

Comparative ecology of seedling recruitment in an oligotrophic wet meadow

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Abstract. For the regeneration niche to contribute to the maintenance of species diversity interspecific differences in sensitivity of seedling recruitment to environmental conditions is assumed. We experimentally tested differences between meadow species for the response of seed germination to chilling, and sensitivity of seedling recruitment to microscale heterogeneity. We also compared the dynamics of seedling recruitment in gaps. Seed germination was tested in standard laboratory germination tests, comparing control seeds with seeds chilled at +4 °C, and at –14 °C for one month. Species responses varied from significant increases in germinability after chilling (e.g. *Cirsium palustre*, *Betonica officinalis*, *Angelica sylvestris*) to significant decreases (e.g. *Hieracium umbellatum*, *Succisa pratensis*, *Selinum carvifolia*). In some species, chilling at +4 °C has a similar effect to chilling at –14 °C, in others the effect of chilling at +4 °C was intermediate, and in some, there was no effect of chilling at +4 °C, but an effect of chilling at –14 °C. Different chilling temperatures also affect timing and speed of seed germination under greenhouse conditions.

The dynamics of seed germination under field conditions was studied by sowing seeds into artificially created gaps and following their germination, both where the seedlings were removed after emergence and where they were not removed. Species differ in their germination dynamics: they all start late April, but then differentiate from an abrupt maximum and early finish of germination (in the second half of May), to prolonged germination without a marked maximum. Seedling removal increased the total number of germinated seeds, with a marked density dependence at this stage. Seeds were also sown into plots with treatments (1) gaps, sod stripped, above-ground vegetation removed; (2) mown, moss layer removed; (3) mown; (4) untouched control. Seedling emergence was monitored for 3 yr. Seedling recruitment decreased from treatments (1) to (4), but sensitivity differed between species. With increasing seed weight, the difference between gaps and other treatments decreased. The results show that there are considerable differences in seedling recruitment sensitivity between species.

Keywords: Chilling; Dormancy; Gap regeneration; Germination; Moss competition.

Nomenclature: Rothmaler (1976).

Introduction

Recent studies into mechanisms that maintain species diversity stress the dynamical nature of processes and species mobility (van der Maarel & Sykes 1993, 1997; Herben et al. 1993, 1997; Huston 1994). Seedling recruitment is an important part of this mobility (e.g. Kalamees & Zobel 1997). Even in communities dominated by clonal plants, certain species are dependent on seedling recruitment and for clonal plants regeneration by seed is important for the maintenance of genetic diversity (Watkinson & Powell 1993). Seedling recruitment requires specific conditions (e.g. Grubb 1977) and is often much more sensitive to environmental conditions and competition than are established plants (Křenová & Lepš 1996; Morgan 1997; Špačková et al. 1998). Such interspecific differences in conditions for seedling recruitment are considered important for the maintenance of species diversity (e.g. Grubb 1977, 1988; Rusch & Fernández-Palacios 1995).

Seedling recruitment usually depends on safe sites, microhabitats suitable for emergence and survival of seedlings. Gaps in the vegetation (i.e. places with decreased competition from established plants) are of vital importance for seedling recruitment (Goldberg 1987; Křenová & Lepš 1996). In gap colonization, the initial colonizer has a competitive advantage, and the process has features of founder control (Yodzis 1978). One important factor determining the identity of the initial colonizer could be emergence time. Identity of the initial colonizer may depend on timing of seed release and phenology of seed germination, with gaps originating in different times of the year expected to be colonized by different species (Hobbs & Mooney 1985, 1991). Such variation could thus promote species coexistence. Similarly, differences in seed sensitivity to chilling may alter the identity of the initial colonizer depending on the weather in a particular year, and might also promote species coexistence. However, these mechanisms depend on the assumption that seedling recruitment is sensitive to environmental variation, mainly temperature and light, and that species differ considerably in their response

(differences in regeneration niche *sensu* Grubb 1977). The aim of this paper is to test this assumption. We compare the sensitivity of seed germination and recruitment to biotic and abiotic environmental factors in several wet meadow species notably to effects of chilling, gaps, moss layer and established vegetation.

Study site

This study site is a wet, oligotrophic, species-rich meadow 10 km southeast of České Budějovice, Czech Republic, 48°57'N, 14°36'E, at 510 m a.s.l. Mean annual temperature is 7.8 °C, mean annual precipitation is 620 mm (České Budějovice Meteorological Station). July is the wettest and warmest month with 102 mm rain and temperatures with mean daily minima and maxima of 11.6°C and 24.1°C. Mean daily minima and maxima in January (the coldest month) are -6.2°C and 0.6°C. Soil nutrient levels are low (total nitrogen 6-8 g/kg dry soil weight, total phosphorus 400-500 mg/kg dry soil weight, C/N ratio 16-20). Phytosociologically, the vegetation belongs to the *Molinietum caeruleae* (*Molinion*) with some elements of the *Violion caninae*. Seeds for all the experiments were collected at this locality.

Methods

Experiment 1: Seed-sensitivity to chilling (Laboratory test of germinability)

Seed germination of 27 species from the study site (Table 1) was tested by germinating 300-3600 seeds of each species in Petri dishes under greenhouse conditions. Before testing seeds were stored dry at +20°C for three months following collection. Three 30-day treatments were applied to test whether chilling is needed for germination: dry seed storage at +4°C, -14°C vs. +20°C. In cold winters, several weeks with temperatures below -10°C occur. After the treatment seeds were sown in Petri dishes on moist blotting paper (50-100 seeds per dish) in the greenhouse at +20°C. The dishes were checked daily and seedlings and rotten seeds counted and removed. Each experiment was terminated when there was no germination for at least one week, but not earlier than 30 days after sowing seeds (line drawings of seedlings at different stages of development obtained from this experiment will be available at: <http://www.bf.jcu.cz/bio/suspa/seedling.htm>). For statistical analyses, each seed was considered an independent observation. Confidence interval limits for germinability were calculated according to Zar (1984, p. 378). Differences in germinability

between treatments were tested using 2×3 contingency tables. Relative germination speed was characterized by the number of days since sowing, median day – when half of the seeds had germinated – and day when the last seed germinated. Treatment effects were tested by a version of the median test: for each species a common median day for all three treatments was determined, and then by the use of 2×3 contingency tables the number of seeds that germinated before and after the common median in the three treatments were compared.

