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Contribution of the soil seed bank to the restoration of temperate grasslands by mechanical sward disturbance

Short title: Seed bank activation by sward disturbance

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Author contributions

DP, MF, TK, UH, NH conceived and designed the research; DM, CJH, DS, VHK performed the field work; DM, CJH, DS, VHK, DP, NH performed the lab work; CJH, VHK analyzed the data and wrote the manuscript; all coauthors contributed to improving the manuscript.

Keywords

Bray-Curtis similarity, diversity enrichment, endangered species, germination, plant species richness, seed ecology

Implications

- Mechanical sward disturbance can be used to boost germination from seed banks in managed mesic grasslands.
- However, the soil seed bank contributes only slightly to the revegetation after sward disturbance due to low numbers of species and viable seeds especially of endangered species.
- Mechanical sward disturbance immediately increases the species richness of arable weeds, while a slight increase in typical grassland species is also possible. If this effect persist on the long run has to be studied further.
- Only in strongly species-impoverished grasslands (i.e. less than 20 plant species at 2 m × 2 m), small-scale sward disturbance can increase the richness of typical grassland species, while species-rich grasslands lose species when this relatively drastic measure is applied.

Abstract

The restoration of grasslands is one of the primary targets of nature conservation. An easy tool to stimulate the growth of plant species currently absent from the aboveground vegetation but hidden in the "dark", is to make use of the soil seed bank. Here, seeds of rare and endangered species may still be present. However, the potential contribution of soil seed banks to effective grasslands restoration still remains unclear, as some but not many valuable species built-up a persistent seed bank. To evaluate the potential of the soil seed bank for grassland restoration we installed an experiment in 73 differently managed grasslands in Germany, where the seed bank was activated by mechanical sward disturbance. We also determined the species richness, the density of viable seeds and the functional composition of seed banks and monitored the regeneration of the aboveground vegetation over two seasons. Our results show that sward disturbance led to an activation of the soil seed banks, which, however, contributed only little to the revegetation after sward disturbance. Additionally, the severe impoverishment of the soil seed bank indicated a restricted potential for the restoration of temperate grasslands. Nevertheless, the activation of the soil seed bank increased not only the richness of arable weeds but also slightly the richness of typical grassland species. We

conclude that only in recently improved and strongly species-impoverished grasslands, sward disturbance alone might be able to slightly increase plant species richness. To achieve a distinct increase in species richness, additional diaspore transfer is needed.

Introduction

Semi-natural central European grasslands are among the most species-rich habitats with high numbers of rare species. However, their conversion to arable fields, abandonment of land use and the increase in management intensity on remaining grasslands have led to a drastic decline in grassland biodiversity (Tscharntke et al. 2005; Wesche et al. 2012). As agricultural intensification is still ongoing in many regions (Kümmerle et al. 2016), different restoration measures have been developed to increase the area and quality of ecologically valuable grasslands in many countries (Muller et al. 1998; Walker et al. 2004; Kiehl et al. 2010). An easy tool for grassland restoration is to make use of the soil seed bank by (locally) breaking-up the existing sward to trigger germination (e.g. Bakker et al. 1996; Bakker & Berendse 1999). Although trampling by livestock also increases bare soil cover, a mechanical treatment with farm machinery can activate the seed bank of the whole topsoil (Schmiede et al. 2012; Klaus et al. 2016).

As many typical grassland species do not produce persistent seeds (Thompson et al. 1997; Bekker et al. 1998; Hölzel & Otte 2004a; Wellstein et al. 2007), by far not all species that have disappeared from the aboveground vegetation may be contained in the seed bank. However, even formerly wide-spread species, which are typical elements of grassland seed banks such as *Daucus carota*, *Hypericum perforatum*, and *Medicago lupulina*, are nowadays absent from many grasslands. Thus, seed bank activation might be a useful and cheap tool to increase plant diversity - at least in particularly species-poor grasslands. However, this approach has not been tested across a broad range of differently managed grasslands.

The soil seed bank consists of seeds produced by the aboveground vegetation and seeds dispersed from adjacent areas (Hutchings & Booth 1996; Hölzel & Otte 2001). The establishment of a persistent seed bank allows plants to bridge temporally unsuitable habitat conditions such as flooding and extreme drought (Bakker et al. 1996; Bossuyt & Honnay 2008). On the contrary, more stable communities of later successional stages produce less long-lived seeds (Bekker et al 1998; Thompson et al. 1998). After large-scale sward disturbance, the secondary succession is often strongly affected by the soil seed bank (Luzuriaga et al. 2005; Pakeman & Small 2005), either positively when ecologically valuable and typical species germinate from the soil seed bank, or negatively when ruderal, competitive or non-native species establish in high numbers (Graham & Hutchings 1988). Together with the soil seed bank, vegetative propagation (clonal spread) and especially the seed rain have been identified as important drivers of gap colonization in grasslands (Hutchings & Booth 1996; Fibich et al. 2013). However, the intertwined roles of seed bank and seed rain during vegetation regeneration after disturbance are not completely understood (Vítová et al. 2017).

