

The impact of seed deficiency on productivity and on negative drought effect in semi-natural grassland

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Abstract

Questions: Vegetative re-sprouting and recruitment from seed determine grassland recovery after severe drought, but the mechanisms determining vegetation composition are not fully understood. We ask how the timing of drought and seed availability modify the drought legacy effects on composition and function in a temperate, semi-natural grassland.

Location: Negrentino, southern Alps, Switzerland.

Methods: Under automated rainout shelters, we simulated extreme seasonal drought events in a late summer and in the following spring, added rainwater to simulate normal conditions in no-drought controls and maintained haymaking at times of normal practice. Towards the end of the summer drought, we added seeds of ten local species in a three-factor split-plot arrangement with seven blocks as the replicated unit. We measured fine-scale variation in soil depth. We assessed the biomass of graminoids and forbs at regular harvesting dates and the species frequencies of established plants and recruits before treatment start, repeating these assessments for three years thereafter. We measured the biomass proportions of post-drought annual recruit cohorts in year 4 after drought.

Results: One-time seed addition augmented recruits, modified species composition and enhanced species diversity; these effects propagated to increased reproductive shoots of recruits and community biomass four years later. Single and repeated seasonal droughts only caused low adult plant mortality but clearly reduced recruitment from seed, while post-drought establishment was slightly enhanced. Seed augmentation compensated the negative spring drought effect on forb recruits and in turn mitigated the negative impact of drought on species diversity after drought.

Conclusions: Our experiment shows that seed deficiency limits productivity and that seed deficiency compensation can help to stabilize diversity and productivity in semi-natural grassland. Releasing the current constraints of management on seed supply in grassland would therefore assist in mitigating negative drought impacts.

KEYWORDS

drought, hay meadow, plant community, rainout shelter, recovery, recruitment, resilience, resistance, seed addition, semi-natural grassland, species diversity, vegetation dynamics

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1 | INTRODUCTION

Low-intensity management in semi-natural grasslands helps to maintain the provision of a wide range of ecosystem services which are highly demanded by humanity (Bengtsson et al., 2019). However, unfertilized grasslands very rich in species at small spatial scale (Wilson et al., 2012) strongly declined during the second half of the 20th century in Europe and their multiple biodiversity-related services were degraded due to land-use change, intensification of management or abandonment (EEA, 2010). As a consequence of climate warming in Central Europe, droughts are increasing in frequency and intensity (IPCC, 2013), added to which are the current threats to biodiversity from eutrophication (Sutton et al., 2011; Roth et al., 2017). Droughts induce changes in grassland vegetation composition which may impair the coexistence of species and this negative impact is often exacerbated by high land-use intensity (Stampfli et al., 2018).

Reduced water availability in soils related to climate and hydrological change causes longer and more severe intermittent drought stress, with the potential to widely modify ecosystem structure and function (Knapp et al., 2015). Species composition may be shifted in communities as limited resources cause differential growth or mortality of dominant species during drought (Hoover et al., 2014) and resource pulses modify biotic interactions after drought (Stampfli et al., 2018). According to the leaf-economic trade-off, slow growth and resource-conservative traits are advantageous under conditions of limited availability of resources, while they are disadvantageous when resources are unlimited (Reich, 2014). Hence, slow-growing, resource-conservative species are often considered to be more drought-tolerant than fast-growing, resource-acquisitive ones (Harrison et al., 2015; Voltaire, 2018). Therefore, community reordering caused by water limitation during drought would favour species with low specific leaf area (SLA), while community reordering caused by a nitrogen-pulse post-drought (Borken and Matzner, 2009) would favour species with high SLA, although community-level responses might be buffered by soil heterogeneity (Fridley et al., 2011). However, the various mechanisms underlying drought-induced changes in grassland communities have received little attention across multiple levels of organization (Felton and Smith, 2017).

In response to extreme droughts, communities have exhibited different maxima of greatest relative change (Ploughe et al., 2019). The different timings of change may be related to the different main drivers as high mortality of established plants may cause maximal change during drought while recruitment from seed may cause maximal change after drought. The largest relative change is expected to take place after drought if the mortality of established species is low and recruitment dynamics drives community reordering (Stampfli and Zeiter, 2004).

Recruitment from seed may compensate for adult plant mortality and operate as a stabilizing process in vegetation after severe drought (Lloret et al., 2012). The idea of enhanced recruitment post-drought due to the release of competition with established vegetation is in line with the theory that the establishment of new species

from seed depends on fluctuating or unconsumed resources left by the established species (Davis et al., 2000; Tilman, 2004). It is also consistent with empirical findings showing that gaps, i.e. open spaces with reduced competition from established plants, provide good opportunities for seedling establishment (Grubb, 1977; Bullock, 2000; Stampfli and Zeiter, 2004; but see Ryser, 1993). However, droughts can also directly hamper seedling recruitment and establishment, as water supply is a critical driver of seed dormancy and germination (Walck et al., 2011) and of seedling survival (Stampfli and Zeiter, 2008). Whether droughts favour or hamper recruitment may depend on limiting conditions and resources for seedling establishment during and after drought such as temperature, water and light. This in turn may affect ecosystem functioning as the rejuvenation of ecosystems appears to increase their sensitivity to climate change (Kröel-Dulay et al., 2015). So far, research on ecosystem responses to climate change has concentrated on cold tundra, boreal forests and treeline ecotones, while plant regeneration is understudied in temperate ecosystems (Walck et al., 2011).

The seasonal timing of drought may have a strong impact on recruitment success as grassland species often exhibit distinct seasonal germination patterns (Thompson et al., 1996; Stampfli and Zeiter, 2008). Reviews have expressed the need for precipitation manipulation experiments to address the role of seasonal timing of droughts on plant communities (Smith, 2011; Wu et al., 2011; Beier et al., 2012), but such studies are relatively rare (Zeiter et al., 2016; Denton et al., 2017).

Post-drought recruitment is not only contingent on water availability (Lloret et al., 2004; Knapp et al., 2008) but also on the availability of propagules which are locally controlled by management of grassland. The density and species diversity of propagules are modified at regional scales as changes in land use have fragmented habitats, altered pastoral systems and impoverished dispersal agents (Poschlod and Bonn, 1998). This has already limited the effectiveness of regional species pools as sources for local colonization (Ozinga et al., 2009). Of course, a lack of seeds is indicated in meadows if harvesting dates precede the time of seed maturation (Smith et al., 1996). As current management practices tend to advance dates of harvests and accelerate the speed of harvesting, seed deficits from dispersal limitation in fragmented habitats may be strongly exacerbated locally by early cutting (Stampfli and Zeiter, 2008).

