Effects of mowing, grazing and fertilization on soil seed banks in temperate grasslands in Central Europe

Journal Article

Author(s):
Klaus, Valentin; Schäfer, Deborah; Prati, Daniel; Busch, Verena; Hamer, Ute; Hoever, Christina J.; Kleinebecker, Till; Mertens, Désirée; Fischer, Erich; Hölzel, Norbert

Publication date:
2018-03-15

Permanent link:
https://doi.org/https://doi.org/10.3929/ethz-b-000249957

Originally published in:
Agriculture, Ecosystems & Environment 256, https://doi.org/10.1016/j.agee.2017.11.008

Publication: Klaus VH, Schäfer D, Prati D, Busch V, Hamer U, Hoever CJ, Kleinebecker T, Mertens D, Fischer M, Hölzel N (2018) Effects of mowing, grazing and fertilization on soil seed banks in temperate grasslands in Central Europe. Agriculture, Ecosystems and Environment 256: 211-217 doi: 10.1016/j.agee.2017.11.008

Effects of mowing, grazing and fertilization on soil seed banks in temperate grasslands in Central Europe

Valentin H. Klaus[1#], Deborah Schäfer[2], Daniel Prati[2], Verena Busch[1], Ute Hamer[1], Christina J. Hoever[1], Till Kleinebecker[1], Désirée Mertens[1], Markus Fischer[2] and Norbert Hölzel[1]

[1] University of Münster, Institute of Landscape Ecology, Heisenbergstr. 2, 48149 Münster, Germany

[2] University of Bern, Institute of Plant Sciences, Altenbergrain 21, 3013 Bern, Switzerland

Corresponding author: v.klaus@uni-muenster.de

current address: ETH Zürich, Institute of Agricultural Sciences, Universitätsstr. 2, 8092 Zürich, Switzerland

Highlights (max 85 characters incl. spaces per point)

- Grassland management has complex, direct and indirect effects on soil seed banks
- Grazing increased species richness of soil seed banks
- Mowing and fertilization decreased species richness and seedling density
- Seed banks are indirectly influenced by factors shaping the aboveground vegetation
- Grazed grasslands might be more resilient towards soil disturbances than mown ones

Keywords (4-6)

Fertilization, grassland restoration, grazing, land-use intensity, mowing, resilience

26

Abstract

Soil seed banks support the recovery of plant species and communities after disturbances. Against the background of increasing disturbance frequencies due to current changes in climate and land use, the importance of seed banks to maintain ecosystem functioning of grasslands is likely to increase. This makes seed bank ecology a highly relevant topic of studies on ecosystem functioning. One of the main drivers of seed bank composition and density in grasslands is the current management. Although highly intensive grassland management can affect soil seed banks negatively, it is still unclear which component of management – fertilization, mowing or grazing – is the most influential. We studied soil seed banks of 73 mesic grasslands, which were arranged along a gradient of land-use intensity, by two seasonally differing surveys. Grasslands generally exhibited rather poor seed banks with low numbers of species and seedlings. Both were even lower in spring compared to autumn sampling. Grassland management turned out to affect soil seed banks in a complex way, via direct and indirect paths. While intensive mowing and fertilization decreased species richness and seedling density, grazing intensity increased species richness in the seed bank. Thus, land-use intensity, an index calculated as a sum of all three components, had highly ambivalent effects on seed banks. We conclude that the positive effect of grazing on soil seed banks makes pastures more resilient towards mechanic disturbances as compared to meadows.

1. Introduction

Land-use changes alter ecosystems in manifold ways. In grasslands, the intensification of land use is responsible for vast losses in diversity, which can in turn affect ecosystem functioning and stability (Allan et al., 2015; Blüthgen et al., 2016; Habel et al., 2013; Tilman and Downing, 1994). For example, the resilience of plant communities has often been connected to the soil seed bank, especially when heavy disturbances destroy established plants and create bare soil (e.g. Thompson et al., 1998). Then, the seed bank supports the recovery of plant communities by contributing species, of which some can be absent from the realized vegetation but still hidden in the soil for many years (Bossuyt and Honnay, 2008; Thompson et al., 1997; Valkó et al., 2011; Vandvik et al., 2016). Regarding the assumed increase in disturbance frequencies due to climate change and land-use intensification (Joyce et al., 2017), the importance of seed banks to support ecosystem functioning of grasslands is likely to increase, highlighting the relevance of seed bank ecology.

Differences in methodology such as the timing of sampling and the procedure for seed extraction potentially influence outcomes of studies on soil seed banks, impacting for example on results on average seed size and longevity (Graham and Hutchings, 1988; Hölzel and Otte, 2004a; Oomes and Ham, 1983; Ter Heerdt et al., 1996). Unfortunately, there is still little information on sampling effects and how comparable different seed bank sampling approaches are, complicating the comparison of different studies and meta-analyses (but see Vandvik et al., 2016). Due to the strong seasonal variability of germinable seeds in the seed bank, repeated measurements can also help to account for seed bank dynamics during the year (Thompson and Grime, 1979).

