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Seed availability in hay meadows: Land-use intensification promotes seed rain but not the persistent seed bank

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ABSTRACT

Intensification of land use in semi-natural hay meadows has resulted in a decrease in species diversity. This is often thought to be caused by the reduced establishment of plant species due to high competition for light under conditions of increased productivity. Sowing experiments in grasslands have found reliable evidence that diversity can also be constrained by seed availability, implying that processes influencing the production and persistence of seeds may be important for the functioning of ecosystems. So far, the effects of land-use intensification on the seed rain and the persistence of seeds in the soil have been unclear.

We selected six pairs of extensively managed (*Festuco-Brometea*) and intensively managed (*Arrhenatheretalia*) grassland with traditional late cutting regimes across Switzerland and covering an annual productivity gradient in the range 176–1211 $\text{g}\,\text{m}^{-2}$. In each grassland community, we estimated seed rain and seed bank using eight pooled seed-trap or topsoil samples of 89 cm^2 in each of six plots representing an area of c. 150 m^2. The seed traps were established in spring 2010 and collected simultaneously with soil cores after an exposure of c. three months. We applied the emergence method in a cold frame over eight months to estimate density of viable seeds.

With community productivity reflecting land-use intensification, the density and species richness in the seed rain increased, while mean seed size diminished and the proportions of persistent seeds and of species with persistent seeds in the topsoil declined. Stronger limitation of seeds in extensively managed semi-natural grasslands can explain the fact that such grasslands are not always richer in species than more intensively managed ones.

1. Introduction

Global maxima of plant-species richness at small spatial scales have been recorded in nutrient-poor semi-natural grasslands (Wilson et al., 2012). Such grasslands, including dry hay meadows and pastures which persist under extensive or low-intensive management (Dietl and Lehmann, 2006), strongly declined over the second half of the 20th century in Europe and their multiple biodiversity-related services were degraded due to changes in land use, such as abandonment or intensification of management (EEA, 2010). This often involved activities such as ploughing, seeding, herbicide and fertilizer applications, and early and high-frequent cutting, resulting in radical replacement of the former plant communities. A more moderate form of land-use change aimed at increasing the quality and quantity of fodder harvested from hay meadows by fertilization in combination with increased cutting frequency and grazing (Dietl and Lehmann, 2006). A decrease in plant-species diversity related with such change is often thought to be caused by reduced establishment success of species due to high competition for light in sites of increased productivity (Hautier et al., 2009). Sowing experiments in grasslands have found reliable evidence that diversity can also be constrained by seed availability (Foster and Tilman, 2003; Turnbull et al., 2000; Zeiter et al., 2006; Zobel et al., 2000), implying that processes influencing the production and persistence of seeds may be important for the functioning of ecosystems. The variation among sites in the population and community response to increased seed availability is strikingly large (Stein et al., 2008; Zeiter et al., 2006) and poorly understood. Unexplained among-site variation might result from differences in the ambient seed rain and the size of the pre-existing seed bank (Clark et al., 2007). In the context of climate change, knowledge of seed rain and seed banks is also important for an understanding of grassland resilience to extreme weather events, as reproductive output and seed persistence can

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be expected to affect community composition (Stampfli and Zeiter, 2004, 2008).

While the effects of the cessation of management on seed availability and the seed banks have been studied in the context of restoration (Bakker et al., 1996; Bekker et al., 1997; Valkó et al., 2011), there is very little information on how land-use intensification affects seed abundance. The maturation of seeds of many species may directly be hampered as an earlier date of starting mowing is often necessary in the context of massive intensification. However, if land-use intensification allows for seed maturation, the seed rain should increase because water and nutrients are less limited. While fertilization of plants grown in greenhouse monocultures has indeed increased seed production (e.g. Manning et al., 2009), fertilization studies in natural communities have revealed mixed results (Burkle and Irwin, 2010; Dainese, 2011; Smith et al., 1996). Land-use intensification also changes the species composition of communities, but the consequences of such change for the seed rain are not known. Lower numbers of seeds may result from the fact that species with larger seeds are common among those European flora which prefer habitats of higher productivity (Pärtel and Zobel, 2007). Moreover, introduced species of grasses in intensively managed grasslands may produce fewer seeds because they have been selected for their clonal growth capacity or bred for forage production (Marshall and Hides, 2000).

In addition to influencing seed-rain density, land-use intensification may also affect the size of the seeds in the seed rain. Seed size may differ across environmental gradients because of a link between two trade-offs, the size-number (Harper et al., 1970; Smith and Fretwell, 1974) and colonization–competition (Armstrong, 1976; Hutchinson, 1951) trade-off. Ideally, larger seed size would be favoured in more fertile habitats in which competition for light is higher, while smaller seed size would be favoured in habitats in which open spaces are created by small-scale disturbances (Pakeman et al., 2008). However, in the face of environmental hazards, the effect of more nutrients stored in reserves may also result in better survival of individuals of species with larger seeds (Westoby et al., 1996). As different processes may select for larger seeds in more or less fertile grasslands, and as more frequent disturbance due to higher cutting frequency would select for smaller seeds in intensively managed grasslands, it remains unclear whether seeds size differs between differently managed grasslands.

