






## Long-term results of initial seeding, mowing and carbon amendment on the restoration of Pannonian sand grassland on old fields

### Langzeiteffekte von Einsaat, Mahd und Kohlenstoffzusatz bei der Renaturierung von pannonischem Sandgrasland auf ehemaligen Äckern

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#### Abstract

Cropland abandonment is an opportunity for semi-natural biodiverse ecosystems to develop through spontaneous succession or active restoration. Spontaneous recovery is limited by the dispersal and capacity of species to establish under the given environmental circumstances. This paper provides an evaluation of three restoration techniques to overcome dispersal and environmental limitations based on revisiting an experiment with the aim to restore Pannonian sand grasslands on old fields, 16 years after its start.

Treatments were carried out in two sites with different date of abandonment, initial soil conditions and landscape features. The experimental design involved ploughing as pre-treatment and eight types of treatments, which consisted of the combinations of initial seeding (2002/2003), mowing (2003–2008), carbon amendment (2003–2008) and control in eight replicates per site. We compared the impact of seeding, mowing, carbon amendment and site on soil available nitrogen, species richness, total vascular plant cover and the relative cover of target and neophyte species with the use of GLM and one-way and two-way ANOVA. We also analysed site-related factors such as soil characteristics and landscape context.

We found a long-term impact of mowing on the total vascular plant cover. We also detected an impact of seeding on the relative cover of seeded species, mainly due to *Stipa borysthénica* (18.5% in seeded and 8.5% in non-seeded plots), and on the relative cover of neophyte species, in particular the annual neophyte *Ambrosia artemisiifolia* (0.07% in seeded and 2.32% in non-seeded plots in one site). Seeded species established and persisted in the long term and also spread into non-seeded plots resulting in 85% and 91% mean cover of target species. Most of the parameters analysed, with the exception of the cover of *Festuca vaginata*, demonstrated a site-specific vegetation development. No long-term impact on soil-available nitrogen was found.

The results of our long-term evaluation of restorative treatments indicate that seeding can successfully assist the restoration of sand grasslands on abandoned fields, while carbon amendment can be used to supplement seeding in the initial years and infrequent mowing in the later years of restoration. The spread of seeded species into non-seeded plots did not allow for the causal analysis of single factors such as mowing or carbon addition in the long term, but it has optimised the restoration process. Based on our results we suggest that restoration of old fields can be triggered with the use of small seed introduction units, also referred to as “establishment windows”.

**Keywords:** carbon amendment, grassland restoration, invasive species, landscape characteristics, long-term monitoring, mowing, nitrogen immobilisation, old-field restoration, sand grassland, seeding

**Erweiterte deutsche Zusammenfassung am Ende des Artikels**

## 1. Introduction

Land abandonment reflects the relationship between humanity and the world’s ecosystems for as long as it has been recorded. According to worldwide records, Europe, South America and China registered land abandonment from the late 19<sup>th</sup> and the early 20<sup>th</sup> century, with a significant increase in the rate of abandonment since the 1960s (HOBBS & CRAMER 2007, ISBELL et al. 2019). According to trends forecasted for the EU between 2015 and 2030, 11% of agricultural land is at high potential risk of abandonment (PERPIÑA et al. 2018). In Europe the rate of abandonment of croplands differs greatly between the West and the East depending on socio-economic determinants. Central and Eastern European state-owned agricultural cooperatives collapsed with the collapse of socialist regimes in the early 1990s (MIHÓK et al. 2017), which accelerated the rate of land abandonment (VALKÓ et al. 2016a). Cropland abandonment provides space for the development of a new cover of vegetation through natural succession. The relationship between past cultivation systems, vegetation, soil, other abiotic and landscape characteristics determines the dynamics of plant community assembly in old fields (CRAMER et al. 2008).

Old fields are a particular kind of ecosystem. Mostly, they are transient ecosystems, which can develop into various ecosystems if there is no post-abandonment management (CLARK 2017). Old fields are normally studied and treated according to their “age” determined by the time elapsed since the cultivation ceased. Spontaneous vegetation development in old fields can result in a new habitat for several native species (SOJNEKOVÁ & CHYTRÝ 2015), which helps to reduce fragmentation in a landscape, but can also result in novel ecosystems with high presence of invasive alien species (CSECSERITS et al. 2011). In general, old fields remain different from primary grasslands for a long time (ISBELL et al. 2019). Old-field succession varies from place to place and from time to time according to the individual history of the site, which involves past agricultural treatments and the abiotic and biotic differences from other sites (KEEVER 1983).

The success of spontaneous recovery after abandonment is limited by certain factors related to the dispersal ability of the species and their capacity to establish and survive under the given environmental circumstances (HOBBS & CRAMER 2007). Excess of nutrients is the most critical local abiotic constraint in old-field succession since it not only affects the ability of native species to survive, but also contributes to the growth and spread of invasive species (BAKKER & BERENDSE 1999). Among the biotic constraints, the most important factor besides competition and invasion is propagule limitation (TÖRÖK et al. 2018). The presence of the required seeds might depend on the timing of abandonment, the size of the field (CRAMER et al. 2008) and the cultivation time prior to abandonment since seed banks

and aboveground vegetation diversity are greatly reduced during long-term cultivation (BAKKER & BERENDSE 1999, KISS et al. 2018). As most grassland species do not form a permanent seed bank in Europe, arable fields contain large amounts of seeds of non-target weed species instead (BAKKER & BERENDSE 1999). Moreover, spatial dispersal of target species is only possible where remnants of the natural vegetation are still present in the landscape (TÖRÖK et al. 2018, HALASSY et al. 2019).

Grassland restoration on old fields can be a strategy to recover biodiversity and ecosystem services, but also to slow down and decrease the spread of weedy and/or invasive species (CRAMER et al. 2008). The most widely used active restoration techniques focus on overcoming specific limitations such as dispersal and abiotic and biotic constraints (HALASSY et al. 2016). Seed introduction can be applied to increase biodiversity where the seed bank is deficient or where barriers prevent the arrival of the required seeds to the site (TÖRÖK et al. 2010, PRACH et al. 2014, KÖVENDI-JAKÓ et al. 2019). A high nutrient content of the soil can be best adjusted with topsoil removal or addition of a carbon source in order to restore nutrient-poor grasslands (PERRY et al. 2010, GILHAUS et al. 2015). Mowing helps increase species diversity through the creation of gaps, which enhance the colonisation of new individuals (MARON & JEFFERIES 2001, WILLIAMS et al. 2007, COLIN 2011, TESEI et al. 2019). However, better results are expected from combining more than one restoration technique (HALASSY et al. 2016).

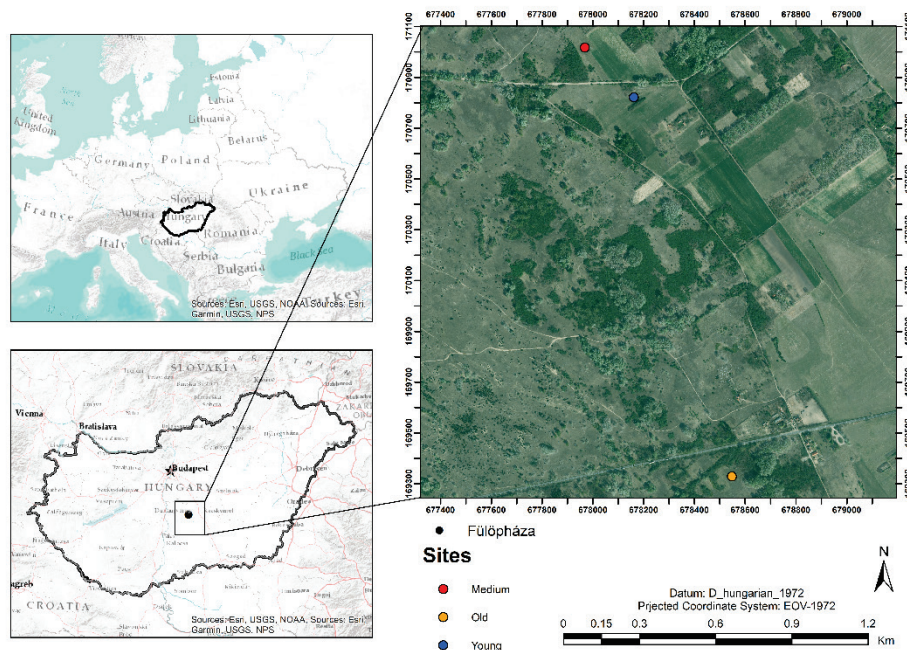
The aim of the present study was to evaluate the long-term impact of seeding, mowing and carbon amendment in the restoration of Pannonian sand grassland on old fields based on revisiting the sites 16 years after the first applications. We addressed the following questions: (1) How do initial seeding, mowing and carbon amendment impact soil nitrogen availability, species richness, total vascular plant cover and the relative cover of target and neophyte species in the long term? (2) How do site characteristics (soil conditions and the surrounding landscape) influence the impact of treatments?

## 2. Materials and methods

### 2.1 Study area

The study area is located in the Kiskun LTER site, Fülöpháza Sand Dunes protected area (N 46.890° E 19.440°), covering an area of 1992 hectares in the Kiskunság National Park, Hungary, Europe (Fig. 1). The average temperature is 10.5 °C with precipitation that reaches a yearly average of 500–550 mm and the frequent occurrence of extensive and severe droughts (KOVÁCS-LÁNG et al. 2008). The soil is calcareous arenosol with a scarce content of humus. The natural vegetation is forest-steppe with endemic open and closed sand grassland and juniper-poplar stands (ERDŐS et al. 2018). During the last centuries, a strong human impact (agriculture and forestry) has caused a significant decrease (92% destroyed) of the Pannonian sand steppe (BIRÓ et al. 2013). From 1980 land abandonment has been widespread as well, particularly in areas of low productivity (CSECSERITS et al. 2011).

The restoration experiment originally involved three sites, which were old fields of different times of abandonment, but the youngest was returned to cultivation in 2008. According to historical aerial photos, the remaining two fields, further referred to as Medium and Old site, were abandoned in the late 1990s (probably 1999) and in the late 1980s (probably 1987), respectively. The Medium and Old sites are located at 105 and 110 m a.s.l., respectively. Physical characterisation of the soil identified silty rough sand and fine sand with some clay and silt in soil layers 120–170 cm deep for the Medium site. The soil of the Old site consisted of rough sand and sand with concretions in soil layers between 260 and 270 cm deep. The measured soil characteristics (moisture, pH and NH<sub>4</sub>-N) of the two sites were similar before treatments, but NO<sub>3</sub>-N availability was significantly higher and substrate-induced



**Fig. 1.** Study area located in the Fülöpháza Sand Dunes protected area, Kiskun LTER site in Hungary. Coloured dots in the aerial photograph show the three sites that were originally part of the experiment: Old site (yellow), Medium site (red) and Young site (blue, not involved in the present analysis).

