

Restoration of a newly created inland-dune complex as a model in practice: impact of substrate, minimized inoculation and grazing

Restitution eines neu aufgeschütteten Binnendünen-Komplexes als Modell für die Praxis: Einfluss von Substrat, Minimal-Inokulation und Beweidung

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Abstract

In Central Europe stands of the *Koelerion glaucae* vegetation complex are threatened and mostly highly fragmented. Knowledge about the impact of abiotic and biotic measures to restore this vegetation complex is crucial. Therefore, an inland sand dune complex (around 2 ha) was created in 2009 as a study model in the Upper Rhine Valley (Germany), which included sites with different substrate conditions as well as grazing impact and minimized inoculation with plant material.

The restoration area is divided into two halves with different substrate conditions (sites 1 and 2), on which inoculation with raked plant material and grazing by donkeys was studied on systematically arranged plots with randomised treatment distribution (32 plots). Additionally the whole area was monitored by a grid-plot approach to show the floristic background (43 plots). Minimized inoculation was conducted with rare *Koelerion glaucae* plant material in small plots covering around 5–7% of the restoration sites. During the four-year study, vegetation development was recorded and examined in relation to the donor site and an older restoration site. Soil seed bank and seed rain in the newly deposited restoration sites were also investigated, as well as the endozoochorous seed-dispersal by donkeys. Target species ratios (TSR) were calculated to estimate the restoration success. We used mixed linear models and detrended correspondence analysis for data evaluation.

Substrate conditions had an impact on the number of target species and on phanerogam and cryptogam cover. Inoculation enhanced both number and, without grazing, cover of target species since the first year. On not-inoculated plots and on grid-plots, target-species numbers increased gradually. Grazing by donkeys did not affect target-species numbers, but had a decreasing effect on target-species cover. Grazing reduced bryophyte cover, especially on inoculated plots. DCA revealed development of the experimental plots towards the donor site, as has occurred on the older restoration site. Soil seed bank and seed rain were characterized by ruderal species, and did not show similarities to the donor site. Endozoochory revealed some target species to be effectively dispersed by donkeys.

Minimized inoculation is suitable to overcome seed limitation and build up starter populations of target species for the colonization of larger restoration sites. However, within four years species composition of the donor site was not achieved. Grazing by donkeys had mainly structural effects for the studied time period.

Keywords: donkeys, endozoochory, soil seed bank, seed rain, target species

Erweiterte deutsche Zusammenfassung am Ende des Artikels

1. Introduction

Central European semi-natural grasslands on calcareous sandy soils are characterized by threatened plant communities and protected by the Fauna-Flora-Habitat directive of the European Union (Natura 2000-Code 6120, SSYMANK et al. 1998). Threats mostly arise from changes in land use such as abandonment or agricultural intensification (POSCHLOD et al. 2005), leading to degradation (e.g. by grass encroachment) or losses of *Koelerio-Corynephoretea* and *Festuco-Brometea* stands with their habitat-typical species. The remaining habitats are highly fragmented and face various threats like reproductive impairment (AGUILAR et al. 2006), genetic depletion and even local extinction (FISCHER & STÖCKLIN 1997). Restoration should therefore concentrate on enlarging and connecting the few remaining sandy grassland sites.

A serious problem for restoration in this habitat type is the almost complete absence of sites with appropriate abiotic conditions. Therefore a higher degree of intervention (WALKER et al. 2014) is necessary “to achieve a desired diversity of species” and communities. Most sites considered for restoration are eutrophicated due to former use as arable fields, constraining the re-establishment of semi-natural grasslands adapted to nutrient-poor soil conditions. Elevated concentrations of both nitrogen and phosphorus in the soil affect restoration adversely. Nitrogen is the limiting factor in our ecosystem type (STORM & SÜSS 2008); enhanced availability was shown to accelerate succession which differed from the typical pathway for sandy grassland (FAUST et al. 2012). The soil phosphate-phosphorus concentration could be related to a decline of the target grass species *Stipa capillata* above a threshold of approximately 20 mg kg⁻¹ in our study area (SÜSS et al. 2004). Target pioneer species can be outcompeted by ruderal species in restoration sites with high soil phosphate concentrations (STROH et al. 2007).

Therefore, the abiotic conditions have to be restored at first to obtain suitable soil conditions for establishment. To reduce nutrients like soil phosphate, topsoil removal was successfully applied in various studies (ALLISON & AUSDEN 2004, JAUNATRE et al. 2014, OLSSON & ÖDMAN 2014), but this technique is cost-intensive (TÖRÖK et al. 2011). An alternative approach - tested in our study area - is the deposition of sand from > 1 m depth, which also creates nutrient-poor soil conditions (EICHBERG et al. 2010).

Beyond the low nutrient concentrations also low seed numbers characterize these deep soil layers (EICHBERG et al. 2010). As seed banks of formerly arable fields are mostly dominated by weedy species (HUTCHINGS & BOOTH 1996) and most target species have only low dispersal distances (JENTSCH & BEYSCHLAG 2003), successful spontaneous establishment requires a target community in the immediate vicinity (DONATH et al. 2003, STROH et al. 2007). To overcome seed limitation various measures to introduce species have been tested (KIEHL et al. 2010). Convincing results were obtained, e.g., by spreading raked or mown plant material onto restoration sites (KIEHL & PFADENHAUER 2007, BAASCH et al. 2012) but also by transfer of seeds of single plant species (FRITSCH et al. 2011). Raked material has the benefit that not only seeds of phanerogams are transferred but also bryophytes and lichens (EICHBERG et al. 2010, JESCHKE 2012). The transferred plant material is usually applied in stripes (HÖLZEL & OTTE 2003, DONATH et al. 2007) or, at smaller restoration sites, on the whole site (EICHBERG et al. 2010).

To enhance and maintain restoration success, follow-up management has to be applied to the restoration site (KIEHL et al. 2010). Calcareous sandy grasslands depend on regular disturbance, which is guaranteed by traditional management (FISCHER et al. 1996, LANGHANS et al. 2009). In our study area management of intact calcareous sandy grasslands comprises

sheep and/or donkey grazing (SÜSS & SCHWABE 2007). Donkeys create gaps in the vegetation by trampling and wallowing (SÜSS & SCHWABE 2007), which is especially important in consolidated sandy grassland, and reduce competitive graminoids (LAMOOT et al. 2005). Apart from the grazing impact, large herbivores can serve as dispersal vectors via epizoochory (COUVREUR et al. 2005) and endozoochory (COSYNS et al. 2005, MOUISSIE et al. 2005a, ROSENTHAL et al. 2012). Little is known about the impact of grazing donkeys on newly created restoration sites.

The aims of the present four-year study were to test the combination of abiotic restoration, minimized biotic restoration and a subsequent grazing by donkeys, to transform a former arable land into calcareous sandy grassland. On the abiotic side, deposition of deep sand on a larger restoration site was so far conducted in one study only (EICHBERG et al. 2010), but the impact of substrate quality was not investigated there. On the biotic side, we tested a minimized application of raked plant material, as donor sites for high quality plant material are small and extensive removal of plant material may negatively affect populations of target species at the donor sites. The use of donkeys for management grazing and their impact as dispersal vectors on newly created restoration sites is almost unknown.

To highlight these aspects for restoration practice the following questions were addressed: (1) What is the impact of substrate condition on development of sandy grassland during four years? (2) Is the inoculation of small plots (= minimized inoculation) sufficient to restore sandy grassland in a larger area in a period of four years? (3) Which role does endozoochorous dispersal by donkeys play for target species? (4) How does grazing by donkeys affect the vegetation development?

2. Methods

2.1 Study area

Located in the northern Upper Rhine Valley, Germany, the study area is characterized by calcareous, nutrient-poor sandy soils (arenosol). The sand originates from aeolian deposits of the Rhine terraces from the late glacial and early postglacial period (AMBOS & KANDLER 1987). Since the Middle Ages anthropo-zoogenic impact (military training areas, pastures) has preserved the open structure of these habitats (ZEHM & ZIMMERMANN 2004). Being in the biogeographic transition zone between subatlantic, subcontinental and submediterranean influence, the co-occurrence of species of these biogeographic zones is remarkable, e.g. *Koeleria glauca* ((sub-) continental), *Corynephorus canescens* (suboceanic) and *Silene conica* (submediterranean). Nowadays, only fragments of the specific vegetation complexes and plant communities *Koelerion glaucae* Volk 1931 (priority habitat 6120 “Xeric sand calcareous grasslands”) and *Allio-Stipetum capillatae* Korneck 1974 (priority habitat 6240 “Sub-pannonic steppic grasslands”) persist (SÜSS et al. 2004, LANGHANS et al. 2009).

The mean annual temperature is 9.7 °C with a mean annual precipitation of 658 mm (data from Frankfurt/Main airport, 1961–1990; Deutscher Wetterdienst, www.dwd.de).

2.2 Restoration sites and abiotic restoration measures

The restoration area (Apfelbachdüne; 8°35' E, 49°56' N; Fig. 1) is situated about 20 km south of Frankfurt/Main. The restoration methods were employed as a compensation measure for a construction project in this area. Until summer 2009 the area was used as arable field; in autumn 2009 it was abiotically restored by depositing deep sand (layer thickness 1–3 m). Restoration site 1 (S1; 1.1 ha; around 20,000 m³ sand) received high-quality sand (assignment criterion Z0 according to LAGA-M 20) of low nutrient status; the adjacent restoration site 2 (S2; 0.8 ha; around 14,000 m³ sand) received sand with



Fig. 1. Arrangement of the study plots and grid plots on the restoration sites. ND = old nature monument 'Apfelbachdüne'; 1 = restoration site 1; 2 = restoration site 2. i- g- = not inoculated, not grazed; i- g+ = not inoculated, grazed; i+ g- = inoculated, not grazed; i+ g+ = inoculated, grazed; GP = grid plot. The aerial photograph of the restoration sites (May 2011) was provided by the 'Hessische Verwaltung für Bodenmanagement und Geoinformation'. Creation of the base map was kindly supported by the 'Amt für Bodenmanagement Heppenheim' (training department).

Abb. 1. Anordnung der Untersuchungsplots und der Rasterpunkte auf den Restitutionsflächen. ND = Naturdenkmal 'Apfelbachdüne'; 1 = Restitutionsfläche 1; 2 = Restitutionsfläche 2. i- g- = nicht inokuliert, unbeweidet; i- g+ = nicht inokuliert, beweidet; i+ g- = inokuliert, unbeweidet; i+ g+ = inokuliert, beweidet; GP = Rasterpunkt. Das Luftbild der Restitutionsflächen (Mai 2011) wurde von der Hessischen Verwaltung für Bodenmanagement und Geoinformation zur Verfügung gestellt. Die Kartengrundlage wurde mit freundlicher Unterstützung des Amtes für Bodenmanagement Heppenheim (Ausbildungsabteilung) erstellt.

partly higher phosphate concentrations. Detailed soil data are given in the 'Results' section. The deep sand was transferred from two construction sites, whereupon the sand of S2 was temporarily stored above-ground and was thereby contaminated with a small amount of silt.

