VARIABILITY OF SEEDLING RECRUITMENT UNDER DOMINANT, MOSS, AND LITTER REMOVAL OVER FOUR YEARS

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Abstract: In a four-year removal experiment we investigated the effect of removing a dominant species (*Nardus stricta*), the litter layer and moss layer on species composition of established vegetation and on numbers and species composition of seedlings. The experiment was conducted in an oligotrophic wet meadow (*Molinion* with some features of *Violion caninae* according to phytosociological classification). After four years of the experiment, the redundancy analysis (RDA) did not reveal any changes in the composition of the established plant community. Seedling numbers and their species composition, however, varied significantly both among treatments and over years. Whereas the differences between treatments were mainly in total seedling numbers, the years differed in both seedling numbers and real representation of individual species. In particular, *Myosotis nemorosa* seedlings highly increased their abundance in a favourable year 1995 constituting one third of all the seedlings found, whereas in other years their abundance was between 4% and 10%. The effects of year and treatment were not additive (significant year × treatment interaction). This means that plots under different treatments are differently affected by climatic conditions of individual years. The greatest increase of seedling recruitment was observed in the plots with the moss layer removed. Microsites with high seedling recruitment persisted in the plots for no more than two years.

Why the established vegetation composition does not differ among treatments even after four years, despite the relatively pronounced and immediate response of seedlings? The number of species with regular seedling recruitment is limited. They are mostly perennials, and consequently, it might take even longer than four years before recruitment limitation affects the population size of established plants.

Keywords: Community structure, Competition, Safe site, Small-scale processes, Species coexistence

Nomenclature: KUČERA & VÁŇA (2003), ROTHMALER (1976)

INTRODUCTION

Most of Central European meadow communities were created by human activities. Despite this, meadows have developed into a natural part of the environment. However, their function, structure and species richness depend strongly on the type of management pursued. This has been known for a long time (STEBLER & SCHRÖTER 1887 in KRAHULEC 1995). For centuries, management practices did not change considerably. In recent decades, however, meadows are often over-fertilized or abandoned. Both fertilization (WILLEMS et al. 1993, FOSTER & GROSS 1998), and abandonment (WILLEMS 1983, BOBBINK & WILLEMS 1987, STAMPFLI 1992, RYSER et al. 1995, DIEMER et al. 2001) lead to changes in species composition and to a loss of species diversity.

Studying this issue and related processes is not only of academic interest, but also of great importance for grassland management. Numerous papers deal with both, mechanistic explanations and conservation and management practices in semi-natural grasslands (e.g. ROELOFS et al. 1996, VAN DUREN et al. 1998, BEKKER et al. 1997, BAKKER & BERENDSE 1999, COULSON et al. 2001, HEGLAND et al. 2001, JENSEN & MEYER 2001, ISSELSTEIN et al. 2002).

The recruitment phase in a plant life cycle is considered crucial for the survival of plant populations and consequently, for the maintenance of community composition and species richness. Compared to the surrounding established vegetation, the seedlings are much more vulnerable to numerous biotic and abiotic factors (e.g., KŘENOVÁ & LEPŠ 1996, FISCHER & MATTHIES 1998, COLLING et al. 2002, ISSELSTEIN et al. 2002).

Factors affecting seedling recruitment vary with the species and with the community studied. The presence and character of the bryophyte layer (KEIZER et al. 1985, WINN 1985, VAN TOOREN 1988, RYSER 1993, KULL 1998, ŠPAČKOVÁ et al. 1998, ZAMFIR 2000, DELACH & KIMMERER 2002), the litter layer (GOLDBERG & WERNER 1983, FACELLI & FACELLI 1993, FOSTER & GROSS 1998, SUDING & GOLDBERG 1999, JENSEN & MEYER 2001), and of the openings in the vegetation turf (GOLDBERG & WERNER 1983, MCCONNAUGHAY & BAZZAZ 1987, WILLIAMS 1992) are most often mentioned in this context.

Long-term field experiments are perpetually needed in ecology. Although longer-term experiments in plant ecology have recently increased in number, information on year to year fluctuation in seedling recruitment is still strongly needed. This fluctuation is considered important for the maintenance of species diversity (GRUBB 1977).

Another factor widely presumed to shape the structure of a community is the presence of a dominant plant species. It is generally expected that the dominant species' abundance, life form or life strategy affect both germination and seedling establishment and the already established plant individuals. Although this view is widely accepted, supporting experiments from grasslands are rare (FOWLER 1981, BOBBINK & WILLEMS 1987, BELSKY 1992, GLENN & COLLINS 1993, HERBEN et al. 1994 and LEPŠ 1999). Among them, LEPŠ (1999) has documented an increase of total biomass, and changes of species composition after dominant removal. FOWLER (1981), BELSKY (1992) and GLENN & COLLINS (1993) demonstrated changes in species abundance, species richness or species numbers respectively. HERBEN et al. (1994) proved changes in species mobility after dominant removal. In each of these experiments, the effect of removal was relatively small. Removal of *Brachypodium* (BOBBINK & WILLEMS 1987), however, resulted in sharp increase in forb biomass and in species number. Results of FOWLER (1981), GLENN & COLLINS (1993) and HERBEN et al. (1994) are based on data from one year, while other studies investigated the effect of experimental treatments for more years.

We have studied the community level effects of several of these factors in a four-year removal experiment in an oligotrophic wet meadow. The aim of this study was (1) to evaluate the importance of a dominant species and the moss and litter layers, and their effects on overall species composition of seedlings and the established plant community, (2) to assess year-to-year differences in species composition and abundance of newly recruited seedlings

and (3) to estimate by indirect evidence the persistence time of microsites favourable for seedling recruitment.

