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Effects of litter on seedling establishment: an indoor experiment with short-lived Brassicaceae species

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Abstract Recruitment by seeds is essential both in vegetation dynamics and in supporting biodiversity in grasslands. The recruitment by seeds is feasible in suitable vegetation gaps from the seed rain and/or by establishment from persistent soil seed banks. Cessation of grassland management results in litter accumulation, which leads to the decline of species diversity by the decreased availability of open patches. Low amounts of litter is often beneficial, while high amounts of litter is detrimental for seed germination and seedling establishment of short-lived species. In a designed indoor experiment, we explored the effect of litter on seedling establishment by germinating six short-lived Brassicaceae species with both increasing seed mass and litter cover. We found that both seed mass and litter had significant effect on germination and establishment of the sown species. Small-seeded species were significantly negatively affected by the 300 and/or 600 g/m^2 litter layers. No negative litter effect was detected for species with high seed masses (*Lepidium* spp.). No overall significant positive litter effect was found, although for most of the species cumulative seedling numbers were not the highest at the bare soil pots. Our results suggest that the negative effects of litter are less feasible on the large-seeded short-lived species than on that of small-seeded ones.

Keywords Biodiversity · Cruciferae · Litter · Seed size · Germination · Weed control

Introduction

Recruitment by seeds is crucial in maintaining biodiversity of grasslands (Zobel et al. 2000). It is feasible in suitable microsites in vegetation gaps from the seed rain and/or by establishment from persistent soil seed banks (Tilman 1993). Although regeneration by seeds is important for both perennial and short-lived species, the most essential is for short-lived ones. Short-lived species considerably contribute to grassland biodiversity and most of them are gap-strategists requiring open patches as microsites for seed germination (Rebollo et al. 2001). Open patches in grasslands were historically provided by free grazing and/or infrequent abiotic disturbances (Pykäla 2000). With increasing human influence, free grazing was replaced by mowing and/or controlled grazing. Recently, large-scale cessation of former management resulted in litter accumulation and the decrease of species diversity by a decreased availability of open patches (Bissels et al. 2006).

Litter accumulation influences species richness, seed germination and seedling establishment both negatively and positively. Litter hampers the seed germination and seedling establishment by (i) forming

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a mechanical barrier (Donath and Eckstein 2010), (ii) by decreasing the solar irradiation (Jensen and Gutekunst 2003) or by changing red-far red ratio of light (Jankowska-Balszczuk and Daws 2007), (iii) by decreasing the fluctuations in temperature (Eckstein and Donath 2005), (iv) or by dissolving of toxic compounds (Ruprecht et al. 2010). Litter affects also positively the germination and seedling establishment (i) by covering seeds from predators (Reader 1993), (ii) by increasing the availability and decreasing the fluctuations in soil humidity (Eckstein and Donath 2005) and (iii) by increasing the nutrient content of the soil (Schlatterer and Tisdale 1969).

Thin litter layer is usually beneficial, while thick litter layer is detrimental for seed germination and seedling establishment of short-lived species (Facelli and Pickett 1991; Xiong and Nilsson 1999). However, studies on the magnitude of these effects on germination and seedling establishment in relation to litter thickness and some seed attributes are scarcely studied (but see Jensen and Gutekunst 2003; Ruprecht et al. 2010). We studied the effect of litter thickness on the germination and seedling establishment of six short-lived *Brassicaceae* species with increasing seed size in an indoor germination experiment. We tested the following hypotheses: (i) low amounts of litter affects positively (positive litter effect), while high amounts negatively (negative litter effect) the germination and seedling establishment of the studied short-lived species. (ii) The ratio of germination and seedling establishment is affected both by the seed mass and amounts of litter; negative litter effect is more feasible for small-seeded species than large-seeded ones (seed size depended effect).

Materials and methods

Six short-lived Brassicaceae species with increasing seed mass were selected for the study (Table 1). The selected species represent the (i) typical seed mass range of Brassicaceae in Europe, (ii) can be easily germinated indoor, (iii) and studying litter effects on the selected species helps us to understand the germination dynamics of characteristic Brassicaceae species of threatened grasslands (e.g., *Teesdalia nudicaulis* or *Arabis nemorensis*; Jentsch and Beyschlag 2003; Hölzel 2005). Seeds were collected from at least 50 plant individuals of each species. Seeds were dry-stored until the germination was started at the end of March 2011. For the selected species long-term seed viability is known under dry storage. Sets of 100 seeds were counted then weighted with an accuracy of 0.01 g.

