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RESEARCH ARTICLE

Vegetation Re-development After Fen Meadow Restoration by Topsoil Removal and Hay Transfer

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Abstract

We investigated the effects of different restoration treatments on the development of fen meadow communities: (1) depth of topsoil removal, with shallow (circa 20 cm) and deep (circa 40 cm) soil removal applied, (2) transfer of seed-containing hay, and (3) access of large animals. We carried out a full factorial experiment with all combinations of these factors and monitored it for 4 years. We studied the effect of seed availability in the soil seed bank on species abundance in the vegetation and compared it to the effect of species introduction by hay. We observed large differences in species composition between different treatments after 4 years. The combination of hay transfer, deep soil removal, and exclusion of large animals resulted in a community with highest similarity to the target vegetation. We found that the transfer of seeds with hay had a larger effect on species abundance than the soil seed bank. Hay transfer appeared to have important consequences on vegetation development because it speeded up the establishment of the target vegetation.

Key words: abiotic conditions, divergent vegetation development, large herbivores, priority effects, soil seed bank.

Introduction

Abiotic site conditions, composition of the soil seed bank, local dispersal, competition, and legacies of past vegetation have all been mentioned as factors affecting vegetation composition after rewetting and topsoil removal (Bakker & Olff 1995; Bakker et al. 1996; Verhagen et al. 2001; Cousins & Eriksson 2002). Several authors treat these factors as abiotic and biotic filters operating on plant community assembly and see restoration as a manipulation of these filters, selecting for certain plant traits and species (e.g., Hobbs & Norton 2004). The combination of filters in a particular situation (Grootjans et al. 2002; Nuttle 2007) and time (Wright & Westoby 1999) can, therefore, be crucial for the restoration outcomes.

Three major filters are regarded as essential in the case of fen meadow restoration (Van Diggelen et al. 2006). The first recognized filter includes the abiotic conditions. Both, the establishment of high water levels (Grootjans et al. 1988; Wassen et al. 1996) and a reduction of nutrient availability appeared to be crucial (Van Duren et al. 1997; Olde Venterink et al. 2002). Topsoil removal can be applied to achieve both wetter conditions and lower nutrient levels (Ramseier 2000; Verhagen et al. 2001; Patzelt et al. 2001). In addition, soil removal also modifies species interactions through the elimination of existing non-target vegetation and its seed bank, thus preventing a quick re-establishment of unwanted species (Jensen 1998; Kiehl et al. 2006).

The second filter is propagule availability (Bakker et al. 1996; Bakker & Berendse 1999). Only a small fraction of the fen meadow species can regenerate from the soil seed bank (Matus et al. 2003) and most have a limited dispersal capacity. Some studies stress the importance of large animals for seed dispersal (Pakeman 2001; Couvreur et al. 2004; Mouissie et al. 2005*a*), but again, only a small fraction of fen meadow species is transferred by zoochory (Pakeman et al. 2002). Seed addition with hay was shown to be an effective measure to overcome this limitation (Hölzel & Otte 2003; Klimkowska et al. 2007*a*). Most likely, early community development is stronger influenced by the amount of availability seeds than later stages of the vegetation development because of the increasing role of the internal community processes.

A third filter is the availability of suitable micro-sites for seedling recruitment (Isselstein et al. 2002; Poschlod &

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Biewer 2005; Rasran et al. 2007). A dense plant cover hampers colonization, whereas gap formation enhances it. The presence of large animals creating gaps therefore might increase species richness after restoration (Sousa 1984; Bakker 1989).

Topsoil removal, hay transfer, and grazing with large herbivores have all been used in wet meadow restoration, but their effectiveness varies from site to site (Klötzli & Grootjans 2001; Grootjans et al. 2002; Klimkowska et al. 2007*a*). We investigated the effects of different combinations of three restoration treatments on the re-establishment of fen meadow vegetation: (1) the depth of the soil removal, (2) species introduction through the transfer of seed-containing hay, and (3) access of large animals. We formulated the following questions:

1. How do abiotic conditions change after top soil removal?
2. To what degree is species abundance in the vegetation related to the amount of seeds transferred with hay and to the amount of seeds available in the soil seed bank?
3. What is the effect of the different treatments on vegetation structure, species richness, and community composition?

