.W. Schultz	Rubiaceae	0.40	95
Inula britannica L.	Asteraceae	0.09	91
Inula salicina L.	Asteraceae	0.16	93
Peucedanum officinale L.	Apiaceae	12.30	75
Silaum silaus (L.) Schinz & Thell.	Apiaceae	2.43	99
Viola elatior Fr.	Violaceae	1.80	95
Viola pumila Chaix	Violaceae	1.08	95

Experimental design

To study the effects of litter and water-addition on seedling emergence, we used 40 pots of 1 l volume (ca. 10 × 10 × 10 cm) for each study species, i. e. 320 pots in total. Pots were filled with commercial potting soil (Fruhstorfer Erde®, Type P, Industrie-Erdenwerke Archut GmbH, Germany) composed of a mixture of peat, clay and humus (pH 5.9; supplemented with 150 mg l^{-1} each of nitrogen and P_2O_5 and 250 mg l^{-1} K_2O). In late December 2003, we sowed 50 seeds of one individual species in each experimental pot. Pots of each study species were then randomly divided into four groups, receiving 0 (control), 2, 4 and 8 g of bench-dry litter per pot on top of the seeds. We used bench-dry litter since oven drying might induce chemical changes or subsequent leaching of litter. These litter levels of 200, 400 and 800 g litter m^{-2} correspond to annual litter production of low, medium and high productive floodplain meadows (Donath et al. 2004). We used dry grass litter cut in the previous year, which came from a mesic unfertilised grassland close to Giessen dominated by the grasses *Poa pratensis*, *Agrostis stolonifera*, *Arrhenaterum elatius*, and *Dactylis glomerata,* but lacking any of the study species. Each litter treatment was divided into two groups that were watered at different frequencies. In a pilot study we found that the soil surface became dry when soil water content (% of soil dry weight) fell below 100%. Half the pots were allowed to become intermittently dry and were watered only when water content of soil in pots without litter cover reached about 70% (in early spring these pots received 50 ml of water about every second week, but they were watered with 130 ml at weekly intervals later in the season when air temperatures and evaporation rates had increased). The soil of the other group of pots was kept constantly humid by watering once a week in early spring and twice a week later in the season. The experiment hence consisted of a combination of eight species × four litter levels × two water-additions (i. e. 64 combinations) each replicated in five pots.

Soil water content was monitored using two replicate pots per litter*water-combination (16 pots) that were filled with a defined soil mass at the start of the experiment and received no seeds. An aliquot of the same soil was dried to determine soil dry mass and initial water content. These soil-water-content-pots were reweighed on several occasions during the experiment to determine soil water content. Additionally, a temperature logger (Tinytalk, Gemini Dataloggers Ltd, UK) recorded hourly temperatures in four additional pots per litter treatment just at soil surface.

All pots (i. e. sown pots, water-content-pots, soil-temperature-pots) were arranged at random in a common garden close to Giessen, Germany (50°32'N, 8°41.3'E, 172 m a. s. l.), surrounded by another row of pots (to reduce desiccation) and protected by a wooden frame covered with thin polyethylene plastic sheet to exclude precipitation. In mid-April, we used quantum sensors (Li-190, Licor Inc., USA) to measure transmittance of photosynthetic active radiation (PAR) to the soil surface in relation to ambient PAR radiation above the polyethylene shield (i. e. relative PAR irradiation) in four pots per litter treatment.

Germination started in early April 2004. Seedlings per pot that emerged above the litter were counted on April 08, 16, 23, May 11 and June 15 and individually marked with non-toxic colour to be able to differentiate newly emerged seedlings from those already present. Since only seedlings that penetrate the litter layer can go on to become established, we considered only emerged seedlings as successfully germinated when calculating the percentage emergence at each date. Since there was no seedling mortality during the experiment, the final percentage of emerged seedlings at the end of the experiment represents cumulative emergence during the germination season.

On June 15, total above-ground biomass was harvested, dried to constant mass at 70°C and weighed to the nearest milligram. From this we derived the mean biomass per individual in each pot.

Data analyses

Univariate four-way ANOVA was employed to analyse for effects of litter, water-addition, species identity and plant family on the dependent variables (final percentage emergence, total biomass per pot and individual mean biomass). For the statistical analysis the factor 'species' was hierarchically nested within the factor 'family' and all factors were considered fixed. Two-way ANOVA was used for the analyses of the effects of litter and water-addition at the species level.

In the case of percentage emergence at each date, we used repeated measures analysis to assess the overall effects of time, each of the factors, and their interactions. In case of the between-subject factors (litter, water, family, species(family)) we carried out univariate repeated measures ANOVA as suggested by von Ende (1993). For the within-subject factor time (and its interactions), MANOVA was used, since data from repeated counting in the same pot rarely meet the assumptions for repeated measures ANOVA, i. e. 'circularity' and 'compound symmetry' (von Ende 1993). In the MANOVA, *P*-values were derived using Pillai's trace, which is more robust than other statistics (Quinn & Keough 2002).

As a measure for the relative contribution of each factor and the interactions to the total variability in final percentage emergence, total biomass and mean biomass per plant, we used the ratio of the sum of squares of the factor or interaction of interest to the total sum of squares (i. e. for all factors, their interactions and the error). If necessary, data were transformed prior to analyses to meet assumptions of analysis of variance (Zar 1999). All statistical analyses were done using SAS 8.2 (Anon. 1999).

Results

Physical effects of litter and water-addition

Average soil temperatures did not differ among litter treatments (one-way ANOVA, ln-transformed data, $F_{3,592} = 0.321$, $P = 0.81$). However, litter cover significantly reduced daily temperature amplitude ($F_{3,592}$ = 11.457, $P < 0.0001$; control pots showed significantly higher amplitudes than all litter treatments), especially during warmer periods such as in late April. In general, control pots had significantly higher daily maximum temperatures than all other litter treatments ($F_{3,592}$ = 18.58, $P < 0.0001$) and maximum hourly temperatures could be up to 10°C higher in control pots than in pots covered with 8 g litter.

Relative PAR irradiation decreased significantly with litter cover (one-way ANOVA, arcsine transformed data $F_{3,12}$ = 307, $P < 0.0001$). Control pots received 64.5% ± 2.5 (mean ± s. e., $N = 4$) of the ambient photon flux density above the polyethylene shield, while seeds below 2, 4 and 8 g litter received only 6.7% ± 0.8, 1.1% ± 0.4 and 0.009% ± 0.006, respectively. Relative photon flux density did not differ significantly between pots covered with 4 and 8 g of litter, but pots covered with 2 g of litter and control pots each differed significantly from all other litter treatments.

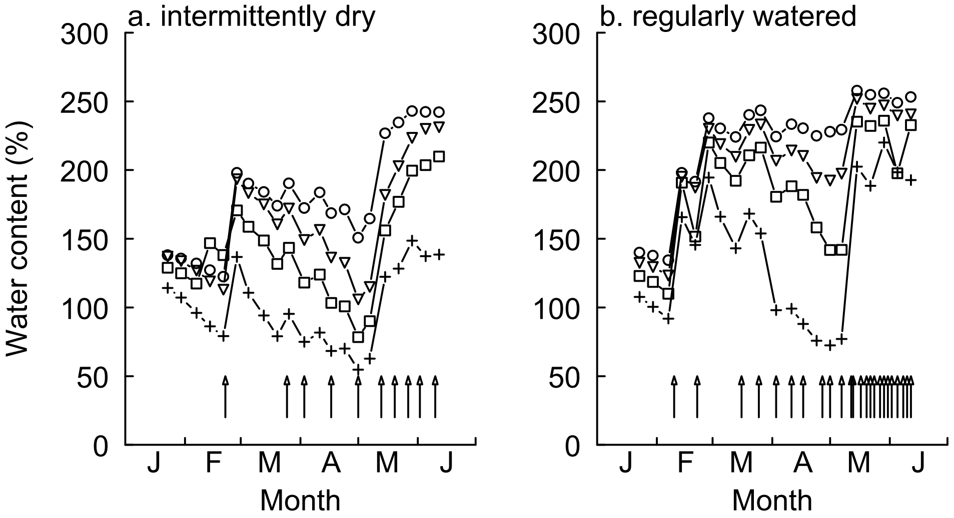

Figure 1. Soil water content (% of soil dry weight) in experimental pots covered with different amounts of litter. Data are means of two replicate pots. Symbols: +, control; squares, 2 g litter; triangles, 4 g litter; circles, 8 g litter. Arrows indicate watering occasions (see Methods).

Owing to rather high temperatures from early-April to early-May, the surface of even the more frequently watered control pots dried out, and differences in soil water content in control pots between watering treatments were therefore small. However, for those pots covered with litter, during the part of the experiment when most seed germination took place (March to early-May), pots that were kept constantly humid contained considerably more water (188% of soil dry weight averaged across litter treatments) than pots that were allowed to become intermittently dry (135%; Figure 1). Additionally, within each water-addition treatment, soil water content increased with litter cover during this period: constantly moist pots contained 44, 49, 39 and 31% more soil water than intermittently dry pots covered with 0, 2, 4, and 8 g litter, respectively (Figure 1). After the watering schedule had been changed in mid-May, differences were much smaller.

Cumulative germination

The effects of water, litter, species, family and their interactions on percentage emergence were highly significant through the whole experiment (Table 2, between-subject factors). MANOVA results, however, showed a clear time effect and also that none of the factors or their interactions showed a consistent pattern throughout the whole experiment (Table 2, within-subject factors, significant factor*time interactions). At the species level, the significance of litter, water-addition, time and their interactions proved that, in most cases, germination changed over time and that species differed in percentage emergence (results not shown). Only in *Viola elatior* and *Silaum silaus* was there a significant litter effect without an effect of water-addition and litter*water-addition interaction.

Final seedling emergence

Final percentage emergence was significantly higher in *Galium* (36.2% ± 2.1, mean ± s. e., *N* = 80) and *Viola* (28.6% ± 2.8) than in *Inula* (18.3% ± 1.6) and the Apiaceae (18.0% ± 1.6; cf. Figure 2) and differed significantly between species within families (Table 3). Across species and water-addition treatments, emergence was twice as high in pots covered with 2, 4, and 8 g litter (about 30% emerged seedlings) than in control pots, and across species and litter treatments water-addition had positive effects on seedling emergence (Table 3). However, there was a significant interaction between litter and water-addition, which itself depended on species identity (significant L*W*S(F) interaction, Table 3). The factor litter, together with its interactions with ‘species’, ‘family’ and ‘water’ accounted for about 35% of the total variation (Table 3), with the litter*family interaction being especially strong.

Table 2. Repeated measurement analysis of the effects of species identity (S), family (F), amount of litter (L) and watering regime (W) on relative germination. In case of the between-subject factors results originate from ANOVA and in case of within-subject factor results originate from MANOVA. T = time; DF_{NUM} = numerator degrees of freedom; DF_{DEN} = denominator degrees of freedom; MQ = mean sum of squares.

Between-subject factors

Source of variation	DF	MQ	*P*
F	3	1.24	< 0.0001
S(F)	4	0.29	< 0.0001
L	3	0.86	< 0.0001
W	1	1.84	< 0.0001
L*F	9	0.49	< 0.0001
W*F	3	0.08	0.0024
L*W	3	0.21	< 0.0001
L*S(F)	12	0.09	< 0.0001
W*S(F)	4	0.08	0.0005
L*W*F	9	0.03	0.0277
L*W*S(F)	12	0.05	0.0004
Error	256	0.02	

Within-subject factors

Source of variation	Pillai's trace	DF_{NUM}	DF_{DEN}	*F*	*P*
T	0.8938	5	252	424.2	< 0.0001
T*F	0.7825	15	762	17.9	< 0.0001
T*S(F)	0.7309	20	1020	11.4	< 0.0001
T*L	0.6227	15	762	13.31	< 0.0001
T*W	0.3101	5	252	22.7	< 0.0001
T*L*F	0.8638	45	1280	5.9	< 0.0001
T*W*F	0.2267	15	762	4.2	< 0.0001
T*L*W	0.2574	15	762	4.8	< 0.0001
T*L*S(F)	0.8033	60	1280	4.1	< 0.0001
T*W*S(F)	0.2001	20	1020	2.7	< 0.0001
T*L*W*F	0.2815	45	1280	1.7	< 0.003
T*L*W*S(F)	0.4567	60	1280	2.1	< 0.0001

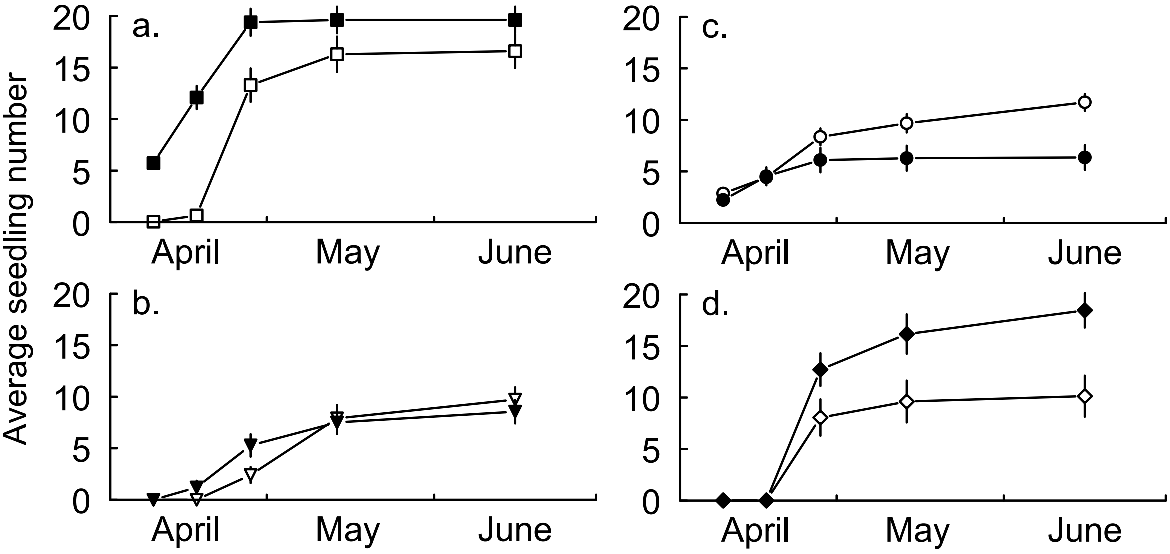

Figure 2. Average cumulative number of seedlings across treatments that emerged out of 50 seeds per pot. Data are means ± s. e. ($N = 40$) in species of *Galium* (a.), *Inula* (b.), *Peucedanum*/*Silaum*(c.) and *Viola* (d.). Symbols: filled squares, *Galium wirtgenii*,; open squares, *G. boreale*; filled triangles, *Inula britannica*; open triangles, *I. salicina*; filled circles, *Silaum silaus*; open circles, *Peucedanum officinale*; filled diamonds, *Viola pumila*; open diamonds, *V. elatior*.

When analysed for each species separately, there was a significant litter*water-addition interaction in six of the eight study species (Figure 3), although in the remaining two (*S. silaus* and *V. elatior*) percentage emergence increased significantly with litter cover irrespective of water-addition. When intermittently dry, germination of all species was significantly higher in pots covered with litter than in control pots. In pots covered with 8 g litter, there was hardly any seedling emergence of *Inula* spp. and emergence decreased considerably in *Galium* spp., but for the Apiaceae and *Viola* spp. seedling emergence was as high or higher than at 2 and 4 g (Figure 3). In contrast, when kept constantly moist, percentage emergence did not differ significantly between controls, 2 and 4 g litter pots in six of eight species with only *G. boreale* and *V. pumila* showing significantly higher emergence in pots with 2 and 4 g litter than in control pots. In *G. wirtgenii* and *Inula* spp. emergence in pots with 8 g litter was significantly lower than in controls, while there was no difference or seedling emergence increased under 8 g litter in all other species.

Table 3. Results of a four-way ANOVA on the effects of species identity (S), family (F), amount of litter (L) and watering regime (W) on relative germination, total biomass and individual biomass. DF = degrees of freedom; MQ = mean sum of squares; vc (%) = relative contribution of individual factors and their interactions to total variation.

Source		**Germination**			**Total biomass**			**Individual biomass**		
of variation	**DF**	**MQ**	***P***	**vc (%)**	**MQ**	***P***	**vc (%)**	**MQ**	***P***	**vc (%)**
F	3	0.36	< 0.0001	14.1	8.84	< 0.0001	4.8	15.69	< 0.0001	5.0
S(F)	4	0.15	< 0.0001	7.8	7.43	< 0.0001	5.4	7.56	0.0001	3.2
L	3	0.30	< 0.0001	11.9	17.60	< 0.0001	9.6	46.82	< 0.0001	14.8
W	1	0.62	< 0.0001	8.1	192.76	< 0.0001	34.9	117.17	< 0.0001	12.3
L*F	9	0.18	< 0.0001	21.3	7.04	< 0.0001	11.5	11.04	< 0.0001	10.5
W*F	3	0.03	0.0052	1.1	3.64	< 0.0001	2.0	1.96	0.1960	0.6
L*W	3	0.05	< 0.0001	2.1	2.72	0.0002	1.5	33.95	< 0.0001	10.7
L*S(F)	12	0.04	< 0.0001	5.8	0.99	0.0044	2.2	1.32	0.3906	1.7
W*S(F)	4	0.03	0.0022	1.5	6.27	< 0.0001	4.5	0.72	0.6774	0.3
L*W*F	9	0.01	0.1878	1.1	1.91	< 0.0001	3.1	5.73	< 0.0001	5.4
L*W*S(F)	12	0.02	0.0014	2.9	0.93	0.0075	2.0	1.60	0.2232	2.0
Error	256	0.0067		22.3	0.3996		18.5	1.2421		33.5

Seedling biomass and density

The most important factor affecting total biomass per pot was water-addition, accounting for 35% of the total variation (Table 3). The effect of water-addition on total biomass increased from control pots to pots covered with 2 g of litter and decreased with further increase in litter cover, leading to a significant litter*water-addition interaction (Table 3). However this interaction further varied among species and families. Biomass per individual plant showed strong negative relations with germination, i. e. the final plant density per pot, in most species. Across species and water-treatments the largest biomass per individual was found at 2 and 4 g of litter, while it decreased in both control pots and at 8 g of litter. Water-addition had positive effects on individual biomass, leading to plants that were kept constantly moist reaching higher biomass at the same density than plants from intermittently dry pots, at least for *Galium wirtgenii*, *Inula* spp., *Peucedanum officinale* and *Viola pumila*. In *Silaum silaus* and *Viola elatior* there were hardly any differences between water-treatments, and in *Galium boreale*, germination and thus density was considerably increased in watered pots. Pots that had no or low germination and low individual biomass, were either infrequently watered (*G. boreale*, *P. officinale*, *Viola* spp.) or covered with 8 g litter (*Inula* spp.). More frequent water-addition led to larger increase in individual biomass in control pots than in pots covered with different amounts of litter (significant litter*water-addition interaction, Table 3). This interaction was independent of species but differed significantly among families (Table 3).

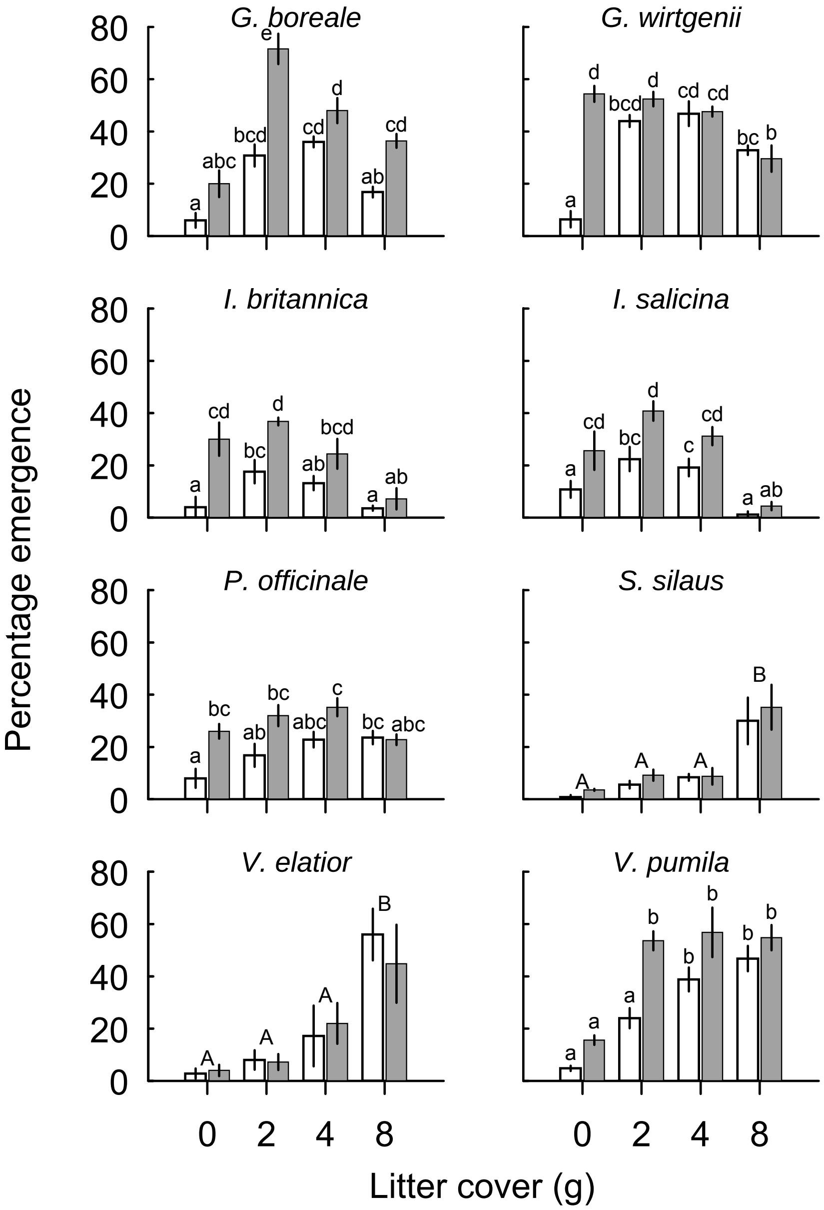

Figure 3. Final percentage seedling emergence of the study species in constantly moist (grey bars) and intermittently dry (open bars) pots six months after sowing. Data are means ± s. e. (*N* = 5). Differences between all means were tested a posteriori using Tukey's HSD test for species with a significant litter*water interaction ($F_{3,32} > 3.12$, $P < 0.04$). For *S. silaus* and *V. elatior* only differences among litter treatments across water-additions were tested, since the main effect of litter was significant ($F_{3,32} > 14.7$, $p < 0.0001$). Bars and litter treatments sharing the same lower or upper case same letter, respectively, are not significantly different ($P > 0.05$).

Discussion

We found evidence for facilitative effects of litter at a lower water-supply rate, where seedling emergence significantly increased when seeds were covered by litter, whereas, in constantly humid pots, germination rarely differed significantly between controls and pots covered with litter (Table 3; Figure 3). Across species, differences in emergence between water-treatments therefore decreased with increasing litter cover, leading to a significant litter*water-addition interaction (Table 3). The facilitative effect of litter on seedling emergence was probably due to improved water conditions (Figure 1) together with reduced thermal stress (significantly lower maximum temperatures; Fowler 1986, Facelli & Pickett 1991b, Xiong et al. 2003). Even though differences in water content in control pots between watering treatments were small during early-April to early-May, and the soil surface dried up in both treatments, relative germination in constantly humid control pots was still higher in most of the species (and significantly higher in four species; Figure 3) than in intermittently dry controls.

For this facilitative effect to occur, litter must lead to a greater alleviation of stressful (i. e. relatively dry) conditions, than it causes deterioration of light quantity and quality (cf. Holmgren et al. 1997). Moisture will enhance germination in most, if not all, grassland species (e. g. Baskin & Baskin 2001). However, since light requirements for germination are related to seed size (Milberg et al. 2000) and differ among species and/or families, the effects of litter and water-addition are not independent of species identity (Table 3; cf. Bosy & Reader 1995). This suggests that the significantly decreased germination under 8 g of litter, irrespective of water-addition, in four of the study species may be related to their small seed size. Similarly, Jensen & Gutekunst (2003) found positive relationships between seed mass (and the proportion of seeds germinating in darkness) and seedling establishment under a litter cover of 3 cm depth. Since light transmittance through increasing amounts of litter approximately follows the Beer-Lambert exponential law (Facelli & Pickett 1991b), photon flux density of PAR under 4 and 8 g of litter was reduced to 1% or less of the ambient (cf. Facelli & Pickett 1991a). After release from the mother plant, smaller seeds are more likely to enter small fissures and cracks and to be incorporated deeper into the soil than large seeds (Milberg et al. 2000, Baskin & Baskin 2001). Since germination from depth leads to high mortality rates, there has probably been strong selection during the evolution of seed size against dark germination in small seeded species (Hodkinson et al. 1998, Milberg et al. 2000, Baskin & Baskin 2001). *Inula britannica* and *I. salicina* which had the lowest seed mass of our study species (Table 1), hardly germinated at a litter cover of 8 g and *G. wirtgenii* and *G. boreale* with seed masses below 0.7 mg also showed considerably reduced germination at 8 g as compared with 2 and 4 g of litter (Figure 3). In contrast, large seeded species, owing to their much larger reserves, are able to germinate in darkness or under a thick litter cover and show higher survival rates under harsh conditions such as drought and deep shade (Bosy & Reader 1995, Turnbull et al. 1999). In *Viola* spp. and *S. silaus*, germination increased monotonically with litter cover and these species showed the highest rates of seedling emergence at 8 g of litter (Figure 3). We suggest that this may due to their relatively hard seed coats which probably need longer exposure to microbiological activity, and thus constantly high moisture below litter, to break physical dormancy (e. g. Baskin & Baskin 2001).

The main causes of seedling mortality are herbivory, drought and fungal attack (Cabin et al. 2000, Moles & Westoby 2004a). Litter on top of germinated seedlings will provide insulation against incoming radiation, decreasing both the temperature amplitude and daily temperature maxima, which may be harmful for seedlings. Higher air humidity under the litter may also prevent seedlings from desiccation and reduce water losses through evapo-transpiration (Fowler 1986; Figure 3, Table 3). Our data thus suggest that these positive effects of litter may operate in mesic grassland during dry seasons, as well as in dry ecosystems (Xiong & Nilsson 1999). Therefore, it seems that dead plant remains exert similar effects on seed germination and seedling establishment to those of living plants, the sign of the interaction (positive and negative) largely depending on the marginal conditions (Foster & Gross 1997, 1998, Holmgren et al. 1997, Tielbörger & Kadmon 2000, Xiong et al. 2003, Eckstein 2005). Partitioning the effects of plant biomass and litter on the recruitment of *Andropogon gerardii* and species richness in successional grassland revealed that the two factors were purely additive (Foster & Gross 1997, 1998). Negative effects of biomass and litter were due to their ability to attenuate incoming light to very low levels. However, litter had the largest negative effects at sites with the highest productivity (Foster & Gross 1997), while effects on germination at low productive sites were not significant.

It should be noted that the presence litter on top of seeds and seedlings will exert diametrically opposite effects from those seen in situations such as abandoned grasslands, where seeds will fall on top of a litter carpet. Here, litter will prevent seeds from coming into contact with the humid soil, reduce germination and result in desiccation of seedlings germinating within the litter (cf. Facelli & Pickett 1991a and references therein). This highlights the fact that, apart from the amount of litter, its position with respect to the shed seeds will determine the outcome of litter-seed interactions. Therefore, the increased production of biomass and thick litter carpets which result from atmospheric nitrogen input and land use abandonment still present a threat to biodiversity conservation (Foster & Gross 1997, 1998, Berendse 1999). Another factor that may be important is the structure, texture and chemical composition of litter, since this will determine litter characteristics that may trigger seed germination and seedling emergence. For example, in contrast to the present study, Jensen & Gutekunst (2003), using rather coarse, heavy *Carex*-litter found predominantly negative effects on seedling emergence.

Additionally, litter may represent protection against herbivory at least in forest systems (Shaw 1968, Sydes & Grime 1981). However, in an old-field, arthropod herbivory on seeds was higher under litter cover than in control plots (Facelli 1994).

In the light of ongoing restoration measures in flood-meadows along the northern Upper Rhine (Hölzel & Otte 2003), our results suggest that the successful establishment of rare species can be enhanced by the transfer of seed-containing plant material, such as litter, from species-rich remnant stands. Compared to the controls an amount of plant material between 0.2 to 0.4 kg m^{-2} seems to be adequate to enhance germination in most species but still avoids suppression of emergence which occurs at higher levels of litter. Thus, as long as appropriate amounts of litter are applied, there is no need to remove the plant material after some time - as suggested by Jones et al. (1995) - since not only will subsequent desiccation of seedlings lower restoration success but additional seeds remaining in the plant material will be removed.