**Abb. 1.** Das Untersuchungsgebiet liegt im Schutzgebiet der Fülöpháza Sanddünen, ein Kiskun LTER Gebiet in Ungarn. Farbige Punkte im Luftbild zeigen die drei Standorte, die ursprünglich Teil des Experiments waren: Alter Standort (gelb), mittlerer Standort (rot) und junger Standort (blau, nicht analysiert).

respiration (SIR) was significantly lower in the Old site than in the Medium site (LLUMIQUINGA 2020). When the experiment started, annual weeds dominated each old field. The Medium site had a high coverage (around 50%) of perennial grasses, and some occurrences of target grassland species were registered in the Old site (HALASSY et al. 2016).

## 2.2 Experimental design

In September 2002 ploughing was applied to a 20 m by 20 m area in each site as a pre-treatment to decrease the effect of the standing vegetation. Inside the ploughed area, 64 plots of 1 m<sup>2</sup> were marked for treatments with 1 m wide paths between the plots. The methods implied eight types of treatments randomly assigned to the eight plots within each row with the eight rows as replicates (Supplement E1). The same design was applied in both sites. Main treatments aimed at the manipulation of the following filters: dispersal limitation, abiotic filter and biotic filter (HALASSY et al. 2016). The treatments were: Control (Co), Seeding (S), Mowing (M) and Carbon amendment (C) plus combinations of Seeding + Carbon amendment (SC), Seeding + Mowing (SM), Mowing + Carbon amendment (CM) and Seeding + Mowing + Carbon amendment (SMC).

Five target species of the open sand grassland community were seeded to overcome dispersal limitation: *Festuca vaginata* (1.55 g/m<sup>2</sup>), *Stipa borysthenica* (1.05 g/m<sup>2</sup>) and *Koeleria glauca* (1.00 g/m<sup>2</sup>) plus two forb species, *Dianthus serotinus* and *Euphorbia seguieriana* (0.20 g/m<sup>2</sup> together) (nomenclature according to KIRÁLY 2009). The intense drought in the summer of 2003 caused the death of the seedlings of *S. borysthenica*. For that reason, it was necessary to re-seed this species in September

2003. This time a seed density of 1.31 g/m<sup>2</sup> was applied. Mowing was applied once a year in September from 2003 until 2008 using a sickle with subsequent hay removal. In the first year, mowing was carried out also in June to control weeds. The dosage used for carbon amendment was 45 g/m<sup>2</sup> of sucrose at a time. The application was carried out every three weeks from April until October, the active period for the vegetation, in each year from 2003 to 2008. Further details are described in HALASSY et al. (2016, 2019).

### 2.3 Monitoring of treatment effects

Changes in soil nitrogen availability were assessed by in situ ion exchange resin (IER) bags following the same methodology as previously during the experiment from 2003 to 2008 (HALASSY et al. 2016). 10 g mixed-bed IER (Aberlite®, Merck, Darmstadt, Germany) was sewed into nylon mesh bags (mesh size approx. 0.2 mm) of 5 cm × 5 cm and attached to a 50 cm long coloured string (SZILI-KOVÁCS et al. 2007). We had three replicates for the main treatments (seeding, mowing, carbon amendment and control), but no measurement in treatment combinations ( $n = 12$  for each site). IER bags were buried in the centre of the plots at a depth of 5–8 cm in April and changed every 6 weeks during the vegetation period until October (four samples per year). NH<sub>4</sub>-N and NO<sub>3</sub>-N were extracted from the resin with 1 M KCl solution after drying and cleaning of the IER bags and measured by the steam distillation method (BREMNER 1965). Total resin-derived N was calculated as the sum of NH<sub>4</sub>-N and NO<sub>3</sub>-N for the four measurements of IER bags within the vegetation period.

To study the changes in the vegetation, the cover of each vascular plant species per plot was estimated visually on the percentage scale in June 4–7 and August 26–28, 2019. We calculated the maximum cover of the spring and autumn estimations for each plant species per plot similar to the previous analysis (HALASSY et al. 2016, 2019). Vegetation indicators assessed for this study were species richness, total cover of vascular plants and relative cover of target and neophyte species. The cover of all species per plot was summed to get the total vascular plant cover. Since species may grow in layers, the calculated total vascular plant cover may exceed 100%. Target species were defined according to previous studies (CSECSERITS et al. 2011, HALASSY et al. 2016) and were further divided into non-seeded and seeded species. Neophyte species were defined by BALOGH et al. (2004) and further divided into annual and perennial species. Finally, the five species seeded and major neophyte species were also assessed individually. See Table 1 for the species involved in the analysis.

### 2.4 Monitoring of site effects

In order to characterise the soil of the sites, we took five samples from the upper 0–20 cm of the soil within the experimental area, but outside the treatment plots, at the four corners and at the centre of each site in June 2019. We analysed the pH, salt content, humus content, calcium and phosphorus content, CaCO<sub>3</sub>, NH<sub>4</sub>-N and NO<sub>3</sub>-N.

In order to characterise the landscapes surrounding the sites, a buffer area of 500 m around the centre of each experimental field was set. A habitat map of the buffers was created based on a previous vegetation map for the area (CSECSERITS et al. 2011) updated according to recent aerial photos and field validation (2019). The buffer areas were classified in seven habitat categories following CSECSERITS et al. (2011): Agriculture (A), Secondary grassland (SG), tree plantations (P), semi-natural grasslands (NG), semi-natural forests (NF), wetlands (W) and settlements (S). The area of each habitat within the buffer was calculated using the software ArcGIS 10.5.

### 2.5 Data analyses

The effect of site, seeding, mowing and carbon amendment and their interaction on species richness was tested by generalised linear models (GLM) using Poisson distribution. In case of total resin-derived nitrogen, total cover and relative cover of target and neophyte species, we used two-way ANOVA. Site was included with two levels (Medium and Old site) in the models. For each main treatment type, we built separate models (indicator ~ site × seeding, indicator ~ site × mowing and indicator ~ site × carbon amendment). For cases with significant interaction, the post-hoc Tukey test was performed. If only

site had a significant impact, one-way ANOVA was carried out for the given factor. The relative cover of non-seeded target species, *S. borysthena*, *K. glauca*, *D. serotinus* and neophyte species were square-root transformed; for *E. seguieriana* we used inverse transformation to meet the assumptions of normality and homoscedasticity.

The soil data of Medium and Old site were also compared through one-way ANOVA. Data processing and testing was performed in the R version 3.6.0 (R CORE TEAM 2019).

### 3. Results

#### 3.1 Changes in resin-derived N

Total resin-derived nitrogen showed a significant ( $F = 20.21, p < 0.001$ ) site impact with the Medium site having  $59.88 \pm 13.83$  mg/l total resin-derived N compared to  $96.44 \pm 26.76$  mg/l at the Old site. No long-term impact on soil-available nitrogen was found.

#### 3.2 Changes in the vegetation

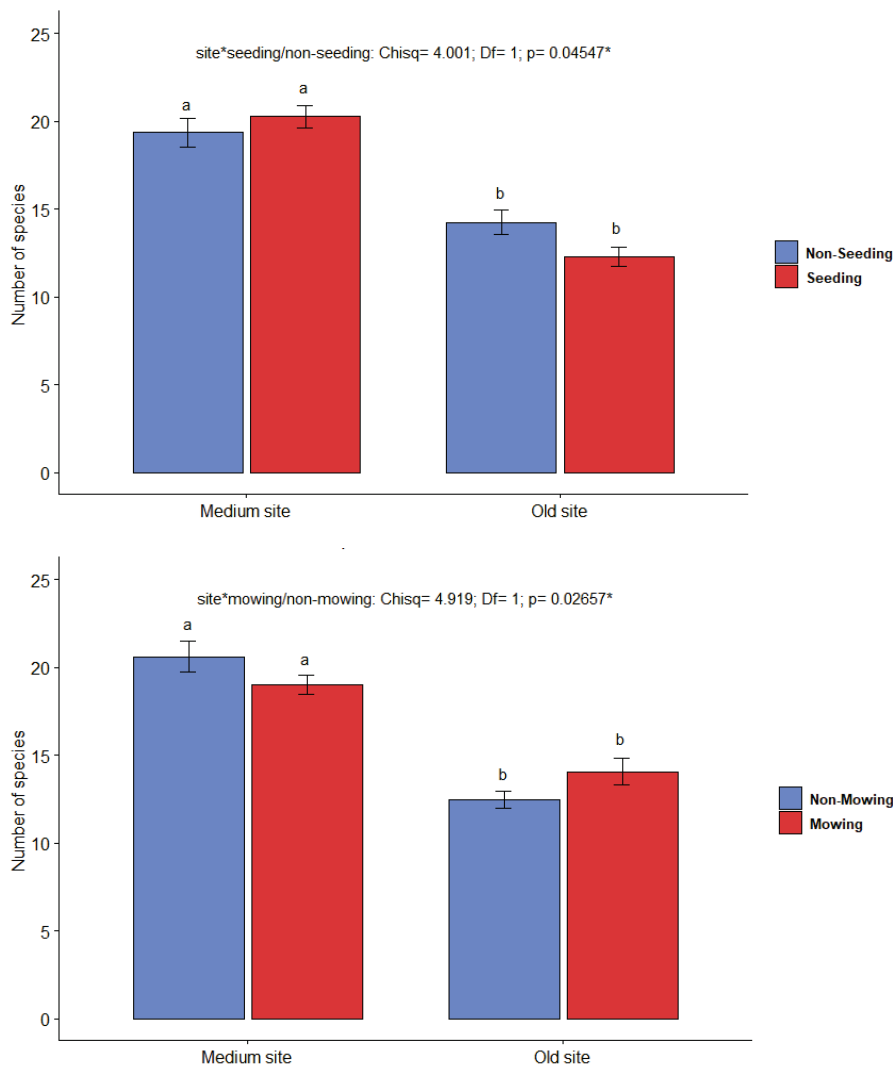
84 vascular plant species were identified in the study, 36 of which were classified as target species and six as neophyte species (Supplement E2).

Site had a strong impact on the number of species (Supplement E3). A significantly higher average number of species was found in the Medium site (20 species/m<sup>2</sup>) compared to the Old site (14 species/m<sup>2</sup>). There was also a significant impact of interaction for site\*seeding ( $Chisq = 4.0, p = 0.04547$ ) and site\*mowing ( $Chisq = 4.9, p = 0.02657$ ). According to the results of the post-hoc statistical tests (Supplement E5–6), both differed only between sites and not within treatments (Fig. 2).

