

SEED BANKS OF MANAGED AND DEGRADED GRASSLANDS IN THE KRKONOŠE MTS., CZECH REPUBLIC

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Abstract: Grassland seed banks are traditionally considered a source of new species in degraded communities. However, many recent studies have shown that the potential of the seed bank to restore many communities is rather limited. Two principal reasons for these limitations, loss of species from the seed bank or inability of the species to create any seed bank, are, however, usually not distinguished.

This study aims to assess the role of seed bank composition and heterogeneity in the restoration of species-rich plant communities. It was carried out in mountain grasslands in the eastern part of the Krkonoše Mountains, Czech Republic. The composition of vegetation and seed bank were recorded and their relationship was assessed in 1.5 m × 1.5 m plots placed in non-degraded and degraded parts of seven grasslands. Vegetation at currently managed sites is not degraded; degraded parts were without management (dominated by *Holcus mollis*, *Bistorta major* or *Rumex alpinus*). The degree of heterogeneity of seed bank and vegetation was tested as the relationship between subplot similarity, distance, and degradation stage.

Degradation had significant effects on composition of both aboveground vegetation and seed bank and increased heterogeneity both in the vegetation and in the seed bank. Species absent from the vegetation of degraded plots were also absent from the seed bank of both degraded and non-degraded plots, indicating that the absence of species from the seed bank is not due to their loss during the degradation process but rather due to the low number of seeds in the seed bank already in the non-degraded communities. Furthermore, the seed bank of the degraded communities largely results from the present vegetation of these communities. This supports the limited role of the seed bank in these communities. Restoration of these sites is thus impossible unless management will include methods with which seeds will arrive at the degraded sites.

Keywords: Degradation, Diversity, Management, Mountain grasslands, Reestablishment, Seed bank, Spatial heterogeneity

Nomenclature: KUBÁT et al. (2002)

Electronic appendix (www.ibot.cas.cz/fofia): Appendix 1, 2, and 3

INTRODUCTION

Land-use changes in the second half of the 20th century in many European countries resulted in irregular management or abandonment of many grasslands with low productivity leading to their degradation and the loss of many rare species (BAKKER 1989, MILBERG 1992, MILBERG & PERSSON 1994, DUTOIT & ALARD 1995, JENSEN 1998, WILLEMS & BIK 1998, KRAHULEC et al. 2001). As a response, many attempts to restore these grasslands have recently been made, resulting in a large number of studies trying to determine the optimal management of these sites (e. g. WILLEMS 1983, BAKKER 1989, SMITH & RUSHTON 1994, RYSER et al. 1995, KRAHULEC et al. 1997, STAMPFLI & ZEITER 1999).

In grasslands that still host a considerable number of species the management regimes should aim to support the spread of disappearing species (e.g. MILBERG & HANSSON 1993, MILBERG & PERSSON 1994, JENSEN 1998, MATUS *et al.* 2003). However, in many cases some species have already been lost (e. g. MILBERG 1995, BAKKER *et al.* 1996, DAVIES & WAITE 1998, WILLEMS & BIK 1998, FALIŇSKA 1999, WAGNER *et al.* 2003). Successful restoration is thus not possible without the reappearance of these species. Still most restoration studies are only concerned with changes in species abundance under different management regimes (e.g. OOMES & MOOI 1981, DUTOIT & ALARD 1995, HADINCOVÁ *et al.* 1997, KRAHULEC *et al.* 2001). Lately there have been several studies dealing with the potential of new species arriving both from the surroundings and from the soil seed bank (e.g. KITAJIMA & TILMAN 1996, McDONALD *et al.* 1996, JENSEN 1998, BEKKER *et al.* 2000, BLOMQVIST *et al.* 2003, MATUS *et al.* 2003).

Existing studies on species composition of the grassland soil seed bank usually compare seed bank and vegetation of plots with different management regimes or of different successional stages (e.g. MILBERG 1992, MILBERG & HANSSON 1993, FALIŇSKA 1999, BEKKER *et al.* 2000, WAGNER *et al.* 2003). A common conclusion of such studies is that for restoration seed bank potential is limited (e.g. GRAHAM & HUTCHINGS 1988, BAKKER *et al.* 1996, McDONALD *et al.* 1996, BEKKER *et al.* 1997, BLOMQVIST *et al.* 2003, MATUS *et al.* 2003). For two different reasons: loss of seeds from the seed bank during the degradation process or the inability of the species to create a seed bank that would be large enough to ensure reappearance of the species. These two alternatives are, however, usually not distinguished. Separating these would enhance our understanding of the seed bank potential in the restoration process.

