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Differential effects of several "litter" types on the germination of dry grassland species

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Abstract

Question: Accumulation of litter can have serious implications on the recruitment of plant species, by modifying the physical, biological and chemical features of the microenvironment or acting as a mechanical barrier for seeds and seedlings. Isolating these different effects has rarely been achieved experimentally.
Location: Transylvanian Lowland, Romania.
Methods: We tested the effects of different "litter" types on the germination of dry grassland species using a controlled pot experiment with three natural litter types, differing in decay state and composition (*Stipa pulcherrima* fresh leaves, partly decomposed leaves and mixed and partly decomposed plant material) and an artificial plastic litter, with two levels of water addition. As a complementary field study, seed sowing was conducted in grassland plots with litter removal and plastic litter application.
Results: Litter effects were mainly positive (intermittent watering) or neutral (frequent watering) under controlled experimental conditions, and mostly negative in the field. Seed size and environmental conditions were the major determinants of litter effects on germination. Significant differences were found in the effect of litter type on germination, much of which could be explained by chemical factors determined by the decay state, as we confirmed a higher concentration of allelopathic compounds in fresh *S. pulcherrima* litter than in the senescing leaf litter.
Conclusions: The effects of litter on seed germination are strongly context dependent, and it is hard to define common rules that apply consistently under various environmental conditions. "Litter" identity and quality matter, i.e. the litter composition and decay state, and influence seed germination.

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Nomenclature: Flora Europaea (Tutin et al. 1964–1980)

Introduction

At a global scale, land-use change is one of the greatest threats to biodiversity and ecosystem functioning (Sala et al. 1996; Chapin et al. 2000). Throughout the last 50 years land-use has changed in Romania, especially through a decrease in livestock number. This has generated a large-scale abandonment of grassland areas, which particularly affected dry grasslands of lower productivity (Enyedi et al. 2008; Ruprecht et al. 2009). A reduction in land-use intensity in grasslands induces biomass accumulation, especially litter, since living plant biomass is not consumed by herbivores or removed by mowing.

Recruitment by seeds is essential for population dynamics and biodiversity in grasslands (e.g. Tilman 1993; Zobel et al. 2000). Litter affects seed germination and seedling establishment and hence may determine the fate of species in a community or govern community processes (reviewed by Facelli & Pickett 1991a; Xiong & Nilsson 1999). Litter acts by modifying microenvironmental conditions for germinating seeds: affecting light availability (Facelli & Pickett 1991b; Jensen & Gutekunst 2003; Weltzin et al. 2005), soil humidity (Fowler 1986; Eckstein & Donath 2005; Wolkovich et al. 2009), temperature and its amplitude (Fahnestock et al. 2000; Eckstein & Donath 2005) and soil nutrient content (Schlatterer & Tisdale 1969; Fahnestock et al. 2000). It can release secondary metabolic or decay compounds with potential toxic effect upon neighbouring species (del Moral & Cates 1971; Bosy & Reader 1995; Ruprecht et al. 2008). In addition, it may protect the seeds from predators (Reader 1993; Facelli 1994) or act as a mechanical barrier for establishing seedlings (Chambers 2000; Rotundo & Aguiar 2005; Donath & Eckstein 2010).

Litter effects are complex and their outcome depends on a delicate balance between facilitative and inhibitory effects (Holmgren et al. 1997), determined by environmental conditions and life history of species (especially seed size). For example, while in a dry ecosystem litter may facilitate seed germination by reducing evaporation and thus increasing water availability, in a mesic environment this influence is of less importance or is even neutral (Fowler 1986; Eckstein & Donath 2005). In addition, this positive effect tends to be more accentuated in the case of species with larger seeds, since these are known to require increased humidity during the breaking of dormancy than smaller seeded species (Baskin & Baskin 2001; Donath & Eckstein 2010). In the same way, light interception by litter and the role of litter as a mechanical barrier have been proven to almost exclusively or most drastically affect species with smaller seed sizes and thus less nutrient reserves, although these factors do not or only more moderately influence larger seeded species (Milberg et al. 2000; Jensen & Gutekunst 2003; Eckstein & Donath 2005).

There are very few experimental studies examining litter effects on plant attributes, in particular on seed germination, that have broken it down into its components. Physical effects, i.e. light interception, has been most successfully isolated from other effects by applying shade cloth and comparing the results with application of natural litter (Amatangelo et al. 2008; Donath & Eckstein 2008; Startsev et al. 2008). Sydes & Grime (1981) used nylon sheets and Rotundo & Aguiar (2005) used plastic fibres, which were expected to exert similar physical effects to natural litter, i.e. on light interception, water availability and as a mechanical barrier, but which did not release nutrients or allelopathic compounds or carry spores of pathogens.

Litter layers in natural habitats usually consist of a complex mosaic of dead plant remains of different species; the word "litter" has been interpreted in various ways in different studies. Experiments have used plant parts of single species or mixtures in different stages of decomposition as "litter", e.g. fresh dried leaves – and stems – of one (de Jong & Klinkhamer 1985; Ruprecht et al. 2008) or several species (Eckstein & Donath 2005), freshly deposited or partly decomposed leaves (Sydes & Grime 1981; Quested & Eriksson 2006) and mixed senesced or partly decomposed plant material (Bosy & Reader 1995; Rotundo & Aguiar 2005; Hovstad & Ohlson 2008). Thus, the important question from these different approaches remains to what extent they represent the real, natural field situation. It is well known that litter of different species may have contrasting properties (e.g. Sydes & Grime 1981; Myster 2006; Quested & Eriksson 2006; Donath & Eckstein 2008). Different litter types, e.g. forb or grass litter, deciduous or evergreen tree leaf litter, can have different physical and chemical properties due to their different decomposition rates (Facelli & Pickett 1991a; Cornelissen 1996; Xiong & Nilsson 1999). Litter state is also not negligible, e.g. partly decomposed leaves are less persistent than fresh leaves (Sydes & Grime 1981). In addition, chemical properties, i.e. the concentration of secondary metabolic compounds, may also differ between plant phenological and decay stages: plant material in early phenological stages may be more phytotoxic than senesced material, and fresh material may contain more allelopathic substances than partly decomposed material (Datta & Sinha-Roy 1975; Bokhari 1978). Therefore, we would expect that chemical effects are stronger in case of fresh compared to partly decomposed litter.

