Journal of Vegetation Science **27** (2016) 238–248

Timing of extreme drought modifies reproductive output in semi-natural grassland

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Keywords
Drought season; Dry spell; Hay meadow; Reproductive shoots; Rooting depth; Seed production; Seed rain; Species-rich grassland

Nomenclature
Lauber & Wagner (1996)

Received 4 May 2015
Accepted 6 October 2015
Co-ordinating Editor: Rasmus Ejrnaes

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Abstract

Questions: Do extreme dry spells in late summer or in spring affect abundance and species composition of the reproductive shoots and the seed rain in the next annual crop? Are drought effects on reproductive shoots related to the rooting depths of species?

Location: Species-rich semi-natural grassland at Negrentino, Switzerland.

Methods: In plots under automated rain-out shelters, rainwater was added to simulate normal conditions and compare them with two experimentally effected long dry spells, in late summer (2004) and in the following spring (2005). For 28 plots, numbers of reproductive shoots per species were counted in 1-m^2 areas and seed rain was estimated using nine sticky traps of 102 cm^2 after dry spells.

Results: The two extreme dry spells in late summer and spring were similar in length and their probability of recurrence. They independently reduced the subsequent reproductive output of the community, while their seasonal timing modified its species composition. Compared to drought in spring, drought in late summer reduced soil moisture more and reduced the number of reproductive shoots of more species. The negative effects of summer drought decreased with species' rooting depth. The shallow-rooted graminoids showed a consistent susceptibility to summer drought, while legumes and other forbs showed more varied responses to both droughts. Spring drought strongly reduced density (–53%) and species richness (–43%) of the community seed rain, while summer drought had only a marginally significant impact on seed density of graminoids (–44%). Reductions in seed number per shoot vs reproductive shoot density distinguished the impacts of drought with respect to its seasonal timing.

Conclusion: The essentially negative impact of drought in different seasons on reproductive output suggests that more frequent dry spells could contribute to local plant diversity loss by aggravating seed deficiency in species-rich grassland.

Introduction

Flowering performance determines the reproductive success and fitness of plants and, therefore, the persistence of species and the composition of communities. Flowering and the production of seeds is also important for the functioning of ecosystems, as limited seed availability has been shown to affect productivity in grasslands (Foster et al. 2004; Zeiter et al. 2006; Stein et al. 2008).

Flowering and seed production in local populations can vary considerably over time, and one contributing factor may be variation in precipitation. Precipitation patterns are expected to change around the world as atmospheric CO_2 concentrations further increase. Various climate change scenarios predict an increase in mean temperature and also an intensification of extreme events. This may result in more strongly drier and wetter years becoming more frequent in the future, both at the global (Meehl et al. 2007) and the regional scale, e.g. Switzerland (C2SM 2011). More extreme rainfall regimes that have longer intervals between rain events, hence stronger variation in soil water availability for plants, are expected to increase

Doi: 10.1111/jvs.12362

the duration and the severity of drought stress in mesic vegetation (Knapp et al. 2008).

Phytomass reduction caused by drought in temperate grassland (Tilman & El Haddi 1992; Stampfli 1995; Wu et al. 2011) implies that established individuals are temporarily reduced in size, as perennial plant species have some capacity to survive drought events (Stampfli & Zeiter 2004; Grime et al. 2008). Drought-induced size reductions may result in reduced flowering and seed production because in many plant populations reproductive output is strongly correlated with plant size (Shipley & Dion 1992).

The importance of water availability for seed production is apparent, at least for annual species, from the worldwide practice of irrigation for crop fields (Tilman et al. 2002). In natural communities of perennial species, the influence of water availability on reproductive output has only been studied for single species (e.g. Fay et al. 2003; Ågren et al. 2008). At the level of whole natural plant communities, the importance of water availability for flower and seed production has only been emphasized in the context of warming experiments and CO_2 enrichment studies, in which manipulations of other factors also caused changes in water availability (e.g. Thürig et al. 2003; Liancourt et al. 2012).

Drought effects on reproductive success are not necessarily negative for all species in a community. As species differ in drought tolerance (Grime et al. 2000), the more drought-tolerant plants could benefit from reduced competition, increase in size and therefore have higher reproductive output. Drought tolerance of species may be positively related to rooting depth, an architectural trait that contributes to the spatial configuration of the root system of a plant, as deep roots allow acquisition of water from deeper soil layers. Indeed, for herbaceous plants in water-limited environments, maximum rooting depth is strongly positively related to site aridity (Schenk & Jackson 2002), and there is further evidence that shallower-rooted herbaceous vegetation is more drought sensitive than vegetation dominated by deeper-rooted woody plants (Debinski et al. 2010). However, so far, the role of root traits in explaining responses to global change has not received much attention (Bardgett et al. 2014) and little is known on the role of rooting depth in explaining drought tolerance patterns within a temperate grassland community composed of mainly herbaceous species (but see Fry et al. 2013). Overall, such species-specific responses to drought could potentially influence the diversity of the reproducing individuals of a community and its future composition. As far as we know, the effect of reduced water availability on reproductive output has not been simultaneously studied for all species in a semi-natural plant community.