We selected 73 temperate grasslands along a gradient of land-use intensity from three regions in Germany to evaluate the potential contribution of the soil seed bank to grassland restoration after mechanical sward disturbance. Therefore, we assessed soil seed banks and aboveground vegetation in control and disturbed plots and monitored the post-disturbance regeneration over two growing seasons. In detail, we hypothesized that:

1) The soil seed bank of a broad range of mesic temperate grasslands is widely dominated by typical grassland species.
2) Mechanical sward disturbance activates the soil seed bank and leads to a decrease in densities of viable seeds in the seed bank and an increase in seedling numbers emerging in the field.
3) The regeneration of the vegetation after sward disturbance strongly depends on the number and species richness of viable seeds in the soil seed bank.

4) At intensively used species-poor grasslands, the disturbance treatment leads to an increase in typical grassland plant species compared to undisturbed control plots.

Methods

Study design

We studied 73 permanent grasslands in three regions in Germany that are part of the *Biodiversity Exploratory* project (Fischer et al. 2010; www.biodiversity-exploratories.de): in northeast Germany (Brandenburg) the UNESCO Biosphere Reserve ***Schorfheide-Chorin***, the exploratory ***Hainich-Dün*** in central Germany (Thuringia) located in the National Park Hainich with surroundings and in southwest Germany (Baden-Württemberg) the UNESCO Biosphere Reserve ***Schwäbische Alb***. For further information on study regions see Table S1. We sampled 25 grasslands in each of *Schorfheide-Chorin* and *Schwäbische Alb*, but just 23 grasslands in *Hainich-Dün*. These were arranged along regional land-use intensity gradients ranging from unfertilized low-intensity grasslands over mesotrophic up to heavily fertilized and intensively managed grasslands. We assessed the intensity of land-use with the help of a farmer's questionnaire reporting on the annual amount of fertilizer applied (kg nitrogen × ha^{-1}), the frequency of mowing (number of cuts) and the grazing intensity (livestock units × grazing days × ha^{-1}) on each grassland from 2006 to 2014 according to Blüthgen et al. (2012). Annual values were averaged to gain a robust long-term mean (Table S1). The land-use gradients are also associated with a plant diversity gradient ranging from 12 to 71 species (per 4 m × 4 m; Klaus et al. 2011).

In 2014, we established a combined disturbance and seed addition experiment at all 73 grasslands, of which we only consider the sward disturbance here (Klaus et al. 2017). The seed bank study was performed on two experimental plots of 7 m × 7 m, one disturbance plot and one untreated control. Disturbance was done with farm machinery using a rotary cultivator or harrow down to 0.1 m at the end of October 2014. After the disturbance, the remnants of plants such as shredded tussocks and roots were left on the plots. For further details on methodology and design see Klaus et al. (2016).

Seed bank and vegetation sampling

The soil seed bank was sampled in March 2015 on all control and disturbance plots, after cold stratification had taken place during winter, using a split tube sampler with an inner diameter of 2.9 cm. Mixed samples of 20 soil cores were collected along two transects on each plot. To overcome spatial dependency of sub-samples, inter-sample distance along transects was set to 50 cm (Plue & Hermy 2012). Thick root networks, litter and the soil below 10 cm depth were removed from the samples. In total, the samples covered 132.1 cm^2 and met the spatial requirements of seed bank sampling for grasslands given by Oomes & Ham (1983). However, for comparison with the aboveground vegetation records, a larger sampling area of the seed bank would have been beneficial (Plue & Hermy 2012), but this was not feasible given the number of sites and replications in our study. To assess germinable seeds, the seedling emergence method was used (Roberts 1981). Mixed soil samples from each plot were spread on two 28 cm × 45 cm trays filled with 3 cm sterilized potting soil. We removed vegetative parts of plants and the soil was crumbled uniformly on the potting soil. Trays were regularly watered and holes in the base of the tray prevented the soil from becoming waterlogged. The trays were placed on the roof of our institute building in Münster. Individual seedlings were recorded and identified to species level regularly from May 2015 to June 2016. All determined seedlings were removed from the trays to prevent competition with newly emerging seedlings. Resprouting roots and rhizomes were removed and excluded from the analysis. Due to the emergence of a few seedlings in control trays, which were filled with sterilized soil only, and to the presence of some adult plants relatively close to the trays, we decided to exclude some species for safety reasons (*Salix* spec, *Erigeron canadensis*, *Epilobium hirsutum* and as from October 2015 also *Poa annua*, *Poa trivialis*, *Sagina procumbens*, *Leucanthemum vulgare*, *Cerastium holosteoides*).

In May 2015 and 2016, aboveground vegetation (including all juvenile plants such as seedlings but also adult plants) was recorded in all three regions before mowing or grazing took place. The percentage cover of all vascular plant species and bare soil was estimated on 2 m × 2 m in each plot. In addition, we identified seedlings to species level when possible and recorded their numbers in four different categories, which are estimations of their abundance and frequency. Categories were: only one individual (transformed to 1 for statistical analysis); two to ten individuals (transformed to 5); 11 to 100 individuals (transformed to 50); more than 100 individuals (transformed to 120). Because it was not possible to distinguish resprouting monocotyledons from those, which were true seedlings, monocotyledons were excluded from the seedling survey in the field.