Experiment 2: Dynamics of seed germination in gaps

Seed germination in artificially created gaps was monitored for 12 species of which seeds were sown in November 1995, and followed over the next growing season. Gaps were created by stripping the sod and removing all of the above-ground vegetation and most of the roots. Seeds of six of the species (*AchPta*, *BetOff*, *LycFlo*, *SanOff*, *SelCar* and *SucPra*) were sown into four gaps each of 0.3m×0.3m. Seedlings were removed as they emerged from two of the plots, and were not removed from the other two plots. Seeds of another six species (*AngSyl*, *CarHar*, *CarPal*, *CirPal*, *HieUmb*, *LycFlo* and *LysVul*) were sown into only two gap plots of 0.3m × 0.3 m and seedlings were removed from both plots. In the central 0.25m × 0.25 m of each gap plot, 1000 seeds of each species were sown (only one species per plot), except for *LycFlo* and *LysVul* for which 2000 seeds were sown (also one species per plot). Seedling emergence was monitored at ca. 10-day intervals. Differences between species were evaluated by repeated measures ANOVA after log(x+1) transformation (Anon. 1996).

Experiment 3: The effect of litter layer and moss layer on seedling establishment

The effect of litter layer and moss layer on seedling establishment was tested in a manipulative field experiment. For six species (*AngSyl*, *BetOff*, *SanOff*, *SelCar*, *SerTin* and *SucPra*) 0.4m×0.4m plots were located in four randomized complete blocks. One additional non-

Table 1. Species with species code used in Experiment 1.

<i>Achillea ptarmica</i>	AchPta	<i>Lysimachia vulgaris</i>	LysVul
<i>Angelica sylvestris</i>	AngSyl	<i>Myosotis nemorosa</i>	MyoNem
<i>Anthoxanthum odoratum</i>	AntOdo	<i>Potentilla erecta</i>	PotEre
<i>Betonica officinalis</i>	BetOff	<i>Prunella vulgaris</i>	PruVul
<i>Carex hartmanii</i>	CarHar	<i>Ranunculus auricomus</i>	RanAur
<i>Carex pallescens</i>	CarPal	<i>Ranunculus flammula</i>	RanFla
<i>Cirsium palustre</i>	CirPal	<i>Sanguisorba officinalis</i>	SanOff
<i>Cynosurus cristatus</i>	CynCri	<i>Scorzonera humilis</i>	ScoHum
<i>Galium boreale</i>	GalBor	<i>Selinum carvifolia</i>	SelCar
<i>Galium uliginosum</i>	GalUli	<i>Senecio rivularis</i>	SenRiv
<i>Hieracium umbellatum</i>	HieUmb	<i>Serratula tinctoria</i>	SerTin
<i>Jacea pratensis</i>	JacPra	<i>Succisa pratensis</i>	SucPra
<i>Luzula campestris</i>	LuzCam	<i>Valeriana dioica</i>	ValDio
<i>Lychnis flos-cuculi</i>	LycFlo		

sown randomized complete block for each species was used as a control for natural seed germination and seedling recruitment. In each block, the following treatments were used:

- (1) gap (sod stripped, all above-ground vegetation removed);
- (2) plot mown and moss layer removed by hand;
- (3) plot mown;
- (4) untouched control.

In late March 1500 seeds of one of the six species were sown (one species in each plot), SelCar and SerTin in 1995, AngSyl, BetOff, SanOff, SelCar, SucPra in 1996 – 750 seeds for SerTin and treatment 2 omitted; SelCar was used in both years to check for differences between years. Seedlings were counted for three years in early summer, midsummer and autumn. Gaps were made in the second half of March before sowing. Treatments (1), (2) and (3) included mowing after counting seedlings in early summer and in midsummer. Litter was removed in treatments (1), (2) and (3) in March of each year.

The data were transformed: $x' = \log(x+1)$ and treated by a repeated-measures ANOVA-model (Anon. 1996). Note that with log-transformed data the interaction between time and treatment reflects differences in relative changes in time. Let $X_{i,t1}$ and $X_{i,t2}$ be the numbers of seedlings in treatment i at times $t1$ and $t2$. When the relative changes are constant, i.e. $X_{i,t2} = X_{i,t1} \cdot k$ for any i (i.e. for all treatments), then $\log(X_{i,t2}) = \log(X_{i,t1}) + \log(k)$ for any i . Then the lines are parallel and there is no interaction between time and treatment. A significant interaction means that values of k differ according to the treatment. If there is no germination, then k is survival (1-mortality) and the interaction then reflects differences in mortality. As $\log(x+1)$ had to be used (because the data contained zeros), the above hold only approximately. Interspecific differences of species sown in 1996 were compared using repeated-measures ANOVA after $\log(x+1)$ transformation. (It would be incorrect to include the two species sown in 1995 because of different census dates for both species and missing treatment (2) for SerTin.

Results

Experiment 1: Seed-sensitivity to chilling (Laboratory test of germinability)

Species differed in germinability, germination speed, relative sensitivity to chilling, and response to different chilling temperatures. Three species did not germinate at all (CarHar, CarPal and ValDio) and germinability of SenRiv was 1% for control seeds and zero after chilling. Germinability of LuzCam and LycFlo was almost 100%.

In 12 of the species chilling had no significant effect,

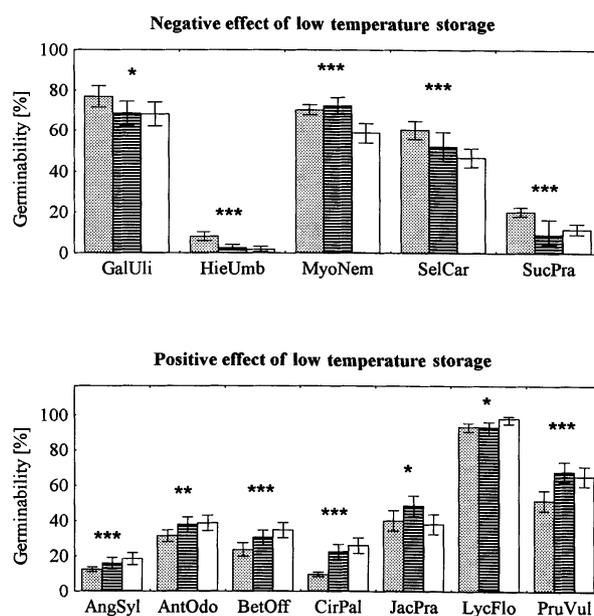


Fig. 1. Germinability under greenhouse conditions with 95 % confidence intervals after dry seed storage at +20°C (dots), after dry chilling at +4°C (hatched) and after dry chilling at -14°C (white). Significance of the effect of seed storage temperature: * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$. For species codes, see Table 1. Species not significantly affected are not shown.