Seed deficiency can affect species composition and constrain both the species richness (Tilman, 1997; Stampfli and Zeiter, 1999; Turnbull et al., 2000; Zobel et al., 2000) and productivity of plant communities (Zeiter et al., 2006; Stein et al., 2008), especially in combination with disturbance (Klaus et al., 2017). Seed deficiency may also limit recovery after drought, as indicated by a long-lasting reduction in species diversity in an observational study (Tilman and El Haddi, 1992). Seed deficiency may be exacerbated as droughts reduce seed production (Dietrich and Smith, 2016; Zeiter et al., 2016). As far as we know, the interactive effects of seed availability and seasonal droughts have not been investigated experimentally.

Here we present the first field experiment to unravel how three crossed factors – drought in late summer, drought in spring

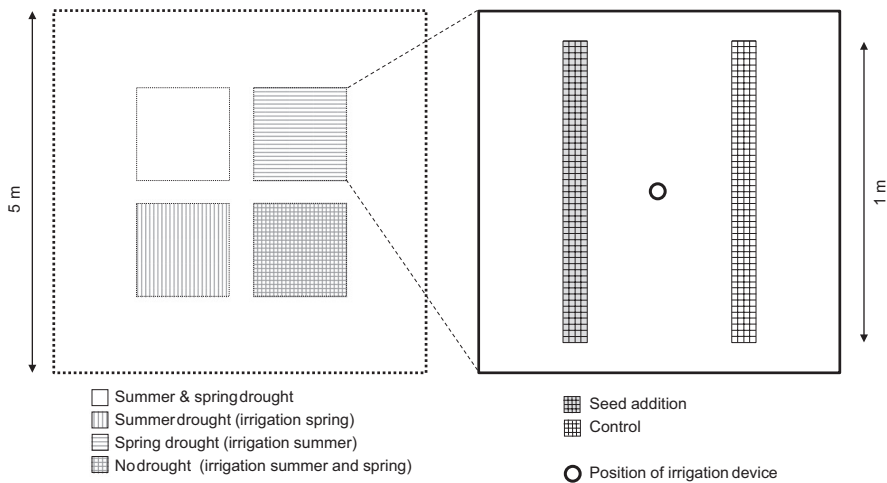


FIGURE 1 Schematic representations of a block (5 m × 5 m), showing four plots with randomly allocated drought treatments, and a plot (1.2 m × 1.2 m) showing two subplots (0.08 m × 1 m) with randomly allocated seed addition and control treatments, each with 200 contiguous quadrats for species frequency recording. Rainout shelters covered whole blocks during rainfall in late summer 2004 and spring 2005 while two irrigation devices per block supplied rainwater to two control plots; one irrigation device per block was shifted between summer and spring

and enhanced seed rain – affect established plants and recruitment and how the legacy effects on recruit cohorts modify community composition and function over five years in semi-natural grassland dominated by perennial species. Our overarching hypothesis was that drought timing and seed availability alter community composition and functioning via effects on post-drought recruitment. Exploring the legacy effects of extreme droughts on single species and plant functional groups, we ask the following questions: (a) is persistence and growth of established vegetation differently affected by droughts in different seasons; (b) is recruitment from seed differently affected by droughts in different seasons and what is the balance between negative effects during and positive effects after droughts; and (c) is community composition and functioning seed-limited and are drought effects modified by seed availability?

2 | METHODS

2.1 | Experimental site

The experiment was performed in semi-natural grassland at Negrentino, southern Switzerland. Located between the valley floor and subalpine pastures at an elevation of 820 m a.s.l., this grassland had been used for bi-annual haymaking during the transhumance economy of the past century. While economic transformations have widely altered land use in the surrounding areas since the 1960s, local subsistence farmers have maintained traditional haymaking in some plots for decades without fertilizing. Although modern machinery has accelerated the speed of haymaking and unified spatial variation between areas harvested by different smallholder families, historical late cutting dates in summer and autumn are currently respected in a few hectares of these extraordinarily species-rich meadows in compliance with management regulations for dry meadows and pastures of national importance by the Swiss federal authorities (TwwV, 2010). These management regulations aim to maintain the extraordinarily high biodiversity via payments for late cutting and the waiving of fertilizer application.

In March 2003 we established our experimental site in an area of ca. 15 m × 25 m with a slope inclination of 11° towards the south-southeast (46.4621 N, 8.9241 E) within species-rich *Mesobromion*-type vegetation (Ellenberg, 1996) and fenced the site in. During our study, we maintained haymaking unvaryingly at two dates, end of June and mid-September, and removed dry leaves from deciduous trees in winter.

The soil is a moderately acid sandy loam (following FAL, 1997) with a high silt content. Soil depth varies in the range of 15 cm to >70 cm. The climate is characterized by a mean annual temperature of 9.8°C (on-site measurements 2005–2009) and a mean annual precipitation of 1,437 mm, with variation between years in the range of 728–2,055 mm (daily precipitation 1961–2010: RhiresD, MeteoSwiss, Zürich). The longest seasonal meteorological droughts defined as sequence of days with a two-day running mean of precipitation < 10 mm based on daily precipitation measurements at Comprovasco since 1893 (MeteoSwiss, Zürich), lasted for 77 days (spring 1942) and 63 days (summer 1957).

2.2 | Experimental design

Treatments simulating extreme seasonal drought events were sequentially applied in late summer 2004 and spring 2005 to seven areas (blocks: ca. 5 m × 5 m) using electrically driven rainout shelters (Appendix S1) which automatically operated during times of precipitation. Our controls mimicked average amounts and frequencies of seasonal rainfall during periods of drought treatment. During summer rain exclusion, two irrigation devices within each block (Appendix S1) gently supplied rainwater to two out of four, randomly allocated areas (plots: ca. 1.2 m × 1.2 m) which served as controls. During spring rain exclusion, one randomly selected irrigation device remained in the same position, while the other device was randomly allocated to one of the two areas which had remained dry in the previous summer. This sequentially nested design therefore resulted in the four plots per block having different drought legacies: one legacy of a late-summer drought followed by a spring drought, two legacies of a single drought, either in late summer or spring, and one legacy of no drought at all

(Figure 1). We chose this design to minimize the side effects of rainout shelters, and to avoid confounding the effects of reduced precipitation with shading, temperature change and wind protection; for technical details of this experimental set-up see Zeiter et al. (2016).

Soil water potential was monitored during periods of drought treatment. In each block, two soil moisture sensors (Equitensimeter EQ15; Ecomatik, Dachau, Germany) were installed at ca. 5.5 cm below the soil surface, one in the plot receiving rainwater (control), the other in the plot receiving no rainwater during both seasons. Topsoil desiccation was slower and more varied across blocks in spring than in summer. Under rainout shelters, water potentials remained below $\psi = -100$ kPa across all blocks for 42 consecutive days in late summer (26 August–6 October 2004) and across most blocks for 22 consecutive days in spring (24 April–15 May 2005). Topsoil never desiccated below $\psi = -63$ kPa in irrigated controls. Progressively declining water potentials during periods of desiccation further indicate that drought treatment plots were not noticeably affected by slope run-off water (Appendix S2). Experimental meteorological droughts lasted for 52 days in late summer 2004 and 59 days in spring 2005, each one mimicking the third longest drought in its season in 111 preceding years (daily precipitation measurements 1893–2003 at Comprovasco; MeteoSwiss, Zürich).