Data analysis

Plant species were categorized according to their main habitat preferences as 'typical grassland species', 'arable species', which are mostly annual weed species such as *Chenopodium* spec., *Sonchus* spec. and *Thlaspi* spec., or 'other species' such as nitrophytic tall herbs and tall woody species like *Urtica* spec. and *Betula* spec. (Ellenberg & Leuschner 2010). The number of endangered species per plot was calculated as the sum of all plant species that are listed as endangered, vulnerable or very rare on the red list of the respective federal state where the grassland was located (Breuning & Demuth 1999; Ristow et al. 2006; Korsch & Westhus 2011). To account for environmental characteristics of grasslands under study, we calculated mean weighted Ellenberg indicator values for soil moisture and nutrient supply from the aboveground vegetation of the control plots (Ellenberg et al. 2001). This will, however, not account for small-scale within-site differences in soil characteristics but give information on the relevance of superior site conditions.

DCA ordination was used to search for effects of study regions, management and environmental gradients on the species composition of the seed bank (Figure S1). Linear mixed effects models (LME) were performed to detect significant differences among control and disturbed plots including disturbance as a fixed factor and grassland as a random factor. To compare the similarity of seed bank and aboveground vegetation, we performed Mantel tests based on Bray-Curtis similarity matrices derived from presence-absence data. The standardized Mantel statistic was tested for significance by using Mantel's asymptotic approximation. In addition, pairwise Bray-Curtis similarity between each seed bank and aboveground vegetation record was calculated. The Mantel test and the similarity calculations were performed with PC-ORD 5 (McCune & Grace 2002). Pearson's correlation coefficient was used to explore relationships between a) species richness and seed or seedling densities from seed banks and aboveground vegetation, and b) the differences in typical grassland species among disturbance and control plots in 2016 and the intensities of fertilization, grazing and mowing at the respective site. To further explore factors influencing the regeneration after disturbance, we used multiple linear regressions including study regions, Ellenberg moisture and nutrient values, and the plant species richness of the aboveground control vegetation as predictor variables. Models were reduced stepwise according to their AIC by using the step-function. To ensure normal distribution of variables, log or square root transformation was applied if necessary. Model assumptions were checked using diagnostic plots. Linear regressions and mixed effects models were carried out with R (R Core Team 2016) using the nlme package (Pinheiro et al. 2016).

Results

Seed bank composition

On average 42.2 seeds germinated from seed bank samples, which corresponds to an average density of 3,192 viable seeds per m^2. These seedlings comprised in total 117 plant species, with a mean richness of 11.6 species and a range of one to 22 species per plot (Table 1). The species richness of the seed bank was positively related to the number of viable seeds ($r = 0.77$; $p < 0.001$). Viable seeds of endangered species were found in only seven out of the 73 grasslands with a maximum of only one endangered species per control plot. Endangered species were mostly typical grassland species, some arable weeds, and just in one case a nitrophytic tall herb (Table S2). The seed bank was widely dominated by typical grassland species (79% of species; 82% of viable seeds), while arable weeds and “other” species occurred with a relatively small share (Table 1). The eleven most abundant species build up almost 60 % of the total number of viable seeds, which were all typical grassland species except one arable weed (Table S2). DCA analysis revealed management types and the presence of fertilization to not affect the species composition of the seed bank, although Ellenberg nutrient values were positively related to numbers of seeds from arable weeds in the seed bank (Figure S1).

Effects of sward disturbance on seed bank and aboveground vegetation

Compared with control seed banks, sward disturbance four months prior sampling decreased the mean density of viable seeds per m^2 by 20 % and the mean species richness by 13 %, especially of typical grassland species (Table 1). The aboveground vegetation was strongly affected by sward disturbance. In 2015, six months after disturbance, the cover of bare soil still showed a drastic increase (+ 764 %) at the cost of grassland species cover (-51 %). On the contrary, disturbance favored the number and species richness of emerging dicot seedlings but also the richness and cover of (adult) arable weeds (Table 1). Species richness and numbers of dicot seedlings in the field were both positively correlated with the proportion of bare soil (numbers: $r = 0.26$, $p < 0.05$; richness: $r = 0.4$, $p < 0.001$). In 2015, a significant increase in plant species richness was due to an increase in the richness of arable weeds only (Table 1, Fig. 1).

In 2016, disturbed plots still showed significantly increased bare soil, higher cover and richness of arable weeds and higher numbers of dicot seedlings, but differences were generally smaller compared to 2015 (Table 1). The initial disturbance effect on the number of dicot seedlings and cover of grassland species already vanished, whereas the disturbance effect on overall species richness was even stronger than before, with significantly higher richness of arable weeds but also in typical grassland species (Fig. 1). The richness of endangered species was generally not affected by sward disturbance and following temporal development (Table 1).

