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SPECIAL FEATURE

ECOLOGICAL CONSEQUENCES OF CLIMATE EXTREMES

Climate extremes initiate ecosystem-regulating functions while maintaining productivity

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Summary

1. Studying the effects of climate or weather extremes such as drought and heat waves on biodiversity and ecosystem functions is one of the most important facets of climate change research. In particular, primary production is amounting to the common currency in field experiments world-wide. Rarely, however, are multiple ecosystem functions measured in a single study in order to address general patterns across different categories of responses and to analyse effects of climate extremes on various ecosystem functions.

2. We set up a long-term field experiment, where we applied recurrent severe drought events annually for five consecutive years to constructed grassland communities in central Europe. The 32 response parameters studied were closely related to ecosystem functions such as primary production, nutrient cycling, carbon fixation, water regulation and community stability.

3. Surprisingly, in the face of severe drought, above- and below-ground primary production of plants remained stable across all years of the drought manipulation.

4. Yet, severe drought significantly reduced below-ground performance of microbes in soil indicated by reduced soil respiration, microbial biomass and cellulose decomposition rates as well as mycorrhization rates. Furthermore, drought reduced leaf water potential, leaf gas exchange and leaf protein content, while increasing maximum uptake capacity, leaf carbon isotope signature and leaf carbohydrate content. With regard to community stability, drought induced complementary plant–plant interactions and shifts in flower phenology, and decreased invasibility of plant communities and primary consumer abundance.

5. *Synthesis*. Our results provide the first field-based experimental evidence that climate extremes initiate plant physiological processes, which may serve to regulate ecosystem productivity. A potential reason for different dynamics in various ecosystem services facing extreme climatic events may lie in the temporal hierarchy of patterns of fast versus slow response. Such data on multiple response parameters within climate change experiments foster the understanding of mechanisms of resilience,

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of synergisms or decoupling of biogeochemical processes, and of fundamental response dynamics to drought at the ecosystem level including potential tipping points and thresholds of regime shift. Future work is needed to elucidate the role of biodiversity and of biotic interactions in modulating ecosystem response to climate extremes.

Key-words: below-ground, competition, decomposition, invasion, leaf chemistry, microbial, phenology, plant–climate interactions, precipitation change, productivity

Introduction

Currently, knowledge about ecological responses to climate change is based largely on effects of climatic trends such as gradual warming, precipitation change and CO_2 enrichment. However, the magnitude and frequency of climate or weather extremes such as severe drought, heat waves, heavy rain and late frost events are expected to increase in the near future (IPCC 2007; O'Gorman & Schneider 2009). Thus, predictions of effects of climate extremes on species, communities and ecosystems have become critical to science and society. Yet, consequences of future climate extremes for ecosystem functions and services are largely unknown and have only recently been addressed by ecological research (Gutschick & BassiriRad 2003; Schröter *et al*. 2005; Jentsch 2006; Jentsch, Kreyling & Beierkuhnlein 2007; Suttle, Thomsen & Power 2007; Knapp *et al*. 2008; Fisher, Turner & Morling 2009; Jentsch & Beierkuhnlein 2010).

There is growing concern that climatic extremes such as severe drought could negatively affect ecosystem functioning and stability. A review of the literature revealed that the focus over the last decade has been primarily on primary productivity (Figure S1a–d and Table S1 in Supporting Information), one of the major common currencies in global ecology. The findings from existing climate change studies on drought effects are highly controversial. While some field experiments showed that natural and simulated drought led to decreases of primary productivity (Olesen & Bindi 2002; Morecroft *et al*. 2004; Penuelas *et al*. 2004; Ciais *et al*. 2005), others did not find any significant effects of locally severe drought manipulations (Fay *et al*. 2000; Kreyling *et al*. 2008c). Generally, evidence suggests that an elongation of inter-rainfall intervals as well as changes in seasonal timing are more likely to cause a reduction of above-ground net primary productivity (ANPP) than reduced total rainfall quantity *per se* (Fay *et al*. 2000; Swemmer, Knapp & Snyman 2007).

However, further aspects confound the debate on ecosystem functioning in the light of climate change. First, the role of biodiversity in ensuring the performance of ecosystem functioning (Balvanera *et al*. 2006; Worm *et al*. 2006; Hector & Bagchi 2007; Suttle, Thomsen & Power 2007) and in enhancing resistance or resilience to drought has been proven to be fundamental (Pfisterer & Schmid 2002; Kahmen, Perner & Buchmann 2005; De Boeck *et al*. 2008; van Ruijven & Berendse 2010). Secondly, multiple ecosystem functions in the face of climate extremes have rarely been addressed simultaneously in experiments (Jentsch, Kreyling & Beierkuhnlein 2007; Jentsch & Beierkuhnlein 2008, 2010). Prevailing response parameters in climate change experiments are above-ground production, soil C:N ratio and soil respiration (Figure S1d, Table S2). However, the interrelationships between above-ground primary production and below-ground nutrient cycling, carbon fixation or water regulation are rarely addressed.

Here, we analyse the effects of recurrent severe drought (local 100-year or1000-year extreme events) on multiple ecosystem properties of a planted grassland in Central Europe in a long-term field experiment (EVENT-I) located in Bayreuth, Germany. Semi-natural European grasslands are widespread, of economic value, provide many ecological services and are important for nature conservation. They have been managed either as hay meadows or pastures in Europe for thousands of years.

Our goal was to assess whether there are general patterns across these different categories of important ecosystem functions including primary productivity, water regulation, carbon fixation, nutrient cycling and compositional stability to climate extremes.