This paper deals with an experiment for which first-year results have already been published (ŠPAČKOVÁ et al. 1998). Having the four-year data series allows us to compare the results obtained in the first year with those in the following three years. The four-year data series also enables us to estimate the year-to-year variability in seedling recruitment, and to test the additivity of effects of year and treatment. Further, with the four-year data available, we were able to estimate the persistence time of favourable microsites.

METHODS

Study site

The study site is located in an oligotrophic, species-rich, wet meadow 10 km southeast of České Budějovice, Czech Republic (latitude 48° 57′ N, longitude 14° 36′ E, altitude approximately 510 m). The mean annual temperature is between 7 °C and 8 °C, and mean annual precipitation is 600–650 mm.

According to phytosociological classification, the vegetation belongs to *Molinion* with some species indicating a transition to *Violion caninae* in drier parts of the meadow, where the experiment was carried out. The vegetation is dominated by two perennial grasses *Molinia caerulea*, and *Nardus stricta*. Other common grasses are *Anthoxanthum odoratum*, *Agrostis canina*, *Briza media*, *Festuca rubra*, *Holcus lanatus*, *Danthonia decumbens*, and a number of sedges, e.g. *Carex panicea*, *C. pallescens*, *C. leporina* and *C. hartmanii*. The most common herbs are *Myosotis nemorosa*, *Potentilla erecta*, *Prunella vulgaris*, *Lathyrus pratensis*, *Cirsium palustre*, *Galium uliginosum*, and *Lysimachia vulgaris*. At the beginning of the experiment, the average number of vascular plant species in 1 × 1 m plots was 26 (including 8 grass species, 4 sedges and 14 forbs), the average number of vascular plant species in 10 × 10 cm subplots was 6. The experimental plots contained 6 bryophyte species, all of them common in meadows in the area (*Aulacomnium palustre*, *Climacium dendroides*, *Hylocomium splendens*, *Rhytidiadelphus squarrosus*, *Pleurozium schreberi* and *Scleropodium purum*).

The total cover of vascular plants was estimated to be about 80%. The average cover of *Nardus stricta* was 25%. Bryophyte cover varied widely and was estimated to average about 35%.

The meadow was regularly mown until the early 1990s, then it was abandoned and was not subjected to any agricultural management prior to the start of the experiment in 1994.

Experimental design

Experimental plots were established in March 1994 shortly after snow melt. The 1 m^2 plots were set up in a randomized complete block design (each block as a row of four adjacent 1 m^2 plots), with each of the four blocks containing the following treatments: (1) a control plot where vegetation remained undisturbed, (2) litter removal, (3) removal of the dominant species (*Nardus stricta*), and (4) litter and bryophyte removal. The moss layer was removed by pulling out individual moss plants by hand, and *Nardus* by carefully pulling out its

individual tillers. We aimed at minimizing soil disturbance. Consequently, small fractions of roots remained in the soil. Nevertheless, the removal of *Nardus* was very successful with nearly no regrowth.

The design of our experiment is not fully factorial. We have removed the litter layer and moss + litter layer, but not the moss layer alone. Unfortunately, it was impossible to remove mosses without considerably disturbing the litter layer. The effect of moss layer can be estimated by comparing the litter removal and the litter and moss removal treatments. The statistical test comparing these two treatments (as planned comparison) is presumably the test of the effect of moss layer itself.

During the summer 1994 a new 0.5 × 0.5 m square was marked out in the middle of each plot and wire rods were inserted into the soil in the corners of each square. A wooden sampling frame with openings fitting into the wire rods was used, thereby improving precision of square placement each year. The sampling frame was divided by metal wires into a grid of subplots 10 × 10 cm each. The covers of established plant species were estimated and the numbers of seedlings of each species were recorded in every 10 × 10 cm subplot. Seedlings were defined as plant individuals grown from seeds that had not yet reached their first winter season.

Every spring, the (very few) newly appearing *Nardus* individuals, mosses and the litter were removed in corresponding treatments and censuses were conducted every summer in mid-season (beginning of July).

Statistical analysis

Total community response (i.e., species composition of established plants and of seedling species) was analyzed by constrained ordinations. Since the vegetation composition was relatively homogeneous (even among the plots of various treatments), the linear response model was appropriate and the redundancy analysis was used (RDA, Lepš & Šmilauer 2003), using the program Canoco for Windows (ter Braak & Šmilauer 2002). Both RDA with and without standardization by sample norm were used. RDA without standardization reflects both the differences in the numbers of seedlings and also the relative proportions of seedling species, while standardized RDA takes into account the proportions of seedling species only. The species that were subjected to experimental manipulations (i.e., *Nardus stricta* and bryophytes) were made passive in order not to influence ordination results. For RDA analyses of species composition of established plant community we used data from the 10 × 10 cm subplots (with permutation test reflecting the hierarchical nature, i.e., permuting the whole 50 × 50 cm plots together, see Lepš & Šmilauer 2003 for discussion of appropriate permutation schemes in designed experiments). For seedling tests, we used the total numbers of seedlings of a particular species in the whole 50 × 50 cm square in order to avoid statistical problems arising from analyses of numerous subplots with no seedlings.

As the treatments form in fact a gradient of disturbance intensity, we can reasonably expect that there will be prevailing unidirectional variation among the treatments. Consequently, we can expect that the test on the first axis will be stronger than the test on the trace, especially when comparing the treatments. We base our analyses on the test on the first axis, but for comparison we report also the test on the trace. For ordination diagrams, the scaling focusing

on intersample distances (thus reflecting correctly the differences between centroids of categorial environmental variables, see LEPŠ & ŠMILAUER 2003) was employed. Only the species with highest fit to ordination axes are shown in the diagrams.

For the univariate response (changes in the total number of seedlings in 50×50 cm squares among treatments and over years) repeated measures ANOVA was used.

