

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

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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 g, 2 g, 4 g and 8 g litter per pot, corresponding to 0 kg m⁻², 0.2 kg m⁻², 0.4 kg m⁻² 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 kg m⁻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 smaller-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 0.2–0.4 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 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.

Key-words: conservation, facilitation, *Galium*, germination, grassland, *Imula*, *Peucedanum*, seed size, *Silaum*, *Viola*

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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; Queded *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 and light quantity and quality; cf. Boser & Reader 1995). Litter accumulation may have similar consequences to those of increased fertilization (Foster & Gross 1997, 1998), which suggests that such 'after-death interactions' may be an important functional link between the higher productivity that 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. Boser & Reader 1995; Špačková *et al.* 1998; Jensen & Meyer 2001; Jensen & Gutekunst 2003), and biomass and species richness (e.g. Foster & Gross 1998; Jensen & Meyer 2001) were predominantly negative. Their 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 (Boser & Reader 1995; Turnbull *et al.* 1999), owing 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 with 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; Xiong & Nilsson 1999, and references therein) there have been relatively few empirical studies simultaneously manipu-

lating 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 because, at least in European ecosystems, seed size and the degree of dark dormancy are closely related (Leishman *et al.* 2000).

As 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, because 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 seedlings' reserves, there should be significant effects of litter treatments on final seedling biomass.

Materials 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), whereas 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 and 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

Table 1 Families, seed mass 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 (mg)	Viability (%)
<i>Galium boreale</i> L.	Rubiaceae	0.68	95.0
<i>Galium wirtgenii</i> F.W. Schultz	Rubiaceae	0.40	95.0
<i>Inula britannica</i> L.	Asteraceae	0.09	91.0
<i>Inula salicina</i> L.	Asteraceae	0.16	93.0
<i>Peucedanum officinale</i> L.	Apiaceae	12.32	75.0
<i>Silaum silaus</i> (L.) Schinz & Thell.	Apiaceae	2.43	99.0
<i>Viola elatior</i> Fr.	Violaceae	1.80	95.0
<i>Viola pumila</i> Chaix	Violaceae	1.08	95.0

Rhine in autumn 2003. These seeds were subsequently dry stored in darkness at room temperature until sowing on 17 December 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).

EXPERIMENTAL DESIGN

To study the effects of litter and water-addition on seedling emergence, we used 40 1-L pots (*c.* 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 P₂O₅ and 250 mg L⁻¹ K₂O). 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 g (control), 2 g, 4 g and 8 g of bench-dry litter per pot on top of the seeds. We used bench-dry litter because oven drying might induce chemical changes or subsequent leaching of litter. These litter levels of 200 g m⁻², 400 g m⁻² and 800 g 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 unfertilized 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 (percentage 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 the 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 8, 16 and 23 April, 11 May and 15 June, and were individually marked with non-toxic colour to be able to differentiate newly emerged seedlings from those already present. Because 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. Because 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 15 June 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.

For the percentage emergence at each date, we used repeated-measures analysis to assess the overall effects of time, each of the factors and their interactions. For 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 as 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 their 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 performed 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 were up to 10 °C higher in control pots than in pots covered with 8 g of litter.

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

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%; Fig. 1). Additionally, within each water-addition treatment, soil water content increased with litter cover during this period: constantly moist pots

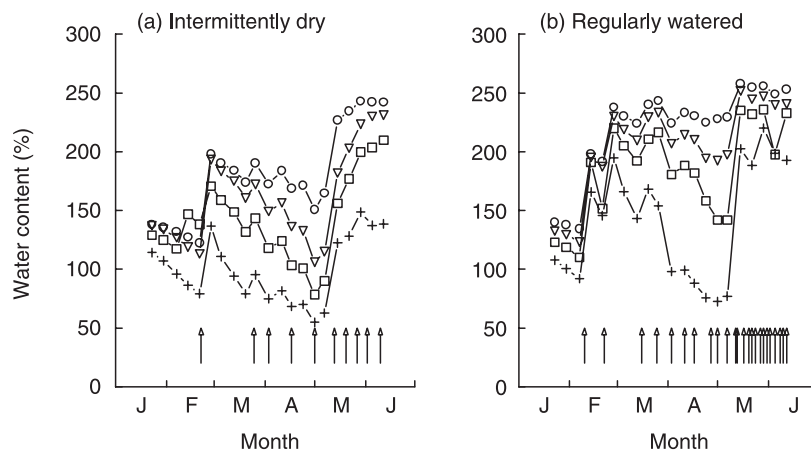


Fig. 1 Soil water content (percentage of soil dry weight) in experimental pots covered with different amounts of litter. Data are presented as means of two replicate pots. Symbols: +, control; squares, 2 g of litter; triangles, 4 g of litter; circles, 8 g of litter. Arrows indicate watering occasions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

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. The between-subject factors results originate from ANOVA and the within-subject factor results originate from MANOVA. T = time; d.f._{NUM} = numerator degrees of freedom; d.f._{DEN} = denominator degrees of freedom; MQ = mean sum of squares.

