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# **Impact of management on diversity of species-rich grasslands**

Ph.D. Thesis

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## **Annotation**

The thesis analyzes the mechanisms of changes in selected community parameters (species richness, species composition, productivity, seedling recruitment) related to management practices on species-rich grasslands. Two processes – secondary succession after abandonment of grasslands and restoration of degraded grasslands, are studied.

## **Declaration [in Czech]**

Prohlašuji, že svoji disertační práci jsem vypracoval samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury.

Prohlašuji, že v souladu s § 47b zákona č. 111/1998 Sb. v platném znění souhlasím se zveřejněním své disertační práce, a to v úpravě vzniklé vypuštěním vyznačených částí archivovaných Přírodovědeckou fakultou elektronickou cestou ve veřejně přístupné části databáze STAG provozované Jihočeskou univerzitou v Českých Budějovicích na jejích internetových stránkách, a to se zachováním mého autorského práva k odevzdanému textu této kvalifikační práce. Souhlasím dále s tím, aby toutéž elektronickou cestou byly v souladu s uvedeným ustanovením zákona č. 111/1998 Sb. zveřejněny posudky školitele a oponentů práce i záznam o průběhu a výsledku obhajoby kvalifikační práce. Rovněž souhlasím s porovnáním textu mé kvalifikační práce s databází kvalifikačních prací Theses.cz provozovanou Národním registrem vysokoškolských kvalifikačních prací a systémem na odhalování plagiátů.

České Budějovice, October 31<sup>st</sup>, 2011

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Dobromil Galvánek

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## List of papers and author's contribution

The thesis is based on the following papers and book chapters (listed chronologically):

- I. **Galváneek D. & Lepš J.** 2008: Changes of species richness pattern in mountain grasslands: abandonment versus restoration. *Biodiversity and Conservation*. 17:3241-3253. (IF = 1.473).  
*Dobromil Galváneek carried out vegetation data sampling, he took part in statistical analyses led by J. Lepš and he wrote major part of the text.*
- II. **Galváneek D. & Lepš J.** 2009: How do management and restoration needs of mountain grasslands depend on moisture regime? Experimental study from north-western Slovakia (Western Carpathians). *Applied Vegetation Science* 12:273-282. (IF = 1.349).  
*Dobromil Galváneek carried out vegetation data sampling, he took part in statistical analyses led by J. Lepš and he wrote major part of the text.*
- III. **Galváneek D.** 2009: The species-rich grasslands of the Zázrivské lazy site in Slovakia. In: Veen P., Jefferson R., de Smidt J. & van der Straaten J. (eds.): *Grasslands in Europe of high nature value*. KNNV Publishing. pp. 196-201.
- IV. **Galváneek D. & Lepš J.:** The effect of management on productivity, litter accumulation and seedling recruitment in a Carpathian mountain grassland. *Plant Ecology*. (submitted).  
*Dobromil Galváneek carried out vegetation data and biomass sampling. He did laboratory works concerning biomass analysis and he took part in statistical analyses led by J. Lepš. He wrote major part of the text.*
- V. **Galváneek D. & Ripka J.:** The establishment of target indicator species on restored sites in Morava River Floodplain. (manuscript).  
*Dobromil Galváneek carried out most of vegetation data sampling. He did statistical analyses and he wrote the text of the manuscript.*

## Introduction

Semi-natural grasslands in Europe belong to the plant communities with the highest species diversity recorded in detailed scale (Kull & Zobel 1991, Klimeš 1997). They may also host a high number of endemic taxa. For example in Slovakia more than 75% of endemic taxa grow in grasslands (Šeffler et al. 2002).

However extensive areas of semi-natural grassland in Europe is a result of human activity, grasslands and their species composition were influenced by co-evolution of human farming and local environment (O'Rourke 2006). Due to continuous management with limited nutrient input and a large portion of human labour, grasslands have become a unique plant community of high conservation value.

After the Second World War European agriculture faced a lot of changes. Due to subsidy support of intensive agriculture on both sides of the Iron Curtain large areas of grasslands were intensified or transformed to arable land and their diversity was significantly reduced. Species-rich grasslands have persisted only in some regions, especially in mountainous areas. The rapid decline of biodiversity led member states of the European Union to the approval of the Habitat Directive protecting threatened habitats and species throughout Europe. The directive covers substantial part of European semi-natural grasslands (EC 2007). Member states are obliged to implement it through the network of protected areas NATURA 2000. Except for NATURA 2000, there are also some other tools for preserving and restoration of grassland biodiversity, e.g. agri-environmental schemes. Several states of EU have started or they plan to start implementation of special measures for conservation management of semi-natural grasslands (e.g. Slovakia, Czech Republic, Poland). However traditional forms of agriculture disappear steadily, so it is necessary to look for alternative methods of grassland management, which could maintain species-rich communities and would be also economically feasible. The ecological research plays an important role as an essential base of knowledge for the formulating of conservation management measures.

Slovakia is a country with a high variety of grassland types compared to other countries of central Europe (Šeffler & Stanová 1999). Although research of grassland vegetation in the country has quite a long tradition, it was mainly oriented on the phytosociological description of grassland vegetation till 1990s

(e.g. Jurko 1974, Maglocký 1979, Ružičková 1986). Later in 1999 a national grassland inventory started and by 2005 more than 90% of the country was mapped. The area of semi-natural and natural grasslands in the country is approximately 300,000 hectares (Galváneš 2006).

The first attempts of an experimental approach to grassland study started in the 90s, when some experiments on grassland management and restoration were established on the Morava River floodplain (Šeffler & Stanová 1999) and on the mountain grasslands of Poloniny National Park (Ružičková et al. 1998). An experimental study of secondary succession was carried out in the Pořana Mts. (Ujházy 2003). In spite of those efforts the knowledge about interaction of grassland management, diversity and species composition was still highly insufficient and it required much more research efforts.

In 2000 DAPHNE-Institute of Applied Ecology started a medium-size project „Central European Grasslands – Conservation and Sustainable Use“. The project covered a huge variety of activities. One of them was an applied research component oriented to the determination of suitable conservation management and restoration of mountain grasslands. Another activity of the project was the finalization of species-rich floodplain meadows on the Morava River floodplain in western Slovakia. The results of management experiments on mountain grasslands and the results of restoration monitoring in the Morava River floodplain are part of this thesis.

A high number of experimental studies on management and restoration of semi-natural grasslands have been published in recent years. They have studied how plant communities react to various management measures like mowing (e.g. Hellstrom et al. 2006, Vanderpoorten et al. 2004, Huhta et al. 2001), grazing (e.g. Kohler et al. 2004, Pykala 2003, Pavlů et al. 2003, de Bello et al. 2007), mulching and mowing (Gaisler et al. 2004) or they compare several management techniques (e.g. Kohler et al. 2005, Stammel et al. 2003, Jantunen 2003, Kahmen et al. 2003). However the experiments are usually established in one vegetation type with the same management history. The locality in Malá Fatra, where we set up our experiment is unique, because it is still traditionally managed by small farms and management is linked to the ownership. Therefore, the abandonment is not driven so much by the quality of the stand, but it is more

driven by the accessibility of the locality. In advance, due to a high heterogeneity, it is possible to compare the performance of vegetation with different moisture status. Grassland vegetation in the surroundings of village Zázrivá (Zázrivské lazy site), where we carried out our experiment was described in the first chapter of the thesis. The site is a typical sample of species-rich mostly calcareous grasslands of the Western Carpathians.

Then we present 3 papers with the results from the experiment.

The first paper (Chapter II) is focusing on the evaluation of species richness, changes after cessation of mowing and after the introduction of restoration mowing. Semi-natural grasslands belong to the plant communities with the highest species richness recorded in a small area and many hypotheses were formulated how to explain this fact (Palmer 1994). In spite of this, we have also to take in mind plant traits of different species and the overall functional diversity of the plant community (de Bello et al. 2006), species diversity is a very important parameter describing the quality of plant community. An interesting task is also spatial dependence of species richness, because it seems to have some relations to management measures applied (e.g. de Bello et al. 2007).

The second paper (Chapter III) tries to evaluate the changes of species composition in our experimental plots. Methods of multivariate analysis (Lepš & Šmilauer 2003) provided a space for comparison of the changes in species composition in contrasting conditions of our experimental plots. Methods of partial direct gradient analysis also enable to determine which ecological variable mostly influence the variance in our data set.

The third paper (Chapter IV) is oriented on the analysis of productivity parameters of grassland as well the recruitment of seedlings in the canopy. Increased nutrient availability weakens nutrient limitation in grasslands and competition for light seems to be much more pronounced (Lepš 1999). The fact has many implications in practical grassland management, because if biomass is not removed from the community by regular mowing or grazing, it may cause eutrophication and thus a reduction of species richness.

Seedling recruitment is also a very important parameter and one of the adaptations, how to promote high species variety in grasslands (van der Maarel 1993 &

Sykes 1993, Herben et al. 1993). However, an accumulation of litter and moss biomass on the ground seems to be aspects limiting recruitment of seedlings (Špačková & Lepš 2004).

Changes in Common Agricultural Policy of European Union have led to some intensification of agriculture in EU. Therefore quite substantial area of arable land is converted into grasslands. The process may have interesting biodiversity conservation consequences so quite a lot of effort is oriented around this kind of research (e. g. van der Putten et al. 2000, Lepš et al. 2007). However the focus is oriented on conservation measures on grasslands in Slovakia is oriented on existing species-rich grasslands; there are some regions or vegetation types, where grassland restoration is urgently needed. The Main focus is namely oriented on floodplain grasslands in lowland areas.

The fourth paper (Chapter V) is focusing on the monitoring results of such restoration applied in Morava River Floodplain. In spite of the fact, experimental research was carried out before the restoration, to find the best methodology for further restoration (Šeffler & Stanová 1999a), restoration itself is an interesting large scale experiment which may bring interesting experience.

If we count the number of studies dealing with the topic of grassland management and restoration, it looks really like the „old evergreen“. However the behaviour of grasslands in different conditions and even in different localities may be totally different (Krahulec 1995), so it looks even generations of ecologists coming after us will have many new questions to ask. The research questions asked in this thesis have usually many practical implications in nature conservation so I hope they will be stimulating for practical conservation activities.