One of the main impacts on the species composition of seed banks in grasslands is their management (Auestad et al., 2013; Wellstein et al., 2007). Although intensive grassland management in general seems to negatively affect seed banks (Bekker et al., 1997), species richness and seedling densities might be differently influenced by specific management practices and previous studies revealed complex or unclear findings (Auestad et al., 2013; Bekker et al., 1998a). Management effects are likely to be indirect via shaping the aboveground vegetation by applying certain management techniques such as mowing and grazing, which will influence seed banks differently as time and

manner of harvest (biomass removal) decide whether seed set is successful and species can contribute seeds to the seed bank or not (Bekker et al., 1997; Wellstein et al., 2007). Fertilization might have two opposing effects on soil seed banks, as on the one hand the addition of further nutritional resources enables plants to invest more in sexual reproduction, but on the other hand fertilization also shifts the aboveground community towards highly productive grass, which are known to poorly contribute to a persistent seed bank (Busch et al., 2017; Thompson and Grime, 1979). Thus, not just presence or absence of a factor, i.e. fertilization, mowing or grazing, but its intensity shapes grassland seed banks (Blüthgen et al., 2012). However, up to now, a comprehensive assessment of the effects of grassland management on seed banks is widely missing (Auestad et al., 2013), although such information may help to identify factors (co-)determining the resilience of this ecosystem (Bossuyt and Honnay, 2008).

We studied soil seed banks of 73 temperate grasslands along a gradient of land-use intensity with two sampling campaigns differing in timing and set-up. After comparing results obtained by both approaches, plant species richness and seedling density of the seed banks were related to fertilization, mowing, grazing and an overall index of land-use intensity. Ellenberg nutrient indicator values were additionally used as a proxy to account for the shift from conservative, rather unproductive to highly competitive and grass dominated plant communities (Ellenberg et al., 2001; Gilhaus et al., 2017a). In detail, we hypothesized that

(1) although the two different sampling approaches yield slightly different results, their species richness and seed densities will be highly correlated;

(2) grazing increases while mowing and fertilization decrease species richness and seedling density;

(3) land-use intensity influences soil seed banks mostly indirectly via the diversity, composition and productivity of the aboveground plant communities.

2. Methods

2.1. Study design

We studied 73 agriculturally managed grasslands in three regions that are part of the *Biodiversity Exploratories* project (Fischer et al., 2010; www.biodiversity-exploratories.de): in northeast Germany the rather flat post-glacial landscape of the UNESCO Biosphere Reserve *Schorfheide-Chorin*, in central Germany the undulating landscape of the *Hainich-Dün* and in southwest Germany the low mountain range of the UNESCO Biosphere Reserve *Schwäbische Alb* (Figure A1 in Supplementary material). For more information on study regions and site conditions see Table A1 in Supplementary material and Gilhaus et al. (2017a). In each region, we sampled 23 to 25 differently managed grasslands ranging from unfertilized, cut or grazed low-intensity grasslands to intensively managed grasslands with heavy fertilization and several cuts per year or a combination of intensive mowing and grazing. This regionally replicated gradient in land-use intensity is representative for grassland management in many parts of Central Europe (Blüthgen et al., 2012). According to this gradient, grassland types varied widely but were representative for large parts of the Central European grassland vegetation (Fischer et al., 2010). Most of the studied grasslands belong to mesic Arrhenatherion elatioris W. Koch 1926 and Cynosurion cristati Tx. 1947 communities, with transitions to Polygono-Trisetion Br.-Bl. et Tx. ex Marschall 1947 nom. invers. propos., CalthionTx. 1937 and Magnocaricion elatae W. Koch 1926 communities depending on altitude and soil moisture. Additionally, rather dry Bromion erecti Koch 1926-communities occurred on very shallow calcareous soils (Klaus et al., 2012).

2.2. Seed bank sampling

We used the seedling emergence method (Thompson et al., 1997) to record soil seed banks of all 73 grasslands. To account for temporal variability and to gain robust mean values, we sampled the seed banks using two approaches differing in timing but also sample preparation and environmental conditions during seedling emergence.

For the first approach, referred to as “autumn sampling”, we sampled a 34 m × 7 m plot on each grassland by taking 20 samples from 0 to 10-15 cm soil depth in October 2014 in three parallel

transects along the longer side of the sampling area. To overcome spatial dependency of sub-samples, inter-sample distance was set to a minimum of 200 cm (Plue and Hermy, 2012). We extracted the samples using a soil corer with an inner diameter of 2.5 cm, resulting in a total sampling area of 98.3 cm^2. For each sample we then roughly removed the aboveground vegetation and bryophytes, but no belowground plant organs. We dried and stored the samples in plastic bags to inhibit regrowth of plant roots. In January 2015, we rehydrated the mixed samples, crumbled and uniformly spread them on two to three 12 cm × 17 cm trays per grassland. The trays were previously filled with 2.5 cm sterilized potting soil and the layer of our samples was about one cm thick. We placed the trays in the Botanical garden of Bern, Switzerland, which is situated at 542 m a.s.l., has a continental climate with a mean temperature of 8 °C and an annual precipitation of 1000 mm. Stratification took place naturally during January and February when trays were outside. We identified all emerging seedlings during 7 months until August 2015. Due to the emergence of seedlings from *Senecio vulgaris* in control trays, which have been filled with sterilized soil only to capture accidentally incoming seeds from outside the trays, this species was excluded from the first sampling approach.