In this study, we compared the effects of three natural litter types, differing in their decay state and composition, on seed germination. We used fresh dried leaves or partly decomposed leaves of a common dominant grass in abandoned dry grassland, *Stipa pulcherrima*, which is known to have allelopathic potential (Ruprecht et al. 2008), and a mixed partly decomposed plant material originating from the same grassland. In a controlled pot experiment, involving eight grassland species with different seed sizes and these three natural litter types, we also manipulated soil moisture in order to evaluate differential litter effects on seed germination under different environmental conditions. In addition, by using plastic litter, we sought to separate physical and chemical effects of litter. As a complementary study, we conducted a seed sowing experiment in a grassland site with litter accumulation (corresponding to the mixed partly decomposed plant material in the pot experiment), where we experimentally removed litter separately and in combination with the application of plastic litter, and followed the germination of seeds of six grassland species. We addressed the following hypotheses:

(H1) The effects of litter on seed germination are predominantly positive under dry conditions and negative or neutral under favourable water supply;

(p) There is an interaction between environmental conditions (watering frequency) and seed size of tested species in the effect of litter on their germination;

(H2) Chemical properties of litter can be equally important to physical properties in determining the success of germination in grasslands;

(p) In the studied dry grassland system litter acts negatively on seed germination due to the allelopathic potential of the dominant species, *S. pulcherrima*;

(H3) "Litter" identity and quality matters: litter composition and decay state influencies seed germination;

(p) Freshly dried leaves of *S. pulcherrima* are more phytotoxic than partly decomposed leaves or mixed partly decomposed plant material in inhibiting seed germination of grassland species.

Methods

Controlled pot experiment

Eight grassland species, which are typical constituents of dry steppe-like grasslands, were selected for an outside, controlled pot experiment on seed germination. The species belonged to different families and had different seed sizes, from very small (0.06 mg) to relatively large (4.13 mg) (Table 1). Seeds were collected in bulk from autochthonous populations in dry steppe-like grasslands in 2008. Seed collection included at least 100 different plant individuals from two to three sites each. Seeds (actually seeds or fruits), cleaned of appendages, were subsequently dry-stored in darkness at room temperature (ca. 20°C) until sowing.

In order to study the effect of different litter types on seed germination we used 10 cm ×10 cm ×10 cm pots, filled with 340 g (dry soil mass: 113 g) of commercial potting soil composed of a mixture of oligotrophic and eutrophic peat with supplemental nitrogen, phosphorus and potassium (pH = 6). On 13 December 2008, we sowed 50 seeds of one species on the soil surface in each pot. Pots of each study species were then randomly assigned to one of the litter treatments on top of the seeds: (1) fresh dried leaves of *S. pulcherrima*, a common dominant grass in abandoned dry grasslands; (2) partly decomposed leaves of *S. pulcherrima*; (3) mixed, partly decomposed plant material, consisting of a mixture of grassland species in decreasing abundance: *S. pulcherrima*, *S. capillata*, *Dichanthium ischaemum*, *Carex humilis*, *Elymus hispidus*, *Eryngium campestre, Teucrium chamaedrys* and *Inula ensifolia*; (4) plastic litter; and (5) no litter. Fresh leaves of *S. pulcherrima*, as well as the mixed partly decomposed plant material, were obtained from the field experiment site in Suatu (see below). Partly decomposed leaves of *S. pulcherrima* were selected from the mixed plant material. Fresh leaves of *S. pulcherrima* were collected in late spring 2008, dried and stored in darkness at room temperature until use. Plastic

Table 1. Family, diaspore (seed or fruit) mass, seed viability, average number of seeds germinated in sown pots (50 seeds sowed) or plots (100 seeds sowed), and spontaneously in pots or plots without seed sowing, respectively, of the dry grassland species selected for the germination experiments. Species are listed in the increasing order of seed mass. Two species were used in both experiments. Three of the study species, i.e. *A. vernalis*, *J. mollis* subsp. *transylvanica* and *S. nutans*, are threatened, red-listed species in Romania (Ruprecht et al. 2009). Data on seed mass are the results of measurements of 500 (5×100) seeds per species. Seed viability of 50 (2×25) seeds per species was tested with a 1% tetrazolium chloride solution. *150 seeds sowed in field plots.