Regeneration of the aboveground vegetation

After sward disturbance, neither the richness nor the number of dicot seedlings emerging in the field related to the species richness and the density of viable seeds in the seed bank ($p > 0.05$). This pattern remained even when grass species were excluded from the analysis, as grass seedlings were not assessed in the field (Fig. 2). In contrast, number and richness of dicot seedlings in the field were positively related to the species richness of the control aboveground vegetation and the Ellenberg nutrient value (Fig. 3, Table S3). The reduction in bare soil from 2015 to 2016 was not related to the density of viable seeds in control seed banks ($p > 0.05$) but to those in disturbed seed banks ($r = 0.29$, $p < 0.05$). Accordingly, but only in 2016, the species richness of the disturbed vegetation was significantly positively related to the species richness of the seed banks (Table S4). In contrast, the species richness of the control vegetation was not or only weakly related to the species richness of the seed banks pointing at very little contribution of the seed bank to the vegetation composition if the sward was left intact (Table S4).

Disturbance and the regeneration of the vegetation did generally not affect the similarity between soil seed banks and aboveground vegetation records, with the latter including both juvenile and adult plants (Table 1). Mantel tests showed that the similarity between disturbed and control vegetation increased from 2015 to 2016 (Table S5), underlining the ongoing post-disturbance regeneration towards the control vegetation.

Effects on plant species richness

When the aboveground vegetation of control and disturbed plots was compared, we found a mean increase of one typical grassland species in the second season after disturbance. This increase was negatively associated with aboveground plant species richness (Table S3). Thus, grasslands at lower levels of plant species richness exhibited a stronger increase compared to already species-rich sites, which even lost species due to the disturbance (Fig. 4). Grasslands with a low richness of up to 20 species per four m^2 on control plots exhibited a mean increase by 2.6 typical grassland species in 2016.

The increase was not significantly correlated with the intensities of fertilization, mowing and grazing at the respective grassland ($p > 0.05$).

Discussion

Seed bank composition

The soil seed banks of the studied grasslands contained in total 117 species only and were, with a mean of 11.6 species per plot, relatively species-poor compared with other grassland studies (Wellstein et al. 2007; Bossuyt & Honnay 2008; Donath et al. 2009). Endangered species were also almost absent from seed banks. Similarly, the number of 3,192 seedlings/m^2 was lower than in studies by Wellstein et al. (2007) and Auestad et al. (2013), but in the range reported for mesic grasslands in the review of Bossuyt & Honnay (2008). Explanations for low species richness and low densities of viable seeds in soil seeds banks of mesic grasslands can mostly be attributed to differences in grassland types, intensive management and methodological details (Graham & Hutchings 1988; Bekker et al. 1997; Hölzel & Otte 2004b). As sampling time was in March, our study will have excluded some transient species, which germinated before sampling (Grime 2001). Furthermore, in line with Graham & Hutchings (1988), outdoor conditions, where trays were set up for germination, might have been relatively harsh decreasing germination rates compared to glasshouse conditions in studies by Wellstein et al. (2007) and Auestad et al. (2013). The depletion of the soil seed banks in seeds and species is likely also the result of the rather high mean land-use intensity when compared to previous studies with mostly unfertilized vegetation (Wellstein et al. 2007; Auestad et al. 2013) as intensive grassland management in general negatively affects seed banks (Bekker et al. 1997). Overall, our study underlines that agriculturally managed temperate grasslands have widely impoverished soil seed banks and that the chance of establishing endangered target species from the soil seed bank is very small, as seed banks were dominated by rather ubiquitous species.

Effects of mechanical sward disturbance on seed bank and aboveground vegetation

Sward disturbance done by a rotary cultivator or harrow affected the aboveground vegetation much stronger than the seed bank. The seed banks just showed an activation indicated by decreased species richness and decreased densities of viable seeds in disturbance plots, assumingly due to increased germination of seeds with a low dormancy level already prior seed bank sampling. Such species with a low dormancy level include grasses and winter annuals typically germinating in autumn and partly in early spring (Grime 2001; Hölzel & Otte 2004c).

Mechanical sward disturbance improved conditions for germination and initial establishment by increasing bare soil, activating the seed bank and simultaneously decreasing competition in the aboveground vegetation. In 2015, the positive relation between seedling numbers and the cover of bare soil underlines the importance of opening the sward (and litter and moss layers) to activate the soil seed bank (Jutila & Grace 2002; Hölzel 2005; Müller et al. 2013). An additional effect of the disturbance might be the release of nutrients from decomposing sward fragments, which promotes seedling emergence (Loydi et al. 2015; Klaus et al. 2016). This is in line with the positive relationship between nutrient supply, seedling numbers and richness found in our study.

Furthermore, sward disturbance immediately changed the aboveground vegetation towards a more ruderal character mainly due to an increase in richness and cover of arable weeds. This also showed the functional importance of arable weeds and other annual plant species to quickly fill gaps after the drastic disturbance (Grime 1998). However, the emergence of ruderal species did not inhibit the recovery of grassland species, as found by Graham & Hutchings (1988). On the contrary, the richness of typical grassland species increased in disturbance plots compared to control plots in 2016.