2.3 Experimental design

In total, 32 systematically arranged plots with randomised treatment distribution were installed on the restoration area (Fig. 1). On each of the restoration sites 1 and 2, eight plots were inoculated with raked plant material and eight were left untreated, respectively. In each case four inoculated and four untreated plots were fenced against grazing by donkeys. To prevent drift of the material by wind, inoculation material was watered with stream water directly after spreading and pressed to the ground with a roller. The plots were 70 m² in extent with a relevé area of 25 m² in the centre. Inter-plot distance was at least 15 m on restoration site 1 and 10 m on restoration site 2 (measured from the outer plot edges).

Additionally, 43 grid-plots ('GP'; à 25 m²) were installed (not inoculated, grazed) covering the whole restoration area systematically (site 1: 23 grid-plots; site 2: 20 grid-plots) to show the 'floristic background' of the whole area.

Since 2010, both restoration sites were grazed separately by a flock of three (autumn 2010) to five (2011–2013) donkeys in summer. Both sites were grazed as long as an acceptable food supply could be assured. The restoration sites were grazed alternately in multiple short-term periods. Total grazing time was from two (2010/13) to ten (2011) weeks on restoration site 1 and from five (2010) to 14 (2011) weeks on restoration site 2.

2.4 Donor site

The donor site for the inoculation material ('D' for Standortübungsplatz; 8°36' E, 49°51' N) is bearing mainly pioneer stages of *Koelerion glaucae* vegetation and is situated about 13 km south of the restoration sites. In March 2010 the donor site was treated with a swather and subsequently the loosened plant material was raked by hand. The collection area for each of the restoration sites was about 290 m² in extent; the raked material was used to inoculate 560 m² on each restoration site. This corresponds to an inoculation area of 5% on S1 and of 7% on S2. The inoculation density was about 740 ± 44 g m⁻² (mean ± SE; *n* = 144) of air-dried inoculation material.

2.5 Soil analysis

Soil samples were collected in December 2009. Sampling points were based on the grid plot approach; 51 samples were taken on S1 and 39 samples on S2. Sampling was conducted using an Eijkelkamp liner sampler (diameter 4.7 cm; Giesbeek, NL); sampling depth was 11–16 cm. The samples were kept cool, sieved (2 mm) within 24 h and frozen (-18 °C) until extraction. Phosphate (P) was measured in calcium acetate/calcium lactate extracts (CAL; 10 g soil + 200 ml) according to VDLUFA (1991). P analysis was carried out photometrically (Segmented Flow Analyser SAN+, Skalar analytical, AA Breda, NL). Total nitrogen (N_{total}) was analysed by elemental analysis (Model 1400, Carlo-Erba, Milan, IT). pH values were measured in 0.01 mol l⁻¹ calcium chloride after centrifugation.

2.6 Soil seed bank

Seed bank samples were taken at the beginning of March 2010 before inoculation of the restoration sites. Per restoration site, 100 samples were taken in a regular grid (based on the grid plots) using an Eijkelkamp liner sampler (see above). Sampling depth was 11–16 cm. By mixing ten individual samples, ten composite samples were obtained per restoration site. Samples were air dried (to eliminate vegetative propagules) and stored at room temperature. To assess the seed contents a seedling emergence method was used (EICHBERG et al. 2006), in which the samples were filled into trays and placed outdoors in the botanical garden of the 'Technische Universität Darmstadt' on a transparently-roofed platform (0.9 m height). The platform was covered by gauze as a protection against aerial seed input and additionally, trays with autoclaved sand were placed between the samples to control for contamination by air-borne seeds. The samples were kept moist and turned every third month. From July 2010 to November 2012, emerging seedlings were identified, counted and removed.

2.7 Seed rain

Seed rain was analysed from May 2010 to May 2012 using funnel traps (KOLLMANN & GÖTZE 1998). Per fenced, non-inoculated plot eight funnel traps were evenly arranged on a 1 m buffer strip around the relevé area. In total, 32 funnel traps were installed per restoration site. Total sampling area per plot was 0.362 m². Trap height was 0.9 m above ground level. To avoid direct seed input into the traps, the vegetation surrounding the traps was cut within a radius of ca. 0.5 m as required. Traps were emptied fortnightly. Trapped seeds were identified and counted; determination was conducted by means of a reference seed collection and literature (BEIJERINCK 1976, CAPPERS et al. 2006).

2.8 Sampling of inoculation material

To test the potential of the inoculation material to transfer species to the restoration sites, samples of the plant material were collected. Per inoculated plot ($n = 16$) ten plastic boards (à 33 cm x 33 cm) were randomly distributed prior to inoculation. Afterwards, the spread plant material was collected from the plastic boards, dried and stored at room temperature. In total, 80 samples were taken per restoration site. One sample of every inoculation plot was dried at 70 °C for 48 h to obtain the dry weight. The remaining samples were divided into three fractions (phanerogams, cryptogams/litter and inorganic material) to assess the percentage by weight of these fractions on inoculation material. After re-unifying the three fractions to the initial sample, the samples were spread on trays with autoclaved sand to assess the seed contents in a seedling emergence experiment. The trays were placed outdoors in the botanical garden (see 'Soil seed bank' section). Trays were watered when samples got dry. Further procedure is as described in the 'Soil seed bank' section.

2.9 Vegetation relevés

Since 2010, vegetation relevés were conducted yearly in spring (May; for therophytes) and summer (July/August) on all experimental plots and on the grid plots. The results of these two relevés were combined into one relevé p.a. For all plant species including bryophytes and lichens both cover-abundance following the extended Braun-Blanquet-scale (BB; according to BARKMAN et al. 1964) and a percentage scale (0.1, 1, 2, ..., 6, 8, 10, 15, ..., 95, 96, ..., 100%) were recorded. Additionally, total cover, cover of phanerogams, cryptogams (bryophytes + lichens) and of bare soil were noted.

Nomenclature follows WISSKIRCHEN & HAEUPLER (1998) for vascular plant species, KOPERSKI et al. (2000) for bryophytes and SCHOLZ (2000) for lichens; syntaxa refer to OBERDORFER (2001).

2.10 Comparison of species composition

The vegetation development was set in context with the donor site 'D' and an older restoration site, which had received inoculation material from the same donor site in 2005 ('RS' for Seeheim; 8°37' E, 49°46' N). Detailed information of the older restoration site is given in EICHBERG et al. (2010). For 'D' vegetation relevés [à 25 m²; BB] were available for the years 2006–2008; they represent relatively stable pioneer vegetation, which was documented by permanent-plot studies (SÜSS et al. 2010). For 'RS' [à 25 m²; BB] we used the first four years since inoculation (2005–2008) for comparison, as this corresponds to the developmental state of the present restoration site. As management measure the 'RS' sites were grazed by donkeys.

2.11 Endozoochory

Dung samples were collected in mid June 2012 on restoration site 1. About eight litres of dung were sampled from different dung accumulations and pooled. The samples were washed with tap water on a sieve (10 mm) to remove potentially adhering sand and seeds. The samples were then coarsely crumbled and dried (40 °C) for seven days. Seed contents were quantified using a seedling emergence method. Pots (22 cm x 15 cm) were filled with a layer of sterile potting soil (4 cm) covered by a thin layer of sterile sand (4 mm); on it, a thin layer of dry dung (10 g, ca. 5 mm) was spread. The pots were placed in a greenhouse (20 °C, 20 h light) for eight weeks; samples were watered every second day. Emerging seedlings were identified, counted and removed. When identification was difficult, seedlings were transplanted to separate pots and grown until identification was possible. After the first growing period, a cold stratification was conducted at 4 °C in the dark for six weeks. Subsequently, a second growing period followed with the same conditions as the first.

2.12 Data analysis

Vegetation data were analysed with detrended correspondence analysis (DCA) using PC-Ord 6.17 (MjM Software, Gleneden Beach, OR, USA). Included were data of the restoration sites and the sites for vegetation comparison ('D', 'RS'), as well as data of soil seed bank and seed rain. For the data of the restoration sites, a mean value of the four replicates was calculated for each treatment type. For seed rain data the sum of both years was used. Braun-Blanquet data were transformed to an ordinal scale beforehand ($r = 1$, $+$ = 2, 1 = 3, 2m = 4, 2a = 5, 2b = 6, 3 = 7, 4 = 8, 5 = 9). Seed bank and seed rain data were transformed to a comparable, weighted scale (1 seed/seedling = 1, 2–9 = 2, 10–49 = 3, 50–99 = 4, 100–499 = 5, 500–999 = 6, 1000–1999 = 7, 2000–2999 = 8, $\geq 3000 = 9$). The analysis was run using the options 'downweight rare species' and 'rescale axes'; the number of segments was 26. To evaluate the percentages of explained variance in the distance matrix, the Relative Euclidean distance was used as recommended by the PC-Ord manual.

Target species ratios (TSR) were calculated as described in EICHBERG et al. (2010), as:

$TSR_{qual} = \text{number of target plant species} / \text{total number of plant species}$, and
 $TSR_{quant} = \text{cover sum of target plant species} / \text{cover sum of all plant species}$.

Target species were defined as species with main occurrence in the classes *Koelerio-Corynephoretea* Klika 1941 and *Festuco-Brometea* Br.-Bl. et Tx. 1943. Ruderal species were defined as species of the classes *Agropyretea intermedio-repentis* Müll. et Görs 1969, *Artemisietea vulgaris* Lohm., Prsg et Tx. in Tx. 1950 and *Chenopodietea* Br.-Bl. 1951.

To test the effects of 'site', 'inoculation', 'grazing' and 'year' on various dependent variables, mixed linear models were used (SAS 9.2, PROC MIXED; SAS Institute Inc., Cary, NC, USA; LITTELL et al. 2006). These models are suitable for analysis of repeated-measures data (LITTELL et al. 1998), as they allow comparison of the goodness of fit of several covariance structures. The best covariance structure was chosen according to the corrected Akaike criterion (AICC). Degrees of freedom were calculated using the Kenward-Roger approximation (SCHAALJE et al. 2002).

To test for effects of distance of the grid plots to the next inoculated plot on target species number and target species ratios, Spearman's rank correlation coefficient was calculated using PROC CORR.

3. Results

3.1 Soil nutrient status

The soils had nearly identical pH values on both restoration sites; only one value (S1) was considerably lower than the mean (Table 1). N_{total} was very low, although on S2 on average three-fold higher than on S1. Phosphate concentrations were very low on S1. S2 had overall higher P concentrations; the values were scattered and covered a relatively wide range.