The data on the total numbers of seedlings in the 10×10 cm subplots were used to analyze the microsite dynamics. Our attempt is based on the assumption that high amounts of seedlings growing in 10×10 cm subplot implies the presence of a microsite favourable for seedling recruitment. In several analyses we correlated the total numbers of seedlings found in 10×10 cm subplots with numbers of seedlings found in the same subplots in all consecutive years. We performed three sets of comparisons. (1) Three comparisons of numbers of seedlings in the same subplot in the consecutive years (time lag of 1 year; 1994/1995, 1995/1996 and 1996/1997), (2) two comparisons with a time lag of 2 years, for data from 1994/1996 and 1995/1997) and (3) one comparison with a time lag of 3 years (1994/1997). We were interested in the correlation of microsites within a (50×50 cm) plot. The measure of dependence within plots was characterized using the ANCOVA framework; plot identity was the grouping variable, and number of seedlings in the previous year was the only covariable. The beta weights (in the within cell regression) are then measures of the within cell correlation between the years under consideration. Their value and meaning is analogous to the correlation coefficient (ranging from -1 to $+1$).

RESULTS

Response of established plants to experimental treatments

We found no effect of treatments on species composition of established vegetation either in the first year (ŠPAČKOVÁ et al. 1998), or in the following three years. Corresponding *P*-values were always higher than 0.68.

Response of seedlings to experimental treatments and its variation in time

The total number of seedlings differed significantly among treatments ($P < 0.01$, $F = 18.71$). The highest number of seedlings was found in plots with the moss + litter removal treatment. The smallest amount of seedlings was recorded in plots with *Nardus* removed, followed by the litter removal treatment and control plots. There were significantly more seedlings in moss and litter removal in comparison with litter removal ($P < 0.01$, $F = 15.45$), indicating a negative effect of moss layer.

The blocks were located very close to each other within homogeneous vegetation. Correspondingly, both in univariate and multivariate analyses, the effect of block was highly non-significant ($P > 0.80$ in all the analyses). Consequently, we decided to omit the block from the subsequent analyses (See Table 1 for the summary of results of all RDA analyses performed.)

The species composition of seedlings differs among treatments. However, only the non-standardized test shows significant results (Fig. 1). Thus, the outcomes reflect

Table. 1. Summary of results of all RDA analyses performed on seedling data. Significant results with $P < 0.05$ are marked in bold. Environmental variable (Env.variable) used in the test: Year (Y), Treatment (Treat), Interaction of Time*Treatment (Y*Treat) is interaction of each level of one variable with each level of the other one. Covariable: Year (Y), Treatment (Treat), Plot Identity (PI). Permutation (type of permutation within plots used in Monte Carlo test) reflects the repeated measurement nature of the experiment (i.e., used split plot design permutation). In the table, we present: permutation on the whole plot level, permutation on the split-plot level. On the split-plot level, freely means also independently across the plots. Sum. Cano: Sum of all canonical eigenvalues. F first, F all, P first, P all: values of F and P respectively on the first ordination axis and on all ordination axes.

Env. variable	Covariable	Permutation	Sum. Cano	F first	F all	P first	P all
Nonstandardized tests							
Y*Treat		freely, freely	0.420	17.17	2.31	**0.002**	**0.002**
Treat	Y	freely, no	0.116	8.576	3.157	**0.008**	**0.028**
Y	Treat	no, freely	0.187	9.674	5.1	**0.002**	**0.002**
Y*Treat	Y, PI	freely, freely	0.117	6.832	1.331	**0.016**	0.070
Standardized tests							
Y*Treat		freely, freely	0.324	5.184	1.533	**0.044**	**0.002**
Treat	Y	freely, no	0.061	2.782	1.522	0.534	0.662
Y	Treat	no, freely	0.180	5.915	4.511	**0.002**	**0.002**
Y*Treat	Y, PI	freely, freely	0.083	2.562	0.878	0.608	0.774

differences in seedling numbers rather than relative proportions among various seedling species themselves.

Regardless of the type of analysis used (standardized and non-standardized RDA), the species composition of seedling community varied significantly between years (both tests $P < 0.01$, see Table 1). The distribution of species over years is shown in Fig. 2. Similarly, the total number of seedlings varies significantly (repeated measures ANOVA, $P < 0.01$, $F = 8.89$) among years. The highest amount of seedlings was found in 1995. It differed considerably from those found in 1994, 1996 and 1997, which had approximately the same number of seedlings.

Fig. 3 displays results of the RDA test with the year × treatment interactions as the only explanatory variables and with no covariables. This corresponds to pooled main and interaction effects in ANOVA. It provides the total explained variability and also a good indication of similarity of individual treatments in individual years. It shows that it was mainly the moss + litter removal treatment in 1995, which differed considerably from the other treatments, but also control and litter removal in this year were different from the rest. The year × treatment interaction itself (note that when testing for interaction without the main effects, the effect of the year and plot identifiers have to be included as covariables) was significant (for the non-standardized RDA). Similarly repeated measures ANOVA provided significant time by treatment interaction ($P < 0.05$, $F = 2.85$). This shows that the effect of year and treatment are not additive. The numbers of seedlings were approximately equal in all years, except for 1995, when seedlings exhibited a pronounced positive reaction especially to the moss + litter removal treatment (Fig. 4). The interaction remained significant even when
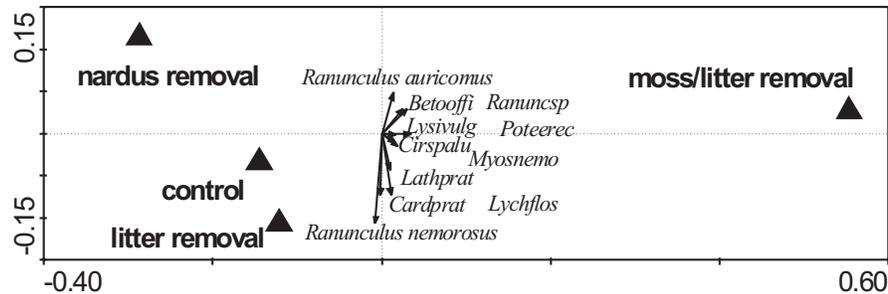
Fig. 1. The distribution of seedling species in plots subjected to various treatments. Result of nonstandardized RDA. Abbreviations of seedling species names: *Betooffi – Betonica officinalis*, *Cardprat – Cardamine pratensis*, *Cirspalu – Cirsium palustre*, *Lathprat – Lathyrus pratensis*, *Lychflos – Lychnis flos-cuculi*, *Lysivulg – Lysimachia vulgaris*, *Myosnemo – Myosotis nemorosa*, *Poteerec – Potentilla erecta*, *Ranuncsp –* undetermined *Ranunculus*. The first and second eigenvalues are 0.106 and 0.007 respectively.