Regeneration of the aboveground vegetation

The number of emerging dicot seedlings after sward disturbance was not related to the density of viable dicot seeds in the seed bank, pointing at the importance that re-sprouting tussocks, root fragments and the seed rain might have had for the regeneration of the vegetation. Although we did not assess monocot seedlings in the field, this result is of vast importance for a broad range of temperate grasslands as the majority of restoration target species are dicot species and a dependency of plant life forms on grassland management intensity has not been found anyways (Auestad et al 2013). Nevertheless, the recovery of the sward from 2015 to 2016 in disturbance plots was slightly positively related to seed densities of the respective soil seed bank. Generally, while the seed bank can be important at the beginning of gap colonization in grasslands (Luzuriaga et al. 2005), the seed rain often gets the strongest influence on vegetation trajectories later on (Pakeman & Small 2005; Vítová et al. 2017). Edwards & Crawly (1999) also found that seedling recruitment in small gaps was nearly solely due to seed rain. However, although the seed bank species richness had no effect on the post-disturbance plant species richness during the first study season, there was a positive relationship between species richness of the aboveground vegetation and that of the seed bank in the second season, especially for arable weeds and grassland species. This relationship could not be found on the control plots, indicating at least a small impact of the seed bank on vegetation development after sward disturbance. Without any sward disturbance such as the impact of heavy grazing and trampling caused by intensive management, the soil seed bank does barely contribute species to the grassland vegetation (Milberg & Persson 1994).

The activation of the seed bank did not increase the similarity between the soil seed bank and the aboveground vegetation. Generally, the similarity was significant but rather low, which is typical of rather stable grasslands with many species producing transient seeds, where the species composition of the aboveground vegetation and the seed bank are quite different (Bossuyt & Honnay 2008; Dölle & Schmidt 2009). Furthermore, the relatively large spatial difference between below and aboveground vegetation records also decreases similarity (Plue & Hermy 2012).

The missing effect of the mechanical sward disturbance on the similarity contrasts finding of Luzuriaga et al. (2005), as a certain proportion of the emerging plants will have developed from the seed bank, especially arable weeds. Presumably, re-sprouting tussocks and root fragments as well as the seed rain might have dominated vegetation regeneration (Bullock et al. 1994; Dölle & Schmidt 2009). In contrast, the grassland in the study by Luzuriaga et al. (2005) was very young and its seed bank still contained high densities of arable weeds, which dominated vegetation composition directly after sward disturbance. The low similarity but also low species richness found in our study will also be an effect of the much larger sampling area of the aboveground vegetation compared to the seed bank, a trend often found in seed bank studies (Vandvik et al. 2016). However, as a high floristic similarity between seed bank and aboveground vegetation is an indication of a high restoration potential (Auestad et al. 2013), our findings indicate a limited possibility to re-establish the aboveground vegetation just from the soil seed bank.

Effects on plant species richness

In 2016, not only arable weeds but also typical grassland plant species had increased in disturbed compared to control plots. While already species-rich grasslands exhibited losses in species, the increase was strongest in the most species-poor communities. Whether this increase, however, will persist or vanish, requires further monitoring. Positively, we did not find a detrimental effect of undesired species germinating from the seed bank, as it is often the case for abandoned arable land (Graham & Hutchings 1988). Nevertheless, practitioners should also consider the danger of invasions e.g. by woody species or other problem species when applying mechanical sward disturbance to larger areas. The 7 m × 7 m size of our experimental plot has been selected due to scientific reasons to guarantee a high number of replications and shall not be seen as a recommendation.

The limited potential of the seed bank documented in our study indirectly underlines the importance of active diaspore dispersal by seeding, in combination with sward disturbance, when a pronounced increase in species richness is desired in grassland restoration (e.g. Walker et al 2004; Donath et al. 2007; Schmiede et al. 2012; Klaus et al. 2016). Despite this, at very species-poor grasslands, even small-scale sward disturbance might be able to slightly increase plant species richness.

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Supporting Information

The following information may be found in the online version of this article:

Table S1. Environmental conditions and plant diversity of the three study regions according to Fischer et al. (2010) and Klaus et al. (2011).

Table S2. Total occurrences and total seed numbers of most abundant and endangered species in seed banks from control and disturbance plots.

Table S3. Multiple linear regression models of seed bank and vegetation characteristics.

Table S4. Linear regression models relating aboveground vegetation in 2016 to seed banks.

Table S5. Mantel tests statistics for seed banks and aboveground vegetation in 2015 and aboveground vegetation in 2016 of control and disturbed plots using Bray-Curtis distance.

Figure S1. DCA ordination of the species composition of the seed banks of three study regions in relation to environmental gradients, grassland management and aboveground vegetation records.

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FIGURE CAPTIONS

Figure 1. Plant species richness of aboveground vegetation (at 2 m × 2 m) separated into species' habitat preferences six months (2015) and 18 months (2016) after mechanical sward disturbance. Asterisks indicate significant differences in groups among disturbed and undisturbed plots within years (*** $p < 0.001$; ** $p < 0.01$).

