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Risk of short-term biodiversity loss under more persistent precipitation regimes

Running title: A novel dimension of rainfall variability

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1. Abstract

Recent findings indicate that atmospheric warming increases the persistence of weather patterns in the mid-latitudes, resulting in sequences of longer dry and wet periods compared to historic averages. The alternation of progressively longer dry and wet extremes could increasingly select for species with a broad environmental tolerance. As a consequence, biodiversity may decline. Here, we explore the relationship between the persistence of summer precipitation regimes and plant diversity by subjecting experimental grassland mesocosms to a gradient of dry-wet alternation frequencies whilst keeping the total precipitation constant. The gradient varied the duration of consecutive wet and dry periods, from 1 up to 60 days with or without precipitation, over a total of 120 days. An alternation of longer dry and wet spells led to a severe loss of species richness (up to -75% relative to the current rainfall pattern in W-Europe) and functional diversity (enhanced dominance of grasses relative to nitrogen (N)-fixers and non-N-fixing forbs). Loss of N-fixers and non-N-fixing forbs in severe treatments was linked to lower baseline competitive success and higher physiological sensitivity to changes in soil moisture compared to grasses. The extent of diversity losses also strongly depended on the timing of the dry and wet periods. Regimes in which long droughts (≥ 20 days) coincided with above-average temperatures showed significantly more physiological plant stress over the experimental period, greater plant mortality, and impoverished communities by the end of the season. Across all regimes, the duration of the longest period below permanent wilting point was an accurate predictor of mortality across the communities, indicating that increasingly persistent precipitation regimes may reduce opportunities for drought stress alleviation. We conclude that without recruitment, which was precluded in this experiment, summer precipitation regimes with longer dry and wet spells will likely diminish plant diversity, at least in the short term.

Keywords: climate change, biodiversity loss, gradient design, grassland diversity, weather persistence, precipitation regimes.

2. Introduction

Global climate change is causing more persistent weather in the mid-latitudes (Coumou et al., 2018; Francis et al., 2020; Pfleiderer et al., 2019). Amplified arctic warming reduces the temperature difference with the equator, resulting in more sluggish circulation patterns and a 'wavier' jet stream (Coumou et al., 2018; Mann et al., 2017). With more stationary weather zones, predominant precipitation patterns are shifting towards sequences of increasingly longer dry and wet spells in the mid-latitudes (Francis & Skific, 2015; van Oldenborgh et al., 2016; Zolina et al., 2013). Single weather extremes such as prolonged dry or wet periods can have profound effects on plant communities and ecosystem functioning (De Boeck et al., 2018b; Grant et al., 2014), and so can altered precipitation regimes (Gherardi & Sala, 2015b; Knapp et al., 2002). Regimes studied so far were characterized by changes in inter- or intra-annual precipitation amounts or more intense peak rainfall interspersed with longer droughts (Fig. 1a) as predicted by global climate models (Felton et al., 2020; Gherardi & Sala, 2015a; Knapp et al., 2002; Suttle et al., 2007). However, the ecological impact of the newly emerging trend of more persistent dry and rainy weather as driven by arctic amplification (sequences of longer blocks of alternating dry and wet periods, Fig. 1b) has barely been studied so far. Consequences for biodiversity are of particular importance, not only because biodiversity loss can lead to declines in ecosystem functioning and services (Isbell et al., 2015b; Kreyling et al., 2017; Rani et al., 2020), but also because diversity can modulate ecosystem responses to climate extremes (Isbell et al., 2015a; Van Ruijven & Berendse, 2010).

Previous studies of altered precipitation regimes have found positive (Gherardi & Sala, 2015a; Knapp et al., 2002), negative (Harrison et al., 2015; Smith et al., 2016; Suttle et al., 2007) or

no effects (Collins et al., 2012; Heisler-White et al., 2009) on biodiversity. In general, the responses are orchestrated by the combination of species traits (e.g. differences in phenology or rooting depth), changing species interactions (e.g. shifts between facilitation and competition) and environmental factors such as temperature, water and nutrient availability (De Boeck et al., 2018a; Grant et al., 2014; Knapp et al., 2002; Pérez-Camacho et al., 2012; Suttle et al., 2007; Winkler et al., 2019; Yang et al., 2011). In mesic grasslands, for example, anomalous reductions in soil water availability can lead to rapid and far-reaching impoverishment of plant diversity through increased dominance of rapidly regenerating grasses at the expense of forbs (De Boeck et al., 2016; Dreesen et al., 2014; Elst et al., 2017; Harrison et al., 2015). Increasing weather persistence may induce novel phenotypic responses by imposing prolonged wet periods in addition to prolonged droughts (Francis & Skific, 2015). Such opposing extremes may have contrasting effects and can favor different species, because of, for example, trade-offs between resistance versus recovery (Cheng et al., 2011; Grant et al., 2014; Hoover et al., 2014). Although temporal variation in weather conditions can help sustain diversity (Adler et al., 2006; Floder et al., 2002; Knapp et al., 2002; Shriver, 2017), evolutionary trade-offs make it highly unlikely that species are well adapted to contrasting extreme environments (Ferenci, 2016; Suding et al., 2003). Opposite adjustments seem needed to withstand longer droughts versus longer wet periods (e.g. deep roots during drought compared to surface roots under waterlogging), both in individual plants (through phenotypic plasticity; Moreno and Bertiller (2015)) and at the community level (through reorganization of species composition; Grant et al. (2014)), which may compromise resistance when such contrasting periods alternately displace the system (the plant or the community) in the opposite direction (Lipiec et al., 2013; Parent et al., 2008; Smith et al., 2016). Eventually, impoverished communities composed mainly of generalist species with a very broad environmental tolerance may be left, leading to ecosystem collapse.

In the current study, we experimentally investigated the consequences of increasingly persistent rainfall patterns on the species richness and composition of temperate grassland mesocosms. Grasslands are ideal model ecosystems for such climate change experiments, because of their high biodiversity, short stature and generally short life cycles of species. Mesocosm studies eliminate much of the natural environmental noise, facilitating identification of otherwise obscured causal relationships between interacting factors (Teuben & Verhoef, 1992). However, they cannot entirely reflect heterogeneous natural landscapes because their soils often have unrepresentative seedbanks and they lack surface topography. For these reasons, patterns of recruitment may be unrealistic, and thus we precluded (re)colonization altogether by weeding. Our goals were (1) to determine if established plant communities respond negatively to an increase in the persistence of dry and wet spells and (2) to disentangle the mechanisms driving the observed responses.

We explored this new dimension (persistence) of changing precipitation variability utilizing a replicated gradient design as suggested by Kreyling et al. (2018) to allow for detection of non-linear responses and tipping points, which improves mechanistic understanding of ecological responses to environmental drivers, ecosystem resilience and acclimation (Beier et al., 2012; Cottingham et al., 2005; Kreyling et al., 2018). This gradient varied the duration of consecutive dry and wet periods while keeping the total precipitation constant over a period of 120 days. Treatments included eight levels ranging from 1 to 60 consecutive days with or without precipitation, starting either with a dry or wet period. Our overarching hypothesis was that more persistent precipitation regimes rapidly induce significant diversity reductions within established plant communities, because adaptations to either extremely dry or extremely wet conditions mutually exclude each other, favoring species with a broad environmental tolerance.

3. Materials and Methods

3.1. Study site and experimental set-up

This experiment was conducted at the Drie Eiken Campus of the University of Antwerp in northern Belgium (51°09'41''N, 04°24'9''E). Belgium has a temperate oceanic climate characterized by mild winters and cool summers, with an average air temperature of 10.6 °C and mean annual precipitation of 848 mm (Royal Meteorological Institute of Belgium, 2019).