3.2 Soil seed bank

The soil seed bank of restoration site 2 had an approximately 5-fold higher seed-density than S1 and comprised more species (19) compared to S1 (three species; Table 2). Most species are ruderal species; target species were only found on S2 with three species and three seedlings. Nearly all species present in the soil seed bank were recorded in the vegetation of the first year of the particular restoration site (Supplement S1), except for one species on S1 (*Cardamine hirsuta*; but detected on S2 in 2011) and three species on S2 (two were recorded since 2011 and one since 2013).

Table 1. Soil data of the restoration sites. Sampling took place in December 2009. Determination was conducted as described in the 'Soil analysis' section (S1: $n = 51$, S2: $n = 39$). min = minimum value; max = maximum value.

Tabelle 1. Bodendaten der Restitutionsflächen. Die Probenahme erfolgte im Dezember 2009. Die Analysen wurden wie im Abschnitt 'Soil analysis' beschrieben durchgeführt (S1: $n = 51$, S2: $n = 39$). min = Minimum-Wert; max = Maximum-Wert.

	Site 1			Site 2		
	mean \pm SE	min	max	mean \pm SE	min	max
PO ₄ ³⁻ -P (mg kg ⁻¹)	6.87 \pm 0.02	1.90	11.33	21.99 \pm 2.00	8.14	53.72
pH	7.51 \pm 0.05	4.89	7.79	7.55 \pm 0.01	7.43	7.67
N _{total} (g kg ⁻¹)	0.03 \pm 0.00	0.01	0.18	0.09 \pm 0.01	0.04	0.29

Table 2. Soil seed bank data, sampled in March 2010 prior to inoculation. The number of seedlings per m² is given (mean \pm SE; $n = 10$). Target species are printed in bold.

Tabelle 2. Diasporenbank im Boden, beprobt im März 2010 vor der Inokulation. Angegeben ist die Keimlingsanzahl pro m² (MW \pm SE; $n = 10$). Zielarten sind in Fettdruck.

Taxa	Site 1	Site 2
<i>Amaranthus retroflexus</i>	12 \pm 12	
<i>Cardamine hirsuta</i>	6 \pm 6	
<i>Chenopodium album</i> agg.	29 \pm 15	63 \pm 29
<i>Chenopodium strictum</i>		35 \pm 15
<i>Conyza canadensis</i>		17 \pm 12
<i>Corispermum leptopterum</i>		6 \pm 6
<i>Digitaria sanguinalis</i>		6 \pm 6
<i>Eragrostis minor</i>		12 \pm 12
<i>Hypericum perforatum</i>		12 \pm 8
<i>Lactuca serriola</i>		6 \pm 6
<i>Medicago lupulina</i>		6 \pm 6
<i>Polygonum aviculare</i> agg.		6 \pm 6
<i>Rumex acetosella</i>		6 \pm 6
<i>Saxifraga tridactylites</i>		6 \pm 6
<i>Setaria pumila</i>		6 \pm 6
<i>Setaria viridis</i>		6 \pm 6
<i>Solanum physalifolium</i>		6 \pm 6
<i>Sonchus oleraceus</i>		6 \pm 6
<i>Taraxacum</i> spec.		6 \pm 6
<i>Urtica dioica</i>		6 \pm 6
<i>Veronica arvensis</i>		6 \pm 6
indetermined		6 \pm 6
Sum	46 \pm 27	225 \pm 73

3.3 Seed rain

During the two years of seed rain investigation, in total seeds of 58 species were trapped (Table 3). Except for seven non-target species, all species were present in the vegetation of at least one of the restoration sites (Supplement S1). On restoration site 1 a total of 29 species were recorded in both investigated years, on restoration site 2 species numbers were higher during the two years (in total 43 species). Target species were underrepresented on the two restoration sites, according to both taxa and seed numbers. The seven trapped target species reflected less than 0.1% of all seeds. Ruderal species dominated the seed rain, accounting for more than 50% of the trapped species and seeds on the particular restoration site. The most frequently trapped seeds on S1 and S2 were those of the ruderal forb *Conyza canadensis*.

Table 3. Seed rain data of both restoration sites, collected via funnel traps (0.9 m above ground) from May 2010 to April 2011 (year 1) and from May 2011 to April 2012 (year 2). The number of seeds per m² is given for each site (mean of the plots \pm SE; $n = 4$). Target species are printed in bold.

Tabelle 3. Diasporenniederschlag der beiden Restitutionsflächen, von Mai 2010 bis April 2011 (year 1) und von Mai 2011 bis April 2012 (year 2) mit Trichterfällern (0,9 m über dem Boden) erfasst. Angegeben ist die Diasporenanzahl pro m² (MW der plots \pm SE; $n = 4$). Zielarten sind in Fettdruck.

Taxa	Site 1		Site 2	
	year 1	year 2	year 1	year 2
<i>Conyza canadensis</i>	1181 \pm 158	886 \pm 160	1821 \pm 1035	3330 \pm 327
<i>Betula pendula</i>	278 \pm 119	1925 \pm 948	54 \pm 4	222 \pm 99
<i>Salix spec.</i>	171 \pm 16	134 \pm 9	241 \pm 18	169 \pm 30
<i>Oenothera biennis</i>	1 \pm 1	73 \pm 32	1 \pm 1	200 \pm 43
<i>Alnus glutinosa</i>	21 \pm 12	126 \pm 90	12 \pm 7	95 \pm 45
<i>Typha latifolia</i>	46 \pm 21	6 \pm 5	70 \pm 21	15 \pm 2
<i>Melilotus albus</i>				133 \pm 46
<i>Chenopodium cf. album</i>	19 \pm 13	1 \pm 1	67 \pm 35	1 \pm 1
<i>Populus spec.</i>	14 \pm 5	6 \pm 2	5 \pm 1	5 \pm 2
<i>Phragmites australis</i>		1 \pm 1	3 \pm 2	23 \pm 10
<i>Sisymbrium altissimum</i>			28 \pm 27	
<i>Pinus sylvestris</i>	6 \pm 3	8 \pm 6	2 \pm 1	3 \pm 1
<i>Oxalis dillenii</i>			2 \pm 2	15 \pm 12
<i>Solanum nigrum</i>	7 \pm 3		7 \pm 3	2 \pm 1
<i>Cirsium arvense</i>	4 \pm 2	1 \pm 1	6 \pm 3	
<i>Epilobium spec.</i>	1 \pm 1	1 \pm 1	1 \pm 1	6 \pm 2
<i>Solidago canadensis</i>		3 \pm 2		3 \pm 2
<i>Tussilago farfara</i>		1 \pm 1	1 \pm 1	2 \pm 1
<i>Hypochaeris radicata</i>	2 \pm 2	1 \pm 1	1 \pm 1	
<i>Solidago gigantea</i>	1 \pm 1	1 \pm 1	1 \pm 1	
<i>Sonchus asper</i>	2 \pm 2	1 \pm 1		
<i>Taraxacum officinale</i> s.l.		2 \pm 1		1 \pm 1
<i>Corispermum leptopterum</i>			2 \pm 1	1 \pm 1

Taxa	Site 1		Site 2	
	year 1	year 2	year 1	year 2
<i>Atriplex sagittata</i>				3 ± 2
<i>Viola arvensis</i>			3 ± 1	
<i>Artemisia vulgaris</i>				3 ± 3
<i>Daucus carota</i>				3 ± 2
<i>Bromus tectorum</i>	1 ± 1	1 ± 1		
<i>Holcus lanatus</i>	1 ± 1	1 ± 1		
<i>Lactuca serriola</i>	1 ± 1	1 ± 1		
<i>Epilobium cf. ciliatum</i>	1 ± 1			1 ± 1
<i>Senecio spec.</i>		1 ± 1		1 ± 1
<i>Cirsium cf. vulgare</i>	2 ± 1			
<i>Vicia hirsuta</i>				2 ± 1
<i>Bryonia dioica</i>	1 ± 1			
<i>Epilobium lamyi</i>	1 ± 1			
<i>Hieracium pilosella</i>	1 ± 1			
<i>Rubus fruticosus</i>	1 ± 1			
<i>Corynephorus canescens</i>		1 ± 1		
<i>Digitaria sanguinalis</i>		1 ± 1		
<i>Rumex acetosella</i>		1 ± 1		
<i>Senecio vernalis</i>		1 ± 1		
<i>Apera spica-venti</i>			1 ± 1	
<i>Cardamine hirsuta</i>			1 ± 1	
<i>Chenopodium spec.</i>			1 ± 1	
<i>Humulus lupulus</i>			1 ± 1	
<i>Papaver dubium/rhoeas</i>			1 ± 1	
<i>Rumex thyrsoiflorus</i>			1 ± 1	
<i>Senecio inaequidens</i>			1 ± 1	
<i>Senecio cf. vulgaris</i>			1 ± 1	
<i>Berteroa incana</i>				1 ± 1
<i>Cerastium semidecandrum</i>				1 ± 1
<i>Erophila verna</i>				1 ± 1
<i>Medicago minima</i>				1 ± 1
<i>Poa compressa</i>				1 ± 1
<i>Rumex obtusifolius</i>				1 ± 1
<i>Sonchus cf. oleraceus</i>				1 ± 1
<i>Verbascum phlomoides</i>				1 ± 1
<i>Vicia cf. angustifolia</i>				1 ± 1
<i>Vicia cracca</i>				1 ± 1
<i>Vicia lathyroides</i>				1 ± 1
indetermined		1 ± 1	1 ± 1	
Sum	1761 ± 235	3184 ± 738	2336 ± 1014	4241 ± 379

3.4 Inoculation material

The air-dried inoculation material consisted by weight of 86% inorganic material (mainly sand), 12% cryptogam phytomass + litter and 2% plant material of phanerogams. By the seedling emergence method in total 50 phanerogam species were detected in the inoculation material. Thereof 26 species (69% of the seedlings) belonged to target species (Table 4); most frequently germinated *Saxifraga tridactylites*, *Arenaria serpyllifolia* agg. and *Koeleria glauca*. Ruderal species accounted for 34% of the species (26% of the seedlings); *Digitaria sanguinalis* was most frequently detected. Seven species (14%; seedlings: 5%) were not classified as target or ruderal. According to functional groups, forbs dominated with 37 species (72%; seedlings: 67%), followed by graminoids with 12 species (24%; seedlings: 30%), one woody plant and one fern (2%; seedlings: 3% and 0.2%, respectively). The fern can most likely be ascribed to a contamination.

Table 4. Plant species detected in the inoculation material. The number of seedlings per m² is given (mean ± SE; n = 16). Target species are printed in bold.

Tabelle 4. Im Inokulationsmaterial erfasste Pflanzenarten. Angegeben ist die Anzahl von Keimlingen pro m² (MW ± SE; n = 16). Zielarten sind in Fettdruck.