Apart from effects of altering the quantity of precipitation on plant growth (Wu et al. 2011), changes in the timing of precipitation can have strong effects (Knapp et al. 2002). Temporal changes in precipitation could, therefore, significantly affect flower and seed production in species-rich managed grasslands, where herbaceous species show specific seasonal patterns of flowering shoot initiation, flowering, seed development and seed ripening (Bommer 1959). Recent reviews have concluded that more precipitation manipulation experiments are needed to address the role of seasonal timing of droughts on plant communities (Wu et al. 2011; Smith 2011), as such studies have very rarely been made (Beier et al. 2012). Specifically, the effect of seasonal timing of drought on flower and seed production has not been investigated.

We treated a semi-natural grassland to experimentally-reduced water availability to investigate the responses of the community to single and sequential droughts in terms of reproductive output. We chose this grassland because species-rich hay meadows have strongly declined due to recent land-use change (Lachat et al. 2010). We explore whether the timing of seasonal drought affects responses at the levels of community and single species, and test whether drought effects on reproductive output are related to the rooting depths of the species.

Methods

Study site

This study was performed at Negrentino (820 m a.s.l., 46°27′51″ N, 8°55′29″ E), southern Switzerland, in semi-natural *Mesobromion*-type grassland (Ellenberg 1996) of high species richness (Stampfli 1992; Zeiter & Stampfli 2012). We maintained a bi-annual management regime of haymaking, which had been common practice for at least the last century, and with no fertilizer application, as had been the case during at least the past three decades. The slope is inclined 11° towards south–southeast. The soil is a relatively deep (mostly >0.7 m), moderately acid sandy loam (*sensu* FAL 1997) with a low nutrient content and a water storage capacity of ca. 60 $dm^3 \cdot m^{-2}$. The climate is temperate humid (Walter & Lieth 1964) with a mean annual temperature of 9.8 °C (on-site measurements 2005–2009) and a mean annual precipitation of 1437 mm, with year-to-year variation in the range of 728–2055 mm (daily precipitation 1961–2010: RhiresD, MeteoSwiss).

Drought experiment

We established 28 plots of 1.5 × 1.5 m arranged in seven blocks of 3 × 3 m. Within each block, we randomly allocated two plots to a drought treatment in late summer 2004, while the two other plots served as (2004) controls.

Later, we randomly allocated one summer-drought and one summer-control plot per block to a drought treatment in spring 2005, while the two other plots served as (2005) controls. This sequentially nested design resulted therefore in the four plots per block having different drought legacies: two legacies of a single drought, either in late summer or spring; one legacy of a late-summer drought followed by a spring drought; and one legacy of no drought at all (Appendix S1). Drought treatments were applied to whole blocks (with a 1-m outer border making them 25 m^2 in area) using rain-out shelters, which operated during times of precipitation. The controls received rainwater via irrigation devices that gently supplied the central 1-m^2 areas of the required plots. We chose this design to minimize side effects of rain-out shelters as far as was possible, and to avoid confounding of effects of reduced precipitation with shading, temperature change and wind protection (Beier et al. 2012; Vogel et al. 2013).

In each block, two soil moisture sensors (Equitensiometer EQ15, Ecomatik, Dachau, DE) were installed at ca. 5.5 cm below the soil surface, one in the plot receiving rainwater (the control), the other in the plot receiving no rainwater (i.e. experimentally droughted) during both seasons. Next to the experiment, we set up a solar-powered weather station consisting of a tipping-bucket rain gauge (ARG100, Delta-T, Cambridge, UK) and sensors to record photosynthetically active radiation (PAR), wind speed, temperature and humidity. A logger (CR10X, Campell Scientific Inc., Logan, UT, US) stored the microclimatic data at intervals of 5 s and enabled the automation of the drought treatments.

The seven rain-out shelters consisted of electrically-driven waterproof screens of white acrylic material (Wintergartenstore Targa PS5000, Stobag, Muri, CH). They were fixed at an angle of 18°, and held at a height of ca. 0.5–1.6 m above ground by four wooden poles. The automatic movement of the screen covers was computer-controlled to minimize coverage time, based on the rainfall, humidity and radiation variables. When a minimum precipitation was detected the screens automatically rolled out over the blocks, and the rain falling on them was conducted away. A few minutes after precipitation ceased, the screens rolled back inwards, to leave the blocks exposed again. PAR recorded above and below the screens (200 and 25 cm above ground) showed that total reduction in radiation due to intermittent coverage by the screen during the periods of simulated drought was just 1.3%.