We assembled 256 identical grassland mesocosms in grey PVC containers (29.5 cm inner diameter and 50 cm depth), and distributed them across eight experimental units (plots), with 32 mesocosms per unit (Fig. S1). Each unit was a plastic, watertight basin of 3 m diameter and 70 cm depth, dug into the soil with the top level at the height of the surrounding ground surface to avoid overheating of the containers, and an automated rain screen overhead that blocked out natural precipitation. The screen closed only during rain events (6-7% of the time in the local climate), so the mesocosms below were fully exposed to ambient conditions most of the time, avoiding shelter artifacts. To allow free drainage, the containers had holes in the bottom lids and were placed on a metal frame 20 cm off the ground. Lining with anti-root canvas prevented root growth through the lids. The distance between containers was minimized and horizontal wooden plates, level with the surface, covered the empty space between containers and the edges of the basin to further avoid radiation hitting the sides of the containers. Containers were filled with sieved (mesh size: 3.5 cm) sandy-loam soil with pH 3.5, 85.5% dry matter (DM), 0.13% N, 2.1% C, 119 mg P kg⁻¹ DM (=total P), 19.4 mg P kg⁻¹ DM (=available P, P-olsen method), collected at 10-60 cm depth from a nature conservation area close to Antwerp, Belgium. On 29 May 2019, all mesocosms were limed with 100 g m⁻² dissolved DCM Groenkalk® (15% MgO, 34% CaO, which modified and stabilized the pH of the top 15 cm soil to between 7.0 and 7.3 four weeks later), and inoculated with a soil biotic community by adding 6.4 mm of top soil from a nearby species rich grassland. The field capacity of the soil was 0.26

$\text{m}^3 \text{ m}^{-3}$ (pF = 2.0) and the permanent wilting point $0.05 \text{ m}^3 \text{ m}^{-3}$ (pF = 4.2) (Bodemkundige Dienst van België, Leuven, Belgium).

Per mesocosm, we planted three individuals of each of 12 common perennial temperate grassland species (36 plants in every mesocosm). These C3 species share a preference for sandy or sandy-loam soil, and covered three functional groups: six grasses (*Agrostis capillaris* L. (AC), *Anthoxanthum odoratum* L. (AO), *Deschampsia cespitosa* (L.) P. Beauv. (DC), *Phleum pratense* L. (PHP), *Poa pratensis* L. (POP), *Holcus lanatus* L. (HL)); three N-fixing forbs (*Lotus corniculatus* L. (LC), *Trifolium pratense* L. (TP), *Trifolium medium* L. (TM)); and three non-N-fixing forbs (*Centaurea jacea* L. (CJ), *Lychnis flos-cuculi* L. (SF), *Plantago lanceolata* L. (PL); referred to as forbs from here on). Species were selected utilizing Ellenberg's moisture and nutrient indices to represent a broad gradient in plant functional traits, with the goal to detect possible trends in the selective pressure exerted by the contrasting extreme events as dry and wet periods would in principle favor different traits. Individuals were planted equidistantly in a grid at 4.5 cm interspace, reflecting spacing *in natura*, and organized to maximize interspecific interactions by avoiding clumping (Fig. S1a). Species positions and north-south orientation of the community were identical in all containers. Seeds were first sown in small seedling containers early April 2019 and transplanted into the mesocosms in the first week of May 2019. Young individuals that died during settlement were replaced until the start of the experiment. Since the mesocosm soil originated from an open forest with grassy undergrowth, the seedbank was unknown and could potentially contain different (non-grassland) species. Therefore, once transplanted, no biocides or fertilizers were added but manual weeding was performed throughout the experiment to prevent non-native colonization. Over the course of the experiment, little weeding was required, indicating limited overall (re)colonization. All mesocosms were equally watered every few days after transplanting to ensure similar starting

points of soil water content (around field capacity) at the start of the experiment (Elst et al., 2017).

Mesocosms were subjected to a gradient ranging from low to highly persistent precipitation regimes (8 levels) during 120 days, from 2 July 2019 to 28 October 2019, either starting with a wet (blue) or dry (brown) period (Fig. 1b, Fig. S1a). Within each regime, dry periods (no irrigation events) alternated with wet periods (one irrigation event on every day) of the same length. The persistency of wet and dry spells was expressed in blocking days, i.e. the number of consecutive days with or without irrigation: 1, 3, 6, 10, 15, 20, 30 and 60 days. The 1-day dry-wet alternation is historically most commonly observed throughout the year and very close to Belgian weather (KMI, 2019a). Irrigation was applied through drippers and programmed to automatically supply water at a fixed time on each irrigation day (between 10:30 and 10:48 a.m.) throughout the entire experimental period. The water volume given during such an irrigation event was constant for all treatments on each irrigation day at 6.87 L/m². This is 1.5 times the average daily Belgian rainfall to compensate for extra evapotranspiration caused by air circulation and higher temperatures around the containers compared to a natural field situation. Compensation for artificially increased evapotranspiration because of design artefacts has been applied in previous experiments (De Boeck et al., 2006). The number of irrigation events and total precipitation during the 120 days of the experiment was the same in all regimes. For practical reasons of irrigation supply, four replicates of all low (1, 3, 6, 10) or high (15, 20, 30, 60) persistence treatments were grouped per plot, but their relative positions were haphazardly assigned across all eight plots to account for possible edge effects (Fig. S1a). This resulted in a total of 16 replicate mesocosms per treatment, equally distributed across four plots.

3.2. Measurements and calculations

Hourly weather records from a nearby weather station in Antwerp (51°13'19"N, 4°27'34"E) were utilized to report average monthly temperatures and vapor pressure deficit (VPD), the latter during daylight hours as a more meaningful measure of air humidity for plant functioning. Average volumetric soil water content (SWC) over 30 cm depth and soil surface temperature were logged automatically every half-hour by a CS650-DS Reflectometer (Campbell® Scientific INC., Logan, Utah, USA) in two to four mesocosms per regime due to practical constraints. For each regime, we calculated the seasonal mean and variability (coefficient of variation, CV) in soil water content of the pooled daily averages. Additionally, for each SWC time series, the total and maximum number of consecutive days below permanent wilting point (of the soil) was calculated utilizing the HeatwaveR package (Schlegel & Smit, 2018).

Three randomly selected replicates per regime (one edge and two center mesocosms, corresponding with the ratio between these positions across all containers) were tracked over the experimental period. In these mesocosms, percentage green cover of the community (entire mesocosm) was estimated to the closest 5% every week as a visual indicator of plant stress, senescence and leaf growth (De Boeck et al., 2016). Estimates were performed by the same observer throughout the season without looking at previous data. At the species level, we recorded greenness and mortality of all individuals in these mesocosms twice a month. The greenness of an individual plant was estimated viewed both from the top and laterally, likewise to the nearest 5%, and combined to get a better overall estimate. For mortality, individual plants were considered dead when they were either completely brown (<2.5% greenness) or had disappeared. Actual mortality might be lower since completely brown plants (especially grasses) can sometimes still recover (Elst et al., 2017; Van Peer et al., 2004). To estimate physiological plant stress, we measured chlorophyll fluorescence with the Plant Efficiency Analyser (Hansatech Ltd, King's Lynn, UK) as an indicator of photosystem II efficiency by

determining the F_v/F_m ratio twice a month on one randomly selected mature green leaf per species in each of the three replicate mesocosm. During each measurement campaign, the same mesocosms were measured at approximately the same time of day (between 9 a.m. and 5 p.m.), but different replicates of the same treatment were measured at different times. Leaves were dark adapted for 30 minutes prior to measurement. When all leaves were brown, F_v/F_m was considered to be zero. Average F_v/F_m values per mesocosm were utilized to express community stress. We also calculated average values of green cover and F_v/F_m over the duration of the experiment per treatment on both community (weighted by the starting densities of all individuals) and species level.

At the end of the experimental period, we recorded mortality of all individuals in 12 out of 16 replicates for each treatment over a period of four days. The resulting presence/absence data were utilized to calculate species richness (R), Shannon-Wiener's diversity index (H), Simpson's diversity index (1- D) and Pielou's evenness index (J) per container with the R package Vegan (p_i , the relative frequency of species i ; S , the total number of species in the community)(Oksanen et al., 2013).

$$H = -\sum_i^S p_i \ln p_i \quad (\text{Eqn 1})$$

$$1 - D = 1 - \sum p_i^2 \quad (\text{Eqn 2})$$

$$J = \frac{H}{\ln S} \quad (\text{Eqn 3})$$

3.3. Statistical analysis

All statistics were performed in R version 3.6.1(R Core Team, 2019). Significance was assumed for p-values below 0.05 and models were optimized through stepwise simplification and comparisons of goodness of fit. Residual checks were utilized to verify underlying model

assumptions (Zuur & Ieno, 2016). All graphs were constructed with the packages dplyr (Wickham et al., 2015) and ggplot2 (Wickham, 2016).