A potential difference in the intensity or predictability of disturbance between extensively and intensively managed grasslands may also influence the persistence of seeds in the soil. According to Fenner and Thompson (2005), the seasonally predictable drought in Mediterranean grassland does not select for annual grasses with a persistent seed bank, while unpredictable disturbances created by grazing tend to favour dicotyledons with persistent seeds. Seed persistence or accumulation in the soil may differ between the two land-use types due to different abundances of seed predators, different seed longevity and dormancy, and different soil characteristics affecting the survival of seeds such as soil moisture (Pakeman et al., 2012) or the presence of fungi (Mordecai, 2012).

Persistence of species in the soil seed bank has been classified according to the vertical distributions of seeds in the soil (Thompson et al., 1997). This method assumes that the depth-distribution of the seeds in the soil reflects seed longevity, in that species with a higher proportion of seeds in lower soil layers have greater seed longevity (Bekker et al., 1998a; Grandin and Rydin, 1998). This assumption, however, does not take into consideration the fact that gain and loss of seeds in soil seed banks are independent processes. A recent seed burial experiment (Saatkamp et al., 2009) found different mortalities of seeds of different species in the soil and showed that estimates of species persistence in a seed bank based on seed densities of different soil layers were in fact correlated with seed production, but not with seed longevity. Therefore, reliable information on the longevity of seeds would need, for example, burial experiments or direct age determination by ^{14}C dating, methods which are lengthy or expensive. A relatively easy way to estimate the proportion of persistent seeds in the soil is to compare the seed densities of soil samples containing the seed rain of the current year and persistent seeds in the soil with the densities of simultaneously caught seeds in traps containing only seed rain of the current year. This approach quantifies the proportion of seeds in the soil which are transient but cannot distinguish between short- and long-term persistent seeds (sensu Thompson et al., 1997). It has rarely been used (but see Jakobsson et al., 2006).

In this study, we simultaneously measured the size and the composition of the seed rain and the topsoil seed bank in twelve permanent grasslands across Switzerland, covering an annual productivity gradient in the range 176–1211 $\mathrm{g\,m^{-2}}$. Within limits of land-use intensification which allow most species to set seed before the first cut, we answer the following questions: (1) Does community productivity influence the density and diversity of the seed rain? (2) Is the size of the seeds in the seed rain related to community productivity? (3) Does community productivity influence density and diversity of the seed bank, or in particular, the proportion of persistent seeds and the proportion of species with persistent seeds in the soil? We further investigated whether productivity effects on seed rain and seed bank are consistent across major plant functional groups.

2. Method

2.1. Study sites

This study was performed in twelve temperate semi-natural grasslands across Switzerland (Table 1, supplementary data, Fig. S1). We chose pairs of extensively managed *Festuco-Brometea* and intensively managed *Arrhenatheretalia*-type grasslands, representing space-for-time substitutes of plant communities transformed by past-to-present land-use intensification (Ellenberg, 1996), in six regions so that each pair had similar climatic conditions. We selected grasslands which have been constantly used for haymaking and not ploughed during the past decades. The extensively managed grasslands are normally not fertilized and are mown once or twice a year. The more intensively managed grasslands, including ‘low intensive’ and ‘moderately intensive’ grasslands sensu Dietl and Lehmann (2006), are regularly fertilized with manure or slurry and mown once to three times a year, often followed by autumn grazing (Table 1). Land use was slightly intensified at one *Festuco-Brometea* site (Pree, fertilization by manure) and slightly extensified at one *Arrhenatheretalia* site (Zollikofen, cessation of fertilization) during the past decade. Our selection of sites excluded ‘highly intensive’ grasslands in which seed set of most species is prevented by early and high-frequent cutting (Dietl and Lehmann, 2006). High species richness qualified several of the extensively managed meadows as sites of national importance (Anonymous, 2010). At the Negrentino site (Stampfli, 1992; Zeiter and Stampfli, 2012), species richness equals current world records at small spatial scales (Wilson et al., 2012).

At each site, a soil profile was sampled using an Edelmann auger (core length 10 cm, diameter 6 cm) to determine soil characteristics (Table 1) such as soil strata, water storage capacity and soil type (sensu FAL, 2002) in spring 2010 (S. Tschumi 2012, unpubl. BA thesis, Universität Freiburg). We also determined pH, measured in water, and C/N ratio, using a C–N analyzer (Vario Macro, Elementar GmbH, Hanau, Germany), of five pooled soil cores (length 6–13 cm, diameter 1.8 cm) sampled with a sharpened tube at each site except at Thun (ten pooled cores of 3 cm in length).

Table 1
Land use and soil characteristics of study sites: geographic location within regions (Reg), Jura (J), Plateau (P), northern Alps (A_N), central Alps (A_C), southern Alps (A_S), southern Prealps (A_{SP}), geographic coordinates (GC), elevation (E), land-use type (T), extensive (e), intensive (i), annual productivity (AP), fertilization applied in period 1990–2009 (F), annual manure of cow, goat, or cow and goat (m_c, m_g, m_{cg}), annual slurry (s), biennial slurry 1991–2000 (s_b), none (-), usual cutting frequency and additional (+) autumn grazing (C), soil strata (Stra), soil type, water storage capacity (WSC), pH and C/N ratio.