At the seed densities used and levels of relative germination observed in this experiment, we found a density-dependent reduction in biomass for most species but no density-dependent mortality due to sibling competition or other causes. The negative relationship between density and biomass per plant was largely an effect of small individual plants at the highest litter cover, since mean percentage emergence did not differ much between pots with 2, 4, and 8 g litter. This suggests that successful emergence through a thick litter cover may deplete the seedling's resources and, in the long run, impair the fitness and fecundity of these smaller seedlings (e. g. Cook 1980).

In summary, our results suggest that facilitation may not only occur between living plants but there may also be positive effects of dead plant remains (i. e. litter) on seedling emergence and establishment. Such 'after-death' interactions may be positive or negative and occur not only in xeric or otherwise extreme ecosystems. The sign of the litter effect on seedling emergence is determined by soil humidity: the threshold value, above which litter effects turn negative, is species and family specific and closely related to seed size. Whether positive litter effects (Xiong & Nilsson 1999; this study) are a consequence of coevolution in grasslands remains to be examined.

Acknowledgements

We want to thank Christiane Lenz-Kuhl and Josef Scholz-vom Hofe for their invaluable help during the experiment and for carrying out the Tetrazolium test. Kai Jensen, Norbert Hölzel, Annette Otte and two anonymous referees offered invaluable comments, which improved earlier versions of this manuscript. Lindsay Haddon kindly corrected our English. This research was funded by the German Federal Agency for Nature Conservation (BfN).

3 Chemical effects of dominant grass on seed germination of four familial pairs of dry grassland species

Ruprecht E, Donath TW, Otte A & Eckstein RL 2008
Seed Science Research 18: 239-248

Abstract

Community composition and ecosystem processes during succession may partly be driven by traits of plant species that attain dominance. Here, we addressed the hypothesis that *Stipa pulcherrima*, the dominant grass of abandoned continental grasslands, control seedling recruitment of co-occurring species through chemical effects of its litter. Eight species with successful and unsuccessful recruitment under field conditions were selected (four familial pairs) to experimentally study effects of leaf leachate under four temperature regimes. Since fungi developed in leachate treated Petri dishes, in another experiment seeds were surface sterilised to remove confounding effects of fungi on recruitment. Leachate affected various stages of seedling recruitment: it significantly reduced seed germination (by 33-94%) and radicle elongation, and it delayed germination of seedlings of all species. In two families, species with unsuccessful field recruitment were more negatively affected than the successful ones. In a third family, the species with successful recruitment was more negatively affected, and in the fourth there was no difference. Similar germination responses after exclusion of fungi through seed surface sterilisation suggested that leachate was responsible for the observed effects on recruitment. Besides other traits and physical or microclimatic effects of accumulating litter, *St. pulcherrima* influences plant community dynamics and may potentially affect ecosystem processes through its secondary compounds.

Introduction

Community assembly during succession may be regulated by (i) environmental conditions and resources, (ii) chance events, and (iii) biotic interactions (Weiher & Keddy 1999). Through the traits of dominant species, the outcome of biotic interactions (herbivory, competition, facilitation) may in turn feedback on abiotic conditions and resource availability and strongly determine the further development of the system. One model system that is currently under great changes is European semi-natural grassland, which carries an exceptionally high diversity of plants (e. g. Ellenberg 1988, Korneck et al. 1998). Diversity and composition of these ecosystems evolved under the prevailing site conditions (Vandvik & Birks 2002) but also in response to continuous human activities over millennia (Ellenberg 1988, Pärtel et al. 1996). Consequently, their characteristic species are adapted to human land use and grassland diversity is strongly related to past and current management practices (Waldhardt & Otte 2003, Wellstein et al. 2007). With agricultural abandonment and intensification, plant diversity in semi-natural grassland communities dramatically changes (Korneck et al. 1998, Luoto et al. 2003, Mitchley & Xofis 2005, Pykälä et al. 2005, Wellstein et al. 2007, Enyedi et al. 2008). The resulting decrease in species numbers is mainly due to (1) an increasing cover and biomass of a few highly productive species, which exert strong competitive effects on subordinate species, and (2) the accumulation of dead plant remains, i. e. litter, which interfere with seed germination and seedling establishment (Bosy & Reader 1995, Virágh & Bartha 1996, Bakker & Berendse 1999, Kahmen et al. 2002, Moog et al. 2002). Litter may affect seed germination by its effects on microclimate, by acting as a mechanical barrier or through chemical effects (Facelli & Pickett 1991a, Bosy & Reader 1995, Jensen & Gutekunst 2003, Eckstein & Donath 2005). Since these effects are litter type specific, the dominant plant species may exert a strong influence on germination and succession (Quested & Eriksson 2006, Myster 2006, Quested et al. 2007, Donath & Eckstein 2008), but also on ecosystem processes (Wardle et al. 1998).

The regeneration of plants from seeds is a process that can be broken down into several conditional components, such as reproductive fertility and seed production, seed viability, germination, seed resource based initial seedling growth (radicle elongation, leaf expansion), autotrophic seedling growth and establishment. As such it is a particularly vulnerable stage in the life cycle of many plant species, and the suppression of seedling recruitment can have serious consequences for population viability and species diversity (e. g. Tilman 1993). In this context allelopathy, i. e. the leaching or volatilization of phytotoxins from plant tissues is considered as one possible mechanism acting on seed germination (Baskin & Baskin 2001), but also on seedling establishment (Schlatterer & Tisdale 1969, Chang-Hung & Chiu-Chung 1975, Werner 1975, Bosy & Reader 1995), and plant growth and distribution (del Moral & Cates 1971, Rice 1972, Newman & Rovira 1975). Chemicals involved are generally secondary metabolites, mainly simply structured, low molecular weight compounds such as coumarins, terpenoids, phenolics or tannins (Rice 1984, Harborne 1993). However, since there are close links between plant secondary compounds, palatability, litter quality and decomposition (Grime et al. 1996), allelochemicals may also affect other components and functions of the ecosystem and thus play an important role in ecosystem regulation (Wardle et al. 1998, Mazzoleni et al. 2007).

Biochemical interactions differ in their strength and may differentially affect the competitive vigour of dominant and subordinate species. Hence, to understand these interactions and the potential influence of dominant species on ecosystem processes, it is necessary to identify plant species in a community with strong allelopathic effects on other species (Chang-Hung & Chiu-Chung 1975, Anaya & del Amo 1978, Anderson et al. 1978), but equally important is to reveal the differential susceptibility of community constituents to these toxins (Datta & Sinha-Roy 1975, Wardle et al. 1993).

Preliminary results of a manipulative field experiment on abandoned grasslands from the Transylvanian lowlands of Romania (Ruprecht et al., 2010a) suggested that secondary compounds of the dominant grass may, among other factors, drive community assembly and succession through strong negative effects on the recruitment of a number of co-occurring plant species. These species rich dry steppe-like grasslands occur on steep south facing slopes with eroded carbonated chernozemic soils on clayish or marly substrate, and are dominated by feather-grass (*Stipa*) species. The main consequences of long-term abandonment in these grasslands are (i) strong dominance by *Stipa pulcherrima*, (ii) litter accumulation, (iii) vegetation composition changes, and (iv) decrease of plant species diversity (Enyedi et al. 2008). Under several experimental treatments consisting of litter and/or biomass removal, establishment success of certain species was very low (Table 1; Ruprecht et al. 2010a), though there were no indications of seed or microsite limitation since plants flowered abundantly and various types of potential safe sites for germination and establishment were created by the treatments. Another group of dry grassland species with abundant flowering and seed production established successfully under the same experimental conditions (Table 1). Therefore, we experimentally investigated possible chemical effects of the dominant species, *St. pulcherrima*, on the germination of seven co-occurring dry grassland species and on itself at two constant and two fluctuating temperature regimes. Previous studies addressing chemical effects of litter have either used one constant (e. g. Anaya & del Amo 1978, Bosy & Reader 1995) or one fluctuating temperature regime (e. g. Rice 1972, Norby & Kozlowski 1980), whereas our experimental setup allows us to address the interaction between litter leachate and temperature across a range of conditions. Our study attempts to link potential chemical effects at the population level with known transformations in ecosystem properties in a dry grassland community as a result of grazing abandonment.

Our main hypotheses were that (1) *Stipa* leachate reduces percentage germination, delays germination, and interferes with radicle elongation, thus exerting strong negative effects on the regeneration through seeds of the eight test species, and that (2) there are species-specific differences in the response of these various components of recruitment to leachate. (3) We hypothesised that the effect size of *Stipa* leachate is related to the germination success under field conditions, i. e. the negative effects are more pronounced in species with unsuccessful than in those with successful field recruitment.

Materials and Methods

Species selection

Eight grassland species, which are typical constituents of dry steppe-like grasslands of the Transylvanian Lowland (Romania), were selected for laboratory seed germination experiments based on the results of previous field experiments (Table 1). The species set consisted of four familial pairs (*Asteraceae*, *Fabaceae*, *Rubiaceae*, *Poaceae*), where one species of each pair was from the group with successful and the other from the group with unsuccessful establishment under field conditions (Table 1). Family pairs were used in order to obtain a phylogenetically balanced data set.

Table 1. Species abbreviations, field germination, establishment success under field conditions, family, diaspore mass, and seed viability of the eight dry grassland species selected for the germination experiments.

Species selected*	**Abbr.**	**Field germ. (seedl. m^{-2})**	**Success**	**Family**	**Diaspore mass (mg)**	**Seed viability (%)**
Serratula radiata (Waldst. & Kit.) Bieb.	Sr	0	–	*Asteraceae*	2.53	80
Jurinea mollis (L.) Rchb. subsp. *transylvanica* (Spreng.) Hayek	Jm	6.2	+	*Asteraceae*	3.45	70
Medicago sativa L. subsp. *falcata* (L.) Arcang.	Ms	1.5	–	*Fabaceae*	1.11	72
Dorycnium pentaphyllum Scop. subsp. *herbaceum* (Vill.) Rouy	Dp	43.3	+	*Fabaceae*	1.56	66
Galium glaucum L.	Gg	0	–	*Rubiaceae*	1.09	70
Asperula cynanchica L.	Ac	42.8	+	*Rubiaceae*	0.63	56
Stipa pulcherrima C. Koch	Stp	2.5	–	*Poaceae*	24.29	92
Stipa capillata L.	Stc	28.7	+	*Poaceae*	5.14	98

Field germination is the sum for the two years of field observations (2006, 2007) of the cumulative number of seedlings counted in two dry grassland sites (Suatu, Puini), expressed as seedlings m^{-2}. Data on seed mass are the results of measurements of 500 (5 x 100) seeds per species. Seed viability of 50 (2 x 25) seeds per species was tested with a 1% tetrazolium chloride solution.

* Plant nomenclature follows Flora Europaea (Tutin et al. 1964-1980).

Seed collection

Propagules of the eight species (called seeds from here on) were collected in bulk from autochthonous populations in dry steppe-like grasslands in the Transylvanian Lowland. Seed collection was carried out between May and August 2007, depending on the time of seed ripening, and included at least 100 different plant individuals from five sites each. Seeds were dry-stored in darkness at room temperature (ca. 20°C) until the start of the experiments in autumn 2007.

Germination experiment 1

The germination experiment was performed in climate chambers (Rumed, Rubarth Apparate GmbH, Laatzen, Germany) at the Department of Landscape Ecology and Landscape Planning, Gießen University, Germany. Fifty seeds of each species were spread on a double layer of filter paper in sterile Petri dishes. A total of forty dishes per species were prepared, randomly distributed into two groups to test for chemical effects of *Stipa pulcherrima*. One group was watered with a leachate prepared from leaves of *St. pulcherrima*, whereas the other group received distilled water (control). Leaves of *St. pulcherrima* were collected in the middle-late phase of the vegetation period (July and August 2007) from two grassland sites (Suatu, Puini) and dried at room temperature. The leachate was prepared by soaking 344.67 g of dry leaves in 3300 ml of distilled water for 48h. These amounts of leaves and water (0.1044 g leaves per ml water) correspond to the average litter quantity in the field (470 g m^{-2}) and the quantity of rain water of an average precipitation event (4.5 l m^{-2}), respectively (Cluj Napoca Meteorological Station). In the leachate and the control treatment, each sample was watered with 10 ml of the leachate solution or 10 ml of distilled water, respectively, once at the start of the experiment. As characteristics that may influence seed germination we assessed the osmotic potential (Osmomat 030, Gonotec GmbH Berlin, Germany) and pH (Lab 860, Schott Instruments, Mainz, Germany) of the leachate.

The twenty dishes of each leachate treatment group were randomly distributed to the four temperature treatments, i. e. two constant (10 and 20°C) and two diurnally fluctuating temperatures (5/15°C and 10/25°C), and incubated in climate chambers. Of each species five Petri dishes were put together into a climate chamber and sealed to reduce evaporation. The light regime was simulating spring day conditions with 12 hours of light and 12 hours of darkness.

Consequently, the experiment comprised the following factors: *species* (factor levels (k) = 8), nested within *field establishment success* (k = 2), *temperature regime* (k = 4), and *leachate* (k = 2). Each combination of factors was replicated five times. Germinated seeds were counted and removed once every week during six weeks. A seed was considered germinated when the radicle was beginning to protrude from the seed coat. At each counting occasion the radicle length of a maximum of five randomly chosen seedlings was measured using a millimeter paper. We used (1) cumulative germination, calculated as the sum of germinated seeds over six weeks in relation to the total number of seeds, (2) the week of maximum germination, and (3) the radicle length of seedlings as dependent variables. Since in our experiment fungi developed in high abundance especially in samples watered with leachate, we visually estimated the cover of developing fungi in the dishes to the nearest 5% and determined fungal genera (or higher order groups).

Germination experiment 2

To separate the possibly confounding effects of leachate and fungi on seed germination, we carried out an additional experiment where we tried to exclude fungi in order to test the effect of leachate alone. The experimental setup was analogous to the above mentioned with the exception that seeds were surface sterilised through the application of NaOCl (3% active chlorine) and the leachate was sterile-filtered in order to exclude spores of fungi and bacteria. We used one diurnally fluctuating temperature regime of 5/15°C (12/12 hours), which had proved to be an optimal germination temperature regime for most of the study species in the first experimental setup. The measures successfully prevented fungal growth in the Petri dishes containing *Jurinea mollis, Medicago sativa, Dorycnium pentaphyllum*, and *Asperula cynanchica* during the first three weeks of germination. Seedlings were counted and removed once every week.

Data analysis

Data on cumulative germination, radicle length, week of maximum germination and fungal abundance were analysed using a four-factorial hierarchical general linear model analysis of variance. The factor *species* was nested within *field germination success* (Table 1) and all factors were considered fixed. In a combined analysis we used data on cumulative germination after three weeks of those four species that showed no fungal growth after the sterilisation treatment. Here we used a three-factorial general linear model analysis of variance with the factors *species*, *leachate* and *seed surface sterilisation*, i. e. sterilisation (no effect of fungi) *vs*. no sterilisation (seeds infected by fungi). Arcsine or square root transformation was used to improve homogeneity of variances. Tukey’s HSD test was applied for *a posteriori* testing of multiple means (Quinn & Keough 2002). All analyses were done using Statistica, ver. 6.0 (StatSoft 2001).

Results

Seed germination

Cumulative percentage germination across temperatures varied significantly among species (Table 2, Figure 1a) and ranged between 1.8% (*St. pulcherrima*) and 67.6% (*S. radiata*). Under experimental conditions, there were no general significant differences between species that showed successful or unsuccessful germination in the field. However, leachate significantly and strongly reduced percentage germination (Figs 1a, 2a); among species, germination was reduced by between 33 and 94% as compared to controls. This factor explained 14.9% of the total variation (Table 2).

Table 2. Effects of species identity, establishment success under field conditions, temperature, and leachate on percentage germination, week of maximum seed germination, and radicle length. SoV = Source of variation, Int = Intercept, Sp(Su) = Species(Success), Su = Success, T = Temperature, L = Leachate.

	Germination				**Week of maximum germination**				**Radicle length**			
SoV	**df**	**MS**	***P***	**vc (%)**	**df**	**MS**	***P***	**vc (%)**	**df**	**MS**	***P***	**vc (%)**
Int	1	70.23	< 0.0001		1	2619.18	< 0.0001		1	1372.13	< 0.0001	
Sp(Su)	6	3.12	< 0.0001	61.40	6	35.33	< 0.0001	22.07	4	4.33	< 0.0001	6.66
Su	1	0.01	0.4808	0.02	1	4.72	0.0258	0.49	1	5.03	< 0.0001	1.93
T	3	0.03	0.0357	0.33	2	6.29	0.0014	1.31	3	16.32	< 0.0001	18.82
L	1	4.55	< 0.0001	14.94	1	260.17	< 0.0001	27.09	1	101.77	< 0.0001	39.12
Sp(Su)*T	18	0.09	< 0.0001	5.15	12	2.05	0.0126	2.55	12	0.73	0.0013	3.35
Sp(Su)*L	6	0.22	< 0.0001	4.35	6	8.06	< 0.0001	5.04	4	1.22	0.0010	1.88
T*L	3	0.05	0.0038	0.53	2	3.74	0.0196	0.78	3	5.59	< 0.0001	6.44
S*L	1	0.01	0.5341	0.02	1	0.08	0.7698	0.01	1	0.79	0.0794	0.30
S*T	3	0.08	0.0003	0.75	2	0.31	0.7214	0.06	3	1.13	0.0049	1.30
S*L*T	3	0.11	< 0.0001	1.08	2	1.91	0.1333	0.40	3	0.23	0.4380	0.27
Sp(Su)*T *L	18	0.03	0.0019	1.62	12	2.09	0.0106	2.61	12	0.41	0.0872	1.91
Error	256	0.01		9.82	383	0.94		37.59	184	0.25		18.00

Data are the results of a four-way hierarchical ANOVA with species nested within establishment success. Temperature = constant temperatures: 10°C, 20°C, fluctuating temperatures: 5/15°C and 10/25°C. Data on relative germination were arcsine transformed, and data on fungal abundance were square root transformed before analysis. Owing to missing data, *St. pulcherrima* and *A. cynanchica* were excluded from the analysis of radicle length, and in the analysis of the week of maximum seed germination the temperature level 20 °C had to be omitted. Abbreviations: df = degrees of freedom; MS = mean sum of squares; vc (%) = percentage of the total variation explained.

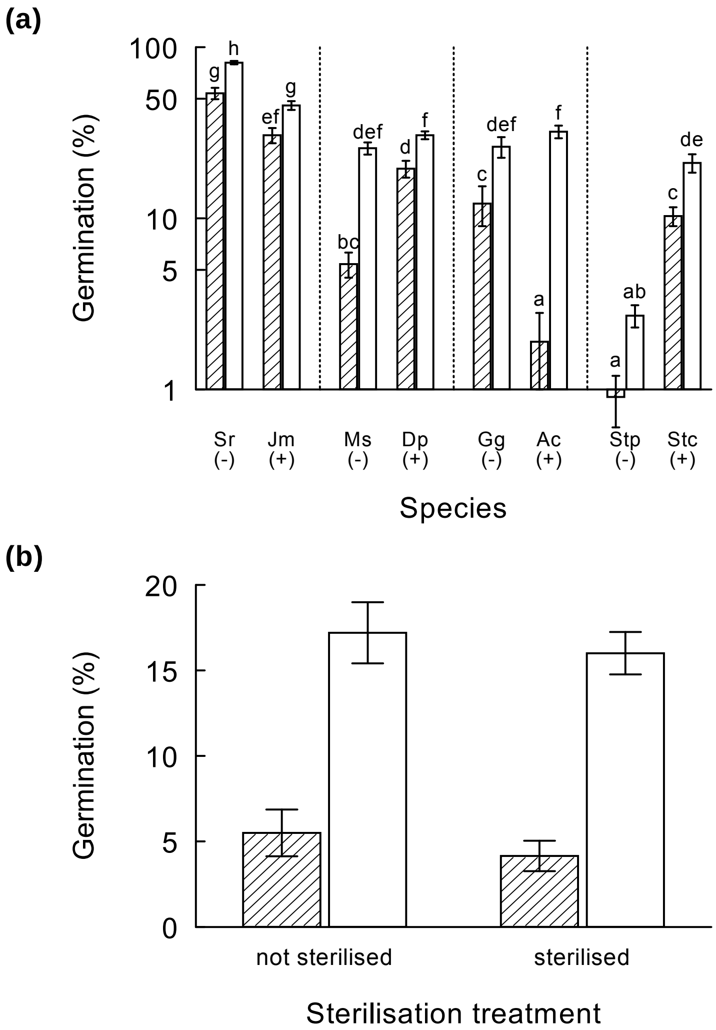

Figure 1. Percentage seed germination in Petri dishes treated with leachate (hatched bars) and distilled water (white bars) across temperature regimes after six weeks (a) and pooled germination of surface sterilised and non-sterilised seeds of four species (*Jm, Ms, Dp* and *Ac*) treated with leachate (hatched bars) and distilled water (white bars) after three weeks (b). For species abbreviations see Table 1. Data are means ± *SE* (*N* = 20). In (a) dotted lines separate family pairs; species with successful and unsuccessful field germination are denoted by (+) and (–), respectively. Note logarithmic y-axis. Means sharing the same letter are not significantly different ($P > 0.05$; Tukey's HSD on the temperature*leachate interaction). Panel (b) shows a comparison of data from the first and second experiment. In the latter fungi were excluded through seed surface sterilisation. The figure depicts the non-significant interaction between the effect of leachate and the presence of fungi ($F_{1,64} = 0.01$, $P = 0.92$).

In both the *Fabaceae* and *Poaceae* the unsuccessful field germinator was more strongly affected by leachate than the successful species, whereas this pattern was reversed in the *Rubiaceae*. Among both *Asteraceae* species the germination response did not differ between the successful and the unsuccessful field germinator. In comparison with the leachate effect, main temperature effects on final germination percentages were rather weak (Figure 2a, Table 2). However, the effect of leachate was significantly lower at 10°C constant temperature than in the temperature regimes with higher daytime temperatures (Figure 2a).

Strong leachate effects on germination were confirmed in the combined analysis of data on cumulative germination after three weeks from both experimental setups. Germination was significantly reduced ($F_{1,64} = 274.39$, $P < 0.0001$) by the application of leachate (Figure 1b). The factor *leachate* alone explained 49.4% of the total variation. In contrast, there was neither a significant main effect of sterilisation ($F_{1,64} = 3.2$, $P > 0.08$) nor a leachate*sterilisation interaction ($F_{1,64} = 0.01$, $P = 0.92$). These results indicate that leachate but not fungal effects were responsible for significantly reduced cumulative germination in experiment 1.

Timing of germination

Although the week of maximum germination also differed among species (Table 2), a large part of the variation (27.1%) was accounted for by the main leachate effect alone. The application of leachate at the start of the experiment significantly delayed the germination peak of the species by about 1.5 weeks.

Radicle length

In contrast to cumulative germination, there was a strongly significant effect of temperature on radicle length ($F_{3,184} = 64.1$, $P < 0.0001$; Figure 2b), which alone explained 18.8% of the variance (Table 2). Leachate significantly reduced radicle length ($F_{1,184} = 399.8$, $P < 0.0001$; explained variance 39%). In addition there was a significant temperature*leachate interaction ($F_{3,184} = 21.9$, $P < 0.0001$), indicating that the negative effect of leachate in comparison to controls was stronger at higher (20°C and 10/25°C) than at lower temperatures (Figure 2b). In general, leachate reduced radicle length to below 5 mm in all temperature regimes, with reduction ranging from 55% at 5/15°C to 74% at 10/25°C.

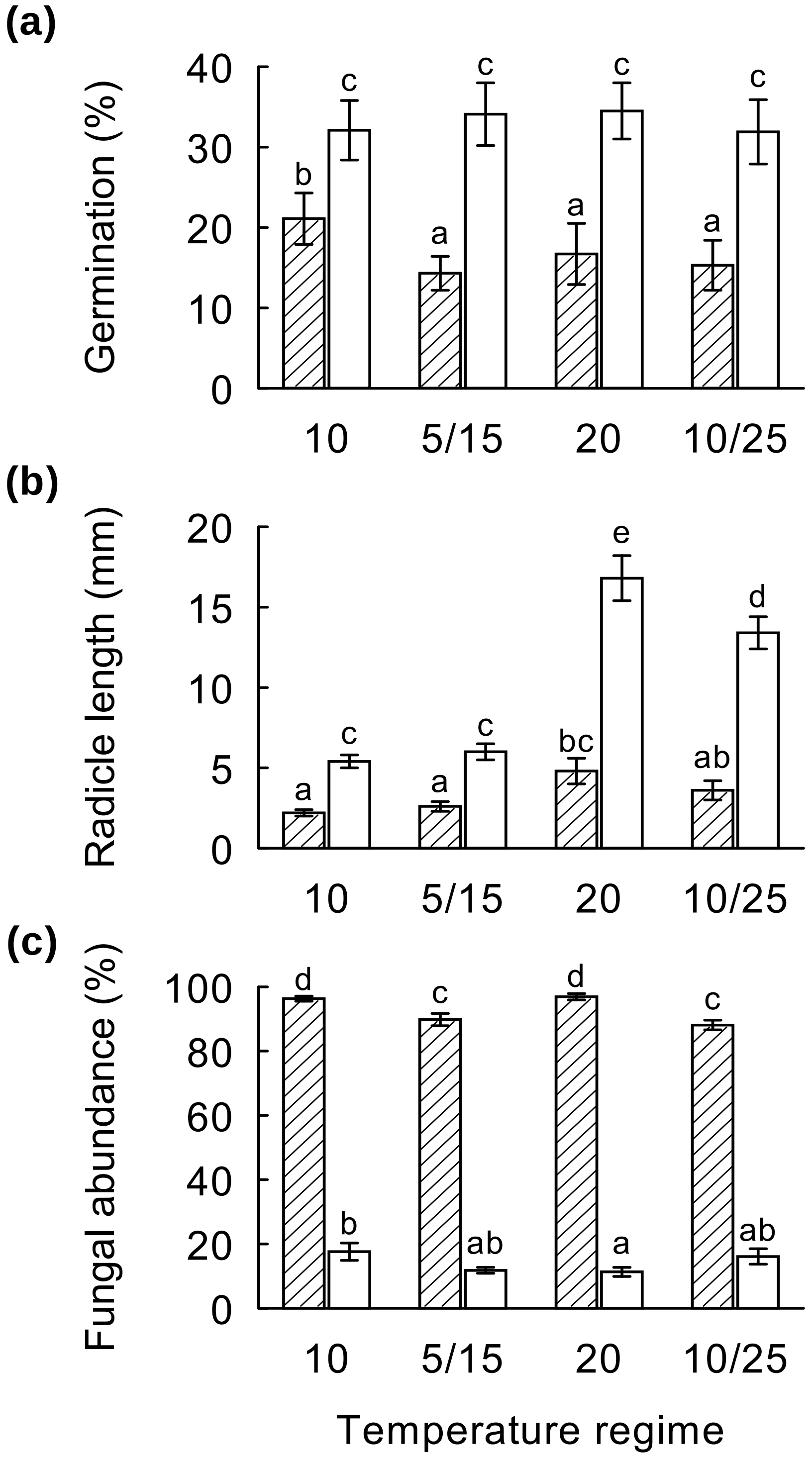

Figure 2. Percentage germination (a), radicle length (b), and fungal abundance (c) in Petri dishes treated with leachate (hatched bars) and distilled water (white bars) at different temperature regimes across species. Data are means ± *SE* (in (a) and (b): *N* = 26-30; in (c): *N* = 40). Means sharing the same letter are not significantly different (*P* > 0.05; Tukey's HSD on the temperature*leachate interaction). *St. pulcherrima* and *A. cynanchica* were excluded from the analysis of radicle length because of missing values at certain temperatures.

Fungal groups and abundance

The distribution of developing fungi in the dishes suggested that these were mostly seed-borne. Application of leachate strongly increased fungal abundance (Figure 2c; $F_{1,256}$ = 6901.5, $P < 0.0001$, explained variance: 88.8%). Compared with the other variables, species effects were small ($F_{6,256}$ = 23.3, $P < 0.0001$, explained variance: 1.8%), which implied that fungal development was not or only weakly species specific. Fungal growth in the leachate treatment was significantly lower under alternating (88.9% ± 1.2, mean ± *SE*, N = 80) than under constant temperature regimes (96.6% ± 0.6, $F_{1,144}$ = 43.3, $P < 0.0001$; Figure 2c). The abundance of fungi (Figure 2c) but not their frequency (Appendix 1) was higher in the leachate treatment than in controls. We classified 14 genera or higher order groups of fungi (Appendix 1). Six of the genera are known to include pathogenic species, which may produce highly active mycotoxins.