Grassland degradation may not only lead to changes in species composition, but also to changes in spatial structure as demonstrated for aboveground vegetation (e.g. KRAHULEC *et al.* 1997, FALIŇSKA 1999). However, hardly anything is known about the spatial structure of the seed bank (but see INGLIS 2000, OLANO *et al.* 2002) and the relationship between the spatial structure of the vegetation and the seed bank. This knowledge is important as not only species composition but also spatial structure can have a great impact on restoration success.

In this study we want to clarify the two above-mentioned issues and answer the following questions:

- (1) What is the seed bank composition of degraded and non-degraded grasslands?
- (2) What is the relationship between the vegetation and seed bank of the degraded and non-degraded grasslands?
- (3) What is the heterogeneity of the seed bank of degraded grasslands and how is it related to heterogeneity of vegetation?
- (4) Are the species missing in the vegetation of degraded grasslands present in the seed bank of degraded and non-degraded grasslands?

To answer these questions, plots were set up in non-degraded and degraded parts of grasslands of the Krkonoše Mountains (Czech Republic) to estimate the effect of degradation, vegetation and soil seed bank, and their relationships.

The Krkonoše (Giant) Mts. are a typical example of a system of both abandoned and managed grasslands at a higher elevation. These grasslands established after cutting down

Table 1. List of grasslands used in this study. Each locality had degraded and non-degraded parts. The cross indicates which degradation stages were found at that grassland. * – Degraded parts dominated by *Holcus mollis*, *Bistorta major* or *Rumex alpinus*.

Grassland	Position		Altitude (m a.s.l.)	Aspect	Slope	<i>Holcus mollis</i> *	<i>Bistorta major</i> *	<i>Rumex alpinus</i> *	Non-degraded part
Černá Voda	N 50°44'8.4"	E 15°48'48.1"	950	SSW and NW	5° and 20°			x	x
Modrý důl	N 50°42'58.1"	E 15°42'23"	1090	S	20°	x	x		x
Novopacká	N 50°46'8.3"	E 15°36'32"	1210	SW	20°			x	x
Severka	N 50°41'41.2"	E 15°42'40"	1050–1075	NE	20°		x		x
Sněžné Domky	N 50°39'18"	E 15°52'1"	990	E	20°	x			x
Svoboda nad Úpou	N 50°38'17"	E 15°48'42.7"	590	S	20°	x			x
Zadní Rennerovky	N 50°42'3.4"	E 15°40'1.1"	1210–1265	SSE	20°		x	x	x

mountain forests in the 16th and 17th centuries and were used for hay production and livestock grazing (LOKVENC 1978). Regular management (hay production, manuring, and grazing) together with species migration from the surroundings gave rise to one of the richest communities in the Krkonoše Mts. After World War II management in these grasslands changed several times resulting in a mosaic of abandoned and managed grasslands.

MATERIALS AND METHODS

Study localities

The study was carried out in seven grasslands in the eastern part of the Krkonoše Mountains, Czech Republic (Table 1). Each grassland has one non-degraded and one or two degraded parts (Table 1). The non-degraded part had species-rich vegetation at currently managed or just recently abandoned sites without any dominant plant species. Degraded parts were sites without management for about twenty to fifty years (KRAHULEC et al. 1997, KRAHULEC, pers. com.) and were dominated by *Holcus mollis*, *Bistorta major* or *Rumex alpinus* (Table 1). In each part of the grassland, three 1.5 m × 1.5 m plots were established and subdivided into 9 subplots (0.5 m × 0.5 m). We do not have any direct data to prove the statement that the degraded plots are degradation stages of the non-degraded plots found at the same locality. All the plots within one locality have almost the same slope, aspect, and bedrock giving no reason to expect that they have been different. However, the reasons for development of one or the other degradation stage are still largely unknown.

Seed bank and vegetation composition

The seed bank was estimated using the seedling emergence method, because this method with stratification is more reliable for determining the species composition of the seed bank of a plant community than elutriation (GROSS 1990). In each subplot, two soil cores of 5 cm in depth (volume 100 cm³, diameter 5 cm) were taken at random in October 2002 from the soil surface. Each sample was treated separately. Litter layer, stones, roots, and rhizomes were

manually removed from the soil samples and the remaining soil was spread in plastic dishes (diameter 13 cm) on a layer of 1.5 cm sterilized sand. When removing litter, care was taken to not remove seeds captured in the litter layer. In this study we collected seed bank samples after the growing season and included the topsoil layer to estimate the composition of both the persistent and transient seed bank and were thus likely to record also species arriving through seed rain to the locality.