Site	Geographic location			Land use				Soil characteristics				
	Reg	GC °N/°E	E m a.s.l.	T	AP $g m^{-2} yr^{-1}$	F	C yr^{-1}	Stra	Soil type	WSC $l m^{-2}$	pH	C/N
Monthey	J	47.07/7.06	650	e	512	–	1+	AC	Regosol	30	6.3	9.9
Combazin	J	47.07/7.05	670	i	535	s_b	1+	AC	Regosol	40	6.5	10.0
Krauchthal	P	47.01/7.57	625	e	462	–	2	AC	Regosol	40	7.0	16.5
Zollikofen	P	47.00/7.46	555	i	754	–	3	ABC	Parabraunerde	125	5.4	8.1
Thun	A_N	46.75/7.59	570	e	153	–	1	AC	Fluvisol	30	6.8	13.9
Erlenbach	A_N	46.66/7.57	830	i	1211	m_c	3+	ABC	Kalkbraunerde	50	6.9	12.6
Bister-Chumme	A_C	46.36/8.08	1110	e	176	–	1	AC	(Trocken-)Regosol	30	7.1	10.7
Bister-Breite	A_C	46.36/8.06	1000	i	550	m_g	2+	ABC	Phaeozem	110	6.9	15.9
Negrentino	A_S	46.46/8.92	820	e	414	–	2	ABC	Saure Braunerde	60	5.2	9.5
Casserio	A_S	46.44/8.94	770	i	1107	s	3+	ABC	Verbraunter Ranker	40	5.4	9.6
Pree	A_{SP}	45.90/9.01	950	e	798	m_{cg}	2+	AC	Rendzina	20	6.0	12.1
Somazzo	A_{SP}	45.88/8.99	575	i	540	m_c	2+	ABC	Braunerde	125	5.5	10.6

2.2. Above-ground vegetation and community productivity

At each site, six plots of 3 × 3 m were established, with a minimal distance of 1.5 m between plots. All species of the above-ground vegetation within plots were recorded immediately before mowing in summer 2010 (supplementary data, Table S1). Rooted frequency of all species in twelve subplots (18 × 36 cm) per plot was also recorded (supplementary data, Fig. S1) and species were listed as being present without reproductive tissue, with flowering stems, or with ripe seeds. The local species pool of a community includes established plants and relates to the area of six plots at a site, while species richness at plot scale was defined by the cumulative number of species in twelve subplots (supplementary data, Table S1). Rooted frequency in subplots was used to calculate Shannon diversity [$H' = -\Sigma\ (p_i \ln p_i)$] at plot scale. Communities of both land-use types differed neither in size of the local species pool ($F_{1,10} = 1.62$, $P = 0.23$) nor in species richness ($F_{1,10} = 0.72$, $P = 0.42$) or Shannon diversity H' ($F_{1,10} = 0.38$, $P = 0.55$) based on ANOVAs.

As a surrogate for community productivity we used the phytomass of the first harvest of subplots (18 × 36 cm) in summer 2010 because this directly reflected the resource availability (nutrients and water) during the period in which the seeds of the sampled seed rain were produced (supplementary data, Table S1). The phytomass, including standing dead plant mass, was cut at 4 cm above-ground with scissors and weighed after drying at 60 °C for 24 h. An analysis of log-transformed phytomass values with ANOVA showed that community productivity (phytomass of the first harvest) was higher ($F_{1,10} = 5.29$, $P < 0.05$) in intensively managed *Arrhenatheretalia* (415 $g m^{-2}$) than in extensively managed *Festuco-Brometea* (264 $g m^{-2}$) grasslands. The phytomass of the first harvest was correlated with the sum of phytomass over consecutive harvests in 2010 (annual productivity, Table 1), based on measurements in the subplots of three out of six plots across sites ($r = 0.94$, $n = 12$).

Frequency sums of typical species of *Festuco-Brometea* and *Molinio-Arrhenatheretea* grasslands (Ellenberg, 1996), contributing 60% to the subplot-based frequency sum over all species and sites, were used as indicators of extensive or intensive land use at the site scale. These indicators were negatively correlated ($r = -0.66$, $n = 12$; supplementary data, Table S1). Frequency sum of *Molinio-Arrhenatheretea* species and community productivity were highly positively correlated over wide ranges ($r = 0.92$, $n = 12$) representing a gradient rather than two distinct types (Fig. 1). This gradient of species composition and resource availability reflects land-use intensification. As community productivity may vary due to small-scale soil heterogeneity or heterogeneous distribution of manure within sites, its relationships with seed-rain and seed-bank variables were analyzed among sites and at the smaller scale of plot within site.

2.3. Design of seed sampling

Seed rain and topsoil seed bank were measured using eight sample pairs, including a seed trap and a soil core of equal dimension, 3.6 cm in diameter and 2.5 cm in height, in the centre of the plots. Seed densities in lower soil layers were not measured as natural seedling emergence from soil depth <1 cm is relatively rare (Traba et al., 2004). The position of sample type was randomly allocated within pairs (supplementary data, Fig. S1). The samples of each measurement type were pooled per plot. With this sampling design, the chance of finding one seed with 95% confidence at a site requires a seed density of 61.3 seeds m^{-2} based on a Poisson distribution of seeds (Thompson et al., 1997).