**Table 1.** Mean relative cover of target species and neophyte species in the two sites in 2019. Bold p-values indicates significant differences between sites based on one-way ANOVA. Asterisks (\*) indicate a significant interaction with the treatments.

**Table 1.** Mittlere relative Deckung von Zielarten und Neophytenarten an den beiden Standorten im Jahr 2019. Fettgedruckte p-Werte zeigen signifikante Unterschiede zwischen den beiden Standorten basierend auf einer einfachen Varianzanalyse (ANOVA) an. Sternchen (\*) kennzeichnen eine signifikante Interaktion mit den Behandlungen.

|                                | Medium site | Old site | F-value  | p-value                             |
|--------------------------------|-------------|----------|----------|-------------------------------------|
| Total target species           | 85%         | 92%      | 12.830   | <b><math>p &lt; 0.001</math></b>    |
| Non-seeded species             | 15%         | 24%      | 17.210   | <b><math>p &lt; 0.001</math></b>    |
| Total seeded species           | 70%         | 68%      | (0.738)  | ( $p = 0.392$ )*                    |
| <i>Festuca vaginata</i>        | 46%         | 44%      | 0.294    | $p = 0.589$                         |
| <i>Stipa borysthena</i>        | 10%         | 17%      | (7.960)  | <b>(<math>p &lt; 0.001</math>)*</b> |
| <i>Koeleria glauca</i>         | 3.50%       | 2.70%    | (0.611)  | ( $p = 0.436$ )*                    |
| <i>Dianthus serotinus</i>      | 6.20%       | 3.10%    | 6.129    | <b><math>p &lt; 0.05</math></b>     |
| <i>Euphorbia seguieriana</i>   | 3.40%       | 0%       | 62.950   | <b><math>p &lt; 0.001</math></b>    |
| Neophytes                      | 4.50%       | 1.23%    | 23.540   | <b><math>p &lt; 0.001</math></b>    |
| Annual neophytes               | 2.01%       | 1.19%    | 14.160   | <b><math>p &lt; 0.001</math></b>    |
| <i>Ambrosia artemisiifolia</i> | 1.52 %      | 1.20%    | (8.329)  | <b>(<math>p &lt; 0.01</math>)*</b>  |
| <i>Conyza canadensis</i>       | 0.49%       | 0%       | (13.700) | <b>(<math>p &lt; 0.001</math>)*</b> |
| Perennial neophytes            | 2.53%       | 0.03%    | 84.880   | <b><math>p &lt; 0.001</math></b>    |
| <i>Oenothera biennis</i>       | 0.87%       | 0%       | 33.020   | <b><math>p &lt; 0.001</math></b>    |
| <i>Asclepias syriaca</i>       | 1.66%       | 0.03%    | 61.930   | <b><math>p &lt; 0.001</math></b>    |



**Fig. 2.** Species richness in **a)** seeding and non-seeding or **b)** mowing and non-mowing plots in the Medium and Old site in 2019. Means and SE are reported based on untransformed data. Significant differences ( $p < 0.05$ ) are indicated by lowercase letters.

**Abb. 2.** Artenreichtum in **a)** gesäten und nicht gesäten bzw. **b)** gemähten und ungemähten Parzellen am mittleren und am alten Standort im Jahr 2019. Mittelwerte und Standardfehler (SE) basieren auf untransformierten Daten. Signifikante Unterschiede ( $p < 0,05$ ) sind durch Kleinbuchstaben über den Balken gekennzeichnet.

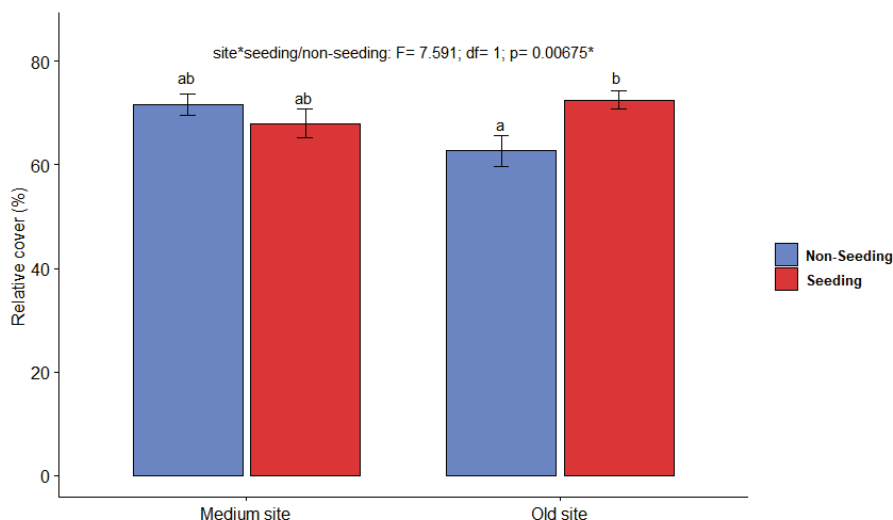
Total vascular plant cover was significantly ( $F = 20.4$ ,  $df = 1$ ,  $p = 1.49e-05$ ) higher in the Medium site (112%) than in the Old site (96%) and was also significantly ( $F = 4.0$ ,  $df = 1$ ,  $p = 0.0479$ ) higher in mowing plots (108%) compared to non-mowing plots (101%).

Statistical models showed a significant impact of the site on the relative cover of target species, non-seeded target species and all seeded species, except for *Festuca vaginata*, which dominated all sites and treatments (Supplement E3). There was a higher relative cover of

target species, non-seeded target species and *Stipa borysthenica* and a lower relative cover of *Dianthus serotinus* and *Euphorbia segueriana* in the Old site compared to the Medium site (Table 1).

From the three treatments, only seeding had a significant impact on the cover of two seeded species: *S. borysthenica* had a significantly higher cover in seeding plots (18.5%) than in non-seeding plots (8.5%), whereas, by contrast, *Koeleria glauca* showed a significantly higher cover in non-seeding plots (3%) compared to seeding plots (2%). Seeding also had a significant impact on the relative cover of seeded species in interaction with site (Supplement E3). The results of the multiple comparison test (Supplement E7) indicated a significant difference between seeding and non-seeding treatment only in the Old site. The highest cover of seeded species (73%) was found in the seeding treatments and the lowest cover (62.5%) in the non-seeding treatments in the Old site. Medium site values for seeding and non-seeding treatments were between the values of the Old site (Fig. 3).

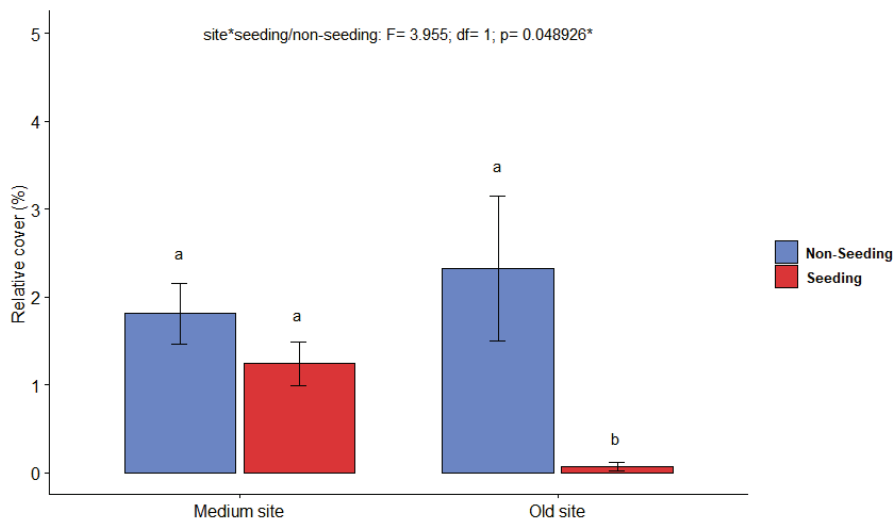
Site had a significant impact on neophyte species for all studied indicators (Supplement E4). The Medium site was more heavily affected by neophytes than the Old site (Table 1). Among the treatments only seeding had an effect on the relative cover of neophyte species, in particular the annual neophyte *Ambrosia artemisiifolia*. The relative cover of neophyte species was significantly ( $F = 5.3, p = 0.0227$ ) lower in case of seeding (1.90%) than without seeding (3.87%), primarily due to the significantly lower ( $F = 10.9, p = 0.00128$ ) relative cover of annual neophytes (0.93% and 2.28% for seeding and non-seeding



**Fig. 3.** Relative cover of seeded species in seeding and non-seeding plots in the Medium and Old site in 2019. Means and SE are reported based on untransformed data. Significant differences ( $p < 0.05$ ) are indicated by lowercase letters.

**Abb. 3.** Relative Deckung der gesäten Arten in gesäten und ungesäten Untersuchungsflächen am mittleren und am alten Standort im Jahr 2019. Mittelwerte und Standardfehler (SE) basieren auf untransformierten Daten. Signifikante Unterschiede ( $p < 0,05$ ) sind durch Kleinbuchstaben über den Balken gekennzeichnet.





**Fig. 4.** Relative cover of *Ambrosia artemisiifolia* in seeding and non-seeding plots in the Medium and Old site in 2019. Means and SE are reported based on untransformed data. Significant differences ( $p < 0.05$ ) are indicated by lowercase letters.

**Abb. 4.** Relative Deckung von *Ambrosia artemisiifolia* in gesäten und ungesäten Untersuchungsflächen auf dem mittleren und alten Standort im Jahr 2019. Mittelwerte und Standardfehler (SE) basieren auf untransformierten Daten. Signifikante Unterschiede ( $p < 0,05$ ) sind durch Kleinbuchstaben über den Balken gekennzeichnet.

plots, respectively). here was a significant site\*seeding interaction for *A. artemisiifolia* (Supplement E4), which had a significantly lower relative cover in seeding plots (0.07%) compared to non-seeding plots (2.32%) in the Old site (Fig. 4)

### 3.3 Soil characteristics of sites

Based on the results of the laboratory analysis of 2019 soil samplings, soil pH was basic in both sites with a slight, but significant difference (Table 2). We can relate this difference to the amount of  $\text{CaCO}_3$ , which was significantly higher in the Old site. At the same time, the humus content was significantly higher in the Medium site. Nitrogen availability was similar in the two sites: The sum of  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  was 15.65 mg/kg in the Medium site and 16.33 mg/kg in the Old site.

### 3.4 Landscape characteristics of sites

The major differences between the two sites were the active agricultural practices still going on nearby the Medium site and the limited share of semi-natural grasslands and forests compared to the Old site (Table 3). Plantations, secondary grasslands and settlements including mostly small farms and roads were of similar extent.

**Table 2.** Results of the soil composition analysis based on the soil sampling in 2019. The reported values for each site are the average of 5 samples collected from 0–20 cm depth. Bold p values indicate significant differences between sites based on one-way ANOVA.