The dishes were placed in a temperature-controlled greenhouse (10–20 °C) and kept moist. One sample from each subplot was set to germinate within two weeks, the second after four-month stratification (left outside in a common garden over winter from November to February). Emerging seedlings were identified using KROPÁČ & NEJEDLÁ (1956), CSAPODY (1968), and LHOTSKÁ et al. (1985). Unidentified seedlings were replanted for later identification. Some closely related species that were difficult to distinguish in the young stage were merged (Table 2). The number of merged species is higher than common in other seed bank studies. Most of the seed bank studies are, however, done only at one locality. Our study was performed in a large number of localities and vegetation types, and we had to ensure that the species names corresponded across all the sites. Thus we often had to merge species due to a few uncertainties at a single site. Where names of genera (except for *Bistorta*, *Holcus*, and *Rumex*) are used in the text, they are shortcuts of merged species (Table 2). Before identification 4.5% of the seedlings died. Therefore, these seedlings could not be included in statistical analyses. Both stratified and non-stratified samples germinated four months. After two months of germination the soil was stirred, because mixing is known to cause more seeds to germinate (ROBERTS 1981 sec. cit. DUTOIT & ALARD 1995). When no more seeds germinated (approximately after four months), germination was ended.

In each subplot, the composition of the aboveground vegetation was recorded in June 2003 on a 1–3 cover scale (< 5%, 5–25%, > 25%).

Data analysis

For all analyses, seedlings that germinated from the seed bank before and after stratification were summed. To obtain data from plots, data from subplots were summed. For analyses comparing seed bank and vegetation, data were transformed to presence/absence of species. We also performed the same analysis without this transformation using standardization by samples to convert vegetation covers and number of seeds to the same units. Since there was no difference between these two types of results, only the results of the former analysis are reported. For the comparison of different degradation stages in grasslands with two types of degraded plots the non-degraded plots were used as reference plots for both types of degraded plots.

All analyses concerned with species composition and species richness were done at both plot and subplot level. However, there was no major difference in the results between the two levels. Showing both results would thus mean showing almost twice the same results. Therefore, only results for plot level are shown further.

All univariate statistical analyses were run under S-PLUS (2000) and all graphs were done using STATISTICA (STATSOFT, INC. 1998). All multivariate analyses were run under CANOCO (TER BRAAK & ŠMILAUER 1998).

Table 2. Species that were difficult to distinguish in vegetation or seed bank in young stage and were merged.

Shortcut	Merged species
<i>Agrostis</i>	<i>A. capillaris</i> , <i>A. stolonifera</i>
<i>Anthoxanthum</i>	<i>A. alpinum</i> , <i>A. odoratum</i>
<i>AvfNast</i>	<i>Avenella flexuosa</i> , <i>Nardus stricta</i>
<i>Campanula</i>	<i>C. bohémica</i> , <i>C. rotundifolia</i>
<i>Carex</i>	<i>C. brizoides</i> , <i>C. canescens</i> , <i>C. nigra</i> , <i>C. ovalis</i> , <i>C. pallescens</i> , <i>C. pilulifera</i>
<i>DafuGyco</i>	<i>Dactylorhiza fuchsii</i> , <i>Gymnadenia conopsea</i>
<i>Epilobium</i>	<i>E. alpestre</i> , <i>E. ciliatum</i>
<i>Gnaphalium</i>	<i>G. norvegicum</i> , <i>G. sylvaticum</i>
<i>Hierlala</i>	<i>Hieracium lachenalii</i> , <i>H. laevigatum</i>
<i>Juncus</i>	<i>J. effusus</i> , <i>J. filiformis</i>
<i>Luzula</i>	<i>L. campestris</i> agg., <i>L. luzuloides</i> , <i>L. pilosa</i>
<i>Poa</i>	<i>P. annua</i> , <i>P. humilis</i> , <i>P. pratensis</i> , <i>P. trivialis</i>
<i>Ranunculus</i>	<i>R. acris</i> , <i>R. auricomus</i> , <i>R. repens</i>

Species richness

Analysis of variance (ANOVA) was used to test the effects of seed bank/vegetation, degradation stage, and their interaction on number of species. Data had normal distribution. Plot was nested within locality.

χ^2 -test with Yates correction was used to assess differences between number of species present in the vegetation and seed bank of degraded and non-degraded plots. All species present in the vegetation of non-degraded plots are included in these analyses.