To test if responses in seasonal mean and CV of soil water content to increasingly persistent precipitation regimes exhibited predominantly linear or non-linear relationships, we first determined which least squares regression model best fits our observations. Because of the observed non-linearity, we then constructed Generalized Additive Mixed Models (GAMM's) to investigate the nature of the relationships between blocking duration and end-of-season species diversity (separate models for R, H, 1-D or J) with the package mgcv (Wood & Wood, 2015). Problems of residual autocorrelation (spatial) were resolved by implementing an appropriate ARIMA model, utilizing the package forecast (Hyndman et al., 2020). Blocking duration and dry or wet start (DW) were fixed effects and plot a random effect. For all GAMM models, differences between interaction smoothers were further explored following Rose et al. (2012). To verify our findings, we constructed four generalized linear mixed models (GLMM's) and applied Analysis of Variance (ANOVA) to evaluate the effects of individual treatments (DW $\times$ blocking duration) on the four diversity indices utilizing the package nlme (Pinheiro et al., 2017), again with plot as a random effect. In these models, we corrected for the observed heteroscedasticity by adapting the variance structure to account for increasing variance with increasingly persistent dry/wet spells. Tukey's HSD post-hoc analysis of significant ANOVA effects was utilized to further explore two-by-two differences between regimes.

Next, we tested for differences in survival responses between plant functional groups (grasses, forbs, N-fixers) by constructing a GAMM (family: binomial, link-function: logit) with survival as the response variable, functional group with dry or wet start as fixed factor and mesocosm nested in plot as random block. A final (GAMM (family: binomial, link function: logit) was constructed to test for differences in survival between individual species, including species

with dry or wet start as fixed factor and mesocosm nested in plot as random block (Pedersen et al., 2018). We then determined two factors to explain differences in individual species survival: baseline competitive success and sensitivity to stress. The observed end of season differences in survival between species for treatments that induced little or no observed stress (1D, 1W, 3D, 3W, 6D, 6W) were assumed to be the result of ‘baseline competitive success’. Here we define baseline competitive success as the ability of the plant to survive within an evolving plant community. Hence, baseline competitive success includes both influences of the (abiotic) growing environment (e.g. nutrient availability) and competition with others on survival. We also constructed a linear model for each species testing species specific end-of-season survival responses to seasonal means of the stress indicator F_v/F_m . The resulting ‘sensitivity to stress’ is defined as the steepness of slope between seasonal means of F_v/F_m and end-of-season survival. Species that maintain survival better as F_v/F_m declines, are considered less sensitive to stress and vice versa.

Finally, underlying mechanisms for the observed patterns were further explored. At the community level, we performed multiple linear regressions to determine the nature and strength of relationships between Shannon diversity or community survival and averages of stress over the experimental period (F_v/F_m , green cover). We also tested the direct effects of SWC on community F_v/F_m for each measurement day (excluding completely brown plants as they no longer respond to changes). Additionally, we investigated the relationships between total survival (community) and soil water availability (mean, CV and days below permanent wilting point). To address significant diversity differences between dry and wet start treatments with the same persistency of dry and wet spells, differences in soil surface temperature, SWC, F_v/F_m and survival between consecutive months were explored by performing non-parametric one-way Kruskal-Wallis tests followed by post-hoc Dunn’s tests with Bonferroni correction for two-by-two comparisons. These models were supplemented with a final GLMM with binomial

error testing for differences in end of month survival between months and treatments with treatment and month as fixed factors and mesocosm nested in plot as random effect.

4. Results

4.1. Patterns in temperature and soil water content

The summer of 2019 was anomalously hot with two heatwaves in the second half of both July and August, and record-breaking daily maximum temperatures above 40 °C (99th percentile) in July (KMI, 2019b). During the experimental period, monthly average air temperatures were comparable but above long term means in July and August and declined thereafter, accompanied by increasing relative humidity and decreasing daytime vapor pressure deficit (Table 1). These differences between months are instrumental in explaining the results.

Throughout the experimental period, temporal patterns of soil water content were strongly influenced by differences in dry/wet spell persistency and air temperature (Fig. 2). During heatwaves, we found evidence of increased evapotranspiration due to high temperatures, as average daily SWC values dropped notably for most treatments (Fig. 2). Temperature and SWC effects were most pronounced at high persistence (Fig. 2; Fig. S2a). During July and August, monthly mean soil surface temperatures were significantly higher in 30-day and 60-day persistence treatments experiencing a drought ($p < 0.001$; Fig. S2a). Additionally, differences in mean soil moisture during drought were observed at 30 days blocking. Here, monthly mean SWC was significantly lower for 30W in August compared to 30D in July ($p < 0.001$; Fig. S2b), despite experiencing the same precipitation regime during these respective periods. Soil water variability responses to increasing weather persistence were best described by a second-degree polynomial, indicating a non-linear trend (Fig. S3). Across the entire experimental period (120 days), daily averages of soil moisture content were less variable in high persistence treatments (Fig. S3; adjusted $R^2 = 0.44$, $p = 0.009$). No clear patterns were found in mean SWC

differences between treatments over the experimental period. Screen malfunctioning caused unexpected rain events to influence SWC in all treatments twice during the experiment, once on the 12th of July (up to 5% increase) and a second time on the 2nd of October (up to 2% increase).

4.2. Patterns in diversity

Increasingly persistent precipitation regimes caused a collapse of plant diversity, starting from a blocking duration of 20 days and progressively accelerating in treatments with a dry start (Fig. 3; Tables S4 and S5). This was mainly driven by species richness, which suffered greater losses than species evenness (Tables S4 and S5). We did not observe significant differences between treatments until 20 days blocking duration for any of the indices (Tables S6, S7, S8 and S9). Persistence and starting type (dry or wet) interacted strongly at 30 and 60 days blocking for all indices, except for species evenness (only at 60 days) (Fig. 3; Tables S6, S7, S8 and S9). For species richness and diversity, losses were significantly greater in 30W and 60D compared to 30D and 60W respectively (Fig. 3; Tables S6, S7 and S9). In spite of reduced species richness in 60W compared to 1D/W, no declines of species diversity or evenness were observed in this treatment (Tables S6, S7, S8 and S9).

Which species or functional groups governed the diversity losses and disappeared from the communities? Survival responses to increasing climate persistence differed between functional groups (grass, forb, N-fixer), species and dry or wet start (Fig. 4 and S11). Overall, grasses were least responsive, and maintained significantly higher survival compared with forbs and N-fixers at high blocking durations (Fig. 4). Despite overlap in confidence intervals, mean survival was consistently higher in forbs compared to N-fixers. All functional groups showed variation in mean survival between starting type (D or W) at 30 and 60 days blocking (cfr. diversity responses) but survival was only significantly lower for forbs in 60D compared to 60W (Fig. 4). A similar trend was observed in the individual species. Although some variation

in mean starting type (D or W) trend was visible at high persistence for most species (with the exception of AC, PHP and SF), only HL and PL showed significantly lower survival in 60D compared to 60W (Fig. S11). By the end of the season, all N-fixers, as well as most forbs (with the exception of PL), had disappeared in the 60D treatment (Fig. 4 and S11). At 30 days blocking, mean survival was found to be lower (albeit not significantly) in 30W compared to 30D for all species except AO, PHP, AC, PL and SF (Fig. S11).