**Tabelle 2.** Bodenzusammensetzung basierend auf der Analyse der Bodenproben aus dem Jahr 2019. Die angegebenen Werte für jeden Standort sind der Durchschnitt von 5 Proben, die aus 0–20 cm Tiefe entnommen wurden. Fettgedruckte p-Werte kennzeichnen einen signifikanten Unterschied zwischen den beiden Standorten basierend auf einer einfachen Varianzanalyse (ANOVA).

|               | pH<br>(H <sub>2</sub> O)                                     | Humus<br>[%]   | CaCO <sub>3</sub><br>[%]                                     | AL - P <sub>2</sub> O <sub>5</sub><br>[mg/kg]         | NH <sub>4</sub> -N<br>[mg/kg]                        | NO <sub>3</sub> -N<br>[mg/kg]                        |
|---------------|--|--|--|---|--|--|
| Medium site   | 7.82   | 0.918  | 6.51   | 68.720  | 12.78  | 2.87   |
| Old site      | 7.95   | 0.742  | 8.61   | 77.254  | 13.71  | 2.62   |
| ANOVA results | <i>F</i> = 11.76<br><i>df</i> = 1<br><b><i>p</i> = 0.002</b> | <i>F</i> = 4.88<br><i>df</i> = 1<br><i>p</i> = 0.404 | <i>F</i> = 14.58<br><i>df</i> = 1<br><b><i>p</i> = 0.001</b> | <i>F</i> = 0.189<br><i>df</i> = 1<br><i>p</i> = 0.699 | <i>F</i> = 2.54<br><i>df</i> = 1<br><i>p</i> = 0.129 | <i>F</i> = 1.13<br><i>df</i> = 1<br><i>p</i> = 0.302 |

**Table 3.** Landscape composition in the surroundings (500 m radius) of the sites. NF – natural forests, NG – natural grasslands, SG – secondary grasslands, W – wetlands, A – agriculture, P – tree plantations, S – settlements based on 2019 aerial photos.

**Tabelle 3.** Landschaftszusammensetzung in der Umgebung (500 m Radius) der beiden Standorte. NF – natürliche Wälder, NG – natürliches Grasland, SG – sekundäres Grasland, W – Feuchtgebiete, A – Landwirtschaft, P – Baumplantagen, S – Siedlungen basierend auf Luftbildern von 2019.

|             | Habitat area (%) |      |       |      |       |       |      |
|-------------|------------------|------|-------|------|-------|-------|------|
|             | NF               | NG   | SG    | W    | A     | P     | S    |
| Medium site | 1.50             | 2.06 | 46.38 | 0    | 13.10 | 34.60 | 2.36 |
| Old site    | 10.78            | 9.41 | 43.10 | 1.50 | 0     | 30.37 | 4.84 |

## 4. Discussion

### 4.1 Long-term impact of initial seeding, mowing and carbon amendment

From the three treatments applied, initial seeding had the strongest impact in the long term: Its effect was still visible in the relative cover of seeded species 16 years after the first applications, mostly due to *Stipa borysthena*, and in the relative cover of neophyte species, mainly the annual *Ambrosia artemisiifolia*. These results correspond to earlier findings; however, some differences between seeded and non-seeded plots have faded with time, such as the increase in species richness, vascular plant cover and target species in the shorter term (HALASSY et al. 2016, 2019). In the long term, the seeded species spread from seeded plots to adjacent areas, resulting in the dominance of target species in both seeded and non-seeded plots in the two sites. The dominance order of species has also changed with time. E.g., *Dianthus serotinus* was the dominant species at the end of the experiment in 2008, its cover reaching up to 69% in some plots that received the combination of seeding and mowing as treatment (HALASSY et al. 2016). By 2019 the dominant grasses became abundant (*Festuca vaginata* 45% on average and *S. borysthena* 13.5% on average), which corresponds to the competition dynamics that forbs and grasses generally develop (SCOTT 2020). It is important to note that early seeding played an important role in preventing the spread of neophyte

species even in the long term. This was mainly due to the suppression of *A. artemisiifolia*, an invasive species causing serious health problems in Europe due to its highly allergenic pollen, whose presence is primarily connected to soil disturbance (KRÖEL-DULAY et al. 2019).

Mowing, which was the second most important treatment in the shorter term (HALASSY et al. 2016, 2019), had only a minor impact in the long term. Many authors (such as DICKSON & BUSBY 2009, MCCAIN et al. 2010, TESEI et al. 2019) note that when grasses exert dominance in a site, a lower diversity of species is observed. Mowing is often used to control dominant species and to open up the sward in order to increase diversity (e.g. MCCAIN et al. 2010). In our case lower cover was obtained in the short term as a result of mowing (HALASSY et al. 2016), but in the long term, mowing had the opposite impact, slightly increasing total cover, which is probably owing to the building-up of grass cover after the cessation of mowing. The expected increase in species richness due to mowing was not confirmed in the longer term, partly due to the opposite trends found for the two sites. This implies that long-term non-frequent mowing can be suggested to maintain species richness (KELEMEN et al. 2014).

An alternative to mowing could be grazing, which is often considered a better management for nature conservation goals (TÄLLE et al. 2016). Sand grasslands in the study region were traditionally grazed by sheep at a low intensity and seldom mown given the low amount of biomass (CSECSERITS et al. 2011). Sheep grazing can support the recovery of native vegetation by facilitating long-range dispersal of target species and by increasing establishment opportunities in microsites created e.g. by trampling (LABADESSA et al. 2020). However, establishment gaps can favour weedy and invasive species, and that can compromise the beneficial impact of either grazing or mowing if invasive pressure is high in the surrounding landscape (REIS et al. 2021).

Carbon amendment reduced mineral-N in the soil and resulted in a decrease in the cover of mosses and an increase of bare ground in the short term (HALASSY et al. 2016). The early differences in soil nitrogen availability were only temporary, as expected (PERRY et al. 2010, HALASSY et al. 2021), and mostly disappeared by 2019 so that no long-term impact of carbon amendment was visible in the vegetation. According to the literature, carbon addition has contradictory results (PERRY et al. 2010), and had no or minor impact on the vegetation in low productive grasslands in Hungary (TÖRÖK et al. 2014, HALASSY et al. 2021) and elsewhere in Europe (STORM & SUSS 2008) if applied to already established vegetation.

The spread of seeded species all over the sites explains the lack of differences between treatments in the long term. An important limitation of long-term studies is that the spatial arrangement of the plots usually allows for an uncontrolled seed exchange between treatments over time (PAKEMAN et al. 2002). It is difficult to avoid this phenomenon, especially in smaller-scale experiments, where it can become visible even after a short time (EICHBERG et al. 2010). Even larger plots (10 m × 10 m) could not prevent the spread of species from adjacent plots after eight years in the study of LEPŠ et al. (2007). This suggests that larger plots sizes and wider guard rows as recommended by PAKEMAN et al. (2002) might still not be sufficient to prevent seeding artifacts in the long term (10+ years), but it might extend the lifespan of such experiments by some extra years. On the other hand, if treatments are carried out on separate old fields, the site effects cannot be separated from the treatment effects since each old field can have a different vegetation development according to its intrinsic characteristics (CRAMER et al. 2008). We suggest to monitor the spread of species from seeded plots, and once they establish in other treatments, care should be taken when

interpreting the results. When the treatments become similar due to seeded species, the site should be regarded as the unit of long-term monitoring, and the treatments are not to be considered separately.

#### 4.2 Site-specific impacts

Our results confirmed site-specific vegetation development. Most studied vegetation indicators showed a significant difference between the two sites. The Medium site showed a higher number of species and a higher total vascular plant cover, but despite the high relative cover of seeded species a lower relative cover of target species due to the presence of neophytes. We found a better spontaneous establishment of target species in the Old site, where the total cover and the relative cover of seeded species were lower. These results are probably related to the age of the old fields, which was the most important determinant of species composition in local studies (CSECSERITS et al. 2011). The Old site was abandoned 16 years and the Medium site 4 years prior treatments, and spontaneous recovery was better in the older old field. However, this impact could not be separated from the landscape impact.

PRACH et al. (2012) and LINDBORG & ERIKSSON (2004) expressed that the species composition of a site may be fully influenced by remnant populations or ancient grasslands in surrounding areas. In our case the surroundings of the Old site contain a higher cover of remnant semi-natural grasslands, which can be an excellent source for the propagules of target species, whereas in case of the Medium site, the presence of agricultural fields poses a threat of spreading neophyte species (CSECSERITS et al. 2016). Although the habitat maps were not created at the time of restoration, land-use changes since 2003 were minor. The most important changes in the landscape are continuing land abandonment and chemical control of alien invasive species, such as *Asclepias syriaca* and *Robinia pseudoacacia* (CSISZÁR & KORDA 2015, BAKACSY & BAGI 2020).

On the other hand, differences between the two sites can also be attributed to abiotic factors, such as soil characteristics, which in turn would influence the vegetation recovery. According to the results of the soil analysis, the Medium site contained a higher amount of humus in the upper soil layer and a higher amount of clay and silt in the deeper soil layers. Both features increase the water-holding capacity of the soil (ANDRADES & MARTÍNEZ 2014, STEVENSON 2014) and show that the Medium site can be better suited for closed sand grassland, whereas the Old site is more suitable for open sand grassland. Open sand grassland species have a better colonisation ability than the specialist species of closed sand grasslands (MOLNÁR & BOTTA-DUKÁT 1998, ŠEFFEROVÁ et al. 2008). Moreover, as groundwater levels fall in the region, closed steppes retreat from drier areas (MOLNÁR et al. 2011).

#### 4.3 Implications for restoration

The treatments applied in this study allowed the development and permanence of grassland species in the long term although the sites differed from each other due to their particular characteristics. Dispersal limitation was successfully overcome since the seeded species were able to establish and colonise adjacent areas. Although the spread of species from the 1-m<sup>2</sup> plots that were seeded to other treatment plots (control, mowing and carbon amendment) compromised our long-term experimental results regarding the different treatments, it did support the restoration process. It is good news for nature conservation since there is no

need to introduce seeds throughout the site when restoring sandy grassland on old fields; rather, it is enough to create smaller establishment windows or stripes from where the species can colonise the whole site (VALKÓ et al. 2016b).

Generally, the successful establishment of introduced species and the diversification of the grassland requires post-sowing management. The study of BARTHA et al. (2003) indicated that the decrease of colonising species after some years of succession coincided with the development of a continuous canopy of perennial species. KELEMEN et al. (2014) found a negative effect of sown grasses on vegetation development in grassland restoration. These authors also found that disturbance resulting from drought events or management decreased the cover of the canopy and created so-called “colonisation windows” where new species could establish. The results of the present study indicate that both mowing and carbon amendment can create colonisation windows, which can enhance vegetation recovery in the long term.