Figure 2. Relationships between a) numbers and b) species richness of dicot seedlings emerging after sward disturbance in the field and viable dicot seeds in control seed banks (all per 1 m^2 in 2015).

Figure 3. Relationship between the species richness of dicot seedlings emerging after disturbance in the field (2015) and the species richness of the control vegetation of the grasslands (2015).

Figure 4. Relationship between the richness of typical grassland species in 2016 disturbance plots and 2016 control plots on 2 m x 2 m. Regression axis (bold) and the 1:1 line (thin) shown. A gain in species can be found in plots above the 1:1 line.

Tables

Table 1. Seed bank and vegetation characteristics of control and disturbance plots. Cover values are sums of single species and can exceed 100% when vegetation was very dense. Significant differences among groups wer tested with linear mixed-effects models and are indicated by *** p < 0.001; ** p < 0.01; * p < 0.05 (see methods section).

		Control			Disturbance effect	Disturbance		
		Mean	SD	Min/Max		Mean	SD	Min/Max
Seedbank (0-10 cm)								
Species richness	All species	11.55	± 4.65	1/22	↓***	10.08	± 4.46	1/20
	Endangered species	0.1	± 0.3	0/1		0.14	± 0.42	0/2
	Grassland species	9.11	± 4.3	0/19	↓***	7.86	± 4.17	0/18
	Arable weeds	2.01	± 1.49	0/6		1.96	± 1.54	0/6
	Others species	0.41	± 0.72	0/3		0.38	± 0.59	0/2
	Grasses	2.36	± 1.5	0/6	↓**	1.95	± 1.52	0/6
	Legumes	1.33	± 1.16	0/5	↓*	1.07	± 0.99	0/4
	Herbs (non-legumes)	7.66	± 3.01	1/14		7.07	± 3.06	1/16
Number viable seeds (m^2)	All species	3191.84	± 2330.64	151.4/11582.1	↓***	2537.51	± 2008	75.7/11657.8
	Endangered species	9.33	± 30.76	0/151.4		22.81	± 85.28	0/529.9
	Grassland species	2626.69	± 2047.08	0/9916.7	↓***	1965.09	± 1743.4	0/10219.5
	Arable weeds	444.87	± 776.3	0/4314.9		484.27	± 711.89	0/4314.9
	Others species	119.25	± 294.79	0/1589.7		84	± 219.82	0/1211.2
	Grasses	630.49	± 679.9	0/2952.3	↓**	447.98	± 605.83	0/4163.5
	Legumes	438.65	± 677.08	0/4390.6	↓**	293.47	± 437.59	0/2271
	Herbs (non-legumes)	2043.9	± 1585.19	75.7/8856.9	↓*	1750.43	± 1356.98	75.7/7040.1
Aboveground vegetation (4 m^2)								
Cover (%)	Grassland species 2015	105.58	± 26.72	48.4/178.8	↓***	53.72	± 34.71	2.3/144.1
	Grassland species 2016	100.22	± 26.66	52.2/167		99.11	± 32.91	41.8/189.6
	Arable weeds 2015	1.67	± 4.19	0/26	↑***	5.79	± 12.84	0/64.6
	Arable weeds 2016	2.38	± 6.88	0/53	↑***	5.92	± 9.43	0/42.1
	Other species 2015	0.34	± 1.43	0/12		0.21	± 0.36	0/2.1
	Other species 2016	0.44	± 1.82	0/15		0.43	± 0.99	0/5.1
	Bare soil 2015	6.46	± 8.26	0.5/60	↑***	49.36	± 27.77	1/99
	Bare soil 2016	9.12	± 12.7	0/60	↑***	19.69	± 16.79	0.5/70
Dicot seedlings	Numbers 2015 (1 m^2)	16.58	± 17.99	0/68.75	↑***	46.71	± 36.03	0/160
	Numbers 2016 (1 m^2)	17.27	± 19.31	0/85.25	↑**	28.09	± 29.95	0/155
	Species richness 2015	3.86	± 2.96	0/12	↑***	8.1	± 4.44	0/22
	Species richness 2016	4.34	± 3.09	0/15		4.41	± 2.9	0/13
Species richness	All species 2015	25.78	± 11.1	7/68	↑*	26.9	± 9.12	10/61
(of adult plants	All species 2016	26.7	± 11.28	8/69	↑***	29.51	± 10.93	12/67
and dicot seedlings)	Endangered species 2015	0.59	± 1.44	0/9		0.59	± 1.16	0/6
	Endangered species 2016	0.53	± 1.39	0/8		0.6	± 1.18	0/5
	Grassland species 2015	23.33	± 10.63	5/58		22.07	± 8.56	6/48
	Grassland species 2016	24.03	± 10.88	6/60	↑**	25.19	± 10.27	7/56
	Arable weeds 2015	1.55	± 1.65	0/6	↑***	3.51	± 2.25	0/8
	Arable weeds 2016	1.92	± 1.78	0/8	↑***	3.34	± 2.83	0/11
	Other species 2015	0.68	± 1.01	0/5		0.95	± 1.13	0/6
	Other species 2016	0.6	± 0.92	0/3		0.84	± 1.15	0/5
Similarity (Bray-Curtis)	Aboveground vs. seed bank 2015	0.34	± 0.13	0/0.59		0.34	± 0.13	0/0.6
	Aboveground vs. seed bank 2016	0.34	± 0.13	0/0.57		0.33	± 0.13	0/0.64