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## CHAPTER I

**Galvánek D. 2009:** The species-rich grasslands of the Zázrivské lazy site in Slovakia. In: Veen P., Jefferson R., de Smidt J. & van der Straaten J. (eds.): Grasslands in Europe of high nature value. KNNV Publishing. pp. 196-201.

(*Druhovo bohaté trávne porasty na lokalite Zázrivské lazy na Slovensku*)

### Abstract

The chapter presents semi-natural grasslands in the site Zázrivské lazy located in the surroundings of village Zázrivá in north-western Slovakia. The village was founded in 16<sup>th</sup> century by Valachian colonists and grasslands have played important role in local agriculture. Nowadays most of the grasslands in the site are managed by co-operative farm, but significant portion of the land is managed also by smaller private farmers. Dominant vegetation types are oligotrophic and mesic pastures of *Violion caninae* and *Cynosurion* alliances and hay meadows of *Arrhenatherion* and *Polygono-Trisetion* alliances. Fen meadows and fens (*Calthion*, *Caricion davallianae*, *Caricion fuscae*) occur only in small fragments. The grasslands with the highest biodiversity are usually located on small traditional farms. The number of those farms is declining rapidly and species-rich grasslands are threatened because of land abandonment or changes in management practices.

### Abstrakt

Kapitola opisuje poloprírodné lúky v území Zázrivské lazy, ktoré sa nachádzajú v okolí obce Zázrivá na severozápadnom Slovensku. Obec založili valašskí kolonisti v 16. storočí a trávne porasty hrali v miestnom poľnohospodárstve dôležitú úlohu. V súčasnosti väčšinu trávnych porastov v území obhospodaruje miestne poľnohospodárske družstvo, významnú výmeru však využívajú aj malí súkromní poľnohospodári. Dominantnými vegetačnými typmi sú oligotrofné až mezotrofné pasienky zväzov *Violion caninae* a *Cynosurion* a kosné lúky zväzov *Arrhenatherion* a *Polygono-Trisetion*. Vlhké a slatinné lúky (zväzov *Calthion*, *Caricion davallianae*, *Caricion fuscae*) sa vyskytujú iba v podobe malých fragmentov. Trávne porasty s najvyššou biodiverzitou sa zvyčajne nachádzajú na malých tradične hospodáriacich farmách. Počet takýchto sa rýchlo znižuje a druhovo bohaté trávne porasty ohrozuje opúšťanie pôdy a zmeny v spôsoboch využívania.

## CHAPTER II

**Galvánek D. & Lepš J. 2008:** Changes of species richness pattern in mountain grasslands: abandonment versus restoration. *Biodiversity and Conservation*. 17:3241-3253. (IF = 1.473).

*(Zmeny druhového bohatstva na horských lúkach: opúšťanie v porovnaní s obnovou)*

Autorský podiel: 60 %

### **Abstract**

Changes in plant species richness at various spatial scales were investigated by manipulative experiment in mountain grasslands. The aim of the research was to compare changes in species richness in newly abandoned sites and sites where restoration measures were applied after 20 years of abandonment. The plots were located in two vegetation types with different moisture regime.

Species richness decreased significantly after abandonment, mainly at the finest spatial scale of 10 × 10 cm. There was significant increase of species richness on restored sites, but it was apparent mainly at a larger scale. However, even 4 years of regular mowing were not sufficient to restore species richness to the level typical for traditionally managed grasslands in the region.

No significant difference was found in the performance of the 2 contrasting vegetation types (wet and dry) in relation to management measures. A significant difference in scale-dependent species richness was only observed. The dry type had a steeper species-area curve, with a lower number of species at the finest spatial scale.

According to the results of the experiment, mountain grasslands are very vulnerable habitats, losing their conservation value quickly after abandonment. Restoration is possible due to an extensive species pool in the region, but return to the original species richness at all spatial scales is quite a long process.

## Abstrakt

Zmeny v druhovom bohatstve rastlín v rôznych priestorových škálach sa sledovali v rámci riadeného experimentu na horských lúkach. Cieľom výskumu bolo porovnanie zmien druhového bohatstva na čerstvo opustených plochách v porovnaní s plochami, kde sa aplikovali obnovné opatrenia po 20 rokoch bez využívania. Plochy sa založili v dvoch vegetačných typoch s rozdielnym vlhkosným režimom. Po opustení došlo k významnému poklesu druhového bohatstva a to najmä v najdetailnejšej škále 10 x 10 cm. K preukaznému zvýšeniu druhového bohatstva došlo aj na obnovovaných plochách, kde sa však prejavilo najmä vo väčšej škále. Avšak ani štyri roky pravidelného kosenia neboli dostatočné, aby sa obnovilo druhové bohatstvo typické pre tradične využívané trávne porasty v regióne. Nezistil sa žiadny významný rozdiel medzi reakciou oboch študovaných typov vegetácie (vlhkého a suchého) vo vzťahu k aplikovanému manažmentu. Zistil sa iba preukazný rozdiel v priestorovom rozložení druhového bohatstva medzi oboma typmi. Suchý typ má menej druhov v najmenšej škále, ale nárast druhov s rastom plochy je tu rýchlejší ako vo vlhkom type.

Výsledky experimentu poukazujú na to, že horské lúky sú veľmi zraniteľným biotopom, ktorí po opustení rýchlo stráca svoju hodnotu z pohľadu ochrany prírody. Obnova je možná vďaka širokému zásobníku druhov v okolí, ale návrat pôvodného druhového bohatstva vo všetkých priestorových škálach je pomerne dlhý proces.

## CHAPTER III

**Galváneek D. & Lepš J. 2009:** How do management and restoration needs of mountain grasslands depend on moisture regime? Experimental study from north-western Slovakia (Western Carpathians). *Applied Vegetation Science* 12:273-282. (IF = 1.349).

*(Ako vlhkostný režim ovplyvňuje nároky horských lúk na manažment a obnovu?)*

Autorský podiel: 60%

### Abstract

*Question:* How does species composition change in traditionally managed meadows after mowing has ceased, and in abandoned meadows after re-introduction of mowing? Are there differences in the dynamics of dry and moderately wet meadows?

*Location:* Zázrivá-Plešivá (19°11'N, 49°16'E), north-western Slovakia, western Carpathians

*Methods:* Pairs of experimental plots (mown and unmown) were established to replicate each combination of dry/wet and traditionally managed/abandoned meadows. Changes in species composition were studied over five years. The data on changes in species composition was analysed by constrained and unconstrained ordinations, and visualized using Principal Response Curves.

*Results:* Species composition of newly abandoned wet grasslands was changing towards the corresponding long abandoned plots even in the first year of abandonment. Similarly, newly established restoration mowing in abandoned dry grasslands rapidly shifted the stand species composition towards that of traditionally managed ones. Nevertheless, four years after re-introduction of mowing, the species composition of the restored plots was still far from target. The effect of mowing in abandoned wet grasslands and abandonment in dry grasslands was much less pronounced and slower.

*Conclusions:* Moisture regime is a very important factor determining the management needs of various grassland types. Wet grasslands are much more sensitive to abandonment, with a fast degradation rate and limited possibilities

for restoration, which can be extremely slow. Even in the dry grasslands, that quickly responded to restoration mowing, restoration is a long-term process.

## **Abstrakt**

*Otázka:* Ako sa mení druhové zloženie tradične využívaných lúk po skončení kosenia a dlhodobo nevyužívaných lúk po obnove kosenia? Sú rozdiely v dynamike suchých a mierne vlhkých lúk?

*Lokalita:* Zázrivá – Plešivá (19°11´ východnej zemepisnej dĺžky, 49°16´ severnej zemepisnej šírky)

*Metódy:* Párové experimentálne plochy (kosené, nekosené) sa založili v replikáciách možných kombinácií suchých/vlhkých a tradične využívaných/opustených lúk. Zmeny druhového zloženia sa sledovali počas piatich rokov. Údaje o zmenách druhového zloženia sa analyzovali pomocou ordinácií s obmedzením aj bez obmedzenia a vizualizovali sa s využitím kriviek hlavnej odpovede druhov.

*Výsledky:* Druhové zloženie čerstvo opustených vlhkých lúk sa od prvého roku od opustenia menilo smerom k zloženiu obdobných dlhodobo nevyužívaných porastov. Podobne obnovné kosenie v suchom type spôsobilo rýchle zmeny smerom k tradične využívaným porastom tohto typu. Avšak aj po štyroch rokoch kosenia bolo druhové zloženie obnovovaných plôch veľmi vzdialené od cieľového stavu. Kosenie dlhodobo zanedbaných vlhkých porastov ako aj absencia kosenia v tradične využívaných porastoch suchého typu, nemali až taký významný vplyv a zmeny tu boli pomalšie.

*Záver:* Vlhkostný režim je významným faktorom, ktorý ovplyvňuje manažmentové nároky rôznych typov trávnych porastov. Vlhké porasty sú oveľa citlivejšie na zanechanie obhospodarovania, keď k degradácii dochádza rýchlo a obnova je veľmi pomalá a jej možnosti sú obmedzené. Aj na suchých lúkach, ktoré rýchlo reagovali na obnovné kosenie, je obnova dlhotrvajúci proces.



## CHAPTER IV

**Galváneek D. & Lepš J.:** The effect of management on productivity, litter accumulation and seedling recruitment in a Carpathian mountain grassland. *Plant Ecology* (prijaté do tlače).

(*Vplyv manažmentu na produktivitu, hromadenie opadu a uchyťavanie semenáčikov na karpatskej horskej lúke*)

Autorský podiel: 60%

### **Abstract**

The management regime may have a significant impact on the productivity and dynamics of grasslands, but the causal relationships influencing grassland conservation value are still not completely understood. Changes of selected community characteristics, such as standing crop, proportion of forbs in the standing crop, litter amount, litter decomposition and seedling recruitment, were investigated in a four year manipulative experiment in a mountain grassland in Slovakia. The aim of the research was to compare changes in newly abandoned sites and sites where restoration measures were applied after 20 years of abandonment. The sites were located in areas containing two vegetation types of the *Arrhenatherion* alliance (wet *Poo-Trisetetum* and dry *Anthoxantho-Agrostietum*) with different moisture regimes. The expected increase of the standing crop after abandonment was rather slow, and more pronounced towards the end of the experiment, and in the wet meadow type (approx. 30 % increase). The restoration mowing promoted forb proportions in the biomass, but it did not decrease the standing biomass in the restored grasslands. Strong litter accumulation after abandonment was observed in subsequent years after abandonment, when the amount of litter increased about 100% in abandoned plots. Decrease in litter was also significant after the start of restoration mowing (a decrease from 258 to 159 g.m<sup>-2</sup> in wet type and from 287 to 147 g.m<sup>-2</sup> in dry type was noted). Accumulated litter was negatively correlated to seedling recruitment ( $r = -0.63$  at the end of the experiment). The litterbag experiment showed that the wet type has a higher rate of decomposition, with 20 % more biomass decomposed during the litter-bag experiment.