Based on our results we suggest that the tested methods can be used to assist the restoration of sand grasslands on abandoned fields. Namely, low-rate seeding of a low-diversity seed mixture, which includes both dominant grasses and subordinate species in “establishment windows” after ploughing followed by some initial management (preferably grazing or mowing) to control dominant species.

Habitat loss and fragmentation is one of the main drivers of the biodiversity crisis. We should consider cropland abandonment as an opportunity for passive or active restoration, which can create new semi-natural habitats and reduce fragmentation for native species and increase related ecosystem services for the benefit of both humans and nature.

## Erweiterte deutsche Zusammenfassung

**Einleitung** – In Mittel- und Osteuropa brachen die staatlichen landwirtschaftlichen Genossenschaften mit dem Zusammenbruch der sozialistischen Regime in den frühen 1990er Jahren zusammen (MIHÓK et al. 2017), was zur Vermehrten Aufgabe von Ackerland führte (VALKÓ et al. 2016a). Prognosen legen zudem nahe, dass zwischen 2015 und 2030 weitere 11 % der landwirtschaftlichen Fläche in der EU brach fallen könnten (PERPIÑA et al. 2018). Die Aufgabe von Ackerland führt zur Entwicklung einer neuen Vegetationsdecke durch natürliche Sukzession und spontane Wiederbesiedlung. Eine solche spontane Vegetationsentwicklung kann neuen Lebensraum für einheimische Arten schaffen (SOJNEKOVÁ & CHYTRÝ 2015) und die Fragmentierung der Landschaft verringern, teilweise aber auch die Präsenz invasiver gebietsfremder Arten fördern (CSECSERITS et al. 2011). Alte Felder, bei denen nach Nutzungsaufgabe eine spontane Wiederbesiedelung stattgefunden hat, unterscheiden sich in der Regel noch lange von primären Grünlandflächen (ISBELL et al. 2019). Die Entwicklung der Brachflächen wird von der historischen Nutzung, der Vegetation, dem Boden, sowie anderen abiotischen und landschaftlichen Merkmalen beeinflusst (CRAMER et al. 2008). Der Erfolg der spontanen Wiederbesiedelung wird zudem durch bestimmte Faktoren begrenzt, die mit der Ausbreitungsfähigkeit der Arten und ihrer Fähigkeit zusammenhängen, sich unter den gegebenen Umweltbedingungen zu etablieren und zu überleben (HOBBS & CRAMER 2007). Bei der Sukzession ehemaliger Äcker ist beispielsweise ein Überschuss an Nährstoffen ein kritischer Faktor, da nicht nur die Überlebensfähigkeit einheimischer Arten beeinträchtigt, sondern auch das Wachstum und die Ausbreitung invasiver Arten gefördert werden kann (BAKKER & BERENDSE 1999). Auch die Ausbreitungslimitierung von Arten spielt eine wichtige Rolle (TÖRÖK et al. 2018). Da die meisten Grünlandarten in Europa keine dauerhafte Samenbank bilden, enthalten Ackerflächen stattdessen große Mengen an Samen von Unkrautarten (BAKKER & BERENDSE 1999). Zudem ist eine räumliche Ausbreitung von Zielarten nur dort möglich, wo noch Reste der natürlichen Vegetation in der Landschaft vorhanden sind (TÖRÖK et al. 2018, HALASSY et al. 2019). Zur aktiven Wiederherstellung der Biodiversität werden brach liegende Äcker deshalb häufig mit bestimmten Zielarten eingesät. Das Ziel dieser Studie war es, Langzeiteffekte von Einsaat, Mahd

und Kohlenstoffzusatz zur Renaturierung von pannonischem Sandgrasland auf ehemaligen Äckern, 16 Jahre nach den ersten Anwendungen zu bewerten. Wir haben folgende Fragen untersucht: (1) Wie wirken sich Einsaat, Mahd und Kohlenstoffanreicherung langfristig auf die Stickstoffverfügbarkeit im Boden, den Artenreichtum, die Gesamtdeckung von Gefäßpflanzen und die relative Deckung von Ziel- und Neophytenarten aus? (2) Welchen Einfluss spielen dabei Standorteigenschaften?

**Methoden** – Das Untersuchungsgebiet befindet sich im Kiskun LTER Gebiet, dem Fülöpháza Sanddünen Schutzgebiet (N 46.890° E 19.440°) im Kiskunság Nationalpark in Ungarn (Abb. 1). Während der letzten Jahrhunderte hat ein starker menschlicher Einfluss (Land- und Forstwirtschaft) zur weitgehenden Zerstörung der pannonischen Sandsteppe geführt (BIRÓ et al. 2013). Seit 1980 ist auch die Aufgabe der Landnutzung weit verbreitet, insbesondere in Gebieten mit geringer Produktivität (CSECSERITS et al. 2011). Unser Renaturierungsexperiment wurde an zwei Standorten durchgeführt, die sich im Aufgabedatum, den Bodenbedingungen und Landschaftsmerkmalen unterschieden. Die Nutzung wurde im als „mittlerer Standort“ bezeichneten Gebiet in den späten 1990er Jahren (wahrscheinlich 1999) aufgegeben und im als „alter Standort“ bezeichneten Gebiet in den späten 1980er Jahren (wahrscheinlich 1987).

Im September 2002 wurde eine 20 m × 20 m große Fläche an jedem Standort als Vorbehandlung gepflügt. Innerhalb der gepflügten Fläche wurden je 64 Untersuchungsflächen mit einer Größe von 1 m<sup>2</sup> für Behandlungen etabliert. Acht unterschiedliche Behandlungsmethoden (Kontrolle, Einsaat (2002/2003), Mahd (2003–2008) und Kohlenstoffzusatz (2003–2008), sowie die Kombinationen aus Einsaat + Kohlenstoffzusatz, Einsaat + Mahd, Mahd + Kohlenstoffzusatz, Einsaat + Mahd + Kohlenstoffzusatz) wurden den acht Parzellen innerhalb jeder Reihe zufällig zugeordnet, wobei die acht Reihen als Wiederholungen dienten (Anhang E1). Eingesät wurden fünf Zielarten des pannonischem Sandgraslandes: Die drei Grasarten *Festuca vaginata*, *Stipa borysthénica*, *Koeleria glauca* sowie die beiden Krautarten *Dianthus serotinus* und *Euphorbia seguérana* (Nomenklatur nach KIRÁLY 2009).

Um die Veränderungen in der Vegetation zu untersuchen, wurde die prozentuale Deckung jeder Gefäßpflanzenart pro Untersuchungsfläche im Juni und im August 2019 geschätzt. Außerdem wurde die Stickstoffverfügbarkeit (mittels Ionenaustauschharz-Beutel) und weitere Bodenuntersuchungen durchgeführt, sowie verschiedene Landschaftsvariablen 500 m um die Zentren der beiden Standorte herum quantifiziert. Details sind in HALASSY et al. (2016) beschrieben.

Wir verglichen die Auswirkungen von Einsaat, Mahd, Kohlenstoffzusatz und Standort auf den verfügbaren Stickstoff im Boden, den Artenreichtum, die Gesamtdeckung der Gefäßpflanzen und die relative Deckung von Ziel- und Neophytenarten mit Hilfe von generalisierten linearen Modellen (GLM) sowie einfachen und Zweiweg-Varianzanalysen (ANOVA). Wir analysierten außerdem welche Effekte standortbezogene Faktoren wie Bodeneigenschaften und Landschaftskontext dabei spielen.

**Ergebnisse** – Wir fanden 84 Gefäßpflanzenarten, von denen 36 als Zielarten und sechs als Neophytenarten eingestuft wurden (Anhang E2). Die durchschnittliche Artenzahl unterschied sich signifikant zwischen den beiden Standorten (Anhang E3; 20 Arten/m<sup>2</sup> am mittleren und 14 Arten/m<sup>2</sup> am alten Standort). Wir fanden einen Langzeiteffekt der Mahd auf die Gesamtdeckung von Gefäßpflanzen (Anhang E3). Die Einsaat beeinflusste die relative Deckung der Zielarten, hauptsächlich von *Stipa borysthénica* (18,5 % in eingesäten und 8,5 % in nicht-eingesäten Untersuchungsflächen), und auf die relative Deckung von Neophytenarten, insbesondere des einjährigen Neophyten *Ambrosia artemisiifolia* (0,07 % in eingesäten und 2,32 % in nicht-eingesäten Untersuchungsflächen; Fig. 4, Anhang E4). Die eingesäten Zielarten etablierten sich und hielten sich langfristig. Sie breiteten sich außerdem in den nicht-eingesäten Untersuchungsflächen aus. Es wurde kein langfristiger Einfluss der Behandlungen auf den im Boden verfügbaren Stickstoff festgestellt.

**Diskussion** – Von den angewendeten Behandlungen hatte die Einsaat den größten Langzeiteffekt, der noch 16 Jahre nach der ersten Anwendung durch eine höhere relative Deckung der angesäten Arten sowie eine niedrigeren relativen Deckung von Neophytenarten sichtbar war. Besonders *Ambrosia artemisiifolia* konnte durch die Einsaat unterdrückt werden, eine invasive Art, die aufgrund ihrer stark allergenen Pollen in Europa ernsthafte Gesundheitsprobleme verursacht und deren Vorkommen vor

allem mit Bodenstörungen verbunden ist (KRÖEL-DULAY et al. 2019). Die eingesäten Arten breiteten sich zudem von den eingesäten Untersuchungsflächen auf die angrenzenden Flächen aus, was zu einer Dominanz der Zielarten an den beiden Standorten sowohl in den eingesäten als auch in den nicht-eingesäten Flächen führte. Die Mahd war der zweitwichtigste Faktor auf kürzere Sicht (HALASSY et al. 2016, 2019). Die erwartete Zunahme des Artenreichtums durch Mahd konnte aber auf längere Sicht nicht bestätigt werden. Die Kohlenstoffanreicherung reduzierte den Stickstoffgehalt des Bodens, führte kurzfristig zu einer Abnahme der Moosdeckung und einer Zunahme des offenen Bodens (HALASSY et al. 2016). Sie hatte aber keinen langfristigen Einfluss auf die Vegetation.

Wir fanden Unterschiede der Vegetationsentwicklung zwischen den beiden untersuchten Standorten mit einer besseren spontanen Etablierung der Zielarten am alten Standort, wo die Gesamtdeckung und die relative Deckung der eingesäten Arten geringer war. Die Zeit seit Nutzungsaufgabe scheint also eine entscheidende Rolle auf die Artenzusammensetzung der Vegetation auf ehemaligen Ackerflächen zu spielen (CSECSERITS et al. 2011). Unsere Ergebnisse legen zudem nahe, dass ein hoher Anteil an naturnahem Grünland in der Umgebung eines Standorts zur Vermehrung von Zielarten und somit zu deren Ausbreitung beitragen kann.