Methods

Study Site and Restoration Setup

The project was started in 2004 in the eastern part of the Całowanie Fen in Central Poland (52°00'40"N, 21°21'00"E). This was a groundwater seepage area, located on the slopes of the Wisła ice-marginal valley (Żurek 1990; Oświt & Dembek 2001). The area was drained for agriculture approximately 50 years ago. Regular mowing led to the development of species-rich fen meadows, which later changed into degraded, species-poor meadows due to drainage (Oświt & Dembek 2001). Species-rich fen meadow vegetation had survived in old, shallow peat-cuts (Podbielkowski 1960; Klimkowska et al. 2007*b*) and the vegetation of such meadows was defined as target vegetation. Abandoned parts of the area changed into shrubs and young forest, providing refuge to wild boar (*Sus scrofa*) and roe deer (*Capreolus capreolus*), which locally foraged on the meadows causing soil disturbance.

We tested the effectiveness of three factors on the restoration of fen meadows in a full factorial design: (1) topsoil removal depth (circa 20 or 40 cm, later referred to as "shallow" and "deep" removal treatments, respectively); (2) seed addition by hay transfer in 2004 and 2005; and (3) impact of large animals (by the use of fences). We established two plots (35 × 35 m), circa 40 m away from one another, both with buffer zones (0.35–1.5 m on the side of each plot, 0.5 m between treatments) in order to avoid edge effects, such as rapid vegetative invasion or nutrient leaching from the surrounding area. Each plot was divided into four strips, two with shallow and two with deep topsoil removal, and half of each plot (with shallow and deep removal) was fenced (in total eight treatment combinations on 8 × 32 m plots). These plots were divided into a grid of 2 × 2 m (4 × 16 = 64 grid cells per treatment). Although such a setup results in pseudoreplication, we chose this approach to avoid a design with many small, separated plots, where edge effects dominate vegetation development instead of treatment (Young 2000). A true replication of the treatments in a large-scale experimental setup was not feasible.

We measured groundwater levels every 2 weeks from April to November in the restoration area, from 2004 to 2007. We took 10 soil samples from the top 10 cm in the shallow and deep removal treatments, degraded meadows, and reference meadows in 2005 and measured total phosphorus (P_{tot}) and nitrate (NO_3^-). P_{tot} content was measured with a colorimetric method, after sample mineralization in HNO_3 and $HClO_4$. NO_3^- content was measured in a 1% K_2SO_4 fresh soil extract, with an auto-analyzer SCALAR.

The hay for the transfer was collected in mid-July 2004 on reference meadows nearby (donor to acceptor ratio, 2:1). This material was partly dried and we prevented seed losses by storing it (for 1.5 month) on agrifibre. Hay was spread in a 5–7 cm thick layer (circa 740 $g\,m^{-2}$ dry weight). In 2005, the hay was collected in the same period, on the same meadows and spread on the same plots but freshly cut hay was transferred. Fences of 1.5 m high were built to exclude wild boar and roe deer. No livestock grazing took place in the area. We found indirect evidences of presence of animals on the plots: animal-created soil disturbance, footprints, and signs of grazing.

Vegetation

We mapped species distribution and abundance (scale 0–absent; 1–present < 5%; 2–5–25%; 3–> 25% cover, dominant) and community structure characteristics (total cover of vascular plants, bare soil, litter, and mosses). We collected data for 4 years, starting from the initial community, 2 months after soil removal. We also recorded species composition in randomly selected sites in degraded meadows (2004, 2006, 2007, 36 plots of 4 m^2) and in the donor vegetation (2004, 2006, 2007, 29 plots of 4 m^2) from where we took the hay. All vegetation records were made at a distance between 20 and 300 m from the restoration plots. In this study, we focused on the vegetation development and the re-establishment of the fen meadow community and not on single species. The vegetation of species-rich fen meadows with water levels not lower than 0.5 m below surface was defined as target vegetation. It was dominated by *Carex nigra, Carex rostrata, Carex diandra, Eriophorum angustifolium, Agrostis canina, Deschampsia cespitosa,* and several wet meadow species (communities of *Caricion nigrae* and *Calthion palustris* alliances). Degraded meadows in the surroundings were severely drained (water levels up to 0.7–1.2 m below surface) with species-poor non-target vegetation dominated by *Festuca rubra, Cardaminopsis arenosa*, *Urtica dioica,* and several other ruderal and common grassland species.

From 2006 onwards, the experimental plots were mown annually in August and hay was removed from the site. We measured aboveground biomass of the newly developing communities at peak standing crop in 2006 and 2007. Eight biomass samples per treatment were collected in 50 × 50 cm plots in random locations, dried at 70°C and weighed.