Species	Family	Diaspore mass (mg)	Seed viability (%)	Sown germination	Spontaneous germination
Controlled pot experiment					
Campanula sibirica L.	*Campanulaceae*	0.06	90	24.30	0.03
Verbascum phoeniceum L.	*Plantaginaceae*	0.14	94	34.62	0
Dichanthium ischaemum (L.) Roberty	*Poaceae*	0.50	98	30.18	0
Potentilla cinerea Chaix ex Vill.	*Rosaceae*	0.55	94	24.62	0
Salvia nutans L.	*Lamiaceae*	1.10	26	7.32	0
Viola hirta L.	*Violaceae*	1.70	54	4.20	0
Dorycnium pentaphyllum Scop. subsp. *herbaceum* (Vill.) Rouy	*Fabaceae*	2.13	94	19.64	0
Jurinea mollis (L.) Rchb. subsp. *transylvanica* (Spreng.) Hayek	*Asteraceae*	4.13	34	26.82	0
Field experiment					
Achillea collina Becker ex Rchb.	*Asteraceae*	0.07	14	4.17*	0.04
Veronica spicata L. subsp. *orchidea* (Crantz) Hayek	*Plantaginaceae*	0.09	98	12.88	1.17
Dichanthium ischaemum (L.) Roberty	*Poaceae*	0.50	90	16.75	0
Dorycnium pentaphyllum Scop. subsp. *herbaceum* (Vill.) Rouy	*Fabaceae*	2.13	41	22.42	2.92
Muscari tenuiflorum Tausch	*Hyacinthaceae*	4.62	88	41.79	0.08
Adonis vernalis L.	*Ranunculaceae*	11.09	75	9.54	0.25

litter was prepared by cutting a light brown cloth made of synthetic fibres into 3-4-mm wide and approximately 10-15-cm long strips. Thus the artificial litter was similar in colour, volume and consistency to natural litters.

All litter types were applied in the same quantity ($4.7\,g\,pot^{-1} = 470\,g\,m^{-2}$), corresponding to the average litter load of a long-term abandoned dry grassland (Ruprecht et al. 2010), and approximately similar particle sizes were attained by cutting. Since natural litter might contain seeds of any of the studied species, we prepared pots with litter applied but without seeds added, as a control.

In order to study the effect of water availability (watering frequency) on seed germination, two groups were created randomly. One group of pots was watered twice as often as the other (frequent watering), and thus kept constantly humid, while the other group of pots was allowed to become intermittently dry (intermittent watering). Frequently watered pots received approximately 70 ml of water about every week in early spring and autumn, and 70-100 ml twice a week during late spring and summer, when air temperatures and evaporation rates had increased (Fig. 1).

Hence, the experiment consisted of a combination of eight species×five litter treatments×two watering frequencies (i.e. 80 combinations), each replicated five times, and five replications for controlling the seed content of the three natural litter types; in total 420 pots. All pots were placed outside, in the University Botanical Garden at Cluj-Napoca. They were arranged at random on wooden sheets, covered with a transparent polyethylene sheet on the top of a frame to exclude precipitation, and surrounded by a metal mesh to protect against animals. There was 20 cm of free space between the top of the pots and the polyethylene sheet, which ensured good air circulation around the pots.

Seeds began to germinate in March 2009. A seed was considered germinated when the radicle began to protrude from the seed coat. Germinated seeds were counted and removed about every second week from 20 March until 6 November 2009. A 2-month summer break (28 June-3 September) was induced after germination had completely stopped to simulate summer drying, when pots were not watered. After the summer break, during autumn, we experienced very low germination (3.06% of all germinated seeds appeared between 3 September and 6 November), while a compact moss layer had developed in several pots (in the frequently watered group). The moss layer could influence our results, thus we decided to omit the autumn period from the analysed data set.

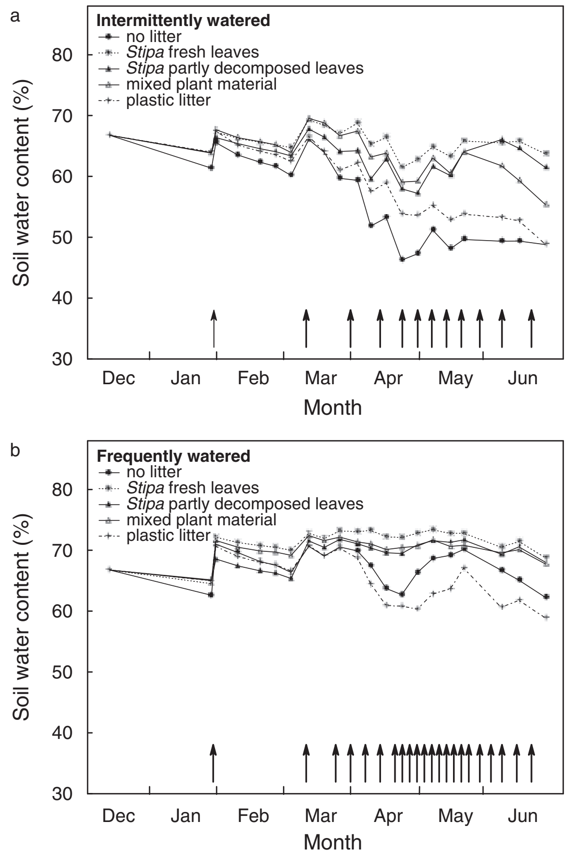

Fig. 1. Soil water content in the intermittently (a) and frequently watered (b) experimental groups. Data are means of five replicate experimental pots subjected to different litter treatments. Arrows indicate watering occasions (see Methods).

We monitored soil water content at approximately weekly intervals during the whole experiment by weighing five pots per litter treatment×watering frequency combination. Soil water content was expressed as a percentage of soil weight ((soil weight − dry soil weight)×soil weight^{-1}×100). In the laboratory, we examined light extinction through the litter types used in this experiment. We used five samples from each litter type in the same quantity as applied during the controlled pot experiment. Samples were placed one after the other in the central 10×10 cm portion of a transparent polyethylene sheet surrounded by the cut upper part of an experimental pot to simulate experimental conditions on the soil surface within the pot. A light source was placed 1 m above the polyethylene sheet and light measurements were taken with a quantum radiometer (Quantitherm light meter, Hansatech, UK) at nine equidistant points (corners of a grid) marked previously (see Facelli & Pickett 1991b). Averages between the nine measuring points for each sample and litter type were used to express the average quantity of transmitted light (PPFD). We also did laboratory temperature measurements in order to compare soil surface temperature and its amplitude between litter treatments at 0, 10, 20 and 30°C . Five measurements were made under the gradually increasing temperatures in one pot per treatment when ambient temperature reached the assigned value. For temperature measurements, pots were filled with moist soil and dry litter was put on top. Amplitude was determined as the magnitude of temperature change in a pot between the four temperatures. In order to determine litter weight loss, as a measure of decomposition rate, we weighed the amount of litter left at the end of the experiment in five pots per litter treatment×watering frequency combination.