We filled 8 cm × 8 cm × 12 cm pots with steam-sterilized potting soil [pH: 5.0–7.0, N-content (m/m %): min 0.5, P_2O_5-content (m/m %): min 1.0, K_2O-content (m/m %): min 0.2; reported by the producer], we sowed 100 seeds of each species separately in each pot (altogether 2,500 seeds of each species were sown). No soil covering was applied. *Festuca pseudovina* litter was laid at the surface after seed sowing in the following densities: 0 g/pot (bare soil), 0.48 g/pot (75 g/m^2), 0.96 g/pot (150 g/m^2), 1.92 g/pot (300 g/m^2) and 3.84 g/pot (600 g/m^2) based on the reported litter ranges in alkali grasslands in the study region (Deák et al. 2011).

There were six species and five treatments with five replicates for each species. We used 20 control pots (filled with steam-sterilized soil only) detecting airborne seed contamination. All pots were placed under natural light on germination shelves ordered randomly. The pots were regularly watered to provide optimal water availability. The germination lasted altogether 29 weeks (from the end of March to early November) with an included watering break between late July and early September to mimic the midsummer drought. Watering was restarted at early September and lasted till the early days of November. Germinated seedlings appeared at the surface of the

Table 1 Seed masses of the germinated Brassicaceae species (mg, mean±SE, $n = 25$)

Species	Collection date	Seed mass
Arabidopsis thaliana	May 14, 2009	0.016 ± 0.001
Erophila verna	April 24, 2010	0.023 ± 0.001
Descurainia sophia	July 10, 2007	0.112 ± 0.001
Capsella bursa-pastoris	May 13, 2009	0.113 ± 0.001
Lepidium perfoliatum	June 15, 2006	0.736 ± 0.004
Lepidium campestre	June 26, 2008	3.056 ± 0.010

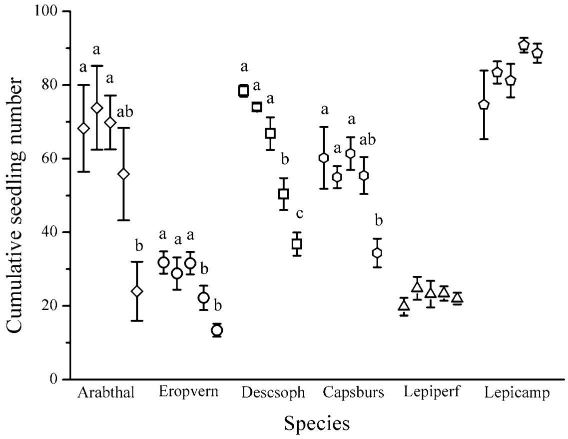

Fig. 1 Cumulative numbers of established seedlings (mean ± SE). *Different superscripted letters* indicate significant differences using One-way ANOVA and Tukey-test ($p < 0.05$). Species are abbreviated using the first four letters of the genus and species name. Increasing litter densities from the *left* to the *right* for each species were the following: 0 g/pot (0 g/m^2, bare soil), 0.48 g/pot (75 g/m^2), 0.96 g/pot (150 g/m^2), 1.92 g/pot (300 g/m^2), and 3.84 g/pot (600 g/m^2)

applied litter-layer were counted and removed in each week.

We performed a univariate GLM, where the litter thickness and seed mass were included as fixed factors. Indicating differences between treatments for each species separately, one-way ANOVA and Tukey-test was calculated. To eliminate the effect of differences in viability rates of different species, all scores were standardized with the means of established seedlings of the pots without litter covering.

Results

We found that the germination and establishment of the sown species were significantly affected both by seed mass and litter. Also a significant interaction between seed mass and litter thickness was detected (Table 2). We found that small-seeded species were negatively affected by thick litter layers (Table 2). A clear negative effect was detected typically from 300 g/m^2 for *Erophila verna* and *Descurainia sophia*, and at 600 g/m^2 litter scores for *Arabidopsis thaliana* and *Capsella bursa-pastoris*. For all formerly mentioned small-seeded species, only one-third to one half of the seedlings found in no litter pots were counted in pots covered with 600 g/m^2 litter (Fig. 1). Conversely, no negative litter effect was detected for the two

Table 2 The effect of seed mass and litter on cumulative germination values (univariate GLM)

Source of variance	df	*F*	*p*
Intercept	1	2395.65	<0.001
Seed mass	5	11.14	<0.001
Amounts of litter	4	11.84	<0.001
Seed mass × amounts of litter	20	2.21	0.004

Lepidium species with high seed masses. For *L. campestre*, the species with the highest seed mass, even a monotonous but only slight increase in seedling establishment was detected.