Seed Identification

We quantified the number of seeds and determined the species composition of the soil seed bank and seeds in the hay. We analyzed the soil seed bank of the top 5 cm in the two topsoil removal depths. We sampled each depth in 10 plots (5 × 5 m), with 10 sub-samples per plot, mixed these sub-samples and processed them together according to Ter Heerdt et al. (1996). We analyzed the seed content of the freshly cut hay in 2004, using a similar method (10 samples, 1,190 g of dry hay). Seeds were separated from the dry material, spread out on trays with sterile soil, and the emerging seedlings were identified and counted. The remaining material was spread on large trays as control for remaining seeds. We assumed that the seed content of the hay in 2005 was similar.

Data Processing and Analysis

In a pre-analysis, we investigated if the two large plots were similar in respect to the measured abiotic conditions (t-test, factorial ANOVA, Fisher LSD post hoc test).

We assessed the impact of the seeds transferred with hay and of the soil seed bank after shallow removal on the abundance of species in subsequent years by means of correlation analysis (data from 2005 and 2007). We used $\ln(x)$ data transformation for the number of seeds in the seed bank and $\ln(x + 1)$ transformation for the seeds in hay to improve the normality. For the species abundance, we used the frequency of species in each treatment combination. Species were used as samples for this correlation analysis. We expected a higher correlation between amount of seeds and the aboveground vegetation for 2005 than for 2007.

We analyzed changes in vegetation structure, average species number, and aboveground biomass with repeated measures (RM) ANOVA. RM ANOVAs with four factors (time, hay addition, fencing, and depth of soil removal) were applied separately to selected dependent variables. In the case of plots without hay transfer, species other than those found in the soil seed bank were assumed to come from colonization. Our aim here was to compare the different treatments and not to measure the absolute numbers of seedlings or abundance of the colonizing plants.

We used multivariate techniques to evaluate the relative effect of the treatments on the development of the species composition over time. We used species abundance records (plant cover classes) for the vegetation composition. We started our analysis with a detrended correspondence analysis (DCA) on the entire data set for preliminary data exploration. Next, we performed a redundancy analysis (RDA) with interactions of time and treatment as the environmental variables (Lepš & Šmilauer 2003) to analyze the effects of the different treatments over time (2004–2007) in comparison to degraded meadows, reference meadows, and species composition of the soil seed bank (2004) and of the seeds in the hay (2004). For DCA and RDA, the data on soil seed bank and seed content of the hay were reclassified (0: < 1; 1: 1–10; 2: 11–100; 3: 101–1000; 4: > 1000 seeds m^{-2}). Data were reclassified in order to compare the seed composition with the vegetation composition in the ordination analysis. Data for the reference and degraded meadow vegetation from 2005 were limited, and therefore, omitted from the time series.

Then, we used a canonical correspondence analysis (CCA) to examine the relative influence of different factors (hay addition, depth of topsoil removal, and fencing) on the species composition in 2007 (last observation year). For testing the factor significance, the Monte-Carlo permutation test was used (Ter Braak & Šmilauer 2002).

We used Statistica 7 (StatSoft, Inc., 1984–2008) for the statistical tests and Canoco for Windows 4.5 (Ter Braak & Šmilauer 2002) for multivariate techniques.

Results

Abiotic Conditions After Topsoil Removal

Topsoil removal resulted in increased wetness of the plots. We measured higher water levels in the restoration plots than in the degraded meadows, whereas water levels in the plots with deep soil removal were similar to those in the reference sites (Table 1). We measured no differences between plots with and without hay, neither with respect to average water level (mean ± standard deviation 35.7 ± 23.1 plot with hay versus 34.5 ± 22.7 plot without hay, values given for the part with shallow removal) nor with respect to elevation (measured with a surveyor's level 1 year after the soil removal, $n = 32$, factorial ANOVA $F = 0.13$, $p = 0.7$). There were significant differences in elevation between plots with shallow and deep removal (factorial ANOVA $F = 96.04$, $p < 0.0001$).

Table 1. Groundwater level characteristics for 2004–2007 (2005–2007 on the restoration plots) in centimeter below surface, negative values indicate water above surface.

	Reference Meadows	*Shallow Soil Removal*	*Deep Soil Removal*	*Degraded Meadows*
Mean ± SD	18.0 ± 19.8	36.4 ± 23.0	24.4 ± 23.0	83.6 ± 25.8
Median	16.2	34.7	22.8	85.0
Minimum	42.4 ± 13.1	58.7 ± 16.9	46.7 ± 16.9	108.5 ± 17.1
Maximum	−5.0 ± 10.3	14.3 ± 23.2	2.3 ± 23.2	59.2 ± 22.3
Amplitude	47.3 ± 14.9	44.4 ± 23.1	44.4 ± 23.1	49.3 ± 10.0

Minimum—average of three lowest values measured each year; maximum—average of three highest values measured each year; amplitude = maximum−minimum.