Species composition

Redundancy analysis (RDA, direct gradient analysis assuming a linear relationship between species abundance and environment; TER BRAAK & ŠMILAUER 1998) was used to assess the relationship between seed bank and vegetation. To estimate significance of the differences, we used a randomization test with a split-plot design. Seed bank and vegetation of the same plot made up a whole plot. Only split plots were randomized. Data were not standardized by species, for standardization by samples, see above.

RDA was also used to assess the effect of degradation (comparison of different degradation stages) separately on the vegetation and seed bank and to assess differences in seed bank after removing the effect of vegetation. This was done using sample positions on the first and second axes from principal component analysis (PCA, indirect gradient analysis assuming a linear relationship between species abundance and environment; TER BRAAK & ŠMILAUER 1998) of vegetation as a covariable in the analysis of seed bank. Unrestricted permutations within blocks defined by localities were used for randomization.

Heterogeneity

Degree of heterogeneity of seed bank and vegetation was tested as subplot similarity between all possible pairs of subplots. For each pair of subplots, Jaccard's similarity index (MORAVEC et al. 1994) was calculated separately for seed bank and vegetation. Each pair of

Table 3. Position of species on the first ordination axis from redundancy analysis (RDA) comparing species composition of seed bank and vegetation. The first ordination axis explained 20.7% of total variation in the dataset. Twenty-five species with strongest affinity to vegetation or seed bank (highest absolute value on the 1st axis) are shown. Species present only in the seed bank or only in the vegetation are set in bold.

Species	1st axis
Prevail in vegetation	
<i>Cardaminopsis halleri</i>	-2.457
<i>Vaccinium myrtillus</i>	-2.215
<i>Ficaria verna</i> subsp. <i>bulbifera</i>	-2
<i>Galeopsis tetrahit</i>	-2
<i>Galium mollugo</i> agg.	-2
<i>Galium saxatile</i>	-2
<i>Hieracium aurantiacum</i>	-2
<i>Imperatoria ostruthium</i>	-2
<i>Melampyrum pratense</i>	-2
<i>Melampyrum sylvaticum</i>	-2
<i>Myrrhis odorata</i>	-2
<i>Phleum rhaeticum</i>	-2
<i>Picea abies</i>, juv.	-2
<i>Pimpinella saxifraga</i>	-2
<i>Plantago lanceolata</i>	-2
<i>Stellaria graminea</i>	-2
<i>Trientalis europaea</i>	-2
<i>Veronica chamaedrys</i>	-2
Prevail in seed bank	
cf. <i>Arabidopsis thaliana</i>	2
<i>Chenopodium album</i> agg.	2
<i>Cirsium heterophyllum</i>	2
<i>Trifolium pratense</i>	2
<i>Deschampsia cespitosa</i>	2.411
<i>Silene vulgaris</i>	2.457
<i>Crepis paludosa</i>	3.486

subplots was assigned to a distance category, indicating the distance between the two subplots within a pair. By definition, each distance category has a different number of pairs (1: 12, 2: 8, 3: 6, 4: 8, 5: 2). Therefore, the average index for each category was calculated. The average similarity between seed bank and vegetation was expressed in percentages.

ANOVA was used to assess the relationship between subplot similarity (expressed as average index for each distance category) and distance, seed bank/vegetation, degradation stage and their interactions. Data had normal distribution. To adjust the number of degrees of freedom in the test of the effect of seed bank/vegetation and degradation stage, plot was used as an error term.

RESULTS

Seed bank

All together 31,290 seedlings germinated from the seed bank. Most of the seedlings (95.5%) were identified to the species or genus level. There were 42% graminoids and 58% forbs. The most abundant species were *Hypericum maculatum* (25%), *Juncus* (24.5%), *Rumex alpinus* (12%), *Agrostis* (6.7%), *Calluna vulgaris* (5.4%), and *Cardaminopsis halleri* (4%). Two of the dominants in the degraded plots occurred only in very low densities in the seed bank,

Holcus mollis 0.6% and *Bistorta major* 0.1%. Their densities were higher when considering only plots where they dominated (*Holcus mollis* 6.5%, *Bistorta major* 0.7%; Appendix 2). The total average seed bank density (\pm s.e.) was $18,108 \pm 23,602$ seedlings/m². Of the 28 species missing in the vegetation of degraded plots, 79% did not form seed bank even in the non-degraded plots (Appendix 1). Only 15 of the 45 species (33%) that survived in the vegetation of the degraded plots from the vegetation of the non-degraded plots did not have a seed bank in the non-degraded plots. This difference was significant ($\chi^2 = 12.38$, $P < 0.001$). Species absent from the vegetation of the degraded plots were also absent from the seed bank of the degraded plots ($\chi^2 = 17.51$, $P < 0.001$; Table 4, Appendix 1).