Taxa	No. of seedlings per m ²	Taxa	No. of seedlings per m ²
<i>Amaranthus retroflexus</i>	0,2 ± 0.1	<i>Petrorhagia prolifera</i>	0,4 ± 0.2
<i>Arenaria serpyllifolia</i> agg.	18,9 ± 2.0	<i>Phleum arenarium</i>	6,4 ± 1.4
<i>Artemisia campestris</i>	0,6 ± 0.2	<i>Phleum phleoides</i>	0,2 ± 0.1
<i>Asplenium ruta-muraria</i>	0,3 ± 0.1	<i>Poa compressa</i>	0,1 ± 0.1
<i>Betula pendula</i>	4,2 ± 1.5	<i>Potentilla argentea</i>	0,2 ± 0.2
<i>Bromus tectorum</i>	0,1 ± 0.1	<i>Rumex acetosella</i>	0,1 ± 0.1
<i>Carex hirta</i>	0,1 ± 0.1	<i>Salix spec.</i>	1,0 ± 0.3
<i>Centaurea stoebe</i> s.l.	0,8 ± 0.2	<i>Salsola kali</i> subsp. <i>tragus</i>	0,6 ± 0.2
<i>Cerastium semidecandrum</i>	5,4 ± 0.7	<i>Saxifraga tridactylites</i>	70,1 ± 6.3
<i>Chenopodium album</i> agg.	0,6 ± 0.2	<i>Sedum acre</i>	0,2 ± 0.1
<i>Comyza canadensis</i>	3,5 ± 0.6	<i>Setaria viridis</i>	35,0 ± 5.3
<i>Corynephorus canescens</i>	0,1 ± 0.1	<i>Silene conica</i>	6,0 ± 1.1
<i>Digitaria sanguinalis</i>	0,1 ± 0.1	<i>Silene otites</i>	0,1 ± 0.1
<i>Diplotaxis tenuifolia</i>	0,1 ± 0.1	<i>Sonchus asper</i>	0,2 ± 0.1
<i>Echium vulgare</i>	0,3 ± 0.1	<i>Sonchus cf. oleraceus</i>	0,3 ± 0.2
<i>Eragrostis minor</i>	0,3 ± 0.1	<i>Stellaria media</i>	0,1 ± 0.1
<i>Erigeron annuus</i>	0,1 ± 0.1	<i>Taraxacum officinale</i> s.l.	0,4 ± 0.2
<i>Erophila verna</i>	0,3 ± 0.1	<i>Urtica dioica</i>	0,2 ± 0.1
<i>Geranium robertianum</i>	0,2 ± 0.1	<i>Verbascum phlomoides</i>	3,1 ± 0.8
<i>Helichrysum arenarium</i>	0,3 ± 0.1	<i>Veronica arvensis</i>	2,9 ± 0.5
<i>Holosteum umbellatum</i>	0,3 ± 0.1	<i>Veronica praecox</i>	0,4 ± 0.2
<i>Hypericum perforatum</i>	0,1 ± 0.1	<i>Veronica verna</i>	0,2 ± 0.1
<i>Koeleria glauca</i>	9,1 ± 1.4	<i>Vicia lathyroides</i>	0,1 ± 0.1
<i>Koeleria macrantha</i>	0,5 ± 0.2	<i>Vulpia myuros</i>	0,1 ± 0.1
<i>Medicago minima</i>	0,3 ± 0.1	indetermined (forb)	2,1 ± 0.3
<i>Oenothera biennis</i>	0,6 ± 0.2	indetermined (graminoid)	0,9 ± 0.4
<i>Ononis repens</i>	0,2 ± 0.1	Sum	178,6 ± 12.9

3.5 Vegetation development

3.5.1 Occurrence of target species

Inoculation had a significant positive effect on the occurrence of target species (Fig. 2; Table 5; Supplement S1). From the first to the second study year the number of target species significantly increased in inoculated plots on both restoration sites; thereafter it remained unchanged. In the first year a total of 16 to 18 (ungrazed/grazed; total: 18) target species were recorded on S1 and 24 to 26 (ungrazed/grazed; total: 27) on S2; the numbers increased to 27 and 25 (ungrazed/grazed; total: 29) on S1 and 32 target species (both ungrazed and grazed; total: 35) on S2 in the last year. Grazing had no significant effect on target species numbers. On S1 the number of target species was lower than on S2 throughout the studied period.

On plots without inoculation, the number of target species increased steadily over the four-year study period (Fig. 2). Already in the first year two target species were recorded on S1 (both on ungrazed and grazed plots), 13 and nine (ungrazed/grazed; total 15) target species were found on S2. In the last studied year, the numbers rose to ten and seven on S1 (ungrazed/grazed; total: 12) and to 20 and 21 on S2 (ungrazed/grazed; total: 24). Most frequently detected target species across all not-inoculated plots and all years were on S1 *Erodium cicutarium* and *Tortula ruraliformis*, and on S2 *Medicago lupulina* and *Trifolium arvense*. On the grid plots almost completely the same target species were detected as on the study plots. One species, *Myosotis stricta*, was exclusively found on grid plots (S2). The number of target species recorded on all grid plots increased from 2010 (S1: 8, S2: 12) to 2013 (S1: 21, S2: 29). Merging the target species numbers of not-inoculated plots and grid-plots in all years, a total of 37 and 42 target species were detected on S1 and S2, respectively (Supplement S1). Of these, two species were exclusively found on S1 and one species on S2. On each of the two restoration sites 11 target species could not be detected outside the inocu-

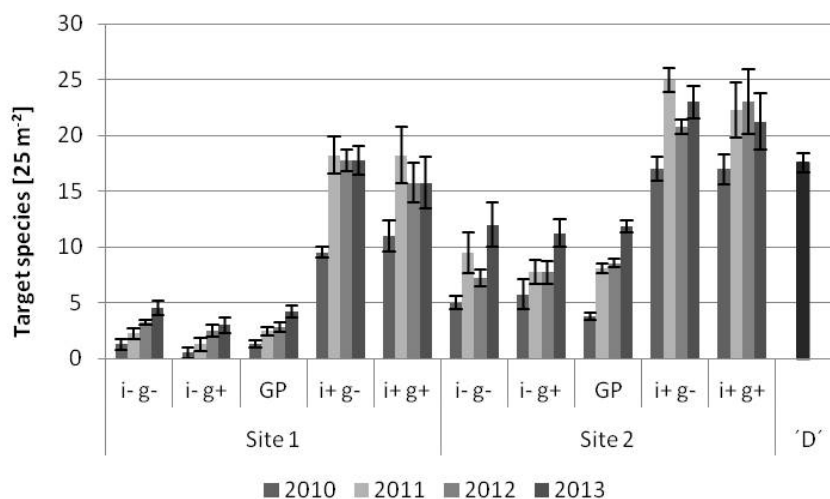


Fig. 2. Target species numbers (mean \pm SE) on the two restoration sites under different treatments (2010–2013). For abbreviations see Figure 1; D = donor site.

Abb. 2. Zielartenanzahl (MW \pm SE) der beiden Restitutionsflächen unter verschiedenen Behandlungen (2010–2013). Für Abkürzungen siehe Abbildung 1; D = Spenderfläche.

Table 5. Effects of site, inoculation with plant material (= inoc), grazing and year on various dependent variables as tested by linear mixed models. TSRqual = qualitative target species ratio; TSRquant = quantitative target species ratio (see 'Methods' section), $F = F$ value, $P =$ significance level. The values of the degrees of freedom numerator (df num) and degrees of freedom denominator (df de) are separated by the effect of 'year'; target species number(df num: 1, 3; df de: 25.7, 72); TSRqual(df num: 1, 3; df de: 28.9, 31.1); TSRquant(df num: 1, 3; df de: 30.4, 43.6); open ground(df num: 1, 3; df de: 24, 22); phanerogam cover (df num: 1, 3; df de: 24, 72) and cryptogam cover (df num: 1, 3; df de: 24, 22).

Tabelle 5. Effekte von Fläche (= site), Inokulation mit Pflanzenmaterial (= inoc), Beweidung (= grazed) und Jahr (= year) auf verschiedene abhängige Variablen, getestet mit gemischten linearen Modellen. TSRqual = qualitativer Zielartenindex; TSRquant = quantitativer Zielartenindex (siehe Abschnitt 'Methods'). $F = F$ -Wert, $P =$ Signifikanzniveau. Die Werte der Zählerfreiheitsgrade (df num) und Nennerfreiheitsgrade (df de) sind durch den Effekt von 'Jahr' getrennt; 'target species number' (df num: 1, 3; df de: 25.7, 72); TSRqual(df num: 1, 3; df de: 28.9, 31.1); TSRquant (df num: 1, 3; df de: 30.4, 43.6); 'open ground' (df num: 1, 3; df de: 24, 22); 'phanerogam cover' (df num: 1, 3; df de: 24, 72) und 'cryptogam cover' (df num: 1, 3; df de: 24, 22).

	target species no.		TSRqual		TSRquant		open ground		phanerogam cover		cryptogam cover	
	F	P	F	P	F	P	F	P	F	P	F	P
site	37.41	<0001	0.4	0.5321	0.27	0.6068	22.21	<0001	10.67	0.0033	31.58	<0001
inoc	188.04	<0001	647.2	<0001	429.19	<0001	10.86	0.0031	0.51	0.4827	43.2	<0001
site*inoc	0.03	0.8707	45.71	<0001	4.83	0.0358	0	0.9923	0.01	0.9275	0.29	0.5976
grazed	0.43	0.5166	20.67	<0001	24.63	<0001	9.09	0.006	1.39	0.25	98.85	<0001
site*grazed	0.04	0.8451	0.03	0.8603	0.03	0.8642	2.75	0.1104	2.05	0.1651	0.05	0.8169
inoc*grazed	0	0.974	1.9	0.1784	35.59	<0001	2.33	0.1397	0.92	0.3465	5.51	0.0274
site*inoc*grazed	0.03	0.8707	4.44	0.0438	3.98	0.0551	1.66	0.2099	2.65	0.1167	0.76	0.3916
year	45.32	<0001	91.78	<0001	3.55	0.022	46.68	<0001	3.47	0.0204	37.75	<0001
site*year	1.14	0.3393	2.41	0.0858	1.4	0.2543	2.5	0.086	5.94	0.0011	7.44	0.0013
inoc*year	11.33	<0001	6.04	0.0023	0.82	0.4916	7.6	0.0012	0.56	0.6422	5.29	0.0067
site*inoc*year	2.29	0.086	0.69	0.5631	3.29	0.0293	0.4	0.7539	0.75	0.5236	1.27	0.3096
grazed*year	1.85	0.1455	4.13	0.0142	5.09	0.0041	27.36	<0001	1.29	0.2853	19.36	<0001
site*grazed*year	1.76	0.1633	2.46	0.0809	1.98	0.1313	3.38	0.0364	1.51	0.2199	1.79	0.1793
inoc*grazed*year	0.2	0.8964	0.48	0.7012	7.4	0.0004	3.06	0.0493	0.49	0.6909	13.38	<0001
site*inoc*grazed*year	0.88	0.4553	0.12	0.9494	1.66	0.1895	1.7	0.1971	1.07	0.3663	2.93	0.0563

lated plots. (Supplement S1). Of these, two species were exclusively found on S1 and one species on S2. On each of the two restoration sites 11 target species could not be detected outside the inoculated plots.

