High-diversity sowing in establishment gaps: a promising new tool for enhancing grassland biodiversity

Ansaat artenreicher Samenmischungen in künstliche Störstellen: eine vielversprechende Methode zur Erhöhung der Diversität im artenarmen Grünland

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Abstract

Halting the loss of grassland biodiversity and restoring degraded ecosystems are high priority tasks in the EU Biodiversity Strategy. Sowing low-diversity seed mixtures is widely used in grassland restoration because of its high predictability and fast, promising results. Generally, the sown perennial grasses establish within a few years and form a dense sward, which effectively suppresses weeds. Unfortunately, these grasslands are often species-poor because the sown grasses hamper the colonisation of target grassland forbs. Our aim was to test a novel approach to increase the diversity of species-poor grasslands. We selected eight 8-year-old grasslands restored by low-diversity seed sowing where we created 32 establishment gaps by breaking up the grass sward and sowing a high-diversity seed mixture (35 native species). Altogether, we established three grazed gaps (1 m × 1 m, 2 m × 2 m and 4 m × 4 m) and one fenced gap (4 m × 4 m) per site and monitored the presence and abundance of sown and non-sown species within a time frame of two years. We asked the following questions: (1) Which target species establish most successfully? (2) What is the effect of establishment gap size on the establishment success of target species and weeds? (3) What is the effect of management (grazed versus not managed) on the species composition of the establishment gaps? Our results showed that by creating establishment gaps and sowing diverse seed mixtures, we were able to overcome microsite and propagule limitation, successfully introducing target species into the species-poor grasslands. We found that all sown species established in the gaps, and the majority of the species maintained or even increased their first-year cover in the second year. Smaller gaps were characterised by lower cover of sown species and a quite stochastic development compared to the larger ones. Weed cover was moderate in the first year and decreased significantly in the second year, regardless of gap size. Therefore, in restoration practice, the use of larger establishment gaps is recommended. We found that the cover of sown species and weeds were similar in the grazed and unmanaged gaps during our study. However, management by extensive grazing might be crucial in the long-term because livestock can disperse target species propagules and create microsites. Our study shows that establishment gaps can serve as biodiversity hotspots. Further studies need to clarify to what extent they can improve the restoration success across the entire grassland.

Keywords: Alkali grassland, grazing, loess grassland, seed mixture, species establishment, weed control

Erweiterte deutsche Zusammenfassung am Ende des Artikels

1. Introduction

Halting the loss of biodiversity and restoring degraded ecosystems are high priority tasks in the EU Biodiversity Strategy (EUROPEAN COMMISSION 2011). Natural and semi-natural grasslands harbour a high species richness of plants and animals (DENGLER et al. 2014), thus their restoration is of high importance (PULLIN et al. 2009, TÄLLE et al. 2015). Grassland restoration in degraded areas, such as former croplands, offers an opportunity to restore biodiversity and ecosystem services, such as weed control, soil protection, carbon sequestration and pollination (TALLIS et al. 2008).

Sowing seeds of target species is widely used in grassland restoration practice (TÖRÖK et al. 2011) as it is a predictable, fast and reliable method (KIEHL et al. 2010, TÖRÖK et al. 2010). A major practical constraint of grassland restoration projects is the limited availability of appropriate seed sources. In Germany, Austria and Switzerland, there are well-developed systems for seed propagation of native species for restoration projects (SCOTTON et al. 2012). However, in other Central-European countries, seeds of most grassland species (especially forbs) are not available on the market. Even though seeds of a few forb species can be purchased, they are usually not of local provenance (TISCHEW et al. 2011, KIRMER et al. 2015). In ecological restoration projects, it is crucial to use seeds from local provenance because these ecotypes are better adapted to local site conditions (BISCHOFF et al. 2010, VAN DER MIJNSBRUGGE et al. 2010). Given the limited availability of seeds, large-scale restoration projects generally use only low-diversity seed sowing in many parts of Central Europe (TÖRÖK et al. 2010). Low-diversity seed mixtures usually contain the seeds of cultivars or a few native competitive grass species, which are characteristic of the target grassland types (TÖRÖK et al. 2011). Generally, these perennial grass species are able to establish within a few years after sowing, and they form a dense canopy, which effectively suppresses weeds (ANDERSON 2007, FOSTER et al. 2007, DEÁK et al. 2011). However, sown grasslands are often species-poor because the sown grasses hamper the establishment of further target grassland species (STAMPFLI & ZEITER 1999, CONRAD & TISCHEW 2011, KELEMEN et al. 2014).

The two major constrains of the establishment of target species are microsite and propagule limitation (MOORE & ELMENDORF 2006). On the one hand, dense vegetation and a high amount of accumulated litter considerably decrease the availability of establishment niches (DEÁK et al. 2011, SENGL et al. 2015). On the other hand, the establishment of target species is hampered by the limited availability of their propagules in seed banks or seed rain. Seed banks of grasslands restored on ex-arable fields are usually impoverished, containing mostly the seeds of arable weeds (HUTCHINGS & BOOTH 1996, BEKKER et al. 1997, TÖRÖK et al. 2012). In intensively used agricultural landscapes, the proportion of natural and semi-natural grasslands is usually rather low, which limits the spatial dispersal of target species (DEÁK et al. 2016). Moreover, the effective spatial dispersal distance of propagules of grassland species is usually less than 100 m (STAMPFLI & ZEITER 1999, 25 m; NOVÁK & KONVIČKA 2006, 50 m; DIACON-BOLLI et al. 2012, 40 m). Therefore, in many cases it is unlikely that target species can establish from the seed rain (WALKER et al. 2004, BURMEIER et al. 2011). Thus it is crucial to improve the biodiversity of sown grasslands and to facilitate the establishment of target species by active restoration measures.