To reveal potential allelopathic content of *S. pulcherrima* leaves and its dependence on the litter phenological stage, leaves collected at the beginning (May) and at the end (October) of the vegetation period were subjected to chemical analyses. A water extract was prepared by soaking 104.4 g of dry leaves in 1000 ml distilled water for 48 h at room temperature (Ruprecht et al. 2008). Leaf material was cut into ca. 5-cm pieces before water extraction. The leachates were vacuum-filtered through Whatman No.1 filter paper. After filtering, a 50-ml sample was lyophilized for water elimination and concentration of potential phenolic acids. The lyophilized sample contained 1-4% water. Further water elimination using ethyl acetate and separation of the two phases was followed by drying with sodium sulphate. The pure organic solution was concentrated until dry on a rotary evaporator. The concentrated and purified samples were analysed with gas chromatography coupled with mass spectrometry (GC-MS). The dried extract was eluted with 0.5 ml methanol, and the solvent used to carry the samples in the column (30 m long, 0.25 mm diameter HP-5 MS non-polar column) was helium. In order to protect the functional groups of the aromatic molecules, we made a derivatization with a pyridine-based reagent (150 μl ml^{-1}, reaction time 30 min, 60°C): Sylon HTP, hexamethyldisilazane+‴ trimethylchlorosilane+pyridine (HMDS+TMCS+Py, 3:1:9). During analysis, the oven temperature was held at 50°C for 0.1 min and raised at 15°C min^{-1} to 280°C.

Field experiment

We conducted a seed sowing experiment at Suatu (46°79′N, 23°97′E, 419 m a.s.l., Transylvanian Lowland, Romania; for details see Ruprecht et al. 2010) in a dry grassland site that was abandoned from grazing 40 years ago. The accumulation of litter after 40 years was 470 g m^{-2}. The grassland was dominated by *S. pulcherrima*, and had a homogeneous species composition. A total of 168 plots of 25 cm×25 cm, with 50 cm between each, were subjected to three different experimental treatments: (1) litter removal, (2) litter removal and plastic litter application, (3) natural litter left intact. The same type of plastic litter was used as in the pot experiment, applied in the same quantity as natural litter (30 g plot^{-1}). In order to compare light interception of natural and plastic litter, we measured photosynthetically active photon flux density (PPFD) on the soil surface and above vegetation in eight plots per each treatment type on 24 May and 15 September 2008, using a quantum radiometer. Averages between eight measuring points within the plots were used as estimates of PPFD (%) reaching the soil surface under different experimental treatments.

Seeds or fruits of six dry grassland species, which had been collected in 2007 from the experimental grassland, were dry-stored in darkness at room temperature and sown in the experimental plots on 1 March 2008. Species belonged to different families and had different seed sizes from very small (0.07 mg) to relatively large (11.09 mg) (Table 1). Species involved in the field experiment only partially corresponded to those used in the pot experiment (there were two common species: *D. ischaemum*, *Dorycnium pentaphyllum*), as we wanted to broaden the validity of our results to a larger set of grassland species. 100 seeds (except for *Achillea collina*, where we applied 150 seeds due to low seed viability; see Table 1) of one species were sown in each plot after litter removal (treatment 1), after litter removal and before the application of plastic litter (treatment 2), and below the natural litter (treatment 3). Each species×treatment combination was replicated eight times, and in addition we installed eight plots per each treatment without any seed addition to control for the natural seed rain, since species used in this experiment were all naturally occurring in the experimental grassland. Species, or their absence, and treatments were assigned randomly to plots. Seed germination was followed from 30 March 2008 until 23 May 2009 by monthly examination, counting and marking germinating seeds or appearing seedlings. Litter removal was gently repeated in the second year on 5 March 2009. Continuing seedling counting beyond the first year was necessary since one of the species, *Muscari tenuiflorum*, only germinated during the second year.

Table 2. Physical and mechanical properties of different litter types used in the controlled pot experiment. Results of transmitted radiation and soil surface temperature are based on laboratory measurements, while soil water content is the average of weekly measurements during the whole experiment. In superscript different letters denote significant differences at $P < 0.05$ between the groups (see results, Tukey HSD test).

	Watering type	Soil water content (%, mean ± SE)	Transmitted radiation (PPFD, mean ± SE)	Soil surface temperature (°C)					Litter weight loss (%, mean ± SE)
				Mean				Amplitude	
				0°C	10°C	20°C	30°C		
No litter	Intermittent	56.45 ± 1.57^{d}	59.29 ± 0.88^{a}	2.2	10.9	17.4	28.9	26.7	
	Frequent	67.35 ± 0.61^{b}							
Stipa fresh leaves	Intermittent	65.62 ± 0.43^{bc}	11.33 ± 0.29^{b}	7.5	11.2	18.8	29.0	21.5	29.15 ± 0.72
	Frequent	71.28 ± 0.49^{a}							54.89 ± 1.89
Stipa partly decomposed leaves	Intermittent	63.46 ± 0.63^{bc}	5.27 ± 0.22^{c}	9.3	10.5	18.2	28.7	19.3	12.55 ± 1.73
	Frequent	69.23 ± 0.48^{ab}							25.74 ± 1.76
Mixed partly decomposed plant material	Intermittent	63.88 ± 0.80^{bc}	4.04 ± 0.34^{d}	5.8	11.3	18.0	28.9	23.1	21.49 ± 4.40
	Frequent	70.16 ± 0.39^{ab}							32.55 ± 4.32
Plastic litter	Intermittent	59.42 ± 1.25^{cd}	9.47 ± 0.34^{b}	4.7	11.1	16.9	28.1	23.4	
	Frequent	65.42 ± 0.83^{bc}							