For the second approach, the "spring sampling", we took 20 samples on each grassland along two 5 m transects within a 7 m × 7 m plot from 0-10 cm soil depth in March 2015, when cold stratification had taken place naturally during the winter period. Transects were placed parallel to the plot borders running from north to south. Inter-sample distance was 50 cm, also overcoming spatial dependency (Plue and Hermy, 2012). We used a split tube sampler with an inner diameter of 2.9 cm, resulting in a total sampling area of 132.1 cm^2. For each sample we removed plant remains, litter but also root networks immediately after the sampling. Mixed samples of each grassland were spread on two 28 cm × 45 cm trays previously filled with 3 cm sterilized potting soil. We then placed the trays on the roof of our six-floored institute building in Münster, Germany. Münster is located 60 m a.s.l. and has an oceanic climate with a mean temperature of 9.2 °C and an annual precipitation of 750 mm. We identified all emerging seedlings during 14 months, from May 2015 to June 2016. Resprouting roots and rhizomes were excluded from the analysis. Due to the emergence of seedlings in control trays, we had to exclude several species from the second sampling (*Salix* spec., *Erigeron canadensis*,

Epilobium hirsutum and as from October 2015 also *Poa annua*, *Poa trivialis*, *Sagina procumbens*, *Leucanthemum vulgare*, *Cerastium holosteoides*).

Trays of both approaches were regularly watered and holes in their base prevented samples from waterlogging. All determined seedlings were removed from the trays in order to prevent competition with newly emerging seedlings. Seedlings, which could not directly be determined were planted into pots and grown until determination was possible.

2.3. Vegetation sampling

From 2009 to 2015, we annually recorded plant diversity and productivity simultaneously in all three regions during May and early June. We recorded cover of all vascular plant species on a permanently marked 4 m × 4 m plot in the central area of each grassland and sampled aboveground biomass, a proxy for grassland productivity, on eight 0.25m^2 quadrats adjacent to the vegetation records. We averaged the annual values for plant species richness, plant cover as well as aboveground biomass to gain a stable mean. For further details on vegetation sampling see Socher et al. (2012).

2.4. Data analysis

To quantify land-use intensity, we used questionnaires to gather information from farmers 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. Vales have been averaged the whole period of nine years to get a stable men. For each plot, an index of land-use intensity (LUI) was calculated by dividing the three components by the mean of the respective component and summing up all three standardized components according to Blüthgen et al. (2012).

As a measure of changes in vegetation composition along environmental gradients, we calculated mean abundance-weighted Ellenberg indicator values for soil moisture and nutrient supply from seed banks aboveground vegetation records (Ellenberg et al., 2001). We also calculated weighted mean values for seed weight of seed bank species by using data from BiolFlor (Kühn et al., 2004) and for

177 seed longevity according to the index introduced by Bekker et al. (1998b) using data from Thompson
178 et al. (1997).

179 Linear mixed effects models with sampling as fixed factor and site as random factor were used to
180 compare the results of both seed bank samplings (Pinheiro et al., 2016). We checked model
181 assumptions using diagnostic plots and log or square-root transformed variables when necessary. We
182 also employed Spearman correlation coefficients to relate species richness and seedling densities of
183 seed banks to each other. To further compare seed bank composition between the two sampling
184 campaigns, we performed a MANTEL test based on Bray-Curtis similarity matrices with Mantel's
185 asymptotic approximation for significance testing (McCune and Grace, 2002).

186 Spearman correlation coefficients were used to relate species richness and seedling densities of seed
187 banks to land-use intensity. Using structural equation modelling (SEM) allowed us to separate direct
188 effects of management on the seed bank from indirect effects acting via composition, diversity and
189 productivity of the vegetation (Shipley, 2002). For these indirect paths, we included the Ellenberg
190 indicator value for nutrient supply, plant species richness and biomass productivity (all from the
191 aboveground vegetation record). See Figure A2 in Supplementary material for the initial full model
192 of SEMs. Non-significant path have been removed from the models to save degrees of freedom for
193 model testing.

194 The MANTEL test was done with PC-ORD 5 (McCune and Grace, 2002). All other analyses were
195 carried out with R (R Core Team, 2016) and the lavaan package (Rosseel, 2012) for the SEMs and
196 the nlme package (Pinheiro et al., 2016) for linear mixed-effects models.

197

198 **3. Results**

199 ***3.1. Seed bank samplings***

200 Sampled grasslands varied widely in land-use intensity, nutrient availability, productivity and plant
201 species richness, and so did the seed bank (Table 1). Both seed bank approaches revealed seed banks
202 to be widely dominated by typical grassland species (Table A2 in Supplementary material). The two

seed bank samplings significantly differed in species richness and seedling densities. The autumn sampling showed 24 % higher richness and 60 % higher densities compared to the spring sampling (Table A3 in Supplementary material). Samplings also exhibited different total numbers of species, with 153 species in autumn and 105 species in spring, although the area covered by the spring sampling was larger than that of the autumn sampling. Autumn sampling also revealed a significantly lower seed longevity, while seed weight as well as both Ellenberg indicator values did not differ among samplings (Table A3 in Supplementary material). Accordingly, results of both samplings were clearly positively correlated (species richness: $r_S = 0.5$; seedling density: $r_S = 0.45$; both $p < 0.01$). Although richness and density of seedlings differed, the MANTEL test showed that the species composition of the two seed bank samplings were significantly similar ($r_S = 0.33$; $p < 0.001$). For further analyses, we therefore pooled the data from the two sampling campaigns and calculate mean species richness and density for each grassland (Table 1).

3.2. Management effects on seed banks

Soil seed banks were significantly associated with grassland management, with species richness being more closely related to management than seedling density (Figure A3 in Supplementary material). Species richness was negatively correlated with fertilization ($r_S = -0.284$; $p < 0.05$) and mowing ($r_S = -0.278$; $p < 0.05$) but positively with grazing ($r_S = 0.271$; $p < 0.05$). Due to these opposing patterns, overall land-use intensity was not significantly related to either seed bank characteristics. Seedling density was negatively correlated with fertilization only ($r_S = -0.234$; $p < 0.05$).