In species-poor grasslands, the installation of small-scale gaps sown with high-diversity seed mixtures could help to overcome both microsite and propagule limitation. Even though the application of stripes can be more cost- and labour-effective (see e.g. DONATH et al. 2007, HEDBERG & KOTOWSKI 2010, RAYBURN & LACA 2013), introduction of target species in small gaps fits better into the landscape as gaps can be placed in a pattern that is closer to nature than a stripe. To decrease the competition by resident grasses, the grass sward must be effectively broken up (PYWELL et al. 2007, SCHMIEDE et al. 2012), similar to natural openings, which are created by droughts, wildfires and ecosystem engineer animals, such as ants, rodents, rabbits and wild boars (BULLOCK et al. 1995, BARTHA et al. 2003, VALKÓ et al. 2014a, ZIMMERMANN et al. 2014). In this way we can suppress the competitive grasses and provide optimal establishment conditions for target species. The next step is to introduce propagules of target species by sowing high-diversity seed mixtures on the disturbed soil surfaces. By using high-diversity seed mixtures, the risk of failure is minimised, ensuring that at least some of the target species can establish (KIRMER et al. 2012).

Within the ProSeed DBU project, we aimed at testing the effectiveness of establishment gaps for introducing characteristic alkali and loess grassland species in species-poor restoration sites in the Hortobágy National Park, East-Hungary (TÖRÖK et al. 2010). We asked the following questions: (1) Which target species establish most successfully? (2) What is the effect of establishment gap size on the establishment success of target species and weeds? (3) What is the effect of management (grazed vs. not managed) on the species composition of the establishment gaps?

2. Materials and methods

2.1 Study sites

The study sites are located in the Hortobágy National Park, East-Hungary, near the towns Tiszafüred and Egyek. This lowland region is characterised by a mean elevation of 88–92 m a.s.l. The climate is continental, the mean annual precipitation is 550 mm, and the mean annual temperature is 9.5 ºC with high interannual fluctuations (LUKÁCS et al. 2015). The natural vegetation of the region harbours alkali marshes (*Bolboschoenetalia maritimi* Hejný in Holub et al. 1967, DEÁK et al. 2014a, 2015a) and alkali meadows (*Beckmannion eruciformis* Soó 1933, DEÁK et al. 2014b) at the lowest elevations and alkali dry grasslands (*Artemisio-Festucetalia pseudovinae* Soó 1968, VALKÓ et al. 2014b, DEÁK et al. 2014c) at higher elevations. The natural vegetation of the most elevated plateaux is loess grassland vegetation (*Festucion rupicolae* Soó 1940, DEÁK et al. 2014c, TÓTH & HÜSE 2014). Large-scale crop production started in the region in the middle of the 19th century. As a result the majority of loess grasslands and a considerable proportion of alkali grasslands were ploughed (VALKÓ et al. 2016).

Our study sites were established on grasslands restored in the frame of the LIFE Nature project ‘Grassland restoration and marsh protection in Egyek-Pusztakócs’ (LIFE 04 NAT HU 119). In this landscape-scale restoration project, the basic species pool of alkali and loess grasslands was restored by sowing of low-diversity seed mixtures on former croplands. In the course of the project, which was one of the largest grassland restoration projects in Europe, 760 hectares of grassland were restored by sowing seeds of native grasses (TÖRÖK et al. 2010). In our study sites, two types of seed mixtures were used: (1) alkali seed mixture (containing seeds of matrix grasses *Festuca pseudovina* and *Poa angustifolia*) in lower lying cropland (below 90 m a.s.l) and (2) loess seed mixture (containing seeds of matrix grasses *Bromus inermis*, *Festuca rupicola* and *Poa angustifolia*) in elevated cropland (above 90 m a.s.l). Seed mixtures were sown in October 2005 at a rate of 25 kg/ha. All sites were managed by mowing once a year in June from 2006 to 2008. After the development of a dense sward of matrix grasses, the traditional management regime, i.e. extensive cattle grazing, was introduced from 2009 onwards. For further details on the landscape-scale restoration project, see TÖRÖK et al. (2010) and DEÁK et al. (2011). The dense sward of the perennial sown grasses was very effective in weed suppression, however, cover and number of target species remained low during vegetation development (TÖRÖK et al. 2010, KELEMEN et al. 2014). This failure provided an incentive for our current study.

2.2 Establishment gaps

2.2.1 Compilation of the high-diversity seed mixture

We compiled a seed mixture using seeds of 35 native species collected in the study region by the authors in the summer of 2013 (Table 1, Fig. 1). Our aim was to cover a broad spectrum of species typical of alkali and loess grasslands. Seeds were air-dried and then cleaned by hand. Seed lots of 100 seeds (three replicates per species) were counted to measure thousand-seed weights using a SARTORIUS 1702 type analytical balance (Table 1). The seed mixture was compiled by carefully mixing all cleaned seed material (Fig. 1).

2.2.2 Establishment of gaps and sampling

We selected eight sites restored by low-diversity seed sowing in 2005. At four sites we sowed an alkali seed mixture, at the other four sites a loess seed mixture. At every site gaps were established in October 2013. Soil preparation included digging over, rotary hoeing and raking to remove all rooting

Fig. 1. The high-diversity seed mixture containing the seeds of 35 species typical of alkali and loess grasslands (Photo: T. Miglécz, 01 October 2013).

Abb. 1. Die verwendete artenreiche Samenmischung enthielt 35 für Alkali- und Lössgrünland typische Pflanzenarten (Foto: T. Miglécz, 1.10.2013).

Table 1. Species composition of the high-diversity seed mixtures together with thousand-seed weights and number of seeds sown per m². For the species marked with an asterisk (*), vegetative diaspores (bulbilli) were collected and sown.

Tabelle 1. Artenzusammensetzung der Ansaatmischung mit Tausendkorngewicht und Anzahl der pro m² ausgebrachten Samen. Mit Sternchen (*) gekennzeichnete Arten wurden in Form vegetativer Diasporen (Bulbillen) ausgebracht.