we compared the moss + litter vs. litter removal ($P = 0.02$, $F = 4.09$), indicating that it was the effect of removed moss layer that enabled the outburst of seedlings in 1995.

Microsite dynamics

Numbers of seedlings in the same subplot in various years are highly significantly correlated for time lags 1 and 2 (beta weights are 0.220, 0.154) and virtually uncorrelated for time lag 3 (beta is 0.012). Similar analyses were made in order to evaluate possible differences in gap persistence in plots subjected to various treatments (Fig. 5). Although we did not find statistical differences between persistence of microsites in various treatments (apparently, the power of the test is low), in Fig. 5 we can see certain trends. While in plots subjected to experimental disturbance (removal of mosses, litter or *Nardus*) the beta values decline from time lag 1 to time lag 3, in the control the correlation between years was always low.

DISCUSSION

In our previous paper we did not find significant changes in species composition of the established plant community. We consequently expressed the assumption that the time frame of one year was too short to exhibit a shift in species composition of established vegetation. But even after four experimental years, our results were still negative.

GOLDBERG & BARTON (1992) in their review of field experiments exploring the role of plant competition, found 62% of experiments to be performed in one year or even less. Considering experiments measuring population response, the longest experiment found by the authors was conducted over three years. Only two experiments tested whole community response using multivariate analyses and both found significant effects on composition of the community, and both were conducted over only one year (GOLDBERG & BARTON 1992). Though since 1992 many more papers describing long term experiments appeared, we are not aware of any investigation that found changes in community attributes as late as after four years.
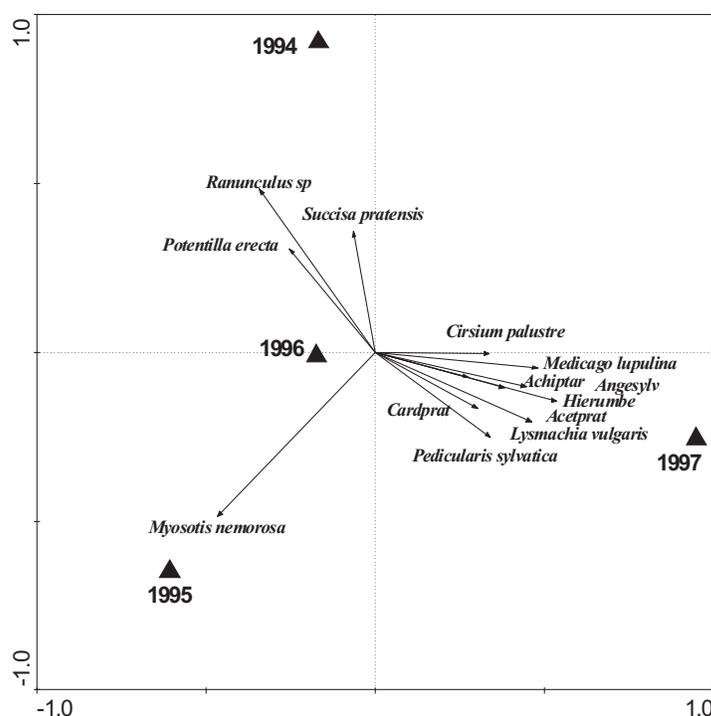
Fig. 2. The distribution of seedling species during different years of the experiment. Results of standardized RDA test. The first and second eigenvalues are 0.088 and 0.066 respectively. *Acetprat – Acetosa pratensis*, *Achiptar – Achillea ptarmica*, *Angesylv – Angelica sylvestris*, *Cardprat – Cardamine pratensis*, *Hierumbe – Hieracium umbellatum*.

In the literature, a pronounced effect of dominant species on the rest of the plant community is often expected. Accordingly, BOBBINK & WILLEMS (1987) confirmed a significant and pronounced effect of removal of *Brachypodium pinnatum*. In contrast, in several other papers (FOWLER 1981, BELSKY 1992, GLENN & COLLINS 1993, HERBEN et al. 1994 and LEPŠ 1999), experimental removal of dominant species provided some changes in community attributes (species composition, species richness or number of species), though the effects were generally less pronounced in comparison with remaining treatments (fertilization, mowing, fire, grazing).