in five species chilling decreased germinability and in seven species germinability was increased (Fig. 1). In species where chilling had a significant effect four different response types were observed:

1. Similar effect of both chilling treatments on germinability (positive for AntOdo, PruVul, CirPal, negative for HieUmb, GalUli, SucPra).
2. Gradual effect (effect of -14°C is greater) of storage temperature on germinability (positive for AngSyl and BetOff, negative for SelCar; LysVul and PotEre showed similar, but nonsignificant responses).
3. Similar effect of seed storage at +4°C and +20°C compared to seed storage at -14°C (LycFlo, in which germinability was highest after seed storage at -14°C, and MyoNem, where storage at -14°C decreased the germinability considerably).
4. Difference ($P = 0.025$) between seeds stored at +4°C and those stored at both other temperatures: only in JacPra; the differences were not highly significant.

Species also differed in their germination dynamics under greenhouse conditions:

- (1) starting day of germination (day 2 after sowing in CirPal, JacPra and LycFlo, day 11 in PotEre and SelCar);
- (2) median day when one half of all germinated seeds of a species had germinated;
- (3) length of the germination period (Fig. 2).

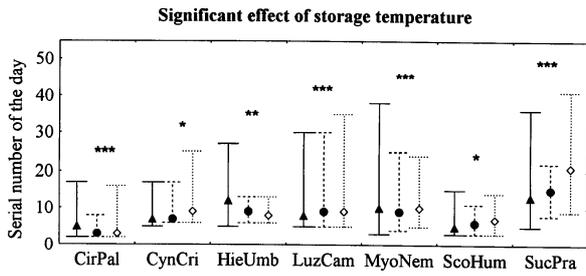


Fig. 2. Effect of seed storage temperature on median day of germination: ▲ = +20°C; ● = +4°C; ◇ = -14°C; * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$. For species codes, see Table 1. Effects on the other species non-significant. Bars extend from the first to the last day of germination.

Dynamics of seed germination under greenhouse conditions, characterized by the day when half of the seeds had germinated, was also affected by chilling. Although differences were small in most species, in some species they were highly significant (Fig. 2). However, biological and statistical significance need not always correspond to each other: if the germination is fast and shows a sharp peak, a one-day difference in median day can be highly statistically significant, but the biological significance may be very small.

Germinability and germination dynamics under greenhouse conditions seem to be independent. The median day and germinability at 20°C are not correlated ($r = -0.148$, $n = 24$, $P = 0.49$). There are four response types: (1) high germinability and early germination lasting for only a short time (e.g. LycFlo), (2) high germinability but slow and late germination (e.g. GalUli), (3) low germinability and early germination lasting for a short time (e.g. AchPta), and (4) low germinability and slow and late germination (e.g. PotEre). Most species are transitional between these four extremes.

The effect of chilling on germinability, and on germination dynamics characterized by the median day seem to be independent. We characterized the effect of chilling on germinability by the ratio between germinability after storage at -14°C and germinability after storage at 20°C and similarly the effect on the dynamics by the ratio of the respective median days. Although the correlation coefficient was negative as expected (increase in germinability is connected with a speeding up of germination), the correlation is weak and nonsignificant ($r = -0.372$, $n = 23$, $P = 0.08$). The effect of dry storage at +4°C tested in a similar way was also nonsignificant ($r = -0.306$, $n = 22$, $P = 0.165$).

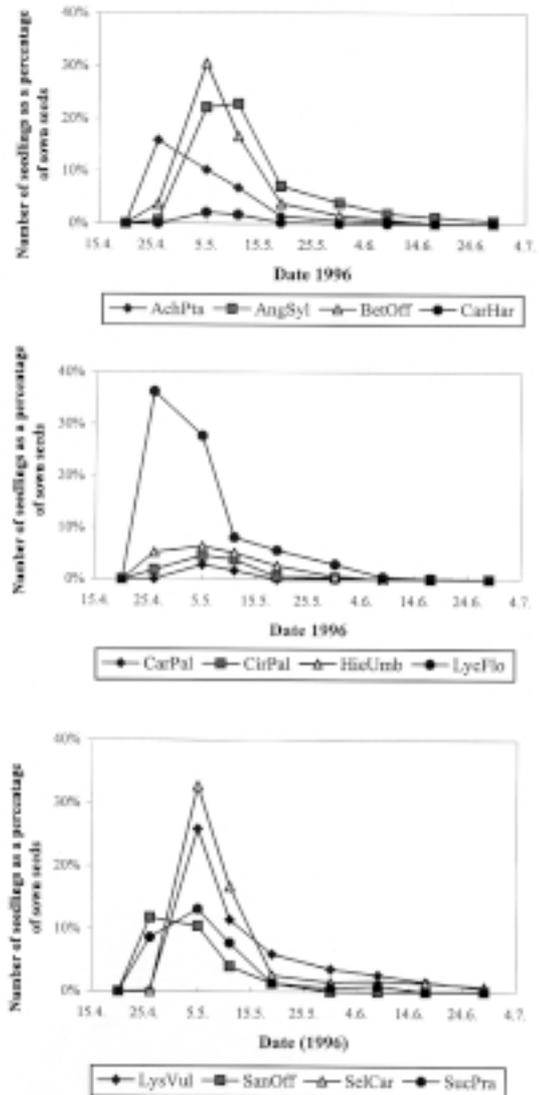


Fig. 3. Dynamics of seed germination in artificially-created gaps with removed seedlings. Dates are given as day.month. For species codes, see Table 1.

Experiment 2: Dynamics of seed germination in gaps

Dynamics of seed germination

Repeated-measures ANOVA of data from plots with regular seedling removal showed highly significant differences between the 12 study species ($P < 10^{-6}$ both for the effect of species and for interaction between species and time). The effect of species corresponds to the mean number of germinated seeds (as the same amount of seeds was sown, this is a test of germinability under field conditions). The interaction between species and time corresponds to differences in germination dynamics.