Within each plot we set two subplots of 8 cm × 100 cm, 56 cm apart (Figure 1). We fixed two grids with 4 cm × 4 cm cells made of 1 mm thick stainless steel to the ground. Cells were further subdivided into 2 cm × 2 cm quadrats so that each subplot was composed of 200 quadrats (Appendix S3).

2.3 | One-time seed addition

We used locally collected viable seeds (Zeiter and Stampfli, 2012) of three grasses, *Bromus erectus*, *Danthonia decumbens*, *Helictotrichon pubescens*, and seven forbs of seven families, *Hypochaeris radicata*, *Primula veris*, *Plantago lanceolata*, *Ranunculus bulbosus*, *Salvia pratensis*, *Sanguisorba minor* and *Scabiosa columbaria* (species names follow Lauber and Wagner, 2001). This species selection represented the proportion of common species between the two functional groups in the community and included species with diverse seasonal germination preferences (Stampfli and Zeiter, 2008).

Between 18 and 20 September 2004 we augmented the density of seeds by 6,250 m⁻² in one randomly selected subplot within each plot by adding one seed of all ten species to each 16-cm² cell (Zeiter and Stampfli, 2012).

2.4 | Data sampling

Subplot grid points were visually inspected at the end of the late-summer drought treatment (mid-October 2004) and one year later to obtain a measure of maximum drought impact on open space and the recovery of green plant cover: open space, i.e. the proportion of space taken by gaps, was estimated as the proportion of 156 grid points not covered with green non-bryophyte plant tissue.

The frequency of species rooted in quadrats of 4 cm² was determined across 200 quadrats per subplot (Figure 1) after the first harvest in July in sequential years, pre-drought (2004) and post-drought (2005–2007). Established plants and recruits were separately recorded. Independent protocols enumerated the seedlings which emerged before July 2004 and additional seedling censuses were performed in late October 2004 and April 2005, 2006 and 2007. New seedlings were marked with coloured ringlets (Appendix S3) to distinguish recruits of three intervals between yearly censuses in July from 2004 to 2007 (cohort 1, cohort 2, cohort 3). In each census, we further recorded the number of recruits which had reached the reproductive stage as unequivocal evidence of recruitment success (Turnbull et al., 2000). In July 2008, we counted the number of reproductive shoots by species and age states (species established in 2004, cohorts 1–4).

Seedling emergence was calculated for every species as the cumulative number of seedlings which had emerged between July 2004 and July 2005. Seedling survival during the spring drought treatment was calculated as the proportion of living seedlings in July 2005 out of the cumulative number of seedlings which had emerged between July 2004 and April 2005.

Species diversity was calculated at the scale of subplots based on rooted frequency in the 200 quadrats (4 cm²) per subplot using the Shannon index [$H' = -\sum (p_i \ln p_i)$] (Figure 1).

Abundance-weighted means of specific leaf area (SLA_{AWM}) were calculated for two functional groups, graminoids and forbs, using frequency data of established species. We obtained SLA from the TRY database (Kattge et al., 2011; Appendix S4), Cerabolini et al. (2010) and Pierce et al. (2007), and calculated averages of SLA by first averaging the values by contributing author to account for disproportionate author contributions to any species, and then averaging the different authors' means.

Biomass was sampled at haymaking dates using an electric lawn mower at 5 cm above ground in sampling areas of 0.09 m × 1 m which slightly exceeded subplots (Figure 1). The biomass of the first five harvests after the start of rainfall manipulation was sorted to graminoids (grasses, sedges and rushes) and forbs (non-gramineous herbs and woody dwarf shrubs). In the final harvest in June 2008, we further measured the contribution of age states to the total biomass. Unmarked seedlings which had emerged since July 2007 were categorized as cohort-4 recruits. Biomass samples were weighed after drying at 80°C for 24 hr.

The soil depth of plots was estimated as the vertical distance from the soil surface to the solid rock. At three regularly spaced points across subplots we measured soil with a pointed steel pin with a diameter of 3 mm to a depth of 67 cm and averaged subplot medians.

2.5 | Data analysis

The design was a three-factor split-plot arrangement with block as replicated unit (Figure 1). The factors late-summer drought (AD, acronym

uses “autumn” as a substitute for “late summer”), spring drought (SD) and seed addition (S) included two levels. The design had four strata: (a) block, $n = 7$; (b) block \times plot pair (two plots allocated to the same AD treatment), $n = 14$ with AD nested within block; (c) block \times plot pair \times plot, $n = 28$ with SD nested within AD; (d) block \times plot pair \times plot \times subplot, $n = 56$ with S nested within drought treatments.

For analyses of the effects of drought on persistence variables (species frequency sum, SLA_{AWM} , species diversity and biomass) of the functional groups of the established plants, we used data from the no-seed-addition control subplot and pre-drought values as covariates, except for biomass because no pre-drought data were available. The covariates were always highly significant (data not shown). Except for recruit data of species used in the seed-addition treatment, the data of two subplots per plot were combined in analyses of drought effects on single species (established plants and recruits) to augment the statistical power. We assume that seed addition did not directly affect the drought sensitivity of established plants or recruits. All other analyses were performed at the subplot level.

All variables were analysed for the time of first measurement. Variables showing a significant treatment effect were also analysed in the following year to assess the temporal persistence of effects.

All statistical analyses were conducted using GENSTAT 18.0 (Payne, 2008). We applied ANOVA to biomass variables and species frequency sums (using log-transformed data to obtain normally distributed residuals and homoscedasticity) and to exponential Shannon diversity indices (Jost, 2006) and a generalized linear model (GLM) with Poisson distribution and log-link function to numbers of emerged seedlings. We applied GLMs with binomial distribution and logit-link to data of seedling survival, frequency of single species and the proportion of space taken by gaps.

Data of single species or functional groups which were rare or showed heterogeneous distribution within the site at the onset of the experiment were not analyzed in order to prevent false-negative results due to insufficient statistical power (for details of criteria used, and the list of variables excluded from statistical analysis, see Appendix S5). Using frequency of established species in 2004 as a covariate for before-drought population size in analyses of recruit data did not generally alter treatment effects on single species (data not shown).

3 | Results

3.1 | Persistence of established plants

Established plants maintained some green cover at the end of late-summer drought but open space was increased (54.3% vs 19.0%, means per treatment; $F_{1,6} = 185.9$, $p < 0.001$). No lag effects of late-summer or spring drought on open space remained one year later (15.8%, mean over all plots; AD: $F_{1,6} = 4.30$, $p = 0.08$; SD: $F_{1,12} = 0.25$, $p = 0.62$; AD \times SD: $F_{1,12} = 1.58$, $p = 0.23$).