In our case, the removal of the dominant species did not have any remarkable effect on the species composition of the established plant community. This can probably be attributed to the low competitive ability of *Nardus stricta*, or the relatively low cover of this species in the plots (the average cover was 25%). In another experiment from the same locality, LEPŠ (1999) found another dominant grass species *Molinia caerulea* to affect the community composition (though the effect was fairly less distinct than other treatments, i.e., fertilization and mowing). Both of these species occur at our site. While *Nardus stricta* prevailed in our experimental plots, *Molinia caerulea* was dominant at the site of the LEPŠ (1999) experiment, and its cover
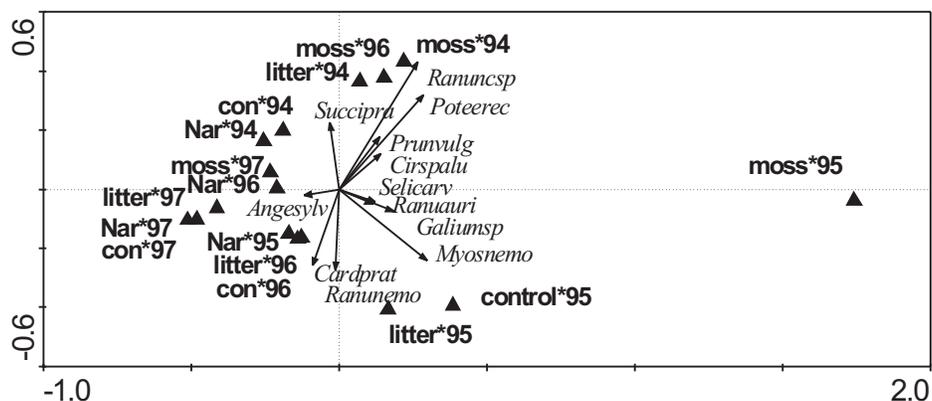
Fig. 3. Results of Year*Treatment interaction analysis (nonstandardized RDA), no covariables used, scaling focused on intersample distances. Abbreviations of seedling species names: *Angesylv – Angelica sylvestris*, *Cardprat – Cardamine pratensis*, *Cirspalu – Cirsium palustre*, *Galiumsp –* undeterminable *Galium*, *Myosnemo – Mysosotis nemorosa*, *Poteerec – Potentilla erecta*, *Prunvulg – Prunella vulgaris*, *Ranuauri – Ranunculus auricomus*, *Ranunemo – Ranunculus nemorosus*, *Ranuncsp –* undeterminable *Ranunculus*, *Selicarv – Selinum carvifolia*, *Succipra – Succisa pratensis*. Each centroid (triangles) represents a treatment in a year. The first and second eigenvalues are 0.263 and 0.060 respectively.

was close to 50%. Both species form dense tufts at their bases, though *Molinia* is generally a taller and larger species, creating also greater amounts of litter. In the experiment of BOBBINK & WILLEMS (1987) the cover of *Brachypodium* was much higher (ca. 70%) than in our case. In the same experiment, removal of *Brachypodium* in a "non-dominant situation" where its cover was lower caused only negligible changes in biomass of remaining species.

The litter layer present on the experimental plots was composed mostly of grass stems while a compact layer was not created. The amount averaged about 155 g/m^2 (during winter). Some other studies that have investigated the significance of the litter layer, found it to be an important factor influencing the plant community. In these studies, litter creates a dense, compact mat, often composed of rosette types of plants (FACELLI & FACELLI 1993, FACELLI & PICKETT 1991, GOLDBERG & WERNER 1983), or the amount of litter was larger than in our case (BOSY & READER 1995, CARSON & PETERSON 1990).

Despite a lack of response from the established community, plants were clearly affected in their seedling stages. The difference between the results of standardized and non-standardized RDA analysis shows that variation in species composition of seedlings among particular treatments is a consequence of the general response of seedlings to moss + litter removal treatment (non-standardized RDA) rather than of changes in seedling species proportions itself (standardized RDA).

Plants were affected mainly by moss + litter removal treatment. The response of seedlings to moss removal in the studied community was generally positive. Both positive (e.g. KEIZER et al. 1985, RYSER 1993) and negative effects (e.g. VAN TOOREN 1990, KEIZER et al. 1985, VAN TOOREN 1988) of the moss layer on seedling appearance have been documented. It was suggested (RYSER 1993) that under favourable conditions, such as greater habitat
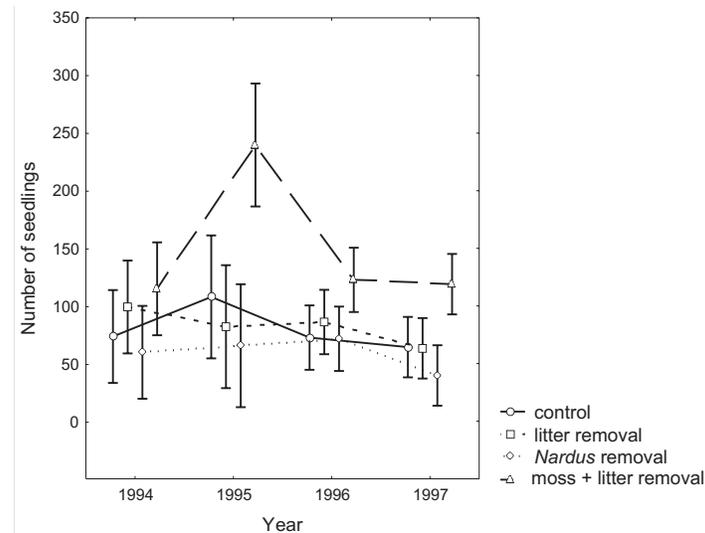
Fig. 4. Average numbers of seedlings in individual treatments over the years. Vertical bars denote 0.95 confidence intervals.

productivity or sufficient water supply, competition by neighboring vegetation largely determines the establishment of seedlings. Conversely, with decreased productivity or in communities with temporal desiccation, the protective role of the moss layer is more important. ZAMFIR (2000) in her greenhouse experiment shows that even in dry conditions the emergence of seedlings is suppressed by a moss layer. However, according to her, under natural conditions the moisture stress can be even more extreme than the greenhouse dry treatment.

In addition to direct competitive effects, the mechanisms of interaction between mosses and seedlings most often considered are lack of light under dense bryophyte turf and changes in red/far red ratio (KEIZER et al. 1985), temperature or air and soil humidity. Mechanical effects ("seed trap" – VAN TOOREN 1988), or indirect biotic effects (predation – VAN TOOREN 1988, DURING & VAN TOOREN 1990) may equally play a role. The allelopathic effects on the germination are also reported from several studies (BROWN 1967, VAN TOOREN 1990). As our study was not designed to reveal these mechanisms, our data do not enable their distinction.