Rain-out shelters created a first dry spell of 52 d in late summer (mid-Aug to mid-Oct) 2004 and a second one of 59 d in spring (mid-Mar to mid-May) 2005. Both dry spells mimicked extreme seasonal events, defined as sequences of days with 2-d running means of precipitation <10 mm, which recur about once in 37 yr (daily precipitation records 1892–2003 at the nearby station Comprovasco of MeteoSwiss). In the control plots we mimicked the precipitation regime of a long-term average season by adding 6–30 mm rainwater weekly as one to three nighttime events of variable duration per week during the simulated dry spells.

Under rain-out shelters the top soil stayed very dry (water potential $\psi < -400$ kPa, median, $n = 7$) for 45 d (26 Aug–9 Oct) in 2014, and for 16 d (30 Apr–15 May) in 2005. During these periods the top soil of the irrigated control plots never dried out ($\psi > -40$ kPa, median, $n = 7$).

Sampling of reproductive shoots and species frequency

We harvested the phytomass of the central 1-m^2 areas of plots at 5 cm above ground using an electric lawn mower, at traditional mowing times, i.e. end of Jun and mid-Sept 2005 (Appendix S1). We counted reproductive shoots of all 64 species found in the hay samples, regarding connected plant parts with seeds, flowers or flower buds as reproductive units. This method was suitable for all species occurring at the site except for three early-flowering (Mar/Apr) species with short reproductive shoots.

To separate drought effects on reproductive allocation from drought effects on vegetative growth, we recorded species frequencies before and after drought treatments, in Jul 2004 and Jul 2005. We recorded for each species, in two gridded strips per plot, the number out of 400 2- × 2-cm quadrats having rooted individuals (Appendix S1).

Seed rain sampling

In the central part of each plot, we installed nine seed traps at stratified random positions within 0.48 × 0.48 m, three traps on 27 May and six traps on 12 Jun 2005 (Appendix S1). The date of trap installation did not affect the number of trapped seeds (1355 vs 1067 seeds·m^{-2}; $t_{27} = 1.8$). Traps consisted of dishes 38 mm in diameter filled with non-drying glue (Tangle-Trap, Andermatt Biocontrol AG, Grossdietwil, CH), and supported 5 cm above the ground on large nails. Traps were black in colour to reduce their attraction to insects (Kollmann & Goetze 1998). We recovered the seed traps on 26 Jun 2005, just before mowing the plots. We counted all seeds per trap and pooled the data of all traps per plot. With this sampling design and based on a Poisson distribution of seeds, a seed density of 294 seeds·m^{-2} is needed in order to have a 95% chance of finding at least one seed per plot. Graminoids and forbs shed between 75 and 80% of their seeds before the end-of-June mowing, and almost no mature seeds were produced at the time of the second harvest (M. Zeiter & A. Stampfli, unpubl. data).

Rooting depth of species

We obtained data on rooting depth from Kutschera & Lichtenegger (1982, 1992) and references therein, and from the TRY database (Kattge et al. 2011), which contain trait data from Fitter & Peat (1994), W. Green (http://bricol.net/downloads/data/PLANTSdatabase/; NRCS: The PLANTS Database, http://plants.usda.gov, 1 Feb 2009), Paula et al. (2009) and V. Lanta (unpubl. data). We selected rooting depth values measured under conditions matching those at our experimental site, i.e. we excluded values from bogs, wet meadows or recently ploughed grasslands. Rooting depth was available for 33 species, from one to eight authors per species (Appendix S2). Single authors contributed up to five values per species. To obtain species' rooting depth, we first averaged the values by contributing author to account for disproportionate author contributions to any particular species, and then averaged the different authors' means.

Data analysis

The design was a two-factor split-plot arrangement with block as replicated unit. The factors late-summer drought (AD, acronym uses 'autumn' as a substitute for 'late summer') and spring drought (SD) included two levels. The design had three strata: (a) block, $n = 7$; (b) block × plot pair (i.e. two plots allocated the same AD treatment), $n = 14$ with AD nested within block and (c) block × plot pair × plot, $n = 28$ with SD nested within AD.

We conducted statistical analysis using GENSTAT 11.0 (Payne 2008). We applied GLM with Poisson distribution and log-link function to count data (number of flowering shoots, species and seeds), and produced accumulated analysis of deviance tables, built up residuals from block × AD for stratum (b) and block × AD × SD for stratum (c), and used quasi-*F* tests (McCullagh & Nelder 1989).