Discussion

Leachate effects on various components of seedling recruitment

Our laboratory germination experiments showed that leaf leachate of *Stipa pulcherrima*, the dominant species of abandoned dry grasslands in Transylvania, exerted strong negative effects on different processes related to regeneration by seeds of all grassland species studied and that the strength of the inhibitory effect differed between test species. This is in line with other studies, which demonstrated that leachate from living plant tissues or dead plant remains inhibited seed germination of co-occurring species (e. g. Schlatterer & Tisdale 1969, Chang-Hung & Chiu-Chung 1975, Werner 1975, Bosy & Reader 1995), but that the degree of susceptibility is species specific. In addition, the leachate effect was prevalent across all constant and fluctuating temperature regimes.

In only two of the species pairs (*Fabaceae* and *Poaceae*) germination of species with unsuccessful field recruitment was significantly more affected by leachate than germination of species with successful germination. The results of our analysis thus led to rejection of our third hypothesis. Our data suggest that chemical effects may only in part be responsible for recruitment failure of the study species under field conditions. Other constraints for successful field recruitment could be very specific requirements for germination (Baskin & Baskin 2001) or other factors related to the life-cycle of the species.

Timing of germination is a species specific characteristic determining the success of species under different habitat conditions (Baskin & Baskin 1988 2001, Olff et al. 1994, Hölzel & Otte 2004). As shown by monthly observations of germination in two grassland sites over two years (Ruprecht et al. 2010a), field germination of dry grassland species occurred mostly in wet periods during the year, with a highly synchronised germination peak in early spring (March or April). According to our experimental results, leachate application may delay seed germination by about 1.5 weeks on average; germination delay ranged from 0.9 weeks (*S. radiata*) to 2.2 weeks (*G. glaucum*). Due to steep slopes, southern orientation and shallow soils with low water holding capacity, environmental conditions in these grasslands are especially harsh. With only a relatively narrow time window favourable for germination, seed germination shortly after precipitation events may present a crucial advantage for seedling establishment. Consequently, missing the favourable period for germination and/or slow seedling growth as a consequence of reduced radicle protrusion in the presence of leachate may be fatal for recruitment from seed and survival of species of this dry grassland system.

Besides specific allelochemical substances, the osmotic potential and pH of litter leachates represent confounding factors that may potentially also inhibit seed germination and seedling development (Wardle *et al,.* 1992). In our study the osmotic potential of the leachates - sterile-filtered as well as unsterilized - was -0.16 ± 0.01 MPa (mean ± *SE*, *N* = 3) and both had a pH value of 5.83 ± 0.01. These values certainly differ from the osmotic potential and pH of distilled water but still lie well within the range where no significant effects on either germination or radicle growth can be expected (Baskin & Baskin 2001, Black et al. 2006). A preliminary HPLC analysis of the leaf leachate indicated the presence of coumarin in *Stipa* leachate. Coumarin is known to have a strong effect on seed germination through irreversibly blocking one or more key physiological events during the early phases of germination (Abenavoli et al. 2006). Additionally, coumarin may affect radicle growth of seedlings (Chon & Kim 2004), which has also been observed for several other allelochemicals (del Moral & Cates 1971, Chou & Young 1975, Norby & Kozlowski 1980). This effect may also have been observed in the present study.

The speed of radicle elongation may be decisive for establishment success, especially in extreme environments where a longer radicle could facilitate an easier water and mineral uptake. In this context the reduction of radicle to below 5 mm by leachate suggests that *Stipa* leachate may not only influence the germination, but also the establishment of dry grassland species.

Were there direct or interactive effects of fungi on germination?

Besides direct effects on seed germination, allelochemicals may also interact with fungi present on seeds, in the litter or in the soil. These interactions may either be positive (secondary compounds promoting spore germination and the development of hyphae) or negative (toxic effects on fungi or, conversely, decomposing activity of fungi; Rice 1984, Rizvi & Rizvi 1992). In addition, seeds may be the direct target of saprophytic and pathogenic fungi, and fungal induced seed mortality or delayed germination may have serious implications for plant demography and community processes (Dalling et al. 1998, Blaney & Kotaten 2001, Mitschunas et al. 2006, Wagner & Mitschunas 2007).

In our experiment the development of fungi was apparently promoted by the leachate. Residues of several plant species produce volatile compounds, which may stimulate spore germination and fungal growth (Menzies & Gilbert 1967, Rizvi & Rizvi 1992). It is thus not unlikely that some secondary compounds of *St. pulcherrima* may have a similar stimulating effect on the development of seed-borne fungi. Alternatively, coumarin, which was found in the aqueous extracts of *St. pulcherrima* leaves, may delay or prevent the recovery of stable membrane configuration after seed imbibition (Abenavoli et al. 2006). This transient perturbation of membrane structures may cause leakage of solutes from the seeds, which in turn may promote fungal growth.

In light of these complex interactions between plant secondary compounds and fungi, high abundance of a diverse set of fungi especially in leachate treated dishes presents a confounding factor for the interpretation of results from our first experiment. However, the fact that we obtained identical germination responses after excluding fungi through seed surface and leachate sterilisation (at least for the first three weeks of germinations) supports the view that fungi had little, if any, effects on germination in our experiment. Additionally, leachate effects on percentage germination and seedling radicle elongation were smaller under lower temperatures, which are more likely connected to the way a chemical compound is operating, whereas fungi developed equally well under low and high temperatures and should thus lead to similar effects across temperature regimes.

Conclusions

Cessation of traditional management is a serious problem for the conservation of species rich dry grasslands in Transylvania. Continuous grazing maintains species rich grassland with an open structure, dominated by *Stipa lessingiana*, a narrow-leaved feather grass species occurring also in semi-desert plant communities of Asia. After abandonment of grazing, soil erosion induced by grazing animals is slowed down, the number and area of bare patches decreases, and the grassland is becoming dominated by *Stipa pulcherrima*, a broad-leaved feather grass with higher biomass production than the former species. The grassland structure is becoming denser, litter is accumulating, and as a long-term consequence species diversity and evenness is decreasing (Enyedi et al. 2008).

Since the aqueous leaf extract of *St. pulcherrima* inhibited germination and establishment of all study species it is not unreasonable to suggest that, besides other traits such as their competitive ability or physical and microclimatic effects of their accumulating litter, chemical litter effects may partly govern the dynamics of dry continental Transylvanian grasslands after abandonment. *St. pulcherrima* itself may evade autotoxic effects through its perennial life-cycle which enables it to maintain itself simply through clonal reproduction. Long hairy awns of its seeds facilitate dispersal over long distances to places where feathergrass abundance and thus autotoxic effects are low.

However, the fact that germination success observed in the field was not consistently related to the germination responses of species to leaf leachate in our experiments indicates that other factors are being equally important for recruitment success in the field. In order to identify these factors and their role for the succession of abandoned dry grasslands further field and laboratory studies are required.

Acknowledgements

We thank Christiane Lenz-Kuhl, Josef Scholz-vom Hofe, Márton Enyedi, Péter L. Pap, Anna Szabó, János Józsa, Mária and Béla Ruprecht for invaluable field and laboratory assistance and Rolf Düring and Uta Kraus for help with the HPLC analysis. We are very grateful to Elke Stein, Karl-Heinz Rexer and Gregor Langen for their help with the determination of fungi and to Hans-Werner Koyro for measuring the osmotic potential of the leachate. Jafargholi Imani is acknowledged for valuable help with the seed sterilization, and Jutta Ahlemeier for statistical advice. Comments of Sandra Burmeier on an earlier version of the manuscript considerably improved this paper. E. R. was supported by a scholarship from the German Academic Exchange Service (DAAD).

Appendix 1. The frequency of fungi (genera or higher order groups) in samples treated with distilled water or leachate under different temperature regimes.

Temperature (°C)		**10**		**5/15**		**20**		**10/25**	
Treatment		**W**	**L**	**W**	**L**	**W**	**L**	**W**	**L**
Fungal genera (groups)	Sapr/Path								
Mucorales (Rhizopus, Mucor)	Sapr	–	40	–	40	15	40	–	40
Alternaria	>> Path*	35	38	39	40	36	35	39	38
Botrytis	Path	38	34	12	13	24	15	39	27
Fusarium	>> Path*	10	25	9	21	9	28	2	27
Epicoccum	Sapr	14	–	4	1	–	–	9	10
Aureobasidium	Sapr	2	–	16	6	–	–	–	–
Sordaria	Sapr	–	3	14	3	–	–	1	8
Rhizoctonia	Path	–	–	3	5	7	7	–	1
Aspergillus	Sapr	–	–	3	–	–	2	1	–
Myxomycetes	Sapr	–	1	–	–	1	–	1	–
Melanospora	Mycoparasite	3	5	–	–	3	–	–	–
cf. Drechslera	>> Path	–	–	–	–	1	–	–	–
Chaetomium	Sapr	–	–	–	–	–	1	1	1
Helminthosporium	Path*	–	–	–	–	–	–	–	1
ND		–	–	–	–	–	–	–	12

Fungal groups may be saprophytic (Sapr) or pathogenic (Path) (>>, mainly pathogenic); some groups are known to produce toxic substances (*); W = water; L = leachate; ND =Not determined.

4 Grass and oak litter exert different effects on seedling emergence of herbaceous perennials from grasslands and woodlands

Donath TW & Eckstein RL 2008 Journal of Ecology 96: 272-280

Summary

1 The effects of litter on seedling emergence, e. g. during succession from grassland to woodland, may not only depend on litter amount and environmental conditions but may also be related to litter types. We tested the effect of litter types, litter cover and soil moisture on the emergence of four congeneric pairs of grassland and woodland species.

2 We carried out a controlled pot experiment (Experiment I) using litter and species from two habitats (grassland vs. woodland), three levels of litter cover (low, medium, high) and two levels of water-addition (leading to constantly humid or intermittently dry substrate). Amounts of litter were adjusted to result in the same relative light reduction for both litter types. Consequently, we applied 2 g, 4 g and 8 g of grassland litter and 0.2 g, 1 g and 2 g of woodland (oak) litter per pot.

3 To separate the shade effect of litter from its mechanical plus chemical effects we exposed additional pots with seeds of the same species to deep shade conditions (Experiment II) corresponding to 4 g and 1 g of grass and oak litter, respectively.

4 Under intermittently dry conditions both litter types increased seedling emergence. In addition, we found a significant interaction between litter type and species origin: seedling emergence of woodland species was significantly lower from beneath grass litter than from beneath oak litter, whereas grassland species emerged equally well from beneath both litter types. Compared with seed germination under a shade cloth, seedling emergence of woodland species from beneath grass litter was reduced by 44%. Litter significantly affected soil humidity and the amplitude of diurnal temperature fluctuations. Differential effects of litter types on woodland and grassland species are probably related to litter structure.

5 **Synthesis:** Our experimental data present evidence that ecosystem specific litter effects slow down succession from grassland to woodland and that the effect size is controlled by the litter amount present and the environmental conditions.

Introduction

The composition of local plant communities is controlled by a series of filters that select those species from the global species pool that (i) reach the local site, (ii) tolerate the local environmental conditions, and (iii) establish successful interactions with other organisms of the same or other trophic levels (Lortie et al. 2004). These interactions include the antagonistic processes of competition and facilitation (Holmgren et al. 1997, Brooker & Callaghan 1998, Bruno et al. 2003). Several studies on plant-plant interactions emphasize that the net effect and sign of interactions depends largely on the vital rate considered and on environmental conditions, e. g. climate, soil depth, age and stage of benefactor and beneficiary (Rousset & Lepart 2000, Hastwell & Facelli 2003, Eckstein 2005, Donath et al. 2006, Lortie & Callaway 2006, Maestre et al. 2005, Michalet 2006). The general rule drawn from these studies is that the presence of facilitative effects in a plant-plant interaction is inversely related to the degree of abiotic stress (Bertness & Callaway 1994, Callaway & Walker 1997).

In this context, several studies have also noted the importance of ‘after death interactions’ mediated through dead plant remains, i. e. litter, on species composition and diversity (e. g. Carson & Peterson 1990, Facelli & Picket 1991a,b, Facelli & Facelli 1993, Foster & Gross 1997, Berendse 1999, Jensen & Meyer 2001, Weltzin et al., 2005, Emery & Gross 2006, Sayer 2006). Plant litter has the potential to interfere with plant performance at various developmental stages through alteration of the chemical (nutrient availability, allelopathy) or physical (light availability, temperature fluctuations, water availability) environment, through either mechanical effects (i. e. acting as a barrier for initial seedling growth; Facelli & Pickett 1991a, Sayer 2006) or by modifying biotic interactions (e. g. decreasing competition and increasing insect herbivory; Facelli 1994).

In a meta-analysis incorporating data from 35 studies worldwide, litter effects on germination and seedling establishment were generally negative (Xiong & Nilsson 1999). However, while the size of the effects of litter in forest ecosystems and of tree leaf litter were significantly negative, the effects of litter in grasslands and of grass litter ranged from strongly negative to slightly positive.

In comparison with other functional types, the germination of grasses is as severely affected by the presence of a litter layer as the germination of forbs (Xiong & Nilsson

1999), while as established plants, grasses suffer more from tree litter than forbs (Sydes & Grime 1981a, b). A distinct effect of litter origin, which was the factor with the largest contribution to total variation in the meta-analysis (Xiong & Nilsson 1999), was recently highlighted by Quested & Eriksson (2006). In their study litter effects were generally positive, although the response of seedling species was additionally modified by litter species' identity. If plants are adapted to the predominant litter species (litter type) of their habitat, we would expect differential effects of litter types on the emergence of species from different habitats, i. e. that seedling emergence of woodland species will be more strongly reduced by grass litter than by tree litter and vice versa.

However, the quantity of litter also determines species responses to the presence of a litter layer. Moderate litter cover exerts positive effects on seedling emergence under dry conditions (e. g. Becerra et al. 2004, Eckstein & Donath 2005, Rotundo & Aguiar 2005, Violle et al. 2006, Quested & Eriksson 2006), whereas heavy or thick litter layers impede emergence (Jensen & Gutekunst 2003, Kostel-Hughes et al. 2005). Therefore, in accordance with conceptual models of interspecific interactions between living plants (Callaway & Walker 1997, Holmgren et al. 1997), we anticipate that the same amount of dead plant material exerts positive effects under stressful abiotic conditions (e. g. drought) and negative effects under more favourable conditions (e. g. high soil humidity).

While a large number of studies have addressed species responses to various amounts of litter cover (for reviews see Facelli & Pickett1991a, Xiong & Nilsson 1999), few have assessed the combined effects of variation in litter cover and abiotic conditions (e. g. Gross 1984, Hamrick & Lee 1987, Xiong et al. 2003, Becerra et al. 2004, Eckstein & Donath 2005), and comparative studies of the effects of different litter types are extremely rare (Schlatterer & Tinsdale 1969, Facelli & Pickett 1991b, Myster 1994, 2006, Quested & Eriksson 2006). To our knowledge the current study presents the first effort to study experimentally the effects of ecosystem specific litter types on representative species of these systems under different environmental conditions. This approach allows us to address the complex interplay between the litter of dominant species and species composition of grasslands and woodlands, and its consequences for possible co-evolution in grassland and woodland plants with respect to the predominant litter type.

We specifically addressed the following questions:

1 Are there ecosystem-specific effects of litter types, i. e. is the emergence of woodland species more negatively affected by grassland litter than by woodland litter and vice versa?

2 Are there, in contrast to Xiong & Nilsson (1999), positive effects not only of grassland but also of woodland litter on seedling emergence?

3 How strong is the microclimatic effect through a shade cloth (including changes in light, temperature and humidity) on seedling emergence in comparison to the additional mechanical plus chemical effects through grass and leaf litter, respectively?

Materials and Methods

Study species

We selected four congeneric pairs of perennial hemicryptophytes, of which one species of the pair was characteristic of open grassland habitats and the other of deciduous woodlands and forests (Table 1). Seeds of the species were collected from several natural populations in grassland and woodlands, respectively, in autumn 2005 and dry stored in darkness at room temperature until sowing on 19 December 2005. This sowing date left enough time for cold stratification which is crucial for successful germination of many plants (Baskin & Baskin 2001). An initial viability test with a 1% tetrazolium-chloride solution (Bennett & Loomis 1949) on additional seed batches showed that except for *Viola reichenbachiana*, almost all seeds (> 86%) used in the experiments were viable (Table 1).

Table 1. Habitat, family, mass per seed (mg) and percentage of viable seeds (Tetrazolium-test) of the study species.

Habitat	Family	Species	Mass per seed (mg)	Viability (%)
Grassland	Rubiaceae	*Galium boreale* L.	0.49	94
	Poaceae	*Poa angustifolia* L.	0.17	94
	Polygonaceae	*Rumex crispus* L.	1.41	100
	Violaceae	*Viola pumila* Chaix	1.25	94
Woodland	Rubiaceae	*Galium odoratum* (L.) Scop.	6.81	86
	Poaceae	*Poa nemoralis* L.	0.11	90
	Polygonaceae	*Rumex sanguineus* L.	0.64	88
	Violaceae	*Viola reichenbachiana* Boreau	1.65	38

Experimental design

In a first experimental setup (Experiment I) we studied the effects of species (factor levels (*k*) = 8, Table 1), original habitat (*k* = 2, grassland vs. woodland), litter type (*k* = 2, grass litter vs. oak litter), litter cover (*k* = 3, low, medium, high) and water addition (*k* = 2, regularly watered vs. intermittently dry) on seedling emergence. We used a completely randomized experimental design. We thus prepared 60 pots of 1 dm^3 volume (ca. 10 × 10 × 10 cm) for each study species, i. e. each litter type × litter cover × water addition combination (12 combinations) replicated five times, plus 10 additional control pots (five regularly watered, five allowed to become intermittently dry) that received no litter.

Pots were filled with commercial potting soil (Fruhstorfer Erde®, Type P, Industrie-Erdenwerke Archut GmbH, Lauterbach, Germany) composed of a mixture of peat, clay and humus (pH_{CaCl2} 5.7, 188 mg l^{-1} nitrogen, 136 mg l^{-1} P_2O_5 and 206 mg l^{-1} K_2O). Soil water content was set to 106 %. On 19th December 2005, we sowed 50 seeds of one individual species in each experimental pot.

We collected dry grass litter from mesic unfertilized grassland lacking any of the study species. Leaves of oak (*Quercus robur* L.) were collected in a mixed deciduous woodland, dried and cut into smaller pieces (4 - 10 cm²) to allow an even application. We used air-dried litter since oven drying might induce chemical changes or subsequent leaching of litter.

Previous studies have shown that litter cover may differentially affect seed germination through its effects on light interception at the ground level, soil temperature and soil moisture (e. g. Facelli & Pickett 1991a, b, Bosy & Reader 1995, Xiong et al. 2003, Eckstein & Donath 2005, Rotundo & Aguiar 2005). Since we were interested in the effects on seedling emergence of comparable degrees of litter cover with respect to light interception, we adjusted the applied amounts of grass and oak litter to result in similar levels of light reduction at the onset of the experiment. Measurements of relative photosynthetically active radiation (PAR, measured with Li-190 quantum sensors, LiCOR Inc., Lincoln, USA) showed that the mass of grass and oak litter resulting in a similar degree of light reduction below the litter cover differed approximately by a factor of four. Therefore, the amounts of litter initially applied to each pot in the current experiment were 2, 4, and 8 g of grass litter and 0.5, 1, and 2 g of oak litter – henceforth denoted as low, medium and high litter cover, respectively – which correspond to initial relative PAR radiation under the litter of about 10% (8% under grass litter / 13 % under oak litter), 1% (0.7% / 1.2%), and 0.1% (0.04% / 0.1%) of ambient. The levels of grass litter applied, i. e. 200 g m^{-2}, 400 g m^{-2} and 800 g m^{-2}, are within the range of low- to high-productive grasslands (Donath et al. 2004). Levels of oak litter manipulation, i. e. 50 g m^{-2}, 100 g m^{-2} and 200 g m^{-2}, are within the range found in deciduous beech forests (e. g. Ellenberg et al. 1986, Wilke et al. 1993) and mixed deciduous forests (Sydes & Grime 1981a). In the latter study, biomass of forest floor species considerably declined above 200 g m^{-2} of leaf litter cover. This agrees with Facelli & Pickett (1991b) who found light penetration below 200 g m^{-2} oak litter was only ~ 0.9% of ambient. Our experimental design allows for comparison between effects of the application of the same amounts of two different litter types (2 g grass vs. 2 g oak litter), which result in different degrees of light interception, with the application of different amounts of litter exerting similar effects on light interception.

Half of the pots were allowed to become intermittently dry. These were watered only when soil water content in pots without litter cover reached about 70%. In the main germination period (early April to mid-May) they received 80 ml of water about once a week, depending on weather conditions. The soil of the other pots was kept constantly humid by watering two or three times a week. Soil water content was monitored using two replicate pots per litter type × litter amount × water-addition combination (24 pots) and four control pots (regularly watered and intermittently dry) that were filled with a defined soil mass at the start of the experiment and received no seeds. An aliquot of the same soil was dried to determine soil dry mass and initial water content. These soil-water-content pots were weighed at least weekly during the experiment to determine soil water content.

In additional soil-temperature pots, temperature loggers (Tinytalk with internal sensor, Gemini Dataloggers Ltd, Chichester, UK) were positioned below the soil surface to record hourly temperatures in two regularly watered pots per litter type × litter cover combination and in two control pots. All pots (sown pots, pots for measuring water content and temperature of the soil) were randomly positioned in a common garden close to Gießen, Germany (50°32'N, 8°41.3'E, 172 m a.s.l.) in late December 2005. An additional row of pots filled only with soil around the experimental pots served as a buffer against desiccation. A wooden frame covered with thin polyethylene plastic sheets protected the experimental setup against precipitation.

Additionally, in a second experiment (Experiment II), we prepared 10 pots per species that received no litter but were watered as in the other experiment (same amounts and frequency of watering). However, these pots were put under a wooden frame covered with shade cloth that resulted in a light reduction as with 4 g and 1 g of grass and oak litter, respectively. A comparison of these three treatment levels allowed us to separate the germination response of species to deep shade from the chemical plus mechanical effects of litter application.

Germination started in early April 2006. Seedlings per pot that emerged above the litter were counted on 20 April, 16 May and 26 June. The main phase of seedling emergence was between 1 April and 16 May, when > 90% of the final emergence was reached. Only seedlings that penetrated the litter layer were considered as successfully emerged. We did not observe any seedling mortality during the experiment. Therefore, we assume that percentage of emerged seedlings at the end of the experiment represents cumulative emergence.

Statistical data analyses

We selected species pairs from the same family (and genus) because they were morphologically very similar but differed in their habitat preference. However, since different subsets of species were nested within each family and each habitat, these latter two factors could not be included into the same statistical model. Since the main aim of this study was to analyse the response to different litter types of species from different habitat types, we accounted for inherent effects of family identity by calculating a one-way ANOVA for the effect of family ($F_{3,556} = 59.3$, $P < 0.001$) and using only the residuals for all further analyses. Percentage seedling emergence was square root arcsine transformed before analysis (Quinn & Keough 2002). Afterwards, ANOVA was employed to analyse for effects of species, original habitat, litter type, litter cover and water-addition on residual final percentage emergence. For the statistical analysis the factor species was hierarchically nested within the factor habitat and all factors were considered fixed. Since pots without litter application served as a control for both litter types and for litter cover, these represent a 'hanging control group' in the statistical analysis. Therefore, we used an overparameterized model and type IV sums of squares (SS; StatSoft 2001). Then, tests of main effects and the interactions proceeded as usual but excluding the control. However, the SS for the error term (and the DF for the error term) was estimated based on the experimental groups specified in the design and the isolated control group. All statistical analyses were performed using Statistica 6.0 (StatSoft 2001).

Results

Effects of treatments on soil temperature and water content

Analysis of temperature data (Table 2; two-way ANOVA, data Loge-transformed before analysis) showed that across litter amounts, daily temperature amplitudes were significantly lower under grassland than under woodland litter, whereas daily mean temperatures did not differ significantly between litter types ($F_{1,308} = 23.3$, $P < 0.001$ and $F_{1,308} = 0.39$, $P = 0.54$, respectively).

Table 2. Daily average temperature (°C) and daily temperature amplitude (°C) in control pots and under low, medium and high cover of grassland and woodland (oak) litter during the main phase of seedling emergence (mean ± *SE*).

Litter type	Amount	Average (°C)	Amplitude (°C)
Control	no litter	13.6 ± 0.6	19.8 ± 1.2
Grassland	low	12.8 ± 0.6	12.8 ± 0.7
	medium	12.6 ± 0.6	13.2 ± 0.8
	high	12.6 ± 0.6	9.9 ± 0.6
Woodland	low	13.2 ± 0.6	17.1 ± 1.0
	medium	12.9 ± 0.6	15.3 ± 0.9
	high	12.9 ± 0.7	14.3 ± 0.8

The main phase of seedling emergence was between 1 April and 15 May (*N* = 45 days). Low, medium and high litter cover refers to 2, 4, and 8 g of grass litter and 0.5, 1, and 2 g of oak litter, which resulted in a similar initial degree of light reduction under both litter types (see Materials and Methods).

Across litter types, daily temperature amplitude, but not daily mean temperature, differed significantly between litter amounts ($F_{2,308} = 5.5$, $P < 0.005$ and $F_{2,308} = 0.12$, $P = 0.89$, respectively). Daily temperature amplitudes were significantly lower under litter cover than in controls, and lower under high than under low litter cover (Tukey's HSD test, $P < 0.05$). Neither average temperature nor amplitude differed between pots covered by the same amount (2 g) of grass and oak litter (low grass vs. high oak cover; Tukey's HSD test, $P > 0.05$).

Statistical analysis of soil water content (Table 3; repeated-measures ANOVA for the main phase of seedling emergence, control pots excluded, $N = 8$ measurements in time), revealed that soil water content increased significantly with litter cover (from 112 to 143%, $F_{2,12} = 42.4$, $P < 0.001$) and was higher under grass than under oak litter (146 vs. 107%, $F_{2,12} = 203.5$, $P < 0.001$). Additionally, there was a significantly litter type × litter cover interaction ($F_{2,12} = 4.1$, $P = 0.044$) owing to an increasing difference in soil water content between litter types with increasing litter amounts. However, the average water content of pots covered with the same amount (2 g) of grass and oak litter did not differ significantly (Tukey's HSD test, $P > 0.05$).

Table 3. Soil water content (% of soil DW; mean ± *SE*) in pots subject to different litter amounts and watering treatments during the main phase of seedling emergence. Pots were weighed at least weekly.

Litter type	Amount	Water	Soil water content (%)
Control	no litter	intermittently dry	71.6 ± 2.4
		permanently moist	111.8 ± 7.4
Grassland	low	intermittently dry	97.7 ± 5.1
		permanently moist	157.3 ± 2.9
	medium	intermittently dry	110.6 ± 4.9
		permanently moist	176.5 ± 1.9
	high	intermittently dry	150.8 ± 4.1
		permanently moist	185.2 ± 3.3
Woodland	low	intermittently dry	73.3 ± 3.5
		permanently moist	119.2 ± 7.3
	medium	intermittently dry	75.0 ± 3.0
		permanently moist	138.3 ± 4.6
	high	intermittently dry	84.0 ± 4.1
		permanently moist	151.7 ± 4.4
Shade	Gauze	intermittently dry	145.6 ± 3.2
		permanently moist	167.2 ± 1.6

The main phase of seedling emergence was between 1 April and 15 May ($N = 8$). For the amounts of litter used see Table 2. Half of the pots were allowed to become intermittently dry and were watered only when water content of soil in pots without litter cover reached about 70%. In the main germination period these pots received 60 ml of water about once a week, whereas the other pots were kept constantly humid (see Materials and Methods).

Seedling emergence (Experiment I)

Seedling emergence ranged between zero and 90% across treatment combinations (Figure 1). Germination percentages of < 30% were found for *G. boreale* and V. *reichenbachiana*, and despite viable seeds, almost no germination was observed in *G. odoratum*. There was a large, significant main effect of species on seedling emergence (Table 4). Additionally, the effects of litter cover, litter type and water-addition varied significantly among species (significant S[H] × factor interactions) and also the interactions litter cover × litter type and litter type × water-addition depended on species identity (significant S[H] × LC × LT and S[H] × LT × W interactions).