Data analysis

A three-way factorial analysis of variance model (ANOVA) was used to analyse the fixed effects of litter treatments, watering frequency and species identity on cumulative germination in the pot experiment. Orthogonal contrasts were calculated between litter treatments and between watering frequency×species×litter treatment combinations.

In the field experiment, a two-way fixed effects ANOVA was performed to test the effects of litter treatment and species identity on cumulative germination. The number of seedlings appearing in 15 month was pooled for each plot (cumulative number of seedlings). Orthogonal contrasts were calculated between the treatments: litter removal versus plastic litter, and natural litter as well as plastic litter versus natural litter separately, for each species.

As a measure of the relative contribution of each factor and their interactions on total variability in cumulative germination, 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). All analyses were done using Statistica, version 6.0 (StatSoft Inc., Tulsa, OK, US).

Results

Physical effects

Soil water content was significantly influenced by the watering frequency and litter treatment, and watering frequency differentially influenced the water content of soils covered by different litter types (repeated measures ANOVA, watering frequency $F_{1,40} = 178.88$, MS = 13 230, $P < 0.001$, litter treatment $F_{4,40} = 25.14$, MS = 1859, $P < 0.001$, watering frequency×litter treatment $F_{4,40} = 3.73$, MS = 276, $P = 0.011$, Error MS = 74; Fig. 1). Natural litter types were more or equally effective in maintaining soil moisture compared to plastic litter or soil without any cover, and the plastic litter had no protective role in retaining soil moisture (Table 2 and Fig. 1).

Light interception was significantly different between the litter treatments (one-way ANOVA, ln-transformed data, $F_{4,20} = 542.56$, MS = 5.53, $P < 0.001$, Error MS = 0.01). The treatment without litter had the highest quantity of light reaching the soil surface (Table 2). Natural litter types differed in their light interception, with *Stipa* fresh leaves transferring the highest quantity of light and the mixed partly decomposed plant material the lowest. Plastic litter mimicked the light regime under fresh *Stipa* leaves (Table 2).

Average soil surface temperature as measured at 0, 10, 20, and 30°C (two-way ANOVA, litter type $F_{4,80} = 129.1$, MS = 13.56, $P < 0.001$, temperature $F_{3,80} = 23\,132.2$, MS = 2431.2, $P < 0.001$, litter type ×temperature $F_{12,80} = 86.5$, MS = 9.09, $P < 0.001$, Error MS = 0.11) and temperature amplitude (one-way ANOVA, litter type $F_{4,20} = 211.68$, MS = 37.09, $P < 0.001$, Error MS = 0.18) differed significantly between litter types applied in pots. Bare soil had the lowest mean temperature and highest amplitude in the measured interval. Natural litter types had similar thermal properties, and plastic litter occupied an intermediate position between bare soil and natural litter types (Table 2).

Persistence of natural litters used in the pot experiment differed between each other and between watering groups. Weight loss, as an estimate of decomposition rate, was consistently higher in the frequently watered group. Fresh *Stipa* leaves had the highest weight loss compared to other litter types, and partly decomposed *Stipa* leaves the lowest (Table 2).

Under field conditions, our treatments created differences in the light regime of differently treated plots (repeated measurement ANOVA, ln-transformed data, treatment $F_{2,21} = 86.52$, MS = 12.89, $P < 0.001$, Error MS = 0.15, time $F_{1,21} = 66.64$, MS = 7.61, $P < 0.001$, treatment×time $F_{2,21} = 4.51$, MS = 0.52, $P = 0.023$, Error MS = 0.11). Natural and plastic litter ensured similar, reduced light conditions for seeds located on the soil surface, compared to litter removed plots (PPFD% mean ± SE: natural litter 1.09 ± 0.16, plastic litter 1.13 ± 0.10, without litter 5.40 ± 0.63), and light interception by natural litter was slightly higher than that of plastic litter in the first part of the vegetation period (orthogonal contrasts of natural litter versus plastic litter: May $F_{1,21} = 4.74$, $P = 0.041$, September $F_{1,21} = 2.09$, $P = 0.163$). Annual precipitation was above or around the average (615 mm) during the two study years (2008: 675 mm, 2009: 594 mm), while mean temperatures were above the average in both years (average: 8.35°C, 2008: 9.8°C, 2009: 10.0°C; data from Cluj-Napoca Meteorological Station).

Phytotoxic constituents of S. pulcherrima leaves

We detected several allelopathic compounds in the spring-collected leaves of *S. pulcherrima*: o-hydroxybenzoic acid, trans-cinnamic acid, 4-hydroxybenzoic acid, 4-hydroxy-3-methoxybenzoic acid (vanillic acid) and 3-hydroxy-4-methoxybenzoic acid (isovanillic acid) (Rice 1995; Blum et al. 1999). In comparison, in the autumn-collected leaves we found succinamic acid, butanedioic acid (succinic acid) and vanillin, which are known to have allelopathic effects (Groner 1974; Harborne 1993; Quayyum et al. 1999), and other compounds, like piperazine or 2,3-dihydrobenzofuran (known as coumarin), which are potential allelopathic agents (Khanh et al. 2008). The number of organic acids detected in the spring leaves was much higher (27) than in the autumn leaves (10).