Table 2. Total phosphorus and nitrate contents of the soil (mean ± SE) in the degraded and reference meadows and after topsoil removal.

	P_{tot} % Dry Weight	NO_3^- mg dm^{-3} Fresh Weight
R^2 ANOVA model	0.89**	0.51**
Location effect	$F = 28.52$**	$F = 12.33$**
Degraded meadows	1.00 ± 0.06^a	35.50 ± 6.02^a
Reference meadows	0.24 ± 0.03^b	2.70 ± 0.8^b
Shallow removal	0.08 ± 0.006^c	20.23 ± 6.22^c
Deep removal	0.16 ± 0.05^{bc}	4.53 ± 0.91^b

ANOVA results with Fisher LSD post hoc test. Significance: **$p < 0.001$; *$p < 0.05$; n.s. not significant. Different letters indicate differences significant at the level $p < 0.05$.

Total phosphorus level (P_{tot}) of the soil was significantly lower in both soil removal treatments than in the degraded meadows (Table 2) but did not differ from levels in the reference meadows (or were even lower than that). The soil nitrate NO_3^- content was also lower after soil removal than in the degraded meadows (Table 2). The deep and shallow removal treatments differed significantly. The nitrate contents of the deep removal plots did not differ from those of the reference meadows.

Species Establishment in Relation to Seed Availability

We found 6788 seeds m^{-2} of 33 common meadow or ruderal species in the soil seed bank after shallow removal and only 83 seeds m^{-2} of five ruderal species in the soil seed bank after deep soil removal. We recorded on average 1174 viable seeds m^{-2} of 41 species that were transferred with hay to the donor area.

The vegetation composition showed a higher correlation with the composition of seeds in the hay than with the composition of the seed bank (Table 3). In 2005, we found a significant relationship between species frequency and seed availability in the hay for both removal depths but in 2007, this relation was found only for the deep removal treatment. The seed bank showed a weak correlation with the species abundance in the shallow removal variant, but only in the plots without hay and only in 2005 (Table 3).

Development of Vegetation Structure

We found significant effects of the treatments, treatment interactions, and interactions with time in all analyzed characteristics of the vegetation structure (Table 4).

The cover of the vascular plants increased steadily over time in all treatments, but this increase was highest in the plots with hay and slightly lower in the plots with deep removal and in the non-fenced plots (Fig. 1). It reached a similar level of 60–80% in all plots after 4 years. The percentage of bare soil in the shallow removal treatment did not differ between sites with and without hay addition after 4 years. In the deep removal treatment, the plot without hay had more open soil than the plot with hay (circa 20% difference). Also, the accessibility for large animals resulted in significantly more open soil (10–20% difference). We observed a decreasing fit over time of the ANOVA model for vascular plant cover and bare soil. The litter cover was strongly determined by hay transfer in the first years (60–100% with hay, 0–10% without hay), and even after 4 years litter cover was 40–50% higher on plots with hay transfer than on plots without hay transfer. We observed a reduction of the litter layer in plots with deep removal and in non-fenced plots. The establishment of mosses was higher in the deep removal treatment (30–50% cover vs. 10% cover in shallow removal treatment) and after transfer of hay. The establishment of mosses was also higher in the fenced plots than in the non-fenced plots (30% cover vs. 3–5% cover).

Aboveground biomass increased over time in all treatments. The effects of removal depth and fencing on biomass were not significant. The biomass on the plots with hay reached a level of 307 ± 81 g m^{-2} (mean ± standard deviation) in 2007, which is close to the values measured in the donor meadow (382 ± 69 g m^{-2}). The biomass on the plots without hay was much lower (157 ± 56.5 g m^{-2}).

Table 3. Correlations (Pearson r, with t-test of significance) between the number of seeds (in the hay and in the seed bank of top 5 cm of soil after shallow removal) and frequency of species recorded in the vegetation.

	2005			2007		
	r	p	n	r	p	n
No. of seeds in hay						
With hay, no fence, shallow removal	0.43	**	26	0.31	n.s.	27
With hay, no fence, deep removal	0.53	***	25	0.41	*	29
With hay, fenced, shallow removal	0.45	**	22	0.21	n.s.	25
With hay, fenced, deep removal	0.47	**	25	0.42	**	30
No. of seeds in seed bank 20–25 cm						
No hay, no fence, shallow removal	0.27	n.s.	28	0.25	n.s.	25
No hay, fenced, shallow removal	0.38	*	28	0.24	n.s.	22
With hay, no fence, shallow removal	0.04	n.s.	26	−0.00	n.s.	24
With hay, fenced, shallow removal	−0.02	n.s.	26	−0.11	n.s.	22

Significance: ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$; n.s. not significant.