Across water treatments seedling emergence at the species level was significantly ($P < 0.05$) higher under at least one level of litter cover than in control pots in *G. boreale* (low, medium and high cover of grass / high cover of oak litter), *P. angustifolia* (high cover of oak litter), *V. pumila* (low, medium and high cover of grass / medium and high cover of oak litter) and *V. reichenbachiana* (medium and high cover of grass / high cover of oak litter). These positive effects of litter cover were especially pronounced under intermittently dry conditions (Figure 1). However, owing to large variation among pots, these effects were not statistically significant in *G. odoratum*, *P. nemoralis*, *R. crispus* and *R. sanguineus*. In *P. nemoralis*, *R. crispus* and *R. sanguineus* seedling emergence was significantly lower under high grass litter cover than in control pots; in *P. angustifolia* this difference was not significant but seedling emergence dropped considerably between medium and high cover of grass litter.

Averaged across the other factors, final seedling emergence was significantly higher in grassland species (32%) than in woodland species (17.6%; significant habitat effect, Table 4) and varied significantly among levels of litter cover. Differences between habitats remained significant even after removing *G. odoratum*, whose very low germination may have biased the results, from the woodland species group (mean ± 1*SE*, 32% ± 1.7 vs. 23.4% ± 1.7; $t = 4.2$, $DF = 488$, $P < 0.0001$, t-test on square root arcsine transformed data). Water-addition also had a strong main effect on seedling emergence (31.1% in permanently moist pots vs. 18.5% under intermittently dry conditions), whereas there was no significant difference between the two litter types. The positive effect of water-addition on seedling emergence was strongest in control pots and decreased with increasing litter cover, independent of species identity (significant LC × W interaction and non-significant S[H] × LC × W interaction).

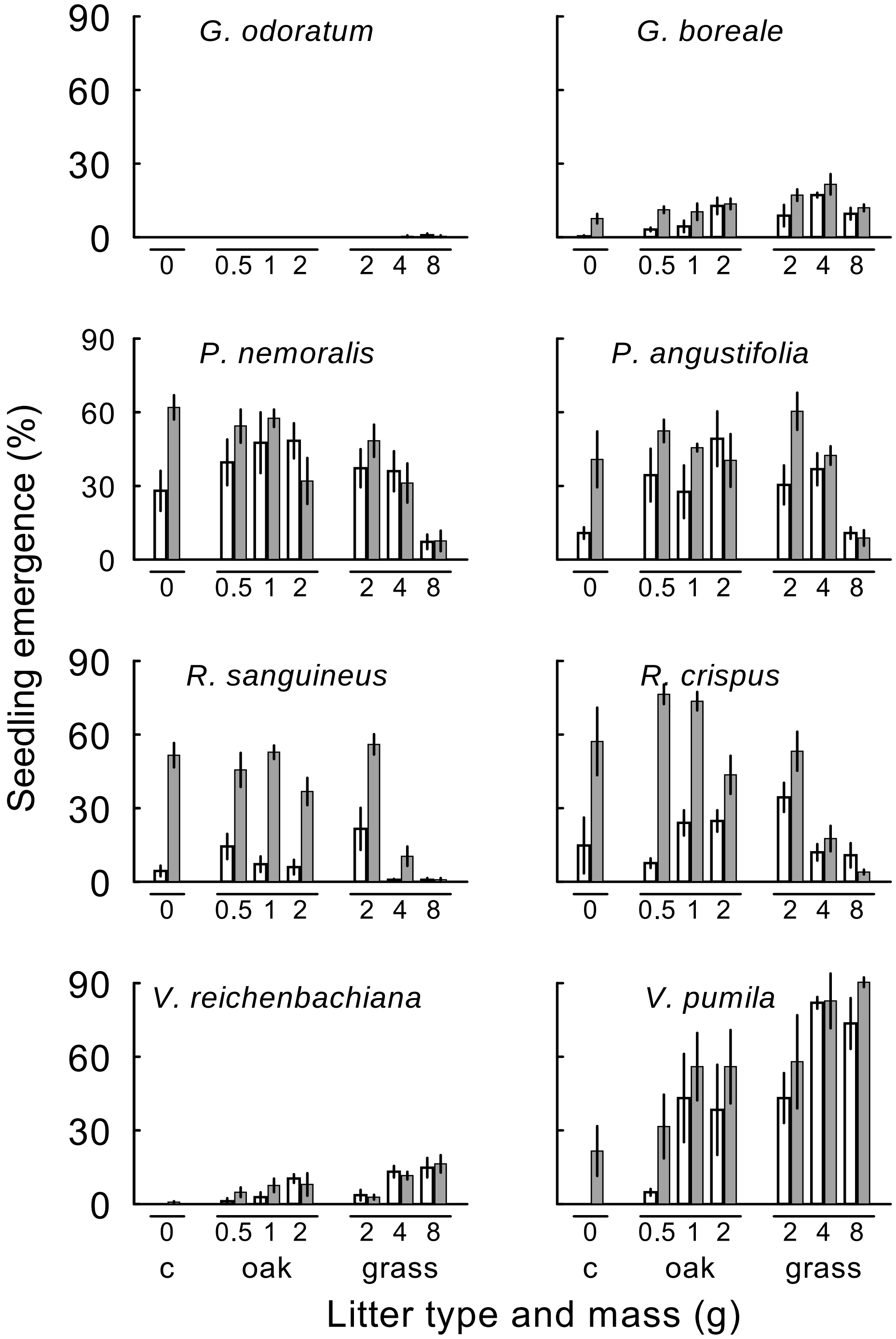

Figure 1. Percentage cumulative seedling emergence of woodland (left panels) and grassland species (right panels) under different amounts of woodland (oak) and grassland litter in constantly moist (grey bars) and intermittently dry pots (white bars). 0.5, 1 and 2 g of oak litter resulted in a similar initial degree of light reduction as 1, 2, and 8 g of grass litter (see Materials and Methods). Data are means ± *SE* (*N* = 5). Abbreviations of genera: G. = *Galium*, P. = *Poa*, R. = *Rumex*, V. = *Viola*.

Finally, seedling emergence of species from woodland habitats was significantly lower under grass litter than under oak litter whereas grassland species emerged equally well (and significantly better than in control pots) under both litter types (significant H × LT interaction; Figure 2). This effect did not depend on species, litter cover or water-addition (Table 4, no significant higher order interactions including H × LT).

Table 4. Results of an ANOVA on the effects of species (cf. Table 1; nested within original habitat), original habitat (grassland vs. woodland), litter cover (litter amount), litter type (grassland vs. oak litter) and water-addition (permanently moist vs. intermittently dry) on final seedling emergence.

Source of variation	**dF**	**MS**	***P***
Intercept	1	0.00	1.0
Species(Original Habitat) [S(H)]	6	1.06	<0.0001
Original Habitat [H]	1	7.80	<0.0001
Litter cover [LC]	2	0.24	0.0004
Litter type [LT]	1	0.11	0.0583
Water addition [W]	1	4.04	<0.0001
S(H) × LC	12	0.42	<0.0001
S(H) × LT	6	0.85	<0.0001
S(H) × W	6	0.35	<0.0001
H × LC	2	0.09	0.0596
H × LT	1	0.38	0.0005
H × W	1	0.26	0.0035
LC × LT	2	0.57	<0.0001
LC × W	2	0.29	<0.0001
LT ×W	1	0.35	0.0008
S(H) × LC × LT	12	0.16	<0.0001
S(H) × LC × W	12	0.03	0.4222
S(H) × LT × W	6	0.11	0.0017
H × LC × LT	2	0.03	0.3828
H × LC × W	2	0.04	0.2588
H × LT × W	1	0.06	0.1732
LC × LT × W	2	0.01	0.7498
S(H) × LC × LT × W	12	0.03	0.4949
H × LC × LT × W	2	0.01	0.7605
Error	448	0.03	
Model R^2		0.6945	<0.0001

Significant effects ($P < 0.05$) are given in bold. Analyses were made on residual seedling emergence after one-way ANOVA with family as factor to account for family-specific effects on germination (see Materials and Methods).

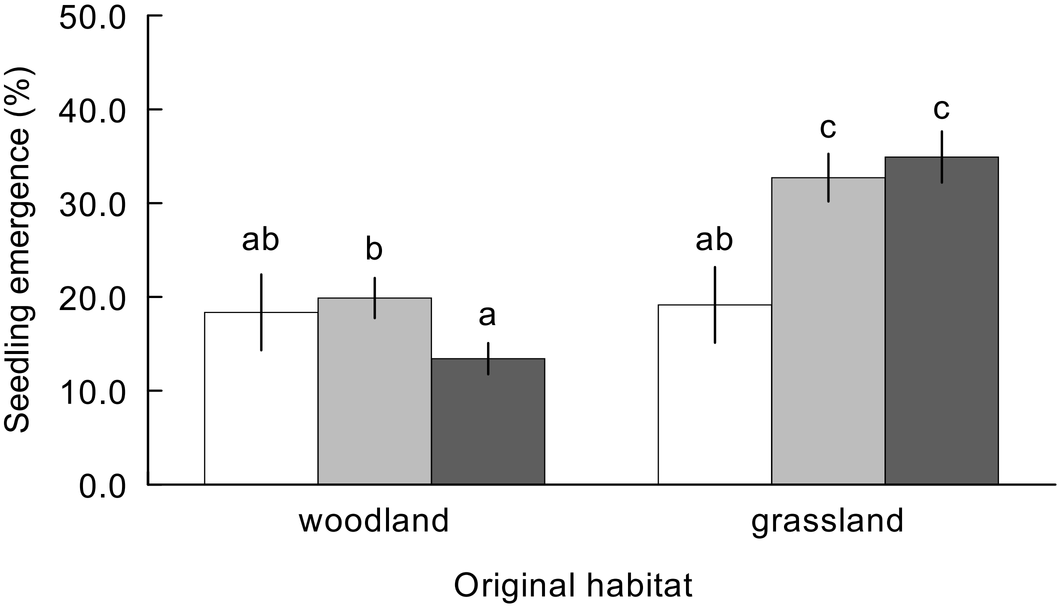

Figure 2. Percentage cumulative seedling emergence of woodland and grassland species (averaged across litter covers and water treatments) in control pots (white bars), under oak litter (light grey bars) and grass litter (dark grey bars). Data are means ± *SE*. The figure depicts the original habitat × litter type interaction ($F_{1,448} = 12.31$, $P = 0.0005$). Bars sharing the same letter are not significantly different ($P > 0.05$).

Chemical and mechanical effects of litter on seedling emergence (Experiment II)

In the analysis of effects of shade conditions, the different treatments (shade vs. grass and oak litter) served to identify different mechanisms through which seedling emergence may potentially be influenced (Bosy & Reader, 1995): while the shade cloth treatment represents the shade effect of litter on seedling environment (i. e. changes in light quantity and quality, temperature and air and soil humidity) excluding chemical and mechanical impacts, the latter come into effect when seeds are covered with oak or grass litter. Grassland species showed significantly higher germination percentages than woodland species under shade cloth (Table 5). In accordance with experiment I, grassland species were less sensitive to litter cover since seedling emergence did not differ significantly between shade cloth and the combined shade plus mechanical and chemical effects through litter. In contrast, the emergence of woodland species from beneath grass litter was significantly reduced by 44% as compared with germination under shade cloth. Seedling emergence of woodland species was also lower from beneath oak litter as compared with the shade cloth (reduction of 5%) but this difference was not significant.

Table 5. Seedling emergence (%; means ± *SE*; *N* = 40) of grassland and woodland species under shade cloth and from beneath oak and grass litter.

Seedling emergence (%)

Treatment	**Grassland species**	**Woodland species**
Shade	32.8 ± 3.5a	25.2 ± 4.1b
Oak litter	35.6 ± 4.6a	23.9 ± 4.4bc
Grass litter	39.1 ± 4.6a	14.1 ± 2.7c

Means sharing the same letter are not significantly different (Tukey's HSD test, $P > 0.05$). There was a significant original habitat × treatment interaction (ANOVA, $F_{2,192} = 9.0$, $P = 0.0002$). This analysis was based on medium amounts of oak and grass litter (1 g and 4 g, respectively) that resulted in similar degrees of light interception at the start of the experiment as under the shade cloth (see Materials and Methods). Seedling emergence in the shade treatment represents the germination response of species to deep shade, while differences in emergence between shade and litter treatments are the chemical plus mechanical effects of litter on germination and initial seedling growth.

Discussion

Our experimental results suggest that more species from grassland than from woodland habitats respond positively to litter cover. Across species, woodland plants showed significantly lower seedling emergence from beneath grass litter than from beneath the litter of their original habitat, whereas grassland species emerged equally well from beneath both litter types. Thus the response to litter of species from different ecosystems varied between ecosystem-specific litter types.

Effects of litter structure

Our data suggest that these differential effects of litter types may in part be caused by differences in litter structure i. e. mechanical litter effects. Grass litter, which consists of senesced threadlike leaves and stems of graminoids, creates dense tightly interwoven mats, whereas oak leaf litter consists of singular leaf units that are moved relatively easily against each other. In addition to morphological adaptations of some woodland forbs that permit shoot emergence of adult plants through thick litter layers (e. g. Salisbury 1916), our results suggest that woodland plants also evolved effective adaptations at the seedling stage.

Depending on the mechanical shoot thrust a seedling may exert after germination (Campbell et al. 1992), emergence from below oak litter may be relatively easier than from below grass litter, at least under dry conditions, because seedling growth may push up and displace oak leaves. We do not know exactly how seedling emergence was influenced by reducing particle size through the cutting of oak leaves but penetration of the litter layer by seedlings is probably more difficult when leaves are whole. This agrees with Xiong et al. (2001) who showed that negative litter effects increased with litter particle size. Although cutting oak leaves may possibly have increased seedling emergence, the experimental treatment still results in two different types of litter with respect to structure: (i) one laminar type consisting of 2-dimensional flat particles (oak leaves) and (ii) an interwoven thread-like type (grass litter). However, absolute litter effects are not crucially important since the aim of the experiment was to compare the response of species from different ecosystems subject to different litter types. The data in Figure 2 and Table 5 present clear evidence for a differential response of woodland vs. grassland species. While grass litter enhanced the emergence of grassland species (Figure 2) and probably all germinated seedlings made their way through the litter (Table 5), a large proportion (44%) of seedlings of typical woodland plants did not manage to successfully penetrate a cover of grass litter.

Mechanical vs. chemical effects

Although mechanical and chemical litter effects cannot be separated in the present experiment, we suggest that chemical effects have been relatively weak. Owing to high concentrations of phenolic compounds that are leached over a long period in oak foliage as compared to other deciduous trees (Kuiters & Sarink 1986), one would expect chemical effects, if any, through the oak litter. However, we found no indication of such effects in our experiment since germination and emergence of grassland species did not differ significantly between oak and grass litter. Additionally, in other studies on the effects of grass litter, chemical effects were mostly weak (e. g. Sydes & Grime 1981b, Bosy & Reader 1995, Rotundo & Aguiar 2005). Reduced emergence of woodland plants from below grass litter are therefore most probably related to mechanical litter effects (litter structure).

Microclimatic litter effects

Significantly lower diurnal temperature fluctuations and higher soil moisture under litter than in control pots as documented in the present study have also been found in earlier studies on the community effects of litter (e. g. Sydes & Grime 1981b, Facelli & Pickett 1991a, Bosy & Reader 1995, Jensen & Gutekunst 2003, Eckstein & Donath 2005, Weltzin et al. 2005). It is well known that large diurnal temperature fluctuations serve as a cue for the detection of gaps (e. g. Thompson & Grime 1983), inducing seed germination in light demanding species and small or ephemeral plants with low competitive ability. The evolution of dark dormancy in small-seeded species (Milberg et al. 2000) results in a close relation between seed size and emergence in both grassland (Jensen & Gutekunst 2003) and woodland plants (Kostel-Hughes et al. 2005). Accordingly, in the present experiment seedling emergence of most small-seeded species (seed mass <1 mg) decreased strongly between intermediate and high litter cover treatments.

Differences in litter structure and density also influenced the microclimatic effects of litter types. When comparing litter amounts with similar effects on the degree of initial light interception, soil moisture beneath oak leaves was considerably lower than under grass litter, and oak litter exerted weaker temperature effects. Additionally, according to Facelli & Pickett (1991b) at the same relative illumination, sites covered with oak litter are characterised by higher proportions of patches with low and high illumination i. e. they experience larger variation of light distribution than grass litter. However, in our study identical amounts of different litter types did not differ in their effects on average temperature and soil humidity. Therefore, in contrast to Xiong & Nilsson (1999), woodland litter also had positive effects on seedling emergence especially under stressful conditions. The selection of litter amounts applied in the present experiment was adjusted by the structural litter effect on relative light interception and was not based simply on litter mass (Facelli & Pickett 1991b), since light quantity and quality (e. g. red/far-red ratio) are important cues for seed germination and seedling growth. Also Sydes & Grime (1981b) showed that spring growth of woodland perennials was more strongly controlled by litter surface area (which is closely related to the degree of light absorption) than by litter mass.

Although the 2 g oak litter treatment is within the range of values found in deciduous forests (Sydes & Grime 1981a, b, Ellenberg et al. 1986), the experimental design did not mimic the accumulation of forest tree leaf litter. Application of higher amounts of oak litter would most probably have decreased seedling emergence dramatically (e. g. Beatty & Sholes 1988, Kostel-Hughes et al. 2005) since virtually no light can penetrate deep forest litter layers (Facelli & Pickett 1991b).

Ecosystem specific litter effects

Strong effects of grass litter on forest species may explain why the succession of abandonment grasslands or old fields to woodland is often a rather slow process (Ellenberg 1986, Facelli & Pickett 1991b, Facelli 1994, Myster 1994). In line with our findings, many grassland herbs will disappear from the above ground vegetation due to litter accumulation during succession (Ellenberg 1986). Under these conditions higher seed mass of woodland species (trees) increases the chance of seedling emergence (Jensen & Gutekunst 2003, Eckstein & Donath 2005). At the same time the probability of large seeded species to be incorporated into litter during colonization of abandoned grassland is - analogous to their movement in the soil (Bekker et al. 1998) - lower than for small seeded species. Grass litter impedes soil contact and shelter of large seeded species and makes these species more prone to herbivory (Shaw 1968). However, after the first woody species have established, various indirect effects of tree leaf litter on competition from co-occurring species may benefit other trees or woodland forbs (Facelli 1994). This suggests that during the colonisation of grassland and old fields, herbaceous woodland species rely on the occurrence of woody plants not only because of changes in microclimatic conditions but also because their seedlings are less likely to emerge from beneath grass litter.

In fertile woodlands, on the other hand, the presence of tree leaf litter prevents grass dominance in the ground flora (Sydes & Grime 1981a,b). Through this mechanism oak litter increases the proportion of herbaceous species and the diversity of the ground layer. In the present experiment seedling emergence of the grasses *P. nemoralis* and *P. angustifolia* was not significantly smaller from beneath 2 g of oak litter (200 g m^{-2}) than in controls. In contrast, Sydes & Grime (1981b) suggested that at the adult stage the basal position of shoot meristems may – in conjunction with differences in microtopography in woodlands as compared to open grassland habitats (Beatty & Sholes 1988, Wilke et al. 1993) – present a selective disadvantage for grasses through seasonal tree leaf shedding and the accumulation of tree litter. Consequently, many grasses that are confined to forests are characterized by a phalanx-strategy of growth. Patches of compact carpets or tussocks built of densely packed tillers will prevent the accumulation of leaf litter and will themselves feedback on the spatial distribution of litter (Sydes & Grime 1981a, Wilke et al. 1993). This effect is reinforced by the persistence of oak leaf litter that usually accumulates over several years on the forest floor and thus also creates dense compact mats that impede establishment of seedlings (Beatty & Sholes 1988). On the other hand under natural conditions dry tree leaf litter is easily redistributed by wind leading to a patchy distribution with areas of accumulation and areas blown bare (Sydes & Grime 1981a, Beatty & Sholes 1988). Thus, forest floor species may either, as it is the case in many forest grasses, rely on clonal reproduction to avoid negative effects of litter on germination or they are confined to patches of bare ground on the forest floor.

In conclusion, our experimental results indicate that the effects of litter may not only vary among seasons, habitats, sites, life-cycle stages, species and litter species (Sydes & Grime 1981a,b, Hamrick & Lee 1987, Carson & Peterson 1990, Facelli & Pickett 1991a,b, Facelli & Facelli 1993, Jensen & Gutekunst 2003, Eckstein & Donath 2005, Violle et al. 2006, Kostel-Hughes et al. 2005, Quested & Eriksson 2006) but there may also be differential effects of ecosystem-specific litter types on species composition in different systems. Our results thus highlight the complex interplay between ecosystem specific litter types (via litter structure), amounts of litter and environmental conditions for species adaptation and its potential influence on species composition of grassland and woodland habitats.

Acknowledgements

We thank Christiane Lenz-Kuhl and Josef Scholz-vom Hofe for invaluable help during the experiment and in the laboratory, Jörg Jacobs and Wolfgang Donath for seed collection, and Jutta Ahlemeyer for statistical advice. Bente Graae, Barney Davies, Bryan Foster, Mike Hutchings and two anonymous referees offered insightful comments that significantly improved the manuscript. All help is gratefully acknowledged.

5 Effects of bryophytes and grass litter on seedling emergence vary by vertical seed position and seed size

Donath TW & Eckstein RL 2010 Plant Ecology 207: 257-268

Abstract

Establishment of plants through seeds is often constrained by the quality of microsites, which is in part controlled by the nature and amount of ground cover. The latter consists of living shoots of vascular plants or bryophytes and/or the dead remains of the dominant species. In the present article, we report the results of a controlled pot experiment with five species characteristic of floodplain grasslands. We manipulated the amounts of grass litter and/or mosses to study (1) differences between ground cover types with respect to their effects on microenvironment and seedling emergence and (2) how these effects interact with seed size and seed sowing position. Increasing amounts of both cover types led to increasing soil humidity, whereas temperature amplitude and illumination were decreased. However, since grass litter decomposed much faster than bryophytes, light conditions for germination under grass litter improved considerably with time. Although seedling emergence varied significantly between species, ground cover types and cover amounts, seed position alone explained about 50% of the variation in the data set. Additionally, we found an important interaction between seed size, seed position and cover type: large-seeded species showed a fitness advantage when seeds were situated beneath a cover, irrespective of cover type, which disappeared when seeds were shed on top of a cover layer. We suggest that this interaction may be ecologically and evolutionarily relevant because it may lead to changes in species composition and diversity of plant communities as a consequence of changes in the amount and type of ground cover.

Introduction

Once viable diaspores reach a site, the dynamic processes of community assembly and invasibility ultimately depend on the availability of safe sites for seed germination (Grubb 1977, Eriksson & Ehrlén 1992, Weiher & Keddy 1999, Donath et al. 2006), which, in turn, is controlled by abiotic conditions, resources and biotic interactions (Davis et al. 2000, Lortie et al. 2004, Ejrnæs et al. 2006). Therefore, changes in the quality and quantity of microsites have a great impact on community composition, diversity and succession through the effects on seed germination and seedling emergence (Davis et al. 2000, Münzbergová 2004, Fenner & Thompson 2005). These changes may be triggered by natural and anthropogenic processes altering the amount and quality of ground cover present. Ground cover can either consist of living plant tissue, such as vascular plants or bryophytes, which would lead to direct plant–plant interactions, or dead plant material, i. e. litter, which leads to “after-death” interactions (sensu Facelli & Facelli 1993). Since the most abundant species within a plant community greatly determine abiotic and biotic conditions for co-occurring species (Grime 1998), dominant ground cover, dead or alive, will have inevitable consequences for the performance of vascular plants through interferences at different life stages (During & Van Tooren 1990, Facelli & Pickett 1991a, Reader 1993, Foster & Gross 1997, Eckstein et al. 2004, Eckstein 2005, Ladd & Facelli 2008, Fayolle et al. 2009).

Since seedling emergence is a particularly vulnerable stage in the life-cycle of many plant species, interference at this stage may be very effective to set the path for evolutionary dynamics of a single species (Stinchcombe & Schmitt 2006) as well as the future development of a whole community (Facelli & Pickett 1991a, Myster 1994, Donath & Eckstein 2008, Fayolle et al. 2009). Although litter and bryophyte cover regularly co-occur in grasslands, there are only few studies that tried to experimentally identify their individual contributions to community assembly and composition through their effects on seedling emergence (e. g. Spacková et al. 1998, Kotorová & Lepš 1999).

In general, litter or moss cover can have chemical, physical and mechanical effects on seeds (During & Van Tooren 1990, Facelli & Pickett 1991a). Although chemical effects through secondary compounds leached from litter (Bosy & Reader 1995, Amatangelo et al. 2008, Hovstad & Ohlson 2008, Ruprecht et al. 2008) or bryophytes (van Tooren 1990) may negatively affect seedling emergence, it seems that these effects are of lower importance when acting in concert with physical and mechanical litter effects (Facelli & Pickett 1991a, Equihua & Usher 1993, Xiong & Nilsson 1999).

Mechanical effects of both litter and bryophytes may be positive when seeds are protected from herbivores through an overlaying cover (During & Van Tooren 1990, Reader 1993, Facelli 1994). However, with increasing thickness, litter or bryophyte cover either interferes with the ability of the radicle to establish soil contact (Mallik et al. 1984, Rotundo & Aguiar 2005) or thick layers present a barrier that hamper seedlings to pierce through the litter from below to reach the light (Fowler 1986, Chambers 2000, Rotundo & Aguiar 2005). This suggests that the relative position of seeds at the time of germination further accentuates how litter or mosses may affect seedling emergence (Facelli & Pickett 1991a, Rotundo & Aguiar 2005). While freshly shed seeds will be situated in the top layers of litter or moss carpets, they will subsequently move downward through decomposition at the base of the cover and input of new plant litter on top or through apical moss growth, respectively.

Changes of the physical conditions may result in increased seedling emergence through the alleviation of water stress due to reduced evaporation and run-off, especially in water-limited situations (Eckstein & Donath 2005, Rotundo & Aguiar 2005). Additionally, litter reduces seed exposure to temperature extremes (Eckstein and Donath 2005), and bryophytes may reduce soil temperature amplitudes through their insulation effect (Van Cleve et al. 1983). This effect may, however, lead to reduced seed germination when temperature amplitudes are below certain thresholds that are needed to induce germination (Thompson & Grime 1983).

Besides ground cover characteristics, traits of the species involved (e. g. seed size) may also affect the outcome of plant–plant interactions. Three mechanisms are related to seed size and may have a strong influence on the performance of seeds (Leishman et al. 2000, Moles & Westoby 2004b): the seedling size effect, the reserve effect and the metabolic effect (Westoby et al. 1996). Although seed size and the related mechanism seem to be phylogenetically biased (Westoby et al. 1992, 1995, Harvey et al. 1995), differences in seed size have, irrespective of phylogenetic components, distinct influence on seed and seedling fate (Leishman et al. 2000, Eckstein & Donath 2005). Seed mobility, which is influenced by seed size and seed surface structure, leads to different starting conditions even before the onset of germination (Bekker et al. 1998). Studies on the formation of soil seed banks have shown that smaller seeds are more likely to be incorporated into the soil seed bank than larger seeds (Bekker et al. 1998, Schmiede et al. 2009). Consequently, we would expect species-specific, seed-size related differences in emergence from above or below a litter or bryophyte cover.

To our knowledge, no previous study has manipulated both the amount of litter and bryophyte cover and the seed sowing position. In the present study, we set up a pot experiment with different amounts of moss or litter alone and a mixture of both, with seeds of different sizes either sown above or beneath the cover. We specifically addressed the following questions:

1 Are there different effects of moss and litter on seedling emergence?
2 Do the effects of litter and moss layer vary with seed size?
3 Is seedling emergence affected by seed position, and how does this response change depending on different cover types and amounts?

Methods

Study species

We selected five species that differed in seed mass: *Arabis nemorensis* (Hoffm.) Koch: 0.06 mg seed^{-1}, *Inula salicina* L.: 0.16 mg seed^{-1}, *Galium wirtgenii* F. W. Schultz (syn. *Galium verum subsp. wirtgenii* (F. W. Schultz) Oborný): 0.40 mg seed^{-1}, *Selinum carvifolia* (L.) L.: 1.06 mg seed^{-1} and *Sanguisorba officinalis* L.: 1.95 mg seed^{-1} (nomenclature: Wisskirchen & Haeupler 1998). Selecting those model species, we were able to focus on effects of cover type and cover amount on seedling emergence in dependence of seed size. These species are common for species-rich flood-meadows along the northern Upper Rhine (cf. Donath et al. 2003), represent characteristic families of dicotyledons of these as well as similar grassland habitats and their seed sizes cover about 70% of the total seed size range of species growing in alluvial meadows (Hölzel and Otte 2004). Seeds were collected between 18 July and 19 September 2007 (depending on the species and their degree of ripeness) in flood-meadows along the northern Upper Rhine. For each species, seeds were collected from at least 20 individuals of at least five populations, with the exception of *S. carvifolia* of which only three populations of sufficient size were available. Seeds were air-dried, manually cleaned and stored in darkness at room temperature (~ 20°C) until sowing on 17 December 2007. This sowing date left enough time for cold stratification during early spring, which enhances germination in several of the study species (Baskin & Baskin 2001, Hölzel & Otte 2004).