Germination

Natural litter used in the pot experiment contained almost no seeds of the involved species, and spontaneous germination in the field plots was also negligible (Table 1), thus not affecting our experimental results.

Table 3. Effects of watering frequency, species identity and litter treatment on cumulative germination in the controlled pot experiment, and species identity and treatment in the field. df = degrees of freedom, MS = mean sum of squares, vc (%) = relative contribution of individual factors and their interactions to total variation.

Source of variation	df	MS	*F*	*P*	vc (%)
Controlled pot experiment					
Intercept	1	184 255.6	5365.04	<0.001	
Watering	1	2176.2	63.37	<0.001	3.4
Species	7	5694.0	165.80	<0.001	62.7
Treatment (litter type)	4	501.1	14.60	<0.001	3.2
Watering×Species	7	279.7	8.15	<0.001	3.1
Watering×Treatment	4	472.9	13.77	<0.001	3.0
Species×Treatment	28	90.4	2.63	<0.001	4.0
Watering×Species×Treatment	28	77.1	2.24	<0.001	3.4
Error	320	34.3			
Field experiment					
Intercept	1	46 260.8	805.8	<0.001	
Species	5	4206.0	73.3	<0.001	59.6
Treatment	2	260.1	4.5	0.013	1.5
Species×Treatment	10	647.7	11.3	<0.001	18.4
Error	126	57.4			

In the pot experiment, cumulative germination across watering frequencies and litter treatments varied significantly and strongly among species (Tables 1 and 3, Fig. 2a and b), and ranged between 8.40% (*Viola hirta*) and 69.24% (*Verbascum phoeniceum*). Watering frequency and litter treatments, as well as their interactions, significantly influenced cumulative germination. At the same time, applied litter treatments had differential effects on germination of the species involved (Table 3, Fig. 2a and b). From the eight dry grassland species used in the controlled pot experiment, very low number of *V. hirta* germinated, thus there were only trends in its germination responses that were not statistically significant.

Under intermittent watering, plastic or natural litters had a positive effect on the germination of seven dry grassland species compared to bare soil, independent of their seed size (Fig. 2a and Table 4); while under frequent watering, the positive effect disappeared in most cases (except *Jurinea mollis*, the largest seeded species) or became negative (in *Campanula sibirica*, the smallest seeded species) (Fig. 2b and Table 4). Except in a single case, *Salvia nutans* in the frequently watered group, plastic litter did not differ from natural litters in terms of germination. However, there were some remarkable differences between the three natural litter types, mainly under frequent watering (Fig. 2a and b, Table 4). The two smallest seeded species, *C. sibirica* and *V. phoeniceum*, reacted differently to the three natural litter types, and

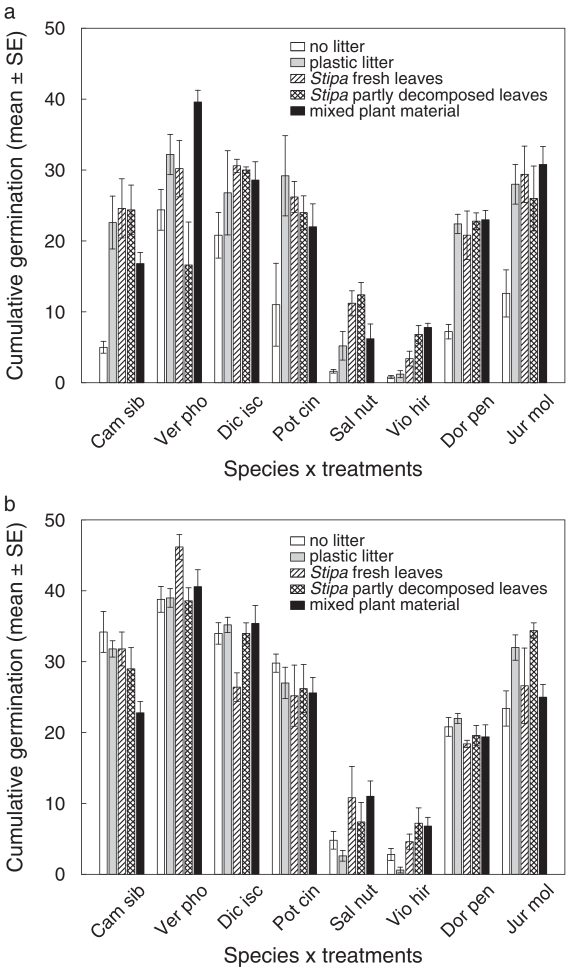

Fig. 2. Effects of different litter treatments on the cumulative germination of eight dry grassland species in the controlled pot experiment under intermittent (a) and frequent (b) watering. Species are plotted in increasing order of seed mass. Results of a three-way factorial ANOVA. Significant differences between the groups are presented in Table 4.

germination of *C. sibirica* was consistently negatively influenced by the mixed partly decomposed plant material under both watering frequencies, while germination of *V. phoeniceum* was lowest under partly decomposed *Stipa* leaves compared to the other two natural litter types. In the case of larger seeded species, as a more general scenario, but only under frequent watering, fresh *Stipa* leaves decreased seed germination of *D. ischaemum* and *J. mollis* compared to the other two natural litter types, and in the case of the poorly germinating *V. hirta* the same trend was observed (Fig. 2a and b, Table 4).