Table 4. Results of RM ANOVA for 2004–2007 data (2006–2007 for biomass).

Variable	*Vascular Plants Cover*	*Bare Soil Cover*	*Moss Cover*	*Litter Cover*	*Aboveground Biomass*	*Total No. of Species 4 m²*	*No. of Species Not Present in Hay or Seed Bank 4 m²*
Intercept	***	***	***	***	***	***	***
Hay	***	***	***	***	***	n.s.	***
Fence	***	***	*	***	n.s.	***	**
Depth	***	***	***	***	n.s.	***	***
Hay × fence	***	***	***	***	*	***	***
Hay × depth	n.s.	***	***	n.s.	*	n.s.	***
Fence × depth	n.s	***	*	**	n.s.	n.s.	n.s.
Hay × fence × depth	***	*	***	***	n.s.	***	n.s.
Time	***	***	***	***	***	***	***
Time × Hay	***	***	***	***	***	***	***
Time × Fence	***	***	***	***	n.s.	*	n.s.
Time × Depth	***	***	***	***	*	***	***
Time × Hay × fence	***	***	***	***	n.s.	n.s.	n.s.
Time × Hay × depth	***	***	***	***	n.s.	***	***
Time × Fence × depth	***	*	n.s.	***	n.s.	*	*
Time × Hay × fence × depth	***	***	***	***	n.s.	***	*
Model R^2 2004*	0.62	0.98	0.39	0.98	—	0.37	0.66
Model R^2 2005*	0.55	0.95	0.43	0.91	—	0.48	0.61
Model R^2 2006*	0.41	0.84	0.18	0.99	0.28	0.18	0.75
Model R^2 2007*	0.32	0.47	0.51	0.71	0.61	0.19	0.63

Significance: ***$p < 0.0001$; **$p < 0.001$; *$p < 0.05$; n.s. not significant. Each column shows results from an RM ANOVA with one dependent variable and four factors (time, hay addition, fencing, and depth of soil removal).

Species Richness in Relation to the Treatments

The total number of species increased in the first year and then remained constant or slightly decreased during the next 2 years (Fig. 2A; Table 4). The plots with shallow removal showed a slightly higher species richness than those with deep removal (mean 21.9 vs. 20.4, respectively, $p < 0.01$). After 4 years, the total species richness in non-fenced plots was slightly higher after hay transfer (mean 22.8 species vs. 18.3 without transfer, $p < 0.0001$), whereas in fenced plots hay transfer had no effect ($p = 0.63$). The number of species that were absent from seed bank and from the hay was higher in the plots with deep removal than with shallow removal (mean 10.6 vs. 6.8, $p < 0.0001$), higher in the plots without hay than with hay (mean 11.7 vs. 5.7, $p < 0.0001$) and slightly higher in the fenced than in non-fenced plots (mean 9.0 vs. 8.4, $p < 0.001$) (Fig. 2B).

Development of the Community Composition

The preliminary DCA showed a gradient length of 5.3 and 3.99 and a cumulative percentage of variance explained of 6.2 and 9.9% for the first and second ordination axes, respectively. The DCA indicated a similar species composition of seeds in the hay and the vegetation of the reference meadow, while the records of the seed bank were more similar to the species composition of the plots without hay transfer in the first year after the topsoil removal.

The RDA analysis showed a change of the species composition over time in comparison to the reference meadows, degraded meadows, soil seed bank, and hay (Fig. 3). Initially, all plots were very similar, but later they developed along different trajectories. The vegetation in the deep removal treatment with hay addition changed most in the direction of the reference vegetation, whereas shallow removal plots with hay