The experiment confirmed a negative role of litter accumulation on seedling recruitment, with the number of seedlings per m<sup>2</sup> decreasing from 413 to

321 individuals in the abandoned wet-type site. This may lead to a decrease in species richness. Mowing along with raking of mowed biomass may be a useful tool to restore degraded mountain grasslands and to remove accumulated litter from the stands.

## Abstrakt

Manažmentový režim môže mať významný vplyv na produktivitu a dynamiku trávnych porastov, ale kauzálne súvislosti týchto parametrov s hodnotou trávnych porastov z pohľadu ochrany prírody nie sú doposiaľ dostatočne preskúmané. V rámci štvorročného manipulatívneho experimentu na horských lúkach na Slovensku sa sledovali zmeny nadzemnej biomasy, množstva opadu, rozklad opadu a uchytávanie semenáčikov. Cieľom výskumu bolo porovnanie zmien v čerstvo opustených porastoch v porovnaní s porastami, kde sa začali aplikovať obnovné opatrenia po 20 rokoch opustenia. Plochy sa založili v dvoch vegetačných typoch zväzu *Arrhenatherion* líšiacich sa vlhkosným režimom (vlhké *Poo-Trisetetum* a suché *Anthoxantho-Agrostietum*). Očakávaný nárast nadzemnej biomasy po opustení lúk bol dosť pomalý a viac sa prejavil až v závere experimentu a vo vlhkom type, kde došlo k nárastu o 30 %. Obnovné kosenie spôsobilo zvýšenie podielu bylín v nadzemnej biomase, nespôsobilo však pokles celkovej nadzemnej biomasy. V čerstvo opustených lúkach došlo v rokoch, nasledujúcich po opustení, k výraznému nárastu množstva opadu, ktoré sa zvýšilo o 100 %. Obnovné kosenie spôsobilo tiež pokles množstva opadu v dlhodobozanedbaných porastoch (z 258 na 159 g.m<sup>-2</sup> vo vlhkom type a z 287 na 147 g.m<sup>-2</sup> v suchom type). Množstvo nahromadeného opadu v negatívne korelovalo s množstvom uchytaných semenáčikov ( $r = -0.63$  v poslednom roku experimentu). Experiment s rozkladovými sáčkami ukázal, že opad sa vo vlhšom type rozkladá rýchlejšie. Za rovnaký čas sa tu rozložilo o 20 % viac biomasy ako v suchom type.

Experiment potvrdil negatívny vplyv nahromadenia opadu na uchytávanie semenáčikov, keď ich množstvo v čerstvo opustenom vlhkom type z 413 na 321 jedincov na meter štvorcový. Tento fakt môže viesť k poklesu druhového bohatstva. Kosenie s následným odstránením biomasy môže byť veľmi významným nástrojom na obnovu degradovaných horských lúk a na odstránenie nahromadeného opadu.

## CHAPTER V

### Establishment of target indicator species on restored sites in the Morava River Floodplain

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#### Abstract

The development of vegetation on ex-arable restored sites was monitored for five years in the Morava River Floodplain in Slovakia. Meadows were restored using a local seed mixture combined with turf transplantation. One site was restored only by the reintroduction of mowing without any addition of seeds. Data on the floristic composition of the sites were recorded once a year along with cover estimates using the 3-degree Tansley's scale. Data on the species composition were analysed by ordination methods (DCA, CCA), and data on the presence of indicator species were tested using general linear models (GLM). The results were compared with the results from a smallscale experiment carried out before large-scale restoration that tested turf transplantation and seed mixture sowing. Significant temporal changes in the species composition were observed at restored sites. The species composition in restored sites was found to be changing slowly towards the target habitats, and the process seemed to be faster wetter sites. Additionally, the presence of ruderal species on restored sites did not decline significantly during the monitoring period. Species of the *Arrhenaterion* alliance and semi-ruderal species became well established in the first years following restoration, whereas the presence of the target species of *Cnidion* alliance did not increase significantly during monitoring. Dominant grass species were successfully recruited, and the establishment of herbs was variable depending on individual species traits. Expectations based on the small-scale experiment high establishment rates of target species on plots with turf transplantation were not confirmed, probably due to the limited dispersal of species from the turf specimens and to high competition of grasses from seed mixture. It is evident that results of small-scale experiments may provide

limited evidence when applied on larger scales, and monitoring of large-scale restoration projects must be incorporated into project planning.

**Keywords:** Grassland restoration, *Cnidion* alliance, turf transplantation, local seed mixture, Slovakia, Central Europe

## Introduction

Alluvial meadows of the *Cnidion venosi* alliance belong to the most threatened habitat types within Europe, and they are protected by an EU Habitat Directive (EC-DG ENV 2007). In the past, these meadows were widespread in lowland areas throughout Europe, but the majority of their area was transformed into arable land or changed to intensively used grasslands (Manchester et al. 1999, Benstead et al. 1999). Several attempts to restore species-rich *Cnidion* meadows have been carried out in European floodplains using various restoration techniques (Bissels et al. 2004, Vécrin et al. 2002, Šeffler et al. 1999a).

Each decision regarding restoration techniques is a compromise among technical, ecological and financial criteria (Manchester et al. 1999). From an ecological point of view, several important aspects must be taken into consideration. Experience with the restoration of species-rich grasslands shows that the process is not straightforward. Simple reintroduction of ecological conditions typical for the target communities does not automatically mean that their successful restoration will occur (Bakker & Berendse 1999).

Seed dispersal appears to be the most limiting factor (Bissels et al. 2004, Bischoff 2002) when seedlings are recruited mostly in close vicinity of parent plants (Bischoff 2002, Donath et al. 2003). Therefore, if the restored sites are isolated from well-preserved grasslands, there is little chance that seeds of target species will reach the restored fields. If restoration efforts are performed in active floodplain, a positive effect of regular flooding on seed dispersal may be expected. However, the results of experimental studies produce controversial results, showing both significant correlations (Rosenthal 2006) and no significant evidence of a relationship between flooding and seed dispersal (Bissels et al. 2004). Experiments addressing seed banks have also that their potential with respect to the restoration of meadows is quite limited. Seed bank on floodplain grasslands is relatively species-poor, and many species typical of floodplain

grasslands are missing (Vécrin et al. 2007). In addition, the seed bank contains high amounts of ruderal species (Vécrin et al. 2002).

All these constraints may be partially overcome using appropriate restoration methods, but the effects appear to vary across individual methods (Klimkowska et al. 2007). Techniques involving propagule transfer are among the most popular methods, but they are limited by different constraints (Donath et al. 2007), and their success strongly depends on many important aspects, e.g., the quality of plant material used for transfer (Hölzel & Otte 2003). Another option is soil (grassland turf) translocation, which seems to be a highly suitable method promoting the establishment of a variety of meadow species (Vécrin & Müller 2003, Šeffler et al. 1999c).

Monitoring following the application of restoration measures is an important part of restoration activities. The trajectory of the expected development of restored sites on ex-arable land is usually estimated from small-scale experiments, where receptor sites are rather small (up to 10 hectares) (Kiehl et al. 2010), and data sampling is carried out on relatively small plots of up to 100 m<sup>2</sup> (Donath et al. 2003, Lepš et al. 2007). However, the real development of restored sites at a large scale may be different from what is expected based on data from small-scale experiments (Underwood et al. 2005, Melbourne & Chesson 2005). Therefore, it is necessary to investigate whether habitat development after restoration follows the expected trajectory (Critchley et al. 2004).

The largest complex of Cnidion meadows in central Europe is located in the lower part of the Morava River floodplain in three countries: Slovakia, Austria and the Czech Republic. A major part of the floodplain grassland is located in Slovakia, covering 1,913 ha of the total 3,450 ha in the area (Šeffler et al. 1999b).

Although most grasslands have been managed as meadows for many decades, some grasslands were ploughed in the 1970s-1980s and managed as intensively used arable land. This type of land management may have many negative consequences when carried out in floodplains with regular floods (e.g., pesticides and fertilisers are discharged to a wide area during floods). Therefore, the decision was made to restore the floodplain meadows in the arable fields on the Slovak side of the floodplain in a 140-ha area (Šeffler et al. 1999a). A 4-year

manipulative experiment was established prior to large-scale restoration to determine the most appropriate methods for local seed sowing and turf transfer. The conclusions of the experiment were rather optimistic, indicating that both sowing of a local seed mixture and turf transfer are suitable methods for the restoration of floodplain grasslands at this locality. The results from plots where turf transfer was performed were especially promising regarding the speed of the whole process (Šeffler et al. 1999c).

Most of the subsequent large-scale restoration was carried out using two principal methods: sowing of a local seed mixture from species-rich floodplain grasslands in the region and translocation of turf from species-rich grasslands in the region to restored fields (Šeffler et al. 1999a).

The aims of this study were to (1) analyse the changes in the species composition on restored sites; (2) test whether these temporal changes were significant; (3) analyse whether these changes led to a species composition typical of the target habitat types; and (4) to compare the results of large-scale restoration with expectations arising from a small-scale restoration experiment performed in the region prior to restoration (Šeffler et al. 1999c).

## **Data and methods**

The study site is located in the middle part of the Morava River Floodplain in Slovakia near the village of Gajary, close to the border with Austria. Large-scale restoration was carried out in six localities (Fig. 1). Three restoration techniques were applied: sowing of a seed mixture, transplantation of turf from a species-rich grassland and mowing twice a year. The application of the different techniques is summarised in Table 1. Because our survey was a component of a practical restoration project, no plot was left as an unrestored control. Therefore, we could only follow the establishment of species on restored plots, but we were not able to accurately evaluate their effectiveness.