In the field experiment, there were also large differences in germination abilities of species sown in grassland plots (Tables 1 and 3, Fig. 3), ranging from 2.78% (*A. collina*) to 41.79% (*M. tenuiflorum*). Treatments applied had different effects on the cumulative germination of sown seeds of grassland species (Table 3). Under field conditions, the germination of smaller seeded species, *A. collina* and *Veronica spicata*, was decreased by both natural and plastic litters, while in the case of larger seeded species litters acted in different ways. Both litter types increased germination of *D. pentaphyllum* and *M. tenuiflorum* compared to the litter removed plots, while natural litter reduced germination of seeds of *D. ischaemum* and *Adonis vernalis* compared to plastic litter and bare soil surfaces.

Discussion

Germination responses in the light of seed size

Species identity was found to account for much of the variation in results, which can be attributed to the highly variable germination success of the tested species, both under controlled and field conditions, governed by species-specific germination requirements and plant traits (Baskin & Baskin 2001).

Seed size is one of the most important plant traits determining the success of plant recruitment (Moles & Westoby 2004, 2006), shaped mainly by environmental factors, which are usually influenced by local biotic conditions, e.g. neighbouring vegetation or accumulated litter (Ryser 1993; Eckstein & Donath 2005; Hölzel 2005; Kahmen & Poschlod 2008). It is well known that small-seeded species are light-demanding during their recruitment (Hodkinson et al. 1998; Milberg et al. 2000; Jensen & Gutekunst 2003; Eckstein & Donath 2005), and we also found that germination of three out of the four small-seeded species (seed mass <0.25 mg) used in our experiments was negatively influenced by the litter cover. The other small-seeded species, *V. phoeniceum*, had distinct germination behaviour, not responding to light supply but to other factors. Its germination increased beneath two of the natural litter types, fresh *Stipa* leaves and mixed partly decomposed plant material, compared to bare soil. These natural litter types lost a high percentage of their weight through decomposition (21.5 to 55%) during the experiment. Nitrate concentrations have been shown to regulate the onset of germination in several plant species (Baskin & Baskin 2001). Thus, nutrients released during decomposition probably exerted a positive effect on seed germination of *V. phoeniceum*. These latter findings stress the importance of background factors in interpreting light dependency of germination (Ryser 1993; Kahmen & Poschlod 2008).

Table 4. Orthogonal contrasts between watering frequency × species × litter treatment combinations in the controlled pot experiment (three-way ANOVA, see Table 3). Species are listed in the increasing order of seed mass. No = no litter, Sl = *Stipa* fresh leaves, Ss = *Stipa* partly decomposed leaves, Ms = Mixed partly decomposed plant material, Nl = natural litter (Sf, Ss, and Ms), Pl = plastic litter; Asterisks denote significant differences at $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$.

Contrasts	Species							
	Cam sib	Ver pho	Dic isc	Pot cin	Sal nut	Vio hir	Dor pen	Jur mol
Intermittently watered								
No versus Nl	***	ns	**	***	**	ns	***	***
No versus Pl	***	*	ns	***	ns	ns	***	***
Pl versus Nl	ns	ns	ns	ns	ns	ns	ns	ns
Sf versus Ss	ns	***	ns	ns	ns	ns	ns	ns
Sf versus Ms	*	*	ns	ns	ns	ns	ns	ns
Ss versus Ms	*	***	ns	ns	ns	ns	ns	ns
Frequently watered								
No versus Nl	*	ns	ns	ns	ns	ns	ns	ns
No versus Pl	ns	ns	ns	ns	ns	ns	ns	*
Pl versus Nl	ns	ns	ns	ns	*	ns	ns	ns
Sf versus Ss	ns	*	*	ns	ns	ns	ns	*
Sf versus Ms	*	ns	*	ns	ns	ns	ns	ns
Ss versus Ms	ns	ns	ns	ns	ns	ns	ns	*

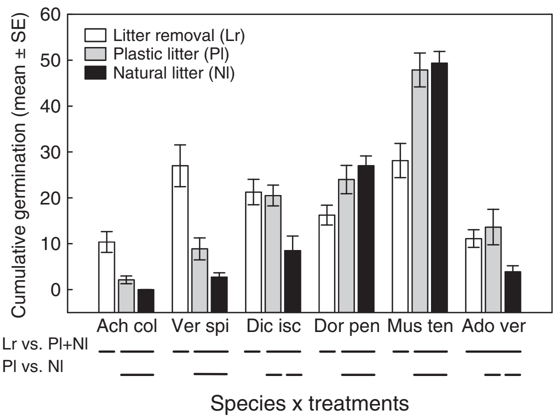

Fig. 3. Effects of treatments on cumulative germination in the field of six dry grassland species. Species are plotted in increasing order of seed mass. Results of a two-way factorial ANOVA. Lines below the bars indicate significance as revealed by orthogonal contrasts between the treatments: litter removal versus plastic and natural litter, plastic litter versus natural litter; broken lines indicate significant differences at $P<0.05$.

It has been shown that larger seeds are better able to establish or survive some hazards, e.g. competition from established vegetation, litter or soil cover, drought and shading, than smaller seeds (reviewed by Moles & Westoby 2004, 2006, but see also Gulmon 1992; Peterson & Facelli 1992; Xiong et al. 2001). Furthermore, larger seeds can benefit from cover by litter, especially in situations of water shortage. Before germination, seeds need to imbibe water in order to reach full turgor for cell elongation. Larger seeds need more time for water absorption than smaller seeds, especially if they are not buried but located on the soil surface, since they have a smaller surface area to mass ratio (Kikuzawa & Koyama 1999). Additionally, water absorption can be retarded by a thick endocarp or seed coat, which is characteristic of larger seeds (Moles et al. 2003). Therefore, larger seeds are expected to require higher and more constant soil humidity prior to germination than smaller seeds. This is in line with results from several field investigations, where litter was shown to have facilitative effects on the germination of large-seeded species under harsh environmental conditions (Fowler 1986; Eckstein & Donath 2005; Albrecht & McCarthy 2009; Donath & Eckstein 2010). Based on our measurements, natural litter treatments provided protection against soil desiccation, which led to a facilitative role of natural litter on germination success of larger seeded species. This effect was intensified under intermittent watering in the pot experiment, where natural litters advanced germination of both larger and smaller seeded species, masking the light reduction effect on small seeds of these litter types. Consequently, environmental conditions, in this particular case less favourable moisture supply during germination, can affect even the most widely accepted relationships between seed size and light requirement (literature cited above), and may rapidly lead to divergent results (e.g. Hölzel 2005; Kahmen & Poschlod 2008).