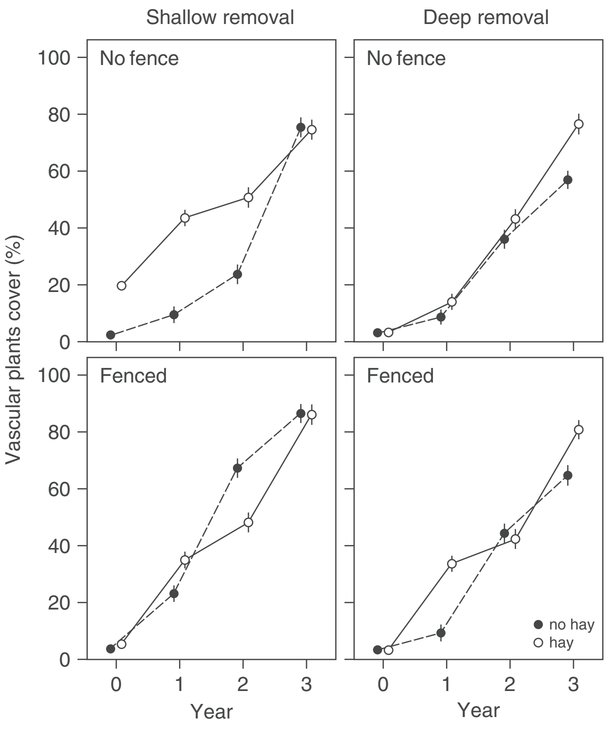

Figure 1. Change of vascular plant cover on 4 m² plots from 2004 (year 0) to 2007 (year 3). Error bars represent 95% confidence interval. Error bars for the first record are very small and not visible on the graph.

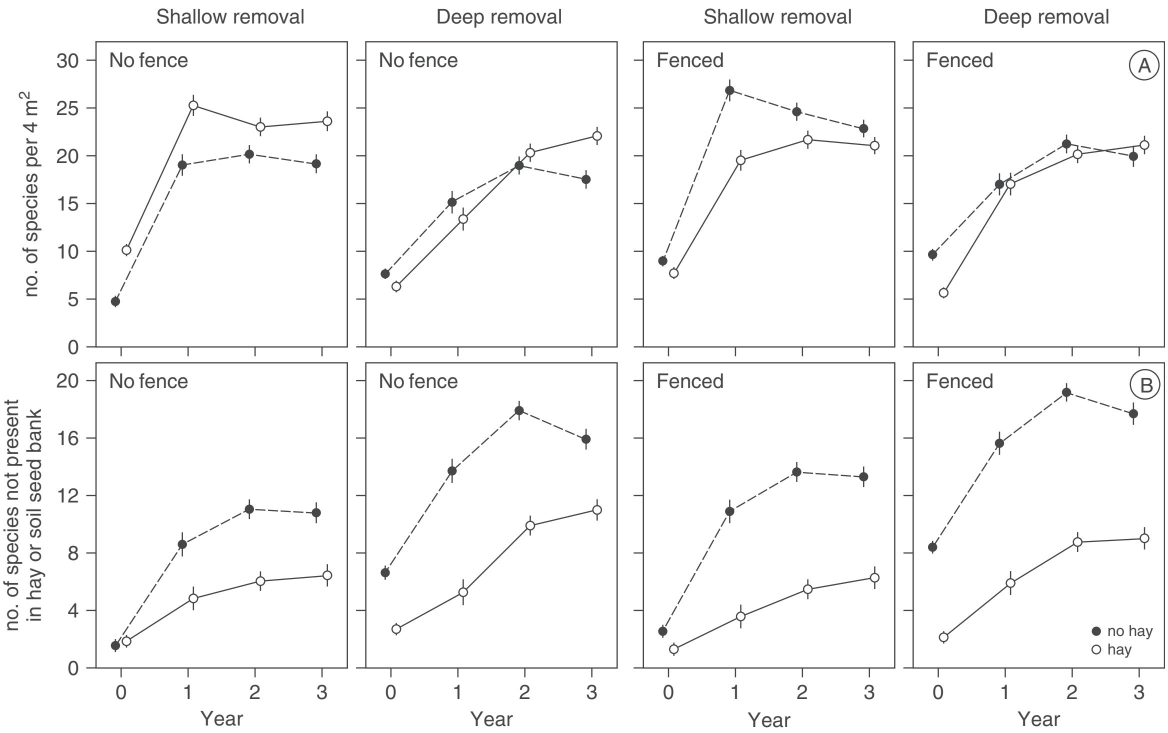

Figure 2. Number of species on 4 m^2 plots from 2004 (year 0) to 2007 (year 3) in the vegetation under various treatments: total number of species (A) and number of species absent from seed bank and from transferred hay (B). Error bars represent 95% confidence interval.

were most similar to the degraded meadows. The plots without hay transfer developed in a very different way, resembling neither reference nor degraded meadow plots. Fenced and non-fenced plots generally developed in a similar way, but fenced plots with deep removal and hay addition became more similar to the reference vegetation than those that were open to animals.

The CCA analysis with the vegetation data from 2007 showed that all three investigated factors (hay transfer, depth of soil removal, and fencing) had a significant impact on species composition (Table 5). Hay addition and removal depth explained more variance in species composition and abundance data than fencing and were of equal importance.

We observed a high abundance of common meadow species and several fen meadow species in the deep removal treatment with hay addition, whereas species from degraded meadows were common on the plots with shallow removal, regardless whether hay had been applied or not. Many pioneer and woody species associated with flooded, organic soils (in the deep removal treatment), or ruderal communities (in the shallow removal treatment) were found in the plot without hay.