A seed mixture was collected using a harvesting machine on a species-rich grassland in the Morava River floodplain. Collection was focused mostly on grass species typical of floodplain meadows in the region. The mixture contained mostly grass species (75 % of the mixture), and the dominant species in the mixture

were *Alopecurus pratensis*, *Poa pratensis* and *Elytrigia repens*. The remainder of the mixture was composed mostly of sedges and herbs. Herbs only represented 8 % of the mixture (Šeffler et al. 1999a). The seed mixture (40-60 kg per ha) was then applied to restored sites.

Turf was translocated from the closest species-rich floodplain meadow, located approximately 3 km downstream from the restored sites. Turf specimens were removed from the source meadow to a depth of 10 cm, then cut into small pieces that measured 10 × 10 cm and translocated to the restored sites, where they were placed on bare soil over an area of 2 × 4 metres (an 8-fold larger area than the turf had covered in the source meadow) (Šeffler et al. 1999a). One turf area of 2 × 4 metres was created per hectare, but the turf specimens were placed randomly, rather than regularly distributed.

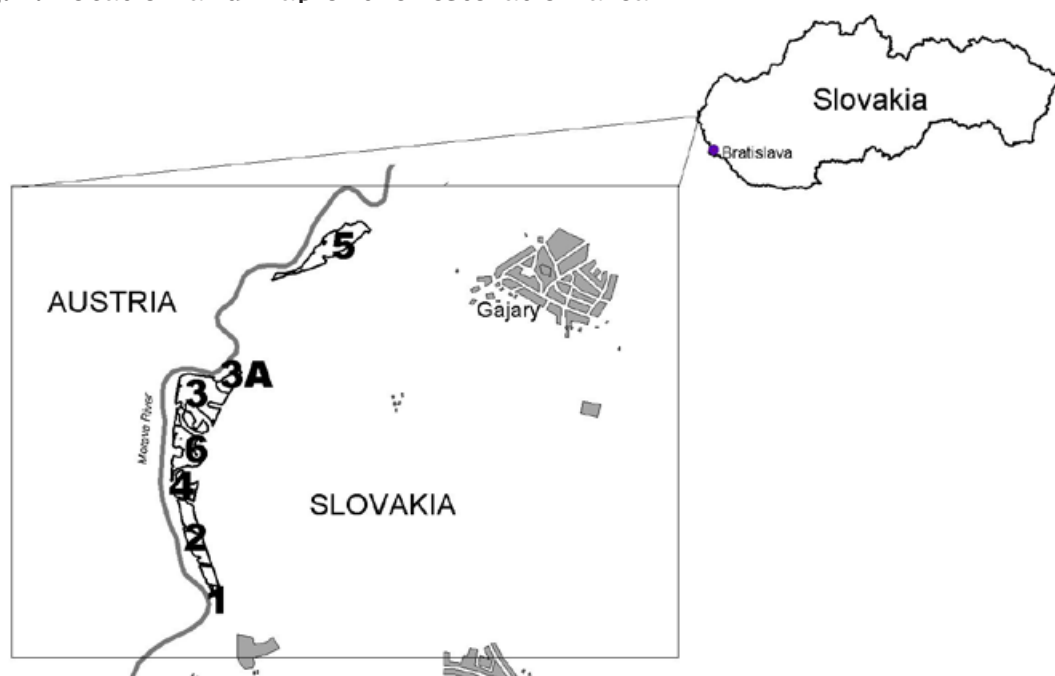
One of the six restored localities (site 5) was restored only by mowing (twice per year), as it was a grassland heavily infested with *Aster novi-belgii* agg. neophytes but still hosted some species typical of floodplain grasslands.

**Table 1:** Restoration techniques applied to different sites.

Site	Seed mixture sowing	Turf trans-plantation	Mowing twice per year	Begin-ning of restoration	Area (ha)	Flood regime
1	yes	yes	yes	1999	7.26	Rarely flooded
2	yes	yes	yes	1999	20.25	Rarely flooded
3	yes	yes	yes	1999	37.91	Occasionally flooded
3A	yes	yes	yes	2000	6.79	Frequently flooded
4	yes	yes	yes	1999	9.80	Rarely flooded
5	no	no	yes	2002	29.54	Frequently flooded
6	yes	yes	yes	2002	21.22	Occasionally flooded

Most of the localities are flooded in some years, but the duration of the floods varies among them. The approximate durations of floods are given in Table 1. This information is based on long-term observations of local farmers (Masarovič in verb.). Frequently flooded sites are flooded regularly each year, up to several times in some years. Occasionally flooded sites are flooded only in certain years during higher flood levels. Rarely flooded sites are flooded only during extremely high floods with many years in between floods. Generally, the part of the floodplain where restoration was performed belongs to the most highly elevated parts of the floodplain area, where floods occur only when the water table in Morava River is rather high. This is probably the reason that the area was used as arable land in the past.

**Fig. 1:** Location and map of the restoration area



Data sampling was carried out using the polygon mapping method (Šeffler et al. 2000), in which the list of higher plant species is recorded in a polygon of homogenous vegetation surrounded by natural or artificial boundaries. The plant cover was estimated using the simple Tansley scale (3 – dominant species, more than 50 %; 2 – frequent species, 1-50 %; 1 – rare species, < 1 %). Each locality was surveyed as a single polygon, except for locality 3, where the wetter portion (polygon 3A) was sampled separately.



A 4-year (1995-98) small-scale experiment was carried out several kilometres to the north of the site of large-scale restoration under conditions very similar to those observed at the restored sites. Sowing of local seed mixtures and transplantation of turf were investigated, but the experiment was not designed as a factorial experiment, so no plots were tested using the combination of both methods (Šeffler et al. 1999c).

## Data analysis

Monitoring began at each site in the first year after the initiation of restoration and continued until 2005. In 2004, data recording was not performed. The data obtained in the field were evaluated by indirect and direct gradient analysis (Lepš & Šmilauer 2003) processed using CANOCO for Windows 4.5 software (ter Braak & Šmilauer 2002). Site 5 was excluded from the direct gradient analysis because it was different from the other sites (no seed sowing or turf transplantation was performed there).

Indirect gradient analysis was performed using the DCA (Detrended Correspondence Analysis) method. The factors characterising the species composition were projected into a graph as supplementary variables. The characteristic species of three alliances (*Cnidion*, *Magnocaricion* and *Arrhenatherion*) recognised as target vegetation for the sites were identified according to national classification overviews (Stanová & Valachovič 2002, Šeffler et al. 2002, 2005) and using local classifications (Stanová et al. 1999). Ruderal species were defined as species occurring mostly in ruderal communities (factor RUDER) according to Jurko (1990) and corrected to local conditions according to Stanová et al. (1999). Species occurring in both ruderal and grassland communities (factor SEMIRUD) were defined using the same published sources. The categorisation of the species into all groups is presented in a table in App. 1. For indirect gradient analysis, all species from different groups present at the sites were counted.

Direct gradient analysis was performed using Canonical Correspondence Analysis (CCA). The factor TIME was tested to detect possible shifts in the species composition on the monitored plots. The significance of the relationship was tested using a Monte Carlo permutation test, with permutations within a block defined as individual localities (localities used as covariables).

The changes of the number of indicator species on the restored sites were analysed by general linear models (GLMs). Only species classified as cover 2 or 3 in Tansley's scale were taken into account. The factor TIME, as consecutive time in years since the beginning of restoration, was considered as a continuous predictor. The factor SITE, identifying particular sites, was a categorical factor. Five indicator species groups were defined. Three groups consisted of characteristic species of the three alliances *Arrhenatherion*, *Cnidion* and *Magnocaricion*. The groups (App. 1) were defined using comprehensive national surveys (Stanová & Valachovič 2002, Šeffer et al. 2002) and local survey from the Morava River floodplain (Stanová et al. 1999). The groups of ruderal species (RUDER) and semi-ruderal species (SEMIRUDER) were identified according to Jurko (1990).

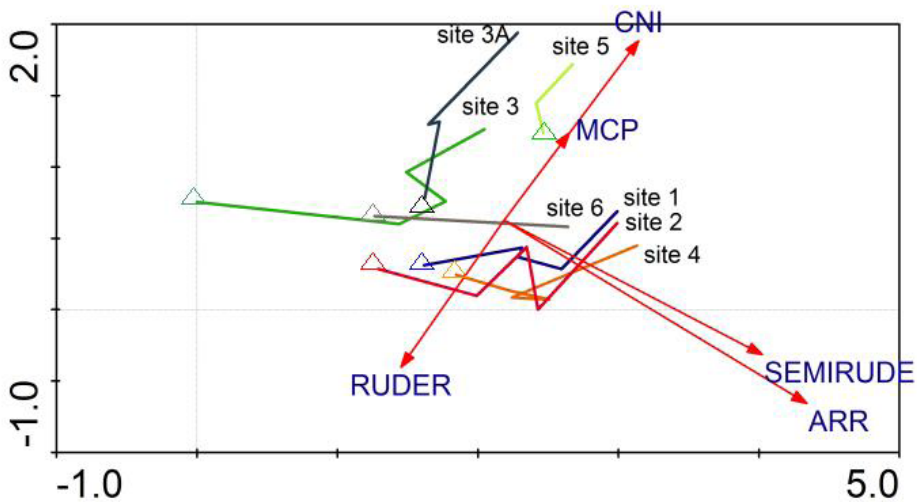
The numbers of species in the five indicator species groups were also calculated for the small-scale experiment, separately for plots with turf transplantation and for the plots with sowing of the seed mixture.