We expected the grassland ecosystem to react sensitively to extreme recurrent drought events, and specifically hypothesized that (i) above-ground productivity would be decreased; and (ii) other ecosystem functions, such as water regulation, carbon fixation, nutrient cycling and compositional stability, would be negatively impacted.

Materials and methods

EXPERIMENTAL DESIGN

The EVENT-I experiment (Jentsch, Kreyling & Beierkuhnlein 2007) is established in the Ecological Botanical Garden of the University of Bayreuth, Germany (49°55′19″N, 11°34′55″E, 365 m a.s.l.) with a mean annual temperature of 8.2 °C and a mean annual precipitation of 724 mm (1971–2000). Precipitation is distributed bi-modally with a major peak in June/July and a second peak in December/January (data: German Weather Service). The experiment was carried out with two fully crossed factors: (i) climate extremes (severe drought, ambient control); (ii) community diversity (two species of one functional group, four species of two functional groups, and four species of three functional groups, monocultures of particular species), representing key species combinations of grassland. The total setup consisted of five replicates of each factorial combination, 60 plots in total of 2×2 m in size. The factors were applied in a split-plot design with the vegetation types and diversity levels blocked and randomly assigned within each drought manipulation (Jentsch, Kreyling & Beierkuhnlein 2007). The originally installed species composition was maintained by periodical weeding. The texture of the previously homogenized and constantly drained soil consisted of loamy sand (82% sand, 13% silt, 5% clay)

with pH = 4.5 in the upper and pH = 6.2 in the lower soil layer (measured in 1 M KCl). Data acquisition was carried out in the central square metre of each plot only, in order to circumvent edge effects.

CLIMATIC EXTREMES

The climate manipulations consisted of extreme drought and ambient conditions for control. Extremeness of drought events was determined by statistical extremity with respect to a historical reference period (extreme value theory) independent of its effects on organisms (Jentsch 2006). In particular, intensity of the treatments was based on the local 100-year extreme event in 2005, 2006 and 2007, and on the local 1000-year extreme event for 2008 and 2009. Vegetation periods (March–September) of 1961–2000 were used as the reference period (data: German Weather Service). Gumbel I distributions were fitted to the annual extremes, and 100-year and 1000-year recurrence events were calculated.

Drought was defined as the number of consecutive days with less than 1 mm daily precipitation. Accordingly, a drought period of 32 days (2005–2007) and of 42 days (2008 and 2009) was applied in the experiment during the peak growing season in June. Maximum values in the historical data set were 33 days without rain during June and July 1976. Drought was induced with the support of rain-out shelters that permitted nearly 90% penetration of photosynthetically active radiation.

Unwanted greenhouse effects were avoided by starting the roof from a height of 80 cm, allowing for near-surface air exchange. After the experimental drought period, the roofs were removed. A lateral surface flow was avoided by plastic sheet pilings around treated plots reaching down to a depth of 10 cm.

The ambient control plots (C) remained without manipulation throughout the entire period. A roof artefact control with five replicates of the rain-out shelters was in place in 2006. Adding the same amount of water as occurred naturally in daily resolution below intact shelters during the drought manipulation period did not result in any significant differences in response parameters, indicating no significant effect from the slightly increased temperature caused by the rain-out shelters.

EXPERIMENTAL PLANT COMMUNITIES

Overall, grasslands are spatially important ecosystems in Central Europe. Five widespread plant species were chosen from the regional flora, i.e. *Arrhenatherum elatius* (L.) P. Beauv. ex J. Presl & C. Presl, *Holcus lanatus* L., *Geranium pratense* L., *Lotus corniculatus* L. and *Plantago lanceolata* L. Species were selected with respect to their affiliation to defined functional groups (grasses, forbs, leguminous forbs), to life span (perennials), to overall importance in nearby and Central European grassland systems, and to the fact that they do naturally grow on substrate similar to the one used in this experiment. One hundred plant individuals per plot in defined quantitative composition were planted in a systematic hexagonal grid with 20 cm distance between individuals in early April 2005. Grass and forb individuals used in the experiment were grown from seeds in a greenhouse in the preceding fall. Thus, all plants were in a juvenile stage during manipulation and data acquisition. All plants had been acclimated on site since February 2005, reaching growth heights of *c.* 15 cm. Biomass at planting amounted to 0.1–0.6 g dry wt per individual. These experimental communities represent naturally occurring species combinations. The grassland plots were established at two levels of species diversity (2 and 4 species) and three levels of functional diversity (1, 2, 3 functional groups), resulting in three species combinations or communities in total (Table 1) plus monocultures of selected species.

RESPONSE PARAMETER

The 32 parameters measured are categorized into five key ecosystem functions (Fig. 1) and are described below in order of their appearance, except for soil moisture, which is presented first. Since complete time series data are not available for all parameters, it is indicated in Table S3 whether data from five consecutive years or from particular years were sampled. All data presented in Fig. 1 are derived from years of maximum drought effects.

SOIL MOISTURE

Soil moisture was recorded by time domain reflectance measurements (Diviner 2000; Sentek Sensor Technologies, Stepney, SA, Australia) at −10 cm in 2005–2007. In 2008–2009, soil moisture was recorded between 2 and 7 cm in one grassland plot per treatment block in 1-h intervals by FD-sensors (Echo.EC-5/k; Decagon Devices, Pullman, WA, USA).