Unsere Ergebnisse zeigen, dass nach dem Pflügen einer Fläche die Einsaat einer Saatgutmischung mit geringer Diversität, welche sowohl dominante Gräser als auch weniger dominante Kräuter enthält, gefolgt von einer anfänglichen Bewirtschaftung (vorzugsweise Beweidung oder Mahd) zur Renaturierung von pannonischem Sandgrasland auf ehemaligen Äckern beitragen und gleichzeitig dominante Arten und Neophyten unterdrücken kann.

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## Author contributions

KT and MH conceived and designed the study; all authors participated in field work; KSZ advised on statistical analysis; YBL, BPR and SN performed statistical analyses for target species, landscape and neophytes, respectively; YBL and MH prepared the first version of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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## Supplements

**Additional supporting information may be found in the online version of this article.**

**Zusätzliche unterstützende Information ist in der Online-Version dieses Artikels zu finden.**

**Supplement E1.** Experimental design.

**Anhang E1.** Experimenteller Aufbau.

**Supplement E2.** List of species found in the study sites with classification as target or neophyte species.

**Anhang E2.** Liste der in den beiden Untersuchungsgebieten gefundenen Arten. Ziel- oder Neophytenarten.

**Supplement E3.** Results of statistical models for species richness, vascular plant cover and relative cover of target species.

**Anhang E3.** Ergebnisse der statistischen Modelle für den Artenreichtum, die Gefäßpflanzenbedeckung und die relative Deckung der Zielarten.

**Supplement E4.** Results of two-way ANOVA for relative cover of neophyte species.

**Anhang E4.** Ergebnisse der Zweifweg-Varianzanalyse (ANOVA) für die relative Deckung der Neophytenarten.

**Supplement E5.** Results of the post-hoc Tukey test for site\*seeding interaction in relation to species richness.

**Anhang E5.** Ergebnisse des Post-Hoc-Tests (Tukey) für die Standort\*Einsaat-Interaktion in Bezug auf den Artenreichtum.

**Supplement E6.** Results of the post-hoc Tukey test for site\*mowing interaction in relation to species richness.

**Anhang E6.** Ergebnisse des Post-Hoc-Tests (Tukey) für Standort\*Mahd-Interaktion in Bezug auf den Artenreichtum.

**Supplement E7.** Results of the post-hoc Tukey test for site\*seeding interaction in relation to relative cover of seeded species.

**Anhang E7.** Ergebnisse des Post-Hoc-Tests (Tukey) für die Interaktion von Standort\*Einsaat in Bezug auf die relative Deckung der angesäten Arten.

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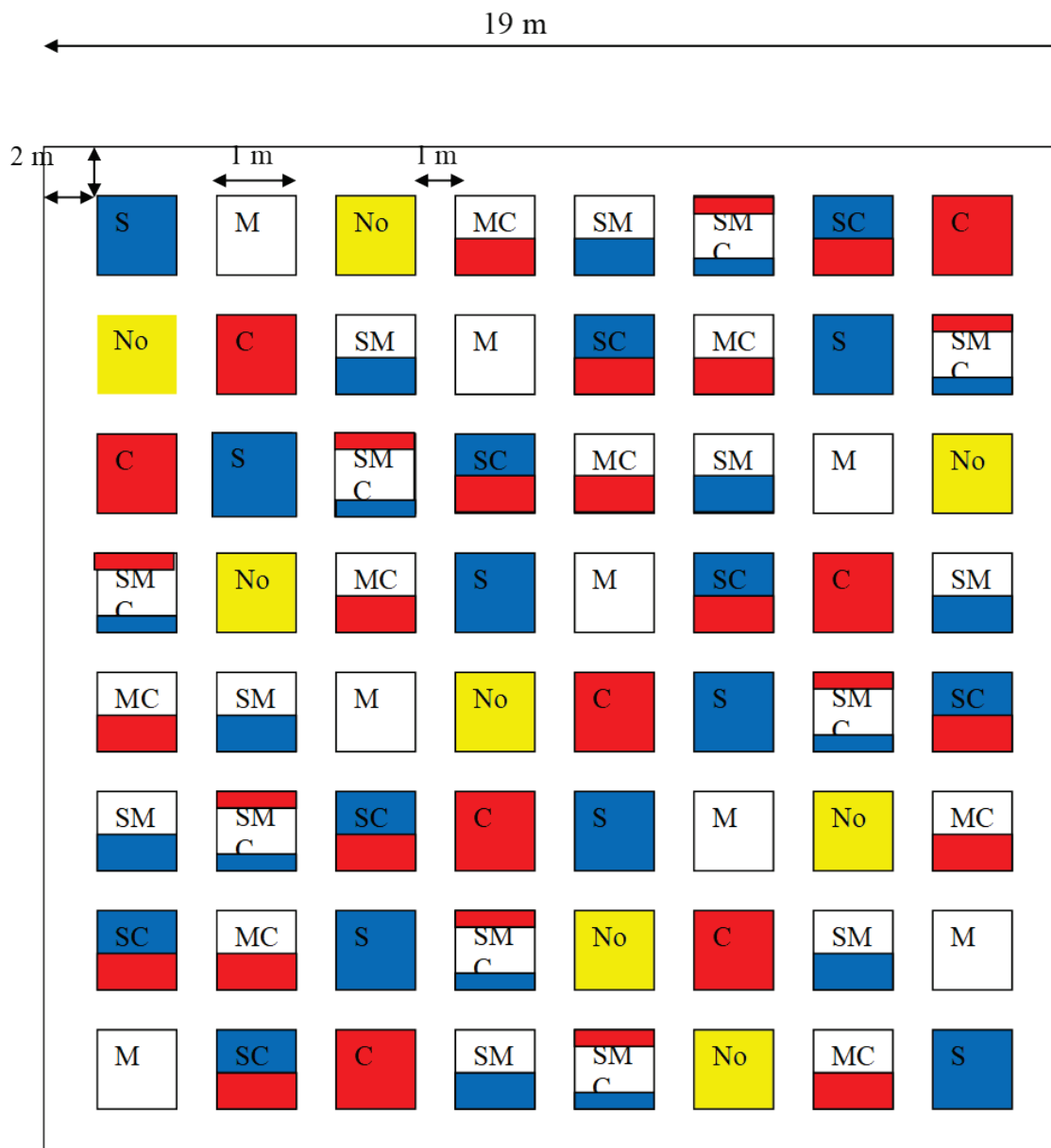
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**Supplement E1.** Experimental design. Abbreviations: Co – control plots, C – carbon amendment, S – Seeding . M – Mowing, SC – seeding + carbon, MC – carbon + mowing, SM – seeding + mowing, SMC – seeding + mowing + carbon. The assigned colours differentiate each treatment: yellow – control, red – carbon amendment, blue – seeding, white – mowing.

**Anhang E1.** Experimenteller Aufbau. Abkürzungen: Co – Kontrollparzellen, C – Kohlenstoffzusatz, S – Einsaat . M – Mähen, SC – Einsaat + Kohlenstoff, MC – Kohlenstoff + Mähen, SM – Einsaat + Mähen, SMC – Einsaat + Mähen + Kohlenstoff. Die zugeordneten Farben unterscheiden die einzelnen Behandlungen: gelb – Kontrolle, rot – Kohlenstoffzusatz, blau – Einsaat, weiß – Mahd.



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**Supplement E2.** List of species found in the study sites with classification as target (T) or neophyte (N) species and mean cover (%) for the two experimental sites. Seeded species are highlighted in bold letters.

**Anhang E2.** Liste der in den beiden Untersuchungsgebieten gefundenen Arten. Ziel- (T) oder Neophytenarten (N) sind gekennzeichnet und die mittlere Deckung (%) für alle Arten ist getrennt für die beiden Standorte angegeben. Eingesäte Arten sind fette Buchstaben hervorgehoben.

|           | Species                             | Classification | Mean cover / % |              |
|-----------|-------------------------------------|----------------|----------------|--------------|
|           |                                     |                | Medium site    | Old site     |
| 1         | <i>Achillea collina</i>             | T              | 1.56           | 8.50         |
| 2         | <i>Alyssum alyssoides</i>           | T              | 0.11           | 0.04         |
| 3         | <i>Ambrosia artemisiifolia</i>      | N              | 1.72           | 1.24         |
| 4         | <i>Anchusa officinalis</i>          |                | 0.01           | 0.53         |
| 5         | <i>Anthemis ruthenica</i>           |                | 0.01           | 0.01         |
| 6         | <i>Apera spica-venti</i>            |                | 0.24           | 0.01         |
| 7         | <i>Arenaria serpyllifolia</i>       |                | 0.64           | 0.38         |
| 8         | <i>Artemisia campestris</i>         | T              | 0.00           | 6.17         |
| 9         | <i>Asclepias syriaca</i>            | N              | 2.61           | 0.26         |
| 10        | <i>Bothriochloa ischaemum</i>       | T              | 2.00           | 0.00         |
| 11        | <i>Bromus squarrosus</i>            | T              | 0.09           | 0.00         |
| 12        | <i>Bromus tectorum</i>              |                | 2.12           | 0.83         |
| 13        | <i>Buglossoides arvensis</i>        |                | 0.00           | 0.01         |
| 14        | <i>Centaurea arenaria</i>           | T              | 2.10           | 4.08         |
| 15        | <i>Cerastium semidecandrum</i>      |                | 0.26           | 0.08         |
| 16        | <i>Chenopodium album</i>            |                | 0.01           | 0.51         |
| 17        | <i>Chondrilla juncea</i>            | T              | 1.95           | 2.24         |
| 18        | <i>Consolida regalis</i>            |                | 0.01           | 2.00         |
| 19        | <i>Convolvulus arvensis</i>         |                | 1.26           | 1.77         |
| 20        | <i>Conyza canadensis</i>            | N              | 1.16           | 0.01         |
| 21        | <i>Corispermum nitidum</i>          | T              | 3.00           | 0.00         |
| 22        | <i>Crataegus monogyna</i>           |                | 4.00           | 0.00         |
| 23        | <i>Crepis rhoedifolia</i>           | T              | 1.82           | 1.03         |
| 24        | <i>Cynodon dactylon</i>             |                | 0.00           | 2.34         |
| 25        | <i>Daucus carota</i>                |                | 0.26           | 0.00         |
| 26        | <i>Descurainia sophia</i>           |                | 0.30           | 0.00         |
| <b>27</b> | <b><i>Dianthus serotinus</i></b>    | <b>T</b>       | <b>7.86</b>    | <b>12.44</b> |
| 28        | <i>Echium vulgare</i>               |                | 0.63           | 1.00         |
| 29        | <i>Elymus repens</i>                |                | 0.31           | 1.02         |
| 30        | <i>Equisetum ramosissimum</i>       | T              | 2.10           | 1.18         |
| 31        | <i>Eragrostis pilosa</i>            |                | 0.00           | 2.00         |
| 32        | <i>Eryngium campestre</i>           | T              | 2.30           | 0.00         |
| 33        | <i>Euphorbia cyparissias</i>        |                | 0.00           | 1.00         |
| <b>34</b> | <b><i>Euphorbia seguieriana</i></b> | <b>T</b>       | <b>5.09</b>    | <b>3.22</b>  |
| 35        | <i>Fallopia convolvulus</i>         |                | 1.03           | 0.26         |
| <b>36</b> | <b><i>Festuca vaginata</i></b>      | <b>T</b>       | <b>50.81</b>   | <b>43.49</b> |
| 37        | <i>Festuca x wagneri</i>            | T              | 1.01           | 0.00         |
| 38        | <i>Galium verum</i>                 | T              | 1.00           | 0.00         |
| 39        | <i>Gleditsia triacanthos</i>        | N              | 0.00           | 0.01         |
| 40        | <i>Gypsophila paniculata</i>        | T              | 5.00           | 3.17         |
| 41        | <i>Kochia laniflora</i>             | T              | 0.01           | 1.36         |
| <b>42</b> | <b><i>Koeleria glauca</i></b>       | <b>T</b>       | <b>4.08</b>    | <b>6.00</b>  |
| 43        | <i>Lathyrus tuberosus</i>           |                | 3.00           | 0.00         |
| 44        | <i>Leontodon hispidus</i>           |                | 1.22           | 0.00         |
| 45        | <i>Linaria genistifolia</i>         | T              | 1.66           | 7.70         |
| 46        | <i>Medicago falcata</i>             | T              | 0.17           | 0.00         |
| 47        | <i>Medicago minima</i>              | T              | 1.84           | 3.95         |
| 48        | <i>Medicago sativa</i>              |                | 0.00           | 1.17         |
| 49        | <i>Melilotus albus</i>              |                | 1.50           | 0.00         |
| 50        | <i>Melilotus officinalis</i>        |                | 8.91           | 0.00         |