The seed traps consisted of plastic pots with drainage holes, filled with sterilized sand of a grain size of <4 mm (Spiel- und Rasensand, Ricoter, Aarberg, Switzerland). The seed traps were placed in spring 2010, level with the ground, after removing a soil core of equal dimensions with a sharpened tube. The traps were covered with a plastic lid to prevent seed loss and contamination when transported to the field and back to the laboratory. The seed traps were collected using forceps in summer 2010, after the first or second cut in the plots, i.e. after an exposure of approximately

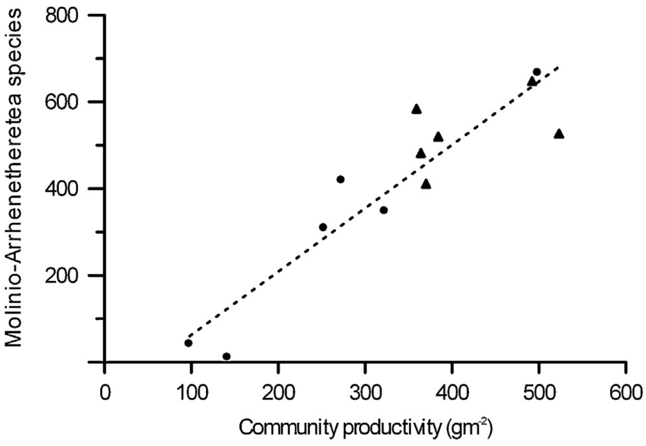

Fig. 1. Positive correlation between frequency sum of typical species of *Molinio-Arrhenatheretea* grasslands (Ellenberg, 1996) and community productivity (phytomass of the first harvest) across extensively (circles) and intensively managed (triangles) grasslands.

three months (supplementary data, Table S2). To mimic traditional hay-making and to enable after-ripening of the seeds, the hay was allowed to dry on the plots for at least 8 h and was turned with a small rake before removal from the plots. Special care was taken to prevent the rake from making contact with the soil surface and the seed traps. Immediately after collecting the traps, the soil samples were cored with a sharpened tube. All samples were stored at room temperature for approximately two months. If the samples were wet or moist due to precipitation preceding their collection, the samples were laid out on plates enclosed within paper bags to prevent contamination, to dry at room temperature.

Sampling was performed approximately simultaneously in the two different grasslands of each region to prevent confounding of effects of land-use intensity and sampling date. The two grassland types had a difference of twelve days in trap exposure resulting from a different timing of the set-up in spring, but this had no effect on the seed rain data because species did not shed seeds during this period (supplementary data, Table S2). It was assumed that most species in both grassland types shed seeds by mid-July.

Thirteen out of 576 seed traps (2%) were lost due to mouse and bird activity (supplementary data, Table S2). At none of the four sites involved did the proportion of missing seed traps exceed 10%. Seed densities measured at these sites were adjusted for the actual number of seed traps. As the total number of missing traps was similar in both land-use types, no attempt was made to correct species richness of the seed rain.

2.4. Seedling emergence method

Seed numbers in the samples were determined using the emergence method of Ter Heerdt et al. (1996). Between 16 September and 8 October 2010, the seeds were concentrated by washing the samples over two sieves. An upper sieve (mesh width 4 mm) was used to remove larger stones and roots, while a lower sieve (mesh width 0.2 mm) ensured that no seeds were lost. The concentrated samples were spread out in a thin layer (3–5 mm) on pots (10 × 10 × 12 cm) filled with seed-free material composed of volcanic particles of different size (HF-Dachterrassensubstrat type E and LTF, Alfred Forster AG, Golaten, Switzerland) topped with a layer of fine diatom sand (Abosan, Alfred Forster AG, Golaten, Switzerland). Single samples expanded over several pots, which were placed in trays in a cold frame and watered regularly from below with tap water. The cold frame was covered with white mesh to reduce seed contamination from the surroundings. Seed contamination from the area surrounding the cold frame was estimated in randomly placed control pots, which added c. 9% to the total pot surface. Of seven species which emerged in the control pots, 206 individuals including 166 individuals of *Salix* sp. were excluded from the seed rain and seed bank estimates. The cold frame was covered with glass panels to avoid seed loss during rain. Glass panels were shaded to avoid high temperatures during sunshine and closed and covered with isolation mats to prevent freezing in winter.

Between 14 October 2010 and 26 May 2011, the positions of trays and pots in the cold frame were randomly shuffled and the emerging graminoid and herb seedlings were recorded during nine regular censuses. A seedling was removed as soon as species identity was apparent. All seedlings were recognized as monocots or dicots; 8.2% died before a more accurate identification was possible (supplementary data, Table S2). In April and May of 2011, only a few seeds emerged and no attempt was made to count potentially remaining viable seeds in the samples.

The temporal set-up of this study mimics natural conditions and allows for a normal seasonal timing of seedling emergence in semi-natural meadows, where most seeds germinate in autumn or spring following seed shedding in summer or natural cold stratification in winter (Stampfli and Zeiter, 2008).