Primary production

ABOVE-GROUND NET PRIMARY PRODUCTION

Above-ground biomass harvests (ANPP) of all standing plant material (dead and alive) in all communities were conducted twice a year (early in July and mid September) in 2005–2009, resembling local

Table 1. Experimental plant communities in the EVENT-I experiment (Jentsch, Kreyling & Beierkuhnlein 2007) representing grassland vegetation in central Europe: three functional diversity levels varied by number of species, growth form and presence/absence of legume

Abbreviation	Vegetation type	Diversity level	Description	Species
$G2^-$	Grassland	A	Two species, one functional group (grass)	*Arrhenatherum elatius*, *Holcus lanatus*
$G4^-$	Grassland	B	Four species, two functional groups (grass, forb)	*Arrhenatherum elatius*, *Holcus lanatus*, *Plantago lanceolata*, *Geranium pratense*
$G4^+$	Grassland	C	Four species, three functional groups (grass, forb, leguminous forb)	*Arrhenatherum elatius*, *Holcus lanatus*, *Plantago lanceolata*, *Lotus corniculatus*

G, grassland; 2/4, number of species; −, without legume; +, with legume

agricultural routines. All biomass was taken out of the central square metre of each grassland plot in order to circumvent edge effects. The harvested biomass was sorted to species and dried to constant weight at 75 °C and weighed (Ohaus Navigator™, Ohaus Corporation, Parsippany, NJ, USA; accuracy ± 0.01 g).

NITROGEN-FIXING LEGUMES

According to the above-mentioned routines, harvested biomass of the legume species *L. corniculatus* was used to determine the performance of nitrogen-fixing plants.

PLANT COVER

Species-specific above-ground cover was quantified using a pin-point method, by recording the presence of plant organs in general and the presence of each species separately at 100 vertically inserted steel needles. These values were then treated as the percentage of cover. The measurement was repeated three times in each vegetation period (May, July and September).

BELOW-GROUND BIOMASS

Root length was used as proxy for below-ground productivity. Root length was acquired by the minirhizotron technique three times a year. One clear plastic tube (5 cm diameter) was installed at a 45° angle in each plot prior to planting. Tubes were installed to a depth of 45 cm. Portions of the tubes exposed at the surface were covered with adhesive aluminium foil and the ends were capped to prevent entry of water, light and heat. Images of 4 cm^2 were collected in the main rooting zone at 15 cm in each tube by a digital camera mounted on an endoscope. Images were analysed for root length using the line intersection method (Tennant 1975) within a systematic grid (10 × 10, with a grid unit of 0.2 × 0.2 cm). Five replicates per sampling date were analysed.

SHOOT-TO-ROOT RATIO

Shoot-to-root ratio was evaluated using the ratio between above-ground cover and below-ground root length at 5 cm soil depth (Kreyling *et al.* 2008b). Both parameters were *a priori* standardized to the same mean and standard deviation.

Water regulation

LEAF WATER POTENTIAL

Predawn (ψ_{pd}) and midday (ψ_l) leaf water potential (ψ_{pd}) were measured on one leaf of *H. lanatus* per plot using a portable pressure chamber (PMS Instruments Co., Corvallis, OR, USA). During measurements, the leaves were cut while enclosed in a plastic bag to reduce further moisture loss during transfer and fixing into the chamber. Moist tissue paper was introduced into the chamber to reduce water loss during the measurements. Measurements were confined to the period between 04:00 and 05:00 hours

LEAF CARBON ISOTOPE SIGNAL

At the end of drought, a set of three fully matured leaves of *A. elatius* from every plot was selected. In each plot, two sun-exposed leaves of five individual plants were sampled and combined. The samples were oven-dried for 48 h at 80 °C. The dry leaves were ball-milled and subsamples of 1 mg analysed for $\delta^{13}C$ with an elemental analyser attached to an isotope-ratio mass spectrometer using ConFlo III interface (Thermo Electron, Bremen, Germany). The carbon isotope composition ($\delta^{13}C$) of a sample was calculated as: $\delta^{13}C = [(R_{sample}/R_{standard}) - 1] \times 1000$, expressed in units of per thousand (‰). ^{13}C:^{12}C ratios were calculated against the P.D. Belemite Standard (precision of 0.2 ‰). The results were compared with other measurements to determine changes associated with shifts in ^{13}C. Every measurement was replicated twice and the accuracy in δ-values was better than 0.1 ‰.

Carbon fixation

EFFICIENCY OF PHOTOSYNTHETIC LIGHT CONVERSION

Chlorophyll *a* fluorescence in the grass species *H. lanatus* was recorded using a pulse-amplitude-modulated photosynthesis yield analyser (PAM 2000 and Mini-PAM; WALZ, Effeltrich, Germany) with a leaf clip holder. The second or third fully expanded leaves were measured on four different tillers of one individual. Four measurements per plant were averaged for further analysis. We obtained predawn fluorescence values at the end of the first drought treatment in May/June and throughout the early recovery period after the second drought. The maximum quantum efficiency of photosystem II was calculated as F_v/F_m. Variable fluorescence (F_v) and maximum fluorescence (F_m) were measured before dawn. Variable fluorescence was calculated as $F_m - F_0$, F_m being the maximum fluorescence of the dark-adapted leaf after applying a saturating light pulse and F_0 being the steady-state fluorescence yield of the dark-adapted leaf (Maxwell & Johnson 2000). To enable a comparison between absolute fluorescence values, a fluorescence standard material was measured before dawn and calculated as F_v/F_m ($F_v = F_m - F_0$) (Maxwell & Johnson 2000). Absolute F_0 and F_m values were taken to separate the effects of photodamage, becoming apparent with an increase of F_0, from the effects of photoprotection related to enhanced non-photochemical quenching, becoming apparent with a decrease in F_m (Walter *et al.* 2011).