Structural equation modelling revealed that grazing had a direct positive effect on the species richness of seed banks (Figure 1). Fertilization, mowing and overall land-use intensity did not have a direct effect on the species richness of seed banks but an indirect negative influence via increasing Ellenberg nutrient indicator values, which were in turn directly negatively associated with species richness (Table 2). Despite being significantly related to nutrient values, neither biomass productivity nor aboveground plant species richness had significant effects on the species richness of the soil seed banks.

230 Seedling density was directly negatively influenced by fertilization and mowing but not (directly) by
231 land-use intensity and grazing. However, land-use intensity but also fertilization and mowing had
232 (also) indirect negative effects on the seedling density of the seed bank by increasing the nutrient
233 values, which were directly decreasing seedling numbers. Additionally, land-use intensity,
234 fertilization and mowing also had positive effects on seedling numbers by decreasing plant species
235 richness of the aboveground vegetation (via enhancing nutrient values) and, in terms of mowing and
236 fertilization, also by enhancing biomass productivity (again via enhancing nutrient values) Figure 1;
237 Table 2). Grazing did not have any significant path in the respective model for seedling density.

238 Although the significance of the majority of paths was consistent among SEMs for different
239 management practices, some paths were less stable and occurred in some models only, for example
240 the positive effect of productivity on seedling densities. When net effects (the sum of positive and
241 negative effects) were calculated (Table 2), only few relatively strong effects remained: grazing
242 increased species richness and mowing and fertilization decreased seedling density.

243

244 **4. Discussion**

245 ***4.1. Seed bank samplings***

246 With an average of about 4,149 seedlings per m^2 soil seed banks were within the range typical for
247 mesic grasslands (Bossuyt and Honnay, 2008). However, these findings are contrasted by
248 considerably higher seedling densities in other grassland studies (e.g. Blomqvist et al., 2003; Hölzel
249 and Otte, 2001). This can be explained by differences in grassland types and vegetation composition,
250 but also current and former land use and the methodology used (Thompson and Grime, 1979). Most
251 studies with highest seedling densities were performed in wet (alluvial) grasslands which generally
252 have a higher proportion of species producing long-lived seeds (Hölzel and Otte, 2004b), while dry
253 calcareous grasslands have considerably poorer seed banks (Jacquemyn et al., 2011). However,
254 Wellstein et al. (2007) found 10,367 seedlings per m^2 in mesic temperate grasslands and Auestad et
255 al. (2013) recorded 11,314 seedlings per m^2 in pastures and road verges. The reason for this is, beside
256 intensive grassland management (see below), mostly methodological, as environmental conditions

during germination in our trays, such as extremes in soil moisture and temperature, might have been less advantageous compared to controlled (glasshouse) conditions as in the studies by Auestad et al. (2013) and Wellstein et al. (2007). Such differences in seedling emergence indoors versus outdoors have also been reported by Graham and Hutchings (1988). However, along with low seedling densities, species richness was also markedly low compared to other studies underlining generally depleted soil seed banks under mesic temperate grasslands.

Autumn and spring sampling differed in species richness and seedling density. We assume the main reason for this to be the sampling time, there were also further small differences among sampling campaigns. Autumn sampling resulted in higher numbers of seeds and species most likely due to the inclusion of more species with a transient seed bank as expressed by shorter seed longevity in autumn samples compared to spring sampling. In spring, these species have already germinated and were thus not included in the analysis (Thompson and Grime, 1997). There was no further unusual or extreme change in abiotic conditions such as flooding or sediment deposition (Hözel and Otte, 2004a) in between sampling times in the environmentally rather stable mesic grasslands. However, the larger area covered by the autumn sampling and the removal of root networks from soil samples of spring sampling may have also led to slightly lower species richness and seedling densities. Furthermore, effects of slightly deeper sampling depth during autumn sampling and differences in environmental conditions during seedling emergence in Bern and Münster cannot completely be ruled out. Yet, mean Ellenberg indicator values did not differ among seed bank samplings, indirectly indicating widely similar conditions for seedling emergence at both locations. In line with the relatively low similarity among seed bank samplings, our results indicate that seed bank composition might also be considerably driven by stochasticity, as many seed bank species occur in very low seedling densities (Thompson and Grime, 1979; Wellstein et al., 2007). This is strengthened by the usually relatively small area covered by seed bank sampling campaigns compared to aboveground vegetation records (Vandvik et al., 2016).

4.2. Management effects on seed banks

The depletion of the soil seed banks in seeds and species is also the result of the rather high mean land-use intensity when compared to previous studies with mostly unfertilized vegetation (Auestad et al., 2013; Wellstein et al., 2007). Hereby, management effects could be clearly separated into positive effects of grazing and negative effects of mowing and fertilization. In line with Jacquemyn et al. (2011), grazing had a direct positive impact on species richness of the seed bank but not on seedling density. In general, grazing can affect seed banks in different ways. First, grazing is often less intensive with small fertilizer inputs when compared to mowing (Blüthgen et al., 2012), and enables a longer period for seed set of different species due to the incomplete biomass removal by livestock and the resulting patchy structure. Second, trampling supports ruderal and annual species by pushing seeds into the ground and by creating open patches for regeneration of annual species (Jacquemyn et al., 2011; Müller et al., 2014). Missing effects of grazing on the seedling density might be due to described positive effects being counteracted by negative effects of grazing before and during seed set, as found by Sternberg et al. (2003). Nevertheless, a certain proportion of seeds eaten by livestock is still able to germinate after excretion and may also become part of the seed bank (Gilhaus et al., 2017b).