Table 4. Two-by-two frequency table for χ^2 -test of number of species in vegetation of degraded plots and in seed bank of non-degraded and degraded plots. All species present in the vegetation of non-degraded plots are included. List of these species is provided in Appendix 1.

		Seed bank of non-degraded plots		Seed bank of degraded plots	
		Absent	Present	Absent	Present
Vegetation of degraded plots	Absent	22	6	23	5
	Present	15	30	13	32

Vegetation versus seed bank

Significant differences between seed bank and vegetation estimated by RDA (Trace = 0.07, $F = 13.805$, $P = 0.005$; Table 3) corresponded to the low average similarity between species composition of the seed bank and vegetation (27%). These differences were mainly due to the absence of many species in the seed bank. Of the 99 species recorded, 9 were present only in the seed bank, and 39 only in the vegetation (Appendix 2).

Species richness

Significant differences in species richness between the degradation stages in both seed bank and vegetation ($F_{3,38} = 5.090$, $P = 0.005$) exist and a significant interaction occurred between seed bank/vegetation and degradation stage ($F_{3,44} = 7.393$, $P < 0.001$). The difference in species richness between seed bank and vegetation was not significant ($F_{1,44} = 1.593$, $P = 0.214$). Non-degraded plots hosted on average 16 species in vegetation per 1.5 m \times 1.5 m; plots dominated by *Rumex* had 12 species, by *Holcus* 11 species, and by *Bistorta* 10 species (Fig. 1). The highest number of species in the seed bank was in the plots dominated by *Rumex* (15), plots dominated by *Bistorta* and *Holcus* and non-degraded plots were almost the same (14, 13, and 13 species, respectively). There were more species in the seed bank than in the vegetation in the degraded plots, but there were more species in the vegetation than in the seed bank in the non-degraded plots (Fig. 1).

Species composition

There were significant differences between the four degradation stages in species composition of aboveground vegetation (Trace = 0.149, $F = 5.09$, $P = 0.005$; Fig. 2A), seed bank (Trace = 0.101, $F = 2.861$, $P = 0.005$), and between the seed bank after removing the effect of aboveground vegetation (Trace = 0.078, $F = 2.478$, $P = 0.005$; Fig. 2B).

In the analysis of vegetation composition, the first ordination axis separated plots dominated by *Rumex* and non-degraded plots (Fig. 2a). There were many species occurring mainly in the plots dominated by *Rumex* (*Cardaminopsis halleri*, *Urtica dioica*, *Alchemilla* spp., *Chaerophyllum hirsutum*, *Carduus personata*, *Epilobium*, and *Chrysosplenium alternifolium*). *Potentilla erecta*, *Vaccinium vitis-idaea*, *Calluna vulgaris*, and *Carex*, however, occurred mainly in the non-degraded plots. Plots dominated by *Holcus* and *Bistorta* were very similar to each other. *Galeopsis tetrahit* is the only typical species of these two types of plots (Fig. 2a).

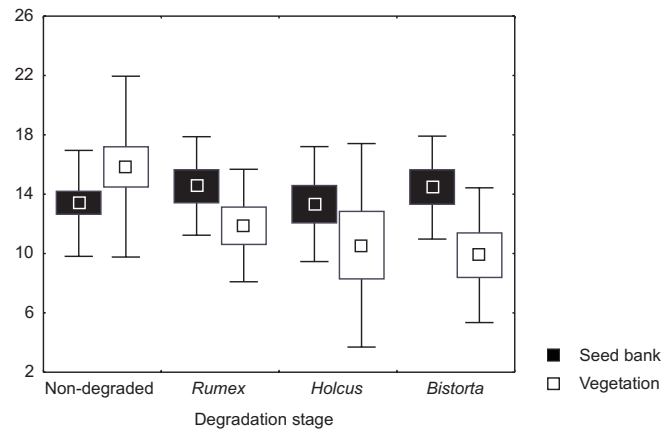


Fig. 1. Differences in species richness between seed bank and vegetation divided into degradation stages. Vegetation was sampled at 1.5 m × 1.5 m plots; seed bank was studied in 1800 cm³ of soil taken from the same plots. The centre of the box represents mean, the box represents standard error, and whiskers represent standard deviation.