## Results

### Changes of the species composition on restored sites

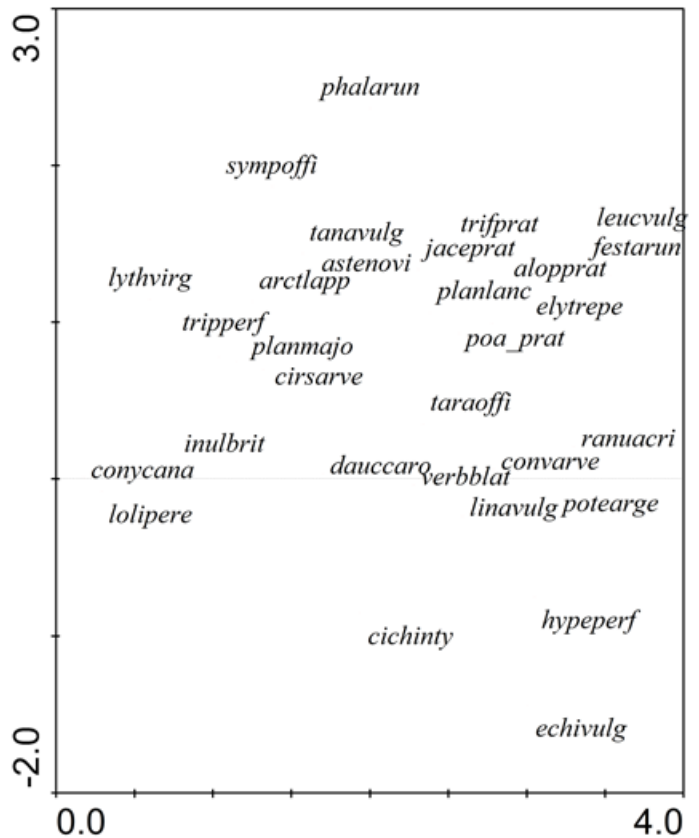
The results of DCA (Fig. 1) show the trajectories of all sites in the ordination space. The first axis may be interpreted as the temporal succession gradient from plant communities with a higher proportion of ruderal species soon after restoration to communities with a higher proportion of grassland species 6 years after restoration. The second axis probably represents the moisture gradient from sites flooded only rarely to sites that are regularly flooded several times per year.

**Fig. 2:** Ordination graph resulting from the DCA (Detrended Correspondence Analysis) method. The records from a single site are connected in a time series. The beginnings of the series are marked by triangles. Arrows represent the number of species of different ecological groups present at the site (ARR – *Arrhenatherion* species, CNI – *Cnidion* species, MCP – *Magnocaricion* species, RUDER – ruderal species, SEMIRUD – species typical of both ruderal and grassland communities). They are projected in the graph as supplementary variables.



A graph from the same analysis with species scores plotted in the ordination space is shown below. The species best fitted to the model are plotted. Species labels represent the approximate locations of the species scores in the ordination space.

alopprat = *Alopecurus pratensis*, arctlapp = *Arctium lappa*, astenovi = *Aster novi-belgii* agg., cichinty = *Cichorium intybus*, cirsarve = *Cirsium arvense*, convarve = *Convolvulus arvensis*, conycana = *Conyza canadensis*, dauccaro = *Daucus carota*, echivulg = *Echium vulgare*, elytrepe = *Elytrigia repens*, festarun = *Festuca arundinacea*, hyperperf = *Hypericum perforatum*, inulbrit = *Inula britannica*, jaceprat = *Jacea pratensis*, leucvulg = *Leucanthemum vulgare*, linavulg = *Linaria vulgaris*, lolipere = *Lolium pe-*



renne, lythvirg = *Lythrum virgatum*, phalarun = *Phalaroides arundinacea*, planlanc = *Plantago lanceolata*, planmajo = *Plantago major*, poa\_prat = *Poa pratensis*, potearge = *Potentilla argentea* agg., ranuacri = *Ranunculus acris*, sympoffi = *Symphytum officinale*, tanavulg = *Tanacetum vulgare*, taraoffi = *Taraxacum officinale*, trifprat = *Trifolium pratense*, tripperf = *Tripleurospermum perforatum*, verbblat = *Verbascum blattaria*

Two groups of the study sites can be recognised in the graph according to their succession trajectories following restoration, with site 6 exhibiting rather specific behaviour. Sites 1, 2 and 4 can be characterised as presenting a step-by-step increase of species typical of the *Arrhenatherion* alliance and species typical for ruderal and grassland communities. Typical species of the *Cnidion* alliance were more frequent in the last year of monitoring, when a decrease of typical ruderal species was observed as well.

Sites 3, 3A and 5 were characterised by a decrease of the number of ruderal species, followed by an increase of *Cnidion* species.

Site 6 is a unique locality where restoration was carried out in 2002, and data sampling began in 2003. Therefore, we have only two records from this site.

The projected species scores in scatter plots confirmed the pattern described. Ruderal species or species dependent on regular disturbance are located on the left side of the graph (e.g., *Coryza canadensis*, *Lythrum virgatum*, *Lolium perenne*, *Tripleurospermum perforatum*, *Inula britannica*). Species that are more typical of closed grasslands are found on the right side of the graph (e.g., *Leucanthemum vulgare*, *Festuca arundinacea*, *Alopecurus pratensis*, *Ranunculus acris*, *Potentilla argentea*). The bottom part of the graph is mostly occupied by species typical of dry stands, such as *Echium vulgare*, *Cichorium intybus* and *Hypericum perforatum*. In the upper part of the graph, typical wetland species, such as *Phalaroides arundinacea* and *Symphytum officinale*, are observed.

Direct gradient analysis using the Canonical Correspondence Analysis method confirmed that the changes in the species composition on the restored plots were highly significant ( $P=0.002$ ). In spite of this, the explained variability is not particularly pronounced (10.6 %).

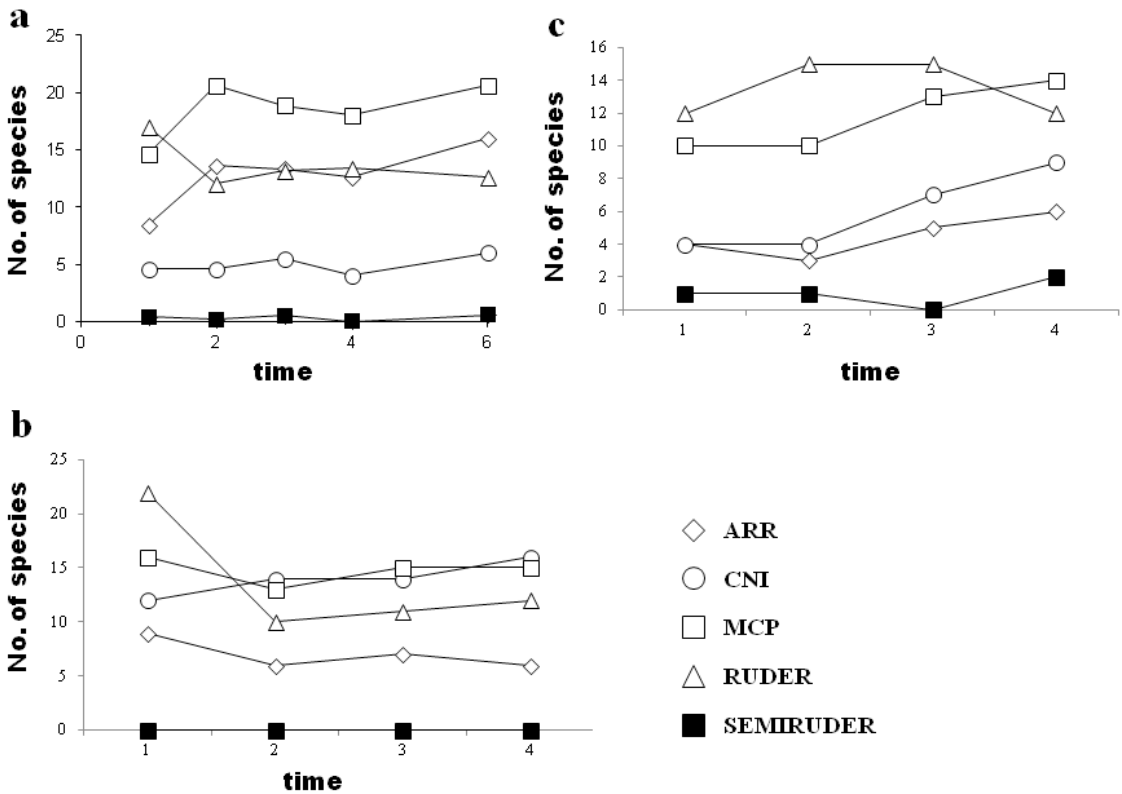
## **Changes in the number of indicator species on restored sites and on experimental plots of the small-scale experiment.**

The changes in the number of indicator species were analysed by general linear models. The overall trend on restored plots is a decrease in the number of ruderal species and an increase in the number of *Arrhenatherion* and semi-ruderal species (Fig. 3). However, only the change in the number of *Arrhenatherion* species is significant ( $P=0.011$ ). The most dramatic changes were observed in first two years after restoration. In the first year following restoration, ruderal species represent the most numerous group on the restored sites; however, the situation changed in the subsequent year.

On the plots of the small-scale experiment where turf was transplanted, there was a relatively constant number of semi-ruderal species, while the number of indicator species of the *Arrhenatherion* alliance was observed to decrease slightly, and that of the *Cnidion* alliance increased slightly. However, the numbers of all of these species were much higher than on plots with the application of seed mixture. Ruderal species dominated the plots only in the first year after restoration, after which they began to decrease in number (Fig. 3).

The experimental plots where the seed mixture was applied show a different pattern. Ruderal species were dominant in the first three years of the experiment, and other species groups representing different grassland types and semi/ruderal species began to increase only from third year of the experiment.

**Fig. 3:** Average numbers of indicator species from different groups (App. 1) in restored plots in consecutive years after restoration; a) large-scale restoration sites (only species with cover degrees of 2 and 3 in Tansley’s scale are considered, and site 5 is not considered); b) small-scale experiment plots with turf transplantation; c) small-scale experiment plots with sowing of the local seed mixture. ARR – characteristic species of the *Arrhenatherion* alliance, CNI – characteristic species of the *Cnidion* alliance, MCP – characteristic species of the *Magnocaricion* alliance, RUDER – ruderal species sensu Jurko (1990), SEMI-RUDER – semi-ruderal species, characteristic of both grassland and ruderal habitats sensu Jurko (1990)



**Table 2:** Results of analysis of changes in the number of indicator species on restored sites by general linear models (GLMs). ARR = number of characteristic species of the *Arrhenatherion* alliance, CNI = number of characteristic species of the *Cnidion* alliance, MCP = number of characteristic species of the *Magnocari- cion* alliance, RUDER = number of ruderal species, SEMIRUDER = number of semiruderal species, TIME = time in years since the beginning of restoration, SITE = identification of restored sites

Factor	ARR	ARR	CNI	CNI	MCP	MCP	RUDER	RUDER	SEMIRUDER	SEMIRUDER
	F	P	F	P	F	P	F	P	F	P
TIME	7.866	<b>0.011</b>	0.860	0.365	0.072	0.792	1.190	0.289	2.323	0.144
SITE	11.176	<b>&lt;0.001</b>	1.507	0.235	4.339	<b>0.008</b>	4.183	<b>0.010</b>	5.875	<b>0.002</b>

### Establishment of indicator species on restored sites compared with those of the small-scale experiment

Analysis of species establishment on the restored sites and the experimental plots showed an uneven ability of floodplain meadow indicator species to be recruited in restored plots. Typical grasses of floodplain meadows (e.g., *Alopecurus pratensis*, *Poa pratensis*) colonised the restored sites quite well. The establishment of sedges was much poorer, as only *Carex praecox* was rarely recorded on restored sites. Some herb indicator species occurred frequently on restored sites, e.g., *Lythrum virgatum* and *Symphytum officinale*, whereas some species were found there only rarely, e.g., *Clematis integrifolia* and *Galium boreale*. There were also species present on transplanted turf that did not occur on restored sites, e.g., *Cnidium dubium*, *Viola pumila* and *Sanguisorba officinalis* (Table 3).