In the first week of the experiment, two trays with 48 pots were accidentally lost, which resulted in a small number of missing values of seed rain and soil samples and the loss of one or two out of six spatial replicates at four sites (supplementary data, Table S2).

Seed mass data of most species were obtained from the BIOLFLOR database (Klotz et al., 2002), except for single species, *Erigeron annuus* (Tackenberg et al., 2011), *Festuca filiformis*, *Hypochaeris radicata*, *Potentilla pusilla* (Stampfli and Zeiter, 2008) and *Poa bulbosa* (Azcárate et al., 2002; species names follow Lauber et al., 2012). Abundance-weighted average seed mass across all species was calculated for seed traps and topsoil samples of each plot. Density of reproductive output in a plot was calculated as the sum over all species of seed mass multiplied by seed abundance.

2.5. Data analysis

All statistical analyses were performed with R (version 2.12.1; R Development Core Team, 2008). Number of seeds per sample was extrapolated to express seed density m^{-2}.

Relationships across sites were analyzed using site means of plots. To analyze the influence of community productivity on seed rain and on the availability of seeds in the topsoil, linear regressions with community productivity as explanatory variate were performed and species richness, species diversity H', log-transformed data of density, seed mass and reproductive output were used as response variates.

In the analysis of the influence of community productivity on the proportions of persistent seeds and of species with persistent seeds in the topsoil, the number of persistent seeds or the number of species with persistent seeds, calculated as the differences between soil cores and traps of corresponding plots, were the response variables and the numbers of seeds or species in the topsoil sample the binomial totals. GLMs (with binomial distribution and logit-link function) were fitted using community productivity as explanatory variate. In a few cases, due to spatial heterogeneity of the seed rain (Peart, 1989) or the seed bank (Thompson, 1986) or both variables, trap data was replaced by soil data, i.e. the number of persistent seeds was set to zero as the number of seeds in traps exceeded the number of seeds in soil cores. This correction was performed at the scale of plots within sites, and site means of the proportions were calculated afterwards.

To analyze the influence of community productivity on seed rain and on the availability of seeds in the topsoil at the scale of plots within sites, generalized linear mixed models (GLMMs) were fitted and evaluated with Poisson error distribution and a log-link function (density and species richness), Gaussian error distribution and identity-link function (species diversity H' and log-transformed seed mass data), or binomial error distribution and logit-link function (proportion of persistent seeds and proportion of species with persistent seeds), and with Laplace approximation (lme4 package; Bates et al., 2010) for maximum likelihood estimation of the parameters (Bolker et al., 2009). Community productivity was coded as a continuous fixed factor, and models fitted a random effect for site. Significance of fixed effects was assessed by removing them from the model and comparing models with likelihood ratio tests.

3. Results

A total of 8153 seedlings of 124 species (67 perennial and 30 annual forbs, 24 perennial and three annual graminoids) emerged, 5090 in the topsoil and 3063 in the seed rain samples. Perennial forbs contributed 36.2% to the total number of recorded seeds, annual forbs 24.1% and perennial graminoids 38.9%, while seeds of

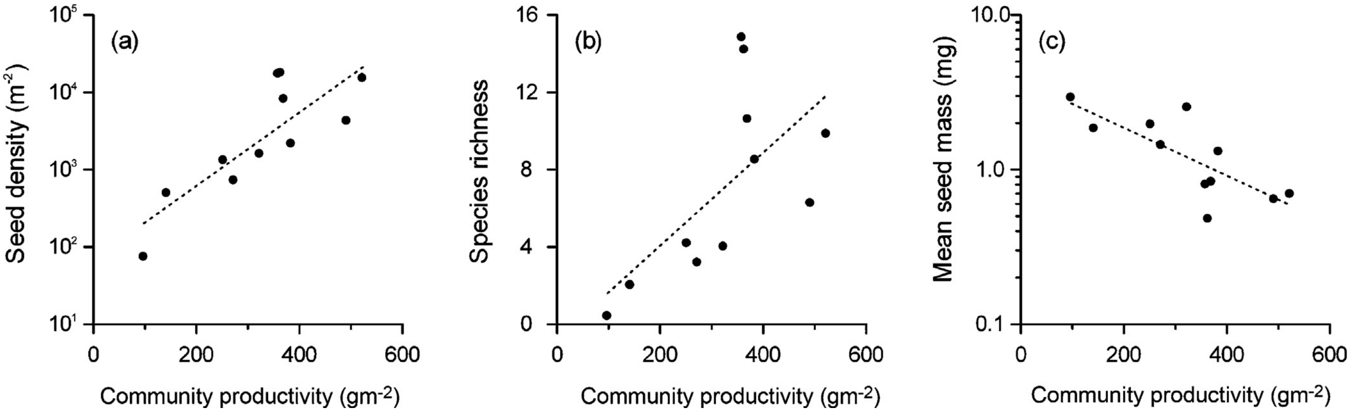

Fig. 2. Relationship between community productivity (phytomass of the first harvest) and seed rain, i.e. (a) seed density, (b) species richness, and (c) mean seeds mass.

annual graminoids were very rare (0.8%). Most species (57%) were represented by small numbers (<10 seeds per species, altogether contributing 2.8% of the total number of recorded seeds). Nearly all seeds in topsoil (98.0%) and seed-rain (99.7%) samples belonged to species of the species pool (supplementary data, Table S2).