Seeds of all 12 species started to germinate over a two week interval at the end of April (Fig. 3). Species differed

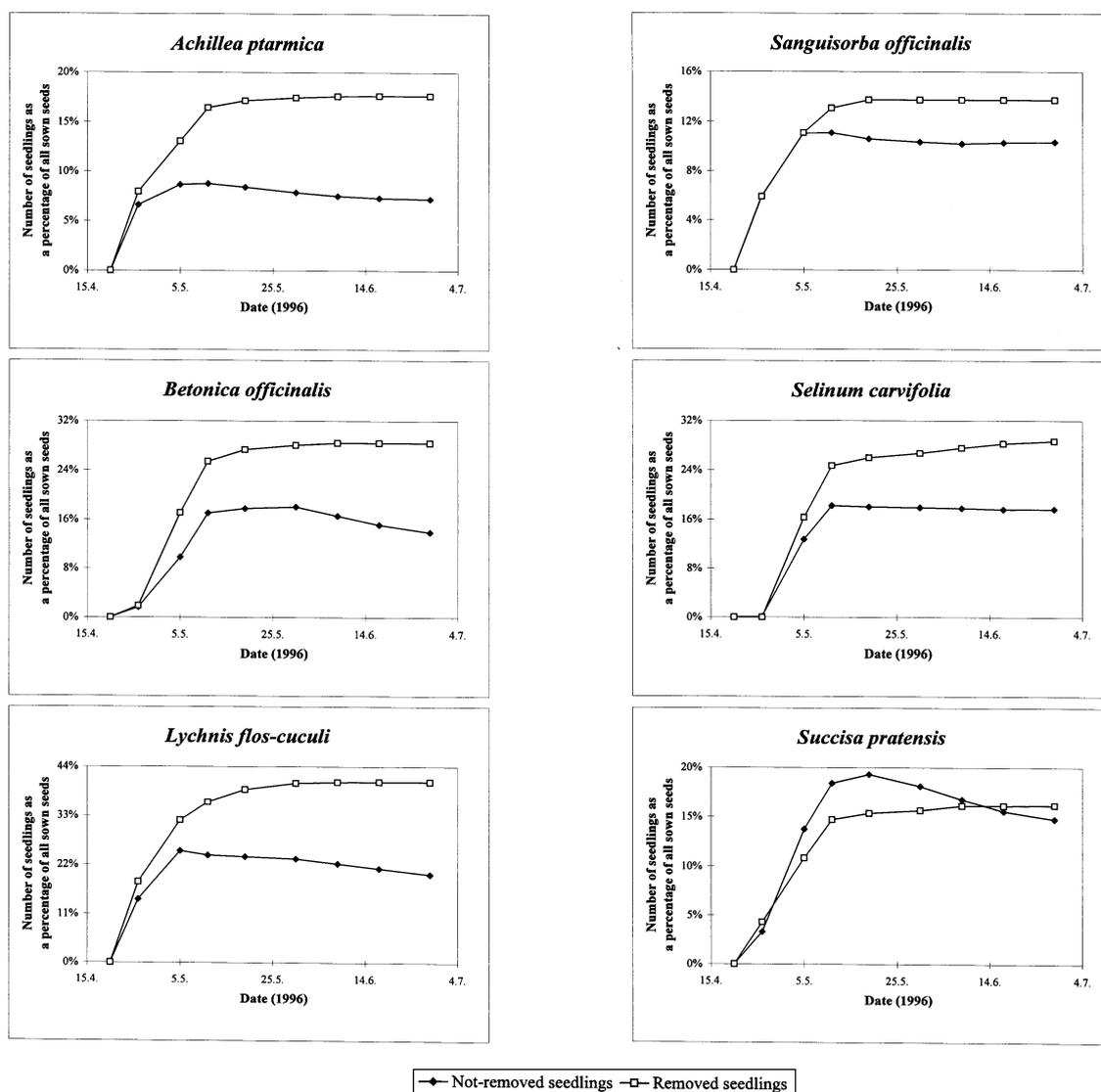


Fig. 4. Dynamics of seed germination in artificial gaps with seedlings removed and not removed (present).

in the timing of germination peak (AchPta and LycFlo had the earliest germination peak). More pronounced are the differences in the sharpness of the germination peak (sharp peak: LycFlo, SelCar; no peak: CarHar, HieUmb) and in the time when germination stopped (CarHar, CarPal and SanOff stopped germinating in the second half of May, while AngSyl, LysVul and SelCar were still germinating by the end of June).

Effects of seedling removal

Germination in gaps in which seedlings were removed was compared with germination in gaps in which seedlings were not removed. This was done for the six species which were sown into four plots each (AchPta, BetOff, LycFlo, SanOff, SelCar and SucPra).

Seedling emergence of five of the six tested species was increased when seedlings were removed (Fig. 4),

indicating competition between seedlings from the beginning of seedling emergence onwards. The number of seedlings over time decreases in plots with seedlings present (not removed) probably due to a self-thinning effect (i.e. density dependent mortality). For SucPra early germination was facilitated by the presence of conspecific seedlings. However, after some time the number of seedlings present decreased by a self-thinning effect.

Comparison of germination in field and laboratory tests

Germinability in the field, estimated as the proportion of the sown seeds that germinated, was correlated with the germinability in Petri dishes ($r = 0.76, 0.77$ and 0.79 for storage temperatures $+20^{\circ}\text{C}$, $+4^{\circ}\text{C}$ and -14°C respectively, $n=12, P<0.01$). Germinability following the lowest temperature chilling provided the best predictor of

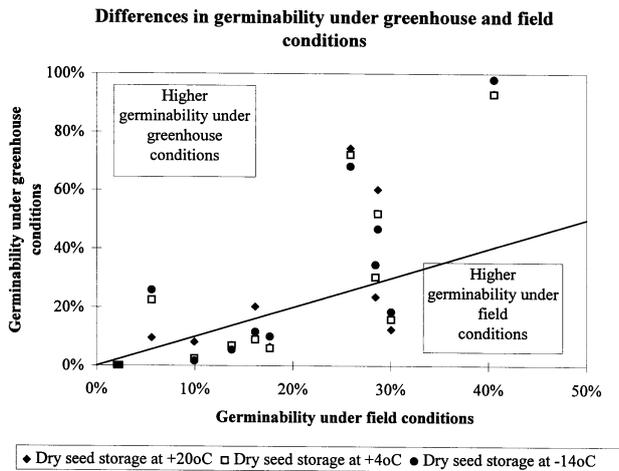


Fig. 5. Comparison of germinability in the field and in the greenhouse. Correlations are $r = 0.76$, 0.77 and 0.79 for storage temperatures $+20^{\circ}\text{C}$, $+4^{\circ}\text{C}$ and -14°C ; $P < 0.01$ in all cases. Differences between correlation coefficients are not significant. The line connects points with the same germinability in the greenhouse and in the field.