We analysed data on the number of reproductive units at the community level, at the level of functional groups (grasses, sedges and rushes, legumes, non-legume forbs) and at the level of single species. At the level of single species, we restricted analyses to those most abundant, i.e. species with a mean number of >1 reproductive unit·m^{-2}. We used species frequency in 2004 (sums of both subplots per plot) as a covariate to account for between-plot differences in species abundance at the onset of the experiment. Nevertheless, data analysis was not feasible for one sedge and three non-legume forbs (Appendix S2) due to high within-block variability in abundance at the onset of the experiment. Thus, final analysis at the level of single species included 35 species. To adjust for drought effects on vegetative growth, we re-ran the analyses and included species abundance after the droughts (Jul 2005) as a second covariate.

Rooting depth data were available for 30 of the 35 species used in single species analyses. To analyse the influence of rooting depth of species on the drought effect on the number of flowering shoots, we performed linear regressions with rooting depth as explanatory variable and the effect size in response to droughts, i.e. response ratio = log (mean number of flowering shoots in drought plots/mean number of flowering shoots in control plots) as a response variable. We fitted separate models for the effect sizes of the late-summer and the spring drought. In addition, we fitted separate models for species with high (≥4 units·m^{-2}) or low (<4 units·m^{-2}) abundant reproductive shoots. We further performed linear regressions with log-transformed before-drought frequency (Jul 2004) of the perennial species as explanatory variable and the effect size in response to droughts as response variable.

Seed rain data were only analysed at the level of the whole community and at the level of graminoids (grasses, sedges and rushes) and forbs (non-legume forbs and legumes), as single-species data and data of functional groups had very low numbers (Appendix S3). Due to these generally low numbers, we used a significance level of $P = 0.1$ for the seed rain data.

Results

A total of 14 653 reproductive shoots from 64 species (20 graminoids, 44 forbs) were found in the hay samples (Appendix S2). The bulk of the reproductive shoots (96.5%) were found in the harvest of the first cut at the end of June (Appendix S2). The density of reproductive shoots increased with species frequency ($r^2 = 0.48$, $P < 0.001$; Appendix S4). In control plots, reproductive shoots of graminoids were slightly more abundant than reproductive shoots of forbs (321 m^{-2} vs 237 m^{-2}). Seeds of 23 species (eight graminoids, 15 forbs) were collected in the seed traps. Seeds of graminoids were much less abundant than seeds of forbs (420 m^{-2} vs 1176 m^{-2}; control plots).

In 53 statistical tests we found 28 significant ($P < 0.05$) spring- or late-summer-drought effects on numbers of reproductive shoots or seed rain but no significant interaction between spring and late-summer droughts in combination with at least one significant main effect (Table 1, Appendix S2). This implies that the effects of the sequentially combined droughts were independent.

Table 1. Effects of droughts in Late-summer 2004 (AD) and spring 2005 (SD) on density and species richness of reproductive shoots and seed rain of semi-natural grassland at Negrentino in summer 2005: species group, density of shoots or seeds in plots of the no drought control, and GLM-F_{quasi} values of late-summer (AD) and spring (SD) drought effects and of interaction; ***$P \leq 0.001$; **$P \leq 0.01$; *$P \leq 0.05$; $^{(*)}P \leq 0.1$.

Species Group	Density				Number of Species			
	Control [m^{-2}]	AD $F_{1,6}$	SD $F_{1,12}$	AD × SD $F_{1,12}$	Control [m^{-2}]	AD $F_{1,6}$	SD $F_{1,12}$	AD × SD $F_{1,12}$
Reproductive shoots								
Grass^1	303.6 ± 54.9	0.43/25.03**	0.20/1.11	0.29/4.28	10.3 ± 0.5	33.63**	0.02	0.02
Sedge & rush	17.7 ± 5.2	6.53*	4.18	0.01	2.4 ± 0.5	0.19	1.69	0.83
Non-legume forb	217.4 ± 37.8	0.41	0.81	0.32	19.0 ± 2.5	3.34	3.55	0.81
Legume	19.9 ± 2.3	1.99	4.29	0	3.9 ± 0.7	5.47	2.64	0.05
Total^1	558.6 ± 70.1	1.16/85.2***	0.01/1.29	0.09/0.25	35.6 ± 1.9	28.0**	7.33*	0.48
Seed rain								
Graminoid^2	420 ± 151	$4.43^{(*)}$	6.67*	2.72	2.0 ± 0.6	1.54	$3.65^{(*)}$	0.10
Forb^2	1176 ± 232	0.15	8.08*	0.01	4.9 ± 0.8	0	7.39*	0.42
Total	1596 ± 268	0.01	11.99**	0.19	6.9 ± 1.0	0.27	16.93**	0.87

[1] Analysis included/excluded *A. capillaris*.