4.3. Underlying mechanisms

The diversity losses were driven by diverging responses of the species (functional groups) to increasingly persistent precipitation regimes (Fig. 4, S11 and S12). The species varied both in their baseline competitive success (end-of-season survival in low stress treatments) and in their sensitivity to stress (slope of declining survival with increasing stress F_v/F_m) (Fig. S12). In general, grasses had the highest baseline competitive success, followed by forbs and N-fixers (Fig. S12a). Forbs were found to be the most stress sensitive species, followed by N-fixers and grasses (Fig. S12b). This explains the observed shift in community composition towards grasses in more persistent precipitation regimes. Community survival responses to increasingly persistent weather were similar to patterns in diversity (not shown). Treatments that caused more stress over the experimental period (seasonal averages) reduced both community survival and diversity by the end of the season (adjusted $R^2 = 0.93 - 0.96$, $p < 0.001$; Fig. 5 and S15). Generally, these high stress levels were caused by the more persistent treatments. However, differences were found between D and W regimes at blocking durations of 30 and 60 days (Fig. 5). At 30 and 60 days blocking duration, monthly F_v/F_m was found to be significantly lower (higher average physiological stress) during the first dry period of 30W and 60D when compared to 30D and 60W respectively (Fig. S2c, $p < 0.001$), resulting in a lower seasonal mean of F_v/F_m .

Why were plants more stressed as precipitation regimes became more persistent? Overall, soil water was pushed further below the PWP in highly persistent treatments, inducing more stress (adjusted $R^2 = 0.63$, $p < 0.001$; Fig. 6a). Higher soil water variability across the season positively influenced community survival (and therefore species diversity), except in 30W (adjusted $R^2 = 0.43$, $p = 0.010$, Fig. S16). Treatments with more days below PWP across the season had lower community survival (adjusted $R^2 = 0.72$, $p < 0.001$; Fig. 6b). However, the length of the longest consecutive period below PWP was found to be the best predictor of community survival, also allowing detection of differences between treatments of the same persistence (adjusted $R^2 = 0.94$, $p < 0.001$; Fig 6c). No significant differences were found in end of month survival between 30W/60W and 30D/60D respectively, but seasonal trends are present (Fig S2d).

5. Discussion

More persistent precipitation regimes featuring longer dry and wet spells greatly impoverished our grassland communities in the short term. They eliminated many of the forbs and N-fixers and shifted the community to a grass-dominated state. These outcomes could be traced to the length of the period during which soil water was below the wilting point, which was generally longer when persistence was high, in turn invoking more physiological stress and ultimately mortality (Fig. 5, 6 and S2). Reduced water availability also lowered the ability of the ecosystem to buffer against extreme temperatures, further aggravating community stress and mortality during hot periods (Fig. 2 and S2). These findings are in line with previous studies, suggesting soil moisture as the main determinant of ecosystem responses to altered rainfall patterns (Felton et al., 2019; Felton et al., 2020; Heisler-White et al., 2009; Jones et al., 2016; Knapp et al., 2002; Suttle et al., 2007). In particular, the length and timing of the dry intervals appear especially important as they relate to more persistent precipitation regimes (Fig. 6).

Throughout the experiment, no signs of waterlogging related stress were found during wet periods, suggesting that the observed patterns resulted from plant responses to drought and temperature. The free drainage of our mesocosms, may in principle have prevented soils from saturating with water, resulting in the absence of waterlogging related stressors (e.g. root hypoxia). Nevertheless, little to no water leaching was observed during periods with high plant mortality and SWC was almost never above field capacity, likely because evapotranspiration was similar to total precipitation, especially in July and August. Although the severity of response is highly ecosystem-dependent, other studies have also shown that ecosystem responses to increasing rainfall variability are often most pronounced during dry periods, with limited effects of increased precipitation on diversity in temperate regions (Collins et al., 2012; De Boeck et al., 2018a; Felton et al., 2020; Hallett et al., 2014; Knapp et al., 2015; Knapp et al., 2002; Pérez-Ramos et al., 2017; Suttle et al., 2007).

5.1. Species responses

The unidirectional loss of species (i.e. towards dominance of grasses) coincides with previous observations of grassland responses to drought (De Boeck et al., 2016; Elst et al., 2017; Hoover et al., 2014; Liu et al., 2018; Pfisterer & Schmid, 2002). This consistency across treatments, despite seasonal variation in the timing of prolonged drought or precipitation, may in part be attributed to the lack of extremely wet soil conditions. Fast-growing species with high water usage would be negatively affected during drought (Elst et al., 2017; Van Peer et al., 2004) but should recover faster once drought stress is alleviated compared to more conservative plants, on the condition they survived the drought extreme. On the other hand, slow-growing species should be affected less by reductions in available soil water (at least initially) since their conservative strategies allow normal growth in more resource-poor environments. If, as suggested here, drought is the main driver of initial plant diversity losses to increasingly

persistent precipitation, it would be expected that the remaining species are either best adapted to cope with dry soil conditions and/or succeeded best in recovering after drought.

The remnant grasses in high persistence treatments are typically very productive, comparatively more deep-rooting and have basal meristems (Elst et al., 2017; Hernandez & Picon-Cochard, 2016; Van Peer et al., 2004). As part of their drought avoidance strategy, they substantially reduce transpiration under reduced water availability through rapid leaf senescence (Munne-Bosch & Alegre, 2004). These traits allow access to water reserves in lower soil layers, prolonged survival during drought and quick regeneration when conditions become favorable again (Elst et al., 2017). In comparison, the lower survival of non-N-fixing forbs at high persistence could be ascribed to their preference for humid soil conditions (excluding PL which had a higher survival) and proportionally lower root densities compared to grasses under drought at depths > 30 cm (Skinner & Comas, 2010). All N-fixers in this experiment are slow growers, especially when competing with grasses (Hernandez & Picon-Cochard, 2016), and remain relatively small (< 45 cm) throughout their lifespan. These traits may have led to relatively low baseline competitive success (Fig. S12a) and could in combination with potentially high drought sensitivity (Fig. S12b) negatively affect their growth at high weather persistence.

Apart from species-specific traits, species interactions may further explain the loss of functional groups. Previous studies have indicated that species interactions in grassland communities may shift from facilitation to competition under increasing temperature and precipitation extremes (Bertness & Callaway, 1994; Grant et al., 2014; Olsen et al., 2016). Fast-growing dominant species with high water usage may for example limit the benefits of slow-growing conservative plant strategies during drought by rapidly consuming the remnant soil water and aggravating drought stress, especially when these conservative species lack drought tolerance. Such effects would be most pronounced in highly persistent treatments where

precipitation is most unevenly distributed across the season, increasing the chance of more and longer drought (and heat) related stresses. Through these mechanisms, slow-growing non-N-fixing-forbs and N-fixers with low drought resistance may be rapidly outcompeted by tall-growing, vigorously regenerating species when subjected to extreme drought, especially in young grassland communities (Dreesen et al., 2015; Elst et al., 2017; Grant et al., 2014).

In contrast with Elst et al. (2017), Ellenberg's indices poorly explained the interspecific differences in survival. Only SF, common in wet temperate grasslands and thus assumed to be drought sensitive, clearly survived less well onwards from about 15 days blocking duration, independent of starting type (Fig. S11). Also in other studies, qualitative functional traits often had limited utility in predicting responses to climate change (Collins et al., 2012). However, differences in survival could be explained by the combination of baseline competitive success and sensitivity to stress. Species with either low baseline competitive success (TM, TP) and/or high sensitivity to stress (SF, TP, HL, LC) suffered the highest mortality in the extreme regimes by the end of the season (Fig. S11).

5.2. Community responses

In highly persistent precipitation regimes, the timing of droughts significantly influenced how much diversity was lost by the end of the season. This highlights the importance of the timing of climate extremes in light of global change (Craine et al., 2012; De Boeck et al., 2011). Differences between 30W and 30D were likely caused by contrasts in community development during the first drought, since total water supply, air temperature and VPD were comparable in the most influential period (July-August; Table 1). Because of a more developed community with higher water usage (Wang et al., 2007), SWC was lower in 30W before and during the first dry period (August) and longer below PWP compared to 30D in July (Fig. 2). This induced more physiological plant stress which resulted in higher community mortality the following month (Fig. 2, 5 and S2). Comparatively, the observed differences between 60W and 60D were

likely related to seasonal effects in addition to community development. The combination of low temperatures and senescence related to phenology reduce plant metabolic activity and resource uptake in autumn (Smakman & Hofstra, 1982). Therefore, the anomalously high end-of-season species diversity for 60W was likely due to a well-established deep-rooting plant community after August and the limited effect of drought on plant functioning at the end of the experimental period. However, an irrigation malfunction that pushed SWC temporarily above PWP around the 5th of October may have had an influence by relieving some of the drought stress.