LEAF GAS EXCHANGE

Carbon dioxide assimilation (A) at the leaf was monitored in *A. elatius* in all the grassland communities. (No data could be obtained from *H. lanatus* in the particular year of data mining due to its leave status.) A series of weekly measurements were carried out using a portable gas-exchange system (LI-6400; LI-Cor, Lincoln, NE, USA). A set of three grass tufts on each plot were identified and marked for measurements. On any measurement day, 2–3 suitable leaf blades selected from each of the tufts per plot were set parallel in the cuvette, with their upper surfaces well exposed so that they were fully illuminated during measurements. Every turn of measurements lasted 1–2 min, when a steady state was attained and a set of 10 readings per measurement logged at 10-s intervals. The selected leaves were marked and similar leaves were monitored either during midday (12:00 to 14:00 hours) or throughout the day (from sunrise to sunset), when diurnal course measurements were conducted. The measured leaves were then excised at the end of the measurement period and the leaf area (LA) of the section of leaf enclosed in the cuvette determined using LA meter. (CI-202 CID; Camas, WA, USA). Leaf area information was then used to standardize the leaf gas-exchange data.

SOIL RESPIRATION

In situ rates of soil respiration were measured using a portable CO_2 infrared gas analyser (EGM-4; PP Systems, Amesbury, USA) linked to a soil respiration chamber (SRC-1; PP Systems). At the beginning of the vegetation period, permanent PVC collars (10 cm diameter, 5 cm height, light grey colour) were installed in every plot with a 1-cm edge above soil surface to realize a closed system when the soil respiration chamber was placed on the collar during measurement. The day before each measurement, all above-ground vegetation was removed from the collar using scissors. During the timeframe of 8:00–12:00 hours, the soil respiration chamber was placed for 240 s on the collar of every plot. An internal fan realized the even distribution of air and the infrared gas analyser monitored the build-up of CO_2 within the system. The rates of soil respiration were determined from this by fitting a quadratic equation to the change in CO_2 concentration with time. For this study, we analysed the soil respiration rates at second 240 of each high-diversity grassland plot including *A. elatius, H. lanatus, P. lanceolata* and *G. pratense* on the last day of drought manipulation.

MAXIMUM LEAF AND CANOPY UPTAKE RATES

Net ecosystem CO_2 exchange (NEE) was measured with chambers on 40 × 40 cm frames established on each of the treatment plots. Daily course of NEE was measured using manually operated, closed gas-exchange canopy chambers. Light–response curves depicting the net photosynthetic CO_2 uptake rate (A) of plants at any measuring time were obtained from leaf-level gas-exchange measurements by fitting an empirical rectangular hyperbola model (Gilmanov *et al.* 2005): $NEE = (\alpha + Q/\alpha Q - \beta) - \gamma$, where α is the initial slope of the light–response curve and an approximation of the canopy light utilization efficiency (mol CO_2 per mol PAR), β is the maximum CO_2 uptake capacity (μmol m^{-2} s^{-1}), Q is the photosynthetically active radiation (PAR, in μmol m^{-2} s^{-1}), and γ is an approximation of the average daytime ecosystem respiration (μmol m^{-2} s^{-1}). An approximation of maximum canopy uptake capacity was extrapolated from leaf-level measurements. Canopy NEE rate was estimated from leaf photosynthetic rate at saturating light intensities (it was shown that A at PAR = 2000 μmol m^{-2} s^{-1} correlates well with canopy NEE). Maximum gross primary productivity (GPP_{max}) was calculated as: $GPP_{max} = NEE_{2000} - R_{eco}$, where A_{2000} is the maximum leaf photosynthetic rate at a saturating level of light intensity and R_{eco} is the corrected respiration term (γ) obtained from the model.

Nutrient cycling

IN SITU DECOMPOSITION RATE OF CELLULOSE

Biological activity of soil fauna and micro-organisms was determined indirectly from the decay of cellulose using mini-container tubes (Kreyling *et al.* 2008a). In total, 864 mini-containers were filled with 0.2 g of cellulose (poor in phosphorus, Schleicher & Schüll, Dassel, Germany) each, closed with a 2-mm mesh, and put into container tubes, consisting of 12 mini-containers each. Two tubes were buried horizontally 1 cm below soil surface in each grassland plot. After 94 days, one tube per plot was harvested, whereas the others were harvested after 186 days. After careful cleaning and drying, the decay of cellulose was determined by subtracting final ashes-free dry mass from initial dry mass (105 °C).

MYCORRHIZAL COLONIZATION

One complete plant individual of *P.lanceolata* was taken from each plot on the last day of drought using a soil core sampler with 5 cm diameter (Eijkelkamp, Giesbeek, the Netherlands). This particular species was chosen, because pre-analysis revealed higher effects of drought on mycorrhizaal colonization of *P. lanceolata* than on that of other species tested. Roots were cut off and fixed in formalin-alcoholic-acid (50% Ethanol, 40% H_2O, 7.5% formalin, 2.5% acidic acid), and stained with 5% blue ink vinegar solution after boiling in 10% KOH. Afterwards, mycorrhization ratios were determined by scanning 15 cm fine roots of each sample for arbuscules and vesicules under a microscope (400×) using the 'magnified intersection method' (McGonigle *et al.* 1990).