Only three out of 31 species tested were affected in frequency by droughts (Appendix S6). The only significant drought effect on

functional groups based on aggregated species frequency data was a -5% reduction in the species diversity of forbs by spring drought (Appendix S7), which persisted in the second summer post-drought (data not shown).

Biomass was only temporarily reduced by drought and fully recovered in the second year after drought (Appendix S8). Late-summer drought reduced the biomass of graminoids (-45%) and forbs (-43%) in the autumn harvest (Appendix S8). Low soil depth marginally exacerbated the negative effect on graminoid but not on forb biomass (Appendix S9). Spring drought reduced biomass of forbs in both summer (-32%) and autumn (-23%) harvests post-drought but only had a marginally significant negative effect on biomass of graminoids in the summer harvest (Appendix S8). These effects were independent of soil depth (data not shown).

3.2 | Recruitment by species

Seedlings of 64 species emerged in the study plots within three years of the start of the experiment, with 99.9% of the frequency sum of recruits representing species which had been established in the plots before that. Recruits of 20 forb and three graminoid perennial species reached reproductive stage within three years (Appendix S6).

One-time seed addition increased the frequency of recruits of all species selected for this treatment (Appendix S6, Appendix S10). Most species showed a positive effect in their cohort-1 and/or cohort-2 recruits while only two species also showed a positive effect in their cohort-3 recruits (Appendix S10). Seed-addition effects on cohort-1 recruits persisted in years 2 and 3 after sowing (Appendix S6). Six out of the ten species reached reproductive stage within three years of having been added (Appendix S10).

Drought affected seedling emergence (the cumulative number of seedlings which emerged) of ten out of 21 species tested one year after start of the experiment (Figure 2; Appendix S6). Late-summer drought had contrasting effects on five forb species, but favoured seedling emergence of three graminoid species (Figure 2a, c). Spring drought hampered the emergence of three forbs but did not affect graminoids (Figure 2b, d). For 14 out of 16 species tested, seedling survival was independent of spring drought (Appendix S6). Thus, for most species with numerous seedlings, the drought responses of cohort-1 recruits in the summer post-drought mirrored drought responses of the cumulative number of seedlings which emerged in the first year after start of the experiment (Appendix S6). For most species tested, cohort-2 and cohort-3 recruits showed no drought legacy effects (Appendix S6).

3.3 | Recruitment by functional group

Across all treatments, most cohort-1 seedlings of graminoids (90%) and forbs (65%) emerged in the autumn after the start of the experiment, while few cohort-1 seedlings of graminoids (3%) and forbs (8%) emerged between April and July (Appendix S6).

FIGURE 2 Effect of droughts in late summer (a, c) and spring (b, d) on seedling emergence of single species in the first year after starting experimental treatments (mean \pm SE, $n = 14$, sums across both subplots per plot). Full line represents species with significant drought effect ($*p < 0.05$). Asterisk indicates species augmented by seed-addition treatment (for full species names, see Appendix S6)

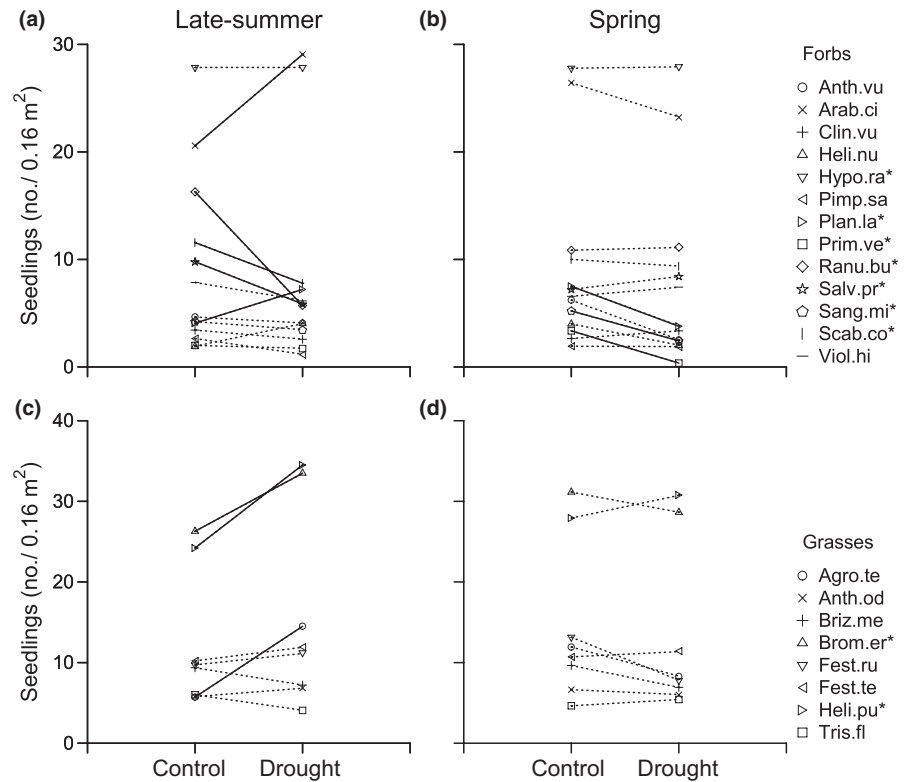
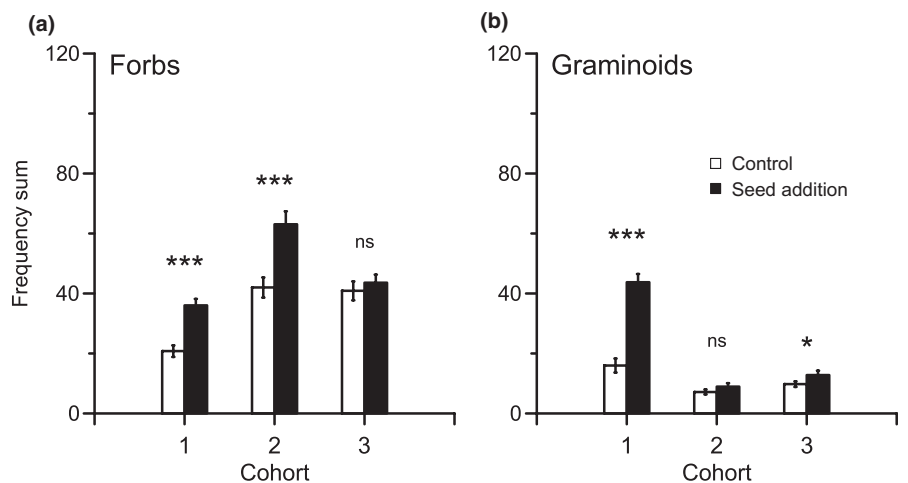


FIGURE 3 Legacy effects of one-time seed addition on species frequency sums of cohort-1-3 recruits of (a) forbs and (b) graminoids which emerged in the first, second or third year after starting the experiment (mean \pm SE, $n = 28$; ***, $p < 0.001$; *, $p < 0.05$; ns, not significant)



Forb recruits of cohort 1 showed a 68% increase due to one-time seed addition (Figure 3a) and a -42% reduction due to spring drought in species frequency sum and equally directed changes in species diversity in the summer of year 1; these effects persisted in years 2 and 3 post-drought (Figure 4a; Table 1). A positive interaction between seed addition and spring drought on species frequency sum indicates that the seed-addition effect mitigated the negative spring drought effect. This interaction effect on species frequency sum faded in years 2 and 3 due to declining numbers of surviving recruits across time (Figure 4a; Table 1) but translated into significant or marginally significant positive interaction effects on reproductive shoots and biomass of cohort-1 forb recruits in year 4 (Figure 4b; Table 1).