Negative effects of fertilization and mowing on the species richness of the soil seed bank were mediated by higher Ellenberg nutrient values being a proxy for changing the plant community composition towards plants with higher individual aboveground productivity which also means towards a more competitive vegetation, which is adapted to high nutrient levels (Ellenberg et al., 2001; Gilhaus et al., 2017a). Species dominating under such conditions are often tall-growing grasses, such as *Dactylis glomerata* or *Arrhenatherum elatius*, which hardly build up a persistent seed bank (Bekker et al., 1997; Thompson and Grime, 1979). Additionally, productive and dense grassland swards decrease the number of gaps for the regeneration of annual species (Jacquemyn et al., 2011). Direct negative effects of fertilization and mowing on seedling density can be explained by the detrimental influence of frequent and early cuts, inhibiting seed set of many species (Dölle and Schmitdt, 2009; Jacquemyn et al., 2011). As in grasslands, fertilization intensity increases with

310 mowing frequency (Blüthgen et al., 2012), which is also true in our study (r_S = 0.636; p < 0.001),
311 fertilization itself might act mainly indirectly via the detrimental impact of mowing (Socher et al.,
312 2012). This is supported by Bekker et al. (1998a), who did not generally find negative effects of
313 fertilizer applications on seed longevity.

314 The overall land-use intensity affected the seed bank only weakly, if at all, because positive and
315 negative effects of the single components mostly cancelled each other out. Nevertheless, the overall
316 land-use intensity showed a slightly closer relationship to mowing and fertilization than to grazing
317 (Blüthgen et al., 2012), explaining in sum negative effects in SEMs. In general, management effects
318 on soil seed banks were a complex mixture of positive and negative effects, and therefore our study
319 highlights the importance of including different land-use components for a comprehensive
320 assessment of the effects of land-use intensity on the soil seed bank.

321 Beside described effects of grassland management, biomass productivity had a positive and
322 aboveground plant species richness a negative effect on seedling density. Very high levels of plant
323 species richness are mostly restricted to low-productive grassland plant communities containing
324 higher proportions of stress tolerating species (Dengler et al., 2014; Klaus et al., 2011), which are
325 known to invest much less in seed production and which often do not build up a persistent seed bank
326 (Thompson and Grime, 1979; Wellstein et al., 2007). At first sight, the positive effect of productivity
327 seems to contradict negative effects of nutrient supply, but this may just indicate that higher
328 productivity also enables plants to produce more seeds (Fujita et al., 2014).

329

330 **5. Conclusions**

331 Depleted seed banks of intensively used temperate grasslands with rather low numbers of viable
332 seeds and species indirectly hint at the importance that vegetative spread generally has for the
333 regeneration of grassland swards (Vítová et al., 2017). As low land-use intensity but intensive
334 grazing increased species richness of seed banks, plant communities of pastoral systems might be
335 more resilient than fertilized meadows. Thus, pastures may have a higher chance to recover after

mechanic disturbance. Taken it the other way round, our study described a higher vulnerability of grasslands communities, which are shaped by mowing only. This should be taken into account when assessing possible impacts of climate and land-use changes on grassland ecosystems.

Acknowledgements

Many thanks go to Judith Hinderling, Stefan Blaser, Svenja Kunze and Melanie Eckardt for their support during field work and data handling. We also thank the managers of the three Exploratories, Kirsten Reichel-Jung, Katrin Lorenzen, Martin Gorke and all former managers for their work in maintaining the plot and project infrastructure; Christiane Fischer forgiving support through the central office, Michael Owonibi for managing the central data base, and Eduard Linsenmair, Dominik Hessenmöller, Ingo Schöning, François Buscot, Ernst-Detlef Schulze, Wolfgang W.Weisser and the late Elisabeth Kalko for their role in setting up the Biodiversity Exploratories project. The work has been funded by the DFG Priority Program 1374 "Infrastructure-Biodiversity-Exploratories" (FI 1246/15-2; HO 3830/2-3; KL 2265/5-1). Field work permits were issued by the responsible state environmental offices of Baden-Württemberg, Thüringen, and Brandenburg (according to § 72 BbgNatSchG).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version…

References

Allan, E., Manning, P., Prati, D., Grassein, F., Alt, F., Binkenstein, J., Blaser, S., Blüthgen, N., Hölzel, N., Klaus, VH., Kleinebecker, T., Morris, E-K., Oelmann, Y., Renner, S.C., Rillig, M.C., Schäfer, M., Schloter, M., Schmitt, B., Schöning, I., Schrumpf, M., Solly, E., Sorkau, E., Steckel, J., Steffen-Dewenter, I., Stempfhuber, B., Trumbore, S., Weiner, C.N., Weisser, W.W., Werner, W., Westphal, C., Wilcke, W., Fischer, M., 2015. Land use intensification alters ecosystem multifunctionality via loss of biodiversity and changes to functional composition. Ecol. Lett. 18, 834–843.

363 Auestad, I., Rydgren, K., Spindelböck, J.P., 2013. Management history affects grassland seed bank build-up.
364 Plant Ecol. 214, 1467–1477.

365 Bekker, R.M., Verweij, G.L., Smith, R.E.N., Reine, R., Bakker, J.P., Sneider, S., 1997. Soil seed banks in
366 European grasslands: does land use affect regeneration perspectives? J. Appl. Ecol. 34, 1293–1310.

