Research Paper

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

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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 to 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