When treatments have affected seedling recruitment, why then was no effect on the established plant community observed? Several species in the locality regularly regenerate by seedlings, but even those are perennials. Consequently, few species might be affected, making the test rather weak. The survival of seedlings also certainly plays a role. As we can see from our microsite dynamics data, the "gaps" last approximately 1 year in disturbed plots. Thus the survival of seedlings should be relatively successful. However, in the case of perennial plant species it takes long time until young plant individual reaches the size enabling the survival in a competitive environment. This time can be longer than one year.
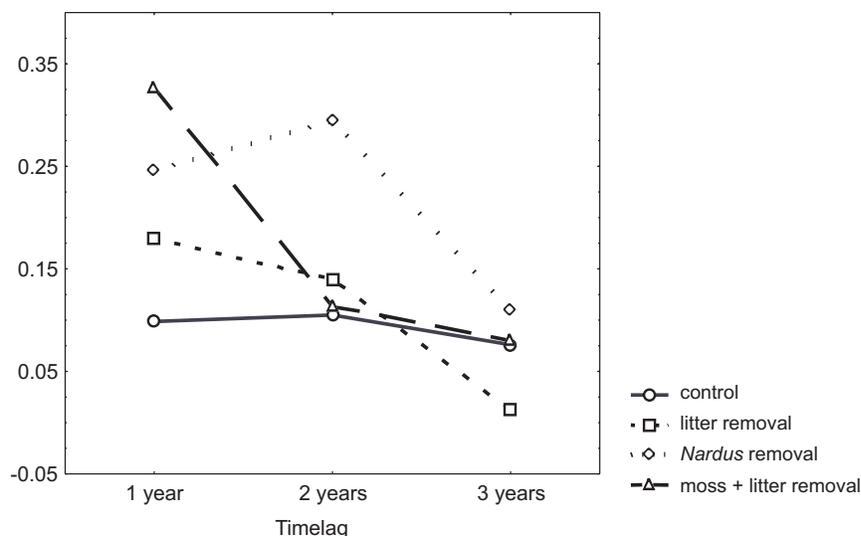
Fig. 5. The values of beta-weights, describing the spatial correlation of seedling numbers over varying time lags, in plots under various treatments.

Another factor might be simply the fact that the meadow was already species rich prior to the start of our experiment. The species pool had no or few species available that could enrich the actual vegetation. Also the structure and species composition of the studied community is stable in time. Thus only minor changes in percentage composition of certain species might occur. These changes, however, are too minor to be demonstrated statistically.

Seedling appearance over experimental years

The number of seedlings was approximately the same in 1994, 1996 and 1997, whereas the seedling appearance in 1995 was considerably higher. As we can see from the time × treatment interaction this positive effect was especially pronounced in plots subjected to moss + litter removal treatment. The majority of experiments investigating seedling recruitment do not involve data from more than one year; the studies that take into account more years usually confirm year by year variation. RABOTNOV (1969) in his review reports considerable year to year variation in seedling emergence. Both OTSUS & ZOBEL (2002) and RAPP & RABINOWITZ (1985) in four-year experiments, report roughly equal numbers of seedlings appearing over three years of their experiments with considerably lower seedling numbers in the fourth year due to severe drought. RYSER (1993) in a two-year study found fluctuation in seedling establishment for two of six sown species. The reasons for these variations are most likely weather conditions or the presence of pathogens and predators (RABOTNOV 1969). While in other noted experiments the "extreme" year meant lower germination, the opposite is true in our case. As the significant year effect in the standardized RDA suggests, 1995 was not favourable for all species; instead, a positive response of a few species was very pronounced. In particular, *Myosotis nemorosa* formed nearly one third of all

the seedlings observed in this year (in the other years, it constituted 4% to 10%). The amount of explained variability is considerably higher for year than for the treatment, the difference being particularly pronounced in the standardized analyses. When confronted with the theory of importance of the regeneration niche for the species coexistence (GRUBB 1977), it seems that in our plots the temporal variation is more important; however, the temporal variation was pronounced only in some microhabitat types.

The second year of the experiment (1995) was indeed different from the others. The significant increase in seedling numbers was mostly in one treatment (moss and litter removal) and especially one seedling species, *Myosotis nemorosa*, increased considerably. This species is known to show considerable fluctuation in year to year performance on our locality (CHALOUPECKÁ, pers. comm). In 1995, May and July were relatively wet months compared to other years, which could contribute to the high germination of *Myosotis*.

We do not have any direct evidence as to how much the seed bank contributed to the seedling outburst in 1995, however, it seems that not too much. In our site, the seedbank consists of many species, mainly sedges. The amount of *Myosotis* seeds (including also visibly damaged seeds) is approximately 5 seeds in one seed bank sample (soil core 6 cm in diameter and 10 cm deep), which is slightly less than the average amount compared to other species (ŠPAČKOVÁ, unpubl. results). Also, we do not expect that this could be the effect of seedlings germinating massively from a seedbank released by the establishment of the experiment. In this case, the effect would have been already seen in 1994, as we established our experiment immediately after snowmelt.

Microsite dynamics

Microsite dynamics is a neglected issue in literature and virtually nothing is known about the persistence of microsites favourable for seedling recruitment (i.e., primarily gaps) in meadow ecosystems. Gap dynamics is often mentioned in forest ecosystem literature. However, we found only two papers dealing with this topic in "herbal" assemblages. BELL et al. (1999) in a paper dealing with seagrass vegetation found larger gaps to survive up to 1.5 years, while smaller gaps persisted for less than 6 months. In another paper, MARRIOTT et al. (1997) measured gap persistence in sown swards using differences in light quality (red/far red ratio). In gaps differing in size and management, their persistence varied from less than 2 weeks to 20–25 weeks. OTSUS & ZOBEL (2002) studied small-scale turnover (in 2×2 cm plots) in a calcareous grassland community. The appearances (both, vegetative and generative) in previously empty plots underwent significant temporal changes and varied in yearly measurements from 79% to 42% during the course of their experiment.