Species	seed weight (g/1000 seeds)	seeds/m²
Achillea collina	0.08	685.06
Aegilops cylindrica	35.37	22.70
Agrimonia eupatoria	20.87	44.85
Agropyron cristatum	1.48	160.94
Allium scorodoprasum*	16.14	20.37
Aster tripolium ssp. pannonicus	0.48	260.57
Atriplex littoralis	3.27	42.02
Atriplex tatarica	3.17	107.31
Bunias orientalis	43.01	21.28
Bupleurum tenuissimum	1.03	48.15
Carthamus lanatus	16.52	21.38
Centaurea jacea ssp. angustifolia	1.16	71.54
Centaurea scabiosa	2.60	89.21
Centaurea solstitialis	1.49	40.25
Dianthus pontederae	0.49	138.13
Falcaria vulgaris	0.62	485.36
Filipendula vulgaris	1.35	863.44
Galium verum	0.27	1654.91
Hypericum perforatum	0.12	464.07
Lathyrus hirsutus	27.43	18.61
Lathyrus tuberosus	31.15	33.66
Lotus corniculatus	1.38	153.42
Plantago media	0.28	171.33
Podospermum canum	3.51	21.46
Potentilla argentea	0.09	607.24
Rapistrum perenne	4.13	9.24
Salvia verticillata	0.49	39.67
Scabiosa ochroleuca	1.22	48.02
Securigera varia	4.59	83.73
Silene viscosa	0.20	164.11
Silene vulgaris	0.60	284.01
Trifolium angulatum	0.44	752.68
Trifolium campestre	0.22	155.86
Trifolium retusum	0.45	154.81
Trifolium striatum	2.33	91.52

plants from the gaps, followed by the the preparation of a fine seedbed by raking. The high-diversity seed mixture was sown at a rate of 10 g/m², which is in accordance with other studies, which used sowing densities between 1 and 10.5 g/m² (see also the review of KIEHL et al. 2010). Before sowing we mixed the seeds with soil in order to prevent wind blowing them away. We established four gaps at every site: (1) 1 m × 1 m, grazed (in total 10 g seed mixture); (2) 2 m × 2 m, grazed (40 g seed mixture); (3) 4 m × 4 m, grazed (160 g seed mixture) and (4) 4 m × 4 m, fenced (160 g seed mixture). The grazed gaps were managed by extensive cattle grazing with a stocking rate of 0.5 LU per hectare, while the fenced ones remained unmanaged. Grazing started in late April, and animals grazed the sites until late October every year. The distance between gaps was at least 50 m to avoid propagule dispersal between gaps. We recorded total vegetation cover and the percentage cover of vascular plant species in each establishment gap ($n = 32$) in June 2014 and 2015. Nomenclature follows KIRÁLY (2009).

2.3 Data processing

Weed species were classified based on TÖRÖK et al. (2012). Adventive competitors (e.g. *Conyza canadensis*), ruderal competitors (e.g. *Cirsium arvense*) and weeds (e.g. *Papaver rhoeas*) were considered as weeds (BORHIDI 1995).

For each of the four types of establishment gaps, cover scores of sown species in the first and in the second year were compared by paired *t*-tests (ZAR 1999). We tested the relationship between the cover and the frequency of the sown species in the first and second year with linear regression. We used linear mixed-effects models (LMEs, ZUUR et al. 2009) to test the effects of gap size (1 m × 1 m, 2 m × 2 m and 4 m × 4 m grazed gaps; fixed factor), year (fixed factor), vegetation type (alkali or loess seed mixture, fixed factor) and site (random factor nested in vegetation type) on vegetation characteristics (dependent variables). We also used LMEs to test the effects of management type (grazed *vs* ungrazed; fixed factor), year (fixed factor), vegetation type (alkali or loess seed mixture, fixed factor) and site (random factor nested in vegetation type) on vegetation characteristics (dependent variables) across the 4 m × 4 m establishment gaps. We used the following vegetation characteristics (dependent variables): total vegetation cover, cover of sown species, cover of sown perennial species, cover of matrix grasses (grasses sown in the low-diversity seed mixtures during the landscape-scale restoration project) and cover of weeds. LMEs were calculated in SPSS 22.0. To compare the species composition of the three types of grazed gaps (1 m × 1 m, 2 m × 2 m and 4 m × 4 m) and that of grazed and fenced 4 m × 4 m gaps, DCA ordination was calculated based on specific cover scores in CANOCO 4.5 (LEPŠ & ŠMILAUER 2003).

3. Results

In total we found 149 species in the establishment gaps. All sown species established either in the first or the second year in at least one establishment gap. In the first year, we recorded 31 sown and 80 unsown species, while in the second year, we recorded 34 sown and 91 unsown species. Out of the 114 recorded unsown species, 60 species were classified as weeds. In the first year, we found several common weeds (weed species having at least 5% cover in at least one establishment gap) across plots. Most common perennial weeds were *Cirsium arvense* (mean cover scores = 5.4%) and *Convolvulus arvensis* (5.0%); most common short-lived weeds included *Bilderdykia convolvulus* (1.5%), *Capsella bursa-pastoris* (0.3%), *Crepis tectorum* (0.9%), *Cynoglossum officinale* (0.6%), *Polygonum aviculare* (4.6%), *Setaria glauca* (2.7%), *Stachys annua* (0.9%) and *Tripleuro-spermum perforatum* (1.3%). In the second year, most common perennial weeds were *Cirs-ium arvense* (2.9%), *Convolvulus arvensis* (3.2%) and *Taraxacum officinale* (2.5%); most common short-lived weeds included *Anthemis arvensis* (0.3%), *Bromus tectorum* (1.0%), *Capsella bursa-pastoris* (2.8%), *Descurainia sophia* (0.3%), *Cynoglossum officinale* (0.7%), *Stellaria media* (0.5%), *Thlaspi arvense* (0.4%) and *Tripleurospermum perforatum* (0.9%).

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Table 2. Mean cover scores (%) of sown species in the first and second year in the four types of establishment gaps compared by paired t-tests. Only species that had significantly increased (upward pointing arrows) or decreased (downward pointing arrows) in at least one type of establishment gap are listed. Significant increases/decreases are indicated by boldface.

Tabelle 2. Mittlere Prozent-Deckungswerte der in den vier Störflächen-Typen angesäten Arten im ersten und zweiten Jahr nach der Ansaat. Lediglich Arten mit signifikanter Zunahme (Pfeile nach oben) oder Abnahme (Pfeile nach unten) in zumindest einem Störflächen-Typ sind dargestellt. Signifikante Veränderungen sind durch Fettdruck gekennzeichnet.