Experimental design

We used a completely randomized experimental design to study the effects of species (factor levels (k) = 5), cover type (k = 3: grass litter, moss and an equal mixture of both), cover amount (k = 3: 2, 4 and 8 g) and seed position (k = 2: above and beneath cover) on seedling emergence. We used 95 pots of 1 dm3 volume (ca. 10 × 10 × 10 cm) per study species, i. e. each cover type × cover amount × seed position combination (18 combinations) was replicated five times, plus five additional control pots that received no cover. Each experimental pot was sown with 50 seeds of one species.

Pots were filled with commercial potting soil (Fruhstorfer Erde®, Type P, Industrie-Erdenwerke Archut GmbH, Lauterbach, Germany) composed of a mixture of peat, clay and humus (pH_{CaCl2}: 6.1, 142 mg l^{-1} nitrogen, 97 mg l^{-1} P_2O_5 and 180 mg l^{-1} K_2O).

The grass litter levels of 2, 4 and 8 g per pot (i. e. 200, 400 and 800 g litter m^{-2}) correspond to annual litter production of low, medium and high productive floodplain meadows (Donath et al. 2004). The grass litter originated from a mesic unfertilized grassland site dominated by *Poa pratensis*, *Agrostis stolonifera*, *Arrhenaterum elatius* and *Dactylis glomerata* that harboured none of the study species. For the moss cover, we used *Rhytidiadelphus squarrosus* (Hedw.) Warnst. collected from an extensive lawn. This species is widespread and commonly found in oligotrophic to mesotrophic habitats, e. g. meadows, fallow land and forests. Also the amount of the moss cover applied (i. e. 200, 400, 800 g litter m^{-2}) was within the range found in these ecosystems (e. g. Simmering et al. 2001 and unpublished data). We used bench-dry grass litter and moss; neither was oven-dried as this might have changed chemical compounds (Shackleton and Rogers 1991) or killed the moss. We mixed an equal mass of bench-dry grass litter and moss to obtain a balanced litter–moss mixture.

Soil water content and soil temperature were monitored using two separate sets of two replicate pots per cover type × cover amount combination each plus two control pots without cover (i. e. 2 × 20 pots) that received no seeds. For water content measurements, these were filled with a defined soil mass. An aliquot of the same soil was dried to determine soil dry mass and initial water content. These pots were weighed weekly during the experiment to determine soil water content as percentage of soil dry weight. For soil temperature measurement, we positioned temperature loggers (Tinytag Transit with internal sensor, Gemini Dataloggers Ltd, Chichester, UK) below the cover to record soil temperatures at hourly intervals.

All pots were arranged at random in a common garden close to Giessen, Germany (50°32′N, 8°41.3′E, 172 m a.s.l.), exposed to open-air conditions and surrounded by an additional row of pots filled with soil to reduce desiccation. In addition to receiving water through precipitation, pots were kept constantly moist by regular watering (once a week in the beginning of the experiment and once a day later in the vegetation period when evaporation had increased).

At the beginning and end of the experiment, we used quantum sensors (Li-190, Licor Inc., USA) to assess the impact of cover amount and cover type on transmittance of photosynthetically active radiation (PAR) to the soil surface in relation to ambient PAR radiation. Additionally, we weighed the amount of cover left at the end of the experiment.

Seeds started to germinate in late March. Emerged seedlings, i. e. those that successfully penetrated through the cover, were counted twice, i. e. on 29 April 2008 and on 24 June 2008. Additionally, we tested for variation in the timing of emergence based on the difference in emergence between the two counting dates ($\Delta_{seedlings}$).

Statistical analysis

ANOVA was employed to analyse for effects of species, cover type, cover amount and seed position on relative seedling emergence and $\Delta_{seedlings}$. Since pots without litter application served as a control for both cover types and for cover amount, these represent an 'isolated control group' in the statistical analysis (Statsoft 2001). Variation between these control pots is accounted for when estimating residual sums of squares and degrees of freedom, whereas tests of main effects and interactions are based on a factorial designs excluding the controls. For the analysis, data on relative seedling emergence were square root arcsine transformed and $\Delta_{\text{seedlings}}$ were log-transformed (ln (x + 6), since the logarithm is not defined for negative numbers, six was added to account for seedling death between counting dates), respectively (Quinn and Keough 2002). As a measure for the relative contribution of each factor and the interactions to the total variability in percentage emergence and $\Delta_{\text{seedlings}}$, we used the ratio of the sum of squares of the factor or interaction of interest to the total sum of squares (i. e. for all factors, their interactions and the error).

The influence of seed size on seedling emergence under the different treatments of the current study and a previous study (Eckstein and Donath 2005) was analysed through linear regression. The latter study included seven species differing in seed mass: *Inula britannica* L.: 0.09 mg seed^{-1}, *Inula salicina* L.: 0.16, *Galium wirtgenii* F. W. Schultz: 0.4 mg seed^{-1}, *Galium boreale* L.: 0.68 mg seed^{-1}, *Viola pumila* Chaix: 1.08 mg seed^{-1}, *Viola elatior* Fr.: 1.8 mg seed^{-1} and *Silaum silaus* (L.) Schinz & Thell.: 2.43 mg seed^{-1}. For the analysis of cover effects on physical variables measured, we used repeated measures ANOVA, in case of temperature, water content and a multi factor ANOVA, in case of PAR. The latter was also used to analyse changes of cover mass during the study. As a post hoc test for differences between groups, we used Tukey HSD test for unequal *N*. All statistical analyses were done using Statistica 6.0 (StatSoft 2001).

Results

Effects on seedling emergence

In a four-way ANOVA, all main effects were highly significant (Table 1). Seed position (SP) alone explained about 50% of the variation in the data set, with significantly lower seedling emergence when seeds were sown on top of a litter and/or moss cover. Across all other factors, the amount of cover (CA) and cover type (CT) explained 16.2 and 7.5% of the total variation, respectively. All three factors, i. e. SP, CA and CT, differed significantly among species (S).

When analysed for the individual species (Figure 1), cover amount explained a higher proportion of the total variation than seed position in *Arabis* (CA = 61.2% vs. SP = 5.3%)

and *Inula* (CA = 54.8% vs. SP = 11.6%), the two small-seeded species, whereas seed position was more important than cover amount in the three species with larger seeds (*Galium* CA = 22.6% vs. SP = 44.3%, *Selinum* CA = 2.1% vs. SP = 69.9%, *Sanguisorba* CA = 8.8% vs. SP = 27.7%). For the whole data set, the S × SP interaction varied among levels of CA. This was because in the two small-seeded species differences in emergence between seed positions were larger at low CA, whereas in the large-seeded ones differences in emergence between seed positions were larger at high CA. Additionally, there was a significant S × SP × CT interaction, i. e. the species-specific effects of seed position on seedling emergence also differed between litter, litter–moss mix and moss.

Table 1. Results of a four-way ANOVA on the effect of species identity (S), cover type (CT), cover amount (CA) and seed position (SP) on percentage emergence and the difference in number of seedlings between 29 April 2008 and 24 June 2008 ($\Delta_{seedlings}$).

	Emergence				**$\Delta_{seedlings}$**			
Source of variation	**df**	**MS**	***P***	**vc (%)**	**df**	**MS**	***P***	**vc (%)**
Intercept	1	119.67	< 0.0001		1	2942.82	< 0.0001	
Species [S]	4	2.32	< 0.0001	12.7	3	6.77	< 0.0001	45.8
Cover type [CT]	2	1.37	< 0.0001	7.5	2	0.41	< 0.0001	2.8
Cover amount [CA]	2	2.95	< 0.0001	16.2	2	1.37	< 0.0001	9.3
Seed position [SP]	1	9.27	< 0.0001	50.8	1	3.42	< 0.0001	23.1
S x CT	8	0.05	0.0035	0.3	6	0.35	< 0.0001	2.3
S x CA	8	0.82	< 0.0001	4.5	6	1.06	< 0.0001	7.2
CT x CA	4	0.29	< 0.0001	1.6	4	0.11	0.0184	0.7
S x SP	4	0.67	< 0.0001	3.7	3	0.83	< 0.0001	5.6
CT x SP	2	0.05	0.0474	0.3	2	0.03	0.4648	0.2
CA x SP	2	0.23	< 0.0001	1.3	2	0.15	0.0169	1.0
S x CT x CA	16	0.04	0.002	0.2	12	0.10	0.0012	0.7
S x CT x SP	8	0.04	0.0122	0.2	6	0.01	0.9124	0.1
S x CA x SP	8	0.08	< 0.0001	0.4	6	0.07	0.0661	0.5
CT x CA x SP	4	0.04	0.0663	0.2	4	0.05	0.2686	0.3
S x CT x CA x SP	16	0.02	0.2926	0.1	12	0.04	0.2649	0.3
Error	380	0.02			288	0.04		

df = degrees of freedom, MS mean sum of squares
vc (%) = relative contribution of individual factors and their interactions to total variation

Effects of seed mass

There was a tendency for higher seedling emergence at low CA and grass litter in small-seeded species. Equal emergence was observed among the levels of CA and CT in *Galium* (intermediate seed size) and higher emergence at high CA irrespective of CT in the two species with the largest seeds (Figure 1). In line with these results we found a significant linear relationships between seed mass and the ratio of emergence from beneath 8 g cover and the non-cover control pots (Figure 2a; grass litter: $y = -0.046 + 4.274 * \text{seed mass}$, $F_{1,3} = 137.7$, $P = 0.0013$; litter–moss mix: $y = -0.563 + 4.481 * \text{seed mass}$, $F_{1,3} = 317.4$, $P = 0.0004$; moss: $y = -0.399 + 3.752 * \text{seed mass}$, F 1,3 = 176.6, $P = 0.0009$). Regression slopes did not differ significantly between cover types (t test of slopes, $P > 0.1$). Additionally, this relationship was the same for a data set on emergence from beneath a grass litter cover from a previous study (Eckstein & Donath 2005) (Figure 2a; $y = -0.850 + 5.002 * \text{seed mass}$, $F_{1,5} = 41.6$, $P = 0.0013$, $N = 7$ species). The latter study contained seven species of the Apiaceae, Asteraceae, Rubiaceae and Violaceae. The slope of the regressions for grass litter did not differ between the present data and the data set from 2005 ($P = 0.4204$). In contrast, the emergence ratio of seedlings sown on top of 8 g of cover relative to non-litter controls (Figure 2b) was only significantly related to seed size for grass litter ($y = -0.021 + 1.950 * \text{seed mass}$, $F_{1,3} = 327.7$, $P = 0.0004$) but not for the other two cover types.

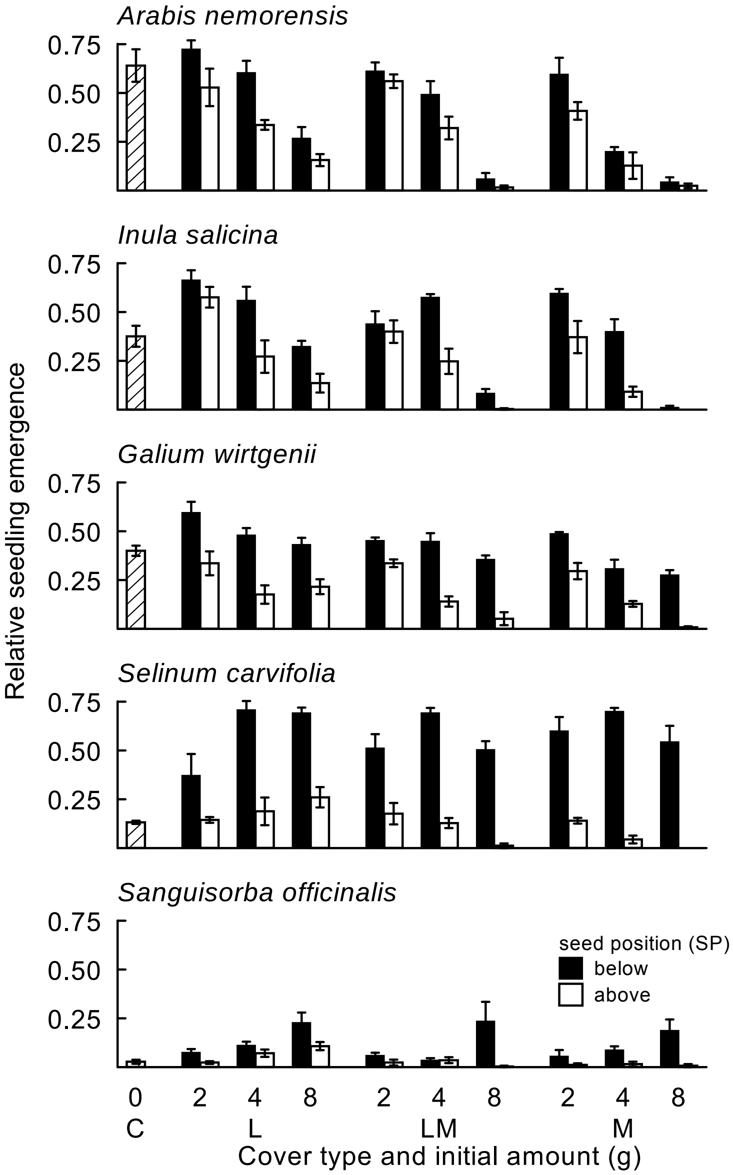

Figure 1. Relative seedling emergence of the study species (arranged according to increasing seed mass) sown beneath (black bars) or above (white bars) different amounts (2, 4, and 8 g) of grassland litter (L), litter–moss mix (LM) and moss (M) cover. The hatched bars denote control pots (C) without any cover. Data are means ± *SE* (*N* = 5).

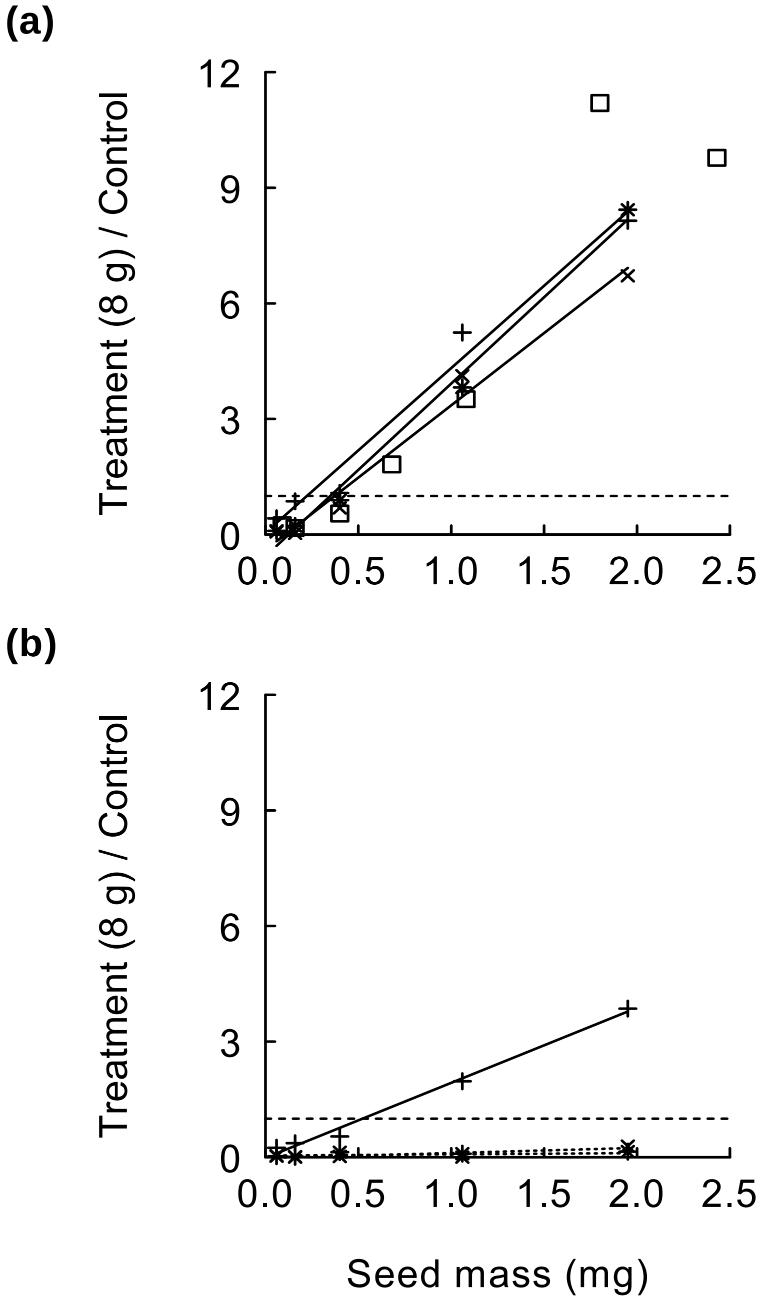

Figure 2. The ratio of seedling emergence in 8 g of cover relative to the control without cover as a function to seed size. **a** The results when seeds are sown beneath the cover and **b** when seeds are sown on top of the cover. Cover types: +, grass litter; *, litter–moss mix; ×, moss cover; in (**a**): □, data from Eckstein and Donath (2005), grass litter, one outlier (*Peucedanum officinale*, seed mass 12.32 mg) excluded. Horizontal dotted line represents a ratio value of 1 between seedling emergence in 8 g of cover relative to the control, i. e. while values < 1 depict inhibition of seedling emergence, values > 1 illustrate facilitation of seedling emergence. Continuous lines depict significant linear regressions for each cover type ($N = 5$, $P < 0.05$). Regression slopes in (**a**) are not significantly different between cover types (*t* test of slope, $P > 0.1$).

Physical effects

While during the main emergence and growth period neither type nor amount of cover had a significant effect on mean soil temperatures (repeated measures ANOVA, ln-transformed data, $F_{2,10} = 0.6$, $P = 0.56$ and $F_{2,10} = 0.4$, $P = 0.69$, respectively), their effects on daily temperature amplitudes were significant ($F_{2,10} = 10.65$, $P = 0.0033$ and $F_{2,10} = 61.44$, $P < 0.0001$, respectively). Temperature amplitudes were significantly lower under moss than under litter and in control pots (Tukey HSD test for unequal *N*, $P < 0.05$, mean ± *SE*, 15.7 ± 0.3°C, 18.9 ± 0.4°C and 20 ± 0.7°C, respectively), but none was significantly different from daily temperature amplitudes under litter–moss-mix (17.9 ± 0.4°C). Across cover types, an increase in the amount of cover was accompanied by a significant decrease in the daily temperature amplitudes (Tukey's HSD test for unequal *N*, $P < 0.01$, 2 g: 21.8 ± 0.4°C, 4 g: 17.3 ± 0.3°C, 8 g: 13.3 ± 0.3°C). However, the temperature amplitude of the control pots (20 ± 0.7°C) was only different from pots covered with 8 g.

Water content was significantly influenced by cover amount, but not by cover type (repeated measures ANOVA, ln-transformed data, $F_{2,10} = 7.7$, $P = 0.01$ and $F_{2,10} = 2.1$, $P = 0.18$, respectively). Average water content reached 125.8 ± 4.8% in the control pots and varied between 134.2 ± 4.3% and 151.9 ± 2.5% of soil dry weight in the treatment pots. Across cover types, water content differed significantly between cover amounts up to 4 g cover, but did not further increase at 8 g (Tukey HSD test for unequal *N*, $P < 0.05$). In contrast to grass litter, water content in the moss treatment did not steadily rise with increasing cover amount but declined at 8 g cover.

In the beginning of the experiment, the percentage of PAR transmittance that reached the soil surface was about the same magnitude within one level of cover amount under the moss as well as the moss-litter-mix, but considerably higher under litter (Figure 3a). At the end of the experiment, the patterns and amounts of relative PAR had changed considerably (Figure 3b). These changes in PAR penetration were affected by both cover type and cover amount (three-way ANOVA, square root arcsine transformed data, $F_{2,198} = 172.2$, $P < 0.0001$, $F_{2,198} = 331.9$, $P < 0.0001$, respectively), and there was a significant interaction between both factors ($F_{4,198} = 16.5$, $P < 0.0001$). Within each level of cover amount, PAR reaching the soil surface differed between litter and moss, and litter and litter–moss mix (Tukey HSD test for unequal *N*, $P < 0.05$). In the moss treatment, light interception changed only slightly with time, whereas it dropped considerably in the litter treatment. In the latter, temporal changes in light interception allowed between twice (at 8 g) and 14-fold (at 2 g) more PAR radiation to reach the soil surface at the end of the experiment. PAR transmittance in the litter–moss mix was intermediate compared to the other cover types.

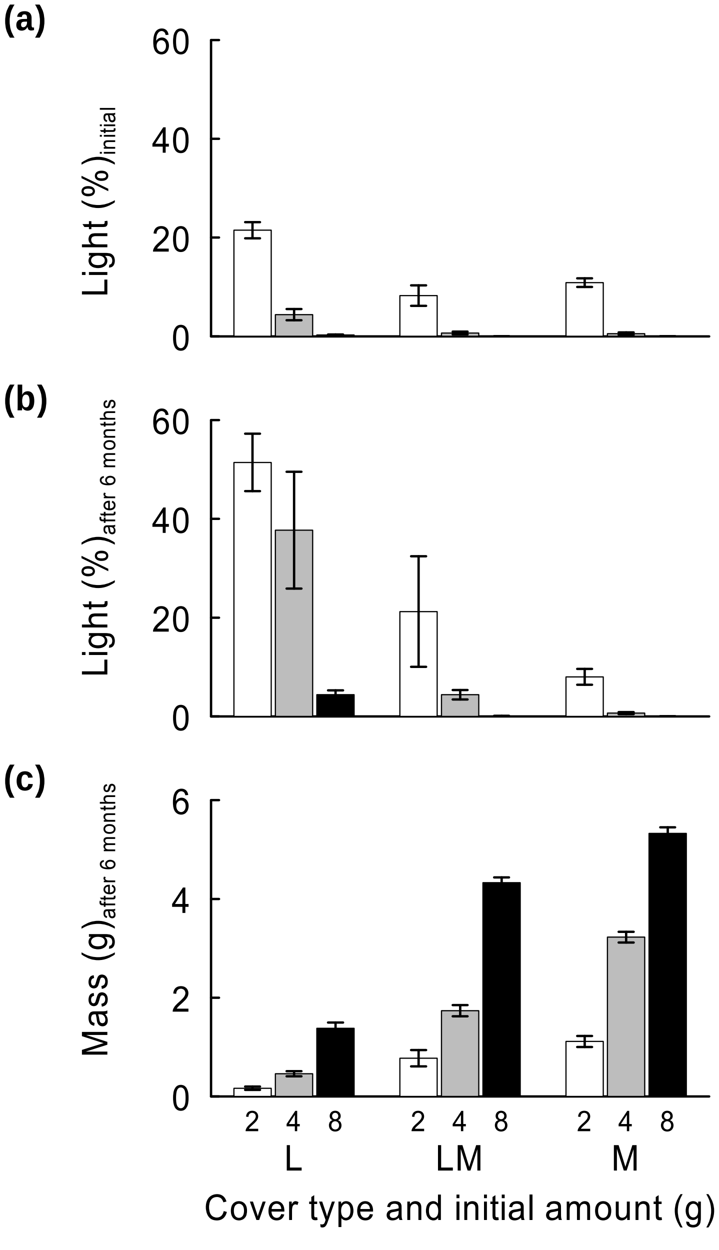

Figure 3. Relative initial (a) and final (after 6 months) (b) amounts of photosynthetically active radiation (% of ambient light) below different amounts and types of cover, and final mass (c) of litter (L), litter–moss mix (LM) and moss (M) cover. Data are means ± *SE* (*N* = 5 in (a) and 3-15 in (b) and (c)).

The mass of litter, litter–moss mixture and moss left at the end of the experiment depended on both starting amount as well as cover type (two-way ANOVA, ln-transformed data, $F_{2,72} = 301.7$, $P < 0.0001$ and $F_{2,72} = 397.2$, $P < 0.0001$, respectively), but the effects of cover type and amount were not additive (significant *cover amount* × *cover type* interaction, $F_{4,72} = 10.9$, $P < 0.0001$). Within each level of cover amount, the quantity not decomposed at the end of the experiment was always significantly different between all three cover types (Tukey HSD test for unequal N, $P < 0.05$), except between moss and mixture at 2 g. While moss only lost 19-45% of its original mass, the litter–moss mix was diminished by 45-61% of the original mass, and the litter layer decreased even by 83-92% of its original mass (Figure 1c). Interestingly, relative losses were always highest at 2 g original cover and, except for moss cover, lowest at 8 g original cover, irrespective of cover type.

Timing of seedling emergence

The difference in emerged seedlings between the two counting dates ($\Delta_{\text{seedlings}}$) varied significantly among species (Table 1; Figure 4), which explained 45.8% of the variation in the data set. *Galium* was excluded from this analysis since it branches already below ground or within the cover layer. Thus, the true number of emerged seedlings could only be verified at the end of the experiment by thoroughly excavating the plants from the pots. Between the two counting dates, significantly more seedlings emerged from beneath a cover layer than from pots where seeds were sown on top of the cover (Table 1). In *Inula*, significantly more seedlings emerged between the counting dates from grassland litter than from both moss or litter–moss mix across all cover amounts (Figure 4a) and across cover types significantly more seedlings of this species and *Arabis* appeared from 2 g and from 4 g of cover than from pots with 8 g cover (Figure 4b).

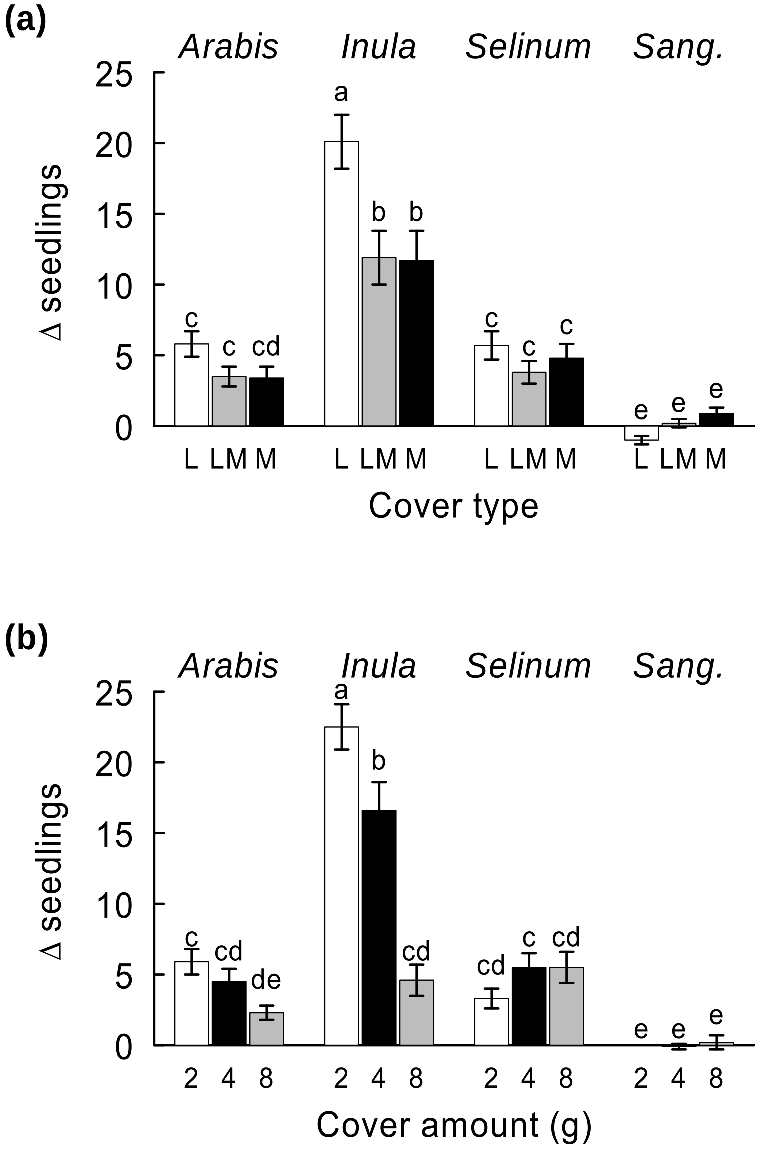

Figure 4. Differences in seedling number between the two counting dates on 27 April 2008 and 24 June 2008 ($\Delta_{\text{seedlings}}$) of *Arabis nemorensis* (*Arabis*), *Inula salicina* (*Inula*), *Selinum carvifolia* (*Selinum*) and *Sanguisorba officinalis* (*Sang.*) from pots with different cover types (**a**) (L grassland litter, LM litter–moss mix, M moss) and different cover amounts (**b**) (2, 4, and 8 g). Data are means ± *SE* ($N = 30$). Different letters denote significant differences at $P < 0.05$ according to Tukey HSD test for unequal *N* (data were ln transformed before analysis). Negative values result from seedling mortality.