[2] Graminoid includes 14% seeds of sedge and rush, forb includes 7.4% legume seeds.

Drought effects on reproductive shoots

Droughts significantly affected reproductive shoot density of almost half of the 35 most abundant species in this community (Fig. 1, Appendix S2). Late summer drought affected twice as many species as spring drought (13 vs six species; Fig. 1); only one species responded to both droughts. The majority of drought effects on species were

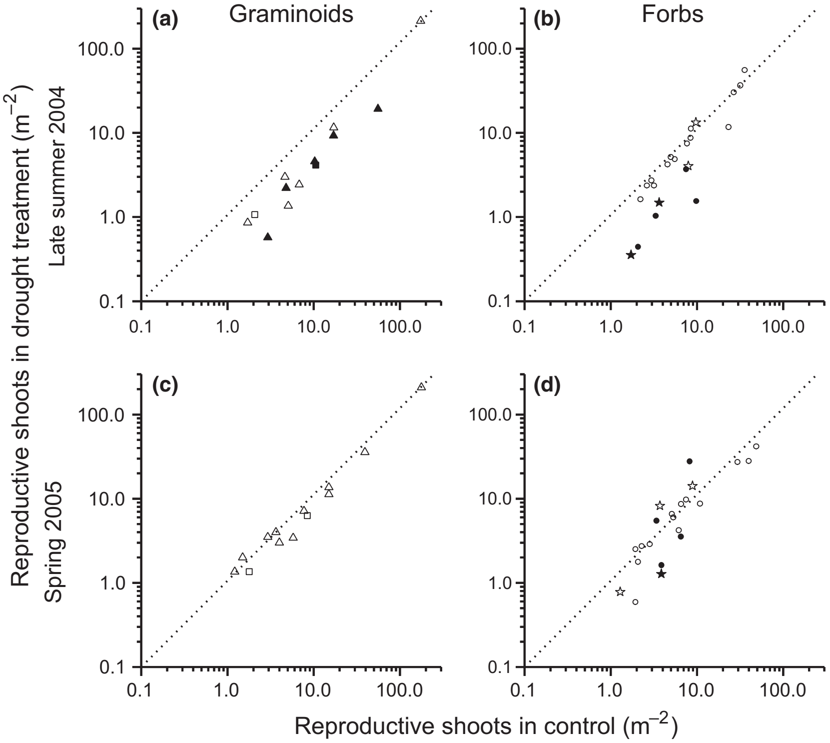

Fig. 1. Effects of droughts in late summer 2004 and spring 2005 on reproductive-shoot density (means, $n = 14$) of graminoids (**a**, **c**) and forbs (**b**, **d**) in summer 2005. Symbols show 35 most abundant species of grasses (triangles), sedges and rushes (quadrats), non-legume forbs (circles) and legumes (stars). A filled symbol below or above the dashed (1:1) lines indicates a significant ($P \leq 0.05$) negative or positive response to drought, respectively. There were no significant interactions between the effects of the two droughts.

negative (–45% to –80%), but two spring drought effects on non-legume forbs were positive (+51%, +216%; Fig. 1, Appendix S2). Most effects did not change when after-drought species frequency was included as a covariate, indicating that declines in reproductive shoots were due to modifications in reproductive allocation rather than plant mortality (Appendix S2). Neither the effect of the late-summer drought ($P = 0.42$, $n = 34$ species) nor the effect of spring drought was related to vegetative abundance of the species ($P = 0.52$, $n = 34$ species), suggesting that dominant and subordinate species were equally susceptible to drought in terms of reproductive shoots. The reproductive output of the far most abundant species, the grass *Agrostis capillaris*, which contributed 37% to the total number of reproductive shoots in the community, was not affected by drought, regardless of timing (Appendix S2).

The negative effect of the Late-summer drought declined with increasing rooting depth of the species ($r^2 = 0.15$, $P < 0.05$, $n = 30$ species; Fig. 2). The relationship was stronger for 17 species with abundant flowering shoots ($r^2 = 0.46$, $P < 0.01$; Fig. 2) and not significant for 13 species with few flowering shoots ($P = 0.54$). The effect of spring drought was not related to rooting depth of the species ($P = 0.95$, $n = 30$ species). Rooting depth significantly differed between graminoids and forbs ($t_{22.8} = 2.84$, $P = 0.009$), with graminoids (40.5 ± 13.8 cm; mean ± SD; $n = 13$ species) having shallower roots than forbs (65.2 ± 32.2 cm; mean ± SD, $n = 17$ species).