SOIL MICROBIAL NITROGEN POOL

Soil microbial nitrogen was extracted from fresh soil according to a modified chloroform fumigation–extraction method (Brookes *et al.* 1985). After chloroform fumigation (24 h at room temperature), dissolved organic and microbial N was extracted with 50 mL 0.5 M K_2SO_4 and quantified (DIMA TOC-100; Dimatec, Essen, Germany). Microbial biomass and relative abundance of microbial groups were measured using phospholipid fatty acid analysis as described (Singh *et al.* 2006).

POTENTIAL SOIL ENZYME ACTIVITIES

For soil enzyme activity measurements, enzymes involved in carbon, nitrogen and phosphorus cycling were selected (Mirzaei *et al.* 2008), thus addressing important microbial soil functions (Waldrop & Firestone 2006). The enzyme activities tested were acid phosphatase cleaving organically bound phosphate, cellobiohydrolase, β-xylosidase and β-glucosidase related to the degradation of plant cell wall components and *N*-acetylglucosaminidase representing chitinases that degrade chitin from fungal or arthropod origin. Soil samples for determining soil enzyme activities were collected immediately after finishing the drought manipulations (Kreyling *et al.* 2008a). Four samples per plot (depth 0–5 cm) were combined, mixed and kept at 4 °C until further processing within 4 weeks after sampling. Soil suspensions (0.4 g fresh soil in 40 mL H_2O) were prepared from each sample. The assay is based on the enzymatic cleavage of the below-detailed methylumbelliferone (MU) coupled substrates and the subsequent detection of MU released during incubation. In brief, 50 μL per well of soil suspensions (three replicates each sample) were dispersed in microplates and 100 μL of substrate solutions were added to start the reactions. After stopping the reaction with 100 μL of 2.5 M Tris buffer and centrifugation, MU concentrations were determined on a fluorescence spectrometer at excitation/emission wavelengths of 365/450 nm respectively. The following enzyme substrates were used with the incubation times given: MUF-phosphate, 20 min; MUF-xyloside, 1 h; MUF-cellobiohydrofurane, 1 h; MUF-*N*-acetyl-β-glucosaminide, 40 min; MUF-β-glucoside, 1 h. Substrate concentrations in the incubation mix were 500 μM except for MUF-cellobiohydrofurane with 400 μM. To account for quenching and to calculate the amount of MUF released, calibration curves were included with 50 μL of soil samples as in the incubation wells and MUF-solutions to give a final amount of 0–500 pmol per well. Negative controls for autofluorescence of substrates were also included. Enzyme activities are expressed as MUF-release per gram soil dry weight per hour.

PLANT-AVAILABLE SOIL NITRATE AND AMMONIUM

Plant-available nitrogen was extracted from four homogenized, sieved (< 2 mm), mixed samples of the upper soil layer (0–10 cm) of each plot sampled in July using a 1 M KCl solution after filtration (Typ 15 A Blauband; Roth, Karlsruhe, Germany) (Kreyling, Beierkuhnlein & Jentsch 2010). Nitrate and ammonium were quantified using flow injection analysis (FIA-LAB; MLE, Dresden, Germany).

LEAF CARBON-TO-NITROGEN RATIO

Leaf carbon (C), leaf nitrogen (N) and C:N ratios were measured from mixed samples of two sun-exposed leaves of five individual plants per species and plot, sampled in July (Kreyling, Beierkuhnlein & Jentsch 2010). The samples were oven-dried for 48 h at 75 °C. The dry leaves were ball-milled and subsamples of 1 mg analysed with an elemental analyser in a mass spectrometer using ConFlo III interface. Plant-available nitrogen was extracted from four homogenized, sieved (2 mm) and filtered (Roth, Germany, Typ 15A Blauband) mixed samples of the upper soil layer (0–10 cm) of each plot using a 1 M KCl solution.

LEAF PROTEIN CONTENT

Total protein content in μg per mg fresh weight was determined as a proxy for nutritive value of the legume key species *H. lanatus*, which was growing in all plots. One leaf sample per plot was taken on the last day of drought treatment, frozen in liquid nitrogen and freeze-dried to determine protein-bound amino acids. Amino acids of the protein fraction were extracted. Amino acid concentrations were measured with an ion exchange chromatograph (amino acid analyser LC 3000; Biotronik SE & Co. KG, Berlin, Germany) and protein content was calculated by pooling the content of each amino acid in the protein fraction.

LEAF NITROGEN ISOTOPE SIGNAL

Equally aged, south-facing leaves of *A. elatius* were collected and oven-dried at 60 °C for 48 h, and then fine-milled. Natural abundance of δ15N and total nitrogen concentration were analysed using an elemental analyser (EA 3000; EuroVector, Italy) coupled online to a ConFlo III interface connected to an isotope-ratio mass spectrometer (MAT 253; Thermo Electron). The δ15N values were calculated as: $\delta 15N\ [‰] = (\text{Rsample}/\text{Rstandard}) - 1 \times 1000$, where R represents the ratio of 15N:14N isotopes. As standard, (nitrogen in) air was used.

Community responses

INVASIBILITY

Invasibility of the experimental communities was recorded three times per year: before and after the drought manipulations in early summer, and in fall (Kreyling *et al.* 2008c). Invading plant individuals were collected from the inner square metre of each plot and subsequently separated by species. Removal took place only after the first true leaves (after the cotyledons) emerged, but most specimens were considerably older than this and clearly established in the stand. At this point in development, we expected that number of individuals give a measure of established invaders rather than chance germinations. For each plot, the number of individuals was determined. The planted target species of the experiment were removed from the subsequent analysis. Tests confirmed that germination from the soil seed bank was negligible after 1 year. Thus, invasibility was only based on species invading from the matrix vegetation.