3.5.2 Target species ratios

The qualitative target species ratio, indicating the proportion of target species to the total species number, was significantly enhanced by inoculation on both restoration sites, although more so on S1 than on S2 (Fig. 3; Table 5). The increase in TSR_{qual} on inoculated plots from 2010 to the next years was significant and came from an increase of target species on S1 and from a combination of increased target species and a decrease in total species numbers on S2. Grazing on inoculated plots resulted in a significantly lower TSR_{qual} on S2 than on S1. The TSR_{qual} of the not-inoculated plots was higher on S2 than on S1, due to a greater proportion of target species on S2. Reasons for the increase in TSR_{qual} of the not-inoculated plots are the same as on inoculated plots. The TSR_{qual} of the grid plots is in the range of the values found on not-inoculated plots of the particular restoration site; on S1 the grid plots have slightly higher values than the not-inoculated, grazed plots.

The inoculation-induced increase of the TSR_{quant} , taking plant cover into account, was more pronounced than of the TSR_{qual} (Fig. 4; Table 5). Inoculation enhanced the TSR_{quant} on S1 significantly more than on S2; on S2 the cover of non-target species was higher overall, which decreased the value. Grazing significantly reduced the TSR_{quant} on inoculated plots dependent on year. To a major extent, this decline was related to a reduction of cover of the bryophyte target species *Tortula ruraliformis*. Not-inoculated plots had very low TSR_{quant} on both restoration sites; grazing on S2 increased the ratio during the four study years. On grid plots, the TSR_{quant} corresponds to those of the not-inoculated plots of the particular restoration site.

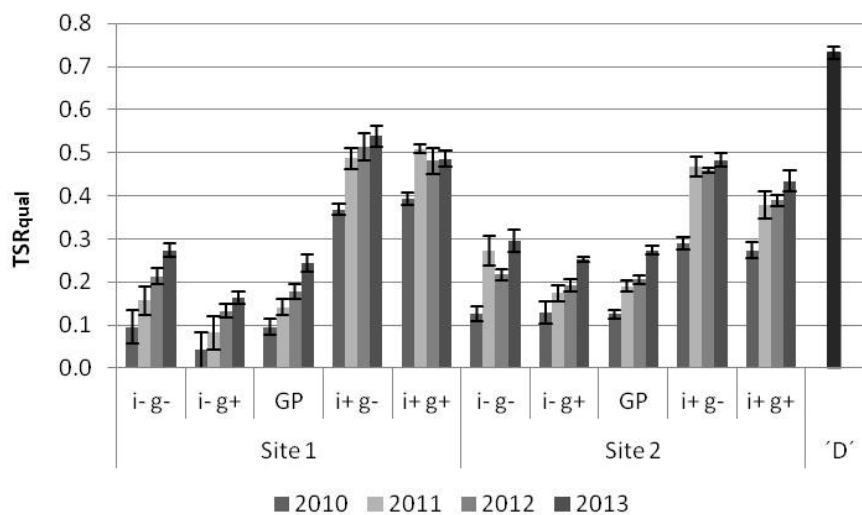


Fig. 3. Qualitative target species ratio (mean \pm SE) of the two restoration sites under different treatments (2010–2013) and of the donor site 'D' (mean of 2006–2008). For abbreviations see Figure 1.

Abb. 3. Qualitativer Zielartenindex (MW \pm SE) der beiden Restitutionsflächen unter verschiedenen Behandlungen (2010–2013) und der Spenderfläche 'D' (MW von 2006–2008). Für Abkürzungen siehe Abbildung 1.

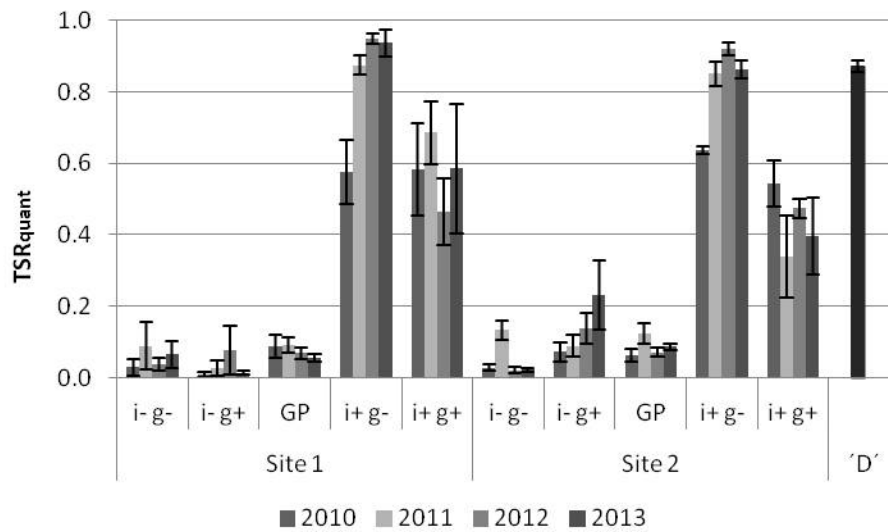


Fig. 4. Quantitative target species ratio (mean ± SE) on the two restoration sites under different treatments (2010–2013) and of the donor site 'D' (mean of 2006–2008). For abbreviations see Figure 1.

Abb. 4. Quantitativer Zielartenindex (MW ± SE) der beiden Restitutionsflächen unter verschiedenen Behandlungen (2010–2013) und der Spenderfläche 'D' (MW von 2006–2008). Für Abkürzungen siehe Abbildung 1.

3.5.3 Cover of functional groups

The proportion of open ground remained high on grazed plots on S1, but significantly decreased on S2 despite grazing during the study time (Fig. 5; Table 5). Grazing on inoculated plots had a significant effect on cover of open ground dependent on year. On S1 grazed plots had similar cover of open ground since the second year, whether they had been inoculated or not. On S2 the cover on inoculated plots was reduced by grazing only in the second year. On not-grazed plots the proportion of open ground declined from the first to the last study year. Grid plots had a proportion of open ground comparable, although slightly higher, to not-inoculated and grazed plots.

The cover of phanerogams on the two restoration sites was only significantly different according to site and year (Table 5). In total, phanerogam cover was higher on S2 than on S1 (Fig. 6). On S1 cover tended to increase during the study (except for inoculated, grazed plots); on S2 the cover fluctuated between the years. Grazing on inoculated plots of S1 reduced cover of phanerogams, whereas the cover on S2 was highest on these plots. On S1 the grid plots had the lowest cover of phanerogams, on S2 were in the range of not-inoculated plots.

Inoculation had already enhanced cover of cryptogams in the first study year (Fig. 7; Table 5). Without grazing, cover significantly increased during the study period, achieving 73% on S1 and 90% on S2 in 2013. Grazing had a significantly decreasing effect on cover of inoculated plots, but the decrease was dependent on year. Since 2012, the cryptogam cover of not-inoculated plots increased on both restoration sites, the increase being significantly

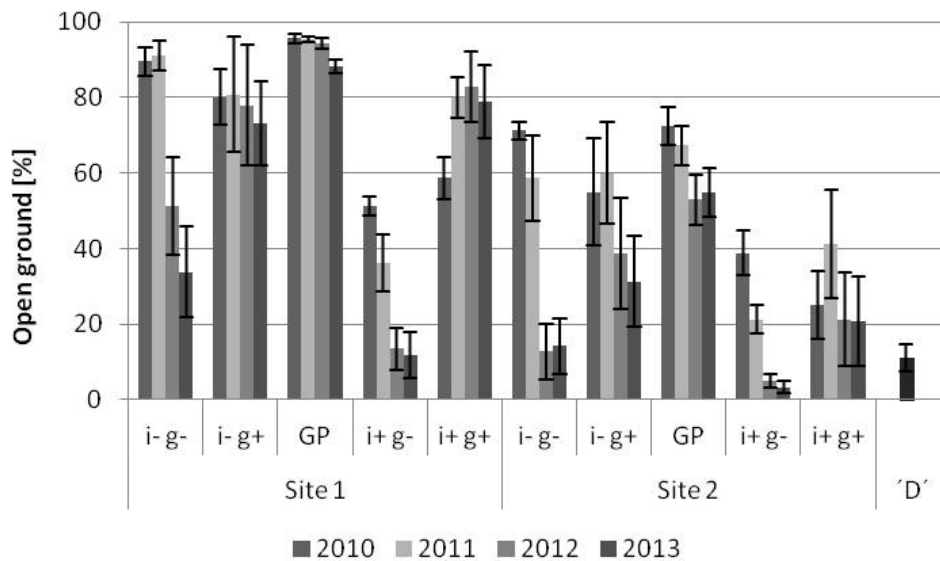


Fig. 5. Cover of open ground (mean ± SE) on the two restoration sites under different treatments (2010–2013) and of the donor site 'D' (mean of 2006–2008). For abbreviations see Figure 1.

Abb. 5. Offenbodendeckung (MW ± SE) der beiden Restitutionsflächen unter verschiedenen Behandlungen (2010–2013) und der Spenderfläche 'D' (MW von 2006–2008). Für Abkürzungen siehe Abbildung 1.

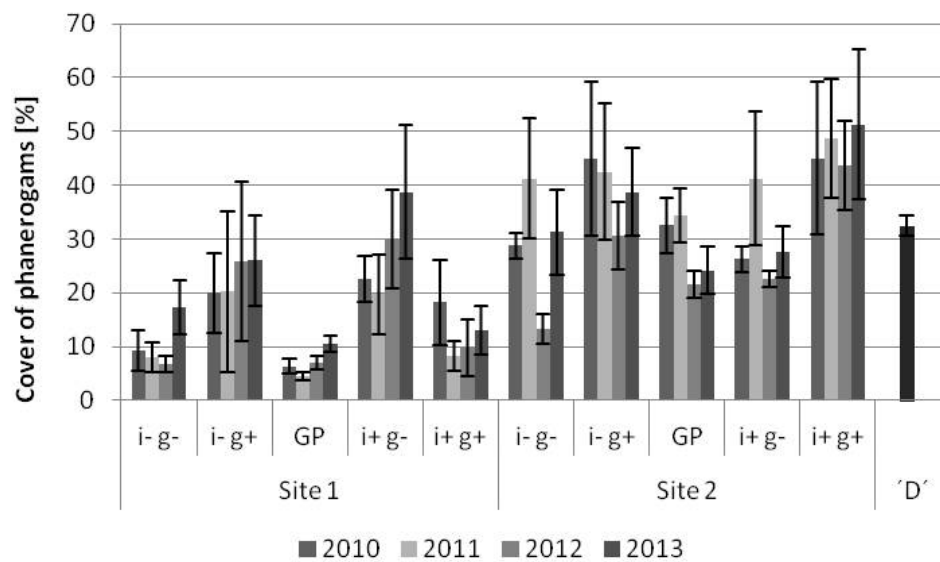


Fig. 6. Cover of phanerogams (mean ± SE) on the two restoration sites under different treatments (2010–2013) and of the donor site 'D' (mean of 2006–2008). For abbreviations see Figure 1.