Gap type	1 m × 1 m grazed			2 m × 2 m grazed			4 m × 4 m grazed			4 m × 4 m fenced		
Year	Year 1	Year 2	*p*	Year 1	Year 2	*p*	Year 1	Year 2	*p*	Year 1	Year 2	*p*
Aegilops cylindrica	**1.5**	**0.2**	**0.010 ↓**	3.1	0.4	0.083	2.4	0.8	0.119	**5.3**	**0.1**	**<0.001 ↓**
Allium scorodoprasum	–	**0.1**	**0.019 ↑**	–	0.1	0.234	–	**0.2**	**0.038 ↑**	–	**0.1**	**0.019 ↑**
Carthamus lanatus	0.1	–	0.442	0.3	0.2	0.878	**0.2**	**0.6**	**0.038 ↑**	**1.6**	**0.1**	**<0.001 ↓**
Centaurea sadleriana	0.8	0.5	0.959	1.6	1.8	0.878	**1.5**	**4.5**	**0.019 ↑**	1.6	1.1	0.574
Dianthus pontederae	–	**0.5**	**0.050 ↑**	**0.1**	**1.6**	**0.050 ↑**	**0.1**	**3.5**	**0.050 ↑**	**0.1**	**0.9**	**0.050 ↑**
Galium verum	1.5	4	0.353	1.8	4	0.234	2.4	5.3	0.087	**0.9**	**2.3**	**0.050 ↑**
Plantago media	0.2	0.8	0.721	**0.3**	**1.2**	**0.050 ↑**	0.4	1.6	0.13	**0.1**	**1.2**	**0.050 ↑**
Podospermum canum	–	0.1	0.645	**0.1**	**0.5**	**0.038 ↑**	0.2	0.7	0.063	–	0.1	0.234
Silene viscosa	–	–	–	–	0.4	0.09	–	**0.5**	**0.010 ↑**	–	**1.1**	**<0.001 ↑**

Fig. 2. Vegetation of a 4 m × 4 m establishment gap in the second year after sowing, with a high cover of sown species, such as *Achillea collina*, *Dianthus pontederae* and *Galium verum* (Photo: S. Radócz, 25 May 2015).

Abb. 2. Vegetation einer 4 m × 4 m-Störfläche im zweiten Jahr nach der Ansaat mit hoher Abundanz von *Achillea collina*, *Dianthus pontederae* und *Galium verum*. (Foto: S. Radócz, 25.05.2015).

3.1 Establishment of sown species

There was a strong positive correlation between the cover of sown species in the first and second year (linear regression, $R = 0.953$, $p < 0.001$). We also detected a positive correlation between the frequency of sown species in the first and second year ($R = 0.670$, $p < 0.001$). The cover of the majority of sown species did not change from the first to the second year. *Dianthus pontederae* was the only species whose cover significantly increased in the second year in all types of gaps (Table 2). We detected a significant increase in the cover of *Allium scorodoprasum*, *Centaurea sadleriana*, *Galium verum*, *Plantago media*, *Podospermum canum* and *Silene viscosa* in at least one type of gap (Table 2). The cover of *Carthamus lanatus* increased in the grazed and decreased in the fenced 4 m × 4 m gaps. The cover of the short-lived grass *Aegilops cylindrica* decreased significantly in the 1 m × 1 m grazed and 4 m × 4 m fenced gaps in the second year (Table 2).

In the DCA ordination, there was a clear distinction between the first- and second-year vegetation, and most species were plotted towards the second year (Fig. 3). Short-lived sown species characteristic of alkali grasslands – such as *Bupleurum tenuissimum*, *Atriplex tatarica*, *A. litoralis* and *Aegilops cylindrica* – were characteristic of the first-year vegetation. Almost all perennial sown species and typical forbs of loess grasslands – such as *Galium verum*, *Filipendula vulgaris*, *Achillea collina*, *Centaurea jacea* ssp. *angustifolia* and *C. sadleriana* – were characteristic of the second-year vegetation (Fig. 3).

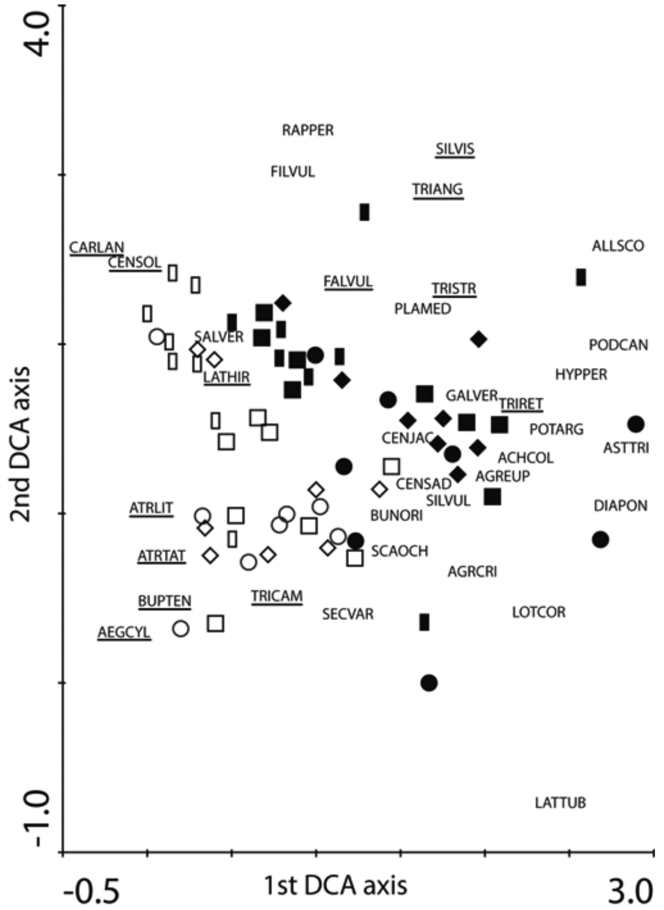

Fig. 3. Species composition of the establishment gaps in the first and second year plotted by a DCA based on specific cover scores. Notations: ○ – 1 m × 1 m grazed establishment gaps, year 1; ● – 1 m × 1 m grazed establishment gaps, year 2; □ – 2 m × 2 m grazed establishment gaps, year 1; ■ – 2 m × 2 m grazed establishment gaps, year 2; ◇ – 4 m × 4 m grazed establishment gaps, year 1; ◆ – 4 m × 4 m grazed establishment gaps, year 2; ▯ – 4 m × 4 m fenced establishment gaps, year 1 and ▮ – 4 m × 4 m fenced establishment gaps, year 2. The 35 sown species are represented by the first three letters of both their genus and species name. Short-lived species are underlined.