Analysis of the seed bank composition clearly separated plots dominated by *Rumex* from all other types. The most common species in the seed bank of the plots dominated by *Rumex* were *Cardaminopsis halleri*, *Chaerophyllum hirsutum*, *Chrysosplenium alternifolium*, *Carduus personata*, *Epilobium*, and *Senecio ovatus*. Non-degraded plots and plots dominated by *Holcus* and *Bistorta* separated just along the second ordination axis. *Galeopsis tetrahit*, *Luzula*, and *Agrostis* dominated the seed bank of plots dominated by *Holcus* and *Bistorta*. *Festuca rubra*, *Ranunculus platanifolius*, *Melampyrum pratense*, *Potentilla erecta*, and *Geranium sylvaticum* dominated the seed bank of the non-degraded plots.

The seed bank composition differed significantly even after removing the differences due to the different composition of vegetation. The percentage of variance of the seed bank explained by the degradation stage declined from 10.1% to 7.8% showing that vegetation composition could account for 2.3% of the variation in species composition of seed banks due to degradation. In this analysis the first axis separated plots dominated by *Holcus* and *Bistorta* and non-degraded plots (Fig. 2b). The species occurring more in the seed bank than in the vegetation of the plots dominated by *Holcus* and *Bistorta* include *Agrostis* and *Galeopsis tetrahit*. The species prevailing in the seed bank of the non-degraded plots included *Melampyrum pratense*, *Geranium sylvaticum*, *Festuca rubra*, *Potentilla erecta*, and *Trifolium pratense*. There were only a few species that occurred more in the seed bank than in the vegetation of the plots dominated by *Rumex* (*Chaerophyllum hirsutum*, *Chrysosplenium alternifolium*, and *Ranunculus platanifolius*; Fig. 2b).

Heterogeneity

Strong differences were found in species composition between neighbouring subplots, and the differences were stronger in the seed bank than in the vegetation (Table 5). The similarity between subplots decreased with distance. The decrease was the same for seed bank and