The graminoid recruits of cohort 1 showed a 174% increase due to one-time seed addition (Figure 3b) and a 30% increase due to late-summer drought in frequency sum in the summer of year 1. The seed-addition effect persisted in years 2 and 3 post-drought (Table 1) and the late-summer drought effect faded due to declining numbers in later years but translated into a significant legacy effect in biomass in year 4 due to the positive interaction of seed addition and late-summer drought on cohort 1 (Appendix S11). Species diversity in this graminoid cohort temporarily decreased as a result of reduced evenness due to the dominance of the added graminoid species in the summer of year 1 (Table 1). The negative spring drought effects

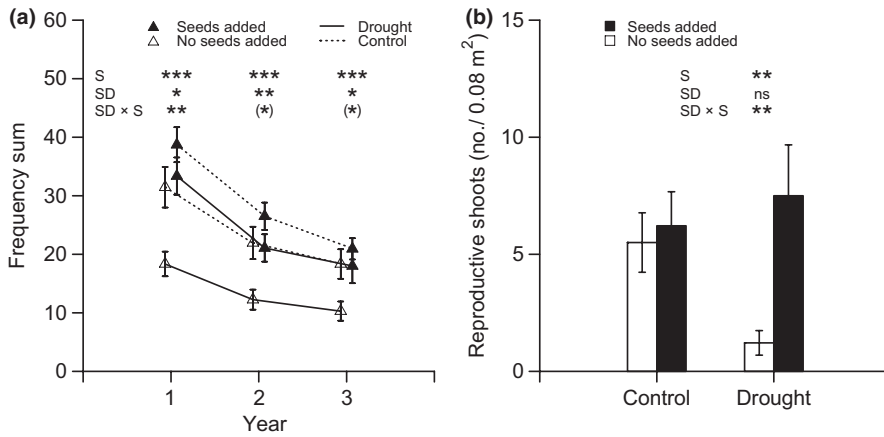


FIGURE 4 Legacy effects of one-time seed addition (S) and spring drought (SD) and of interaction (SD × S) on cohort-1 forb recruits. (a) Species frequency sums in summer of years 1–3. (b) Number of reproductive shoots in summer of year 4 after seed addition (***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; (*) $p < 0.1$; ns, not significant)

on diversity of graminoid recruits of cohort 1 were only transient (Table 1).

Cohort-2 forb recruits and cohort-3 graminoid recruits were increased in species frequency sum by one-time seed addition (Figure 3; Appendix S12). Cohort-2 forb recruits showed a ~15% reduction in diversity due to spring drought (Appendix S12, Appendix S13). Cohort-3 forb recruits showed a positive three-year legacy effect of late-summer drought in species diversity (Appendix S12); this effect mirrored the positive effect on the second generation of *Arabis ciliata* recruits (Appendix S14).

3.4 | Change in community composition and functioning

In the subplots of the no-seed-addition control, the sum of recruits which emerged from seed after the start of the experiment accounted for 22% of total species frequency sum in year 3 (Appendix S15). In year 4, recruits accounted for 15% of the total number of reproductive shoots in the community and for 15% of community biomass (Appendix S15). Forb recruits comprised the majority (>81%) in all variables.

TABLE 1 Summary of statistical analyses of multiple-year legacy effects of late-summer drought (AD) and seed addition (S) in year 0 and of spring drought (SD) in year 1 on frequency sum, species diversity, number (N) and species richness (SR) of reproductive shoots and biomass of cohort-1 forb recruits and on frequency sum, species diversity and biomass of cohort-1 graminoid recruits in summer

| Variable | Year | AD | SD | AD × SD | S | S × AD | S × SD | S × AD × SD |
|------------------------|------|--------------|----------------|------------|------------------------------|---------------------------|-------------------|-------------------|
| | | $F_{1,6}$ | $F_{1,12}$ | $F_{1,12}$ | $F_{1,24}$ | $F_{1,24}$ | $F_{1,24}$ | $F_{1,24}$ |
| FORBS | | | | | | | | |
| Frequency sum | 1 | 0.24 | 9.01* | 0.41 | 57.87*** | 0.58 | 7.99** | 0.40 |
| | 2 | 0.11 | 12.16** | 0.59 | 31.42*** | 0.02 | 3.16 | 0.58 |
| | 3 | 0.08 | 5.48* | 0.35 | 16.90*** | 0.02 | 3.02 | 1.37 |
| Diversity (exp[H']) | 1 | 0.59 | 14.36** | 1.58 | 139.50*** | 0.02 | 0.61 | 1.09 |
| | 2 | 0.13 | 7.65* | 1.74 | 73.15*** | 0.00 | 0.01 | 1.43 |
| | 3 | 0.00 | 8.79* | 2.43 | 63.15*** | 0.22 | 0.03 | 1.15 |
| Reproductive shoots N | 4 | 0.14 | 1.05 | 1.93 | 10.22** | 0.42 | 12.56** | 1.21 |
| Reproductive shoots SR | 4 | 0.12 | 3.38 | 1.87 | 14.89** | 0.06 | 2.99 | 0.09 |
| Biomass | 4 | 0.00 | 0.42 | 0.67 | 31.07^{o,***} | 0.17 ^o | 3.00 ^o | 1.62 ^o |
| GRAMINOIDS | | | | | | | | |
| Frequency sum | 1 | 7.01* | 0.78 | 2.74 | 71.00*** | 2.30 | 1.89 | 0.93 |
| | 2 | 0.81 | 0.05 | 0.67 | 59.27*** | 0.58 | 1.18 | 0.61 |
| | 3 | 0.94 | 0.17 | 0.22 | 63.08*** | 2.50 | 1.22 | 1.00 |
| Diversity (exp[H']) | 1 | 0.00 | 11.62** | 0.47 | 15.85*** | 0.02 | 2.96 | 2.65 |
| | 2 | 0.10 | 5.89* | 2.10 | 0.13 | 0.07 | 0.49 | 2.11 |
| | 3 | 0.05 | 2.93 | 0.07 | 0.00 | 2.12 | 0.24 | 1.75 |
| Biomass | 4 | 2.00 | 0.03 | 0.40 | 19.29^{o,**} | 7.31^{o,*} | 0.63 ^o | 0.44 ^o |

Cohort-1 recruits emerged from seed between July of year 0 and July of year 1. ANOVA- F or GLM- F_{quasi} values of AD, SD, S effects and their interactions (***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$). ^o, degrees of freedom adjusted due to outlier ($F_{1,23}$).