Abb. 3. Gradientenanalyse (DCA) der Artenzusammensetzung auf Störflächen im ersten und zweiten Jahr nach der Ansaat. In die Analyse gingen die prozentualen Deckungen der Arten ein. Die Symbole bedeuten: ○ – beweidete 1 m × 1 m-Störfläche im 1. Jahr; ● – beweidete 1 m × 1 m-Störfläche im 2. Jahr; □ – beweidete 2 m × 2 m-Störfläche im 1. Jahr; ■ – beweidete 2 m × 2 m-Störfläche im 2. Jahr; ◇ – beweidete 4 m × 4 m-Störfläche im 1. Jahr; ◆ – beweidete 4 m × 4 m-Störfläche im 2. Jahr; ▯ – unbeweidete 4 m × 4 m-Störfläche im 1. Jahr; ▮ – unbeweidete 4 m × 4 m-Störfläche im 2. Jahr. Die Position der 35 angesäten Zielarten ist im DCA-Diagram mit jeweils den ersten drei Buchstaben des Gattungs- und Artnamens dargestellt. Kurzlebige Arten sind unterstrichen.

3.2 Effects of gap size

The size of gaps affected the total vegetation cover and the cover of sown and perennial sown species (Table 3). All sown species and sown perennial species had the highest cover scores in the largest establishment gaps in both years (Table 3 and 4). The total vegetation cover and the cover of sown perennial species increased from the first year to the second. The cover of matrix grasses increased significantly from the first year to the second, and similar scores were detected in the differently sized gaps (Table 3 and 4). The vegetation type had a significant effect only on the cover of matrix grasses: They had higher cover scores on sites formerly restored by loess grass seed mixture. The cover of weeds was not different in the differently sized gaps and decreased from the first year to the second (Table 3 and 4).

The DCA ordination showed that the differently sized grazed gaps had a similar species composition in the first year (Fig. 3). In the second year, the dissimilarity of species composition increased in grazed 1 m × 1 m gaps, while it decreased in grazed 2 m × 2 m and 4 m × 4 m gaps. The species composition of the first and second year was more similar in larger gaps (2 m × 2 m and 4 m × 4 m) than in the smallest gap (1 m × 1 m, Fig. 3).

3.3 Effects of grazing exclosure

Total vegetation cover was significantly lower and sown perennial cover higher in grazed 4 m × 4 m gaps than in fenced 4 m × 4 m gaps (Table 4 and 5). Differences in cover of sown species, matrix grasses and weeds were not significant between grazed and ungrazed variants. The cover of perennial sown species and matrix grasses increased from the first year to the second, regardless of the management type.

The DCA ordination showed that in the first year, fenced gaps were characterised by the sown short-lived prickly species (*Carthamus lanatus* and *Centaurea solstitialis*). In the second year, several short-lived sown species (*Silene viscosa*, *Trifolium angulatum*, *T. striatum* and *Falcaria vulgaris*) were typical for the fenced 4 m × 4 m gaps. The dissimilarity of species composition in the grazed 4 m × 4 m gaps was lower than in the fenced ones in the second year (Fig. 3).

Table 3. Effects of establishment gap size (fixed factor), year (fixed factor), vegetation type (alkali or loess grassland, fixed factor) and site (random factor nested in vegetation type) on the cover of species groups in the grazed establishment gaps tested by linear mixed-effects models (LMEs). Significant differences are indicated by boldface.

Tabelle 3. Einfluss der Störflächengröße, des Jahres, des Vegetationstyps (Alkali- *vs*. Lössgrasland) und der Lokalität auf die Deckung der Artengruppe in den beweideten Störflächen. Die Störflächengröße, das Jahr und der Vegetationstyp gingen in die linearen gemischten Modelle (*linear mixed-effect models*, LME) als feste Faktoren und die Lokalität (eingeschachtelt in den Vegetationstyp) als zufälliger Faktor ein. Signifikante Effekte sind durch Fettdruck gekennzeichnet.

	Gap size		Year		Vegetation type		Site	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Total cover	**3.31**	**0.048**	**4.41**	**0.043**	0.30	0.606	**4.85**	**<0.001**
Sown cover	**8.65**	**<0.001**	1.63	0.210	2.35	0.176	1.44	0.226
Sown perennial cover	**8.18**	**0.001**	**9.25**	**0.004**	0.93	0.371	**3.95**	**0.003**
Matrix grass cover	1.58	0.219	**13.35**	**<0.001**	**7.97**	**0.030**	1.40	0.241
Weed cover	0.68	0.511	**6.64**	**0.014**	0.46	0.524	**2.56**	**0.036**

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Table 4. Cover of species groups (%, mean ± 1 × standard error) in the four types of establishment gaps in the first and second year after sowing.

Tabelle 4. Prozent-Deckungen der Artengruppen (Mittelwert und einfacher Standardfehler) für die vier Störflächen-Typen im ersten und zweiten Jahr nach der Ansaat.

Gap type	Year 1				Year 2			
	1 m × 1 m grazed	2m × 2 m grazed	4m × 4 m grazed	4 m × 4 m fenced	1 m ×1 m grazed	2 m × 2 m grazed	4 m × 4 m grazed	4 m × 4 m fenced
Total cover	60.6 ± 5.7	65.6 ± 3.3	74.1 ± 2.3	80.4 ± 2.6	68.8 ± 8.4	76.8 ± 2.9	74.6 ± 3.1	79.4 ± 3.6
Sown cover	28.6 ± 6.0	38.6 ± 4.5	51.8 ± 7.0	66.3 ± 5.5	30.9 ± 8.6	15.8 ± 1.1	59.2 ± 6.8	50.8 ± 10.3
Sown perennial cover	11.7 ± 4.0	18.7 ± 5.0	24.5 ± 5.1	8.2 ± 2.2	20.0 ± 6.4	25.4 ± 3.6	42.9 ± 7.7	20.7 ± 5.0
Matrix grass cover	4.8 ± 2.2	3.7 ± 0.9	2.2 ± 0.3	1.6 ± 1.0	9.6 ± 2.7	6.9 ± 1.6	7.2 ± 1.2	8.1 ± 3.5
Weed cover	26.0 ± 4.0	29.2 ± 5.6	30.7 ± 4.6	18.5 ± 4.4	19.3 ± 6.2	16.2 ± 4.8	23.3 ± 3.3	19.2 ± 7.8

Table 5. Effects of management type (grazed/ungrazed, fixed factor), year (fixed factor), vegetation type (alkali or loess grass seed mixture, fixed factor) and site (random factor nested in vegetation type) on the cover of species groups in the grazed and fenced 4 m × 4 m establishment gaps, tested by linear mixed-effects models (LMEs). Significant differences are indicated by boldface.