Discussion

Microclimatic effects of litter and moss cover

In general, litter and moss cover exerted similar microenvironmental effects leading to lower temperature amplitudes, higher soil moisture and lower light levels with increasing mass (and thickness) of the cover. We found that, in line with other studies, increasing cover amount decreased temperature amplitude but not mean temperature below the cover (Jensen & Gutekunst 2003, Eckstein and Donath 2005). In addition to attenuating temperature extremes, the presence of ground cover also lowered water stress. However, the decrease in water content between 4 and 8 g of moss cover may be an indication that poikilohydric mosses, which are well adapted to repeated cycles of drying and re-wetting, compete with seedlings for water (Mallik et al. 1988, Serpe et al. 2006).

Photosynthetically active radiation beneath the cover decreased sharply with the amount applied (Figure 3a). In line with other studies (Facelli and Pickett 1991b, Eckstein and Donath 2005), which found an exponential decline of PAR with an increasing amount of ground cover, the thickest cover (8 g) lead to almost complete darkness (< 0.5% of ambient PAR).

Due to the observed differences in decomposition of moss and litter (Figure 3c), there were also temporal changes in illumination (Figure 3a, b). Since decomposition of mosses was partly compensated for by re-growth, light interception through the moss cover had hardly changed, whereas much more PAR reached the soil beneath litter and litter–moss mixture treatments.

Effects of ground cover type on seedling emergence

Our data suggest that seedling emergence was more severely affected by a cover of bryophytes than by the same amount of grass litter, i. e. 22.6 ± 0.02% vs. 34.6 ± 0.02% (mean ± *SE*). We propose that this is related to differences in structure between cover types, which may influence seed movements and penetration of the ground cover by seedlings (Sydes & Grime 1981, Campbell et al. 1992, Donath and Eckstein 2008). Although the main effect size of cover type was relatively small in comparison to other factors included in the experiment (Table 1), our results highlight the importance of higher decomposition rate in grass litter, compared to bryophytes. This is in line with

Nilsson et al. (1999), who found lower decomposition rates of bryophyte cover than tree and dwarf shrub litter from boreal forest sites. In our experiment, the amount of grass litter decreased at a rate of 0.29, 0.56 and 1.05 g month^{-1} in the 2, 4 and 8 g treatment, respectively, under outdoor conditions, whereas the layer of *Rhytidiadelphus* showed a decrease of only 0.14, 0.12 and 0.42 g month^{-1} (Figure 3a). Consequently, small-seeded species such as *Inula* and *Arabis* that demand a high supply of light (Milberg et al. 2000, Baskin & Baskin 2001) and relatively high temperatures for germination (Hölzel & Otte 2004) and thus show relatively slow, delayed germination (Eckstein & Donath 2005) may emerge later in the growing season after the cover of grass litter has diminished (Figure 4). A thick layer of bryophytes, however, will considerably reduce reproduction through seeds of these small-seeded species. Our study thus suggests that the cover type may significantly influence species assembly and species composition of grassland communities. This is in line with the results from field investigations. In wet grasslands, for example, bryophyte encroachment after abandonment exerts strong effects on plant species diversity (Kotorová & Lepš 1999) and regeneration through seeds (Oostermeijer et al. 1994, Spacková et al. 1998).

Effects of seed size and seed position

Within a range of seed sizes from 0.06 to 1.95 mg, we found a linear relationship between seed size and seedling emergence from beneath a thick layer of moss and/or grass litter (Figure 2a). That this relationship is not simply an effect of species identity could be verified through comparison with data on emergence from beneath a grass litter cover from a previous study (Eckstein & Donath 2005; Figure 2a), where the slope of the regressions for grass litter did not differ between the present data and the data set from 2005. Also among 35 grassland plants (seed size: 0.01 to 4.8 mg) seedling emergence from beneath 3 and 8 cm of *Carex* litter increased with seed size (Jensen & Gutekunst 2003). A similar trend has been observed in forest species, where seedling emergence through accumulated litter is also controlled by seed size (Seiwa & Kikuzawa 1996, Kostel-Hughes et al. 2005). Since larger seed sizes generally lead to larger seedling sizes (e. g. Jurado & Westoby 1992), it has been suggested that seedlings of large-seeded species possess a better ability to penetrate a layer of litter, bryophytes or soil and can establish under more unfavourable, i. e. shady or competitive conditions, than small seedlings (Leishman et al. 2000). Large-seeded species may have an advantage over small-seeded species not only since they profit of the higher moisture content associated with increasing cover (Eckstein & Donath 2005) but also since the protective effect of litter against seed predation increases with seed size (Reader 1993).

Seed position (beneath or on top of the cover) was the single factor that explained half of the variation in the data set on seedling emergence. Also Rotundo & Aguiar (2005) found strongly reduced emergence of *Bromus pictus*, a species of arid grass–shrub steppe in Argentina, when sown within a litter cover. The importance of seed position for germination and seedling fate was already pointed out by Facelli & Pickett (1991a, and references therein). When seeds are trapped on top or within a layer of litter and/or bryophytes they do not have contact with the soil. Those seeds that remain dry and therefore do not germinate may, depending on environmental conditions such as air and soil temperatures, eventually enter dormancy (Baskin & Baskin 2001). In this case, only long-term persistent seeds maintain the possibility for successful establishment when microsite demands are met in the future. In contrast, short-term persistent seeds are in danger of burial by too large amounts of litter or are overgrown by mosses during the short period they are germinable. Alternatively, if seeds become imbibed, e. g. during periods with sufficient precipitation, seedlings will have to establish soil contact to avoid lethal desiccation when the litter and/or moss layer dries out and to penetrate above the cover layer to reach sufficient light. Depending on the thickness of the cover, resources necessary for the elongation of the radicle or hypocotyl may be substantial. However, the more reserves are depleted simply for penetration of the cover the fewer resources are left for successful establishment in competition with other plants (Hamrick & Lee 1987). Under these conditions, the radicle of larger seedlings (i. e. species with larger seeds) may have a higher capability to establish this vital soil contact than the radicle of small-seeded species. However, our data suggest that in large-seeded floodplain grassland species (>0.4 mg) seed position explained a higher proportion of the variance in the data than cover amount, whereas in small-seeded species (seed size $\ll$0.4 mg) cover amount was more important than seed position. Thus, counterintuitively, differences in seedling emergence between seed position treatments were smaller in small-seeded than in large-seeded species. This can be explained as follows: irrespective of seed size, relatively few seedlings emerged successfully after seeds were sown on top of the cover, whereas more seedlings of large-seeded species managed to penetrate a thick cover layer when sown beneath the cover. In contrast, at low cover amounts, seedling emergence of small-seeded species was higher than that of large-seeded species both from beneath and above the ground cover (cf. Figure 1). The fact that seedling emergence in large-seeded species is higher when seeds are positioned beneath the cover than when seeds are shed on top of a bryophyte layer or litter–moss mixture indicates that the seeds are entrapped in the ground cover and do not reach the soil surface. In contrast, initial positioning of the seed had no effect in combination with grass litter. Small seeds, on the other hand, are more mobile (Chambers et al. 1991) and therefore “percolate” through the cover layer. When they reach the soil surface, the seeds may either not germinate or fail to overcome the cover layer. However, since small seeds are more prone to be incorporated into the soil than large seeds (Bekker et al. 1998, Schmiede et al. 2009) and small seedlings will probably not be able to penetrate even through a thin overlaying cover of soil and die off (Burmeier et al., 2010), there has been strong selection against dark germination in small-seeded plant species (Milberg et al. 2000). Thus, there appears to be an interaction between seed size, seed position and cover type (Figure 2).

Our data suggest that large-seeded species have an edge over small-seeded ones when seeds are situated beneath a cover, irrespective of cover type (Figure 2a). In contrast, when seeds are shed on top of a cover, the advantage of large seed size remains only on grass litter but not on mosses or litter–moss mixtures (Figure 2b).

Conclusions

Our results imply that large-seeded species will have a selective advantage at moderate amounts of grass litter cover, whereas some small-seeded species may adapt to these litter amounts through the timing of germination. If litter amounts increase, diversity decreases because there will be selection against small-seeded species, commonly not able to persist under such conditions.

In contrast, bryophyte encroachment in meadows does not entail a selective advantage for large-seeded species, given that seeds are shed on top of the bryophyte layer. Our study suggests that bryophytes have the potential to decrease plant diversity irrespective of seed size. However, those individuals that manage to establish under these conditions may potentially benefit from higher humidity levels within a bryophyte layer (Oostermeijer et al. 1994, Eckstein et al. 2004).

Interestingly, the range of cover amounts where seedling emergence reached its optimum (this study, Eckstein & Donath 2005) lies within the ascending segment (200–600 g m^{-2}) of the humped-backed curve relating above ground biomass and species diversity (Al-Mufti et al. 1977, Grime 2002). In this context, our results suggest that moderate amounts of grass litter as found in regularly mown, extensive flood-meadows (200–500 g m^{-2}; Donath et al. 2004) support a high plant species diversity (Carson & Peterson 1990, Hovstad & Ohlson 2008). This relationship may be driven by ecologically and evolutionarily relevant interactions between seed size, cover types and seed positions. Thus, in concert with changes in cover quality and quantity during natural processes, e. g. succession, or through human impact, e. g. management changes, serious long-term consequences on community structure and ecosystem functioning may occur (Xiong & Nilsson 1999, Ruprecht 2006, Klanderud & Totland 2007, Bruun & Ten Brink 2008).

Acknowledgements

We thank Christiane Lenz-Kuhl and Josef Scholz-vom Hofe for their support during the experiment and in the laboratory and Sandra Burmeier for seed collection. Sandra Burmeier, Annette Otte, Eszter Ruprecht and two anonymous referees offered helpful comments that significantly improved the manuscript. All help is gratefully acknowledged.

6 Litter effects on seedling establishment interact with seed position and earthworm activity

Donath TW & Eckstein RL Plant Biology accepted
doi: 10.1111/j.1438-8677.2011.00490.x

Abstract

Seedling establishment is influenced by cover of litter and by seed predators but little is known about interactions between these two factors. We tested their effects on the emergence of five typical grassland species in a microcosm experiment. We manipulated the amounts of grass litter, seed sowing position and earthworm activity to determine i) whether the protective effect of litter against seed predation depends on cover amount and seed sowing position, i. e. on top or beneath the litter, ii) if seed transport by earthworms changes the effect of seed sowing position on seedling emergence and (iii) if seeds transported into deeper soil layers by earthworms are still germinable.

Litter cover and presence of earthworms lowered seedling emergence. The impact of seed position increased with seed size. Emergence of large-seeded species was reduced when sown on top. Additionally, we found an important seed position × earthworm interaction related to seed size. Emergence of large seeded species sown on top of the litter was up to three times higher when earthworms were present than without earthworms. Earthworms also significantly altered the depth distribution of seeds in the soil and across treatments on average six per cent of the seeds germinated after burial. In contrast to the seed position effect, we found no size effect in mobility and germinability of seeds after burial into the soil. Nevertheless, the fate of differently sized seeds may differ. While burial will remove large seeds from the regeneration pool, it may enhance the seed bank build up in small seeded species. Consequently, changes in the amount of litter cover and in the invertebrate community play a significant role for plant community composition.

Introduction

The distribution of viable seeds is controlled through abiotic and biotic processes acting during the pre- and post-dispersal phase of seeds (Chambers & Macmahon 1994, Fenner & Thompson 2005). Once seeds are being dispersed, their success depends on the presence and quality of safe sites, which are controlled by abiotic conditions, resources and biotic interactions (Davis et al. 2000, Ejrnæs et al. 2006, Lortie et al. 2004, Wurst et al. 2005). The latter include negative food-web effects through pathogens (Blaney & Kotanen 2001) or seed predators such as arthropods (Chambers & Macmahon 1994, Facelli 1994), which may kill seeds. In this context, Eisenhauer et al. (2008) revealed that earthworms also seem to have decisive effects on seed fate; seeds merely passing the gut had in some cases even higher germination percentages than seeds not ingested (Eisenhauer et al. 2008), earthworms can also act as seed predators. Depending on seeds characteristics earthworms digest up to 100% of the ingested seeds (Eisenhauer et al. 2008, Willems & Huijsmans 1994). Seeds may be protected from these perils through burial into the soil or into a layer of dead plant remains, i. e. litter.

The litter will attenuate adverse extremes in temperature and moisture (Eckstein & Donath 2005, Rotundo & Aguiar 2005). In addition to these physical effects, litter will also change the chemical environment of seeds (Ruprecht et al. 2010b) and present a mechanical barrier (Facelli & Pickett 1991). While there is evidence that chemical effects of secondary compounds leached from litter are of lower importance, physical and mechanical effects of litter may be decisive for seedling emergence (Facelli & Pickett 1991, Xiong & Nilsson 1999). Positive mechanical effects of a litter or soil cover may be due to protection from predation. This protective effect of litter seems to increase with cover amount (Facelli 1994, Reader 1993, Shaw 1968, Sydes & Grime 1981). Similarly, seeds buried into the soil are less likely to be detected than those on the soil surface (Crawley & Long 1995). Additionally, the importance of the protection of seeds from predation by a soil or litter cover is related to the spatial arrangement of seeds, for instance seeds at higher density or clumped seeds are more likely to be eaten than isolated seeds at low density (Cabin et al. 2000).

Since the position of seeds has an impact on both the protection from predation and seedling establishment, the positioning of seeds on top or below a litter cover or a soil cover will also directly affect germination and seedling emergence (Burmeier et al. 2010, Donath & Eckstein 2010, Facelli & Pickett 1991, Rotundo & Aguiar 2005). In abandoned grasslands the yearly input of biomass increases the thickness of the litter layer (Ruprecht et al. 2010b). With increasing thickness of the layer covering the seedlings, growth is retarded since the young plants are forced to pierce through a thicker cover of soil (Burmeier et al. 2010, Zhang &Maun 1990) and/or litter (Chambers 2000, Fowler 1986) to reach the light. Conversely, with increasing thickness of the litter layer beneath seedlings, the ability of the radicle to establish soil contact is diminished (Donath & Eckstein 2010, Mallik et al. 1984, Rotundo & Aguiar 2005). A seed's position will also change during time. While freshly shed seeds will be situated in the top layers of litter, they will subsequently move downward as a consequence of decomposition at the base of the cover and input of new plant litter on top and by redistribution through for example wind or rain.

The activity of soil-dwelling invertebrates such as anecic earthworms may change the speed of seed movement in both the litter cover and the soil during secondary dispersal (cf. Chambers & MacMahon). Seeds may either be merely co-carried with the litter into the soil, or may be selectively targeted as food by earthworms (Zaller & Saxler 2007). When not digested the final depth at which seeds come to rest in the soil after secondary dispersal will, to a large extent, determine the chance of successful germination and emergence.

The response of seeds to soil cover, burial and predation is influenced by plant traits such as seed size. In general, seedling performance is closely related to seed size (Leishman et al. 2000, Moles & Westoby 2004b). Additionally, seed mobility, which is influenced by seed size and seed surface structure, leads to different starting conditions even before the onset of germination (Bekker et al. 1998, Burmeier et al. 2010). While large seeds are caught more persistently in a litter cover, small seeded species percolate through the litter cover (Donath & Eckstein 2010). Analogously, studies on the formation of soil seed banks have shown that smaller seeds are more likely than larger seeds to be incorporated into the soil seed bank (Bekker et al. 1998, Burmeier et al. 2010, Schmiede et al. 2009). Studies on vertical seed transport by earthworms showed that they bury smaller seeds deeper into the soil than larger seeds and that seeds with a rough, though not spiky, surface are preferred over seeds with a slick surface (Regnier et al. 2008).

However, earthworms also preferably ingest and digest smaller seeds (Eisenhauer et al. 2010), which will counteract their positive contribution to the formation of a persistent seed bank. For small seeds, which are more likely to be persistent (Thompson et al. 1993), burial speeds up the formation of a persistent seed bank (Schmiede et al. 2009). Large seeds, on the other hand, will most likely be removed from the regeneration pool through decay or fatal germination after burial deep into the soil (Burmeier et al. 2010).

Although litter cover and earthworms regularly co-occur in grasslands, we are not aware of any study that tried to experimentally identify their individual contributions to seedling emergence. In this context, we developed an experimental setup, in which we manipulated both the amount of litter and the seed sowing position in concert with earthworm-induced dynamics to assess their effect on seedling emergence of plant species with different seed size. Subsequently, we also followed the post burial fate of seeds.

We specifically addressed the following questions:

1. Does the protective effect of litter against seed predation depend on cover amount and seed sowing position?
2. Does seed transport by earthworms change the effect of seed sowing position on seedling emergence and do these effects vary with differences in seed sizes?
3. If seeds are transported into deeper soil layers by earthworms, will they still be germinable and will their fate depend on seed size?

Material and Methods

Study species

We selected five species of the Apiaceae family with differing seed mass and seed size but similar in the morphology of the fruit. Thus, it is reasonable to assume that the seeds of these species are equally well captured by earthworms (Willems & Huijsmans 1994). Seed mass ranged between 0.77 mg seed^{-1} (*Pimpinella saxifraga* L.) and 6.23 mg seed^{-1} (*Heracleum sphondylium* L.). These species also had the smallest and largest seed size, respectively (Table 1). Seeds of *Peucedanum officinale* were collected in the field in autumn 2009 while all other seeds were obtained from a supplier of regional wildflower seeds (Rieger-Hofmann GmbH, Blaufelden-Raboldshausen, Germany). Seeds were manually cleaned and stored in darkness at room temperature (~ 20°C) until sowing on February 8th 2010. An initial viability test with a 1% tetrazolium chloride solution (Bennett & Loomis 1949) on additional seed batches showed that viability was, except for *Angelica sylvestris*, well above 70% (Table 1). Unfortunately, *Heracleum sphondylium* completely failed to germinate and was thus excluded from all analyses.

Earthworms used in the experiment belonged to the species *Lumbricus terrestris*. These were purchased from a commercial supplier (faunatopics GmbH, Marbach am Neckar, Germany). As described by Fründ et al. (2010) we checked earthworms for health before introduction into the experimental pots.

Table 1. Diaspore characteristics, percentage of viable seeds (Tetrazolium-test) and germination percentage under the control treatment (no litter, no earthworms) of the study species (mean ± *SE*).

Species	Mass (mg)	Length (mm)	Width (mm)	Thickness (mm)	Viability (%)	Germination (%)
Pimpinella saxifraga L.	0.77	1.99 ± 0.06	0.98 ± 0.02	0.71 ± 0.02	98	28.94 ± 2.72
Angelica sylvestris L.	1.48	5.14 ± 0.12	3.40 ± 0.14	0.65 ± 0.019	90	5.63 ± 1.04
Pastinaca sativa L.	3.75	5.47 ± 0.17	3.82 ± 0.11	0.57 ± 0.01	78	58.13 ± 2.97
Peucedanum officinale L.	4.61	5.07 ± 0.08	3.54 ± 0.10	0.63 ± 0.03	100	22.69 ± 1.59
Heracleum sphondylium L.	6.23	7.46 ± 0.25	5.68 ± 0.19	0.53 ± 0.02	82	0 ± 0.0

Experimental design

Seedling emergence

We used a completely randomized experimental design to study the effects of species (factor levels (*k*) = 4), cover amount (*k* = 3: 0 g dm^{-2}, 4 g dm^{-2}, 8 g dm^{-2}), seed position (*k* = 2: above vs. beneath cover) and presence of earthworms (*k* = 2: present vs. absent) on seedling emergence (experiment I). As microcosms we used 96 pots of 3 dm^3 volume (ca. 14 × 14 × 23 cm) per study species, i. e. each cover amount × seed position × earthworm combination (12 combinations) was replicated eight times. Each experimental pot was sown with 100 seeds of one species on top or below grass litter, respectively.

Pots were filled with top soil from an arable field (pH$_{KCL}$: 6.8, 0.19 % total nitrogen, 13.5 mg * 100 g^{-1} phosphorus and 23 mg * 100g^{-1} potassium), leaving about 4 cm up to the pot rim. The material was steam-sterilized prior to the experiments to kill any plant seeds and earthworms potentially present in the substrate (6 h at 80 °C, Sterilo 1 K, MAFAC/Schwarz, Alpirsbach, Germany).

The grass litter levels of 4 and 8 g litter dm^{-2} correspond to annual productivity of intermediately and highly productive grasslands, respectively (Donath et al. 2004, Ruprecht et al. 2010b). The grass litter originated from a mesic unfertilized grassland site dominated by *Poa pratensis*, *Agrostis stolonifera*, *Arrhenaterum elatius* and *Dactylis glomerata* that harboured none of the study species. We used bench-dry grass litter, that is, it was not oven-dried as this might have changed chemical compounds (Shackleton & Rogers 1991). At the end we observed – apart from some burial and mixing with casts – a more or less intact litter cover in all litter treatments.

To allow cold stratification of seeds, which enhances germination especially in Apiaceae (Baskin & Baskin 2001, Hölzel & Otte 2004), sown pots were exposed to free air conditions until March 17th (average daily mean (T_{mean}), minimum (T_{min}) and maximum (Tmax) air temperature (mean ± *SE*): T_{mean} = 3.1°C ± 0.7°C; T_{min} = -3.9 ± 1.1°C, T_{max} = 15.7°C ± 1.25°C). After stratification, pots were randomly placed in a greenhouse with automatic watering (twice a week), controlled temperature (T_{min} ~ 15°C, T_{max} ~ 23°C), light ($L_{6:00-22:00}$ ≥ 10 klx) and air humidity (~ 70%) in Giessen, Germany. In the greenhouse, three adult individuals of *Lumbricus terrestris* per pot were added to pots of the earthworm treatment. This is at the upper limit of naturally occurring earthworm densities (cf. Evers et al. 2010, Fründ et al. 2010). Escape of earthworms was prevented by covering pots with white plastic gauze (mesh size 0.35 mm) at the top and bottom. To avoid microclimatic differences between treatments, pots without earthworms were covered with gauze as well.

Seeds started to germinate in late March. Emerged seedlings, that is those that successfully penetrated through the cover, were counted and removed thrice, i. e. on April 7th, on May 10th and on May 31st 2010. At the last counting date, we also checked pots for earthworms still present and found that despite the gauze some earthworms had escaped their original pots or may have died. On average 2.2 ± 0.07 (mean ± *SE*) earthworms were left per pot on May 31st 2010. However, this did not seem to have an influence on the outcome of the experiment (cf. results), probably since earthworms escaped or died well after the germination peak on April 7th, when 71% of the seedlings had already emerged.

Soil temperature was monitored using two separate pots per cover amount (3 x 2 pots) that received no seeds. For soil temperature measurement we positioned temperature loggers (Tinytag Transit with internal sensor, Gemini Dataloggers Ltd, Chichester, UK) below the cover to record soil temperatures at hourly intervals (Table 2).

Table 2. Daily average temperature, average minimum temperature, average maximum temperature and temperature amplitude (mean ± *SE*) in the control treatment (0 g dm^{-2}) and beneath 4 g dm^{-2} as well as 8 g dm^{-2} of litter cover during the main germination period in experiment I (March 18th until April 15th).

Litter amount (g dm^{-2})	Average (°C)	Minimum (°C)	Maximum (°C)	Amplitude (°C)
0	18.2 ± 0.1	14.8 ± 0.1	23.5 ± 0.4	8.5 ± 0.5
4	17.5 ± 0.1	15.1 ± 0.1	21.2 ± 0.3	6.1 ± 0.3
8	17.0 ± 0.1	15.5 ± 0.1	18.5 ± 0.1	3.0 ± 0.1

Germinability after burial

After germination ceased, we additionally assessed to which depth germinable seeds were transported (experiment II). Due to logistic constraints (limited space in the greenhouse for placement of seed trays) the number of samples had to be reduced for this analysis. Thus, we included only pots without litter cover, with 8 g litter dm^{-2} (seeds sown beneath) and with 8 g litter dm^{-2} (seeds sown on top of the litter). For each of the three treatments we randomly selected pots with and without earthworms. Since we expected a higher variability in seed burial in the presence of earthworms, we choose five replicates in this case vs. three replicates when earthworms were absent. After removal of the litter, the content of each pot was divided into five consecutive layers. The two top layers were 2.5 cm thick, the three bottom layers were 5 cm thick. These soil layers were thinly spread into Styrofoam trays (28 * 18 cm) and again arranged at random under greenhouse conditions (cf. experiment I) on June 7th. Emerging seedlings were counted twice, on June 28th and July 22nd.

From this experimental setup, we also excluded pots with seeds of *Heracleum sphondylium* since this species completely failed to germinate. However, since it was the species with the largest, most visible seeds, we compared the number of seeds left on top of the soil between control pots (0 g litter dm^{-2}) with and without earthworms to estimate their effect on seed predation and displacement.

Statistical Analysis

A four-way ANCOVA was employed to analyse effects of species, cover amount, seed position, and earthworms on seedling emergence in experiment I. In this and the following analyses, we included the number of earthworms at the end of the experiment in each pot as a predictor variable to account for the escape or mortality of some earthworms. Since in case of the treatment level 0 g dm^{-2} (litter free treatment) there was only one seed position, we excluded this treatment level and analysed the effects of seed position only for the cover amounts of 4 and 8 g dm^{-2}. Additionally, to also include the litter free treatment in the analysis we split the data set into three further meaningful subsets and calculated (i) a two-way ANCOVA to test for the effects of species and earthworms within the 0 g dm^{-2} treatment, (ii) a three-way ANCOVA to analyse for differential effects of species, cover amount and earthworms between the 0 g dm^{-2} and the litter treatments (4 and 8 g dm^{-2}) when seeds were sown beneath a litter cover, and (iii) the same three-way ANCOVA but for seeds sown on top of a litter cover.

For the analysis of germinability after burial (experiment II), we carried out two ANOVAs. In this case, we employed a repeated measure analysis to account for the dependence between depth and pots and present Huynh-Feldt corrected *P*-values (Quinn & Keough 2002). In the first analysis, we focused on the effect of cover amount and thus we compared the treatments no litter and 8 g litter dm^{-2} (seeds sown beneath litter) to test the effects of species, cover amount, earthworms and depth on seedling emergence. In the second analysis, we focused on the seed position effect and therefore we compared 8 g litter dm^{-2} (seeds sown beneath litter) with 8 g litter dm^{-2} (seeds sown on top of litter) to test for the effects of species, seed position, earthworms and depth on seedling emergence. To account for seeds already germinated, we set the seedlings emerging from various depths in relation to the number of non-germinated seeds, calculating the difference between the number of seeds sown in the beginning (100 seeds/pot) and the number of seedlings that emerged before we split the soil layers (May 31st 2010).

Since all dependent variables were proportion data, they were square root arcsine transformed (Quinn & Keough 2002). As a measure for the relative contribution of each factor and the interactions to the total variability we used the ratio of the sum of squares of the factor or interaction of interest to the total sum of squares. All statistical analyses were done using Statistica 8.0 (StatSoft 2008).

Results

Seedling emergence

In all ANCOVAs of seedling emergence, main effects proved to be highly significant but the number of earthworms left at the end of the experiment, which was used as an additional predictor variable, was far from reaching significance (Table 3).

Table 3. Results of a four-way ANCOVA on the effects of species identity (S), seed position (SP), cover amount (CA; 4 and 8 g dm^{-2}), and earthworm presence (E) on percentage emergence of seedlings. The number of earthworms present at the end of the experiment was used as covariable. Abbreviations: df = Degrees of freedom, MQ = mean sum of squares, F = variance ratio, *P* = error probability, vc (%) = relative contribution of individual factors and their interactions to total variation.