germinability in the field, although differences between correlation coefficients are small and not significant. Species with low germinability germinated relatively better in the field (CarHar, CarPal, HieUmb, AchPta, SanOff), whereas species with high germinability germinated relatively better under greenhouse conditions (LysVul, LycFlo; Fig. 5). The speed of germination in Petri dishes, characterized by the median day of germination, is positively correlated with the speed of germination in the field, which is characterized by the percentage of seeds germinated by the second census (Fig. 6). Similarly, the percentage of seeds which germinated before the end of May in the field can be predicted by the day when the last seed germinated in the greenhouse (Fig. 7). However, the correlations were only significant for characteristics of control seeds, the correlations for both chilled treatments were weaker and nonsignificant. Also, the sensitivity to chilling (characterized by the ratios of germinability and median day in chilled and control seeds) is not correlated with any characteristic of the germination speed in the field. Correlation coefficients are nonsignificant, $P > 0.20$ for all the eight correlation coefficients between germination speed characterized by percentages of germinated seeds at the end of April and at the end of May, and ratios of median day and germinability between control and both chilling treatments.

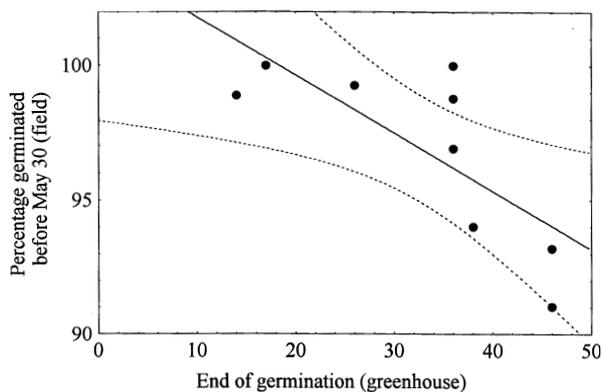


Fig. 7. Relationship between germination speed in the laboratory (expressed as the day when the last seed germinated) and germination speed in the field expressed as percentage of the seeds having germinated before May 30 of all the seeds germinated during the whole experiment ($r = -0.744$, $P = 0.022$). Regression line (percentage = $103.96 - 0.216$ end) with 95% confidence band is shown.

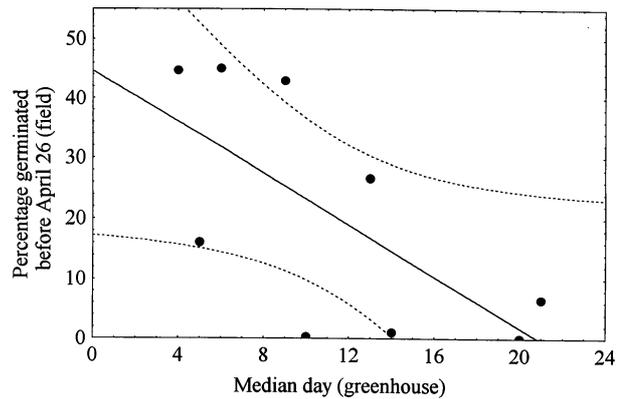


Fig. 6. Relationship between germination speed in the laboratory (expressed as the median day for control seeds) and in the field expressed as percentage of the seeds having germinated before April 26 of all seeds germinated during the whole experiment ($r = -0.670$, $P = 0.048$). Regression line (percentage = $44.6 - 2.14$ median day) with 95% confidence band is shown.

Experiment 3: The effect of litter layer and moss layer on seedling establishment

Experiment 3: The effect of litter layer and moss layer on seedling establishment

Establishment was highest in gaps for all species, followed by mowing+moss removal, mowing and control (Figs. 8 and 9). Data from the natural seedling recruitment controls were not included, because the number of seedlings of all the tested species was zero or close to zero. This shows that the results are not affected by the natural seed rain. The treatment effect was highly significant in all species and the interaction between time and treatment was significant in almost all cases