Most of the observed species present similar behaviour in the small-scale experimental plots and the large-scale restored plots, with some exceptions. A number of species do not occur in the area where the small-scale experiment was established, e.g., *Alisma lanceolatum* and *Gratiola officinalis*. Therefore, they are not present in the experimental plots. Additionally, there are a number of

species that were recruited very successfully to the experimental plots but that did not appear on restored sites, or were very rarely found there, e.g., *Cnidium dubium*, *Serratula tinctoria*, *Viola pumila* (Table 3).

**Table 3:** Comparison of the establishment of floodplain meadow species in the plots of the small-scale restoration experiment and the monitored sites. (x – the species is present in the seed mixture or at the donor site; f – frequent occurrence in the experiment plots or the monitored sites; o – occasional presence in the experiment plots or the monitored sites; r – rare presence in the experiment plots or the monitored sites; cover of more than 50% at the donor site; 2 – cover between 1 and 50% at the donor site; 1 – cover of less than 1% at the donor site)

	Restored sites	Seed mixture large-scale restoration	Donor meadow for turfs large-scale restoration	Seed mixture experiment	Sown plots experiment	Donor meadow for turfs experiment	Transplanted plots experiment
<i>Alisma lanceolatum</i>	o		1				
<i>Allium angulosum</i>	r		2	x	r	x	r
<i>Alopecurus pratensis</i>	o	x	3	x	o	x	f
<i>Aristolochia clematitis</i>			2				
<i>Cardamine matthioli</i>			1				
<i>Cardamine pratensis</i>			2			x	f
<i>Carex acutiformis</i>			1				
<i>Carex distans</i>			2				
<i>Carex melanostachya</i>			2				r



	Restored sites	Seed mixture large-scale restoration	Donor meadow for turfs large-scale restoration	Seed mixture experiment	Sown plots experiment	Donor meadow for turfs experiment	Transplanted plots experiment
<i>Carex praecox</i>	r		2			x	o
<i>Carex riparia</i>	r						
<i>Clematis integrifolia</i>	r		2	x	r		r
<i>Cnidium dubium</i>			2			x	f
<i>Elytrigia repens</i>	f	x	2		o	x	f
<i>Festuca pratensis</i>			1				r
<i>Festuca rupicola</i>	r						
<i>Filipendula ulmaria</i>			2				
<i>Filipendula vulgaris</i>	r						
<i>Fragaria viridis</i>	r						
<i>Galium boreale</i>	r		2	x	r		o
<i>Galium palustre</i>					o		
<i>Gratiola officinalis</i>	o		2				
<i>Inula britannica</i>	f						
<i>Iris pseudacorus</i>	r		1				
<i>Lathyrus pratensis</i>	o		2		f	x	f
<i>Lychnis flos-cuculi</i>	o		2			x	f
<i>Lysimachia nummularia</i>	o					x	f
<i>Lythrum salicaria</i>			2	x	o		r
<i>Lythrum virgatum</i>	f						
<i>Phalaroides arundinacea</i>	f		2				
<i>Plantago altissima</i>			2				

	Restored sites	Seed mixture large-scale restoration	Donor meadow for turfs large-scale restoration	Seed mixture experiment	Sown plots experiment	Donor meadow for turfs experiment	Transplanted plots experiment
<i>Poa palustris</i>	o		2		o	x	o
<i>Poa pratensis</i>	f	x			f	x	f
<i>Poa trivialis</i>	o		2		o		o
<i>Populus nigra</i>	o						
<i>Potentilla reptans</i>	f		2		o		o
<i>Pseudolysimachion longifolium</i>	o		2				o
<i>Ranunculus acris</i>	o		2				r
<i>Ranunculus auricomus</i>			1			x	o
<i>Ranunculus repens</i>	o		2		f	x	f
<i>Rorippa austriaca</i>	r		1		o		f
<i>Rorippa × armoracioides</i>							r
<i>Rumex crispus</i>	o		2		o	x	r
<i>Sanguisorba officinalis</i>			2	x			r
<i>Scutellaria hastifolia</i>	r						
<i>Serratula tinctoria</i>			2	x		x	f
<i>Symphytum officinale</i>	f		2			x	f
<i>Thalictrum flavum</i>			2				o
<i>Viola pumila</i>						x	o

## Discussion

Monitoring of restoration projects is an invaluable tool because the success of these (often very costly) efforts can only be evaluated when they are monitored. However, restoration projects differ from experimental research, as only one method, considered to be the best one, is usually applied in these projects, and no control is generally available. Consequently, it is very difficult to disentangle the effect of sowing from turf transfer in the present study because both were applied together. Similarly, the only site where these measures were not applied differed initially from the other sites, so it cannot serve as a “control”. Consequently, we are limited to a rather informal interpretation of the temporal trends observed during five years of monitoring.

Each monitoring programme must solve the problem of how to address complex parameters, such as species composition, especially in habitats that are relatively rich in species. Approaches oriented toward target species are quite common (Rosenthal 2003) and very useful. The phytocoenological classification of non-forest vegetation in Central Europe is relatively well developed, providing lists of indicator species based on analyses of large datasets of vegetation relevés (e.g., Chytrý & Tichý 2003). As the natural vegetation of the Morava River floodplain has been very well surveyed (Šeffler & Stanová 1999), the target vegetation for the restored sites can be estimated relatively precisely.

The polygon mapping method involving the recording of species of vascular plants has particularly been used for large inventories of grassland vegetation (Šeffler et al. 2002, Sarbu et al. 2002), but we have also found it suitable for restoration monitoring. This method is able to detect species changes if a relatively high species turnover is expected, which is the case in the present study. The species record per polygon is comparable with that of several relevés performed on a site (Filipová et al. 2005), and it is much less time consuming.

Making a decision regarding the methodology to be used for restoration at the study locality required taking into account several important factors, which could limit the effectiveness of restoration activities. The main problem reported with respect to similar restoration efforts throughout Europe is dispersal limitation (Donath et al. 2003, Bischoff 2002, Bissels et al. 2004). In our case, we

proposed to overcome this issue using two methods: sowing of a seed mixture from species-rich floodplain grasslands and transplantation of turf from the same type of grasslands. Sowing of seed mixtures with high diversity may be a suitable technique for the restoration of species-rich grasslands. The use of such mixtures suppresses natural colonisation but promotes higher productivity in the first years following restoration (Lepš et al. 2007). Translocation of turf also seems to be a method promoting higher diversity on restored plots and better establishment of rarer species (Vécrin & Muller 2003).

No flooding model is available for the part of the floodplain where restoration was performed, but under analogous ecological conditions, floodplain meadows of the *Cnidion venosi* alliance are known to exist, as well as of the *Magnocaricion* alliance. Higher elevation areas of the floodplain, which are flooded irregularly, host vegetation associated with the transition to mesic grasslands of the *Arrhenatherion* alliance. Hence, the vegetation of these three alliances may be considered as target vegetation for our restored sites. Comparison of the establishment of indicator species for various potential target grassland alliances showed that species of the *Arrhenatherion* alliance appear to be the most successful. Many species typical of this alliance are semi-ruderal species, which are able to persist in both grassland and ruderal communities. They were also found to be typical of the early stages of succession in former arable fields by Filipová et al. (2005). In contrast, species typical of alluvial meadows of the *Cnidion* alliance did not significantly increase in number on restored sites. This lack of increase might be caused by the limitation of seed germination by low moisture and the lack of flooding at some sites, but it can also result from the specific seed germination traits of some *Cnidion* species (Hölzel & Otte 2004).

The results of the small-scale experiment (Šeffer et al. 1999c) indicated that we should expect a decrease in the number of ruderal species two years after restoration on plots with turf transplantation and four years after restoration on plots sown with the seed mixture. Non-ruderal grassland species began to dominate the restored sites two years after restoration, but no significant decrease in the number of ruderal species was observed. Donath et al. (2003) and Bissels et al. (2004) also reported a fairly significant presence of ruderal species on restored sites in the Rhine floodplain in first years after restoration.

Ruderal species probably benefit from the fact that dominant grasses are not able to cover ground completely in the first year after restoration, and gaps are still present. In addition, several very dry years occurred following restoration; in particular, 2003 was an extremely dry year. Such extreme droughts may suppress dominant grass species and even promote the colonisation of invasive or ruderal species (Morecroft et al. 2004). They also cause high mortality rates of young seedlings (Bissels et al. 2006).

There are two groups of sites at our study locality (Fig. 1), which exhibit slightly different performances concerning their species composition. One group is represented by sites 3, 3A and 5, consisting of sites that are frequently, or at least occasionally flooded. The second group is represented by the rest of the sites (1, 2 and 4), where flooding is rare (except for site 6). Regularly flooded sites exhibit a faster transition towards species-rich floodplain grasslands after restoration. This situation may be a result of increased soil moisture, which has a positive effect on seed germination (Eckstein & Donath 2005). It could also be expected that more frequent, regular floods could promote the transfer of diaspores from well-preserved meadows, but it appears that the flooding regime does not play as important a role as dispersal and microsite limitation (Bissels et al. 2004).

One of the techniques used in this study was sowing of a seed mixture of local origin. We conclude that, 6 years after restoration, dominant grass species (e.g., *Alopecurus pratensis*, *Poa pratensis*) from the mixture had become well established on the restored sites, and represented the dominant species on most of the sites.