Abb. 6. Deckung der Phanerogamen (MW ± SE) der beiden Restitutionsflächen unter verschiedenen Behandlungen (2010–2013) und der Spenderfläche 'D' (MW von 2006–2008). Für Abkürzungen siehe Abbildung 1.

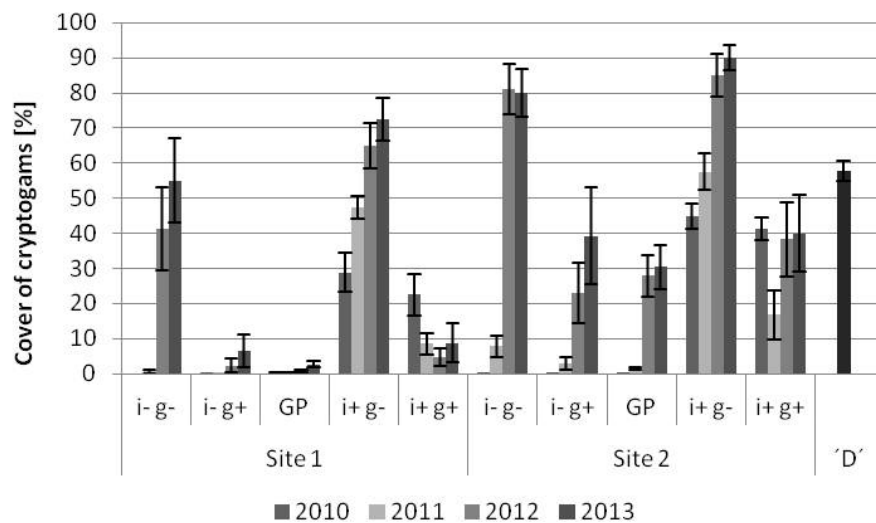


Fig. 7. Cover of cryptogams (mean \pm SE) on the two restoration sites under different treatments (2010–2013) and of the donor site 'D' (mean of 2006–2008). For abbreviations see Figure 1.

Abb. 7. Deckung der Kryptogamen (MW \pm SE) der beiden Restitutionsflächen unter verschiedenen Behandlungen (2010–2013) und der Spenderfläche 'D' (MW von 2006–2008). Für Abkürzungen siehe Abbildung 1.

stronger on S2. Again, the cover was negatively affected by grazing; the impact was greater on S1 than on S2. According to the grid plots the cover of cryptogams corresponds to the cover on not-inoculated, grazed plots of the particular restoration site.

3.5.4 Spreading patterns

The total number of target species was higher on grid plots of S2 than of S1 in 2013 (Fig. 8a), but on both restoration sites no significant effect was found for the distance from the grid plots to the next inoculated plot in any year. Distance had also no significant effect on the qualitative TSR regarding restoration site or year; the TSR_{qual} was very similar on both restoration sites in 2013 (Fig. 8b). Only with regard to the quantitative TSR (Fig. 8c) did distance from the grid plots to the next inoculated plot lead to a significant decrease of the TSR_{quant} on S1 in 2012 ($r_s = -0.4314$, $p = 0.0398$) and 2013 ($r_s = -0.5191$, $p = 0.0111$), but not in the previous years and not on S2.

3.6 Comparison of species composition

Nearly all species (35 species, 90%) recorded on the donor site (vegetation relevés of 2006–2008) were found in the inoculated plots (Supplement S1); only four species were not detected in the inoculated plots (two target species: *Alyssum alyssoides* and *Myosotis stricta*). An additional 20 target species were exclusively found on the recipient plots, whereof two were only found on S1 and ten on S2. Three of these 20 species were on the Red List (S1: one species; S2: two species; KORNECK et al. 1996).

Comparing the target species ratios of the restoration sites and the donor site revealed differences in qualitative and quantitative TSRs. The mean TSR_{qual} of inoculated plots, with values of 0.54 to 0.49 (ungrazed/grazed) on S1 and 0.48 to 0.43 (ungrazed/grazed) on

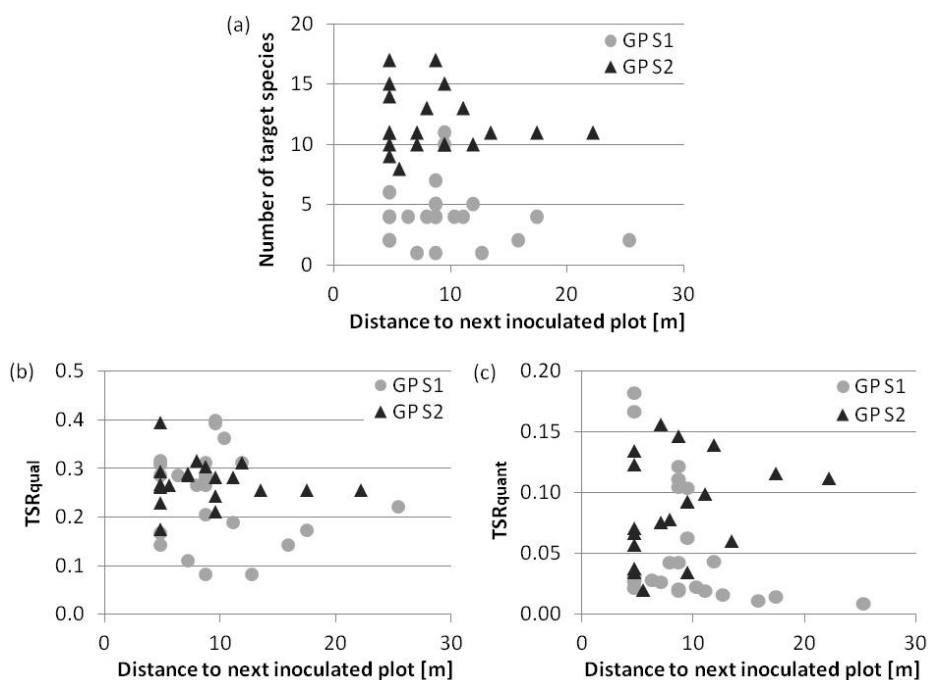


Fig. 8. Relations of (a) target species number; (b) TSR_{qual} ; and (c) TSR_{quant} of the grid plots to distance to the next inoculated plot for the year 2013. GP S1 = grid plots restoration site 1; GP S2 = grid plots restoration site 2.

Abb. 8. Beziehung von (a) Zielartenanzahl; (b) TSR_{qual} ; und (c) TSR_{quant} der Rasterpunkte zu der Entfernung zum nächsten inokulierten Plot im Jahr 2013. GP S1 = Rasterpunkte Restitutionsfläche 1; GP S2 = Rasterpunkte Restitutionsfläche 2.

S2 (in 2013), was still lower than that on the donor site (0.73; mean of 2006–2008; Fig. 3). In contrast, the TSR_{quant} of inoculated and ungrazed plots had values equal to (S2: 0.86) or even higher than (S1: 0.94) those of the donor site (0.87; mean of 2006–2008; Fig. 4).

Detrended correspondence analysis revealed a clear separation of the donor site, the restoration sites (S1, S2 and RS) and the seed bank and seed rain along the first axis (Fig. 9). The plots of the donor site are closely grouped together on the left side, seed bank and seed rain of the restoration sites are arranged at the opposite side. All restoration sites are located in between, the inoculated plots and the older restoration site being grouped closer to the donor site than not-inoculated plots. The trajectories of both inoculated (incl. RS) and not-inoculated plots tend towards the donor site. The changes in community structure are most pronounced from 2010 to 2011. The second axis separates the two restoration sites; especially according to inoculation and seed rain. The not-inoculated plots of S1 are clearly separated from the inoculated plots and those of S2.

3.7 Endozoochory

A total of 88 seedlings of 10 species emerged from the endozoochory samples (8 l; dry weight 1740 g). 60% of these were target species, accounting for 54% of the seedlings. The recorded target species were (ordered by frequency) *Arenaria serpyllifolia* agg., *Rumex ace-*

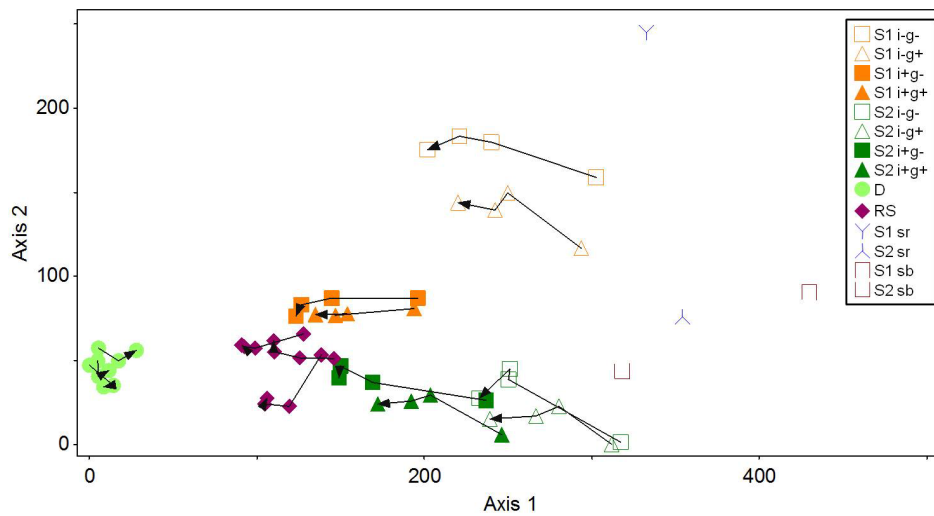


Fig. 9. Detrended correspondence analysis (DCA) of the restoration sites S1 and S2 under different treatments (2010–2013), soil seed bank and seed rain data of the restoration sites, the donor site 'D' (2006–2008) and another restoration site ('RS'; 2005–2008). Time points of experimental plots, donor site and 'RS' are connected by trajectories. Eigenvalues and in parentheses the percentage of explained variance: axis 1: 0.45 (54.7%), axis 2: 0.15 (5.1%), axis 3: 0.12 (10.7%). For abbreviations see Figure 1; sr = seed rain, sb = seed bank.