As predicted by Pfleiderer et al. (2019), increased weather persistence substantially worsened the effects of summer heat extremes. From 20 days blocking onwards, species diversity was found to be significantly lower in most treatments (excluding 60W), and mortality was highest in months when a drought period coincided with a heatwave (Fig. S2d). The lack of differences between treatments at lower blocking durations (Tables S6, S7, S8, S9) also suggests that the studied grassland communities were relatively resistant to mild changes in the persistence of dry and wet spells. The observed diversity plateau at low persistence and the following accelerating decline may indicate an ecological threshold of which exceedance leads to rapid and far-reaching changes in plant community composition. Grassland communities may not respond negatively to small or moderate increases in the persistence of precipitation regimes where exceedance of critical soil moisture thresholds is rare and always of short duration (Fig. 6). Indeed, small temporal changes in weather conditions and increased soil water variability (as primarily seen in low persistence treatments; Fig. S16) can promote diversity and impede competitive exclusion (Adler et al., 2006; Floder et al., 2002; Knapp et al., 2002). In addition, while one day precipitation blocks are the most prevalent pattern in Belgium, longer blocks of variable duration are common (KMI, 2019a). Consequently, species assemblages are likely naturally adapted to a range of variable weather conditions, which in turn facilitates

coexistence. However, as rainfall persistence increases, SWC is more likely to be pushed below critical thresholds for longer (Fig. 6). This results in the rapid accumulation of stress tolerated by progressively less species and higher mortality, the longer the system remains in such a critical state.

In that regard, the differences between pulse (short period with no precipitation) and press (long term precipitation deficits) type drought events are pertinent. Pulse droughts lead to relatively short periods (weeks) of intense stress followed by complete stress alleviation upon extensive rewetting (De Boeck et al., 2018a; Dreesen et al., 2014; Hoover & Rogers, 2016; Zhou et al., 2016). Press events result in moderate drought stress for months or even years, with lower than normal precipitation, but with brief moments of recovery. Since both drought types affect plant communities and ecosystem resistance in different ways, this distinction may be of particular importance when ecosystems are subjected to multiple extremes (Anderegg et al., 2020). High predictability of plant mortality by the time spent below PWP (Fig. 6b,c), suggests that the period during which pulse droughts occur is the main determinant of their outcome (e.g. drought during summer and/or heatwaves resulted in longer periods below PWP and higher end-of-season mortality). In addition, these findings indicate that effects of press droughts may highly depend on the timing of ‘rain relief’ (drought rescue) (Felton et al., 2020). Diverse plant communities may be able to persist for prolonged times during press droughts, given they receive precipitation inputs before SWC dips below PWP for too long (e.g. 60W in September-October). In such instances, productivity would likely decline but transgression of ecological survival thresholds should be limited, resulting in little diversity loss.

Although (re)colonization was prevented in the current experiment, non-clonal colonization in dense grassland is usually limited during the high summer and autumn (Bullock et al., 1995), as also suggested by the limited amount of weeding necessary in our mesocosms. We therefore consider the rapid decline of diversity we observed a realistic short term effect of more

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persistent precipitation regimes. On the longer term, gap formation during drought and the potential ensuing empty niches would give opportunity to other species (or ecotypes) to establish, as already observed in a variety of ecosystems (Evans et al., 2011; Liao et al., 2015; Winkler et al., 2019). Yet, if the weather pattern persists, there is also a filter for the success of these colonizers since many seedlings require benign soil conditions (i.e. not too dry or wet) for prolonged times in order to settle (Wellstein, 2012). Eventually, the community could shift towards a new dynamic equilibrium, emerging from increased temporal niche partitioning (e.g. through diversification of species phenologies related to predictable timings of dry/wet periods) (Adler et al., 2006; Blackford et al., 2020; Shriver, 2017), the inclusion of the toughest long-lived generalists (K-species), and/or the ephemeral presence of different short-lived species (r-species) related to the current environmental conditions. Similarly dynamic plant compositions, reversely changing according to variations in environmental conditions can be seen throughout the phenological phases of many ecosystems (e.g. booming of short-cycle annuals in desert ecosystems after rain) (Aronson et al., 1992; Encinas-Viso et al., 2012; Sakai, 2001). However, reaching such a new dynamic equilibrium following far-reaching changes in precipitation regimes could be a lengthy process during which ecosystem services related to diversity are diminished, because ecosystem adaptation to environmental conditions outside of the phenotypic tolerance of most native species requires heterogenous landscapes with high (re)colonization rates, diverse seedbanks and/or many generations (Rowland et al., 2011; Walsworth et al., 2019).

6. Conclusion

In this study, we demonstrated that increasingly persistent precipitation regimes may have rapid and profound consequences for temperate grasslands by reducing plant diversity. In the biosphere, a non-random loss of functional plant diversity does not only influence plant communities, but also interactions between different trophic levels of the ecosystem which can

result in cascading effects (Suttle et al., 2007). Across all treatments, end-of-season community mortality could be accurately predicted by the length of the longest period below wilting point, integrating how persistency of dry/wet spells and timing of extreme events interact to determine severity of drought. Consequently, drought stress alleviation may be key to limit the adverse effects of more persistent summer precipitation regimes for plant communities and ecosystems as a whole.

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8. Conflict of interest

The contributing authors declare no conflict of interest.

9. References

- Adler, P. B., HilleRisLambers, J., Kyriakidis, P. C., Guan, Q. F., & Levine, J. M. (2006). Climate variability has a stabilizing effect on the coexistence of prairie grasses. *Proceedings of the National Academy of Sciences of the United States of America*, 103(34), 12793-12798. doi:10.1073/pnas.0600599103
- Anderegg, W. R. L., Trugman, A. T., Badgley, G., Konings, A. G., & Shaw, J. (2020). Divergent forest sensitivity to repeated extreme droughts. *Nature Climate Change*, 1-5. doi:10.1038/s41558-020-00919-1
- Aronson, J., Kigel, J., Shmida, A., & Klein, J. (1992). Adaptive phenology of desert and Mediterranean populations of annual plants grown with and without water stress. *Oecologia*, 89(1), 17-26. doi:10.1007/BF00319010
- Beier, C., Beierkuhnlein, C., Wohlgemuth, T., Penuelas, J., Emmett, B., Korner, C., . . . Hansen, K. (2012). Precipitation manipulation experiments--challenges and recommendations for the future. *Ecol Lett*, 15(8), 899-911. doi:10.1111/j.1461-0248.2012.01793.x
- Bertness, M. D., & Callaway, R. (1994). Positive interactions in communities. *Trends Ecol Evol*, 9(5), 191-193. doi:10.1016/0169-5347(94)90088-4
- Blackford, C., Germain, R. M., & Gilbert, B. (2020). Species Differences in Phenology Shape Coexistence. *Am Nat*, 195(6), E168-E180. doi:10.1086/708719
- Bullock, J. M., Hill, B. C., Silvertown, J., & Sutton, M. (1995). Gap Colonization as a Source of Grassland Community Change - Effects of Gap Size and Grazing on the Rate and

Mode of Colonization by Different Species. *Oikos*, 72(2), 273-282.

doi:10.2307/3546229

Cheng, Y., Tsubo, M., Ito, T. Y., Nishihara, E., & Shinoda, M. (2011). Impact of rainfall variability and grazing pressure on plant diversity in Mongolian grasslands. *Journal of Arid Environments*, 75(5), 471-476. doi:10.1016/j.jaridenv.2010.12.019

Collins, S. L., Koerner, S. E., Plaut, J. A., Okie, J. G., Brese, D., Calabrese, L. B., . . .