Tabelle 5. Einfluss der Nutzung (Beweidung *vs*. Brache), des Jahres (Jahr 1 *vs*. Jahr 2 nach Ansaat), des Vegetationstyps (Alkali- *vs*. Lössgrasland) und der Lokalität auf die Deckung von fünf Artengruppen in den beweideten Störflächen. Die Nutzung, das Jahr und der Vegetationstyp gingen in die linearen gemischten Modelle (*linear mixed-effect models*, LME) als feste Faktoren und die Lokalität (eingeschachtelt in den Vegetationstyp) als zufälliger Faktor ein. Signifikante Effekte sind durch Fettdruck gekennzeichnet.

	Management		Year		Vegetation type		Site	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Total cover	**4.31**	**0.049**	0.01	0.926	0.02	0.902	2.13	0.091
Sown cover	0.17	0.687	0.30	0.587	0.71	0.433	1.61	0.190
Sown perennial cover	**19.15**	**<0.001**	**12.22**	**0.002**	1.63	0.249	2.55	0.050
Matrix grass cover	0.01	0.930	**10.94**	**0.003**	1.57	0.257	1.59	0.198
Weed cover	3.10	0.092	0.53	0.474	0.04	0.842	2.55	0.051

4. Discussion

4.1 Establishment of sown species

Many authors stated that high-diversity sowing enables the establishment of multiple target species by overcoming propagule limitation (e.g. LEPŠ et al. 2007, KIRMER et al. 2012, PRACH et al. 2013). But even when seed sources are nearby, microsite limitation can hamper successful colonisation for longer periods of time (e.g. ÖSTER et al. 2009). In our study the use of establishment gaps proved to be an effective tool to overcome microsite and propagule limitation and support the establishment of characteristic subordinate species in restored species-poor grasslands. A similar approach is recommended by COIFFAIT-GOMBAULT et al. (2012) and TÖRÖK et al. (2010), who suggested that founder or matrix species should be established first on former arable land in order to facilitate the establishment of later sown or spontaneously immigrating target species. In our study region, grasslands restored by low-diversity sowing are characterised by a high cover of sown perennial grasses hampering the establishment of target species. TÖRÖK et al. (2010) reported that cover scores of sown grasses were higher than 75% three years after restoration, while KELEMEN et al. (2014) found that their cover was still between 65 and 71% six years after restoration. Similarly to PYWELL et al. (2007) as well as SCHMIEDE et al. (2012) and JOHN et al. (2016), we created suitable establishment conditions for sown target species by a severe disturbance of the grass sward. In the first two years, the cover of resident matrix grasses (*Bromus inermis*, *Festuca pseudovina*, *F. rupicola* and *Poa angustifolia*) was below 10% in all types of gaps, resulting in high niche availability and low competition, thus facilitating the establishment of sown target species. A similar effect was reported by ZOBEL et al. (2000) as well as PYWELL et al. (2007). In our study all sown target species were able to establish successfully in the first and/or in the second year.

The cover of sown species was similar both in alkali and loess sites, which suggests that the use of a highly diverse seed mixture increases the chance for several species to establish under various site conditions. Similar to JOHN et al. (2016), we found that the first year was

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crucial for the establishment of sown species, indicated by a strong correlation between first- and second-year cover and frequency of sown species. Most species that were successful in the first year had similar or even higher cover scores in the second year. This indicates that the majority of established species can persist in the gaps and thus get the chance to disperse into the sown grasslands in the future. Other authors stated that almost 90% of introduced species had been able to spread into the surrounding grasslands after 7–8 years (BURMEIER et al. 2011). During our study we observed the establishment of *Centaurea solstitialis* (a native target species protected in Hungary) in the grasslands near to the establishment gaps already in the second year.

We found a shift in life history spectra of the sown species from the first to the second year. Short-lived sown species were more frequent in the first year, while perennials were more frequent in the second year. This is in line with the findings of MIGLÉCZ et al. (2015), who found similar trends in vineyard inter-rows sown with high-diversity seed mixtures. Some short-lived sown species typical in the first year (*Atriplex litoralis*, *A. tatarica* and *Bupleurum tenuissimum*) are characteristic stress tolerant species in the secondary succession on alkali soils in the study region (DEÁK et al. 2015b). Second-year vegetation was mainly characterised by perennial species and species typical of loess grasslands (TÓTH & HÜSE 2014).

4.2 Effects of gap size

Our results showed that larger gaps are more appropriate for the establishment of target species than smaller ones. On the one hand, we found that large establishment gaps were characterised by higher total vegetation cover and higher cover of sown species (see Table 4). On the other hand, vegetation development in small (1 m × 1 m) gaps was stochastic compared to larger gaps, as shown by the DCA ordination (see Fig. 3). Small gaps are characterised by a lower surface/perimeter ratio, thus the surrounding dense canopy and root system of competitive grasses likely have a larger effect on their vegetation development (DEÁK et al. 2011). This is also confirmed by results of ECKSTEIN et al. (2012) and LUDEWIG et al. (2015), who found that very small gaps (between 10 cm × 10 cm and 32 cm × 32 cm) are quickly recolonised by the surrounding resident vegetation. Thus by establishing larger gaps, site managers can expect a better development of targeted vegetation.