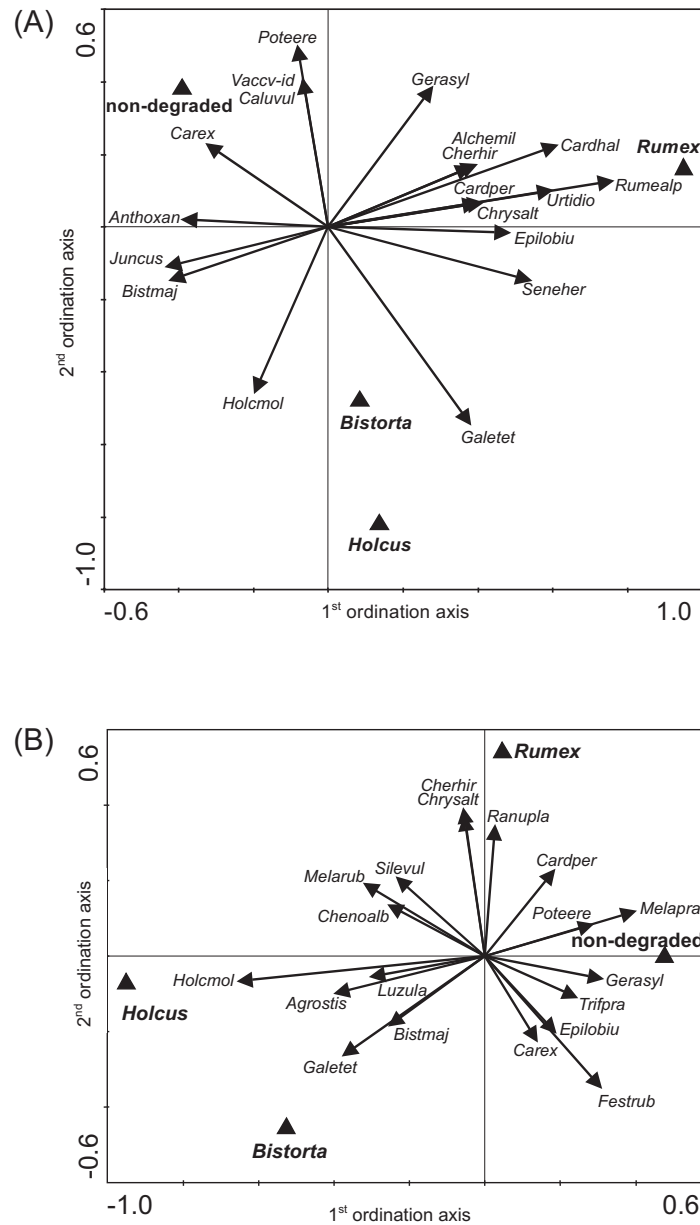


Fig. 2. Results of redundancy analysis (RDA) comparing: (A) Species composition of vegetation of degraded and non-degraded plots. First ordination axis explained 14.7%, second ordination axis explained 8.5% of the total variation in the dataset. (B) Species composition of seed bank of degraded and non-degraded plots after removing the effect of vegetation. First ordination axis explained 8.3%, second ordination axis explained 4.9% of the total variation in the dataset. *Holcus* – plots dominated by *Holcus mollis*, *Bistorta* – plots dominated by *Bistorta major*, *Rumex* – plots dominated by *Rumex alpinus*, non-degraded – plots without any strong dominant. Arrows show direction in which species cover increases. List of the shortcuts of all species used in our study is in Appendix 3.

Table 5. Results of ANOVA testing differences in heterogeneity between seed bank and vegetation, between different distances, and between different degradation stages.

	d.f.	error d.f.	<i>F</i>	<i>P</i>
Degradation stage	3	38	1.621	0.200
Distance	4	176	14.746	< 0.001
Distance × Degradation stage	12	176	2.989	< 0.001
Bank/Vegetation	1	44	78.742	< 0.001
Bank/Vegetation × Degradation stage	3	44	1.281	0.293
Bank/Vegetation × Distance	4	176	1.448	0.220
Bank/Vegetation × Distance × Degradation stage	12	176	2.470	0.005

vegetation but differed between degradation stages, being the weakest in the non-degraded plots (Fig. 3). This indicates that degradation increases heterogeneity and the increase can be detected both in the vegetation and in the seed bank (Fig. 3, Table 5).

DISCUSSION

In this study we present an approach to separate two principal causes for absence of species from the seed bank. The inability of species to form a seed bank and species loss from the seed bank during the degradation process. We do this by comparing seed banks and vegetation of pairs of degraded and non-degraded grasslands within the same localities.

Our results showed that species missing in the seed bank of the degraded plots were also missing in the seed bank of the non-degraded plots. If, however, the species missing from the vegetation of degraded plots were present in seed bank of the non-degraded plots, but not of the degraded plots, it would be worth exploring the time since abandonment of these grasslands and thus the time over which the seeds disappear from the seed bank. In our case, the time since abandonment, and thus the longevity of seeds in the seed bank, is not expected to affect the results; the species of interest do not form any detectable seed bank.

Non-degraded plots hosted more species than any of the degraded plots, but there were not such large differences in the seed bank, and surprisingly the highest number of species in the seed bank was in the plots dominated by *Rumex*. Here, there were more species in the seed bank than in the vegetation, possibly due to the increased effect of competition on germination (TILMAN 1993, KITAJIMA & TILMAN 1996), which is a common effect of degradation.

In spite of similar species number there were significant differences in species composition of the seed bank between degraded and non-degraded plots. The differences are mainly due to the higher proportion of graminoids and ruderal species in the seed bank of degraded plots. The species lost from the seed bank of degraded plots are mainly perennial herbs (e.g. *Geranium sylvaticum*, *Potentilla erecta*) and two grass species (*Festuca rubra*, *Anthoxanthum*). These species persist in the vegetation of degraded plots, but are missing from the seed bank. This is probably because these species are already rare in the seed bank of non-degraded plots, and lowered seed production at the degraded plots is not enough to support a significant seed bank. This corresponds well to the commonly reported transitivity of the seed banks of many grassland species (RICE 1989, FENNER 1992, THOMPSON et al. 1997, BASKIN & BASKIN 1998). In plots dominated by *Rumex* there were