The frequency sum of species and species diversity of the total community were persistently increased after one-time seed addition and these effects translated to a 13% increase in grassland biomass in year 4 after seed addition to 0.08 m² subplots (Table 2; Figure 5). Cohort-1 recruits mainly contributed to this seed-addition effect (Appendix S15).

Species diversity fell for two years after spring drought. A positive interaction between seed addition and spring drought after three years indicates that seed addition mitigated this negative effect of spring drought on diversity (Table 2, Figure 6). Late-summer drought showed no legacy effect on community composition and functioning (Table 2).

4 | Discussion

Our experiment with the crossed-treatment seed augmentation and precipitation exclusion in different seasons showed only transient drought-induced reductions in plant biomass. Barely occurring negative effects on established vegetation indicated high community resistance of semi-natural grassland under extreme droughts in different seasons. Recruitment, however, showed opposing species responses to drought, whereby the negative effects via inhibited emergence of seedlings during spring drought clearly outweighed the positive effects via enhanced establishment post-drought. Seed augmentation had a strong and long-lasting effect on community composition, which translated into increased community productivity in later years. Moreover, seed augmentation mitigated the negative effect of spring drought on species diversity via the increased recruitment of forbs.

4.1 | Growth and persistence of established vegetation

Late-summer drought caused stronger relative biomass reduction than spring drought (Appendix S8), even though duration of precipitation exclusion and extremity of the resulting meteorological drought was similar for both treatments. This can be explained by a faster decline in soil moisture due to higher temperatures in summer (Appendix S2), as was shown in a mesocosm study (De Boeck et al., 2011), and by biomass recovery and compensatory growth which probably reduced the effect size of the spring drought in our study during a six-week period between the end of spring drought and the traditional cutting date. The recovery and compensatory growth of relatively drought-resistant plants showing plasticity in timing of growth also explained smaller effects of early-season compared to late-season drought on annual net primary productivity (ANPP) in tallgrass prairie (Denton et al., 2017). Our study further confirms the capacity of many grasslands for fast plant biomass recovery after drought (Mariotte et al., 2013; Hoover et al., 2014; Yang et al., 2016; Kreyling et al., 2017; Stampfli et al., 2018; Mackie et al., 2019). Graminoids and forbs showed similar resistance in biomass to late-summer drought (Appendix S8). The stronger reduction in biomass of forbs than of graminoids after spring drought reflects faster recovery and compensatory growth of graminoids post-drought, consistent with findings across multiple temperate grasslands (Stampfli et al., 2018), rather than a difference in drought resistance between the two functional groups. In fact, forb biomass showed higher resistance after late-summer drought than graminoid biomass in plots of lower soil depth (Appendix S9), consistent with findings across multiple temperate grasslands (Mackie et al., 2019).

TABLE 2 Summary of statistical analyses testing legacy effects of droughts in late summer (AD) and subsequent spring (SD) and of seed addition (S) on community composition and functioning, i.e. frequency sum, species diversity, biomass and number of reproductive shoots (N) and species richness (SR) in summer, for 1–4 years after treatments

| Variable | Year | AD | SD | AD × SD | S | S × AD | S × SD | S × AD × SD |
|------------------------|------|-----------|-----------------|------------|-----------------|--------------|--------------|-------------|
| | | $F_{1,6}$ | $F_{1,12}$ | $F_{1,12}$ | $F_{1,24}$ | $F_{1,24}$ | $F_{1,24}$ | $F_{1,24}$ |
| Frequency sum | 1 | 0.45 | 0.91 | 2.01 | 48.91*** | 1.85 | 4.60* | 0.36 |
| | 2 | 1.15 | 2.79 | 0.01 | 36.32*** | 0.01 | 0.33 | 0.10 |
| | 3 | 4.42 | 0.31 | 0.28 | 30.64*** | 1.81 | 0.00 | 2.09 |
| Diversity (exp[H']) | 1 | 2.09 | 36.35*** | 0.02 | 42.13*** | 2.96 | 1.73 | 3.21 |
| | 2 | 0.24 | 8.18* | 0.92 | 40.07*** | 5.89* | 1.75 | 3.15 |
| | 3 | 0.88 | 3.46 | 2.38 | 17.95*** | 0.85 | 6.21* | 3.75 |
| Biomass | 1 | 0.64 | 20.81*** | 1.17 | 0.31° | 0.13° | 0.86° | 1.46° |
| | 2 | 0.76 | 0.01 | 4.23 | 0.12° | 1.38° | 0.02° | 0.11° |
| | 3 | 4.40 | 1.11 | 1.75 | 0.07° | 1.53° | 0.08° | 0.07° |
| | 4 | 0.00 | 2.33 | 2.39 | 6.75* | 0.42° | 0.12° | 0.25° |
| Reproductive shoots N | 4 | 0.92 | 1.25 | 0.54 | 0.02 | 1.09 | 0.49 | 0.21 |
| Reproductive shoots SR | 4 | 1.58 | 0.08 | 0.00 | 0.40 | 0.29 | 0.26 | 0.42 |

ANOVA-F or GLM-F_{quasi} values of summer and spring droughts and of seed addition and their interactions (***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$). The covariable (pre-drought values) was always highly significant for community composition variables (data not shown). °, degrees of freedom adjusted due to outlier ($F_{1,23}$).