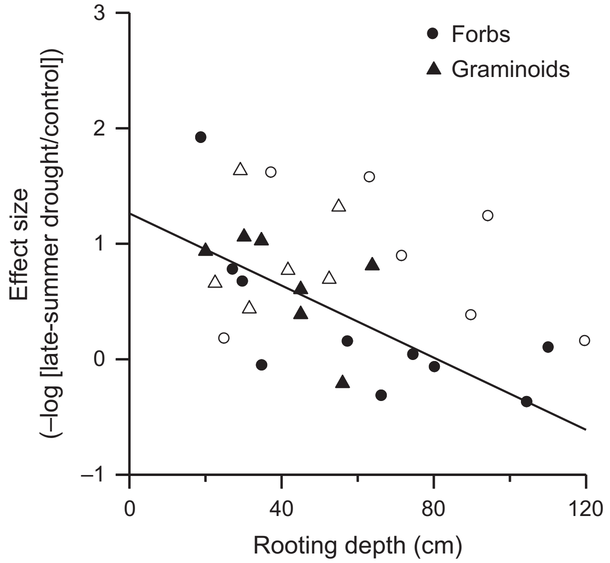

Fig. 2. Relationship between rooting depth and effect size of late-summer drought on reproductive-shoot density of most abundant species (means, $n = 14$ plots). Filled symbols indicate species with abundant reproductive shoots ($R^2 = 0.46$, $P < 0.01$, $n = 17$ species), unfilled symbols indicate species with only a few reproductive shoots ($P = 0.54$, $n = 13$ species).

Late-summer drought negatively affected reproductive shoots of six out of 13 graminoid species (Fig. 1, Appendix S2), consistently reducing grasses (–57%, after exclusion of *A. capillaris*; Table 1) and sedges and rushes (–111%; Table 1). Late summer drought also reduced the total number of grass species with reproductive shoots (–32%; Table 1). Although droughts affected ten out of 22 forb species (Fig. 1, Appendix S2), inconsistent responses of individual species balanced out at the level of functional groups, and thus droughts did not affect total density and species richness of reproductive shoots of non-legume forbs and legumes (Table 1).

At the community level, late-summer drought reduced total density (–26%, after exclusion of *A. capillaris*) and species richness (–13%) of reproductive shoots and spring drought reduced species richness (–8%) of reproductive shoots (Fig. 3, Table 1).

Drought effects on seed rain

Spring drought strongly reduced density (–53%) and species richness (–43%) in the seed rain (Table 1), while late-summer drought had no effect (Fig. 3). Reductions included density (–63%) and species richness (–52%) of graminoids and density (–50%) and species richness (–40%) of forbs (Table 1). Late-summer drought only reduced density of graminoids in the seed rain (–44%, $P < 0.1$; Table 1).

Discussion

In species-rich grassland, two extreme dry spells in late summer and spring, which were similar in length and probability of recurrence, strongly and independently reduced the reproductive output of the community, while seasonal timing of the dry spell modified its species composition. Late-summer drought had a stronger effect on density and diversity of reproductive shoots than spring drought, and its negative effect decreased with rooting depth of the single species. The shallow-rooted graminoid species showed a consistent susceptibility to late summer drought. Legume and non-legume forb species showed more varied responses to both droughts. Spring drought strongly reduced density and species richness of the community seed rain while summer drought only reduced the seed density of graminoids. Reductions in seed number per shoot or reproductive-shoot density distinguished impacts of summer and spring droughts.

Effects of sequential droughts are not currently well understood. While abiotic stress caused by an extreme event is thought to reduce resilience of ecosystems towards recurrent stressful events (Scheffer et al. 2001), plants often show high phenotypic plasticity and are able to