Establishment gaps can provide proper conditions not only for the sown target species, but also for weeds. After soil disturbance in former croplands, a high level of weed infestation can be expected, emerging from persistent seed banks (HUTCHINGS & BOOTH 1996, BEKKER et al. 1997, HÖLZEL & OTTE 2003, DONATH et al. 2007, TÖRÖK et al. 2012). Our results suggest that the establishment of weeds in the gaps is only a small-scale and temporal phenomenon, which poses no real threat for restoration (see also WARREN et al. 2002). In our study there was only a moderate weed encroachment (weed cover between 18.5 and 30.7%) in the first year. As a comparison, TÖRÖK et al. (2012) found a weed cover of 64–70% in the first year after sowing low-diversity grass seed mixtures in the landscape-scale restoration project. The likely reason is that due to the long-term post-restoration management (mowing from 2006 to 2008 and grazing thereafter), weeds were not able to produce seeds so that their seed banks impoverished (KELEMEN et al. 2014). Another reason is that in the present study we applied high-density sowing (corresponding to 100 kg/ha as opposed to 25 kg/ha used in the landscape-scale restoration project), which could have been more effective in weed suppression. In our study most of the weeds were short-lived species, which are not problematic from the restoration ecological viewpoint. Though two perennial weeds, *Cirsium arvense* and *Convolvulus arvensis*, were common in the first year, their cover decreasedin the second year. We found that, regardless of gap size, the cover of weeds decreased significantly from the first year to the second, caused by the establishment of more competitive perennial species emerging from the seed mixture. This is important because farmers were afraid of weed infestation in larger gaps. Since weed emergence in our gaps did not pose a threat for the grassland area, the use of 4 m × 4 m gaps can be recommended for future restoration projects.

4.3 Effects of management type

We found that total vegetation cover was lower in grazed sites, likely because of the continuous biomass removal (see also PAVLŮ et al. 2007, TÖRÖK et al. 2014). The cover scores of sown species, matrix grasses and weeds were similar in the grazed and unmanaged gaps in the first two years after sowing. These results might suggest that management does not play a crucial role in the early vegetation developement of the establishment gaps. However, grazing might prove to be an important long-term conservation tool. First, in the absence of biomass removal, litter accumulation and decreased microsite availability can be expected in the fenced gaps, which can lead to the disappearance of the sown species in the long run (ESKELINEN & VIRTANEN 2005, KELEMEN et al. 2014, LEPŠ et al. 2007). Second, grazing livestock can disperse target species propagules to the remaining grassland area (WESSELS et al. 2008, ROSENTHAL et al. 2012, FREUND et al. 2014). Finally, in a closed sward, livestock can create small-scale disturbances, which can act as microsites for the establishment of target species (ESKELINEN & VIRTANEN 2005, MANN & TISCHEW 2010, ROSENTHAL et al. 2012, FREUND et al. 2015, TÖLGYESI et al. 2015).

In the second year, the dissimilarity of species composition in grazed 4 m × 4 m gaps was lower than in fenced gaps. The stochastic vegetation development in fenced gaps might indicate that a lack of management can lead to multiple pathways of vegetation development (see also MOOG et al. 2002, KÖHLER et al. 2005). The DCA ordination showed that short-lived species were characteristic of fenced gaps, with sown short-lived prickly species (*Carthamus lanatus* and *Centaurea solstitialis*) prevailing in the first and short-lived sown species (*Silene viscosa*, *Trifolium angulatum*, *T. striatum* and *Falcaria vulgaris*) dominating in the second year. The likely reason for this result is that short-lived species had a higher chance to set seeds in the fenced gaps in the first year, thus they could germinate in higher numbers in the second year compared to grazed gaps (ABOLING et al. 2008). However, this effect might be temporary due to an increasing competition by perennial species in subsequent years.

5. Conclusions

We used a novel approach to enhance the biodiversity of species-poor grasslands by sowing high-diversity seed mixtures in patches prepared by sward disturbance. Our results showed that the use of establishment gaps is a promising tool for increasing the diversity of species-poor sown grasslands. This method is especially useful in cases where the spontaneous establishment of target species is improbable due to lack of appropriate diaspore sources in the surroundings and high competition by resident grasses. While soil preparation increases the availability of microsites, high-diversity sowing introduces the propagules of multiple target species. Based on our results, larger gaps (4 m × 4m) are more effective than smaller ones.

Given the high establishment success of the sown target species, establishment gaps can serve as biodiversity hotspots. Further studies need to clarify to what extent target species can disperse from gaps to adjacent areas, establish persistent populations and consequently increase species richness across the entire grassland. Small-scale disturbances created by rodents and wild boars, which are abundant in the study area, could create crucial microsites by breaking up the closed grass sward. Livestock grazing could achieve a similar effect and increase the dispersal of target species propagules. In grassland restoration practice, grazing is not a widely used management tool in the first years after sowing because it can lead to weed encroachment by creating open surfaces favouring the germination of weeds. However, our results suggest that in case of small establishment gaps embedded in the matrix of already recovered grasslands, grazing can well be a suitable management tool even from the first year onwards since grazing did not hamper the establishment of sown species and did not lead to weed encroachment.

Erweiterte deutsche Zusammenfassung

Einleitung – In der Europäischen Union wird der Renaturierung degradierter Ökosysteme hohe Priorität beigemessen. Die Ansaat von artenarmen Gräsermischungen ist eine weit verbreitete Renaturierungspraxis, durch die schnelle und gut vorhersagbare Resultate erzielt werden können. Die angesäten mehrjährigen Gräser bilden innerhalb weniger Jahre eine dichte Grasnarbe, die effektiv unerwünschte Ruderalarten unterdrückt und landwirtschaftlich genutzt werden kann, die aber auch die Einwanderung von krautigen Zielarten behindert. In vielen landwirtschaftlich geprägten Regionen sind zudem kaum noch Diasporenquellen wichtiger Grünlandarten vorhanden, deshalb ist das aktive Einbringen von Zielarten eine wichtige Maßnahme zur Artenanreicherung.