Nonaka, E. (2012). Stability of tallgrass prairie during a 19-year increase in growing season precipitation. *Functional Ecology*, 26(6), 1450-1459. doi:10.1111/j.1365-2435.2012.01995.x

Cottingham, K. L., Lennon, J. T., & Brown, B. L. (2005). Knowing when to draw the line: designing more informative ecological experiments. *Frontiers in Ecology and the Environment*, 3(3), 145-152. doi:10.1890/1540-9295(2005)003[0145:Kwtdtl]2.0.Co;2

Coumou, D., Di Capua, G., Vavrus, S., Wang, L., & Wang, S. (2018). The influence of Arctic amplification on mid-latitude summer circulation. *Nat Commun*, 9(1), 2959. doi:10.1038/s41467-018-05256-8

Craine, J. M., Nippert, J. B., Elmore, A. J., Skibbe, A. M., Hutchinson, S. L., & Brunsell, N. A. (2012). Timing of climate variability and grassland productivity. *Proc Natl Acad Sci U S A*, 109(9), 3401-3405. doi:10.1073/pnas.1118438109

De Boeck, H. J., Bassin, S., Verlinden, M., Zeiter, M., & Hiltbrunner, E. (2016). Simulated heat waves affected alpine grassland only in combination with drought. *New Phytol*, 209(2), 531-541. doi:10.1111/nph.13601

De Boeck, H. J., Bloor, J. M. G., Kreyling, J., Ransijn, J. C. G., Nijs, I., Jentsch, A., . . .

Wardle, D. (2018a). Patterns and drivers of biodiversity-stability relationships under climate extremes. *Journal of Ecology*, *106*(3), 890-902. doi:10.1111/1365-2745.12897

De Boeck, H. J., Dreesen, F. E., Janssens, I. A., & Nijs, I. (2011). Whole-system responses of experimental plant communities to climate extremes imposed in different seasons. *New Phytol*, *189*(3), 806-817. doi:10.1111/j.1469-8137.2010.03515.x

De Boeck, H. J., Hiltbrunner, E., Verlinden, M., Bassin, S., & Zeiter, M. (2018b). Legacy Effects of Climate Extremes in Alpine Grassland. *Front Plant Sci*, *9*, 1586. doi:10.3389/fpls.2018.01586

De Boeck, H. J., Lemmens, C. M. H. M., Bossuyt, H., Malchair, S., Carnol, M., Merckx, R., . . . Ceulemans, R. (2006). How do climate warming and plant species richness affect water use in experimental grasslands? *Plant and soil*, *288*(1-2), 249-261. doi:10.1007/s11104-006-9112-5

Dreesen, F. E., De Boeck, H. J., Horemans, J. A., Janssens, I. A., & Nijs, I. (2015). Recovery dynamics and invasibility of herbaceous plant communities after exposure to experimental climate extremes. *Basic and Applied Ecology*, *16*(7), 583-591. doi:10.1016/j.baae.2015.05.002

Dreesen, F. E., De Boeck, H. J., Janssens, I. A., & Nijs, I. (2014). Do successive climate extremes weaken the resistance of plant communities? An experimental study using plant assemblages. *Biogeosciences*, *11*(1), 109-121. doi:10.5194/bg-11-109-2014

Elst, E. M., De Boeck, H. J., Vanmaele, L., Verlinden, M., Dhliwayo, P., & Nijs, I. (2017). Impact of climate extremes modulated by species characteristics and richness.

Perspectives in Plant Ecology Evolution and Systematics, 24, 80-92.

doi:10.1016/j.ppees.2016.12.007

Encinas-Viso, F., Revilla, T. A., & Etienne, R. S. (2012). Phenology drives mutualistic network structure and diversity. *Ecol Lett*, 15(3), 198-208. doi:10.1111/j.1461-0248.2011.01726.x

Evans, S. E., Byrne, K. M., Lauenroth, W. K., & Burke, I. C. (2011). Defining the limit to resistance in a drought-tolerant grassland: long-term severe drought significantly reduces the dominant species and increases ruderals. *Journal of Ecology*, 99(6), 1500-1507. doi:10.1111/j.1365-2745.2011.01864.x

Felton, A. J., Knapp, A. K., & Smith, M. D. (2019). Carbon exchange responses of a mesic grassland to an extreme gradient of precipitation. *Oecologia*, 189(3), 565-576. doi:10.1007/s00442-018-4284-2

Felton, A. J., Slette, I. J., Smith, M. D., & Knapp, A. K. (2020). Precipitation amount and event size interact to reduce ecosystem functioning during dry years in a mesic grassland. *Glob Chang Biol*, 26(2), 658-668. doi:10.1111/gcb.14789

Ferenci, T. (2016). Trade-off Mechanisms Shaping the Diversity of Bacteria. *Trends Microbiol*, 24(3), 209-223. doi:10.1016/j.tim.2015.11.009

Floder, S., Urabe, J., & Kawabata, Z. I. (2002). The influence of fluctuating light intensities on species composition and diversity of natural phytoplankton communities. *Oecologia*, 133(3), 395-401. doi:10.1007/s00442-002-1048-8

Francis, J. A., & Skific, N. (2015). Evidence linking rapid Arctic warming to mid-latitude weather patterns. *Philos Trans A Math Phys Eng Sci*, 373(2045), 20140170. doi:10.1098/rsta.2014.0170

Francis, J. A., Skific, N., & Vavrus, S. J. (2020). Increased persistence of large-scale circulation regimes over Asia in the era of amplified Arctic warming, past and future. *Sci Rep*, 10(1), 14953. doi:10.1038/s41598-020-71945-4

Gherardi, L. A., & Sala, O. E. (2015a). Enhanced interannual precipitation variability increases plant functional diversity that in turn ameliorates negative impact on productivity. *Ecol Lett*, 18(12), 1293-1300. doi:10.1111/ele.12523

Gherardi, L. A., & Sala, O. E. (2015b). Enhanced precipitation variability decreases grass- and increases shrub-productivity. *Proc Natl Acad Sci U S A*, 112(41), 12735-12740. doi:10.1073/pnas.1506433112

Grant, K., Kreyling, J., Heilmeyer, H., Beierkuhnlein, C., & Jentsch, A. (2014). Extreme weather events and plant-plant interactions: shifts between competition and facilitation among grassland species in the face of drought and heavy rainfall. *Ecological Research*, 29(5), 991-1001. doi:10.1007/s11284-014-1187-5

Hallett, L. M., Hsu, J. S., Cleland, E. E., Collins, S. L., Dickson, T. L., Farrer, E. C., . . . Suding, K. N. (2014). Biotic mechanisms of community stability shift along a precipitation gradient. *Ecology*, 95(6), 1693-1700. doi:10.1890/13-0895.1

Harrison, S., Gornish, E. S., & Copeland, S. (2015). Climate-driven diversity loss in a grassland community. *Proc Natl Acad Sci U S A*, 112(28), 8672-8677. doi:10.1073/pnas.1502074112

Heisler-White, J. L., Blair, J. M., Kelly, E. F., Harmoney, K., & Knapp, A. K. (2009).

Contingent productivity responses to more extreme rainfall regimes across a grassland biome. *Global Change Biology*, 15(12), 2894-2904. doi:10.1111/j.1365-2486.2009.01961.x

Hernandez, P., & Picon-Cochard, C. (2016). Presence of *Trifolium repens* Promotes

Complementarity of Water Use and N Facilitation in Diverse Grass Mixtures. *Front Plant Sci*, 7, 538. doi:10.3389/fpls.2016.00538

Hoover, D. L., Knapp, A. K., & Smith, M. D. (2014). Resistance and resilience of a grassland

ecosystem to climate extremes. *Ecology*, 95(9), 2646-2656. doi:Doi 10.1890/13-2186.1

Hoover, D. L., & Rogers, B. M. (2016). Not all droughts are created equal: the impacts of

interannual drought pattern and magnitude on grassland carbon cycling. *Global Change Biology*, 22(5), 1809-1820. doi:/10.1111/gcb.13161

Hyndman, R. J., Athanasopoulos, G., Bergmeir, C., Caceres, G., Chhay, L., O'Hara-Wild, M.,

. . . Wang, E. (2020). Package 'forecast'. Retrieved from

<https://github.com/robjhyndman/forecast>

Isbell, F., Craven, D., Connolly, J., Loreau, M., Schmid, B., Beierkuhnlein, C., . . .