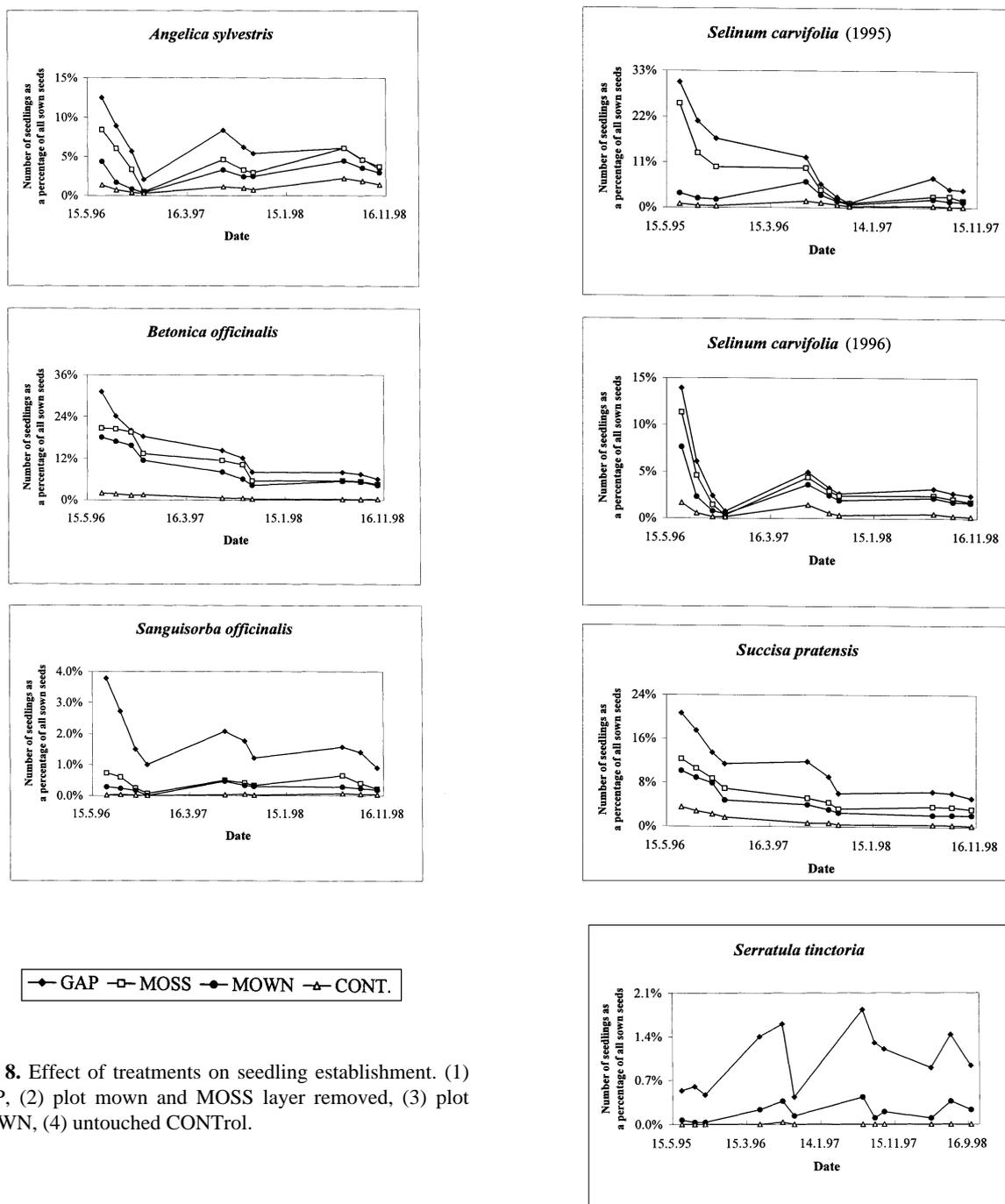


Fig. 8. Effect of treatments on seedling establishment. (1) GAP, (2) plot mown and MOSS layer removed, (3) plot MOWN, (4) untouched CONTROL.

(except BetOff, $P=0.051$ and SucPra, $P= 0.138$). However, species differ in their relative sensitivity to these factors (Figs. 8, 9, Tables 2 and 3). In the repeated measures ANOVA of $\log(x+1)$ transformed data (Table 2), comparing the dynamics of the five species sown in 1996, all terms are highly significant. The effect of species corresponds to differences in mean species germination over all the treatments (i.e. species differ in the number of germinated seeds).

The significant interaction between species and

treatment reveals differences in species sensitivity to particular treatments. Significant interaction between species and time reveals interspecific differences in germination phenology. Significant interactions between treatment and time reveals differences in seedling recruitment dynamics among treatments. Significant interactions between species, treatment, and time reveals interspecific differences in rates of change under various treatments. Differences in sensitivity to various treatments among species can be clearly demonstrated

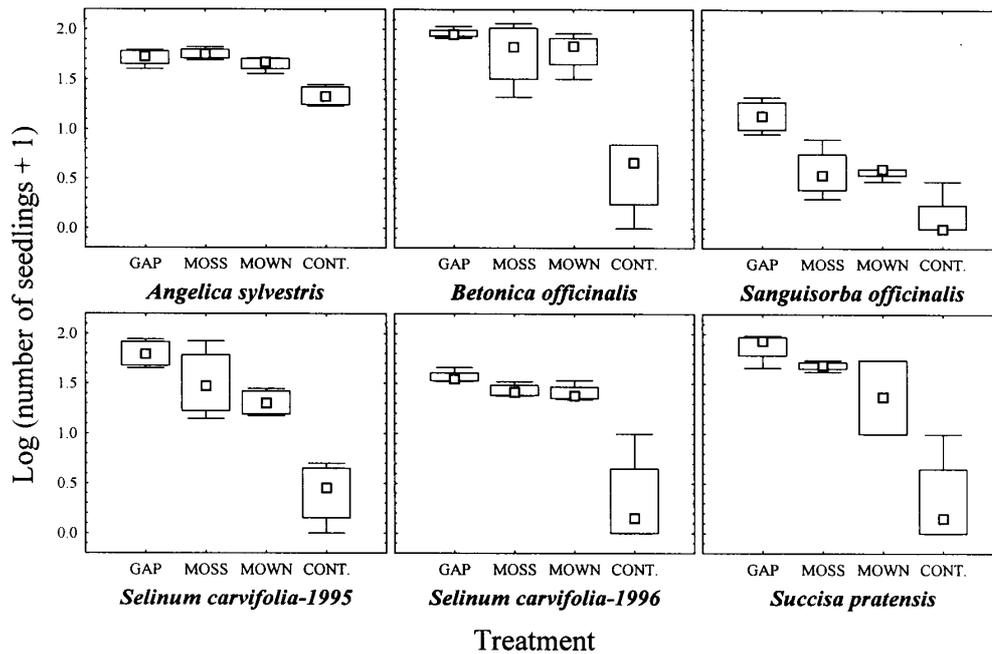


Fig. 9. Box and whisker plot displaying median, interquartile range (box), and range (whiskers) of numbers of seedlings of five species sown in 1996 in four treatments; GAP, plot mown and MOSS layer removed, plot MOWN, and untreated CONTROL, at the end of the first season.

by comparison of the five species (AngOff, BetOff, SanOff and SucPra, and SelCar sown in both 1995 and 1996) after the third season (Fig. 9). The recruitment of BetOff was successful in all treatments except the control. A similar result was obtained after the third season for SelCar, whereas recruitment of SanOff was very low

Table 2. Results of the repeated-measures ANOVA-model comparing seedling recruitment of five species sown in 1996 (AngSyl, BetOff, SanOff, SelCar, SucPra) in gap, mown+moss removal, mowing and control treatments. Species (S) and treatment (Tr) are the fixed factors, block (B) is the random factor the effect of which is not tested. Time (Ti) is the repeated-measures factor. Interaction terms with block are used as error terms.