Seed densities in topsoil samples of the twelve grassland sites ranged from 1695 seeds m^{-2} (Thun) to 25,116 seeds m^{-2} (Zollikofen, supplementary data, Table S2). Seed rain in the current year varied between 74 seeds m^{-2} (Thun) and 17,782 seeds m^{-2} (Somazzo, supplementary data, Table S2). The trap-soil comparisons revealed proportions of persistent seeds in the topsoil of between 0.05 in Casserio and 0.95 in Thun.

Out of all species with flowers, most reached seed maturity by the time of the first cut at each site, except at Pree where only 9% of the flowering species had mature seeds (supplementary data, Table S1). Pree was excluded from further analysis because its seed rain was affected by too early a cutting date (supplementary data, Table S1). Linear regression revealed no effect of the date of first cutting on the proportion of species with mature seeds at the other sites ($r^2 = 0.02$, $P = 0.30$, $n = 11$).

3.1. Productivity effects on seed rain

At the scale of sites, seed density ($r^2 = 0.61$, $P < 0.01$, $n = 11$), reproductive output ($r^2 = 0.56$, $P < 0.01$, $n = 11$), species richness ($r^2 = 0.35$, $P < 0.05$, $n = 11$) and diversity H' ($r^2 = 0.42$, $P < 0.05$, $n = 11$) were positively related, and seed mass in the seed rain ($r^2 = 0.56$, $P < 0.01$, $n = 11$) negatively related to community productivity (Fig. 2). The seed densities of graminoids, annual forbs and perennial forbs were positively related to community productivity (Table 2). Graminoids showed the strongest increase in seed density with community productivity. Species richness of perennial forbs in the seed rain was positively related, but species richness of annual forbs was not related to community productivity (Table 2). A positive relationship of species richness of graminoids in the seed rain with community productivity was only marginally significant (Table 2).

Most across-site relationships between seed-rain variables and community productivity were confirmed by GLMMs accounting for within-site variability (supplementary data, Tables S3, S4).

3.2. Productivity effects on seed bank

Seed densities in the topsoil and in the seed rain were correlated ($r = 0.89$, $P < 0.001$, $n = 11$) and species richness in the topsoil and in the seed rain were correlated ($r = 0.96$, $P < 0.001$, $n = 11$). Seed density ($r^2 = 0.34$, $P < 0.05$, $n = 11$) was positively related to community productivity and species richness in the topsoil showed only a marginally significant relationship with community productivity ($r^2 = 0.23$, $P = 0.08$, $n = 11$; Fig. 3a and b).

The proportion of persistent seeds ($F_{1,9} = 10.31$, $P < 0.05$, $n = 11$) and the proportion of species with persistent seeds in the soil ($F_{1,9} = 12.83$, $P < 0.01$, $n = 11$) were negatively related to community productivity (Fig. 3c and d). The proportion of persistent seeds of annual forbs and perennial forbs were negatively related, but the proportion of persistent seeds of graminoids in the seed bank was not related to community productivity (Table 2). The proportion of species with persistent seeds of annual forbs and perennial forbs were negatively related, but the proportion of species with persistent seeds of graminoids in the seed bank was not related to community productivity (Table 2).

Most across-site relationships between seed-bank variables and community productivity were confirmed by GLMMs accounting for within-site variability (supplementary data, Tables S5, S6, and S7).

4. Discussion

Our selection of extensively managed (*Festuco-Brometea*) and intensively managed (*Arrhenatheretalia*) grasslands provided a wide gradient of community productivity, which also partly reflects

Table 2

Results of linear regressions (seed rain) or GLMs (proportion of persistent seeds in topsoil seed bank) with seed density and species richness of plant functional groups as response variates and community productivity as explanatory variate. Intercept (Inter), slope, r^2, and P-values (significant values in bold type).

	Seed density				Species richness			
	Inter	Slope	r^2	P	Inter	Slope	r^2	P
Seed rain								
Graminoids	−0.30	0.0040	0.53	**0.007**	−0.66	0.0115	0.25	0.066
Annual forbs	−0.23	0.0027	0.41	**0.019**	−0.06	0.0039	0.12	0.156
Perennial forbs	0.04	0.0031	0.59	**0.003**	−0.04	0.0087	0.48	**0.011**
Seed bank								
Graminoids	1.83	−0.0077		0.242	1.28	−0.0056		0.187
Annual forbs	4.16	−0.0096		**0.030**	4.12	−0.0107		**0.012**
Perennial forbs	2.57	−0.0083		**0.002**	1.67	−0.0048		**0.041**