Eisenhauer, N. (2015a). Biodiversity increases the resistance of ecosystem productivity to climate extremes. *Nature*, 526(7574), 574-577.

doi:10.1038/nature15374

Isbell, F., Tilman, D., Polasky, S., & Loreau, M. (2015b). The biodiversity-dependent

ecosystem service debt. *Ecol Lett*, 18(2), 119-134. doi:10.1111/ele.12393

Jones, S. K., Collins, S. L., Blair, J. M., Smith, M. D., & Knapp, A. K. (2016). Altered rainfall patterns increase forb abundance and richness in native tallgrass prairie. *Sci Rep*, 6, 20120. doi:10.1038/srep20120

KMI. (2019a). Klimatologisch jaaroverzicht, jaar 2019.

KMI. (2019b). Klimatologisch seizoenoverzicht, zomer 2019.

Knapp, A. K., Carroll, C. J. W., Denton, E. M., La Pierre, K. J., Collins, S. L., & Smith, M. D. (2015). Differential sensitivity to regional-scale drought in six central US grasslands. *Oecologia*, 177(4), 949-957. doi:10.1007/s00442-015-3233-6

Knapp, A. K., Fay, P. A., Blair, J. M., Collins, S. L., Smith, M. D., Carlisle, J. D., . . . McCarron, J. K. (2002). Rainfall variability, carbon cycling, and plant species diversity in a mesic grassland. *Science*, 298(5601), 2202-2205. doi:10.1126/science.1076347

Kreyling, J., Dengler, J., Walter, J., Velez, N., Ugurlu, E., Sopotlieva, D., . . . Jentsch, A. (2017). Species richness effects on grassland recovery from drought depend on community productivity in a multisite experiment. *Ecol Lett*, 20(11), 1405-1413. doi:10.1111/ele.12848

Kreyling, J., Schweiger, A. H., Bahn, M., Ineson, P., Migliavacca, M., Morel-Journel, T., . . . Larsen, K. S. (2018). To replicate, or not to replicate - that is the question: how to tackle nonlinear responses in ecological experiments. *Ecol Lett*, 21(11), 1629-1638. doi:10.1111/ele.13134

- Liao, J. B., Bogaert, J., & Nijs, I. (2015). Species interactions determine the spatial mortality patterns emerging in plant communities after extreme events. *Scientific reports*, 5, 11229. doi:10.1038/srep11229
- Lipiec, J., Doussan, C., Nosalewicz, A., & Kondracka, K. (2013). Effect of drought and heat stresses on plant growth and yield: a review. *International Agrophysics*, 27(4), 463-477. doi:10.2478/intag-2013-0017
- Liu, H., Mi, Z., Lin, L., Wang, Y., Zhang, Z., Zhang, F., . . . He, J. S. (2018). Shifting plant species composition in response to climate change stabilizes grassland primary production. *Proc Natl Acad Sci U S A*, 115(16), 4051-4056. doi:10.1073/pnas.1700299114
- Mann, M. E., Rahmstorf, S., Kornhuber, K., Steinman, B. A., Miller, S. K., & Coumou, D. (2017). Influence of Anthropogenic Climate Change on Planetary Wave Resonance and Extreme Weather Events. *Sci Rep*, 7, 45242. doi:10.1038/srep45242
- Moreno, L., & Bertiller, M. B. (2015). Phenotypic plasticity of morpho-chemical traits of perennial grasses from contrasting environments of arid Patagonia. *Journal of Arid Environments*, 116, 96-102. doi:10.1016/j.jaridenv.2015.02.007
- Munne-Bosch, S., & Alegre, L. (2004). Die and let live: leaf senescence contributes to plant survival under drought stress. *Funct Plant Biol*, 31(3), 203-216. doi:10.1071/FP03236
- Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'hara, R., . . . Wagner, H. (2013). Package 'vegan'. Retrieved from <https://github.com/vegandevs/vegan>
- Olsen, S. L., Topper, J. P., Skarpaas, O., Vandvik, V., & Klanderud, K. (2016). From facilitation to competition: temperature-driven shift in dominant plant interactions

affects population dynamics in seminatural grasslands. *Glob Chang Biol*, 22(5), 1915-1926. doi:10.1111/gcb.13241

Parent, C., Capelli, N., Berger, A., Crèvecoeur, M., & Dat, J. F. (2008). An overview of plant responses to soil waterlogging. *Plant stress*, 2(1), 20-27.

Pedersen, E. J., Miller, D. L., Simpson, G. L., & Ross, N. (2018). *Hierarchical generalized additive models: an introduction with mgcv*. *PeerJ*, 7:e6876. Retrieved from <https://doi.org/10.7717/peerj.6876>

Pérez-Camacho, L., Rebollo, S., Hernández-Santana, V., García-Salgado, G., Pavón-García, J., & Gómez-Sal, A. (2012). Plant functional trait responses to interannual rainfall variability, summer drought and seasonal grazing in Mediterranean herbaceous communities. *Functional Ecology*, 26(3), 740-749. doi:10.1111/j.1365-2435.2012.01967.x

Pérez-Ramos, I. M., Díaz-Delgado, R., de la Riva, E. G., Villar, R., Lloret, F., Marañón, T., & Zanne, A. (2017). Climate variability and community stability in Mediterranean shrublands: the role of functional diversity and soil environment. *Journal of Ecology*, 105(5), 1335-1346. doi:10.1111/1365-2745.12747

Pfisterer, A. B., & Schmid, B. (2002). Diversity-dependent production can decrease the stability of ecosystem functioning. *Nature*, 416(6876), 84-86. doi:10.1038/416084a

Pfleiderer, P., Schleussner, C. F., Kornhuber, K., & Coumou, D. (2019). Summer weather becomes more persistent in a 2 degrees C world. *Nature Climate Change*, 9(9), 666-+. doi:10.1038/s41558-019-0555-0

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., Heisterkamp, S., Van Willigen, B., & Maintainer, R. (2017). Package 'nlme'. Retrieved from <https://svn.r-project.org/R-packages/trunk/nlme/>

R Core Team. (2019). R: a language and environment for statistical computing, R v.3.6.1. Retrieved from <https://www.R-project.org/>

Rani, G., Kaur, J., Kumar, A., & Yogalakshmi, K. (2020). Ecosystem Health and Dynamics: An Indicator of Global Climate Change. In *Contemporary Environmental Issues and Challenges in Era of Climate Change* (pp. 1-32): Springer.

Rose, N. L., Yang, H. D., Turner, S. D., & Simpson, G. L. (2012). An assessment of the mechanisms for the transfer of lead and mercury from atmospherically contaminated organic soils to lake sediments with particular reference to Scotland, UK. *Geochimica et Cosmochimica Acta*, 82, 113-135. doi:10.1016/j.gca.2010.12.026

Rowland, E. L., Davison, J. E., & Graumlich, L. J. (2011). Approaches to evaluating climate change impacts on species: a guide to initiating the adaptation planning process. *Environ Manage*, 47(3), 322-337. doi:10.1007/s00267-010-9608-x

Sakai, S. (2001). Phenological diversity in tropical forests. *Population Ecology*, 43(1), 77-86. doi:10.1007/PI00012018

Schlegel, R., & Smit, A. (2018). heatwaveR: A central algorithm for the detection of heatwaves and cold-spells. Retrieved from <https://robwschlegel.github.io/heatwaveR/>

Shriver, R. K. (2017). Rainfall variability and fine-scale life history tradeoffs help drive niche partitioning in a desert annual plant community. *Ecol Lett*, 20(10), 1231-1241. doi:10.1111/ele.12818

Skinner, R. H., & Comas, L. H. (2010). Root Distribution of Temperate Forage Species Subjected to Water and Nitrogen Stress. *Crop science*, 50(5), 2178-2185.