We found no significant positive litter effect, although for most of the species, the maximum of cumulative seedling numbers were not detected in the "bare soil" pots (for *Arabidopsis thaliana* and *Lepidium perfoliatum* the maximum was at 75 g/m^2, for *Capsella bursa-pastoris* at 150 g/m^2, and for *Lepidium campestre* at 300 g/m^2).

Discussion

The interaction between litter and seed mass is plausible, because high litter amounts suppressed small-seeded species, while no negative litter effect was feasible for large-seeded ones. An indoor experiment is particularly useful for determining the effect of litter, because the environmental factors are controlled by the experiment. In a real field situation, there are a lot of uncontrolled factors, for example, spatial variation in soil moisture, seed predation, or fungal infection. Our results clearly suggested that among species in Brassicaceae family with similar life strategy, the increase in seed mass provides an establishment advantage to a respective species in case of litter accumulation. Similar results were obtained in other multiple and single species studies. In studies using species belonging to different plant families, a negative effect of litter was detected for small-seeded species and much moderate or no negative effect for large-seeded ones (Jensen and Gutekunst 2003; Donath and Eckstein 2010).

Several mechanisms were reported to be responsible for the germination and establishment advantage of large-seeded species. Large seeds contain higher amounts of nutrient storage tissues which enables them to establish over a wider range of abiotic and biotic conditions (Grundy 2003; Jensen and Gutekunst 2003). From large seeds generally large seedlings emerge, which have higher rates of seedling survival (Harel et al. 2011), and can more successfully compete and tolerate litter or soil coverage than small-seeded ones (Bond et al. 1999). Larger seeds can have even benefits from litter covering, especially under water stressed conditions. Larger seeds need more time for water absorption than smaller seeds because of smaller seed surface/mass ratio, thus, they require higher and more constant soil humidity prior to germination (Kikuzawa and Koyama 1999).

Former findings detected a positive litter effect in arid environments where small amounts of litter might increase the water retention of soil providing higher water availability (Boeken and Orenstein 2001). We found no positive litter effect on germination and seedling establishment of the sown species. The most likely reason for the non-detection of a positive litter effect was that we provided optimal water availability for germination in our study; thus, the water retention was not an issue. These results correspond with the findings of Xiong and Nilsson (1999), where much stronger effects of litter on germination was detected in field than in the greenhouse experiment.

There were high differences in the amount of litter that affected negatively the germination and establishment of the sown species in our and in some of the mentioned studies. In our study, no negative litter effect was detected for most of the sown species even at litter amounts of 300 g. This contradicts with the findings of Jensen and Gutekunst (2003) where a negative litter effect was feasible for most of the sown species even at a much lower litter mass of 170 g/m^2 (out of 35 species of various genera sown with seed masses ranging from 0.01 to 4.8 mg, 83 % of species were suppressed). Similarly, to our study, no negative litter effect was found for 200–400 g/m^2 litter (which corresponded 1–2 cm-thick litter layer, Donath et al. pers. comm.) using mixed litter composing mostly narrow-leaved grass species (e.g., *Poa pratensis*, *Agrostis stolonifera*, *Arrhenatherum elatius,* and *Dactylis glomerata*; Donath and Eckstein 2010). The most likely reason for the detected differences could be that Jensen and Gutekunst (2003) used the litter of a broad-leaved species *Carex acutiformis*, which is less compact and fluffy but more voluminous than that of the narrow-leaved *Festuca pseudovina* in our study and the mixed litter in Donath and Eckstein (2010). Thus, the weight of a 3-cm-thick litter layer mentioned in the study was only 170 g/m^2, whereas in our study and also in the study of Eckstein and Donath (2005) it was around 600 g/m^2. These findings points out that the germination suppression effect of litter is likely be regulated by thickness (litter acts as a physical barrier and/or decreases light irradiance); which is a parameter rarely measured and reported in most of the studies investigating litter effects in various grasslands and scales (but see Dalling and Hubbel 2002 or Jensen and Gutekunst 2003).

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