 In our case, the estimates of favourable microsite persistence were based on numbers of seedlings found in the same 10×10 cm subplots in subsequent years. Beta weights decline from the first and second year steeply and are very low in the third year. This would imply that the persistence of favourable microsites in our site is one to two years. Management and disturbance generally plays an important role in meadow ecosystems; in our case in managed plots (i.e., in plots subjected to one of our experimental treatments) beta weights exhibit higher correlation for time lag 1 and 2, i.e., microsites favourable for seedling recruitment endure for at least one, but probably even two years (Fig. 5). In contrast, in control plots, the

correlation was very low for any time lag. The importance of continuous management for the endurance of microsites favourable for seedling recruitment is here evident. The significance of gaps or safe sites for germination of seedlings is widely accepted. However, the position of gaps in the vegetation changes in time. This spatial instability is probably less important for the germination of seedlings, as there are always at least some gaps in the vegetation. However, the persistence of a gap is important for seedling survival, as the seedling needs to reach maturity before the gap closes up.

REFERENCES

BAKKER J.P. & BERENDSE F. (1999): Constraints in the restoration of ecological diversity in grassland and heathland communities. *Trends Ecol. Evol.* 14: 63–68.

BEKKER R.M., VERWEIJ G.L., SMITH R.E.N., REINE R., BAKKER J.P. & SCHNEIDER S. (1997): Soil seed banks in European grasslands: does land use affect regeneration perspectives? *J. Appl. Ecol.* 34: 1293–1310.

BELL S.S., ROBBINS B.D. & JENSEN S.L. (1999): Gap dynamics in a seagrass landscape. *Ecosytems* 2: 493–504.

BELSKY J.A. (1992): Effects of grazing, competition, disturbance and fire on species composition and diversity in grassland communities. *J. Veg. Sci.* 3: 187–200.

BOBBINK R. & WILLEMS J.H. (1987): Increasing dominance of *Brachypodium pinnatum* (L.) BEAUV. in chalk grasslands – a threat to a species rich ecosystem. *Biol. Conservation* 40: 301–314.

BOSY J.L. & READER R.J. (1995): Mechanisms underlying the suppression of forb seelding emergence by grass (*Poa pratensis*) litter. *Funct. Ecol.* 9: 635–639.

BROWN R.T. (1967): Influence of naturally occurring compounds on germination and growth of Jack Pine. *Ecology* 48: 542–546.

CARSON W.P. & PETERSON C.J. (1990): The role of litter in an old-field community: impact of litter quantity in different seasons on plant species richness and abundance. *Oecologia* 85: 8–13.

COLLING G., MATTHIES D. & RECKINGER C. (2002): Population structure and establishment of the threatened long-lived perennial *Scorzonera humilis* in relation to environment. *J. Appl. Ecol.* 39: 310–320.

COULSON S.J., BULLOCK J.M., STEVENSON M.J. & PYWELL R.F. (2001): Colonization of grassland by sown species: dispersal versus microsite limitation in responses to management. *J. Appl. Ecol.* 38: 204–216.

DELACH A. & KIMMERER R.W. (2002): The effect of *Polytrichum piliferum* on seed germination and establishment on iron mine tailings in New York. *Bryologist* 105: 249–255.

DIEMER M., OETIKER K. & BILLETER R. (2001): Abandonment alters community composition and canopy structure of Swiss calcareous fens. *Appl. Veg. Sci.* 4: 237–246.

DURING H.J. & VAN TOOREN B.F. (1990): Bryophyte interactions with other plants. *Bot. J. Linn. Soc.* 104: 79–98.

FACELLI J.M. & FACELLI E. (1993): Interactions after death – plant litter controls priority effects in a successional plant community. *Oecologia* 95: 277–282.

FACELLI J.M. & PICKETT S.T.A. (1991): Plant litter: light interception and effects on an old field plant community. *Ecology* 72: 1024–1031.

FISCHER M. & MATTHIES D. (1998): Experimental demography of the rare *Gentianella germanica*: seed bank formation and microsite effects on seedling establishment. *Ecography* 21: 269–278.

FOSTER B.L. & GROSS K.L. (1998): Species richness in a successional grassland: effects of nitrogen enrichment and plant litter. *Ecology* 79: 2593–2602.

FOWLER N. (1981). Competition and coexistence in a North Carolina grassland. II. The effect of the experimental removal of species. *J. Ecol.* 69: 843–854.

GLENN S.M. & COLLINS S.L. (1993): Experimental analysis of patch dynamics in tallgrass prairie plant communities. *J. Veg. Sci.* 4: 157–162.

GOLDBERG D.E. & BARTON A.M. (1992): Patterns and consequences of interspecific competition in natural communities – a review of field experiments with plants. *Amer. Naturalist* 139: 771–801.

GOLDBERG D.E. & WERNER P.A. (1983): The effects of size of opening in vegetation and litter cover on seedling establishment of goldenrods (*Solidago* spp.). *Oecologia* 60: 149–155.

GRUBB P.J. (1977): The maintenance of species richness in plant communities: the importance of the regeneration niche. *Biol. Rev.* 52: 107–145.

HEGLAND S.J., VAN LEEUWEN M. & OOSTERMEIJER J.G.B. (2001): Population structure of *Salvia pratensis* in relation to vegetation and management of Dutch dry floodplain grasslands. *J. Appl. Ecol.* 38: 1277–1289.