PLANT COMPOSITIONAL CHANGE

The measurements of above-ground species-specific cover (see above) were used to evaluate shifts in the species abundance distributions of the artificial plant assemblages. Compositional change of each individual plot was evaluated by comparing the species abundance distribution at each time step to the initial species abundance distribution (5 weeks after planting) by the Bray–Curtis index.

COMPETITIVE EFFECT/FACILITATIVE EFFECT

The relative neighbour effect (RNE) calculates the effect of neighbours relative to the plant with the greatest performance: $\text{RNE} = P_{\text{contr}} - P_{\text{mix}}/x$ with $x = P_{\text{contr}}$ if $P_{\text{contr}} > P_{\text{mix}}$ and $x = P_{\text{mix}}$ if $P_{\text{mix}} > P_{\text{contr}}$, where RNE = Relative neighbour effect ($-1 \le \text{RNE} \le +1$), P_{contr} = performance per plant for a plant growing alone, P_{mix} = performance per plant for a plant growing in mixture. Negative values indicate facilitation, and positive values indicate competition (Markham & Chanway 1996).

SENESCENCE

Tissue die-back was quantified by cover measurements of standing-dead plant organs (Kreyling *et al.* 2008d). A pin-point method was applied, recording the presence of plant organs in general and the presence for each species separately at 100 vertically inserted steel needles. These values were treated as percentage cover. The measurement was repeated four times over the course of the vegetation period.

VARIABILITY IN LENGTH OF FLOWERING

For each species, weekly observations of the flowering status of four individuals per plot and species were carried out (Jentsch *et al.* 2009). Individuals were counted as 'flowering' when the anthers were visible in at least one flower. Flowering length was calculated as the difference between the dates of the 25th and 75th percentiles of the flowering curve over time. Variability in length of flowering was obtained as the standard deviation between all species for each treatment (drought and control) separately. Statistical significance of difference in variability was evaluated by the Levene test.

VARIABILITY IN FLOWER PHENOLOGY

Flower phenology was obtained from the same data as length of flowering (see above). As a surrogate, the mid-flowering date was calculated for each species and plot, i.e. the date of the 50th percentile of the flowering curve over time. Variability in flower phenology was expressed as the standard deviation between all species for each treatment (drought and control) separately. Statistical significance of difference in variability was evaluated by the Levene test.

RESISTANCE TO HERBIVORY (PHENOL CONTENT)

For analysis of total soluble carbohydrates and total phenolics, three mixed samples of at least two plants per plot were taken at the end of the drought period, immediately frozen in liquid nitrogen and lyophilized ($n = 15$). Thirty milligrams were extracted in 50% methanol. Total soluble carbohydrates were analysed using the anthrone

method with glucose as a standard. Extinction was measured at 620 nm. Total phenols were analysed using Folin Ciocalteu's reagent and catechin as a standard and measuring extinction at 750 nm.

PRIMARY CONSUMER ABUNDANCE

Richness was sampled in June in one circular area (40 cm diameter) in each grassland plot using a D-Vac suction sampler (ecotech GmbH, Bonn, Germany). For each plot, the sampling bag was removed and all sampled material was stored in ethanol. Arthropod samples were quantified as the total number of individuals and identified at least to order level. However, some taxa were identified to the family level (families within the *Coleoptera, Hemiptera*, most *Hymenoptera*) and in one case to genus level (*Psylliodes Chrysomelidae*). The use of higher taxonomic levels has been shown to produce a good approximation of total species richness (Biaggini *et al.* 2007).

STATISTICAL ANALYSES

Linear Models combined with ANOVA were applied to test for significant differences between groups at single points of time, while taking the split-plot design into account. Homogeneous groups of factor combinations (drought manipulation, vegetation type, diversity level) were identified by Tukey's HSD *post hoc* comparisons. Level of significance was set to $P < 0.05$. Statistical significance of difference in variability of length in flowering was evaluated by the Levene test.

For time series, Linear Mixed-Effects Models were employed to test for effects of drought manipulation and diversity and their respective interactions while taking the split-plot design and the repeated measures into account (time used as random factor). When no significant interaction was found, the model was simplified by using only the drought manipulations as fixed effects and time as random effect. Significance of differences ($P < 0.05$) was evaluated by Markov Chain Monte Carlo sampling of 1000 permutations. Linear Mixed-Effects Models were conducted with the function 'lmer' (Bates & Sarkar 2007).

Prior to statistical analysis, data was log- or square-root-transformed, if conditions of normality were not met, or to improve homogeneity of variances. Both characteristics were tested by examining the residuals versus fitted plots and the normal qq-plots of the linear models. All statistical analyses were performed using R.

Results

The effects of drought on all measured ecosystem properties are summarized in Fig. 1 using response ratios to standardize the effect size of the severe drought treatment.

WATER REGULATION

Severe drought significantly reduced soil moisture during the manipulation periods in all years (Figs 1 and 2). A high variability both within years and between years is evident due to inter-annual variability of precipitation (Table 2). Even though absolute minima in soil moisture were similar for drought and control in most years, soil moisture of the drought plots remained considerably longer below the approximate permanent wilting point (pF = 4.2) for the soil substrate. The manipulation effect vanished within days for all years except 2009, where a lag phase of about 2 months until August occurred. Further, drought decreased leaf water potential, while increasing leaf carbon isotope signal in some species (Fig. 2).