Source of variation	**df**	**MQ**	**F**	***P***	**vc (%)**
Intercept	1	3.55	747.61	< 0.0001	
Earthworms finally present	1	0.01	1.60	0.2066	0.11
Species [S]	3	1.05	221.00	< 0.0001	46.74
Seed position [SP]	1	1.02	215.29	< 0.0001	15.18
Cover amount [CA]	1	0.23	47.60	< 0.0001	3.36
Earthworm [E]	1	0.03	7.15	0.0081	0.50
S × SP	3	0.28	59.38	< 0.0001	12.56
S × CA	3	0.04	7.39	0.0001	1.56
SP × CA	1	0.01	2.21	0.1388	0.16
S × E	3	0.03	6.34	0.0004	1.34
SP × E	1	0.11	22.15	< 0.0001	1.56
SA × E	1	0.0002	0.05	0.8233	0.004
S × SP × CA	3	0.004	0.76	0.5190	0.16
S × SP × E	3	0.02	3.90	0.0096	0.83
S × CA × E	3	0.001	0.30	0.8251	0.06
SP × CA × E	1	0.01	1.60	0.2075	0.11
S × SP × CA × E	3	0.001	0.22	0.8831	0.05
Error	223	0.005			15.72

In general, across all other factors a litter cover of 4 and 8 g m^{-2} led to a decreased seedling emergence compared to the litter free treatment (mean ± *SE* of 0, 4 and 8 g m^{-2}: 21.6% ± 1.8, 6.8% ± 0.9, 3.6% ± 0.5, respectively) and seedling emergence was higher when seeds were sown beneath as opposed to on top of a litter cover (8.2% ± 0.9 vs. 2.2% ± 0.3).

In case of the litter free treatments, earthworms significantly reduced seedling emergence by 50% (mean ± *SE*, 28.8% ± 2.6 vs. 14.4% ± 2.1; two-way ANCOVA of 0 g dm^{-2} treatments only, $F_{1,119} = 48.7$, $P < 0.0001$, Figure 1). In contrast, in the litter treatments, reduction of seedling emergence caused by earthworms was much smaller or their presence even resulted in higher seedling emergence (Figure 1). Their impact on seedling emergence also decreased with increase of cover amount and was smaller when seeds were sown on top than when sown beneath a litter cover. As a consequence, across species, earthworms only significantly lowered emergence when seeds were positioned below a 4 g dm^{-2} litter cover (12.8% ± 2.3 vs. 8.4% ± 1.9, $P < 0.05$, Tukey HSD test post hoc a significant earthworm × cover amount interaction, three-way ANCOVA (ii above), results not shown) but did not significantly reduce seedling emergence when seeds were on top of a litter cover.

The four-way ANCOVA of seedling emergence in the litter treatments revealed the main effects to be highly significant, still only species identity (S) and seed position (SP) explained a considerable amount of the total variation in the data set, i. e. 46.7% and 15.2%, respectively. In contrast, cover amount (CA) and earthworms (E) explained only 3.4% and 0.5%, respectively, of the total variation. All three factors, SP, CA and E, differed significantly between species (S).

Interestingly, the interaction between earthworms and seed position depended on species identity (significant three-way interaction S × SP × E). Consequently, when analysed for each species separately, we found a highly significant seed position × earthworm interaction only in case of the three larger seeded species, *A. sylvestris*, *P. sativa* and *P. officinale*. In these species, the presence of earthworms even enhanced seedling emergence after sowing on top of the litter cover whereas emergence of seedlings grown from seeds sown below the litter cover was reduced (Figure 1). In *P. saxifraga*, the species with the smallest seeds, not the seed position × earthworm interaction was significant but only the earthworm main effect, which also explained a relatively high proportion of the total variation of 19.3% (Figure 1). Moreover, there was a shift in the proportion of explained variance from small to large seeded species. While in case of the small seeded species *P. saxifraga*, cover amount and earthworms draw most of the total variation, the large seeded species *P. sativa* and *P. officinale* seem much more sensitive to seed sowing position than to cover amount (see Figure 1).

Germinability after burial

In pots with and without earthworms most (61.5 % ± 4.4 and 94.1 % ± 3.1, respectively) of the germinable seeds were found in the upper 2.5 cm of the soil (Figure 2). Except for *A. sylvestris*, across treatments there were higher percentages of germinable seeds in this upper layer in pots without earthworms than in pots with worms. For the deeper soil depths (> 2.5 cm) seed germination, with very few exceptions, occurred only in pots that had contained earthworms. Accordingly, the percentage of seeds of *H. sphondylium* that were displaced from the soil surface in the litter free pots at the end of the experiment was significantly higher in pots with earthworms added (84.7% ± 4.9 vs. 9.1% ± 2.1, $t_{30} = 13.8$, $P < 0.0001$, $N = 32$; t-test).

In the comparison between controls and pots with 8 g litter dm^{-2} (seeds sown beneath litter; Figure 2, white vs. grey bars), there was a significant main effect of earthworms ($F_{1,47} = 4.5$, $P < 0.05$), however, the effect of earthworms varied significantly with soil depth and cover amount ($F_{4,188} = 2.6$, $P_{H\text{-}F} < 0.05$).

In contrast, the comparison of seed position (below vs. on top) in pots with 8 g litter dm^{-2} revealed no significant main effect of earthworms on percentage emergence ($F_{1,48}=0.007$, $P=0.93$). But we found a highly significant interaction between earthworms and seed sowing position ($F_{1,48} = 12.4$, $P < 0.001$), which significantly interacted with soil depth ($F_{4,192} = 2.5$, $P_{H\text{-}F} < 0.05$). Thus, when seeds were originally sown on top of litter, there was very little germination in pots that had been without earthworms (0.65% ± 0.43, across depths and species, $N = 20$), whereas germination was significantly higher in pots with earthworms (1.62% ± 0.49). When seeds were originally sown beneath litter, there were no significant differences between pots with (1.52% ± 0.57) and without worms (2.99% ± 1.69). In addition, higher percentage germination was detected from depth > 2.5 cm in pots with earthworms than in pots without.

Interestingly, we found no indication for species specific differences in seed mobility, as the analyses revealed no significant two-way interaction between species and depth both in the case of comparison between controls and pots with 8 g litter dm^{-2} (seeds sown beneath litter) ($F_{12,188} = 0.56$, $p_{H\text{-}F} = 0.87$) and comparison of seed position (below vs. on top) ($F_{12,192} = 1.2$, $p_{H\text{-}F} = 0.30$).

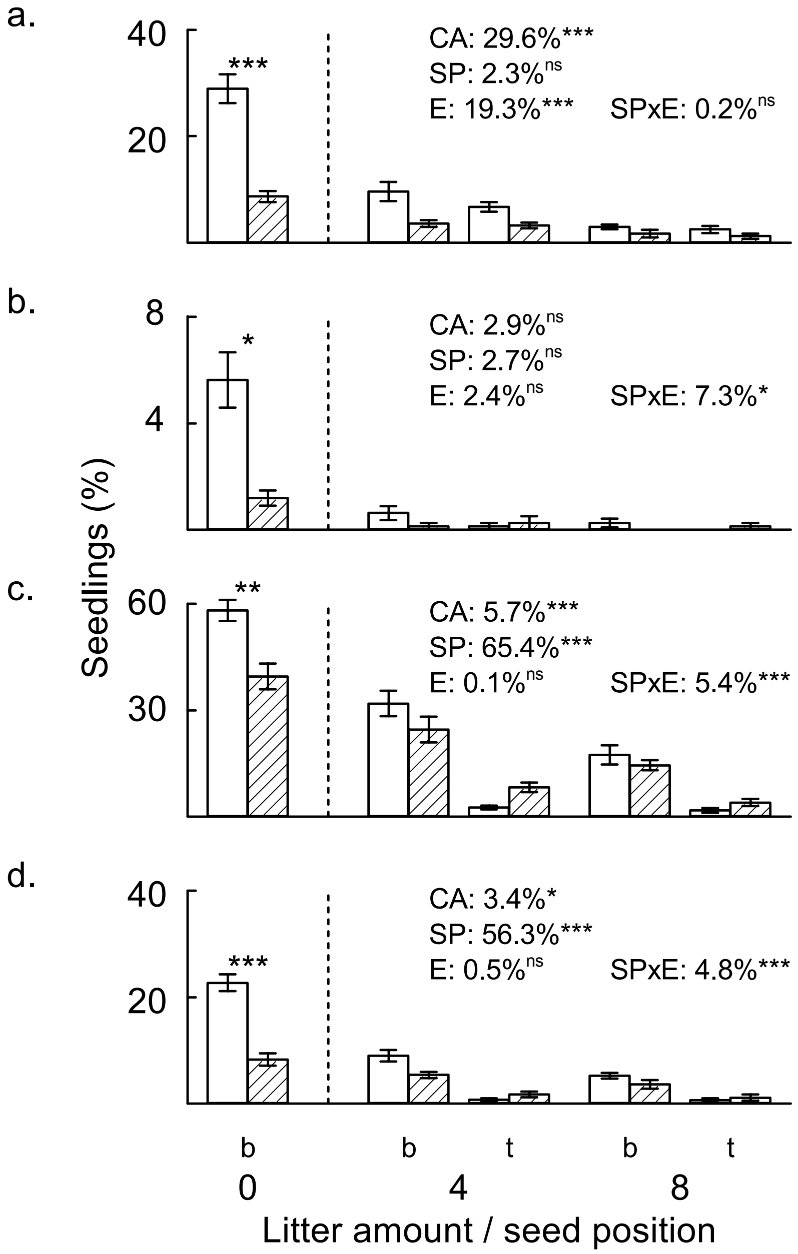

Figure 1. Percentage of seedlings of *Pimpinella saxifraga* (a.), *Angelica sylvestris* (b.), *Pastinaca sativa* (c.) and *Peucedanum officinale* (d.) emerging at different litter amounts (0 [controls], 4 and 8 g dm^{-2}) and seed sowing positions (b, beneath litter layer; t, on top of litter) in pots without (white bars) and with (hatched bars) worms added. Data are means ± S.E. (*N* = 16 for controls and *N* = 8 for all other treatments). For controls, asterisks indicate significant differences between means according to a one-way ANCOVA (see Material and Methods). Inset numbers denote the percentage of explained variance and asterisks indicate significance of factors according to a three-way ANCOVA testing for the effects of litter amount (CA), sowing position (SP) and earthworms (E). Only effects and interactions are given that were significant in at least one of the study species. ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, not significant ($P > 0.05$).

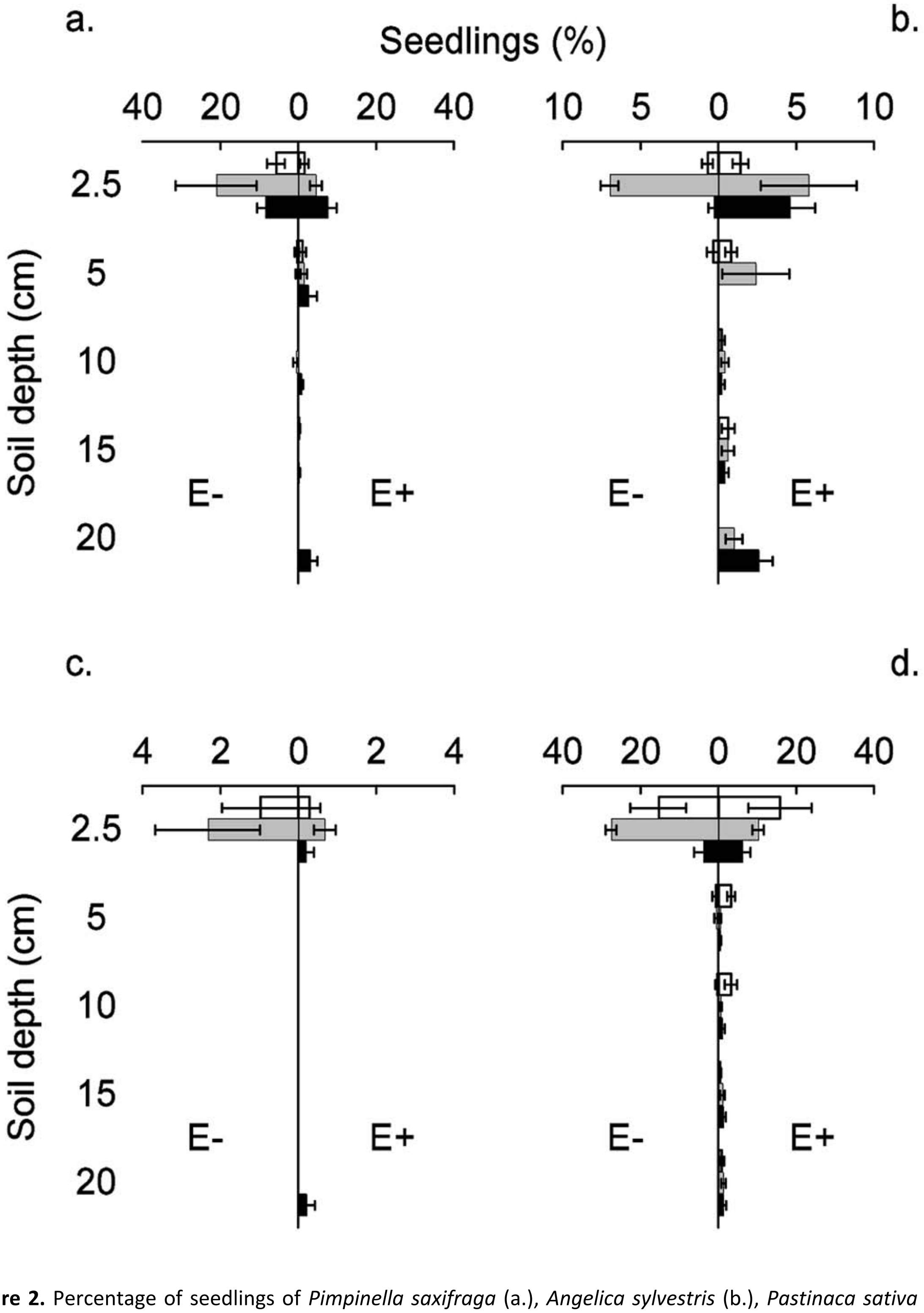

Figure 2. Percentage of seedlings of *Pimpinella saxifraga* (a.), *Angelica sylvestris* (b.), *Pastinaca sativa* (c.) and *Peucedanum officinale* (d.) emerging at the end of the experiment II in different soil layers (lower limit: 2.5, 5, 10, 15 and 20 cm) in pots without (E-, bars to the left) and with (E+, bars to the right) worms added. Data are means ± S.E. (*N* = 3 for E- and *N* = 5 for E+). Treatments shown are controls (white bars), 8 g dm^{-2} of litter with seeds sown beneath (gray bars) and 8 g dm^{-2} of litter with seeds sown on top (black bars).

Discussion

Seedling emergence

Our data suggests a context-dependent effect of earthworms on seedling emergence. In the litter-free treatment, seedling emergence was significantly higher in pots without earthworms than when earthworms were present. This was because earthworms either consumed seeds or translocated them to greater depths from which they did not emerge. In the litter treatments, seedling emergence was generally lower than that without litter. However, in the presence of a litter cover also differences in seedling emergence between pots with and without earthworms were always lower compared to the litter-free treatment. This general pattern is in line with the observations that in the field both litter cover and seed predation lower seedling emergence (Facelli & Pickett 1991, Xiong & Nilsson 1999). The negative effect of a litter cover on seedling emergence in the present study highlights the importance of marginal conditions on the size and sign of litter effects (Donath & Eckstein 2008). While under dry conditions moderate litter amounts facilitate seedling emergence (Xiong & Nilsson 1999, Eckstein & Donath 2005), under more benign moisture conditions, like in the present greenhouse experiment with regular watering and high air humidity, the negative effects outweigh the positive effects of litter (Xiong & Nilsson 1999). Accordingly, also the general negative effect of seed burial and predation through earthworms was evident only in case of small seeded species but was significantly modified by seed position in the three larger seeded species (Figure 1). In the latter cases, we found a significant interactive effect of earthworm activity and seed position (Table 3). Contrary to the reduction of seedling emergence from below litter in the presence of earthworms we found equal or higher seedling emergence in the presence of earthworms when species were sown on top of the litter cover (Figure 1).

When accessibility of seeds is similar, there seems to be little effect of seed size on seed loss to predation (Fröborg 2001, Mittelbach & Gross 1984, van Tooren 1988). Differences in accessibility, on the other hand, may be influenced by relative size of seeds, litter and predators, respectively. Reader (1993) found that the effect size of the *litter* × *seed predation* interaction depended on seed size. While small seeded species did only benefit from removal of soil cover, the importance of protection against seed predation increased in larger seeded species, resulting in equal seedling numbers for large and small seeded species as a consequence of either seed predation or suppression through litter. This may be more pronounced when seed size is considerably larger than particle size of the matrix, i. e. litter and soil, and thus large seeds are more easily detected than small seeds. Apart from seed size, seed selection behavior of earthworms is found to be determined by taste, smell and surface texture of a seed (Regnier et al. 2008, Willems & Huijsmans 1994). Also the absolute and relative sizes of the predators seem to influence the impact of predation (Eisenhauer et al. 2009, Saska 2008). Also the number of earthworms present will affect their impact on seed and seedling fate. In the present study we used a density of about 150 earthworms per m². Although, this number is at the upper limit of densities found regularly in the field (Evers et al. 2010, Fründ et al. 2010), our results show that the mechanisms acting influence seed and seedling fate. However, field experiments would be needed to further elucidate their relevance under natural conditions. While litter tends to protect against rodents and large invertebrates (Helliwell 1965, Sydes & Grime 1981), litter may even enhance seed mortality by increasing numbers of arthropods (Facelli 1994, Saska 2008) or fungal or bacterial pathogens (Blaney & Kotanen 2001, Dalling et al. 2010).

In the current experimental setup, differences in seedling emergence between the earthworm treatments tended to decrease with increasing litter amount, which is in line with the pattern that seeds are better protected with increasing cover amount (Cintra 1997). However, increased seed predation at low amount of litter cover may also be due to the lower availability of litter as an alternative food source. This pattern is further affected by the relative position of seeds which showed a strong effect on seedling emergence (cf. Table 3). At the species level, the importance of the position effect (measured as percentage of explained variance) increased with seed size (Figure 1). Conversely, cover amount loses influence with increasing seed size. The present experiment confirms a pattern found in a former study of Donath & Eckstein (2010). The smaller the seeds, the more easily seeds from the top of the litter percolate deeper into the litter resulting in similar starting conditions for seeds from on top and beneath the litter cover when germination starts. In contrast, large seeded species are not so easily moved into the litter cover. When these seeds are trapped on top or within a layer of litter they cannot establish contact with the soil. Seeds that remain dry and therefore do not germinate may eventually enter dormancy. However, if seeds imbibe moisture, seedlings have to establish soil contact to avoid lethal desiccation when the litter layer dries out. Thus, while initial seed position loses importance in small seeded species, importance of seed position prevails in large seeded species. The latter are less mobile in a litter layer and either do not germinate due to moisture levels beneath a certain threshold or because seedlings are not capable to establish vital soil contact. These effects are attenuated by earthworm activity, which moves part of the seeds deeper into the litter or close to the soil surface where moisture conditions are more benign and soil contact more easily reached (Figure 1).

Post burial fate

It is generally acknowledged that small seeds are more likely to be moved into deeper soil layers (Bekker et al. 1998, Schmiede et al. 2009). However, in accordance with other studies on seed transport by earthworms (Eisenhauer et al. 2009, Regnier et al. 2008), the present study revealed no clear size effect on seed translocation. This may be because in our experiment no extremely small or large seeds were included. Thus, even the largest seeds of *Heracleum spondylium* were almost completely buried by earthworms. Our results highlight the potential of earthworms to speed up seed transport into deeper soil layers. This may have considerable influences on plant community assembly. While seeds of all sizes seem to partly germinate after burial, the proportion of seeds that finally emerge increases with seed size and decreases with burial depth (Burmeier et al. 2010, Maun & Lapierre 1986, Zhang & Maun 1990). The evolution of dark dormancy in small seeded species (Milberg et al. 2000) reduces fatal germination and leaves them viable after burial (Burmeier et al. 2010). Thus, rapid seed burial by earthworms very likely contributes to the buildup of a seed bank in small seeded species and, in contrast to large seeded species, they are not lost from the regeneration pool but may contribute to the above-ground plant community in the future. This process is again partly influenced by earthworm activity since they may also move seeds upwards onto the soil surface where they can germinate (Milcu et al. 2006, Willems & Huijsmans 1994). Furthermore, earthworms seem to reduce the likelihood of emergence after germination through selectively feeding on seedlings (Eisenhauer et al. 2010, Shumway & Koide 1994). If belowground larger seedlings are more likely to be eaten by earthworms than seedlings of small seeded species, predation by earthworms may be an important driver in plant community assembly above and below ground.

Our results indicate that the impact of litter layers on seedling emergence cannot be determined solely by the assessment of their chemical, physical and mechanical effects. In addition, plant-animal interactions will further determine the sign and strength of the impact of these after-death plant-plant interactions and ultimately their potential influence on species assembly in plant communities.

Acknowledgements

We thank Christiane Lenz-Kuhl, Josef Scholz-vom Hofe and Eszter Ruprecht for their support during the experiment and in the laboratory. Annette Otte, Eszter Ruprecht, Susanne Wurst and three anonymous referees offered helpful comments that improved the manuscript. Gregory Mahone polished our English. All help is gratefully acknowledged.

7 Panel discussion

The net and gross effects of biotic and abiotic interactions of plants shape plant communities. In case of after-death interactions through litter, a meta-analysis revealed that litter effects on seedling emergence were predominately negative (Xiong & Nilsson 1999), with some variability due to e. g. study method, experimental duration, litter quantity and quality. Similarly, direction and size of the effect of a ground cover by mosses seems to vary in different contexts (During 1990, Eckstein et al. 2011, Soudzilovskaia et al. 2011). The studies presented in this compilation show that positive effects of a litter cover are closely linked to the marginal conditions and the respective amount of cover prevailing. In general, the studies emphasize that ameliorative effects of litter on the physical, mechanical and chemical environment for seedling emergence diminish with increasing litter covers, while negative effects seem to prevail. In addition, under more benign environmental conditions, negative cover effects override positive cover effects at much lower cover quantity.

One important physical effect of ground cover results from its influence on water availability (chapter 2). This study revealed that the facilitative effect of litter occurs at low water-supply rates, where a litter cover significantly increases successful emergence. Under more constant moisture regimes, in contrast, germination rarely increases in the presence of a litter cover (chapter 6; Xiong et al. 1999). This was also shown for deciduous forests by Albrecht & McCarthy (2009), where under the more benign conditions of north-eastern oriented slopes litter removal increased seedling recruitment while positive litter effects were found when water availability was critical at southward oriented slopes or due temporal water shortages.

In addition, ground cover affects also diurnal temperature fluctuations (Sydes & Grime 1981b, Facelli & Pickett 1991a, Jensen & Gutekunst 2003, Weltzin et al. 2005) which in turn influence seed germination (e. g. Thompson & Grime 1983). In line with other studies, the studies presented in this compilation show that increasing cover amount decreased the temperature amplitude but not the mean temperature below the cover (chapters 2, 4, 5, 6; Jensen & Gutekunst 2003). Large diurnal temperature fluctuations serve as a cue for the detection of gaps, inducing seed germination in light demanding species and small or ephemeral plants with low competitive ability. In these cases, differences in the composition of the ground cover lead to specific response patterns through altered temperature dynamics (Thompson & Grime 1983). The study on effects of oak and grass litter revealed that the former exerted weaker temperature effects than grass litter if amounts with similar effects on the degree of initial light interception are compared (chapter 4). However, identical amounts of different litter types did not differ in their effects on average temperature and soil humidity. In case of a moss cover, findings by Soudzilovskaia et al. (2011) show species-specific changes of the temperature amplitude beneath a cover by different moss species and that this pattern is closely linked to species-specific thickness of moss cushions. Their study corroborates the effects of the bryophyte cover on the temperature amplitude in the study presented in chapter 5. There, the temperature amplitudes beneath a moss cover decreased with increasing cover amount. Interestingly, the temperature amplitude beneath the moss cover was also lower than under the grass litter cover, probably since grass litter has a higher mass density than the moss and thus probably a less insulating effect.

The quantities and qualities of light that reach a seed increase with the density of the litter cover (chapter 5; Keizer et al. 1985, Jensen & Meyer 2001,). The evolution of dark dormancy in small-seeded species (Milberg et al. 2000) results in a close relation between seed size and emergence in both grassland (Jensen & Gutekunst 2003) and woodland plants (Kostel-Hughes et al. 2005). Accordingly, seedling emergence of most small-seeded species decreases with increasing litter cover (chapter 2, Hölzel 2005). When comparing different soil covers (e. g. litter and moss cover; chapter 5) differences in the decomposition pattern of moss and litter led to cover-specific temporal variations in illumination. In contrast to dead litter, mosses compensate for decomposition by re-growth. Consequently, while light interception through mosses changes only marginally, PAR (photosynthetically active radiation) that penetrates a litter cover increases over time (chapter 5).

Mechanical litter effects were revealed when comparing litter cover of different structure (grass vs. leaf litter; chapter 4). This study showed that seedling suppression in woodland species through grass litter was significantly higher than through litter of their original habitat; whereas grassland species emerged equally well from beneath both litter types. These differences may be partly due to different mechanical litter effects that result from specific structural differences of litter types. While grass litter consists of senesced threadlike leaves and stems of graminoids, which can create tightly interwoven mats, oak leaf litter consists of singular leaf units that are moved more easily by seedlings. Since emergence of woodland species was higher from below oak than from below grass litter, woodland species seem to have developed adaptions to the cover quality prevailing in their ecosystem. As a result, differences in the shoot thrust seedlings are able to exert during seedling emergence may have evolved (Campell et al. 1992). Consequently, while probably all germinated seedlings of grassland species made their way through grass litter, a large proportion of seedlings of typical woodland plants did not manage to successfully penetrate a cover of grass litter (chapter 4).

Compared to physically and mechanically induced effects on seedling emergence chemical effects seem of lower importance. Still, in case of the dominant species (*Stipa pulcherrima*) in abandoned Transylvanian dry grasslands these were experimentally confirmed (chapter 3). In this study leaf leachate of *Stipa pulcherrima*, exerted, apart from species-specific responses, a negative effect on different processes of seed germination and seedling emergence of co-occurring grassland species (chapter 3). This finding corroborates other studies, which demonstrated that leachate from living plant tissues or dead plant remains suppresses seed germination of co-occurring species (e. g. Schlatterer & Tisdale 1969, Chang-Hung & Chiu-Chung 1975, Werner 1975, Bosy & Reader 1995, Li et al. 2011). These chemical effects are much harder to detect when they interact with mechanical and physical effects (chapter 4). Similarly, Soudzilovskaia et al. (2011) found that, under field conditions, the effects of phenolic substances leached from moss cushions on seedling emergence seemed to be mostly overridden by physical and mechanical effects. Still, under laboratory conditions their study revealed a strong decrease in seedling emergence with increasing amount of phenolic leachate from moss cushions. One possible explanation for this pattern might be a much faster degradation of phenolic compounds under common garden and field conditions than under laboratory conditions. Accordingly the study presented in chapter 4, which compared effects of grass and oak litter, suggests that chemical effects are indeed of lower importance. Despite high concentrations of phenolic compounds leaching from oak foliage (Kuiters & Sarink 1986) neither in grassland nor in woodland species a dose-response relationship between phenolic leachate and seedling emergence was revealed. Nevertheless, under field conditions even small litter induced variations in seedling emergence might be decisive for seedling recruitment. This is implied by the fact that the timing of germination is a species-specific characteristic determining the success of species under varying habitat conditions (Baskin & Baskin 1988 & 2001, Olff et al. 1994, Hölzel & Otte 2004). The germination peak is often highly synchronized with soil moisture dynamics (Ruprecht et al. 2010a). In this context, the experimental results imply that a delay of germination through secondary compounds by about 1.5 weeks (chapter 3) will lead to increased seedling mortality under natural conditions (Li et al. 2011). This will be especially true when water supply is restricted, due to southward oriented slopes and/or shallow soils, and leaves only a relatively narrow time window for successful germination and emergence (Ruprecht et al. 2010).