In Ungarn wurden im Hortobágyi-Nationalpark zwischen 2005 und 2008 im Rahmen eines LIFE-Projektes 760 ha ehemaliges Ackerland durch Ansaat von zwei bis drei Grasarten in artenarmes Grünland umgewandelt und anschließend beweidet (TÖRÖK et al. 2010, 2011). Durch den Mangel an geeigneten Diasporenquellen konnten sich dort bisher allerdings kaum krautige Zielarten etablieren. In der vorliegenden Studie wurde deshalb eine neue Methode zur Diversifizierung von artenarmen Grünland getestet. Dazu wurden in dichten Grasbeständen kleinräumige Störflächen geschaffen und in diese eine standortgerechte Samenmischung aus 35 krautigen Zielarten ausgebracht. Konkret stellten wir folgende Fragen: (1) Welche der angesäten Zielarten etablieren sich in den kleinräumigen Störflächen am erfolgreichsten? (2) Wie wirken sich die Größe der Störflächen auf den Etablierungserfolg der Zielarten sowie auf die Abundanz von unerwünschten Arten aus? (3) Wie beeinflusst das Management (mit und ohne Beweidung) die Artenzusammensetzung der Störflächen?

Material und Methoden – Die Untersuchung wurde auf ehemaligen Ackerflächen im Hortobágyi-Nationalpark in Ost-Ungarn durchgeführt, die vor ca. acht Jahren durch die Ansaat von zwei bis drei Wildgrasarten in artenarmes Grünland umgewandelt worden waren. Die Flächen waren durch eine hohe Deckung der angesähten ausdauernden Matrix-Grasarten und eine dichten Grasnarbe gekennzeichnet. An acht Lokalitäten wurde die Grasnarbe dieser Flächen durch intensives Hacken nachhaltig gestört und in den so entstandenen Störstellen im Oktober 2013 35 heimische Wildkrautarten mit einer Dichte von 10 g/m² angesät (Tab. 1, Abb. 1). Pro Lokalität wurden drei beweidete Störflächen mit unterschiedlicher Größe (1 m × 1 m, 2 m × 2 m und 4m × 4 m) und eine eingezäunte 4 m × 4 m-Störfläche ohne Beweidung eingerichtet. Eine bzw. zwei Vegetationsperioden später (im Juni 2014 und 2015) wurde auf allen Störflächen die Deckung aller Gefäßpflanzen prozentgenau erfasst. Der Einfluss der Größe der Störflächen mit Beweidung (1 m × 1 m *vs*. 2 m × 2 m *vs*. 4 m ×4 m), der Nutzung (Beweidung *vs*. Brache auf den 4 m × 4 m-Flächen), des Jahres (Jahr 1 nach Ansaat *vs*. Jahr 2 nach Ansaat), des Vegetationstyps (Alkali- *vs*. Lössgrasland) und der Lokalität auf die Etablierung der Zielarten sowie auf allgemeine Vegetationsmerkmale wurde mit linearen gemischten Modellen überprüft (ZUUR et al. 2009). Die Größe der Störflächen, die Nutzung, das Jahr und der Vegetationstyp gingen in die statistischen Modelle als feste Faktoren und die (in den Vegetationstyp eingeschachtelte) Lokalität als zufälliger Faktor ein. Die Etablierung der angesäten Zielarten sowie die allgemeinen Vegetationsmerkmale waren die abhängigen Variablen. Zusätzlich wurde die Artenzusammensetzung der vier verschiedenen Störflächentypen mit Gradientenanalyse (DCA) untersucht.

Ergebnisse – Grundsätzlich konnten sich alle angesäten krautigen Zielarten etablieren und die Mehrzahl der Zielarten wies im zweiten Jahr nach der Ansaat höhere Deckungswerte auf als im ersten Jahr (oder zumindest ähnlich hohe wie im ersten Jahr). Die prozentuale Deckung der angesäten Arten war auf kleinen Störflächen niedriger als auf großen Störflächen. Ferner waren kleine Störflächen im Vergleich zu größeren durch eine heterogenere Artenzusammensetzung gekennzeichnet (Tab. 3 und 4, Abb. 3). Die Gesamtdeckung und die Deckung der mehrjährigen angesäten Arten nahm vom ersten zum zweiten Jahr zu (Tab. 3 und 4). Die Deckung unerwünschter Arten war im ersten Jahr mäßig und nahm dann zum zweiten Jahr unabhängig von der Flächengröße signifikant ab. In den beweideten 4 m × 4 m-Flächen waren die Gesamtdeckung und die Deckung der angesäten mehrjährigen Arten signifikant höher als in den eingezäunten 4 m × 4 m-Flächen. Die Deckungen der angesäten Arten, der Matrixgräser und der Unkräuter unterschieden sich zwischen beweideten und ungenutzten Flächen nicht.

Diskussion – Unsere Ergebnisse zeigen, dass durch die Ansaat artenreicher, standortgerechter Samenmischungen in künstliche Störflächen in artenarmen Grünland sowohl der Mangel an Etablierungsnischen kompensiert als auch Ausbreitungsbarrieren überwunden werden können. Die Störung der Grasnarbe führte zu einer nachhaltigen Reduktion der Gräserdeckung (in beiden Versuchsjahren < 10 %), so dass sich die angesäten Zielarten erfolgreich etablieren konnten (PYWELL et al. 2007, SCHMIEDE et al. 2012, JOHN et al. 2016).

Die meisten angesäten Arten, die sich im ersten Jahr erfolgreich etablieren konnten, hatten auch im zweiten Jahr höhere oder zumindest ähnlich hohe Deckungswerte, wobei größere Störstellen (4 m × 4 m) sich als effektiver erwiesen als kleinere.

Aufgrund unserer Ergebnisse schlussfolgern wir, dass sich die Ausbringung von artenreichen, standortgerechten Samenmischungen in größere Störstellen gut zur Schaffung von kleinräumigen Biodiversität-Hotspots eignet. Interessant war auch, dass diese Flächen bereits im ersten Jahr beweidet werden können, ohne dass es zu einer Beeinträchtigung der angesäten Zielarten durch die Weidetiere oder zu einem erhöhten Aufkommen von Unkrautarten kommt. Allerdings sind weitere Studien notwendig, um zu klären, in welchem Ausmaß Zielarten aus diesen kleinräumigen Biodiversität-Hotspots in die artenarmen Grünlandflächen einwandern können. Dieser Prozess kann mit hoher Wahrscheinlichkeit durch eine Störung der dichten Grasnarbe beschleunigt werden, wobei Weidetieren, aber auch Wildschweinen, eine wichtige Rolle zukommen kann, da diese nicht nur Diasporen von Zielarten ausbreiten, sondern auch kleinflächige Etablierungsnischen schaffen können.

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