|    | Species                          | Classification | Mean cover / % |              |
|----|----------------------------------|----------------|----------------|--------------|
|    |                                  |                | Medium site    | Old site     |
| 51 | <i>Minuartia glomerata</i>       | T              | 0.75           | 0.00         |
| 52 | <i>Muscari comosum</i>           |                | 0.00           | 1.00         |
| 53 | <i>Oenothera biennis</i>         | N              | 2.19           | 0.00         |
| 54 | <i>Papaver rhoeas</i>            |                | 0.07           | 0.00         |
| 55 | <i>Petrorhagia prolifera</i>     |                | 0.05           | 0.01         |
| 56 | <i>Picris hieracioides</i>       |                | 1.22           | 0.00         |
| 57 | <i>Pimpinella saxifraga</i>      | T              | 0.40           | 0.00         |
| 58 | <i>Plantago indica</i>           | T              | 1.00           | 0.00         |
| 59 | <i>Plantago lanceolata</i>       |                | 0.26           | 0.00         |
| 60 | <i>Poa angustifolia</i>          | T              | 8.48           | 4.50         |
| 61 | <i>Poa bulbosa</i>               | T              | 10.00          | 0.00         |
| 62 | <i>Polygonum arenarium</i>       | T              | 0.00           | 0.51         |
| 63 | <i>Rhinantus serotinus</i>       |                | 1.36           | 0.00         |
| 64 | <i>Robinia pseudoacacia</i>      | N              | 22.00          | 0.00         |
| 65 | <i>Scabiosa ochroleuca</i>       | T              | 6.00           | 0.00         |
| 66 | <i>Secale cereale</i>            |                | 0.00           | 4.34         |
| 67 | <i>Secale sylvestre</i>          | T              | 0.00           | 0.01         |
| 68 | <i>Setaria viridis</i>           |                | 0.20           | 0.00         |
| 69 | <i>Silene alba</i>               |                | 1.00           | 1.45         |
| 70 | <i>Silene conica</i>             |                | 0.22           | 0.20         |
| 71 | <i>Sisymbrium orientale</i>      |                | 1.00           | 0.00         |
| 72 | <i>Solidago virgaurea</i>        |                | 1.00           | 0.00         |
| 73 | <b><i>Stipa borysthenica</i></b> | <b>T</b>       | <b>13.10</b>   | <b>17.31</b> |
| 74 | <i>Stipa capillata</i>           | T              | 1.13           | 4.00         |
| 75 | <i>Syrenia cana</i>              | T              | 0.55           | 0.01         |
| 76 | <i>Thesium ramosum</i>           | T              | 0.01           | 0.15         |
| 77 | <i>Tragopogon dubius</i>         |                | 0.38           | 0.51         |
| 78 | <i>Tragopogon floccosus</i>      | T              | 0.77           | 0.51         |
| 79 | <i>Verbascum lychnitis</i>       | T              | 6.23           | 10.84        |
| 80 | <i>Veronica arvensis</i>         |                | 0.03           | 0.00         |
| 81 | <i>Vicia angustifolia</i>        |                | 0.84           | 0.01         |
| 82 | <i>Vicia hirsuta</i>             |                | 1.00           | 0.00         |
| 83 | <i>Vicia villosa</i>             |                | 3.90           | 4.51         |
| 84 | <i>Viola arvensis</i>            |                | 0.01           | 1.01         |

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**Supplement E3.** Results of statistical models for species richness, vascular plant cover and relative cover of target species. The number of species was tested by generalised linear models (GLM) using Poisson distribution. Cover data were tested by two-way ANOVA.

**Anhang E3.** Ergebnisse der statistischen Modelle für den Artenreichtum, die Gefäßpflanzenbedeckung und die relative Deckung der Zielarten. Die Anzahl der Arten wurde durch generalisierte lineare Modelle (GLM) unter Verwendung der Poisson-Verteilung getestet. Die Deckungsdaten wurden , mittels Zweiweg-Varianzanalyse (ANOVA) analysiert.

| Indicators                   | Treatments                   | Site   | Treatment                                  | Site*Treatment                                   |
|------------------------------|------------------------------|--|--|--|
| Species richness             | Seeding   Non-Seeding        | Chi <sup>2</sup> =86.834 df=1<br><b>p&lt; 2.2e-16***</b> | Chi <sup>2</sup> =1.723 df=1<br>p=0.189    | Chi <sup>2</sup> =4.001 df=1<br><b>p=0.04547</b> |
|                              | Mowing   Non-Mowing          | Chi <sup>2</sup> =83.319 df=1<br><b>p&lt; 2.2e-16***</b> | Chi <sup>2</sup> = 0.200 df=1<br>p=0.65460 | Chi <sup>2</sup> =4.919 df=1<br><b>p=0.02657</b> |
|                              | Carbon   Non-Carbon          | Chi <sup>2</sup> =83.184 df=1<br><b>p&lt; 2.2e-16***</b> | Chi <sup>2</sup> =0.255 df=1<br>p=0.6136   | -  |
| Vascular plant cover         | Seeding   Non-Seeding        | F=20.316 df= 1<br><b>p=1.49e-05***</b>                   | F=3.274 df=1,<br>p=0.0728                  | -  |
|                              | Mowing   Non-Mowing          | F=20.430 df= 1<br><b>p=1.49e-05***</b>                   | F=3.992 df= 1<br><b>p=0.0479 *</b>         | -  |
|                              | Carbon   Non-Carbon          | F=19.801 df= 1<br><b>p=1.88e-05 ***</b>                  | F=0.024 df= 1<br>p=0.877                   | -  |
| Target species               | Seeding   Non-Seeding        | F= 11.359 df= 1<br><b>p=3.45e-06***</b>                  | F=1.347 df= 1<br>p=0.248                   | -  |
|                              | Mowing   Non-Mowing          | F=23.37 df= 1<br><b>p=3.85e-06***</b>                    | F=0.00 df= 1<br>p=0.995                    | -  |
|                              | Carbon   Non-Carbon          | F=23.43 df= 1<br><b>p=3.75e-06***</b>                    | F=0.33 df= 1<br>p=0.567                    | -  |
| Non-seeded target species    | Seeding   Non-Seeding        | F= 22.752 df= 1<br><b>p=5.08e-06***</b>                  | F=1.033 df= 1<br>p=0.311                   | -  |
|                              | Mowing   Non-Mowing          | F=22.446 df= 1<br><b>p=5.77e-06***</b>                   | F=0.594 df= 1<br>p=0.442                   | -  |
|                              | Carbon   Non-Carbon          | F=22.354 df= 1<br><b>p=6.01e-06***</b>                   | F=0.077 df= 1<br>p=0.782                   | -  |
| Seeded species               | <b>Seeding   Non-Seeding</b> | F=0.780 df= 1<br>p=0.37887                               | F=1.613 df= 1<br>p=0.20644                 | <b>F=7.591 df= 1<br/>p=0.00675 **</b>            |
|                              | Mowing   Non-Mowing          | F=0.734 df= 1<br>p=0.393                                 | F=0.325 df= 1<br>p=0.569                   | -  |
|                              | Carbon   Non-Carbon          | F=0.736 df= 1<br>p=0.393                                 | F=0.655 df= 1<br>p=0.420                   | -  |
| <i>Festuca vaginata</i>      | Seeding   Non-Seeding        | F=0.296 df= 1<br>p=0.587                                 | F=1.771 df= 1<br>p=0.186                   | -  |
|                              | Mowing   Non-Mowing          | F=0.292 df= 1<br>p=0.59                                  | F=0.008 df= 1<br>p=0.93                    | -  |
|                              | Carbon   Non-Carbon          | F=0.293 df= 1<br>p=0.590                                 | F=0.306 df= 1<br>p=0.581                   | -  |
| <i>Stipa borystenica</i>     | <b>Seeding   Non-Seeding</b> | F=11.22 df= 1<br><b>p=0.00107 ***</b>                    | F=28.41 df= 1<br><b>p=4.43e-07 ***</b>     | -  |
|                              | Mowing   Non-Mowing          | F=9.147 df= 1<br><b>p=0.00302 **</b>                     | F=0.000 df= 1<br>p=0.99739                 | -  |
|                              | Carbon   Non-Carbon          | F=9.166 df= 1<br><b>p=0.003 **</b>                       | F=0.259 df= 1<br>p=0.612                   | -  |
| <i>Koeleria glauca</i>       | <b>Seeding   Non-Seeding</b> | F=12.219 df= 1<br><b>p=0.000655***</b>                   | F=6.074 df= 1<br><b>p=0.015074 *</b>       | -  |
|                              | Mowing   Non-Mowing          | F=11.723 df= 1<br><b>p=0.000835***</b>                   | F=0.758 df= 1<br>p=0.385719                | -  |
|                              | Carbon   Non-Carbon          | F=11.730 df= 1<br><b>p=0.000833***</b>                   | F=0.826 df= 1<br>p=0.365142                | -  |
| <i>Dianthus serotinus</i>    | Seeding   Non-Seeding        | F=20.596 df= 1<br><b>p=1.31e-05***</b>                   | F=0.035 df= 1<br>p=0.853                   | -  |
|                              | Mowing   Non-Mowing          | F=20.673 df= 1<br><b>p=1.27e-05***</b>                   | F=0.502 df= 1<br>p=0.48                    | -  |
|                              | Carbon   Non-Carbon          | F=20.656 df= 1<br><b>p=1.28e-05***</b>                   | F=0.398 df= 1<br>p=0.529                   | -  |
| <i>Euphorbia seguieriana</i> | Seeding   Non-Seeding        | F=62.454 df= 1<br><b>p=1.22e-12 ***</b>                  | F=0.002 df= 1<br>p=0.966                   | -  |
|                              | Mowing   Non-Mowing          | F=64.032 df= 1<br><b>p=7.16e-13 ***</b>                  | F=3.159 df= 1<br>p=0.0779                  | -  |
|                              | Carbon   Non-Carbon          | F=62.674 df= 1<br><b>p=1.13e-12 ***</b>                  | F=0.441 df= 1<br>p=0.508                   | -  |