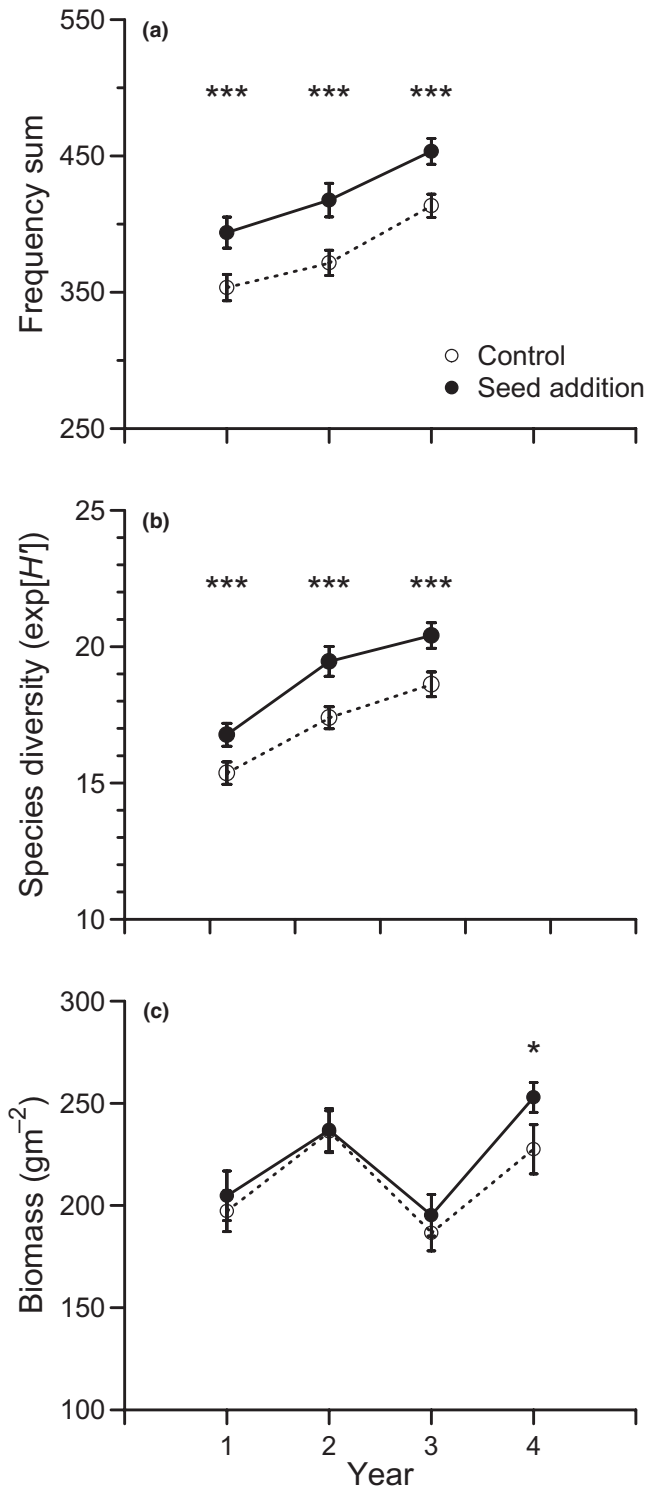


FIGURE 5 Legacy effects of one-time seed addition on community composition and functioning over four years after seed addition: (a) frequency sum; (b) species diversity; and (c) biomass of summer harvest (mean \pm SE, $n = 28$; ***, $p < 0.001$; *, $p < 0.05$). Note that data show an 11% biomass increase in year 4 based on samples of 0.09 m^2

The high persistence of most single species, together with very small or lacking negative effects on the structure of the established vegetation (Appendix S7) implies low drought-induced mortality of

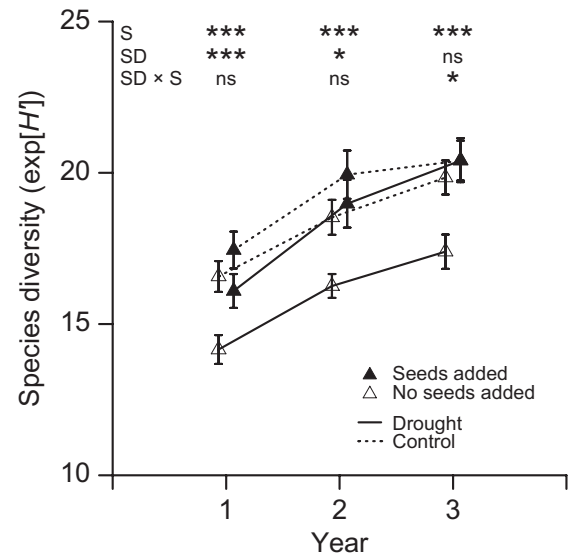


FIGURE 6 Legacy effects of one-time seed addition (S) and spring drought (SD) and of their interaction (SD \times S) on species diversity (exp[H']) over three years after experimental treatments (means \pm SE, $n = 14$; ***, $p < 0.001$; *, $p < 0.05$; ns, not significant)

established plants. Likewise, only minor species loss was found in response to recurrent experimental summer droughts of approximately similar length in a low-productive pasture (Grime et al., 2008). Obviously, seasonal precipitation exclusion in temperate climate is not long enough to induce severe soil moisture droughts which could push most grassland species beyond mortality thresholds (Poirier et al., 2012; Volaire et al., 2014).

4.2 | Opposing drought effects on recruits

Whereas seedling emergence of many graminoid and forb species was reduced by droughts in both seasons, seedling survival was not sensitive to spring drought (Appendix S6); seedling survival in response to late-summer drought was not studied because of the selected timing of our seed-addition treatment. Our finding that germinating seeds show higher vulnerability to drought than seedlings corroborates findings from studies in Mediterranean shrubland (Lloret et al., 2004) and in the greenhouse (Fay and Schultz, 2009). Germinating seeds lying on top of soil rely completely on water availability at the soil surface, which makes them much more sensitive to drought than seedlings which have reached soil moisture in deeper soil layers due to fast root growth in their first weeks after germination (Cerletti, 1997). The effect of drought on seedling survival may be underestimated in our study due to the relatively long intervals between seedling censuses during which emerged seedlings may have died without being noticed. Nevertheless, as most cohort-1 seedlings had already emerged in the autumn after seed addition, our conclusion that seedling survival is not sensitive to spring drought holds for many ca. five-month-old seedlings at the start of the spring drought treatment.

Droughts in both seasons reduced seedling emergence in several single species (Figure 2), but only spring drought showed a long-lasting negative effect on recruitment at the functional-group level in this experiment (Figure 4). Likewise, results from semi-natural grassland in the close vicinity of our study site showed that seedling emergence is negatively related to the duration of low soil moisture in spring but not in autumn (Stampfli and Zeiter, 2008). The temporal pattern of abundant seedling emergence in autumn but only minor emergence between mid-April and time of first cutting found in our study is in line with earlier observations across five years (Stampfli and Zeiter, 2008) and implies that compensation of direct negative effects of drought on recruitment is partly possible after late-summer droughts, but unlikely after spring droughts. Early summer is less favourable for recruitment than autumn due to higher solar radiation, which desiccates the soil surface faster and prevents seedling emergence unless the weather is very wet. Such conditions enhance standing crops and the competitive effects on recruits by limiting light availability. Hence the germination season in temperate grassland is related to the temporal occurrence of favourable light conditions (Kahmen and Poschlod, 2008).

Very few single species, two forbs and three grasses, showed increased seedling emergence after late-summer drought, which is consistent with the expectation that biomass reduction and increased open space (gaps) would enhance recruitment post-drought (Figure 2). Recruitment enhancement due to reduced competition was exemplified in this study by the most positively affected *Arabis ciliata*, a relatively short-lived species, which showed an increased number of reproductive individuals and abundance of next-generation seedlings in post-drought years (Appendix S14). This is in line with the drought-induced enhanced abundance of "ruderal" species in ex-arable grassland (Morecroft et al., 2004) and shortgrass steppe (Evans et al., 2011). The largely lacking evidence for recruitment enhancement after spring drought is consistent with the generally unfavourable conditions for seedling establishment in early summer.