Using plastic material to compare chemical and physical litter effects

Using plastic fibres of a cloth developed for shading as a so-called "artificial litter", is a rarely used in plant ecology studies to address and separate the various roles of living and dead biomass on plant recruitment (Grime & Jeffrey 1965; Rotundo & Aguiar 2005; Amatangelo et al. 2008). The plastic litter used in the present experiment was not expected to release nutrients and inhibitory chemical substances, thus offering a good possibility to reveal the chemical effects of natural litters. At the same time, its physical properties, apart from light interception, were far from those of natural litters. Even so, we found almost no differences in the effect of plastic and natural litters on germination of grassland species in the pot experiment, while plastic litter represented a more suitable environment compared to natural litter for seed germination of two out of the six species sown in field plots. Thus, contrary to our expectations and former findings (Ruprecht et al. 2008), physical or mechanical (seed protection against predators) litter effects were proved to be more important than chemical effects in determining germination success.

Differences between natural litter types

Based on our measurements, natural litter types originating from the same dry grassland and used in the pot experiment differed in their light interception and persistence, but created a similar soil surface temperature and had similar water-holding capacity. Contrary to the relatively low variance in physical properties, significant differences were found in their effect on germination, much of which can be explained by chemical factors. Corresponding to our expectations and to results presented by Datta & Sinha-Roy (1975) and Bokhari (1978), GC-MS analysis of *Stipa* leaf extracts detected more phenolic compounds with known allelopathic effects in freshly dried than in senescent leaves. This may demonstrate that more allelopathic substances are produced in the first part of the vegetation period, while towards autumn plant synthesis could switch to storage, with the appearance of glycerine or arabinitol in autumn leaves of *S. pulcherrima*. Under frequent watering, probably leading to more intense leaching of chemical substances, fresh *Stipa* leaves exerted a significant negative effect on germination of two larger seeded species, *D. ischaemum* and *J. mollis*, and a similar trend was observed in the germination response of *V. hirta*, compared to the other natural litter types. This result, although substantially weaker, is in line with the findings of a former laboratory experiment, which demonstrated that an aqueous extract of *S. pulcherrima* leaves inhibited seed germination and radicle elongation of all the tested dry grassland species (Ruprecht et al. 2008).

During decomposition, nutrient release from natural plant material can stimulate seedling growth (Schlatterer & Tisdale 1969), and we found increased germination of *V. phoeniceum* under the more rapidly decaying litter types, which amplifies differences in chemical properties of natural litters. These results emphasize possible differences between natural litter types, determined mainly by chemical differences attributable to the decay state (see also Datta & Sinha-Roy 1975; Bokhari 1978), thus the importance of the deliberate selection of "litters" used in the experiments.

Comparison of effects of litter between pot and field experiments

In natural systems, neighbouring plant species or unpredictable circumstances (e.g. seed predators, pathogens and altering weather) can influence the effects of litter, the outcome of the so-called "after death interactions" (Reader 1993; Ryser 1993; Hölzel 2005; Kahmen & Poschlod 2008), which could be the cause of the conflicting results experienced between pot and field experiments (e.g. Sydes & Grime 1981; Rotundo & Aguiar 2005). In our case, such a comparison needs cautious examination, since the species used in the two experiments only partially overlapped. However, a careful comparison can be made based on species traits, e.g. seed size. Under field conditions, natural litter exerted mostly negative effects on seed germination of the test species, positive effects were experienced only in two larger seeded species, *D. pentaphyllum* and *M. tenuiflorum*, while under controlled pot conditions litter effects were mainly positive (under intermittent watering) or neutral (under frequent watering), except for the germination inhibiting effect of fresh *Stipa* leaves on three out of the eight test species. Small-seeded species reacted more intensely, by reduced cumulative germination, to the same quantities of litter under field than in pot experiments. It is probable that in field plots light interception of the litter was intensified through effects of the surrounding vegetation. Foster & Gross (1998) also found that litter and living above-ground biomass have additive effects on seedling emergence.

Conclusions

The findings of this study underline the great significance of species-specific requirements for microsite quality during recruitment. These different demands may govern changes in succession after field abandonment and accumulation of plant litter (Myster 1994; Jensen & Schrautzer 1999). The obtained results also suggest that facilitating or inhibiting effects of litter on seed germination are strongly context-dependent, and it is difficult to define common rules that apply under differing environmental conditions. Our results are in concert with other studies on dry grasslands, which found that physical or mechanical impacts of litter are more important than its chemical effects in shaping recruitment success and community structure (e.g. Rotundo & Aguiar 2005; Amatangelo et al. 2008). At the same time, chemical litter effects should not be underestimated, since we found differential effects of fresh *S. pulcherrima* leaves on the germination of four out of the eight tested species under controlled conditions (see also Ruprecht et al. 2008). Differences between natural litters were mainly attributable to decay state and less to composition. Hence, our study stresses the importance of litter type used in germination tests (Myster 2006; Quested & Eriksson 2006; Donath & Eckstein 2008).

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