Abb. 9. Detrended correspondence analysis (DCA) der Restitutionsflächen S1 und S2 unter verschiedenen Behandlungen (2010–2013), von Diasporenbank des Bodens und Diasporenniederschlag der Restitutionsflächen, der Spenderfläche 'D' (2006–2008) und einer weiteren der Restitutionsfläche ('RS'; 2005–2008). Trajektorien verbinden die untersuchten Jahre von Untersuchungsplots, Spenderfläche und 'RS'. Eigenvalues und in Klammern der Anteil der erklärten Varianz: Achse 1: 0,45 (54,7 %), axis 2: 0,15 (5,1 %), axis 3: 0,12 (10,7 %). Für Abkürzungen siehe Abbildung 1; sr = Diasporenniederschlag, sb = Diasporenbank des Bodens.

tosella s.l., *Potentilla argentea* agg., *Cerastium semidecandrum*, *Silene conica* and *Phleum arenarium*. Ruderal species accounted for 30% of the species and 44% of the seedlings; *Bromus tectorum* most frequently emerged, followed by *Poa angustifolia*. Forbs accounted for 56% of all seedlings, grasses had a proportion of 44%. All species detected in the samples (except for *Plantago major*) were present in the vegetation of restoration site 1.

4. Discussion

4.1 What is the impact of substrate condition on development of sandy grassland during four years?

Substrate condition could be shown to have impacts on phanerogam and cryptogam cover, cover of open ground and on the number of detected target species. Thereby, open ground was reduced and the other parameters were enhanced on restoration site 2 compared to S1. These differences in development of the two restoration sites may be caused by the differences in nutrient status or in the seed bank of the soil substrates.

However, the investigated soil parameters were, except for phosphate on S2, in the range of or even below values measured in target communities of our study area and hence suitable for the re-establishment of calcareous sandy grassland. The pH in both deposited substrates was within the range of values measured in stands of *Jurineo-Koelerietum* and *Allio-Stipetum* (SÜSS et al. 2004). Total nitrogen differed on the two restoration sites with slightly higher values on S2, but overall values were below the $0.28 \pm 0.12 \text{ g kg}^{-1}$ (mean \pm SD; 10–30 cm) measured in sandy grasslands in this region (SÜSS et al. 2004). The same applies to mineral N values quantified by LINGEN (2013) on the restoration sites (0.7 to 0.8 kg N ha^{-1} on S1 and S2, respectively), which were below 3.8 kg N ha^{-1} (0–10 cm) reported by STORM et al. (1998) in stands of *Allio-Stipetum*. Only the concentration of phosphate-P, which is typically below $15\text{--}20 \text{ mg kg}^{-1}$ in our study area (STORM et al. 1998), exceeded this threshold on average slightly on S2, but some samples achieved values up to 50 mg P kg^{-1} . Above $40\text{--}50 \text{ mg kg}^{-1}$, P is considered to be no more a limiting nutrient in agriculture (SCHEFFER & SCHACHTSCHABEL 2002).

The effects of higher P concentrations on vegetation can be, e.g., increased biomass, loss of diversity, especially of endangered species and a facilitation of competitive graminoids (CARROLL et al. 2003, SÜSS et al. 2004, WASSEN et al. 2005, HEJCMAN et al. 2010). As yet none of these effects can be definitely ascertained for S2 for the investigated time period. The P concentration may have had an influence on the higher vegetation cover on S2 in the first years, e.g. by facilitating legumes (BOBBINK 1991, JANSSENS et al. 1998) like *Melilotus albus* which achieved high cover values. Competitive graminoids were detected on both restoration sites; the frequency and cover increased slightly on S2 during the last study years (data not presented, but see Supplement S1).

The factor with a probably stronger differentiating effect for the two substrates in the initial stages is the seed bank. The soil seed bank of S1 was, as expected (EICHBERG et al. 2010), extremely poor in species and quantity of seeds. Deep sand of below 1 m depth should be nearly free of seeds as the number of viable seeds declines with soil depth (GODEFROID et al. 2006). S2 had an around five-fold higher seed density and more species than S1; at the same sampling depth in *Koelerion glaucae* stands there were comparable seed densities and even fewer species (EICHBERG et al. 2006). The temporary above-ground storage almost certainly caused a contamination of the substrate with seeds, since nearly all species detected in the seed bank were recorded in the first-year vegetation of S2. Even though a strikingly high number of target species was detected on this site - even without inoculation - the high proportion of ruderal species is counterproductive for restoration efforts. Admittedly, the first years of many restoration projects are characterized by weedy annuals (e.g. JONGEPIEROVÁ et al. 2007) but a high proportion of non-target species remained on S2 even in the fourth year.

4.2 Is the inoculation of small plots sufficient to restore sandy grassland in a larger area in a period of four years?

Application of plant material resulted in a punctual increase of target species number and qualitative and quantitative target species ratios on the inoculated plots since the first studied year. In comparison, on the whole restoration area (i.e. on not-inoculated plots and on grid-plots) these parameters, despite their increasing values, remained much lower, showing that the large-scale restoration of sandy grassland cannot be achieved with this approach in a four-year period but certainly takes more time.

That the application of plant material following abiotic restoration enhances the occurrence of target species was already reported in many other restoration projects (HÖLZEL & OTTE 2003, KIEHL et al. 2006, DONATH et al. 2007, EDWARDS et al. 2007). Quantity and cover of target species on inoculated plots corresponded to the values of the donor site (cf. EICHBERG et al. 2010). Inoculated plots developed in the direction of the donor site, but differences remained after four years. Not-inoculated plots developed in the same direction, though the distance to the donor site was greater than in the case of inoculated plots. Approximation of these plots to the donor site may be related to an immigration of target species from inoculated plots to not-inoculated plots (and the surrounding area). During the four study years on both restoration sites 70% (S1) to 73% (S2) of all recorded target species were at least once detected outside of inoculated plots. The target species which were not recorded outside of inoculated plots were mostly species found only with few individuals. BURMEIER et al. (2011) described as well rather slow colonization velocities in a flood-meadow restoration project, although almost 95% of species had spread from plant material strips 7–8 years after restoration.

In contrast to BURMEIER et al. (2011) the distance between inoculated plot and 'receiver'-plot was not found to be a determining factor for the number of target species, which can have different explanations. On the one hand, the soil seed bank can be - even though only few target species were recorded on S2 - a source for target species irrespective of inoculation. Also, plant material that fell down during the inoculation can be considered as source for target species. These two sources can explain the occurrence of target species which were detected outside of inoculated plots already in the first year. On the other hand, the analysed dispersal vectors can contribute to an unpredictability of dispersal away from inoculation plots. Via endozoochory seeds can be transported over several kilometres by large herbivores (PAKEMAN et al. 2002). Seed rain is also an undirected vector, but it was, in accordance with other restoration projects (STROH et al. 2002, FREUND et al. 2014), dominated by non-target species. Nevertheless, the inoculated plots may serve as a propagule source for target species even though seeds (or dispersal units of bryophytes) were not detected in the seed rain. As the dispersal distances of many target species are only low (JENTSCH & BEYSCHLAG 2003), propagules might not have reached the seed traps.

Another factor relevant for evaluating the restoration success is, apart from the quantity of target species, their cover. During the four investigated years the increase in quantity of target species on not-inoculated plots had nearly no effect on enhancing their proportion in total cover. However, this may be linked to the observation in the grid-plot approach that the quantitative TSR declined with distance to the next inoculated plot (at least on S1 in the last two years). In the near vicinity of inoculated plots it might be more likely that target species reach higher cover values, because of the above mentioned low dispersal distance of many target species. This might especially apply to many therophytic and/or early successional target species, e.g. *Silene conica*, as they have only low growth-heights or rather small canopies. High cover values can be reached by these species only when occurring in high abundance. Tall-growing target species forming greater canopies emerged almost exclusively on inoculated plots, e.g. *Centaurea stoebe* and *Artemisia campestris*. The high target-species cover reported on the donor site is correlated with a very low quantity and cover of non-target species. Once again, time will probably play a crucial role in enhancing target-species cover especially of phanerogams. For enhanced target bryophyte cover the grazing regime has to be adapted (see section 4.4 below).

4.3 Which role does endozoochorous dispersal by donkeys play for target species?

Dung of free-ranging herbivores is an important dispersal agent for various plant species both in terms of quantity of seeds and of dispersed species (MOUISSIE et al. 2005b). In our study only about 10% of species recorded in the actual vegetation were detected in the dung samples, whereas in other studies around one-quarter (COSYNS et al. 2005; horse, cattle) to one-third (STROH et al. 2012; horse) of species present in the background vegetation were recorded in dung. Because our dung samples were collected only at a single sampling date, a prolonged sampling period would presumably have had enhanced the number of detected species and thus have displayed the overall endozoochorous dispersal potential more accurately. A study conducted in a dune system by BAKKER et al. (2008) revealed a maximum of species (and seed density) detected in cattle dung in July to September. Nevertheless, a high proportion of target species found in the dung samples is remarkable and the sampling date may have contributed to this. At least for two target species recorded in endozoochory samples, establishment next to dung accumulations was observed on the restoration sites (personal observation). These species, *Silene conica* and *Phleum arenarium*, certainly originated from the inoculation material and were only recorded outside of inoculated plots since the second to third study year. The high proportion of open ground on the restoration sites may contribute to improved establishment following dispersal (see COSYNS et al. 2006).

4.4 How does grazing by donkeys affect the vegetation development?

After four study years grazing had effects on vegetation cover of the restoration sites mainly by maintaining a high proportion of open ground and hampering the establishment of a bryophyte layer (mainly acrocarpous bryophytes). The reduction of a pleurocarpous moss, forming dense mats in later successional stages of sandy grassland, was observed by STROH (2006) and EICHBERG et al. (2010) and evaluated positively. However, on inoculated plots grazing led to a severe reduction of target bryophyte cover and thus had a negative effect on total cover of target species. We infer from this that the grazing period was too long and/or the stocking density was too high. In the case of our restoration sites the sandy substrate, which is not really solid, has to be taken into account; the donkeys always sank in a bit while grazing. Especially on frequently trampled sites this might also reduce the establishment success of seedlings.

Effects on vegetation composition like enhanced species diversity or facilitation of desired species (RASRAN et al. 2007, PLASSMANN et al. 2010) could so far not be observed. In later successional stages opening of a dense sward and the creation of gaps probably have a positive effect on diversity of species and habitats, but in our initial successional stage gaps for establishment are not a limiting factor. An effect of grazing on the quantity of target species or their proportion of total species number was not detected. Additionally, grazing did not result in enhanced cover of target species in relation to total cover as described by EICHBERG et al. (2010). A reduction of (high-growing) ruderal species as reported by STROH et al. (2007; for sheep) could not be ascertained. In contrast, two dominant ruderal species, *Oenothera biennis* (S1) and *Melilotus albus* (S2), were only slightly grazed by donkeys, which prefer a diet of graminoids (COSYNS et al. 2001). In contrast, sheep are known to graze these species preferentially (STROH et al. 2002), therefore a mixed grazing management might have been useful to reduce ruderal species more intensively.