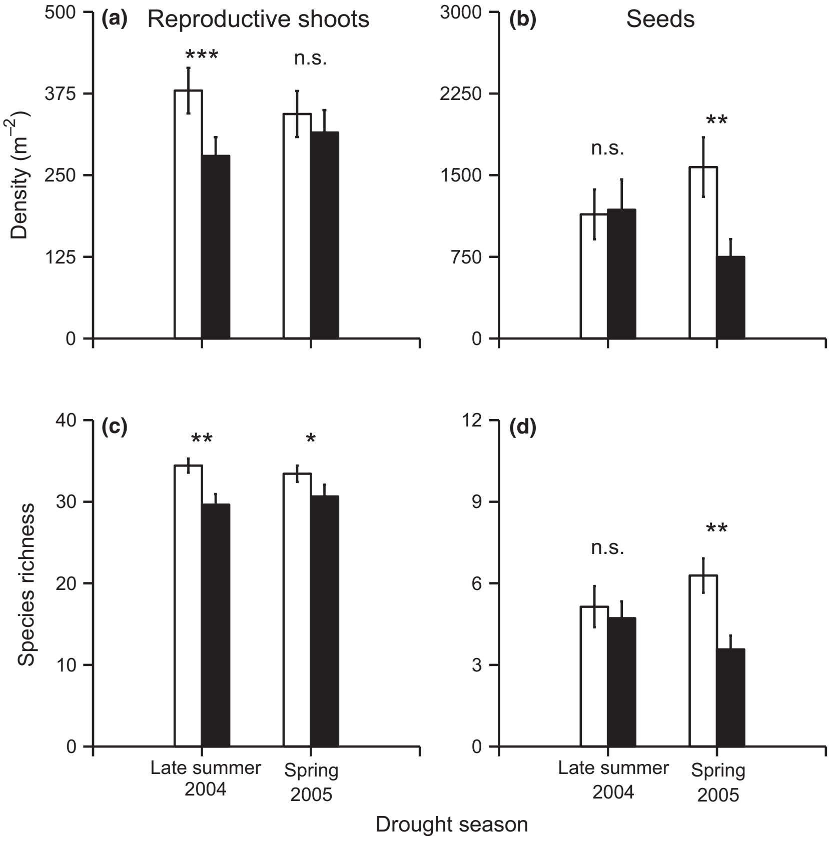

Fig. 3. Effect of drought in late summer 2004 and spring 2005 (filled bars) on community reproductive output in summer 2005, compared to controls (open bars), in terms of (**a**, **b**) density, and (**c**, **d**) species richness, of reproductive shoots and of seeds (mean ± SE). Density of reproductive shoots after excluding *A. capillaris*. *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; n.s., not significant. The interactions between late-summer drought and spring drought were not significant.

acclimate to stressful abiotic conditions (Bruce et al. 2007), e.g. by increasing root growth in the case of water shortage. Our study of a naturally assembled community did not find interactive carry-over effects of two sequential droughts on reproductive output, while other studies using experimentally assembled grassland (Zavalloni et al. 2008) or one single grass species (Walter et al. 2011) showed mixed results. Apparently, drought strength and the length of the time span between events can influence carry-over effects of sequential droughts.

Effect of dry spells on reproductive shoot density

Late-summer drought affected twice as many species as did spring drought. The differences in effect sizes on flowering between late-summer and spring dry spells can be explained by a faster decline in soil moisture due to higher temperatures in summer, as was shown in a mesocosm study (De Boeck et al. 2011). Nevertheless, vulnerability to late-summer drought also seems to be influenced by the flowering phenology of species. Floral primordia of many temperate grass species are already initiated in autumn (Heide 1994) and consistently late-summer drought negatively affected reproductive-shoot density of several grass species, while spring drought had no effect. Moreover, species-specific timing of flower initiation of grass species (Bommer 1959) might explain their different susceptibility to late-summer drought. Grass species that initiate flowering already in autumn or winter showed reduced reproductive-shoot densities in our study, while species that initiate flowering much later had time to recover from late-summer drought and showed no effect.

Although summer drought strongly reduced the flowering of many of the less abundant grasses, it did not affect the relatively deep-rooted *A. capillaris*, the most frequent species in this community. This is consistent with a study conducted in a native Great Plains' grassland by Fay et al. (2003), who found that the dominant grass species was tolerant to rainfall variability, while flowering of a subdominant grass species was reduced. Recurrent reductions of a community's reproductive output, which affect subordinate species but not dominant ones, may result in loss of species from the community.

The fact that effects of dry spells on reproductive-shoot density were more pronounced and more often negative for the shallower-rooted grass species than for the deeper-rooted forbs, hints at rooting depth as a potential key aspect explaining drought-sensitivity differences in reproductive output between temperate grassland species. This is in contrast to other temperate grassland studies that did not find different drought responses in biomass production of grasses and forbs (Morecroft et al. 2004; Grime et al. 2008). Deep roots were probably of little advantage for water acquisition in these studies performed on relatively shallow soil. Alternatively, different drought effects on vegetative growth and on reproductive output are not modified by the same plant traits. While plant tolerance was positively related to rooting depth under long precipitation exclusion in our study, Fry et al. (2013) showed that shallow-rooted species, especially annuals, were more resistant to precipitation reduction than deeper-rooted perennial forbs. Apparently, deep roots are important under extreme dry spells when the upper soil layers lose soil moisture, while shallow roots favour the acquisition of water under scenarios of reduced but recurrent precipitation supplying only upper soil layers with water (Schwinning & Sala 2004).