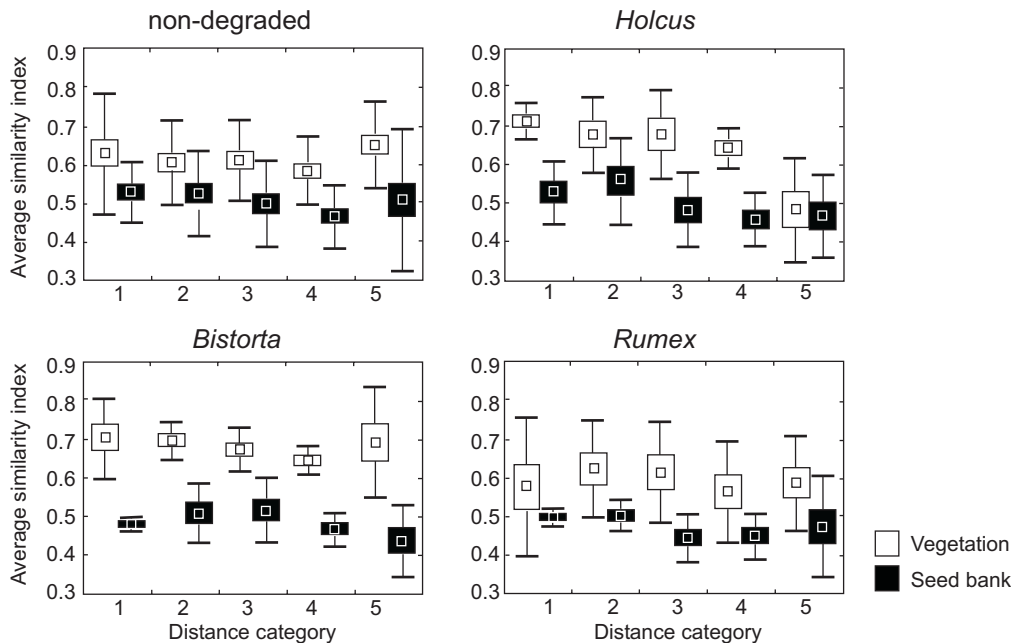


Fig. 3. Differences in heterogeneity (expressed as average similarity index) between seed bank and vegetation divided into five distances and four degradation stages. The centre of the box represents mean, the box represents standard error, and whiskers represent standard deviation. Results of the analysis are in Table 5.

several other species in the seed bank not present elsewhere in the grasslands (*Chaerophyllum hirsutum*, *Chrysosplenium alternifolium*).

Methodological issues

One of our conclusions is based on the argument that rare species are absent from the seed bank even of the non-degraded communities. This may also be a result of a small sample size. The volume of our sample per degradation stage per grassland (5.4 l) exceeds the suggested volume for determining seed bank species composition in grasslands (1.0–1.2 l; HUTCHINGS 1986). The volume corresponds to the sample size used in other studies of seed bank composition (e.g. MILBERG & HANSSON 1993, BEKKER et al. 2000, WAGNER et al. 2003). Still it covers only 1.6% of the plot and we might have missed some rare species. However, given the low germination percentage of species in natural conditions (e.g. JAKOBSSON & ERIKSSON 2000, FRANZÉN 2001, MÜNZBERGOVÁ 2004), the number of seeds present that we missed would be anyhow so low that they would not make a major contribution to restoration.

Another reason for missing rare species may be that the conditions in our greenhouse were not suitable for their germination or survival (4.5% of seedlings died before identification). However, the average number of seedlings (18,108 seedlings/m²) was equal to or higher than the number of seedlings in other studies (e.g. MILBERG & HANSSON 1993, MILBERG 1995,

WILLEMS & BIK 1998, FALIŇSKA 1999), indicating that the conditions in our greenhouse reached the standard conditions.

Species in the seed bank may be also missing due to different spatial heterogeneity of the seed bank and the vegetation. In our experiment, differences between neighbouring plots in species composition were stronger in the seed bank than in the vegetation. Differences between plots increased with distance and differences between distances were bigger in degraded plots than in non-degraded plots. This shows that the lower number of species found in degraded plots may also be partly due to the coarser grain of heterogeneity of these grasslands. However, the sampling design distributing the seed bank samples evenly over the experimental plots should reduce this problem.

Options for restoration of Krkonoše mountain grasslands from the seed bank

There were more species in the seed bank than in the vegetation of degraded plots. Almost none of the species missing in the vegetation of degraded plots were present in the seed bank of these plots. Moreover, these species were also absent from the seed bank of non-degraded plots. This means that management primarily aimed at the suppression of dominant species cannot restore species diversity without external seed import, as was found for example by BEKKER et al. (1997), JENSEN (1998), and BLOMQVIST et al. (2003).

It has been suggested that seed rain from outside the community not seed bank may be the most important source of new species (JEFFERSON & USHER 1989, PEART 1989, JENSEN 1998). In this study we collected both persistent and transient seed banks and were thus likely to record also species arriving through seed rain to the locality. Thus we can conclude that even seed rain cannot be expected to bring new species unless a long period of time is available or there is between year variation in seed production and we did not capture the year with high seed production.

All the above information indicates that new species have to be added by deliberate sowing; alternatively, grazing animals might serve as seed vectors (KRAHULEC et al. 2001). These conclusions are also supported by the data on heterogeneity of seed bank and vegetation. Strong changes in spatial structure of both seed bank and vegetation of the degraded plots when compared to the non-degraded plots support the view that the seed bank of the degraded plots is more a result of the current vegetation of the degraded plots than of the previous non-degraded vegetation.

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