5. Concluding remarks

Our results show that, on the abiotic site, the deposition of deep sand can be used as an effective restoration measure in sand ecosystems to reduce soil nutrients and undesired seed bank species. On the biotic site, we could show that using only small amounts of plant material applied in distributed patches can be a very useful tool to overcome seed limitation and establish source or 'starter' populations for target species in restoration projects. Grazing by donkeys should be carefully applied with respect to trampling, but has the potential to disperse high proportions of target species when grazing management is planned according to the peak season of target species' seed ripening.

Erweiterte deutsche Zusammenfassung

Einleitung – In Zentraleuropa sind Bestände des *Koelerion glaucae*-Vegetationskomplexes bedroht und zumeist hochgradig fragmentiert. Sie haben einen hohen Schutzstatus durch die Fauna-Flora-Habitat-Richtlinie der Europäischen Union (Natura 2000-Code 6120). Kenntnisse zur Wirkung abiotischer und biotischer Restitutionsmaßnahmen sind essentiell für die Restitution dieses Vegetationskomplexes.

Daher wurde für eine Modellstudie nördlich von Darmstadt (Hessen) im Jahr 2009 ein Dünenkomplex aus basenreichem Tiefensand im Rahmen von Ausgleichsmaßnahmen (u.a. durch ein Bauprojekt in der Nähe) geschaffen, der aus zwei verschiedenen Substratqualitäten besteht. In unserem Feldexperiment war es möglich, auf beiden Substrattypen die Behandlungen „Inokulation mit Pflanzenmaterial von Leitbildflächen“ und „Beweidung durch Esel“ zu untersuchen. Dabei wurde der Ansatz der „Minimal-Inokulation“ auf nur kleinen Flächen erprobt. Hintergrund dafür ist, dass die raren Vorkommen des *Koelerion glaucae*-Komplexes im Gebiet der nördlichen hessischen Oberrheinebene nur sehr eingeschränkt zur Entnahme von Pflanzenmaterial zur Verfügung stehen.

Die Fragestellungen dieser Arbeit sind: (1) Welchen Einfluss hat die Substratqualität auf die Entwicklung von Sandrasen über einen Zeitraum von vier Jahren? (2) Ist die Inokulation von kleinen Flächen (Minimal-Inokulation) ausreichend, um Sandrasen eines größeren Dünenkomplexes zu restituieren? (3) Welche Rolle spielt Endozoochorie durch Esel für die Ausbreitung von Zielarten? (4) Wie beeinflusst die Beweidung durch Esel die Vegetationsentwicklung?

Material und Methoden – Die Restitutionsfläche 'Apfelbachdüne' (8°35' E, 49°56' N, Fig. 1) wurde mit einem künstlich aufgebauten Dünenkomplex aus Tiefensand, der aus laufenden Bauprojekten stammte, geschaffen. Eine Teilfläche des Komplexes (S1) erhielt Sand mit niedrigem Nährstoffstatus (1,1 ha), ein weiterer (S2; 0,8 ha) besteht aus Sand mit höheren Phosphatwerten.

Insgesamt wurden 32 Plots in systematischer Verteilung auf der Restitutionsfläche errichtet; auf jeder Teilfläche konnten 8 mit *Koelerion glaucae*-Rechgut inokulierte und 8 unbehandelte Flächen installiert werden, von denen jeweils 4 beweidet und 4 nicht beweidet wurden. Die Behandlungen wurden randomisiert verteilt. Die inokulierte Fläche umfasst etwa 5–7 % der Gesamtfläche. Pflanzensoziologische Aufnahmen der verschieden behandelten Untersuchungsflächen (à 25 m²) wurden über vier Jahre mit einer Prozentskala durchgeführt. Um den floristischen Hintergrund der gesamten Fläche darstellen zu können, konnten zusätzlich 43 Aufnahmen aus gleichmäßig verteilten Rasterpunkten (Grid-Plots 'GP'; à 25 m²) aus dem gleichen Zeitraum einbezogen werden (alle Flächen beweidet). Die Beweidung der Restitutionsfläche erfolgte durch eine Eselgruppe von 3–5 Tieren.

Als Donorfläche für die Minimalinokulation diente ausgerechtes Pflanzenmaterial aus einem nahe gelegenen *Koelerion glaucae*-FFH-Gebiet.

Die Vegetationsentwicklung wurde mit multivariaten Methoden und mit gemischten linearen Modellen (SAS 9.2, PROC MIXED, SAS Institute Inc.) ausgewertet, auch unter Einbeziehung der Donorfläche und einer älteren Restitutionsfläche. Qualitativer und quantitativer Leitarten-Index („Target-species ratio“; TSR) wurden zur Bestimmung der proportionalen Anteile von Leitarten berechnet. Desweiteren fanden Untersuchungen von Bodenparametern (N- und P-Gehalte, pH-Werte), sowie des

Potentials des Inokulationsmaterials, der Diasporenbank und des Diasporen-Niederschlags statt. Zudem konnten Untersuchungen zur Endozoochorie durchgeführt werden, indem die keimfähigen Diasporen im Eseldung mit der Auflaufmethode untersucht wurden.

Ergebnisse – Die Substratqualität hatte einen Einfluss auf die Zahl der Leitarten sowie auf die Deckung von Phanero- und Kryptogamen, wobei das P-reichere Substrat von S2 jeweils die höheren Werte aufwies (Abb. 2, 6, 7). Auch in Bezug auf die Diasporenbank wirkte sich die Substratqualität aus (Tab. 2); S2 wies eine etwa 5-mal höhere Diasporendichte auf als S1, wobei die Diasporenbank beider Restitutionsflächen von Ruderalarten dominiert wurde.

Die Inokulation führte ab dem ersten Untersuchungs-jahr zu einer Erhöhung der Anzahl von Leitarten und damit auch zu einer Erhöhung des qualitativen Leitarten-Index (Abb. 2, 3). Gleichzeitig kam es bei Weideausschluss zu einer deutlichen Zunahme des Deckungsanteils von Leitarten, d.h. des quantitativen Leitarten-Index (Abb. 4). Fast alle auf der Spenderfläche erfassten Arten konnten auf inokulierten Flächen nachgewiesen werden (Beilage S1). Auf nicht inokulierten Flächen und den Rasterpunkten kam es zu einer kontinuierlichen Zunahme der Leitartenzahl, wohingegen deren Deckungsanteil gering blieb (Abb. 2, 3, 4). Die Entfernung zwischen inokulierten Flächen und Rasterpunkten hatte kaum Effekte auf den Anteil von Leitarten oder deren Deckung (Abb. 8).

Die Beweidung hatte keinen Einfluss auf die Leitartenzahl, führte jedoch zu strukturellen Änderungen (Reduktion der Moosdeckung, Abb. 2, 4, 7).

Die Ausbreitung einiger Leitarten mittels Endozoochorie durch Esel konnte nachgewiesen werden. Dagegen wurden im Diasporen-Niederschlag der Restitutionsflächen vornehmlich Ruderalarten erfasst, Leitarten waren mit lediglich 0,1 % aller Diasporen unterrepräsentiert (Tab. 3).

Die Ordination (DCA) zeigt die Entwicklung der untersuchten Plots genau wie die der Vergleichsfläche in Richtung der Spenderfläche (Abb. 9).

Diskussion – (1) Die verschiedenen Bodenparameter lagen bis auf Phosphat (S2) im Bereich oder sogar niedriger als in Leitbildsystemen, so dass von dieser Seite die Entwicklung von *Koelerion glaucae*-Vegetation gegeben ist. Bekannte Effekte höherer P Konzentrationen wie der Verlust von (Leit-) Artendiversität oder die Zunahme kompetitiver Graminoider konnten bislang nicht eindeutig auf S2 beobachtet werden. Einen stärker differenzierenden Effekt hatte sicherlich die initiale Diasporenbank der Substrate, wobei S1 die erwartete geringe Diasporendichte von Tiefensand aufwies, wohingegen S2 vermutlich durch oberirdische Lagerung mit Diasporen kontaminiert wurde.

(2) Die punktuelle Zunahme der Leitartenzahl durch Inokulation mit Pflanzenmaterial ist ein aus anderen Studien bekannter Effekt. Die Ausbreitung von Leitarten von den inokulierten Flächen in die Gesamtfläche verlief langsam und war durch verschiedene mögliche Faktoren (Diasporenbank, -niederschlag, Zoochorie) nicht distanzabhängig. Eine leichte Entfernungsabhängigkeit in Bezug auf die Leitartendeckung kann mit der zumeist geringen Ausbreitungsdistanz vieler Leitarten erklärt werden.

(3) Der Anteil endozoochor transportierter Arten lag bei unserer Probennahme bei etwa 10 % aller erfassten Arten (davon 60 % Leitarten). Dieser Anteil ließe sich sicherlich durch mehrere über die Vegetationsperiode verteilte Probennahmen erhöhen. Doch dürfte aus phänologischen Gründen der Zeitpunkt im Juni für die Erfassung von endozoochor ausgebreiteten Leitarten besonders günstig gewesen sein.

(4) Die Beweidung wirkte sich im Untersuchungszeitraum vor allem durch die Erhaltung eines hohen Offenbodenanteils und eine gehemmte Entwicklung einer Mooschicht auf den Restitutionsflächen aus. Die Vegetationszusammensetzung wurde bislang durch die Beweidung nicht beeinflusst, es wurden beispielsweise keine Steigerungen der Artendiversität oder eine Förderung von Leitarten beobachtet.

Schlussfolgerungen – Die Ergebnisse zeigen, dass die Minimal-Inokulation geeignet war, das Fehlen eines Diasporen-Reservoirs zu überwinden und Startpopulationen aufzubauen. Die Methode eignet sich, wenn nur geringe Materialmengen von gut entwickelten Donorflächen entnommen werden können. Die floristische Struktur der Donorfläche konnte allerdings in vier Jahren noch nicht erreicht werden. Die Beweidung führte in der Untersuchungsperiode vor allem zu strukturellen Änderungen.

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Supplements

Supplement S1. Presence table for all species growing on the different treatment plots of the two restoration sites ($n = 4$; the number of plots with the species is given) in the years 2010 to 2013.

Beilage S1. Stetigkeitstabelle aller Arten, die auf den verschiedenen Behandlungen ($n = 4$; die Anzahl der Plots mit der Art ist angegeben) der beiden Restitutionsflächen in den Jahren 2010 bis 2013 erfasst wurden.

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