FIGURES

Figure 1

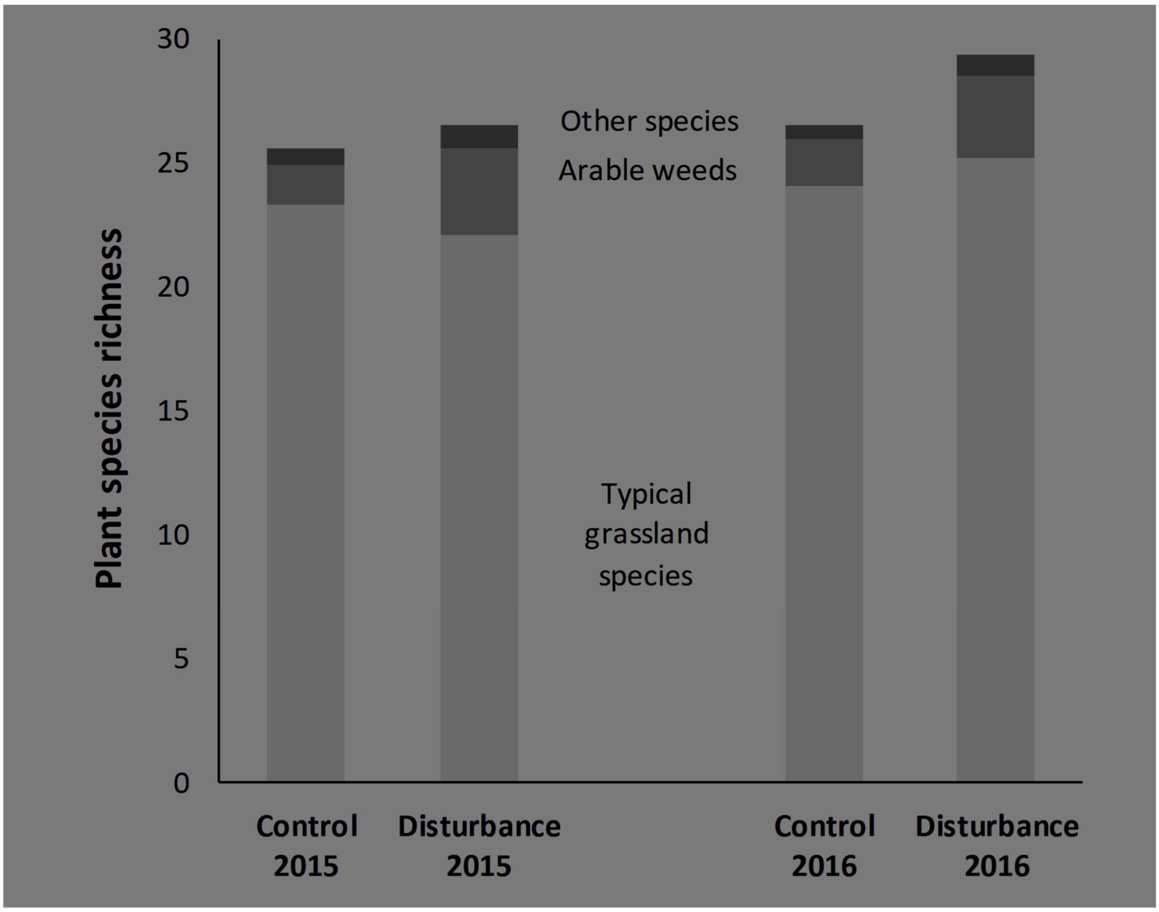

Figure 2

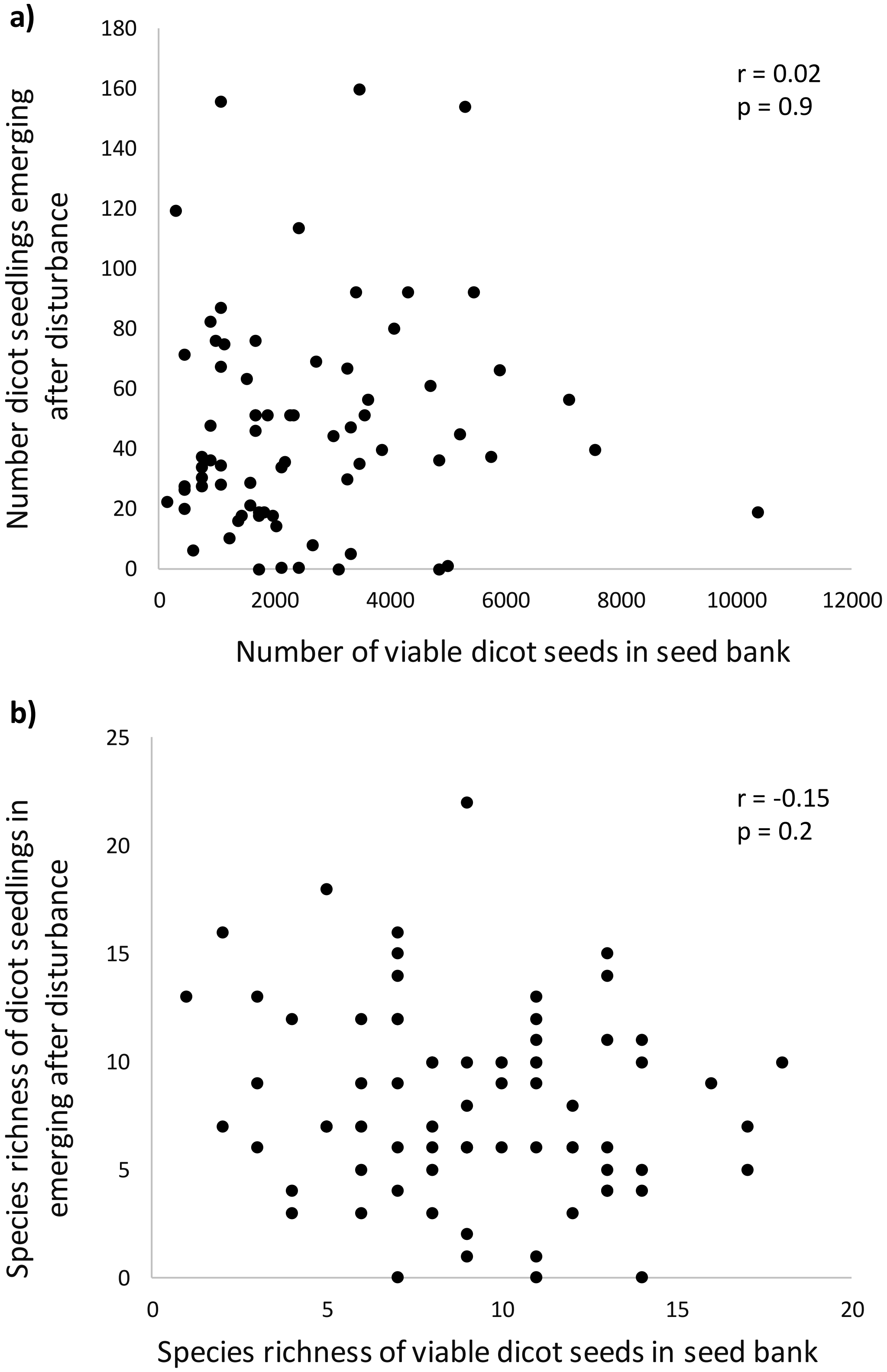
a)
r = 0.02
p = 0.9
Number dicot seedlings emerging after disturbance
Number of viable dicot seeds in seed bank