Depending on a seed's relative position, the effect size of chemical, physical and mechanical processes varies. This effect is highlighted in chapter 5, where seed position (beneath vs. on top of the cover) in a litter or moss cover explained a high amount of the variation in seedling emergence (chapter 4). Facelli & Pickett (1991a, and references therein) already surmised that in the presence of a litter cover seedling emergence might be influenced by a seed's relative position. A layer of grass litter, leaves or bryophytes impedes seeds trapped on top or within to have contact with the soil. Those seeds that remain dry and therefore do not germinate may eventually enter dormancy (Baskin & Baskin 2001). In this case, only long-term persistent seeds maintain a chance for successful establishment when microsite demands are met in the future. In contrast, short-term persistent seeds are in danger of burial by too large amounts of litter or overgrowth by mosses during the short period they are germinable. Imbibed seeds on top, in contrast, will have to establish soil contact to avoid lethal desiccation when the soil cover dries out, whereas seeds within or below the cover have to penetrate the layer to reach sufficient light. Under these conditions, the radicle of larger seedlings (i. e. species with larger seeds) may have a higher capability to establish this vital soil contact than the radicle of small-seeded species. Opposite to these expectations the results presented in chapter 5 suggest that in large-seeded floodplain grassland species (>0.4 mg) seed position explained a higher proportion of the variance in the data than cover amount, whereas in small-seeded species (seed mass <0.4 mg) cover amount was more important than seed position (chapter 4). While emergence of small-seeded species showed a continuous decrease with increasing cover amount irrespective of sowing position, a higher portion of seedlings of large seeded species tended to emerge from below the soil cover irrespective of cover amount. This pattern was further accentuated by structural differences between the cover layers (grass vs. moss). Seedling emergence from large seeds that are shed on top of a bryophyte layer or litter–moss mixture was lower than when seeds were positioned beneath the cover suggesting that large seeds are more easily trapped above the soil surface. Borchet et al. (1986) found a similar pattern in case of *Quercus douglasii* where seedling establishment failed when seeds where situated on top of thick layers of dead grass. Analogously, Schramm & Ehrenfeld (2010) revealed that early establishment and seedling survival of *Microstegium vimineum* (seed mass > 1 mg; Flory et al. 2011), an invasive non-native grass species in mixed hardwood forests in Northern America, is strongly reduced when seeds are shed on top of an intact layer of forest tree litter compared when placed beneath or in areas where the litter cover is disturbed. In contrast, small seeds are more mobile and therefore more easily reach the soil surface (Chambers et al. 1991) and thus differences in emergence between seeds initially sown on top or beneath a soil cover are small (chapter 5).

As anticipated, seed size seems to be one key factor determining seedling emergence in the presence of litter. This is in accordance with findings in an experimental screening by Jensen & Gutekunst (2003). This study revealed that seedling emergence of 35 grassland plants from beneath *Carex* litter increased with seed size. Similarly, several authors observed a similar trend in forest species, where seedling emergence through accumulated litter is also influenced by seed size (Seiwa & Kikuzawa 1996, Kostel-Hughes et al. 2005). Beneath a litter cover, large-seeded species experience advantages over small-seeded species since they are able devote higher resources to penetrate a layer of litter, bryophytes or soil and thus can establish under more unfavourable, i. e. shady or competitive, conditions than small seeds' seedlings (Leishman et al. 2000). There has probably been strong selection during the evolution of seed size against dark germination in small seeded species (Hodkinson et al. 1998, Milberg et al. 2000, Baskin & Baskin 2001), since smaller seeds are more likely to move to deeper layers of a ground cover (chapter 5) or of the soil than large seeds (Milberg et al. 2000, Baskin & Baskin 2001) and subsequent germination will lead to high mortality rates (Burmeier et al. 2010).

The effect size and direction of the processes and mechanisms discussed above also varies between ecosystems (Xiong & Nilsson 1999). In contrast to their meta-analysis, the study presented in chapter 4 revealed positive ecosystem-specific effects of tree litter on seedling emergence. Woodland species reacted especially positive towards cover by oak litter but not by grass litter. An experimental study by Baker & Murray (2010) corroborates the existence of positive effects of tree litter on early growth of a co-occurring plant species. They found that seedlings of *Pinus radiata*, a non-native but highly invasive species in natural eucalypt woodlands in Australia, build up higher biomass at low levels of tree litter in comparison to no cover. Ecosystem-specific reactions of forest species on grass litter like those presented in chapter 4 may explain why the succession of abandoned grasslands or old fields to woodland is often a rather slow process (Ellenberg 1986, Facelli & Pickett 1991b, Facelli 1994, Myster 1994, Flory & Clay 2010). However, succession may speed up after the establishment of the first woody species that enhance establishment of typical woodland forbs through specific litter effects (Facelli 1994). This suggests that apart from changes in microclimatic conditions herbaceous woodland species also rely on the occurrence of woody plants because their seedlings are less likely to emerge from beneath grass litter during succession (chapter 4).

Effects patterns of seed size, litter type and quantity on seedling emergence are further altered by interactions across trophic levels. Chapter 6 presents an experimental study on the influence of earthworms on seedling emergence in the presence or absence of a litter layer. The results show that in the litter-free treatment seedling emergence was significantly higher in earthworm-free pots than when earthworms were present. With a litter cover, differences in seedling emergence between pots with and without earthworms were mostly lower compared to the litter-free treatment. This general pattern is in line with two common observations, i. e. that both litter cover and seed predation lower seedling emergence (Facelli & Pickett 1991, Xiong & Nilsson 1999). Accordingly, Reader (1993) found that the effect size of the *litter* × *seed predation* interaction depended on seed size. While small seeded species only benefitted from removal of ground cover, the importance of protection against seed predation increased in larger seeded species. When the accessibility of seeds is similar, there seems to be little effect of seed size on seed loss to predation (Mittelbach & Gross 1984, van Tooren 1988, Fröborg 2001). Differences in accessibility, on the other hand, may be influenced by relative size of seeds, litter and predators, respectively. Detection of seeds probably increases with increased ratio of seed size and particle size of litter or soil. Presence of earthworms also interacts with cover amount. In general, differences in seedling emergence between the earthworm treatments decreased with an increase of litter amount (chapter 6), which suggests the occurrence of higher protective effects at increased cover amounts (Cintra 1997). Additionally, earthworms affect seedling emergence by merely increasing the dynamics in a system. The importance of this effect is highlighted in studies about the impact of non-native invasive earthworms on northern deciduous forests in North America. Part of their influence is caused through an acceleration of nutrient cycling (Bohlen et al. 2004). Earthworms speed up mineralisation of nutrients from forest floor material, e. g. leave litter, by consuming it and mixing it with the mineral soil. This loss of the litter cover in concert with other effects is responsible for severe changes – even the loss of species - in the plant community composition below-ground and above-ground (Bohlen et al. 2004, Eisenhauer et al. 2009). Another mechanism might be that earthworms alter seed mobility. In general, small seeds show a higher mobility than large seeds (Bekker et al. 1998). When these seeds are trapped on top or within a layer of litter they cannot establish soil contact and are lost to fatal germination (chapter 5). Consequently, large seeds would profit the most if their mobility was enhanced and seeds were actively moved into the litter cover where moisture conditions are more benign and soil contact more easily reached. In line with this prediction, earthworms enhanced seedling emergence in large seeded species when seeds were sown on top (chapter 6). Thus, apart from negative effects of earthworms on seedling emergence through seed and seedling digestion they are capable to attenuate negative effects of litter covers. This will be the case when seeds are merely co-carried with litter into the soil or seeds are ingested but not digested and subsequently - still germinable - egested (Eisenhauer et al. 2009).

Relevance for nature conservation and restoration

Chemically, physically and mechanically induced litter-related processes on seedling emergence and establishment are also of practical relevance. This is acknowledged in several studies on the restoration of species-rich semi-natural grassland communities either through the reintroduction of regular management in abandoned grasslands (Lepš 1999, Ruprecht et al. 2010a) or via the transfer of seed-containing plant material (Donath et al. 2007, Klimkowska et al. 2007, Kiehl et al. 2010, Török et al. 2011).

Semi-natural grasslands evolved under continuous (from today's perspective) non-intensive anthropogenic influence (Ellenberg 1996). Consequently, in cases where the intensity of human activities alters, changes in the plant community composition can be observed, both under intensification and abandonment of management (Korneck et al. 1998, Enyedi et al. 2008). In case of abandonment, the accumulation of a cover by dead plant litter is one of the driving forces of species loss (Jensen 1998, Korneck et al. 1998, Lepš 1999). The thickness of a litter cover that accumulates in grassland depends on the duration of abandonment, the productivity of the site and the prevailing disturbance regimes of the grassland system. The negative effects that go along with an accumulation of litter during abandonment (chapter 2) reduce the availability of safe sites for the regeneration of most plant species (Gross & Werner 1982, Bergelson 1990). This microsite limitation is often mentioned as the prime cause of recruitment failure in grassland species (Špačková et al. 1998, Bissels et al. 2006). The re-introduction of the management might be one possibility to stop and reverse this process (Kotorová & Lepš 1999) but several studies showed that in these cases merely removing the litter cover and re-introducing regular management leads to the re-establishment of only a limited number of species (Pfadenhauer & Maas 1987, Bossuyt & Honnay 2008, Valkó et al. 2010). In a review, Bossuyt and Honnay (2008) assessed that across different grasslands rare and habitat-specific species are very unlikely to re-emerge solely by the re-introduction of regular grassland management. This limitation is even more pronounced after periods of intensified grassland or arable management (Bakker et al. 1996, Donath et al. 2003, Bossuyt & Honnay 2008). Still, Ruprecht et al. (2010a) found that the experimental removal of litter in combination with cutting and removal of the above ground biomass not only enhanced seedling emergence and survival in steppe-like grasslands in Romania but did also lead to the re-emergence of formerly lost species.

When species are not present in the above-ground vegetation or in the seed-bank, they might re-colonise from the surroundings (Donath et al. 2003, Kirmer et al. 2008). In those cases, where dispersal limitation does not impede re-colonisation the degree of invasibility will be crucial. The invasibility of plant communities is closely linked to the availability of suitable microsites which in turn is determined by the biotic and abiotic interactions. In restoration projects, suitable microsites for species re-establishment from remnant stands nearby restoration site are limited in time. While in the early years of grassland succession short-lived species with high turn-over rates often dominate, perennial species prevail already after 2-3 years (Donath et al. 2007, Kiehl et al. 2010). Thus, in grassland succession invasibility will be higher in the beginning and decreases with the closing of the sward. This, in conjunction with a low seed pressure built up by target species will inevitably lead to low re-colonisation rates.

In cases where typical grassland species are not present in the soil seed bank or in the above ground vegetation of the restoration sites, active measures of species re-introduction are needed (Kiehl et al. 2010). In these cases, the transfer of seed-containing plant material proved to be very effective and was increasingly applied (Klimkowska et al. 2007, Kiehl et al. 2010, Török et al. 2011). The method was applied in a wide variety of grassland types such as mesic grasslands (Jones et al. 1995, Molder, 1995), wet and fen meadows (Patzelt et al. 2001, Poschlod and Biewer 2005, Rasran et al. 2007, Klimkowska et al. 2010), calcareous grasslands (Pfadenhauer et al. 2000, Kiehl et al. 2006, Edwards et al. 2007), sandy grasslands (Kirmer and Mahn 2001, Stroh et al. 2002, Eichberg et al. 2010) and flood meadows (Hölzel and Otte 2003, Donath et al. 2007). In these and similar studies, the amount of plant material applied varies considerably. According to the experimental results of the studies included in the present compilation, it is regularly assumed that small plant material cover enhances seedling emergence and establishment under harsh site conditions, i. e. dry sites or bare soils, and that with increasing amounts of plant material applied especially small seeded, light demanding species are hampered (Kiehl et al. 2010). Consequently, the optimal amount of applied plant material of low productive calcareous grasslands was between 300-600 g m^{-2} while the application of higher amounts led to a decrease in establishment success (Kiehl et al. 2006). In contrast, in highly productive flood meadows the application of 750-1500 g m^{-2} plant material seemed not to hamper the re-establishment of species-rich plant communities (Donath et al. 2007, Kiehl et al. 2010). This seems to contradict the general findings presented in chapter 2 where large seeded flood meadow species showed higher seedling emergence with increasing litter cover, whereas small-seeded species with higher light demands for germination already reached their optimum at 200 – 400 g m^{-2} plant material cover. On the one hand, plant species of these relative productive plant communities might be adapted to the presence of higher living or dead biomass, on the other hand the decay of the litter cover under field conditions is relatively high, i. e. not only does the plant material settle and decompose rather fast, but the disturbance by wild boar, moles or disturbance even at a smaller scale through earthworms (chapter 6) also creates suitable microsites where small seeded, light demanding species find optimal conditions for establishment (Burmeier et al. 2011, Schmiede et al. in press). Thus, in line with the experimental results, the heterogeneity of plant material applied in restoration projects provides a wide range of microsites that match a wide range of species-specific demands during early establishment under various marginal conditions.

8 Summary

The articles in this work describe and discuss the results of a series of microcosm experiments that tried to untangle the mechanical, physical and chemical impacts of different soil covers, i. e. litter and moss, on seedling emergence under different environmental conditions.

The results presented in chapter 2 reveal positive effects of dead plant remains (i. e. litter) on seedling emergence and establishment. These positive interactions between living and dead plants do not only act in xeric or otherwise extreme ecosystems but also under mesic site conditions. Still, also in the latter the direction and size of litter effects on seedling emergence is regulated by soil humidity. In addition, the threshold value of litter amount, above which litter effects turn negative, is species- and family-specific and seems to be related to seed size.

Chapter 3 shows that in dry grassland secondary chemical compounds of the dominant grass species *Stipa pulcherrima* affect seedling emergence of co-occurring species at various stages. Litter leachate delayed seed germination and also reduced radicle elongation. In dry grassland, with a rather narrow temporal gap for successful seedling emergence, the delay of germination and reduction of radicle elongation will jeopardize species regeneration. These negative chemical effects in concert with biotic and abiotic interactions may enhance and maintain species loss.

Litter shows also ecosystem-specific effects (chapter 4). The experimental results presented here suggest that species from woodland habitats are more negatively affected by litter than species from grassland. Across species, woodland plants showed significantly lower seedling emergence from beneath grass litter than from beneath the litter of their original habitat. In contrast, grassland species emerged equally well from beneath both litter types. Thus, the response of species towards the presence of litter is ecosystem-specific. These results highlight the potential influence of ground cover on species composition of grassland and woodland habitats through a complex interplay between ecosystem specific litter types, amounts of litter, environmental conditions and species adaptation.

Chapter 5 confirms the dependence of ground cover effects (grass litter vs. moss) on species-specific traits such as seed size. In this experiment the effect of relative seed position (on top vs. beneath) was explored, too. It seems that large-seeded species have a selective advantage at moderate amounts of grass litter cover, whereas some small-seeded species may adapt to these litter amounts through delayed germination. If litter amounts increase, diversity decreases because there will be selection against small-seeded species, commonly not able to persist under such conditions. The results also suggest that - irrespective of cover type - large-seeded species have an advantage over small-seeded ones when seeds are situated beneath a cover. In contrast, when seeds are shed on top of a cover, the advantage of large seed size remains only on grass litter but not on mosses. Thus, bryophytes may decrease plant diversity irrespectively of the plant's seed size.

The influence of soil invertebrates, e. g. earthworms, on after-death plant-plant interactions is highlighted in chapter 6. These results indicate that the impact of litter layers on seedling emergence cannot be determined solely by the assessment of their chemical, physical and mechanical effects. This study shows that natural disturbance, e. g. through animals, have the potential to even change the direction of the seed position effect on seedling emergence. While in general, earthworms reduce seedling emergence, their presence did increase successful seedling establishment of large seeded species sown on top of the litter up to three times compared to pots without earthworms.

The studies included in this compilation show that a soil cover has chemical, mechanical and physical effects on the invasibility of plant communities and their species composition. The effect size and direction depends on the prevailing abiotic and biotic conditions. These general litter-related processes that influence seedling emergence are also of practical relevance in restoration ecology. Concerning the restoration of species-rich semi-natural grassland through reintroduction of regular management in abandoned grasslands or transfer of seed-containing plant material, the type and quantity of litter has decisive effects on the success of restoration efforts.

9 Zusammenfassung

Diese Habilitationsschrift umfasst mehrere Artikel über die Ergebnisse von Mikrokosmos-Experimenten zur Keimlingsetablierung. Ziel der Experimente war es, die physikalischen, mechanischen und chemischen Einflüsse unterschiedlicher Bodenauflagen wie Laub- und Grasstreu sowie Moos auf die Keimlingsetablierung zu untersuchen.

Die in Kapitel 2 beschriebenen Untersuchungen zeigen, dass die Bedeckung von Samen mit Grasstreu positive Effekte auf die Keimlingsetablierung hat. Diese sind nicht nur unter Extrembedingungen wie Trockenheit festzustellen, sondern auch unter mesischen Standortbedingungen nachzuweisen. Unabhängig von den allgemeinen Standortbedingungen wird die Effektgröße und -richtung durch die vorherrschende Wasserverfügbarkeit im Boden bestimmt. Darüber hinaus ist die Streumenge, ab der die positiven Effekte der Streu auf die Keimlingsetablierung sich ins Negative verkehren, art- und gattungsspezifisch und mit der Samengröße korreliert.

Für Kapitel 3 wurden mögliche Wirkungen sekundärer Inhaltsstoffe einer dominanten Grasart (*Stipa pulcherrima*) steppenähnlicher Trockenrasen des transsilvanischen Tieflands (Rumänien) auf die Keimung und Keimlingsentwicklung mehrerer subdominanter Gräser und Kräuter untersucht. Bei diesem Experiment zeigte sich, dass das Eluat aus der Streu von *Stipa pulcherrima* die Samenkeimung der anderen Arten verzögerte und auch ihre Wurzelentwicklung negativ beeinflusste. Im Grasland trockener Standorte, wo günstige Bedingungen für die Samenkeimung nur während eines kurzen Zeitfensters gegeben sind, kann bereits eine Verzögerung der Keimung und Wurzelentwicklung um wenige Tage die Keimlingsetablierung und damit die generative Regeneration der Arten gefährden. Diese negativen chemischen Effekte einer Streudecke, wie sie sich zum Beispiel in Grünlandbrachen ausbildet, können zusammen mit biotischen und abiotischen Interaktionen den beobachtbaren Artenverlust im Brachland forcieren.

Streudecken zeigen auch ökosystemspezifische Effekte auf die Keimlingsetablierung (Kapitel 4). Die experimentellen Ergebnisse weisen darauf hin, dass Waldarten während der Keimlingsetablierung deutlich sensibler auf eine Streuabdeckung reagieren als Graslandarten. Insgesamt zeigten Waldarten eine deutlich stärkere ökosystemspezifische

Reaktion während der Keimlingsetablierung: Grasstreu verringerte die Anzahl der Keimlinge deutlich stärker als die waldtypische Blattstreu. Im Gegensatz dazu war die Keimlingsetablierung der Graslandarten unabhängig von der Art der Streuabdeckung.

Der Versuch, der in Kapitel 5 dargestellt wird, zeigt die Bedeutung der artspezifischen Eigenschaft Samengröße, für die Keimlingsetablierung bei verschiedenen Bodenauflagen (Grasstreu vs. Moos). In diesem Experiment wurde auch die Bedeutung der relativen Samenposition auf die Keimlingsetablierung untersucht. Während großsamige Arten einen besseren Etablierungserfolg bei mittleren Streumengen aufweisen, verzögert sich bei kleinsamigen Arten die Keimung, was als mögliche Anpassungsstrategie an hohe Streuabdeckungen gedeutet werden kann. Die Ergebnisse geben auch Hinweise darauf, dass großsamige Arten eine höhere Keimlingsetablierung als kleinsamige Arten erreichen, wenn sie eine Streu- oder Moosschicht durchstoßen müssen – und das unabhängig von der Art der Bodenauflage (Grasstreu oder Moos). Wenn allerdings die Samen auf die Streu- oder Moosschicht fallen zeigen großsamige Arten nur im Falle der Gras-, nicht aber bei Moosstreu weiterhin eine höhere Keimlingsetablierung als kleinsamige Arten.

Interaktionen zwischen der Bodenmakrofauna, in diesem Fall Regenwürmer, und Bodenauflagen und deren Auswirkungen auf die Keimlingsetablierung sind Thema in Kapitel 6. Die Ergebnisse dieser Untersuchungen zeigen, dass der Einfluss von Grasstreu auf die Keimlingsetablierung nicht nur auf deren physikalische, mechanische und chemische Effekte reduziert werden sollte. Die Anwesenheit von Regenwürmern beispielsweise kann die Richtung des Effektes der Streuabdeckung in Abhängigkeit von der relativen Samenposition verändern: Während im Allgemeinen Regenwürmer die Keimlingsetablierung reduzierten, führte ihre Anwesenheit bei großsamigen Arten, dann zu erhöhten Keimlingszahlen , wenn die Samen zu Versuchsbeginn auf der Streu lagen.

Die experimentellen Arbeiten, die dieser Habilitationsschrift zu Grunde liegen zeigen, dass eine Bodenauflage durch physikalische, mechanische und chemische Effekte die Keimlingsetablierung, die Invasibilität von Pflanzengemeinschaften und deren Artenzusammensetzung beeinflussen. Die Effektgröße und -richtung hängt dabei von den vorherrschenden abiotischen und biotischen Bedingungen ab. Diese experimentell belegten streubedingten Effekte sind auch von praktischer Bedeutung für die Grünlandrenaturierung mittels Wiedereinführung eines adäquaten Managements oder

der Aufbringung diasporenhaltigen Mahdgutes. Bei diesen Maßnahmen ist die Qualität und Quantität der Streu oder des Mahdgutes entscheidend für die Vegetationsentwicklung in Folge der Renaturierungsbemühungen.

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11 Publications

English publications

1. Donath TW & Eckstein RL: Litter effects on seedling establishment interact with seed position and earthworm activity. Plant Biology: accepted
   doi: 10.1111/j.1438-8677.2011.00490.x
2. Schmiede R, Otte A & Donath TW: Enhancing plant biodiversity in species-poor grassland through plant material application – the impact of sward disturbance. Applied Vegetation Science: accepted
3. Shakeri Z, Marvie-Mohadjer MR, Simberloff D, Etemad V, Asadi M, Donath TW, Otte A & Eckstein RL: Community composition, diversity and invasive plants of Caspian *Fagus orientalis* forests: which are the main driving factors? Phytocoenologia: accepted
4. Burmeier S, Eckstein RL, Donath TW & Otte A 2011 Plant pattern development during post-restoration succession in grasslands – a case study of *Arabis nemorensis*. Restoration Ecology 19: 648-659
5. Burmeier S, Eckstein RL, Otte A & Donath TW 2011 Spatially-restricted plant material application creates colonization initials for flood meadow species. Biological Conservation 144: 212-219
6. Hensgen F, Albrecht C, Donath TW, Otte A & Eckstein RL 2011 Distribution of gastropods in floodplain compartments and feeding preferences for river corridor plant species: is there an effect of gastropod herbivory on the distribution of river corridor plants? Flora 206: 534-543
7. Jung LS, Eckstein RL, Donath TW & Otte A 2011 A physiological approach to reduce population densities of *Colchicum autumnale* in extensively managed grasslands. Grassland Science in Europe 16: 67-69
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13. Kiehl K, Kirmer A, Donath TW, Rasran L & Hölzel N 2010 Species introduction in restoration projects - evaluation of different techniques for the establishment of semi-natural grasslands in Central and Northwestern Europe. Basic and Applied Ecology 11: 285-299
14. Ruprecht E, Enyedi NZ, Eckstein RL & Donath TW 2010 Restorative removal of plant litter and vegetation 40 years after abandonment enhances re-emergence of steppe grassland vegetation. Biological Conservation 143: 449-456
15. Schmiede R, Donath TW & Otte A 2009 Seed bank development after the restoration of alluvial grassland via transfer of seed-containing plant material. Biological Conservation 142: 158-167
16. Donath TW & Eckstein RL 2008 Grass and oak litter exert different effects on seedling emergence of herbaceous perennials from grasslands and woodlands. Journal of Ecology 96: 272-280
17. Ruprecht E, Donath TW, Otte A & Eckstein RL 2008 Chemical effects of dominant grass on seed germination of four familial pairs of dry grassland species. Seed Science Research 18: 239-248
18. Donath TW, Bissels S, Hölzel N & Otte A 2007 Large scale application of diaspore transfer with plant material in restoration practice - impact of seed and microsite limitation. Biological Conservation 138: 224-234

19. Donath TW, Hölzel N & Otte A 2006 Influence of competition by sown grass, disturbance and litter on recruitment of rare flood-meadow species. Biological Conservation 130: 315-323
20. Bissels S, Donath TW, Hölzel N & Otte A 2006 Effects of different mowing regimes on seedling recruitment in alluvial grasslands. Basic and Applied Ecology 7: 433-442
21. Bissels S, Donath TW, Hölzel N & Otte A 2005 Ephemeral wetland vegetation of irregularly flooded arable fields: the importance of persistent soil seed banks. Phytocoenologia 35: 469-488
22. Eckstein RL & Donath TW 2005 Interactions between litter and water availability affect seedling emergence in four familial pairs of floodplain species. Journal of Ecology 93: 807-81
23. Bissels S, Hölzel N, Donath TW & Otte A 2004 Evaluation of restoration success in alluvial grasslands under contrasting flooding regimes. Biological Conservation: 118/5: 641-650
24. Donath TW, Hölzel N, Bissels S & Otte A 2004 Perspectives for incorporating biomass from non-intensively managed temperate flood meadows into farming systems. Agriculture, Ecosystems and Environment 104: 439-451
25. Donath TW, Hölzel N & Otte A 2003 The impact of site conditions and seed dispersal on restoration success in alluvial meadows. Applied Vegetation Science 6: 13-22

- Jung LS, Eckstein RL, Otte A & Donath TW: Trade-offs in management of meadow saffron Colchicum autumnale L. in extensively managed grasslands: mowing for population control or low toxicity in hay: submitted

German publications

1. Schmiede R, Otte A & Donath TW: Artenanreicherung durch Mahdgutübertragung in artenarmen Grünlandbeständen – welche Störungsintensität der Grasnarbe ist nötig? Naturschutz und Biologische Vielfalt: accepted
2. Handke K, Otte A & Donath TW: Alternierend spät gemähte Randstreifen fördern die Wirbellosenfauna in Auenwiesen: Naturschutz und Landschaftsplanung 43: 280-288
3. Schmiede R, Harnisch M, Otte A & Donath TW 2010 Stromtalwiesen am hesssichen Oberrhein - aktuelle Renaturierungsmaßnahmen und Perspektiven für ihre Erhaltung. Collurio 28: 69-81
4. Donath TW, Schmiede R, Harnisch M, Burmeier S, Eckstein RL & Otte A 2009 Renaturierung von Auenwiesen - Perspektiven für die langfristige Entwicklung. ANL Laufener Spezialbeiträge 2/09: 122-132
5. Burmeier S, Eckstein RL, Donath TW & Otte A 2008 Samenbankentwicklung und -dynamik in Stromtalwiesen am hessischen Oberrhein. Treffpunkt Biologische Vielfalt 8: 133-138
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Acknowledgements

First of all, I want to thank Prof. Dr. Dr. Annette Otte for giving me the opportunity to work on an interesting and wide variety of topics, and for her support and advice during the genesis of this habilitation thesis.

This thesis could not have been accomplished without the net and gross effects of biotic and abiotic factors as well as their interactions under various marginal conditions at the Division of Landscape Ecology and Landscape Planning and neighbouring institutes which included the pleasant and inspiring atmosphere, discussions and coffee breaks. Indispensable was also the help of former and current colleagues, collaboration partners and co-authors; I am indebted to their support. I am especially grateful for the continuous and fruitful cooperation with Lutz Eckstein, who contributed a lot more to this habilitation than mere co-authorship on papers. Also Eszter Ruprecht deserves many thanks; she widened the scope of the series of experimental studies on litter ecology to the Transylvanian dry grasslands of Romania.

Ideas and designs for experiments might be good, still they will not lead to scientific findings without thorough care: many thanks to Josef Scholz-vom Hofe for his support during practical planning, set up and conducting of the experiments; also Christiane Lenz-Kuhl was a great help. Further projects during my habilitation time were successful through the highly motivated work of the doctoral students Sandra Burmeier, Linda Jung, Ralf Schmiede and Bianka Zelle and several BSc and MSc students that took or take part in the projects. Matthias Harnisch deserves many thanks for his constant care during the restoration projects and the inspiring email exchange. Not to forget Dietmar Simmering who is a continuous well of fruitful and entertaining scientific discussions at its best; and who commented in this spirit on this thesis. Several others who contributed assistance are acknowledged in the publications herein.

Last but not least, I thank all family members and friends who followed my work with interest and gave me moral support. I especially appreciate Astrid’s comments on this thesis and the papers therein and her support of my scientific work. Finally, I thank Julie, Anneli and Milan for providing the best possible distraction from scientific work.