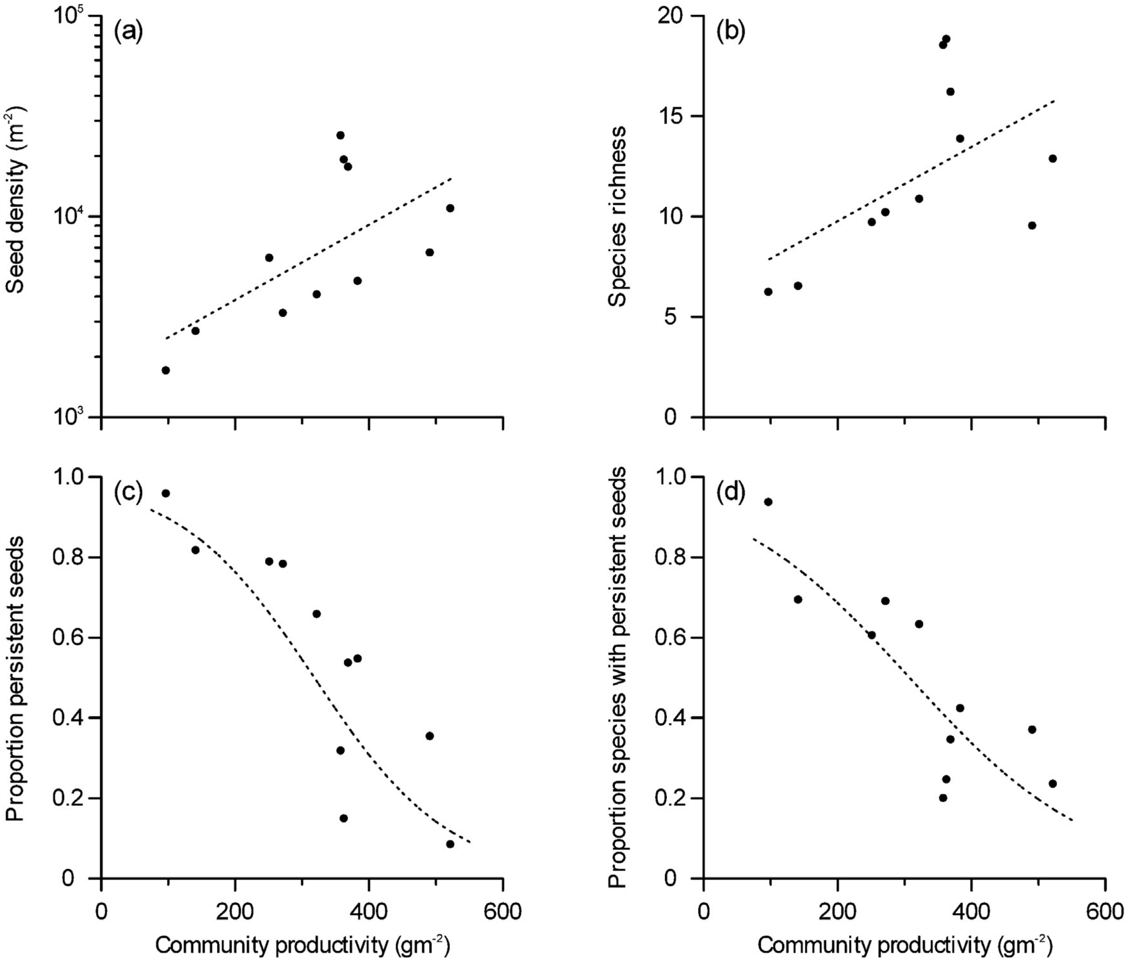

Fig. 3. Relationship between community productivity (phytomass of the first harvest) and topsoil seed bank, i.e. (a) seed density, (b) species richness, (c) proportion of persistent seeds, and (d) proportion of species with persistent seeds.

the effects over time of adding manure or slurry, increased frequency of cutting and occasional seeding of grasses in a successional process of land-use intensification since the 1950s. By selecting regions of different climatic conditions across Switzerland and choosing land-use types pair wise within regions, the effect of variable weather conditions on the seed-rain and topsoil seed-bank estimates was balanced across the gradient. With increasing land-use intensity, seed traps revealed increasing density and diminishing size of the seeds in the seed rain, while the soil-trap comparison showed a declining persistent-seed proportion in the topsoil. These findings are valid as long as land-use intensification does not preclude the maturation or distribution of seeds.

The consistent relationships within and across sites implies that small-scale variability of community productivity and land-use intensification enhancing productivity and changing species composition have positive effects on seed production that are principally driven by resource supply.

4.1. Influence of land-use intensity on seed rain

Seed-rain densities in hay meadows across Switzerland were in the same order of magnitude as seed-rain densities measured in a species-rich perennial grassland in Sweden (3820 seeds m^{-2}, Jakobsson et al., 2006) or in a calcareous grassland in England (10,949 seeds m^{-2}, Leishmann, 2001). Differences in seed-rain densities among grassland communities not only reflect differences in community productivity, but also the effects of the methods applied. While seed-rain estimates based on the exposure of pots with sterilized soil in the field, as in this study and in Jakobsson et al. (2006) underestimate seed production because samples are exposed to potential seed predators (Chabrerie and Alard, 2005; Kollmann and Goetze, 1998), the use of sticky traps as in Leishmann (2001) may overestimate the density of viable seeds in the seed rain because viable and dead seeds cannot be distinguished.