Between-subject factors					
Source of variation	d.f.	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	d.f. _{NUM}	d.f. _{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	13.3	< 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

contained 44%, 49%, 39% and 31% more soil water than intermittently dry pots covered with 0 g, 2 g, 4 g and 8 g of litter, respectively (Fig. 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 (data 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.

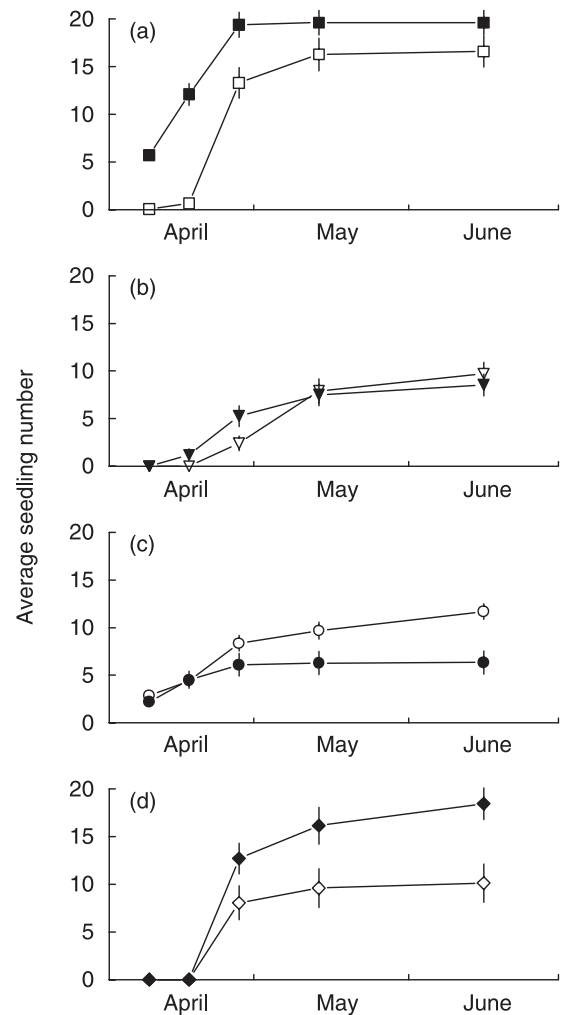


Fig. 2 Average cumulative number of seedlings across treatments that emerged out of 50 seeds per pot. Data are presented as means ± SE ($n = 40$) in species of *Galium* (a), *Inula* (b), *Peucedanum/Silaum* (c) and *Viola* (d). Symbols: filled squares, *Galium wirtgenii*; open squares, *G. boreale*;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

In the species with the smallest seeds, i.e. *Inula salicina* and *I. britannica*, seedling emergence occurred over the whole duration of the experiment, whereas in the other species emergence had already levelled off by the end of April. Furthermore, emergence patterns in *Viola* spp. were different from the general trend, with the peak of seedling emergence being more pronounced, i.e. with no seedlings at the first two counting dates, but most germination taking place by the end of April.

FINAL SEEDLING EMERGENCE

Final percentage emergence was significantly higher in *Galium* ($36.2 \pm 2.1\%$, mean ± SE, $n = 80$) and *Viola* ($28.6 \pm 2.8\%$) than in *Inula* ($18.3 \pm 1.6\%$) and the Apiaceae ($18.0 \pm 1.6\%$; cf. Fig. 2) and differed significantly between species within families (Table 3). Across species and water-addition treatments, emergence was

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. d.f. = degrees of freedom; MQ = mean sum of squares; vc (%) = relative contribution of individual factors and their interactions to total variation

Source of variation	d.f.	Germination			Total biomass			Individual biomass		
		MQ	<i>P</i>	vc (%)	MQ	<i>P</i>	vc (%)	MQ	<i>P</i>	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

twice as high in pots covered with 2 g, 4 g and 8 g of 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.