367 Bekker, R.M., Knevel, I.C., Tallowin, J.B.R., Troost, E.M.L., Bakker, J.P., 1998a. Soil nutrient input effects
368 on seed longevity: a burial experiment with fen-meadow species. Func. Ecol. 12, 673–682.

369 Bekker, R.M., Bakker, J.P., Grandin, U., Kalamees, R., Milberg, P., Poschlod, P., Thompson, K., Willems,
370 J.H., 1998b. Seed size, shape and vertical distribution in the soil: indicators of seed longevity. Func.
371 Ecol. 12, 834–842.

372 Blüthgen, N., Dormann, C., Prati, D., Klaus, V.H., Kleinebecker, T., Hölzel, N., Alt, F., Boch, S., Gockel, S.,
373 Hemp, A., Müller, J., Nieschulze, J., Renner, S.C., Schöning, I., Schumacher, U., Socher, S.A., Wells,
374 K., Birkhofer, K., Buscot, F., Oelmann, Y., Rothenwöhrer, C., Scherber, C., Tscharntke, T., Weiner, C.,
375 Fischer, M., Kalko, E.K.V., Linsenmair, K.E., Schulze, E.-D., Weisser, W.W., 2012. A quantitative
376 index of land-use intensity in grasslands: integrating mowing, grazing and fertilization. Basic Appl.
377 Ecol. 13, 207–220.

378 Blüthgen, N., Simons, N., Jung, K., Prati, D., Renner, S., Boch, S., Fischer, M., Hölzel, N., Klaus, V.H.,
379 Kleinebecker, T., Tschapka, M., Weisser, W.W., Gossner, M.M., 2016. Land use imperils plant and
380 animal community stability through changes in asynchrony rather than diversity. Nat. Commun. 7,
381 10697.

382 Blomqvist, M.M., Bekker, R.M., Vos, P., 2003. Restoration of ditch bank plant species richness: The
383 potential of the soil seed bank. Appl. Veg. Sci. 6, 179–188.

384 Bossuyt, B., Honnay, O., 2008. Can the seed bank be used for ecological restoration? An overview of seed
385 bank characteristics in European communities. J. Veg. Sci. 19, 875–884.

386 Busch, V., Klaus, V.H., Penone, C., Schäfer, D., Boch, S., Prati, D., Müller, J., Socher, S.A., Niinemets, Ü.,
387 Peñuelas, J., Hölzel, N., Fischer, M., Kleinebecker, T., 2017. Nutrient stoichiometry and land use rather
388 than species richness determine plant functional diversity. Ecol. Evol., doi: 10.1002/ece3.3609.

389 Dengler, J., Janišová, M., Török, P., Wellstein, C., 2014. Biodiversity of Palaearctic grasslands: a synthesis.
390 Agric. Ecosys. Environ. 182, 1–14.

391 Dölle, M., Schmidt, W., 2009. The relationship between soil seed bank, above-ground vegetation and
392 disturbance intensity on old-field successional permanent plots. Appl. Veg. Sci. 12, 415–428.

393 Ellenberg, H., Weber, H.E., Düll, R., Wirth, V., Werner, W., 2001. Zeigerwerte von Pflanzen in
394 Mitteleuropa. Scr. Geobot. 18, 1–262.

395 Fischer, M., Bossdorf, O., Gockel, S., Hänsel, F., Hemp, A., Hessenmöller, D., Korte, G., Nieschulze, J.,
396 Pfeiffer, S., Prati, D., Renner, S., Schöning, I., Schumacher, U., Wells, K., Buscot, F., Kalko, E.K.V.,

Linsenmair, K.E., Schulze, E.-D., Weisser, W.W., 2010. Implementing largescale and long-term functional biodiversity research: the biodiversity exploratories. Basic Appl. Ecol. 11, 473–485.

Fujita, Y., Olde Venterink, H., van Bodegom, P.M., Douma, J.C., Heil, G.W., Hölzel, N., Jabłońska, E., Kotowski, W., Okruszko, T., Pawlikowski, P., de Ruiter, P.C., Wassen, M.J., 2014. Low investment in sexual reproduction threatens plants adapted to phosphorus limitation. Nature 505, 82–86.

Gilhaus, K., Hölzel, N., Kleinebcker, T., Prati, D., Boch, S., Fischer, M., Schmitt, B., Rupprecht, D., Klaus, V.H., 2017a. Grassland management in Germany: effects on plant diversity and vegetation composition. Tuexenia 37, 379–397.

Gilhaus, K., Freitag, M., Kunze, S., Hölzel, N., 2017b. High fodder value and feeding likelihood favor endozoochorous plant dispersal. J. Veg. Sci. 28, 357–367.

Graham, D.J., Hutchings, M.J., 1988. A field investigation of germination from the seed bank of a chalk grassland ley on former arable land. J. Appl. Ecol. 25, 253–263.

Habel, J.C., Dengler, J., Janišová, M., Török, P., Wellstein, C., Wiezik, M., 2013. European grassland ecosystems: Threatened hotspots of biodiversity. Biodivers. Conserv. 22, 2131–2138.

Hölzel, N., Otte, A., 2001. The impact of flooding regime on the soil seed bank of flood-meadows. J. Veg. Sci. 12, 209–218.

Hölzel, N., Otte, A., 2004a. Restoration of a species-rich flood meadow by topsoil removal and diaspore transfer with plant material. Appl. Veg. Sci. 6, 131–40.