Turf transplantation is considered to be a suitable method to overcome seed dispersal limitation (Kiehl et al. 2010). Although negative experiences have also arisen from using this method (Kardol et al. 2009), it is generally assumed to represent a suitable means of restoring species-rich grasslands. Turf transplantation was applied as an additional method to enhance the overall diversity on restored sites and to promote the establishment of rare species that may not be included in the seed mixture. The source meadow hosted vegetation typical of *Cnidion* floodplain meadows associated with the presence of several indicator species. The performance of these indicator species on restored sites appears to be quite species specific. Species such as *Alium angulosum*, *Carex praecox* and

*Vicia cracca* were only recorded on restored sites very soon after restoration, and they then disappeared. The species *Poa palustris* exhibited similar performance only at drier localities, whereas it was recruited well to the wet locality of site 6. In contrast, the species *Galium boreale*, *Ranunculus acris* and *Symphytum officinale* were recorded for the first time several years after restoration. These findings provide support for the conclusion that species performance is highly individual, and some species may be limited by site conditions or by higher competition in later successional stages.

The results of the small-scale experiment showed a very promising pattern of target species establishment on the plots with turf transplantation (Fig. 3). Such a pattern was not confirmed for the large-scale restoration sites, perhaps because only species associated with cover of greater than 1% were considered. Some additional indicator species of floodplain meadows were recorded on restored sites, but with only an individual occurrence being observed. We are not able to precisely determine whether transplanted turf had a positive effect on species establishment, though some positive effect might be expected. Nevertheless, this effect seems to be very limited on a larger scale. Species of floodplain meadows exhibit very limited dispersal (Bischoff 2002), and thus, turf may only influence the very close surroundings of the site where it is transplanted. The small-scale experiment performed in an area of several square metres therefore provided an overly optimistic view. This issue could be overcome by employing higher density turf application, but such an approach is not feasible from a technical and financial point of view.

The establishment of species from turf could also be limited by competition from sown grasses. The amount of the seed mixture used in large-scale restoration was high (40-60 kg of seeds per square meter) compared with the usual recommendations (see Kiehl et al. 2010) and might result in higher competition among species during their establishment. With respect to other possibilities regarding seed dispersal to the study localities, dispersal via wind and flood waters may be considered. The role of wind dispersal appears to be very limited, because restored sites are mostly surrounded by arable fields or by floodplain forests. The closest well-preserved floodplain grasslands are several kilometres from the restored sites. Therefore, these well-preserved grasslands probably did not play as significant a role as dispersal sources.

It may be expected that some propagules could also be transported to restored sites by floods. Our results show that sites that are flooded more frequently show faster development toward species-rich alluvial grasslands. However, Bissels et al. (2004) found no evidence that the flooding regime influence input of diaspores. We expect that the positive influence of flooding is more important with respect to its effect on seed germination than the input of new seeds itself. Regular flooding may positively influence moisture conditions within a locality and, thus, improve conditions for seed germination.

Our findings related to monitoring the large-scale restoration of floodplain grasslands in the Morava River floodplain show that the development of restored sites at a large scale can be different from that expected from small-scale experiments. Although restored sites develop in the expected direction towards species-rich alluvial meadows, the process is slower than expected from small-scale experiments. Turf transplantation appears to be the more useful of the tested methods to overcome dispersal limitation, but the effect of this method is very limited at a larger scale.

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## Appendix 1: Categorisation of indicator species present on restored sites

Species	Alliance indicator species (ARR- <i>Arrhenatherion</i> , CNI- <i>Cnidion</i> , MCP- <i>Magnocaricion</i> )	Ruderal (RUDER)/ semi-ruderal species (SEMIRUD)
<i>Acetosa pratensis</i>	ARR	
<i>Acetosella vulgaris</i>	ARR	SEMIRUD
<i>Agrimonia eupatoria</i>	ARR	
<i>Achillea millefolium</i>	ARR	SEMIRUD
<i>Alisma lanceolatum</i>	CNI	
<i>Allium angulosum</i>	CNI	
<i>Alopecurus geniculatus</i>	MCP	
<i>Alopecurus pratensis</i>	CNI	
<i>Ambrosia artemisiifolia</i>		RUDER
<i>Anchusa officinalis</i>		RUDER
<i>Anthoxanthum odoratum</i>	ARR	
<i>Arctium lappa</i>		RUDER
<i>Aristolochia clematitis</i>		RUDER
<i>Armoracia rusticana</i>		RUDER
<i>Arrhenatherum elatius</i>	ARR	SEMIRUD
<i>Artemisia absinthium</i>		RUDER
<i>Artemisia campestris</i>		SEMIRUD
<i>Artemisia vulgaris</i>		RUDER
<i>Aster novi-belgii</i> agg.		RUDER
<i>Atriplex</i> sp.		RUDER
<i>Avena sativa</i>		RUDER
<i>Berteroa incana</i>	ARR	SEMIRUD
<i>Bidens frondosa</i>		RUDER
<i>Bidens tripartita</i>		RUDER
<i>Bromus hordeaceus</i>	ARR	SEMIRUD
<i>Bromus inermis</i>		SEMIRUD
<i>Bromus sterilis</i>		RUDER

<b>Species</b>	<b>Alliance indicator species (ARR-Arrhenatherion, CNI-Cnidion, MCP-Magnocaricion)</b>	<b>Ruderal (RUDER)/ semi-ruderal species (SEMIRUD)</b>
<i>Calamagrostis epigejos</i>	ARR	SEMIRUD
<i>Capsella bursa-pastoris</i>		RUDER
<i>Carduus acanthoides</i>		RUDER
<i>Carduus nutans</i>		SEMIRUD
<i>Carex praecox</i>	CNI	
<i>Carex riparia</i>	CNI	
<i>Centaureum erythraea</i>	ARR	
<i>Cichorium intybus</i>		SEMIRUD
<i>Cirsium arvense</i>		RUDER
<i>Cirsium vulgare</i>		RUDER
<i>Clematis integrifolia</i>	CNI	
<i>Convolvulus arvensis</i>		RUDER
<i>Conyza canadensis</i>		RUDER
<i>Cota tinctoria</i>		SEMIRUD
<i>Dactylis glomerata</i>	ARR	SEMIRUD
<i>Datura stramonium</i>		RUDER
<i>Daucus carota</i>		SEMIRUD
<i>Dianthus armeria</i>	ARR	
<i>Echinochloa crus-galli</i>		RUDER
<i>Echium vulgare</i>	ARR	SEMIRUD
<i>Elytrigia repens</i>		RUDER
<i>Equisetum arvense</i>	ARR	SEMIRUD
<i>Erdoium cicutarium</i>		SEMIRUD
<i>Eryngium campestre</i>	ARR	SEMIRUD
<i>Festuca rupicola</i>	ARR	
<i>Filipendula vulgaris</i>	ARR	
<i>Fragaria viridis</i>	ARR	
<i>Galium boreale</i>	ARR	
<i>Galium verum</i>	ARR	SEMIRUD
<i>Glechoma hederacea</i>		SEMIRUD
<i>Gratiola officinalis</i>	CNI	

Species	Alliance indicator species (ARR- <i>Arrhenatherion</i> , CNI- <i>Cnidion</i> , MCP- <i>Magnocaricion</i> )	Ruderal (RUDER)/ semi-ruderal species (SEMIRUD)
<i>Helianthus annuus</i>		RUDER
<i>Hordeum murinum</i>		RUDER
<i>Hypericum perforatum</i>	ARR	SEMIRUD
<i>Chaiturus marrubiastrum</i>		RUDER
<i>Chenopodium album</i>		RUDER
<i>Inula britannica</i>	CNI	
<i>Iris pseudacorus</i>	CNI	
<i>Jacea pratensis</i>		SEMIRUD
<i>Lactuca serriola</i>		RUDER
<i>Lactuca</i> sp.		RUDER
<i>Lamium album</i>		RUDER
<i>Lathyrus pratensis</i>	CNI	SEMIRUD
<i>Lathyrus tuberosus</i>		RUDER
<i>Leucanthemum vulgare</i>	ARR	SEMIRUD
<i>Linaria genistiifolia</i>		SEMIRUD
<i>Linaria vulgaris</i>		RUDER
<i>Lolium perenne</i>		SEMIRUD
<i>Lotus corniculatus</i>	ARR	SEMIRUD
<i>Lycopus exaltatus</i>		SEMIRUD
<i>Lychnis flos-cuculi</i>	CNI	
<i>Lysimachia nummularia</i>	CNI	SEMIRUD
<i>Lysimachia vulgaris</i>		SEMIRUD
<i>Lythrum virgatum</i>	ARR	SEMIRUD
<i>Matricaria discoidea</i>		RUDER
<i>Medicago sativa</i>		SEMIRUD
<i>Melilotus officinalis</i>		RUDER
<i>Mentha aquatica</i>		SEMIRUD
<i>Odontites vulgaris</i>	ARR	SEMIRUD
<i>Pastinaca sativa</i>	ARR	SEMIRUD
<i>Persicaria amphibia</i>	MCP	

<b>Species</b>	<b>Alliance indicator species (ARR-Arrhenatherion, CNI-Cnidion, MCP-Magnocaricion)</b>	<b>Ruderal (RUDER)/ semi-ruderal species (SEMIRUD)</b>
<i>Persicaria dubia</i>		RUDER
<i>Persicaria hydropiper</i>		RUDER
<i>Phalaroides arundinacea</i>		SEMIRUD
<i>Phleum pratense</i>	ARR	SEMIRUD
<i>Phragmites australis</i>		RUDER
<i>Picris hieracioides</i>		SEMIRUD
<i>Plantago lanceolata</i>	ARR	SEMIRUD
<i>Plantago major</i>		RUDER
<i>Poa palustris</i>	CNI	SEMIRUD
<i>Poa pratensis</i>	ARR	SEMIRUD
<i>Poa trivialis</i>	CNI	SEMIRUD
<i>Polygonum aviculare</i>		RUDER
<i>Potentilla anserina</i>		RUDER
<i>Potentilla argentea</i> agg.	ARR	SEMIRUD
<i>Potentilla reptans</i>	CNI	SEMIRUD
<i>Prunella vulgaris</i>	ARR	SEMIRUD
<i>Pseudolysimachion longifolium</i>	CNI	
<i>Ranunculus acris</i> agg.	ARR	
<i>Ranunculus repens</i>		SEMIRUD
<i>Rorippa amphibia</i>	MCP	SEMIRUD
<i>Rorippa austriaca</i>	CNI	SEMIRUD
<i>Rorippa palustris</i>		RUDER
<i>Rorippa sylvestris</i>		RUDER
<i>Rubus</i> sp.	ARR	
<i>Rumex crispus</i>		SEMIRUD
<i>Rumex obtusifolius</i>		SEMIRUD
<i>Salvia pratensis</i>	ARR	
<i>Scrophularia nodosa</i>		SEMIRUD
<i>Scutellaria hastifolia</i>	CNI	
<i>Securigera varia</i>	ARR	SEMIRUD