doi:10.2135/cropsci2009.08.0461

Smakman, G., & Hofstra, R. J. J. (1982). Energy-Metabolism of *Plantago-Lanceolata*, as Affected by Change in Root Temperature. *Physiologia plantarum*, 56(1), 33-37.

doi:10.1111/j.1399-3054.1982.tb04896.x

Smith, N. G., Schuster, M. J., & Dukes, J. S. (2016). Rainfall variability and nitrogen addition synergistically reduce plant diversity in a restored tallgrass prairie. *Journal of Applied Ecology*, 53(2), 579-586. doi:10.1111/1365-2664.12593

Suding, K. N., Goldberg, D. E., & Hartman, K. M. (2003). Relationships among species traits: Separating levels of response and identifying linkages to abundance. *Ecology*, 84(1), 1-16. doi:10.1890/0012-9658(2003)084[0001:RASTSL]2.0.CO;2

Suttle, K. B., Thomsen, M. A., & Power, M. E. (2007). Species interactions reverse grassland responses to changing climate. *Science*, 315(5812), 640-642.

doi:10.1126/science.1136401

Teuben, A., & Verhoef, H. (1992). Relevance of micro-and mesocosm experiments for studying soil ecosystem processes. *Soil Biology and Biochemistry*, 24(11), 1179-1183.

doi:10.1016/0038-0717(92)90069-A

van Oldenborgh, G. J., Philip, S., Aalbers, E., Vautard, R., Otto, F., Haustein, K., . . . Cullen, H. (2016). Rapid attribution of the May/June 2016 flood-inducing precipitation in France and Germany to climate change. *Hydrol Earth Syst Sci Discuss*, 10.

doi:10.5194/hess-2016-308

- Van Peer, L., Nijs, I., Reheul, D., & De Cauwer, B. (2004). Species richness and susceptibility to heat and drought extremes in synthesized grassland ecosystems: compositional vs physiological effects. *Functional Ecology*, 18(6), 769-778. doi:10.1111/j.0269-8463.2004.00901.x
- Van Ruijven, J., & Berendse, F. (2010). Diversity enhances community recovery, but not resistance, after drought. *Journal of Ecology*, 98(1), 81-86. doi:10.1111/j.1365-2745.2009.01603.x
- Walsworth, T. E., Schindler, D. E., Colton, M. A., Webster, M. S., Palumbi, S. R., Mumby, P. J., . . . Pinsky, M. L. (2019). Management for network diversity speeds evolutionary adaptation to climate change. *Nature Climate Change*, 9(8), 632-+.
doi:10.1038/s41558-019-0518-5
- Wang, Y., Yu, S., & Wang, J. (2007). Biomass-dependent susceptibility to drought in experimental grassland communities. *Ecol Lett*, 10(5), 401-410. doi:10.1111/j.1461-0248.2007.01031.x
- Wellstein, C. (2012). Seed-litter-position drives seedling establishment in grassland species under recurrent drought. *Plant Biol (Stuttg)*, 14(6), 1006-1010. doi:10.1111/j.1438-8677.2012.00635.x
- Wickham, H. (2016). *ggplot2: elegant graphics for data analysis*: Springer.
- Wickham, H., Francois, R., Henry, L., & Müller, K. (2015). dplyr: A grammar of data manipulation. Retrieved from <https://dplyr.tidyverse.org/>

Winkler, D. E., Belnap, J., Hoover, D., Reed, S. C., & Duniway, M. C. (2019). Shrub persistence and increased grass mortality in response to drought in dryland systems. *Glob Chang Biol*, 25(9), 3121-3135. doi:10.1111/gcb.14667

Wood, S., & Wood, M. S. (2015). Package 'mgcv'. Retrieved from <https://cran.r-project.org/web/packages/mgcv/mgcv.pdf>

Yang, H. J., Li, Y., Wu, M. Y., Zhang, Z., Li, L. H., & Wan, S. Q. (2011). Plant community responses to nitrogen addition and increased precipitation: the importance of water availability and species traits. *Global Change Biology*, 17(9), 2936-2944. doi:10.1111/j.1365-2486.2011.02423.x

Zhou, S. X., Medlyn, B. E., & Prentice, I. C. (2016). Long-term water stress leads to acclimation of drought sensitivity of photosynthetic capacity in xeric but not riparian Eucalyptus species. *Ann Bot*, 117(1), 133-144. doi:10.1093/aob/mcv161

Zolina, O., Simmer, C., Belyaev, K., Gulev, S. K., & Koltermann, P. (2013). Changes in the Duration of European Wet and Dry Spells during the Last 60 Years. *Journal of Climate*, 26(6), 2022-2047. doi:10.1175/Jcli-D-11-00498.1

Zuur, A. F., & Ieno, E. N. (2016). A protocol for conducting and presenting results of regression-type analyses. *Methods in Ecology and Evolution*, 7(6), 636-645. doi:10.1111/2041-210x.12577

10. Tables

Table 1. Monthly averages of mean, maximum and minimum air temperature (T_{air}), and relative humidity and vapor pressure deficit (VPD) during daytime for each month of the experimental period in 2019 (KMI, 2019b). The long-term temperature averages (1981-2010) are given in parentheses.

Month	T_{air} mean (°C)	T_{air} max. (°C)	T_{air} min. (°C)	Relative humidity (%)	VPD (kPa)
July	19.7 (18.5)	24.4 (23.2)	14.6 (13.8)	54.3	1.38
August	19.4 (18.2)	24.4 (23.1)	14.3 (13.2)	58.4	1.22
September	15.5 (15.1)	19.7 (19.7)	11.3 (10.6)	60.4	0.86
October	12.3 (11.3)	15.3 (15.3)	9.6 (7.4)	72.2	0.47

11. Figure legends

Figure 1. Schematic representation of our gradient design (b) of precipitation regimes compared with previous designs (a). Earlier studies have inquired how patterns with altered precipitation amounts or more concentrated rainfall interspersed with longer droughts (Knapp et al., 2002) can influence grassland diversity. The current experiment investigated the effects of more persistent precipitation patterns by alternating increasingly longer dry and wet periods.

Figure 2. Evolution of soil water content across the experimental period by treatment, averaged over 30 cm depth. Persistence of the wet/dry spells is expressed as the number of days with or without irrigation followed by the letter ‘W’ or ‘D’ for treatments starting with a wet or dry period, respectively. Bars above each window show the approximate predetermined dry (white) and wet (colored) periods per treatment. The dashed line indicates the permanent wilting point (PWP). Heatwaves, indicated in orange, were defined as a period of three or more days with average temperature >25 °C (KMI, 2019b).

Figure 3. Effect of more persistent precipitation regimes on end-of-season Shannon-Wiener's Diversity index (a), Simpson's diversity index (b), Pielou's evenness index (c), and species richness (d). Full lines represent GAMM model outcomes (means) for individual smoothers per starting type and their approximate 95% point-wise CI's (shaded area) on the response scale. Treatments starting either with a dry or wet period are indicated in red and blue respectively. Each symbol is an individual mesocosm.

Figure 4. Effect of more persistent precipitation regimes on end-of-season survival of plant functional groups for treatments starting with either a dry (a) or a wet period (b). Full lines represent GAMM model outcomes (means) for individual smoothers and their approximate 95% point-wise CI's (shaded area) on the response scale.

Figure 5. Relationships (and adjusted R^2) between the seasonal mean of community Fv/Fm (physiological stress indicator) and (a) end-of-season survival or (b) Shannon-Wiener's diversity index. Error bars and shaded area represent ± 1 SE on the mean. The color gradient indicates the number of blocking days corresponding to each treatment either with dry start (D, circle) or wet start (W, triangle).

Figure 6. Regression model outcomes of (a) the effect of soil water content (SWC) on Fv/Fm of the community (excluding completely brown plants) per measurement day, and the relationships between end-of-season survival and (b) the total number of days below permanent wilting point (PWP) or (c) length of the longest period below PWP throughout the experimental period. Error bars and shaded area represent ± 1 SE of the mean. The color gradient indicates the number of blocking days corresponding to each treatment either with dry start (d, circle) or wet start (W, triangle). The dashed line highlights the PWP.