When analysed for each species separately, there was a significant litter × water-addition interaction in six of the eight study species (Fig. 3), whereas 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 of 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 g and 4 g (Fig. 3). In contrast, when kept constantly moist, percentage emergence did not differ significantly between controls, 2-g 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 g and 4 g of litter than in control pots. In *G. wirtgenii* and *Inula* spp. emergence in pots with 8 g of litter was significantly lower than in controls, whereas there was no difference or seedling emergence increased under 8 g of litter in all other species.

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 g and 4 g of litter, whereas 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 *Silva silaus* and *Viola elatior* there were hardly any differences between water-treatments, and in *Galium boreale* germination and thus density were 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 of litter (*Inula* spp.). More frequent water-addition led to a 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).

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, Fig. 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).

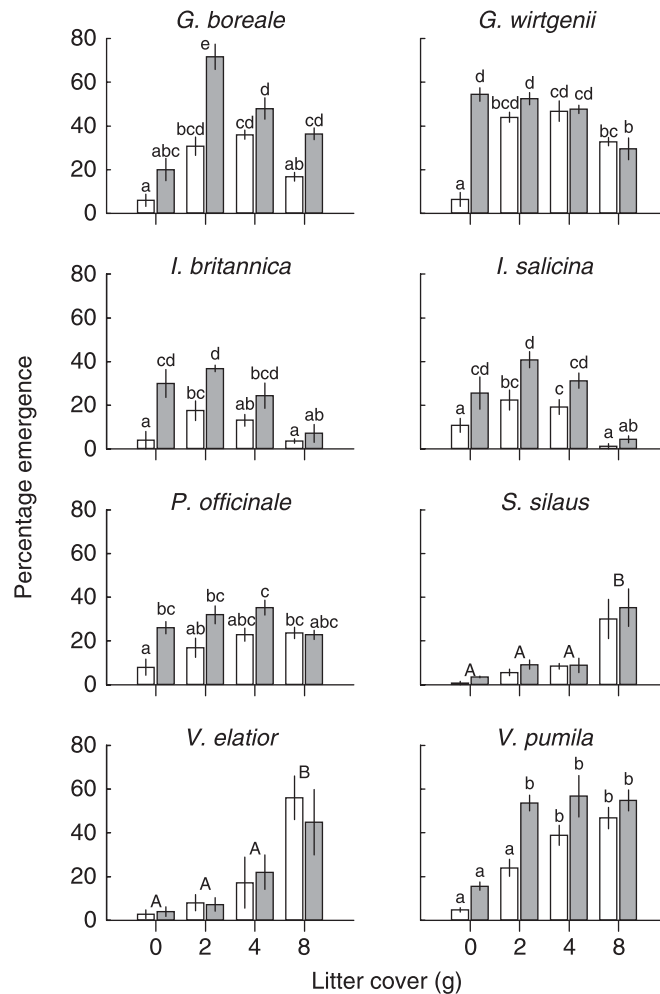


Fig. 3 Final percentage seedling emergence of the study species in constantly moist (shaded bars) and intermittently dry (open bars) pots 6 months after sowing. Data are presented as means \pm SE ($n = 5$). Differences between all means were tested a posteriori using Tukey's HSD test for species with a significant litter \times 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, because 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 letter, respectively, are not significantly different ($P > 0.05$).

The facilitative effect of litter on seedling emergence was probably due to improved water conditions (Fig. 1) together with reduced thermal stress (significantly lower maximum temperatures; Fowler 1986; Facelli & Pickett 1991b; Xiong *et al.* 2003). Although 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; Fig. 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, because 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. Boser & 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 & Gutkunst (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. Because light transmittance through increasing amounts of litter approximately follows the Beer–Lambert exponential law (Facelli & Pickett 1991b), photon flux density of PAR under 4 g and 8 g of litter was reduced to 1% or less of ambient levels (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). As 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 at 2 g and 4 g of litter (Fig. 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 (Fig. 3). We suggest that this may be 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 2004). Litter on top of germinated seedlings will provide insulation against incoming radiation, decreasing both the temperature amplitude and the 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; Fig. 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), whereas effects on germination at low-productivity sites were of 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 that 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, as 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 with controls, an amount of plant material of 0.2–0.4 kg m⁻² seems to be adequate to enhance germination in most species but still avoids suppression of emergence that 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) – because 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, as mean percentage emergence did not differ much between pots with 2 g, 4 g and 8 g of 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 become 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.

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