Hölzel, N., Otte, A., 2004b. Assessing soil seed bank persistence in flood-meadows: The search for reliable traits. J. Veg. Sci. 15, 93–100.

Jacquemyn, H., Van Mechelen, C., Brys, R., Honnay, O., 2011. Management effects on the vegetation and soil seed bank of calcareous grasslands: an 11-year experiment. Biol. Conserv. 144, 416–422.

Joyce, C. B., Simpson, M., Casanova, M., 2017. Future wet grasslands: ecological implications of climate change. Ecosys. Health Sust. 2, doi:10.1002/ehs2.1240.

Klaus, V. H., Kleinebecker, T., Hölzel, N. Blüthgen, N., Boch S., Müller, J., Socher, S. A., Prati, D., Fischer, M., 2011. Nutrient concentrations and fibre contents of plant community biomass reflect species richness patterns along a broad range of land-use intensities among agricultural grasslands. Persp. Plant Ecol. Evol. Sys. 13, 287–295.

Klaus, V.H., Kleinebecker, T., Boch, S., Müller, J., Socher, A.S., Prati, D., Fischer, M., Hölzel, N., 2012. NIRS meets Ellenberg's indicator values: Prediction of moisture and nitrogen values of agricultural grassland vegetation by means of near-infrared spectral characteristics. Ecol. Indic. 14, 82–86.

Kühn, I., Durka, W., Klotz, S., 2004. BiolFlor: a new plant-trait database as a tool for plant invasion ecology. Divers. Distrib. 10, 363–365.

McCune, B., Grace, J.B., 2002. Analysis of Ecological Communities. MjM Software Design, Gleneden Beach, Oregon.

Müller, J., Heinze, J., Joshi, J., Boch, S., Klaus, V.H., Fischer, M., Prati, D., 2014. Influence of experimental soil disturbances on the diversity of bryophytes and vascular plants in agricultural grasslands. J. Plant Ecol. 7, 509–517.

Oomes, M.J.A., Ham M., 1983. Some methods of determining the seed bank. Acta Bot. Neerl. 32, 244.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., R Core Team, 2016. nlme: Linear and Nonlinear Mixed Effects Models.

Plue, J., Hermy, M., 2012. Consistent seed bank spatial structure across semi-natural habitats determines plot sampling. J. Veg. Sci. 23, 505–516.

R Core Team, 2016. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.r-project.org (accessed 17. March 2017).

Rosseel, Y., 2012. lavaan: An R Package for Structural Equation Modeling. J. Stat. Softw. 48, 1–36.

Shipley, B., 2002. Cause and correlation in biology: a user's guide to path analysis, structural equations and causal inference. Cambridge Univ. Press, Cambridge.

Socher, S., Prati, D., Müller, J., Klaus, V.H., Hölzel, N., Fischer, M., 2012. Direct and productivity-mediated indirect effects of fertilization, mowing and grazing intensities on grassland plant species richness. J. Ecol. 100, 1391–1399.

Sternberg, M., Gutman, M., Perevolotsky, A., Kigel, J., 2003. Effects of grazing on soil seed bank dynamics: an approach with functional groups. J. Veg. Sci. 14, 375–386.

Ter Heerdt, G.N.J., Verweij, G.L., Bekker, R.M., Bakker, J.P., 1996. An improved method for seed-bank analysis: seedling emergence after removing the soil by sieving. Func. Ecol. 10, 144–151.

Tilman, D., Downing, J.A., 1994. Biodiversity and stability in grasslands. Nature 367, 363–365.

Thompson, K., Grime, J.P., 1979. Seasonal variation in the seed banks of herbaceous species in ten contrasting habitats. J. Ecol. 67, 893–921.

Thompson, K., Bakker, J.P., Bekker, R.M., 1997. The Soil Seed Banks of North West Europe: Methodology, Density and Longevity. Cambridge University Press, Cambridge, UK.

Thompson, K., Bakker, J.P., Bekker, R.M., Hodgson, J.G., 1998. Ecological correlates of seed persistence in the soil in the north-west European flora. J. Ecol. 86, 163–169.

Valkó, O., Török, P., Tóthmérész, B., Matus, G., 2011. Restoration potential in seed banks of acidic fen and dry-mesophilous meadows: Can restoration be based on local seed banks? Rest. Ecol. 19, 9–15.

Vandvik, V., Klanderud, K., Meineri, E., Måren, I.E., Töpper, J., 2016. Seed banks are biodiversity reservoirs: species-area relationships above versus below ground. Oikos 125, 218–228.

463 Vítová A., Macek P., Lepš J., 2017. Disentangling the interplay of generative and vegetative propagation
464 among different functional groups during gap colonization in meadows. Func. Ecol. 31, 458–468.

465 Wellstein, C., Otte, A., Waldhardt, R., 2007. Seed bank diversity in mesic grasslands in relation to vegetation
466 type, management and site conditions. J. Veg. Sci. 18, 153–162.