HERBEN T., KRAHULEC F., HADINCOVÁ V. & PECHÁČKOVÁ S. (1994): Is a grassland community composed of coexisting species with low and high spatial mobility. *Folia Geobot. Phytotax.* 29: 459–468.

ISSELSTEIN J., TALLOWIN J.R.B. & SMITH R.E.N. (2002): Factors affecting seed germination and seedling establishment of fen-meadow species. *Restor. Ecol.* 10: 173–184.

JENSEN K. & MEYER C. (2001): Effects of light competition and litter on the performance of *Viola palustris* and on species composition and diversity of an abandoned fen meadow. *Pl. Ecol.* 155: 169–181

KEIZER P.J., VAN TOOREN B.F. & DURING H.J. (1985): Effects of bryophytes on seedling emergence and establishment of short lived forbs in chalk grassland. *J. Ecol.* 73: 493–504.

KRAHULEC F. (1995): Species coexistence in temperate grasslands. *Folia Geobot. Phytotax.* 30: 113–116.

KŘENOVÁ Z. & LEPŠ J. (1996): Regeneration of a *Gentiana pneumonanthe* population in an oligotrophic wet meadow. *J. Veg. Sci.* 7: 107–112.

KUČERA J. & VÁŇA J. (2003): Check- and Red List of bryophytes of the Czech Republic (2003). *Preslia* 75: 153–222.

KULL T. (1998): Fruit-set and recruitment in populations of *Cypripedium calceolus* L. in Estonia. *Bot. J. Linn. Soc.* 126: 27–38.

LEPŠ J. (1999): Nutrient status, disturbance and competition: an experimental test of relationships in a wet meadow copy. *J. Veg. Sci.* 10: 219–230.

LEPŠ J. & ŠMILAUER P. (2003): *Multivariate analysis of ecological data using CANOCO*. Cambridge University Press, Cambridge.

MARRIOTT C.A., FISHER J.M., HOOD K.J. & SMITH M.A. (1997): Persistence and colonization of gaps in sown swards of grass and clover under different sward managements. *Grass Forage Sci.* 52: 156–166.

MCCONNAUGHAY K.D.M. & BAZZAZ F.A. (1987): The relationship between gap size and performance of several colonizing annuals. *Ecology* 68: 411–416.

OTSUS M. & ZOBEL M. (2002): Small-scale turnover in a calcareous grassland, its pattern and components. *J. Veg. Sci.* 13: 199–206.

RABOTNOV T.A. (1969): Plant regeneration from seed in meadows of the USSR. *Herbage Abstr.* 39: 269–277.

RAPP J.K. & RABINOWITZ D. (1985): Colonization and establishment of Missouri prairie plants on artificial soil disturbances. 1. Dynamics of form and graminoid seedlings and shoots. *Amer. J. Bot.* 72: 1618–1628.

ROELOFS J.G.M, BOBBINK R., BROUWER E. & DE GRAAF M.C.C. (1996): Restoration ecology of aquatic and terrestrial vegetation on non-calcareous sandy soils in The Netherlands. *Acta Bot. Neerl.* 45: 517–541.

ROTHMALER W. (1976): *Exkursionsflora für die Gebiete der DDR und der BRD. Kritischer Band.* Ed. 4. Volk und Wissen, Berlin.

RYSER P. (1993): Influences of neighbouring plants on seedling establishment in limestone grassland. *J. Veg. Sci.* 4: 195–202.

RYSER P., LANGENAUER R. & GIGON A. (1995). Species richness and vegetation structure in a limestone grassland after 15 years management with six biomass removal regimes. *Folia Geobot. Phytotax.* 30: 157–167.

ŠPAČKOVÁ I., KOTOROVÁ I. & LEPŠ J. (1998): Sensitivity of seedling recruitment to moss, litter and dominant removal in an oligotrophic wet meadow. *Folia Geobot.* 33: 17–*30.*

STAMPFLI A. (1992): Year-to-year changes in unfertilized meadows of great species richness detected by point quadrat analysis. *Vegetatio* 103: 125–132.

SUDING K.N. & GOLDBERG D.E. (1999): Variation in the effects of vegetation and litter on recruitment across productivity gradients. *J. Ecol.* 87: 436–449.

TER BRAAK C.J.F. & ŠMILAUER P. (2002): *CANOCO Reference manual and CanoDraw for Windows user's guide: Software for Canonical Community Ordination (version 4.5)*. Microcomputer Power, Ithaca.

VAN DUREN I.C., STRYKSTRA R.J., GROOTJANS A.P., TER HERERDT G.N.J. & PEGTEL D.M. (1998): A multidisciplinary evaluation of restoration measures in a degraded *Cirsio-Molinietum* fen meadow. *Appl. Veg. Sci.* 1: 115–130.

VAN TOOREN B.F. (1988): The fate of seeds after dispersal in chalk grassland: the role of the bryophyte layer. *Oikos* 53: 41–48.

VAN TOOREN B.F. (1990): Effects of a bryophyte layer on the emergence of seedlings of chalk grassland species. *Acta Oecol.* 11: 155–163.

WILLEMS J.H. (1983): Species composition and above ground phytomass in chalk grassland with different management. *Vegetatio* 52: 171–180.

WILLEMS J.H., PEET R.K. & BIK L. (1993): Changes in chalk grassland structure and species richness resulting from selective nutrient additions. *J. Veg. Sci.* 4: 203–212.

WILLIAMS R.J. (1992): Gap dynamics in sub-alpine heathland and grassland vegetation in south-eastern Australia. *J. Ecol.* 80: 343–352.

WINN A.A. (1985): Effects of seed size and microsite on seedling emergence of *Prunella vulgaris* in four habitats. *J. Ecol.* 73: 831–840.

ZAMFIR M. (2000): Effects of bryophytes and lichens on seedling emergence of alvar plants: evidence from greenhouse experiments. *Oikos* 88: 603–611.