<b>Species</b>	<b>Alliance indicator species (ARR-Arrhenatherion, CNI-Cnidion, MCP-Magnocaricion)</b>	<b>Ruderal (RUDER)/ semi-ruderal species (SEMIRUD)</b>
<i>Senecio jacobaea</i>		SEMIRUD
<i>Setaria pumila</i>		RUDER
<i>Silene latifolia</i>		RUDER
<i>Silene vulgaris</i>	ARR	SEMIRUD
<i>Spergularia rubra</i>		SEMIRUD
<i>Stachys palustris</i>		RUDER
<i>Stellaria graminea</i>	ARR	SEMIRUD
<i>Stenactis annua</i>		RUDER
<i>Symphytum officinale</i>	CNI	SEMIRUD
<i>Tanacetum vulgare</i>		RUDER
<i>Taraxacum officinale</i>	CNI	SEMIRUD
<i>Tithymalus cyparissias</i>		SEMIRUD
<i>Trifolium arvense</i>	ARR	SEMIRUD
<i>Trifolium campestre</i>	ARR	SEMIRUD
<i>Trifolium hybridum</i>	ARR	SEMIRUD
<i>Trifolium pratense</i>	ARR	SEMIRUD
<i>Trifolium repens</i>	ARR	SEMIRUD
<i>Tripleurospermum perforatum</i>		RUDER
<i>Urtica dioica</i>		RUDER
<i>Verbascum blattaria</i>		RUDER
<i>Verbascum lychnitis</i>	ARR	
<i>Veronica scutellata</i>	MCP	
<i>Veronica verna</i> agg.		RUDER
<i>Vicia cracca</i>	ARR	SEMIRUD
<i>Vicia hirsuta</i>		RUDER
<i>Vicia tetrasperma</i>		RUDER
<i>Xanthium strumarium</i>		RUDER
<i>Zea mays</i>		RUDER



## General discussion

The attempt to conserve high nature-value (HNV) grasslands in Europe has become one of the main topics for European nature conservation. The loss of these areas has accelerated in recent decades, especially in the Central and Eastern European countries (Küster & Keenleyside 2009). The total area in HNV grasslands is changing (e.g., Kaligarić et al. 2006). Moreover, their species composition is being altered and influenced by management changes in local and landscape contexts (Halada et al. 2008).

Hay meadows are one of the most threatened and most interesting grassland habitats. They are of conservation interest because of their extremely high species richness on a very small scale (Klimeš 2008), which results in part from local natural conditions but is also the result of long-time extensive management with low nutrient input.

The Carpathian Mountains are one of the most important centres of European plant diversity and endemism (Webster et al. 2001). Species-rich grasslands are not a dominant habitat covering a large area of the region, but they are of high value (Šeffler et al. 2002, Sarbu et al. 2004). Their conservation and restoration is a very urgent task that requires information on the ways in which different management and restoration techniques have influence grassland diversity and species composition. Such information promotes cost-effective and efficient conservation measures. Despite particular efforts (Başnou et al. 2009, Klimeš et al. 2008, Halada et al. 2001), experimentally developed management recommendations based on long-term research in the region are still lacking.

The assessment of species richness must incorporate the aspect of scale (Rosenzweig 1995, Magurran 2004). A number of studies have shown that the changes of species richness in grasslands are scale dependent (Huber 1994, de Bello et al. 2006). Our data confirmed this finding and showed that the decrease of species richness after the cessation of mowing is most pronounced on a very detailed scale (Chapter II). In contrast, the re-introduction of mowing promotes an increase of species richness that occurs primarily on a larger scale. These changes may occur because the density of species decreases step-by-step after abandonment and the absence of species is first detected on a detailed scale.

Consequently, the re-appearance of the species in restored grasslands is gradual, and they are first detected in larger plots.

Species-rich grasslands have existed continuously since the Neolithic in central Europe (Klimeš 2008). Although our study area was colonised much later, beginning in the 16th century, the grasslands in the area also have a remarkable degree of species richness. Due to extensive use, which is balanced with the local abiotic conditions, high numbers of species may coexist together in a small space. However, the results of our study show that the balance is fragile. If mowing is absent, the conditions for coexistence in a small space are lost.

It is probable that several mechanisms play a role in the reduction of species richness after grassland abandonment.

The first mechanism is litter accumulation. Our study confirmed that litter accumulation after the cessation of mowing is rapid and has a negative impact on species richness (e.g., Foster & Gross 1998). The negative correlation between litter accumulation and seedling recruitment was pronounced even from the first year after abandonment (Chapter IV). Litter also physically limits the growth of young plants during the springtime (Janeček & Lepš 2005).

The second mechanism is a lack of standing biomass removal. Mowing removes a significant portion of the plants, but its effect is not even (Klimeš & Klimešová 2002). Higher broad-leaf plants lose a higher proportion of their body. They are harmed more than smaller plants growing close to the ground. Mowing seems to maintain a necessary competitive balance that promotes the co-existence of species with various ecological strategies.

The third mechanism could be the absence of small topsoil disturbances caused by mowing and subsequent raking. These disturbances might be significant, especially for seedling recruitment (Zobel et al. 2000, Kotorová & Lepš 1999). However, soil disturbances are still present on grasslands after abandonment (e.g., due to ants, wild pigs or moles). Nevertheless, their distribution is usually rather patchy, and they have a different character from the disturbances caused by mowing and raking.

Our results showed that the simple re-introduction of mowing on long-time abandoned grasslands is not a guarantee that the former species composition and species richness will be restored in a short time (Chapter III).

The main constraint on rapid restoration appears to be a lack of propagules of the grassland species that were lost during the period of abandonment (Bakker & Berendse 1999). Grassland species have different mobilities (Herben et al. 1993), but most of these species have only limited dispersal abilities over longer distances (Stein et al. 2008, Franzén & Eriksson 2003). In addition, they usually lack a long-term persistent seed bank (Davies & Waite 1998, Handlová & Münzbergová 2006). If they become absent from the local species pool due to successional changes, their return is usually very slow and unpredictable. A lack of propagules may also be caused by changes on the landscape level. Traditionally farmed landscape was a very heterogeneous mosaic of differently used small patches. Grasslands covered a much larger area and were less fragmented. Domestic animals were continuously present on grasslands during the vegetation season. Such land use promoted permanent seed dispersal and created refuges for weakly competitive species (Kleyer et al. 2007).

The current landscape is considerably different from traditionally farmed landscape. Grasslands are more fragmented because of successional changes. Traditional small-scale management is disappearing and is being replaced by the large-scale management practices of large agricultural companies.

Dispersal limitation may be overcome by several methods of diaspore transfer used in grassland restoration projects (Kiehl et al. 2010). Our case study from the Morava River floodplain seeks to evaluate grassland restoration on ex-arable land using a local seed mixture and turf transfer. Its results show that the full restoration of the species composition and diversity of species-rich meadows is a very complex and long-term process (Chapter V). Different restoration methods have their technical, spatial and financial limits. Turf transfer appeared to be a very efficient method according to the results of a small-scale experiment, but its effect might be limited on a larger scale due to the limited ability of species to disperse from transferred turfs. It is theoretically possible to transfer more turfs if the donor locality is sufficiently large, but practical restoration

projects must also consider financial limitations. These limits may determine the feasibility of different restoration methods.

Moisture is one of the most important ecological factors influencing grassland diversity in central Europe (Šeffer et al. 2002). We have shown in our study that the position of a particular grassland type on a moisture gradient may influence the requirements for its regular management and the possibilities for its restoration (Chapter III). Moisture may play an important role for several reasons. Wet grasslands are more productive than dry ones. At our study site, wet grasslands have approximately 1.5 times more standing crop than dry grasslands. If they are not mown, more litter accumulates on the ground. The negative consequences of litter accumulation were discussed above. Although litter decomposition in wet grasslands is more rapid than in dry grasslands, the difference in the decomposition rates is not as high in relative terms as the difference in the amount of standing crop, and it does not compensate for the higher productivity of wet grasslands.

At our study site, the wet grasslands have more broad-leaved grasses and forbs with bigger leaves in their species pool. If they are not suppressed by mowing, they are able to invest more resources in leaf growth and thus monopolise the light reaching the grassland canopy. Small species growing near the ground are suppressed and later excluded from the canopy. These strong competitors are less frequent in dry grasslands. In addition, the growth of plants in dry grasslands is limited much more strongly by droughts (Stampfli & Zeiter 2004), which limit the growth of stronger competitors.

The results of our study support the conclusion that wet and more eutrophic types of mountain grasslands are more threatened by abandonment and by successional changes and that their management should therefore be prioritised in plans for the use of species-rich grasslands (Chapter III). Even a short period without mowing may have a very negative impact on species composition, and the changes are barely reversible.

This consideration has already been reflected, in part, in a number of national rural development programmes. For example, certain countries, such as Slovakia and Poland, introduced agri-environmental schemes for species-rich grasslands

with differentiated obligations and payments. This trend is surely positive, but the real effectiveness of environmental payments (e.g., agri-environment, NATURA 2000 payments) appears to be very questionable, and the results of studies of the impact of such payments on biodiversity are controversial (Kleijn & Sutherland 2003). It is possible that these payments are not more effective because they provide a very attractive incentive for larger farms but are of less interest to smaller traditional farms (Schmitzberger et al. 2005). The fulfilment of an extensive list of obligations related to all environmental and hygienic laws imposes very high fixed costs on the farmers, and the payments are not sufficiently high to meet these costs. The size of a farm that is still viable from an economic perspective and still offers a number of biodiversity benefits is approximately 20-60 hectares in our region (Čierna-Plassmann 2010).

It is unlikely that EU regulations and the resulting national laws will be significantly modified to improve the position of small farms. Accordingly, it is probable that alternative solutions to preserve traditional farming must be identified, e.g., establishing unions of small private farmers that offer common machinery and subsidy services to the members.

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