Our finding of an increasing seed-rain density with intensification of grassland management contradicts a fertilization experiment in an upland hay meadow in northern England (Smith et al., 1996), but is in line with results from a fertilization experiment in a subalpine meadow in the Rocky Mountains (Burkle and Irwin, 2010). The duration of fertilizer application may explain the differences between the studies, as longer fertilization application might not only result in a higher availability of nutrients but also in a change in the species composition. In our study, such an influence of land-use intensification on species composition is indicated by differences between plant functional groups, a marginally significant positive effect on species diversity and species richness, and a negative effect on seed size in the seed rain. A stronger effect of land-use intensification on grasses than on annual and perennial forbs is consistent with studies showing that seed production of grasses is especially favoured by fertilization (Burkle and Irwin, 2010; Smith et al., 1996).

The increase of species richness and diversity in the seed rain with increasing land-use intensity might partly be due to the higher probability of detecting a species when its seeds are more abundant, as seed densities increased with increasing land-use intensity. At the lower end of the productivity gradient, seed densities in the seed rain turned out to be very low. A higher sample size would be needed to correctly estimate species richness and to disentangle a land-use effect from a density-related artefact. The low seed densities in the seed rain of the extensively managed grasslands, together with the very low proportion of seeds originating from outside the 9-m^2 plots, imply that species in nutrient-poor grasslands have low dispersal potentials. This is in line with an earlier study showing that spontaneous long-distance dispersal followed by successful establishment is rather unusual for grassland species (Stampfli and Zeiter, 1999).

4.2. *Influence of land-use intensity on seed size*

The decrease in seed size with increasing community productivity in our study contradicts the expectation that a high-fertility site should favour species with larger seeds. This is in contrast to a study that supported this hypothesis (Manning et al., 2009). In our study, the increase in site fertility with increasing land-use intensity is linked with an increase in disturbance. As increase in fertility and increase in disturbance are opposing selecting forces regarding seed-size optimization, their relative strengths might differ along the land-use intensity gradient. If establishment rates are generally low in sites of high productivity due to strong competition for light caused by the established vegetation (Hautier et al., 2009), a larger seed size might only marginally promote establishment success. Optimizing dispersal ability to increase establishment success during intermittent phases of reduced competition due to small-scale disturbances would then be a more successful strategy for plants in more productive grasslands. In extensively managed, nutrient-poor grasslands, establishment success is often not strongly related to competition for light imposed by standing biomass (Houseman and Gross, 2006; Zeiter and Stampfli, 2012). From low densities of simultaneously emerging seedlings found in such grasslands (Stampfli and Zeiter, 2008; Zobel et al., 2000), competition between seedlings does not appear to be a major structuring force (Moles and Westoby, 2004). Rather than being an advantage in the seedlings' competition for light, larger seed size might entail the advantage of greater survival in the face of environmental hazards such as droughts (Westoby et al., 1996). Even short droughts have been shown to reduce the establishment success of seedlings (Stampfli and Zeiter, 2008).

Although Pärtel and Zobel (2007) showed that species with larger but fewer seeds generally prefer habitats of higher productivity in their study across all the species of European flora including rare ones, our result suggests that, in more productive grasslands, locally abundant species produce more but smaller seeds.

4.3. *Influence of land-use intensity on topsoil seed bank*

The correlation we found between seed densities in the topsoil and in the seed rain is consistent with the conclusion of Saatkamp et al. (2009) that seed density in the topsoil reflects seed production. Consequently, a seed rain consisting of seeds of plant species with equal persistence traits would result in a constant proportion of persistent seeds across the productivity gradient. In our study, the proportion of persistent seeds was not maintained but declined with community productivity, while absolute seed densities in the soil increased. This is clear evidence of a higher persistence of the seeds in the topsoil of less productive grasslands. As density of viable seeds in the soil is probably not related to the concentration of nitrogen (Kitajima and Tilman, 1996) or nutrient input (Bekker et al., 1998b) in the soil, we suggest that different seed traits of species across the land-use intensity gradient, such as inherent longevity or dormancy, result in different persistence of seeds in the soil. Nevertheless, other environmental factors such as soil moisture (Mordecai, 2012; Pakeman et al., 2012) or the activities of seed predators may have variously affected the seeds in the topsoil along the productivity gradient.

5. Conclusion

Low seed densities at the lower end of the productivity gradient suggest that the species richness of communities is more seed limited in extensively managed than in intensively managed grasslands. This is an explanation for why species richness is not negatively related to land-use intensity in our grasslands, as would be expected from other studies (Kleijn et al., 2009; Socher et al., 2012; Zechmeister et al., 2003). A test of this expectation requires that measurements of seed densities in the soil are combined with seed-addition experiments to estimate establishment rates in the corresponding communities (Clark et al., 2007). As Kitajima and Tilman (1996) showed for an unmanaged old-field, a lower species richness of more productive plant communities may also result from the inhibition of germination and not from a lower density of seeds in the soil or from lower species richness in the seed bank. So far, the bulk of evidence for the relative importance of natural seed availability in the soil compared to other factors affecting seedling recruitment is from single sites. Multi-site studies along environmental or land-use gradients are needed to more generally elucidate the relative roles of natural seed availability, dispersal limitation and biotic interactions in causing diversity patterns in grasslands.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.agee.2013.03.009.

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