PRIMARY PRODUCTION

At the level of the grassland community or ecosystem, respectively, local, annually recurrent 100-year and 1000-year extreme drought events had no significant effect on various processes that contribute to primary production in any of the 5 years from 2005 to 2009 (Figs 1 and 3). Surprisingly, neither ANPP, nor green cover of vegetation or below-ground production recorded as root length in the main rooting horizon were affected by drought (Figs 1 and 3). Further, there was no significant drought effect on biomass production of the nitrogen-fixing plant *L. corniculatus* (Fig. 1).

CARBON FIXATION

Drought increased the maximum uptake capacity (GPPmax) in grassland by 36% (Fig. 1). The soil respiration rate (R_{eco} calculated by the model was lower under drought than under ambient conditions. Soil respiration was slightly but not significantly decreased at the end of the drought.

NUTRIENT CYCLING

Nutrient cycling in soil was clearly affected by drought (Fig. 1). The annually recurrent drought events increased ammonium content in soil, whereas soil microbial N was decreased. Overall turnover rates were reduced, indicated by decreased decomposition rate of cellulose and potential enzymatic activities. The relative abundance of different microbial groups except for arbuscular mycorrhiza remained unchanged.

Remarkably, despite stability in biomass production, drought decreased leaf protein content and the leaf nitrogen isotope signature and increased C:N ratio and carbohydrate content in leaves, thus decreasing feed value of plant tissue.

COMMUNITY RESPONSES

Some ecosystem properties associated with community stability were positively affected by drought. For example, annually recurrent drought events reduced the invasibility of plant communities and, thus, increased community stability. Remarkably, recurrent severe drought did not cause any shift in the absolute abundance of species, thus, it did not cause any compositional change within 5 years (Fig. 4), although it induced complementary, species-specific plant–plant interactions resulting in shifts in species-specific biomass contribution to overall community production. For example, the competitive effect of neighbouring plants on *L. corniculatus* was increased by drought as well as the facilitative effect of neighbouring plants on *A. elatius*. Still, a significant difference between

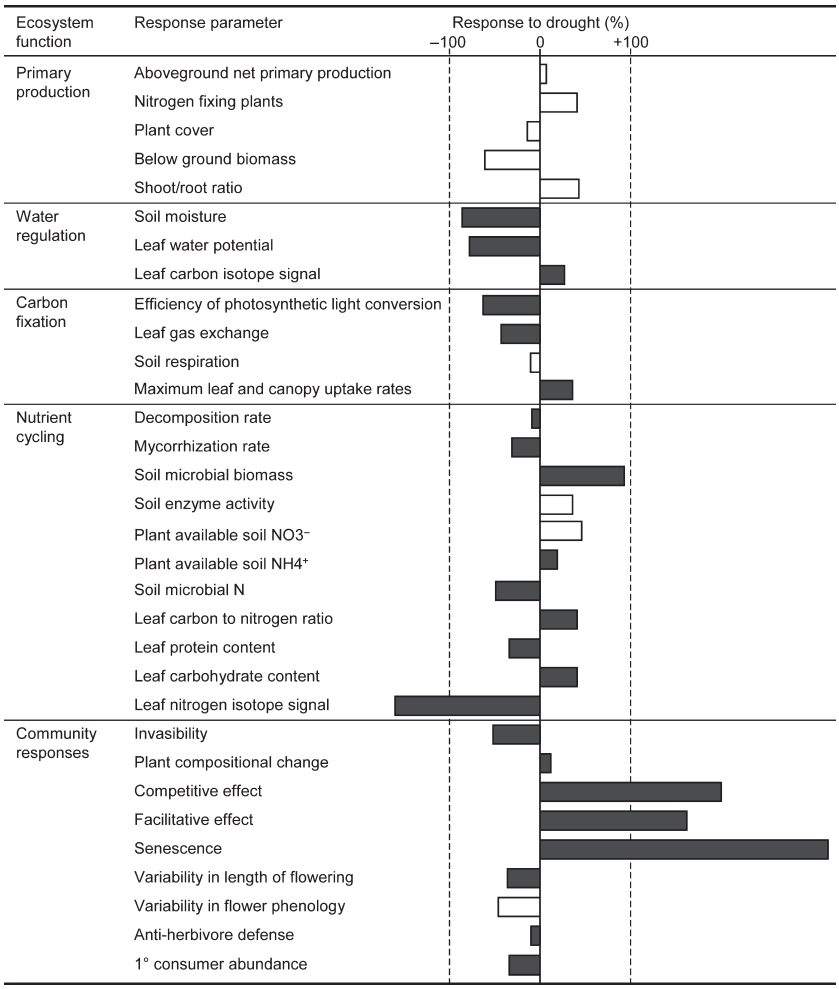

Fig. 1. Effects of recurrent severe drought events on 32 response parameters organized into ecosystem functions. All data were collected at the EVENT-I experimental site (Jentsch, Kreyling & Beierkuhnlein 2007) in Central Europe during the years 2005–2009. A parameter is marked as significant (filled black bar) if data of at least 1 year showed significant differences between drought and ambient conditions (ANOVA). Data shown represent maximum effects from years with highest drought effects, averaged over all three experimental grassland communities. For references of published details please refer to Materials and methods section.

drought and control was found in community composition when comparing the species abundance distribution at each time step to the initial species abundance distribution by a similarity index. Further, drought increased leaf senescence and caused shifts in flower phenology with regards to variability in length of flowering and mid-flowering day of particular species in some years (for detailed results on shifts in phenology see Jentsch *et al.* 2009). According to the decreased feed value of plant tissue, primary consumer abundance was decreased by drought.