Factor	Effect		Error		F	P
	df	MS	df	MS		
S	4	30.24	12	0.78	38.80	0.0000
B	3	0.31				
Tr	3	48.24	9	0.18	269.00	0.0000
Ti	9	3.28	27	0.08	43.42	0.0000
S*B	12	0.78				
S*Tr	12	1.22	36	0.35	3.48	0.0018
B*Tr	9	0.18				
S*Ti	36	0.77	108	0.04	21.10	0.0000
B*Ti	27	0.08				
Tr*Ti	27	0.06	81	0.04	1.73	0.0312
S*B*Tr	36	0.35				
S*B*Ti	108	0.04				
S*Tr*Ti	108	0.06	324	0.03	2.00	0.0000
B*Tr*Ti	81	0.04				
S*B*Tr*Ti	324	0.03				

in all treatments, except the gap treatment (Fig. 9).

The observed number of seedlings changed considerably over time (Fig. 8). The number of SerTin seedlings increased steadily from the first season onwards, because the density of seedlings was very low, as was intraspecific competition, and new seedlings appeared each growing season (no seedlings of SerTin were found in plots with only natural recruitment). Increases in seedling numbers at the beginning of each new season were also observed for AngSyl, SanOff and SelCar, but the final number of seedlings was lower than in the first season. Decreases in number of seedlings of BetOff and SucPra were gradual. This was caused by the fact that seedlings from the first growing season were big enough to inhibit new seed germination at the beginning of the next growing seasons. An explanation for this effect may be the fact that seedlings of BetOff and SucPra have perennial leaves, whereas the leaves of seedlings of AngSyl, SanOff, SelCar and SerTin die in the autumn.

Differences in seedling numbers between SelCar sown in 1995 and in 1996 in the nine corresponding dates are not significant ($F=6.733$; $df=1,3$; $P=0.081$), even though the census dates were not exactly the same. This indicates reproducibility of the obtained results. However, differences suggest caution in interpretations and importance not only of spatial, but also temporal replications of the field experiments.

The number of seedlings in various treatments expressed as a percentage of seedlings present in gaps can

Table 3. Effect of treatments on seedling recruitment in differently treated plots expressed as percentages of seedlings in the treatment as compared to number of seedlings in gaps (more than 100% means that the recruitment was more successful than in gaps). Four treatments were used: GAP, mowing+MOSS layer removed, MOWN, and CONTROL. Two of the species were observed in 1995 (*Selinum carvifolia*: SelCar-95, *Serratula tinctoria*: SerTin - treatment 2 is missing for this species), five of the species (*Angelica sylvestris*: AngSyl, *Betonica officinalis*: BetOff, *Sanguisorba officinalis*: SanOff, *Selinum carvifolia*: SelCar-96, *Succisa pratensis*: SucPra) were observed in 1996. The last two rows present the correlation coefficient (r) of the relative success vs. seed weight and the corresponding significance value. For the calculation, the average of the two values for *Selinum carvifolia* is used.

	Seed weight (mg/seed)	Number of seedlings as a percentage of seedlings present in gaps at the end of:								
		first season			second season			third season		
		MOSS	MOWN	CONT.	MOSS	MOWN	CONT.	MOSS	MOWN	CONT.
SelCar-95	0.929	59.06	11.61	2.10	69.33	60.00	25.33	37.45	31.87	3.19
SerTin	0.516	—	7.14	0.00	—	30.77	0.00	—	16.67	0.00
AngSyl	1.215	59.12	14.71	7.06	54.32	45.68	13.58	108.78	86.34	40.98
BetOff	0.979	97.51	78.44	6.88	68.18	52.48	3.72	75.21	70.25	3.86
SanOff	0.887	16.67	11.11	1.11	27.40	24.66	1.37	24.07	20.37	3.70
SelCar-96	0.929	60.42	32.64	6.25	92.41	72.78	12.66	72.41	68.97	6.90
SucPra	1.026	65.01	58.56	17.00	53.44	41.87	5.51	61.41	40.51	3.22
r	—	0.279	0.328	0.582	0.077	0.402	0.545	0.918	0.803	0.686
P	—	0.650	0.522	0.225	0.902	0.429	0.264	0.027	0.054	0.133

be taken as a measure of competitive effects on seedling recruitment. The effect of competition decreases with seed weight: when the seed weight is higher, the relative differences between gaps and other treatments are smaller. The correlation between seed weight and relative recruitment success is positive for all the treatments in all three years (Table 3), but often not significant. However, the power of the test is small, because only six species (five in moss removal plots) are used and the range of seed weight is small.

Discussion

The investigated species differ in response to all of the studied characteristics. However, the results should be interpreted with caution. For example, the two *Carex* species (*C. hartmanii*, *C. pallescens*) did not germinate in laboratory tests but they germinated under field conditions (albeit poorly). They probably require chilling in a wet environment. Our simple germination tests cannot cover all the possible demands for breaking dormancy in various species (Nikolaeva et al. 1985). Nevertheless, field and laboratory germinations are highly correlated: about 60% (r^2 , r being between 0.76 and 0.79, Fig. 5) of between-species variability in germinability under field conditions can be explained by germinability under greenhouse conditions. Species with low germinability (in both tests) usually germinate relatively better in the field, whereas the opposite is true for species with high germinability. Low germinability is probably connected with dormancy. Our simple tests were not able to mimic complex field conditions, which could be necessary for breaking dormancy. The negative effect of pathogens,

predators, etc., in the field is evident as a decrease in germinability. However, this effect is more than compensated in species where field conditions increase germination by breaking dormancy, usually species with low greenhouse germinability. Consequently, we were only able to detect this in the species whose germinability had not been considerably increased under field conditions, usually those with high germinability in the greenhouse.

We found also large interspecific differences in germination dynamics, both under greenhouse and field conditions. Germination speed in the greenhouse provides a rough estimate for the germination dynamics in the field. Species with a fast germination in Petri dishes started to germinate in the field early and germinated quickly (at the end of April), whereas species of which some seeds germinated more than one month after sowing seeds into Petri dishes germinated in the field still in June. However, the relationship is weak.

In Experiment 2 the difference between the total number of seedlings that germinated in plots with seedlings removed, and the number of seedlings present in plots with seedlings not-removed can be explained by seedling mortality and by suppression of seed germination caused by the presence of seedlings. Although these effects cannot be separated in our data, field observations indicate that both are important: although the newly emerged seedlings cannot be unequivocally distinguished and counted in the non-removal plots, a rough estimate based on seedling size suggested that more new seedling appeared during the late intervals in removal plots. We expect that this is not only a result of direct resource competition, but that other effects related to dense seedling cover (e.g. changes in red/far-red

ratio) may play a part as well. This shows that population size can be strongly density-regulated. Mortality of seeds in the soil is usually not considered to be a density dependent factor (e.g. Begon et al. 1986, p. 558), but when the number of germinating seeds decreases with increasing seedling density, total seed mortality probably increases (any period of time seeds remain in the soil without germinating increases their chance of being eaten, rotting, etc.).