b)
r = -0.15
p = 0.2
Species richness of dicot seedlings in emerging after disturbance
Species richness of viable dicot seeds in seed bank

Figure 3

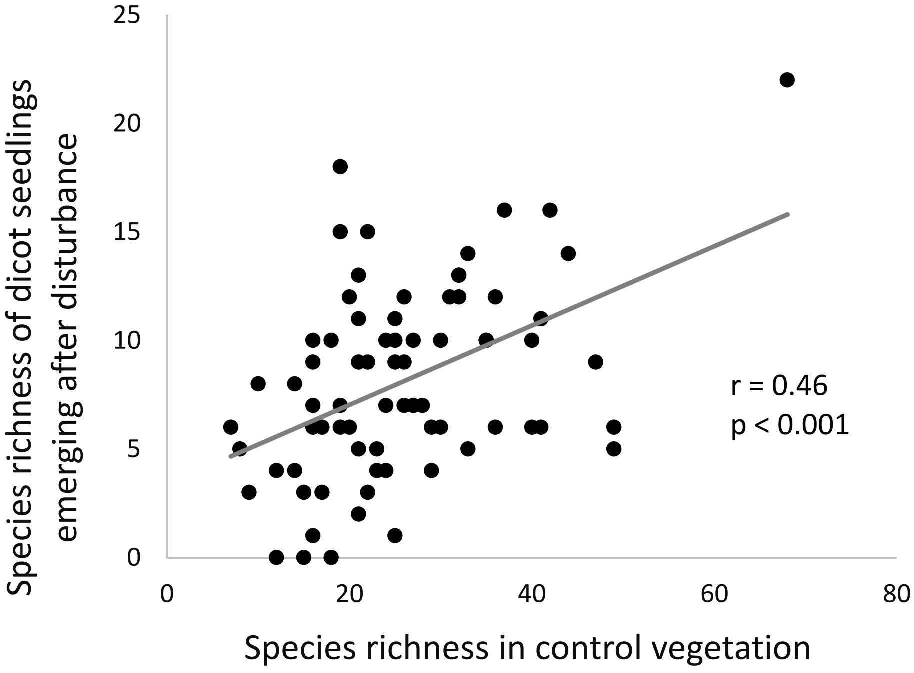

Figure 4