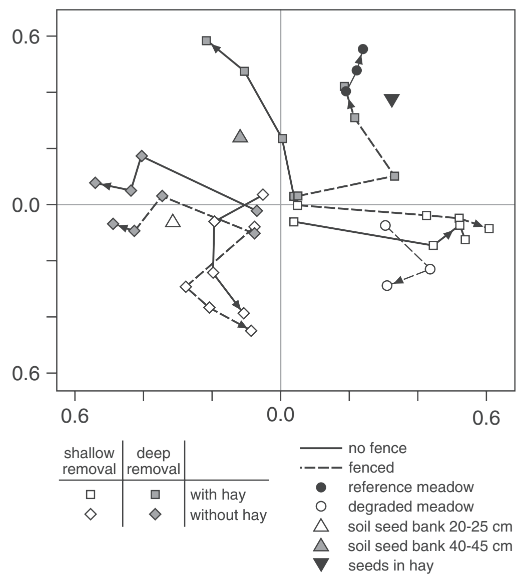

Figure 3. Changes in the species composition over time in various treatments (recorded annually in years 2004–2007), degraded meadows and the reference (RDA ordination graph). First ordination axis: $\lambda = 0.10$, cumulative percentage of variance explained 12.4%; second ordination axis: $\lambda = 0.055$, cumulative percentage of variance explained 19.1%. Test of all canonical axes: $F = 30.3$, $p = 0.002$.

Discussion

Our study showed that the best effects in fen meadow restoration could be achieved rather fast by a combination of deep removal and hay transfer, in which both hay application and soil removal depth had large influence on vegetation development. This is in accordance with other studies (Patzelt et al. 2001; Hölzel & Otte 2003; Klimkowska et al. 2007*a*; Rasran et al. 2007). Combinations of these two measures

Table 5. Relative importance of the factors analyzed by CCA.

Relative Importance of Factors Tested in the Forward Selection Procedure			
Faction	*F*	*P*	*Absolute % Explained Variance*
Hay addition	33.23	0.002	6
Depth of soil removal	32.92	0.002	5.7
Fence	7.12	0.002	1.3
CCA results			
	Axis 1	*Axis 2*	
Eigenvalues	0.315	0.250	
Cumulative % variance explained	6.8	12.2	
Total inertia	4.624		

The explained variance expresses the proportion of the randomness in species composition, which is explained by the factors.

resulted in optimal abiotic conditions, supplied seeds and favorable environmental conditions for the establishment of fen meadow vegetation, eliminated the non-target vegetation and its seed bank, and probably prevented the establishment of wind-dispersed non-target species. Good restoration results were achieved already after a few years, whereas vegetation development after re-introduction of management or soil disturbance without species introduction may stay different from the original even after 25–30 years (Hirst et al. 2005).

Seed Addition and Soil Seed Bank in Relation to Species Establishment

We found that the transfer of hay had a strong effect on species abundance in the vegetation. The majority of species introduced with the hay were strong competitors (e.g., *Deschampsia caespitosa*) that were also abundant in the surroundings and could easily colonize new sites. Competitive and quickly spreading species often profit from restoration actions, such as seed addition (Pywell et al. 2003; Klimkowska et al. 2007*a*). Therefore, it is important to collect hay for transfer from stands with a high proportion of target species and during the time when they have ripe seeds, to maximize the establishment chances of such species (Rasran et al. 2006; Kiehl et al. 2006). The relationship between the number of seeds in the hay and species abundance in the vegetation decreased over time, probably due to an increasing influence of internal community dynamics.

In contrast to hay transfer, the seed bank did not contribute much to vegetation development, even in the shallow removal treatment. A reason for this could be the relatively low seed density of circa 6800 seeds m^{-2} as compared to circa 50,000 seeds m^{-2} in the top 0–10 cm soil layer in degraded meadows (A. Klimkowska, unpublished data). Fen meadow species typically form only short-term persistent soil seed banks (Jensen 1998; Matus et al. 2003), which limits regeneration from the seed bank after a long period of degradation. On the contrary, a high density of long-term persistent seeds of ruderal species in the soil is a legacy of degraded meadows. Removal of this seed bank with the topsoil is an effective measure to limit non-target species re-establishment (Tallowin & Smith 2001; Pywell et al. 2003) and to enhance conditions for less competitive stress-tolerant fen species (Lenssen et al. 1998; Isselstein et al. 2002). This mechanism may be especially important in the beginning of community development and in the seedling stage.