Only two out of 35 species showed positive drought effects, suggesting that the flowering of a high proportion of species is more constrained by water availability than by competition for light.

Effect of dry spell on seed rain

The density and diversity of the seed rain of graminoids and forbs was strongly reduced by the dry spell in spring, while the density of reproductive shoots was not affected. This implies that spring drought mainly constrained the number of seeds per shoot, through either abortion of flowers or of seeds.

The strong negative effect of late-summer drought on reproductive shoots of many species, especially grasses, did not translate into a negative effect on community seed rain (Fig. 3a,b), as particularly grasses had very low numbers of seeds in the seed rain despite their copious flowering. In nearby grassland plots, repeated low graminoid seedling densities were explained by timing of seed maturation, being distinctly later for many grasses than for most forbs (Stampfli & Zeiter 2004, 2008).

Using seed traps, our estimates of drought effects on seed density may include seeds that were dispersed from irrigated control plots or the surrounding vegetation, although seeds are often only dispersed over short distances in nutrient-poor semi-natural grasslands (Diacon-Bolli et al. 2013). Our approach may, therefore, have underestimated negative drought effects on seed rain. The apparently low number of seeds found in the seed rain is not an idiosyncrasy of a particular year of study. Seed rain density is inherently low in nutrient-poor grasslands (Zeiter et al. 2013). Thus, a much higher number of seed traps would be needed to determine drought effects on the seed density of single species. Seed availability and to some degree also the ratio in reproductive output between major functional groups such as grasses and forbs can be increased by postponing the traditional cutting date in hay meadows (Smith et al. 1996; Stampfli & Zeiter 2008). Thus, the negative effects of more frequent dry spells in a future climate on reproductive output in semi-natural grassland could be mitigated by management changes.

Implications for community composition

Limited seed availability can constrain plant species richness in grassland (Zobel et al. 2000; Foster et al. 2004; Zeiter et al. 2006). A more frequent occurrence of long droughts in the future might aggravate seed deficiency and this may increase the risk of time-delayed declines of species that rely on regular regeneration by seed, or even cause their extinction ('extinction debt' *sensu* Tilman et al. 1994). Our observational study in nearby plots in which some grass species have seed-limited populations due to their late phenology of seed maturation and early cutting, showed that declines in relative abundance of species over a period of 4 yr after extremely dry summers in three sequential years were related with poor recruitment from seed (Stampfli & Zeiter 2004).

Under specific circumstances negative drought effects on species richness via seed deficiency may be balanced or overcompensated by positive effects on subsequent seed germination and establishment via a temporary release from light limitation. However, positive effects on light may be offset by greater losses in soil moisture, creating a stressful environment for seedling establishment and growth (Suding & Goldberg 2001). Apparently, environmental conditions and processes that determine establishment success and the reassembly of communities after drought are still poorly understood.

Flower diversity is also important for pollinator diversity (Fründ et al. 2010). Negative drought effects on flowering performance might cause cascading effects on pollinators. As pollinator diversity and abundance can influence the seed set of plants (Fontaine et al. 2006), drought effects on flowering could result in a negative feedback on the plant species, which rely on continuous regeneration by seed to maintain their populations.

Conclusion

Dry spells in late summer and in spring reduced community-level flower and seed production in the species-rich semi-natural grassland, and the seasonal timing of the drought had distinct effects on graminoids and forbs and on single species mediated by their rooting depth. Increased frequency of dry spells related to climate warming can aggravate seed deficiency and potentially reduce species diversity in semi-natural grassland, with possible cascading effects on species of other trophic levels and on ecosystem functioning.

Acknowledgements

This work was supported by the Swiss National Science Foundation (grant nos. 31003A-100612, 31003A-112639, 310030-149862). The Stiftung zur Förderung der Wissenschaftlichen Forschung an der Universität Bern provided technical equipment. The study was supported by the TRY initiative on plant traits (http://www.try-db.org). The TRY initiative and database is hosted, developed and maintained by J. Kattge and G. Bönisch (Max Planck Institute for Biogeochemistry, Jena, DE). TRY is currently supported by DIVERSITAS/Future Earth and the German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig. We thank M. Zimmermann for constructing the seed traps and K. Mackie-Haas and two anonymous reviewers for their helpful comments on an earlier version of the manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Design showing one out of seven blocks.

Appendix S2. Species-level effects of droughts in late summer 2004 and spring 2005 on reproductive shoot density.

Appendix S3. Species-level effects of droughts in late summer 2004 and spring 2005 on seed rain.

Appendix S4. Relationship between species frequency and reproductive-shoot density of the most abundant species.