Regardless of the mode of dispersal, the distribution of seeds within a plot is not uniform (Willson 1992) and is often very clumped. This means that if there is high seedling density in a gap, then the seedlings often belong to one species. This phenomenon increases the effect of intraspecific competition (Pacala 1986). Germination suppression and seedling mortality during self-thinning is then affected mainly by the density of the species.

Environmental and biotically-generated heterogeneity and their effect on seedling recruitment are often considered to be important for the maintenance of species diversity (Grubb 1977, 1988; Rusch & Fernández-Palacios 1995). Interspecific differences in sensitivity to environmental conditions are necessary for these mechanisms to operate. For example, when gaps in the community are colonized with subsequent founder control (Yodzis 1978), the time of gap creation is important if germination time differs between species (Hobbs & Mooney 1985). Also, weather can influence the colonization of gaps (Hobbs & Mooney 1991). Particular species (and their ecotypes) germinate in waves variously situated in time (Gulmon 1992; Masuda & Washitani 1992). Differences between species in timing of germination in our study are probably too small to cause large differences in gap colonization due to variation in timing of gap creation. However, differences can be important when we consider weather conditions: late frosts appear in the study area as late as the first half of May, which is the time when species differ considerably in percentage of germinated seeds. Those differences can greatly influence seedling recruitment. Earlier germination can give an advantage to a species if there is no frost, but is fatal for seedlings if there is a frost. This might be the cause of the large variability in both numbers and composition of seedlings over the years in the same locality (Špačková 1998). Similar phenomena were also observed in other habitats (Titus & del Moral 1998).

Genetically fixed differences in response to chilling exist between species, between populations and also within populations (Meyer & Kitchen 1994). However, the relationship is probably complex. We have not found any relationship between species response to chilling and timing of seed germination in the field. Similarly, Schütz & Milberg (1997) concluded that their

data from laboratory experiments on the germination of various populations of *Carex canescens* could not be used to predict differences in timing of seedling emergence in the field.

Generally, few meadow species form long-term persistent seed banks (Bekker et al. 1998). This corresponds to our results: the majority of seeds sown in our experiments germinated during the season following seed production. However, some of the remaining seeds germinated in subsequent seasons.

Seedling recruitment is probably the most sensitive part of the life cycle, sensitive to both the effect of living vegetation and litter. Whereas the effect of living biomass is often found on both established plants and seedlings, the effect of litter is usually more important for seedling recruitment and growth (Foster & Gross 1997). Litter was removed at the beginning of each season from all plots except the untreated control, and the control has the lowest number of seedlings for all the species.

Seed size was found to be positively correlated with the relative success of seedling recruitment in competition-influenced plots in comparison with gaps. Smaller seeds are usually associated with lower recruitment under more competitive situations (Gross 1984; Westoby et al. 1996). This is not surprising, because the energy stored in large seeds enables these seeds to overcome difficult conditions under litter and dense vegetation (reserve effect, Westoby et al. 1996). The correlations were not always significant, probably because of the low number of species tested and small range of seed weights. In a similar study (Křenová & Lepš 1996), *Gentiana pneumonanthe*, the species with much smaller seeds was found to be completely dependent on gap regeneration. On the contrary, in old fields in Michigan, larger-seeded species were not significantly more likely to emerge in undisturbed vegetation than were smaller-seeded species (Goldberg 1987). However, the emergence studies in Michigan included both the effect of seed density and probability of germination, whereas in our experiment, the seed density was constant. The recruitment of the species with the largest seeds (*Angelica sylvestris*) was the least suppressed in comparison with gap plots. This is in good agreement with a parallel study at the locality where *Angelica sylvestris* was found to perform best in unmown plots (Lepš 1999). However, in that study the number of seedlings of *Sanguisorba officinalis* and *Betonica officinalis* were found to be lower in mown plots than in unmown plots, whereas here these species were found to be considerably suppressed in the unmown control. Mowing influences not only conditions for seed germination and seedling establishment, but also seed production: *Sanguisorba officinalis* and *Betonica officinalis* are late flowering species, and this effect overcompensates for the positive effect of mowing

on seedling recruitment.

Mosses usually have small or no effect on established plants, however, they influence seedling recruitment. Mechanisms influencing germination include changes in quantity and quality (red/far-red ratio) of light under the bryophyte layer, mechanical prevention of seeds from reaching the soil surface, and allelopathic effects (van Tooren 1990; During & van Tooren 1990). The role of the moss layer in seed germination in grassland communities can be both protective (e.g. Ryser 1990) and inhibitive (Keizer et al. 1985; van Tooren 1990; Špačková et al. 1998). For example, the moss layer can protect seeds from predation (van Tooren 1988). The protective role is more important in dry grasslands (Ryser 1990) or in communities with temporal desiccation (During & van Tooren 1990). The effect of the moss layer on vascular plants may change from the seed to the seedling stage. Our results also show a significant negative effect of the moss layer on germination, but the intensity of the effect varies between species, and changes over time. In nature, the moss layer cover is positively influenced by similar factors as for seedling recruitment (e.g. by mowing, Lepš 1999). This is the reason why moss layer and seedling occurrence might be positively correlated (i.e. found in similar microhabitats) even when moss suppresses the seedling recruitment.

All of the species tested for the effect of treatments on seedling recruitment are polycarpic perennials, except *Angelica sylvestris* which is often monocarpic (Grime et al. 1988). Seedling recruitment suppression in polycarpic perennials will first change the population demographic structure and only after that will the population decline, depending on species longevity (none of the tested species exhibit pronounced clonal lateral spread). *A. sylvestris* is very well equipped for seed regeneration: the seeds are relatively large, enabling germination in the shade. The flowering plant produces large quantities of winged seeds that are released through the winter, so that the seeds can disperse on the surface of the snow.

Seedling recruitment is usually positively affected by disturbance, and the positive effect increases with disturbance intensity and differs among species. However, the same disturbance might have negative effects on growth and seed production, and the magnitude of these effects is also species specific. These differences between species together with disturbance heterogeneity are important factors in promoting species coexistence.

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