The species with a high seed density in the soil seed bank were also abundant in the surroundings and could easily disperse into the plots. Most likely the layer of hay hampered their germination and/or establishment from the seed bank, that would explain the lower contribution of seed bank-related species in the hay application treatment, which was also found in other studies (Xiong et al. 2003; Eckstein & Donath 2005; Donath et al. 2006).

Vegetation Structure and Species Richness in Relation to Restoration Measures

We found an increase of vascular plant cover and a decrease of bare soil in all treatments, probably resulting in a decreasing colonization probability over time. The addition of hay resulted in a higher total species richness, but also in less species colonizing the plots from sources other than the seed bank or the hay (possibly by inhibiting seeds of pioneers and woody species). If adjacent areas are dominated by non-target, weedy, quickly dispersing species (as in our case) this inhibiting effect of hay may play an important role in the early community assembly process. The establishment of new species decreased with time to zero, except for the deep removal treatment with hay, where we observed new species entering the vegetation even after 4 years. Other studies also reported a prolonged local establishment, despite a decline in bare soil, thus suggesting that even after a few years there is still enough recruitment space (Schächtele & Kiehl 2004; Kiehl & Wagner 2006). The establishment of mosses was higher in the deep removal treatment and after transfer of hay, probably due to vegetative spread (Poschlod & Biewer 2005).

Disturbance by large animals led to a continuous creation of new gaps and resulted in a higher total species richness on non-fenced plots, especially when combined with hay transfer. Lower litter accumulation in non-fenced plots is also likely to be caused by biomass removal by animals. Small-scale soil disturbances were reported to be important for species establishment in productive sites, whereas seed availability is a bottleneck in low productive sites (Foster 2001; Foster et al. 2004). However, we did not observe any positive effect of large animals (mainly wild boar) on target vegetation establishment (Rasran et al. 2007). Their effect was mainly related to soil disturbances, allowing for species establishment from the persistent soil seed bank (mostly of non-target species) on plots with hay (Kotanen 1995; Thompson et al. 1998; Schmidt et al. 2004). Moreover, wild animals may transport seeds of non-target species to restored areas, when they forage in highly productive communities (Mouissie et al. 2005*b*). In our experiment, exclusion of such disturbances resulted in a vegetation composition more similar to the target fen meadow.

Aboveground biomass increased over time in all plots, which was probably related to the increase in vegetation cover. This increase was strongest after hay transfer, due to a high contribution of grasses and sedges, which were introduced with the hay. Light competition probably becomes an important constraint for target species establishment in later stages, (Kotowski & van Diggelen 2004), but after 4 years the canopy was still open.

The Importance of Starting Conditions

The increasing differences in species composition between the different treatments in our study imply that early conditions may have a strong impact on later vegetation development, possibly due to priority effects (Ross & Harper 1972; Suding et al. 2004). Other studies also showed priority effects in plant communities, either driven by plant litter (Facelli & Facelli 1993), soil microbial communities (Kardol et al. 2007) or random processes, such as community history or sequence of species arrival (Chase 2004).

In our study, the seed availability was likely the most important for the occurrence of priority effects. The amount of seeds of non-target species in the soil seed bank or reaching the site from the surroundings was much higher than the number of seeds of target vegetation. Changing this ratio by seed transfer may cause a priority effect. This ratio may be changed further by suppressing the germination of non-target species. Hay transfer may lower germination and seedling survival due to litter accumulation (Jensen 1998; Eckstein & Donath 2005), chemical inhibition (Muller 1969) or pathogen attacks (Moles & Westoby 2004), but may also facilitate seedling establishment by conserving soil moisture (Cobbaert et al. 2004; Eckstein & Donath 2005). Our results suggest that hay application may restrict unwanted colonization from the surroundings and inhibit the germination of non-target species from the seed bank (Eckstein & Donath 2005; Donath et al. 2006).

Implications for Practice

- Fen meadow restoration can be achieved by applying topsoil removal followed by species introduction (by hay addition).
- Topsoil removal and hay addition should be conducted together and immediately at the beginning of the restoration. Otherwise, the restoration might be delayed because of unanticipated vegetation development.
- Hay transfer from various mowing dates and several species-rich stands (multiple hay transfer) could be recommended, in order to facilitate target community development. Accumulation of thick hay layers, however, should be avoided to allow the establishment of light-demanding target species.
- Large animals, such as wild boar, did not enhance the dispersal and establishment of target vegetation in restored fen meadows. Excluding soil disturbances by large animals, at least in the first years, resulted in a vegetation more similar to that of the reference meadows.
- In the long run, regular mowing will be necessary to avoid shrub encroachment and dominance of competitive species.

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