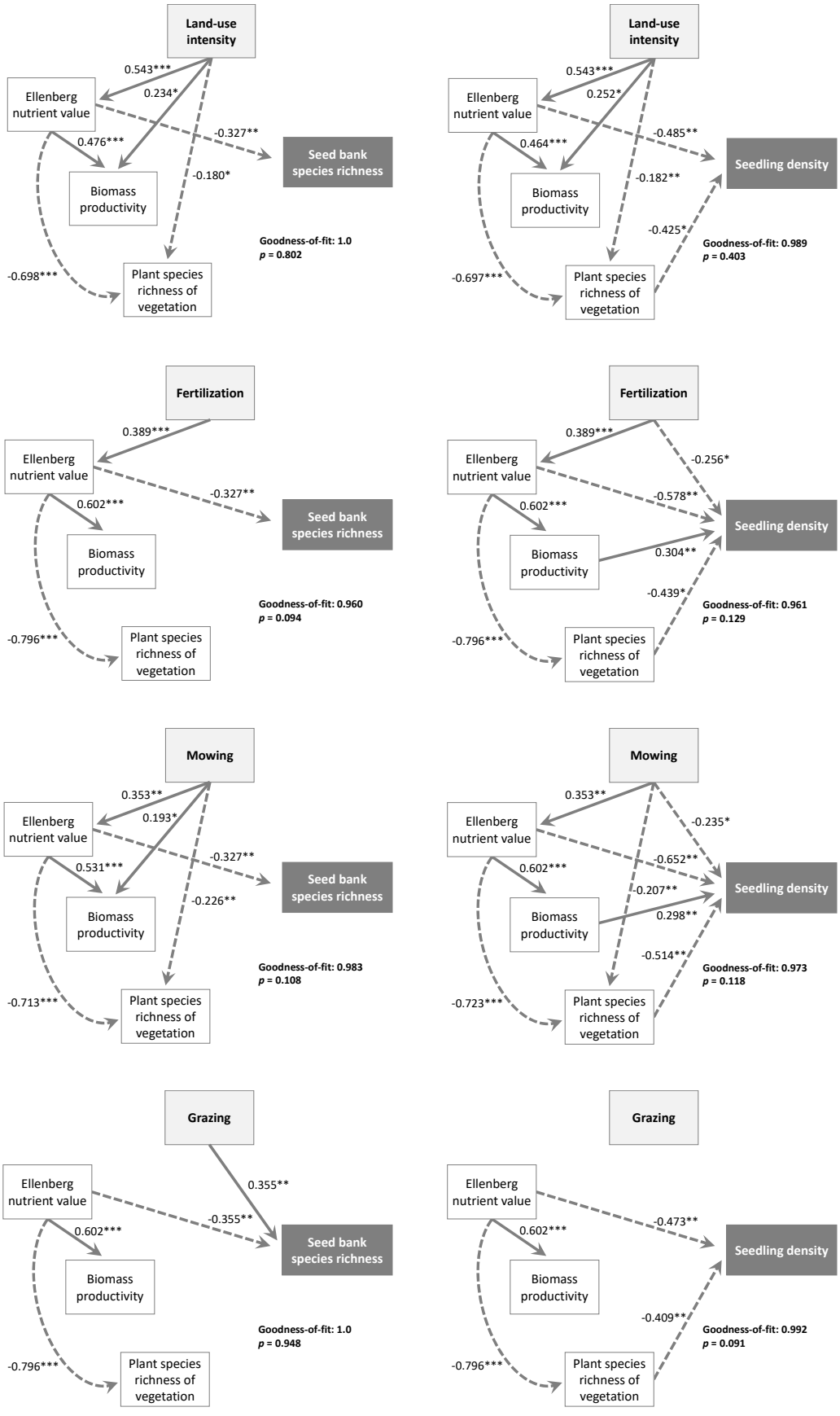
Table and figures

Table 1. Characteristics of seed banks, grassland vegetation and land use. For seed banks mean values of both sampling approaches are shown. See methods section for further details. Plant species richness is a multi-year average of the aboveground plant community. Grazing intensity is given in livestock units*grazing days*year^{-1}; Mowing intensity is given as cuts*year^{-1}; Fertilization intensity is given as the amount of nitrogen in fertilizer in kg*ha^{-1}*year^{-1}; the land-use intensity index (LUI) integrates all three components after individual standardization.

		Mean	SD	Min/Max
Seed bank	Species richness	12.91 ±	4.37	3.5/25
	Seedling density (m^2)	4149 ±	2405	556/11597
Grassland vegetation	Plant species richness (16 m^2)	28.78 ±	11.15	13.4/69.4
	Biomass productivity (m^2)	242.46 ±	86	42.5/447.9
	Ellenberg nutrient value	5.81 ±	1.1	2.46/7.16
Land use	Grazing intensity	143.76 ±	180.45	0/865.1
	Mowing intensity	1.01 ±	0.91	0/3
	Fertilization intensity	34.71 ±	48.35	0/208.1
	Land-use intensity (LUI)	1.67 ±	0.6	0.49/3.57

Table 2. Single standardized path coefficients and net effects of grassland management on seed banks in structural equation models (Fig 1). Indirect effects have been calculated by multiplying respective direct path coefficients. Nutrient IV = Ellenberg indicator value for nutrient supply, calculated from the aboveground plant community. Plant species richness also refers to the aboveground vegetation. LUI = land-use intensity index. Path with β > 0.2 are given in bold.

		Species richness				**Seedling density**			
		LUI	Fertilization	Mowing	Grazing	LUI	Fertilization	Mowing	Grazin
	Direct effect	0	0	0	0.355	0	-0.256	-0.235	0
Indirect effects	via nutrient IV	-0.178	-0.127	-0.115	0	-0.263	-0.225	-0.23	0
	via productivity	0	0	0	0	0	0.071	0.063	0
	via plant species richness	0	0	0	0	0.161	0.136	0.144	0
	Total effect	-0.178	-0.127	-0.115	**0.355**	-0.103	**-0.274**	**-0.257**	0



472

473 Figure 1.