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**Supplement E4.** Results of two-way ANOVA for relative cover of neophyte species. In case of no significant interactions at  $p < 0.05$ , we re-ran the models with main factors only. If none of the treatments was significant, one-way ANOVA was performed for site (Table 2).

**Anhang E4.** Ergebnisse der Zweifweg-Varianzanalyse (ANOVA) für die relative Deckung der Neophytenarten. Wenn keine signifikanten Interaktionen ( $p < 0,05$ ) auftraten, berechneten wir die Modelle nochmals, allerdings nur unter Verwendung der Hauptfaktoren. Wenn keine der Behandlungen signifikant war, wurde eine einfache Varianzanalyse (ANOVA) für den Standort

| Indicators                     | Treatments                              | Site                                     | Treatment                                | Site*Treatment                          |
|--------------------------------|---|--|--|---|
| Neophyte species               | Seeding   Non-Seeding                   | F=24.346, df=1,<br><b>p=2.51e-06 ***</b> | F=5.324, df=1,<br><b>p=0.0227 **</b>     | -                                       |
|                                | Mowing   Non-Mowing                     | F=23.402, df=1,<br><b>p=3.79e-06 ***</b> | F=0.271, df=1,<br>p=0.604                | -                                       |
|                                | Carbon amendment   Non-Carbon amendment | F=19.801, df=1,<br><b>p=1.88e-05 ***</b> | F=0.024, df=1,<br>p=0.877                | -                                       |
| Annual species                 | Seeding   Non-Seeding                   | F=15.27, df=1,<br><b>p=0.000152 ***</b>  | F=10.86, df=1,<br><b>p=0.001280 **</b>   | -                                       |
|                                | Mowing   Non-Mowing                     | F=14.168, df=1,<br><b>p=0.000256 ***</b> | F=1.047, df=1,<br>p=0.308287             | -                                       |
|                                | Carbon amendment   Non-Carbon amendment | F=14.256, df=1,<br><b>p=0.000245 ***</b> | F=1.833, df=1,<br>p=0.178240             | -                                       |
| <i>Ambrosia artemisiifolia</i> | Seeding   Non-Seeding                   | F=9.285, df=1,<br><b>p=0.002824 **</b>   | F=12.503, df=1,<br><b>p=0.000572 ***</b> | <b>F= 3.955, df=1,<br/>p=0.048926 *</b> |
|                                | Mowing   Non-Mowing                     | F=8.297, df=1,<br><b>p=0.00467 **</b>    | F=0.514, df=1,<br>p=0.47471              | -                                       |
|                                | Carbon amendment   Non-Carbon amendment | F=8.344, df=1,<br><b>p=0.00456 **</b>    | F=1.225, df=1,<br>p=0.27054              | -                                       |
| <i>Conyza canadensis</i>       | Seeding   Non-Seeding                   | F=13.594, df=1,<br><b>p=0.000337 **</b>  | F=0.005, df=1,<br>p=0.942270             | -                                       |
|                                | Mowing   Non-Mowing                     | F=13.699, df=1,<br><b>p=0.00032 ***</b>  | F=0.971, df=1,<br>p=0.32636              | -                                       |
|                                | Carbon amendment   Non-Carbon amendment | F=13.723, df=1,<br><b>p=0.000317 ***</b> | F=1.191, df=1,<br>p=0.277179             | -                                       |
| Perennial species              | Seeding   Non-Seeding                   | F=84.508, df=1,<br><b>p=1.06e-15 ***</b> | F=0.443, df=1,<br>p=0.507                | -                                       |
|                                | Mowing   Non-Mowing                     | F=84.673, df=1,<br><b>p=1.01e-15 ***</b> | F=0.687, df=1,<br>p=0.409                | -                                       |
|                                | Carbon amendment   Non-Carbon amendment | F=84.213, df=1,<br><b>p=1.16e-15 ***</b> | F=0.005, df=1,<br>p=0.947                | -                                       |
| <i>Asclepias syriaca</i>       | Seeding   Non-Seeding                   | F=61.87, df=1,<br><b>p=1.48e-12 ***</b>  | F=0.88, df=1,<br>p=0.35                  | -                                       |
|                                | Mowing   Non-Mowing                     | F=61.492, df=1,<br><b>p=1.69e-12 ***</b> | F=0.106, df=1,<br>p=0.745                | -                                       |
|                                | Carbon amendment   Non-Carbon amendment | F=61.767, df=1,<br><b>p=1.54e-12 ***</b> | F=0.666, df=1,<br>p=0.416                | -                                       |
| <i>Oenothera biennis</i>       | Seeding   Non-Seeding                   | F=32.765, df=1,<br><b>p=7.29e-08 ***</b> | F=0.025, df=1,<br>p=0.874                | -                                       |
|                                | Mowing   Non-Mowing                     | F=33.610, df=1,<br><b>p=5.17e-08 ***</b> | F=3.249, df=1,<br>p=0.0739               | -                                       |
|                                | Carbon amendment   Non-Carbon amendment | F=32.891, df=1,<br><b>p=6.93e-08 ***</b> | F=0.505, df=1,<br>p=0.479                | -                                       |



**Supplement E5.** Results of the post-hoc Tukey test for site\*seeding interaction in relation to species richness. Abbreviations: Old – Old site, Med – Medium site, NoM – non-mowing, M – mowing. Significant interactions at  $p < 0.05$  are highlighted by grey background.

**Anhang E5.** Ergebnisse des Post-Hoc-Tests (Tukey) für die Standort\*Einsaat-Interaktion in Bezug auf den Artenreichtum. Abkürzungen: Alt – Alter Standort, Med – Mittlerer Standort, NoM – ungemäht, M – gemäht. Signifikante Interaktionen ( $p < 0,05$ ) sind durch grauen Hintergrund hervorgehoben.

| Groups being compared | Estimate | Std. Error | z value | p value |
|-----------------------|----------|------------|---------|---------|
| Med:NoS – Old:NoS     | 5.094    | 1.025      | 4.971   | <0.0001 |
| Med:S – Med:NoS       | -0.906   | 1.112      | -0.815  | 0.8476  |
| Old:S – Med:NoS       | 7.062    | 0.994      | 7.104   | <0.0001 |
| Med:S – Old:NoS       | -6.000   | 1.038      | -5.779  | <0.0001 |
| Old:S – Old:NoS       | 1.969    | 0.911      | 2.162   | 0.1339  |
| Old:S – Med:S         | 7.969    | 1.008      | 7.903   | <0.0001 |

**Supplement E6.** Results of the post-hoc Tukey test for site\*mowing interaction in relation to species richness. Abbreviations: Old – Old site, Med – Medium site, NoM – non-mowing, M – mowing. Significant interactions at  $p < 0.05$  are highlighted by grey background.

**Anhang E6.** Ergebnisse des Post-Hoc-Tests (Tukey) für Standort\*Mahd-Interaktion in Bezug auf den Artenreichtum. Abkürzungen: Alt – Alter Standort, Med – Mittlerer Standort, NoM – Nicht-Mähen, M – Mähen. Signifikante Interaktionen ( $p < 0,05$ ) sind durch grauen Hintergrund hervorgehoben.

| Groups being compared | Estimate | Std. Error | z value | p value |
|-----------------------|----------|------------|---------|---------|
| Med:NoM – Old:NoM     | 8.12     | 1.016      | 7.993   | <0.0001 |
| Med:M – Med:NoM       | 1.59     | 1.112      | 1.433   | 0.4788  |
| Old:M – Med:NoM       | 6.53     | 1.041      | 6.276   | <0.0001 |
| Med:M – Old:NoM       | -6.53    | 0.992      | -6.586  | <0.0001 |
| Old:M – Old:NoM       | -1.59    | 0.911      | -1.750  | 0.2977  |
| Old:M – Med:M         | 4.94     | 1.016      | 4.858   | <0.0001 |

**Supplement E7.** Results of the post-hoc Tukey test for site\*seeding interaction in relation to relative cover of seeded species. Abbreviations: Old – Old site, Med – Medium site, NoM – non-mowing, M – mowing. Significant interactions at  $p < 0.05$  are highlighted by grey background.

**Anhang E7.** Ergebnisse des Post-Hoc-Tests (Tukey) für die Interaktion von Standort\*Einsaat in Bezug auf die relative Deckung der angesähten Arten. Abkürzungen: Alt – Alter Standort, Med – Mittlerer Standort, NoM – Nicht-Mähen, M – Mähen. Signifikante Interaktionen ( $p < 0,05$ ) sind durch grauen Hintergrund hervorgehoben.

| Groups being compared | Diff   | Lower  | Upper | p value |
|-----------------------|--------|--------|-------|---------|
| Med:NoS – Old:NoS     | -0.088 | -0.178 | 0.001 | 0.0542  |
| Med:S – Med:NoS       | -0.036 | -0.126 | 0.054 | 0.7203  |
| Old:S – Med:NoS       | 0.009  | -0.080 | 0.099 | 0.9928  |
| Med:S – Old:NoS       | 0.052  | -0.037 | 0.142 | 0.4272  |
| Old:S – Old:NoS       | 0.098  | 0.008  | 0.188 | 0.0263  |
| Old:S – Med:S         | 0.046  | -0.044 | 0.135 | 0.5497  |

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