We only found transient evidence of enhanced recruitment post-drought at the functional-group level, limited to grasses used in the seed addition treatment (Appendix S11), but no evidence at the whole-community level. Apparently, positive drought-induced effects on recruitment of single species by means of reduced competition from established plants were outweighed by the negative direct effect of water shortage on seedling emergence. This implies that competition from established plants is of minor importance in this grassland (Zeiter and Stampfli, 2012). Physical hazards, such as drought and frost, and pathogens therefore control seedling establishment to a greater extent than competition by neighbouring plants in grasslands of low productivity (Ryser, 1993).

Interestingly, evidence for changes in community composition via enhanced recruitment post-drought comes from circumstances in which droughts occurred sequentially in multiple years (Morecroft et al., 2004; Stampfli and Zeiter, 2004; Evans et al., 2011). Similar droughts in sequential years could potentially reduce the recovery speed of the established vegetation and provide prolonged opportunities for recruitment and establishment post-drought. In our study, however, sequential droughts in late summer and spring did not

result in community composition change because recruit emergence was partially diminished by the late end of the late-summer drought and severely hampered by the spring drought.

The decline in species diversity of forb recruits in the year post-drought (Appendix S13) probably reflects a negative spring drought impact on seed production. A drought-induced reduction of the reproductive output of grassland herbs is known from other studies (Dietrich and Smith, 2016; Zeiter et al., 2016).

4.3 | Role of regeneration in vegetation dynamics

For two reasons, the hypothesis that enhanced post-drought recruitment from seed operates as a stabilizing process in vegetation by compensating for adult mortality (Lloret et al., 2012) was not supported by our experiment. Firstly, in our study, adult plants of most species were able to persist across extreme seasonal droughts via vegetative re-sprouting. Secondly, extreme spring drought caused a persistent reduction of species diversity by inhibiting the emergence of forb recruits. Nevertheless, this implies that similar to the drought-induced vegetation dynamics of forests (Martínez-Vilalta and Lloret, 2016) and consistent with our overarching hypothesis, recruitment plays a key role in temperate semi-natural grassland dominated by perennial species. This is further in line with the conclusion that climate change is more likely to influence population growth via effects on recruitment than via effects on survival from a long-term demographic study of relatively short-lived species in prairie (Dagleish et al., 2010).

4.4 | Importance of seed availability

One-time seed addition had long-lasting effects on both community composition and functioning in our study (Figure 5). This is in line with multi-species seed addition experiments performed in various grasslands which have shown that communities are unsaturated with species in the sense that local ecological processes, such as competition or herbivory, and the availability of seeds constrain species richness (Turnbull et al., 2000; Zobel et al., 2000; Foster and Tilman, 2003).

Nevertheless, our findings of a small-scale increase in species richness and diversity due to seed augmentation may also be interpreted as an effect of limited short-distance dispersal of seeds. The fact that 99.9% of all recruits observed over five years belonged to species which were already present in the plots at the onset of the experiment suggests that species in this semi-natural grassland have low dispersal potentials. The same conclusion resulted from a seed-rain study in twelve Swiss hay meadows (Zeiter et al., 2013). This is in line with global estimates of very short dispersal distances for the majority of herbaceous species (Tamme et al., 2014).

While dispersal limitation was found to have constrained productivity in several studies after introducing new species (Zeiter et al., 2006; Stein et al., 2008), our study shows that seed deficiency constrained productivity after augmentation of the seeds of resident species. This is remarkable because our seed augmentation treatment simulated a

seed rain of normal density (Clark et al., 2007) for hay meadows with late cutting dates in summer (Zeiter et al., 2013). The effect of seed augmentation on community productivity was independent of drought. This further supports our previous conclusion that droughts which cause low mortality in established plants only marginally reduce competition on recruits. The delayed increase in biomass which developed over four years after seed augmentation (Figure 5c) reflects the slow establishment of long-lived perennial species in semi-natural grassland (Zeiter et al., 2006; Zeiter and Stampfli, 2008).

The stronger effect size of seed addition on graminoids compared to forbs in the year following seed addition (Figure 3) is in line with low graminoid seedling densities repeatedly observed in nearby grassland plots cut at similar dates. This is due to the timing of seed maturation, as seeds of many species of grasses mature later than seeds of most forb species (Stampfli and Zeiter, 2004, 2008). On the other hand, forb recruits showed more pronounced delays in seed addition legacy effects than graminoid recruits, at both single-species and functional-group levels (Figure 3). This is consistent with a lower proportion of persistent graminoid seeds in the topsoil of Swiss hay meadows (Zeiter et al., 2013).

4.5 | Implications for management of high nature value grassland

The predominant practice of early, fast or high-frequency grassland harvesting with the objective of delivering high-quality fodder implies that seed deficiency is a prevalent problem for biodiversity conservation and maintenance which also includes semi-natural grasslands in agricultural landscapes today. At times of subsistence farming, smallholders were aware of the relationship between regular early mowing and longer-term yield reduction (Babai and Molnár, 2014). Similarly, at Negrentino, variability in mowing dates between years or deliberate rotation of mowing dates among local areas may have ensured the likelihood of seed maturation and dispersal of bulk grasses and enough hay yield in the long run.

As increasing drought frequency exacerbates seed deficiency in high nature value (HNV) grasslands, the practical question of whether seed deficiency compensation would mitigate negative drought effects is timely. Our experiment shows that seed deficiency compensation can indeed support rejuvenation in a critical phase during extreme drought and in turn moderate the negative impacts of drought on species diversity and stabilize grassland functioning in the long run (Figure 6). This implies that the augmentation of seed supply from resident species could effectively counteract semi-natural grassland degradation in a future climate with more frequent droughts. Local knowledge is required to ensure the opportunities for maturation and dispersal of seeds supplied from resident species.

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AUTHOR CONTRIBUTIONS

AS and MZ equally shared all tasks.

DATA AVAILABILITY STATEMENT

Field protocols and primary data files containing spatiotemporally explicit information of established plants and recruits by species, and integrated data files used for the analyses are stored with the authors. Data are available upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

Appendix S1. Photographs showing rainout shelters and irrigation devices

Appendix S2. Time graph of simulated rain and soil water potential resulting from treatments

Appendix S3. Photograph showing grid cells with subdivisions

Appendix S4. Reference list for SLA data obtained from TRY database

Appendix S5. Selection criteria of variables for statistical analysis

Appendix S6. Effects on single species

Appendix S7. Statistical table of drought effects on persistence of established vegetation

Appendix S8. Drought effects on biomass

Appendix S9. Relationship between soil depth and biomass in drought and control treatments

Appendix S10. Seed-addition effects on species frequency of recruit cohorts 1-3

Appendix S11. Legacy effects on cohort-1 graminoid recruits

Appendix S12. Statistical table of drought effects on cohort-2 and cohort-3 recruits

Appendix S13. Drought legacy effect on year-2 forb recruits

Appendix S14. Legacy effect of late-summer drought on cohort-3 recruits of forbs

Appendix S15. Proportions of established plants and recruit cohorts

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