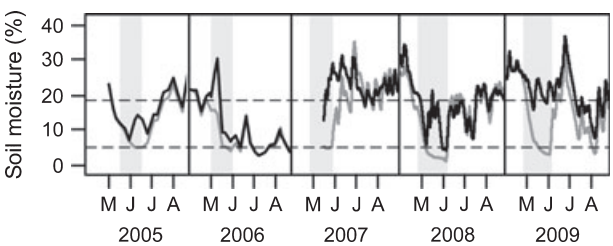

Fig. 2. (a) Above-ground Net Primary Production (ANPP), (b) cover of green biomass, and (c) root length over five growing seasons (mean ± SE over all species compositions in grassland, $n = 15$ per data point).

Discussion

Our experimental approach has the ambitious goal to search for a synthesis of the wide range of drought responses collected in a single study. Our goal is to see whether general patterns about different categories of responses can be drawn within a single temperate grassland study system. In the following, we first discuss particular drought responses, and then suggest potential reasons why the responses may differ among the five major ecosystem functions.

WATER REGULATION

Soil moisture dynamics and other soil-related parameters integrate how biological systems respond to climate change (Emmett *et al.* 2004). Soil water content was significantly reduced by drought in our experiment, but there were strong differences between years (Fig. 2). Natural precipitation during the manipulation periods is of importance here, as the years 2005–2008 all included some natural dry spells and effect size of the drought manipulation therefore was bigger in 2009 when no such natural event occurred. Still, it is not completely clear how precipitation regimes translate into variation of the soil moisture regime (Weltzin *et al.* 2003; Potts *et al.* 2006; Dermody *et al.* 2007). There is a growing number

Table 2. Temperature and precipitation sums (added daily amount) for each year until the start of the drought manipulation and the respective alteration from the long-term mean (1971–2000, data: German Weather Service station Bayreuth)

Year	Temperature sum (1 January to start of manipulation)	Relative change of temperature sum compared to long-term mean (%)	Precipitation sum (1 January to start of manipulation)	Relative change of precipitation sum compared to long-term mean (%)
2005	824.7	−3	259.7	−9
2006	394.7	−38	208.3	+10
2007	978.7	+77	258.6	+9
2008	757.6	+40	282.2	+19
2009	574.9	+4	246.4	+4

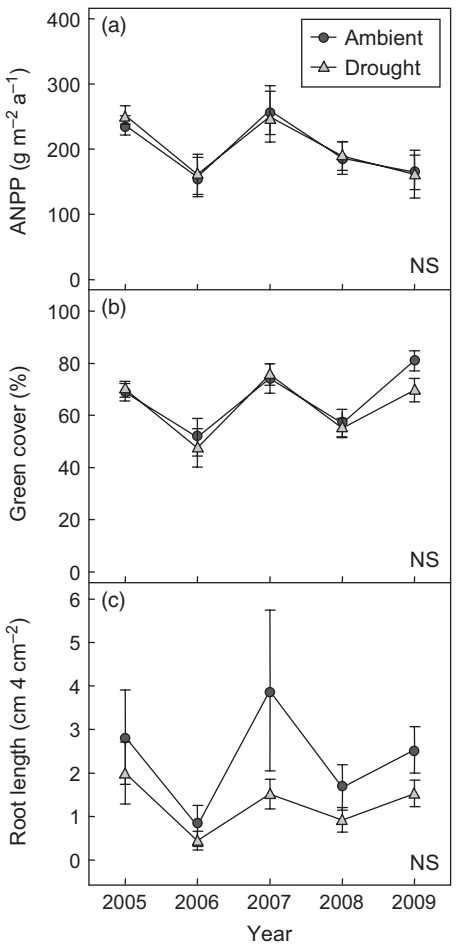

Fig. 3. Soil moisture in the EVENT experiment at −2 to −7 cm during manipulation (light grey boxes) and recovery after extreme drought for control (black line) and drought (grey line). MJJA = May, June, July, August. Plant-available water is shown between the dashed lines: permanent wilting point (pF = 4.2) and field capacity (pF = 1.8). See Materials and methods for technical details.

of studies explicitly addressing time lags between precipitation manipulation and the soil moisture regime (Dermody *et al.* 2007; Sherry *et al.* 2007), soil moisture storage (Potts *et al.* 2006) or soil hydrological properties as affected by interacting climatic drivers (Bell, Sherry & Luo 2010). However, re-wetting dynamics (Xiang *et al.* 2008), soil drying (St Clair *et al.* 2009) and potential carry-over effects between recurrent heavy rainfall or drought events have not been analysed in much detail. The transformation of precipitation pulses to increased soil water contents available to plant roots and soil biota for uptake can be complex: soil depth, soil texture, parent material, organic matter content, vegetation type, presence of plant functional types, LA index and soil surface characteristics all affect the partitioning between interception, run-off, infiltration and subsequent hydraulic re-distribution, soil evaporation, plant water uptake and seepage (Loik *et al.* 2004; Bell, Sherry & Luo 2010).

Amount, frequency and seasonal timing of soil water available for plants, soil fauna and soil microbes will basically determine much of the ecosystem response to more extreme precipitation regimes. While in this experiment we only manipulated the amount of soil water available to plants, seasonality issues appear to be an emerging research frontier. Yet, the major remaining challenge is to assess how future precipitation regimes with more extreme precipitation events affect – due to alterations in soil moisture – biogeochemical cycles, biotic interactions and ecosystem functions.

PRIMARY PRODUCTION

We found that drought has resulted in pronounced effects in the functional performance such as carbon fixation and nutrient cycling of plant communities and of individual species as well as in fluxes and pools. However, all ecosystem properties related to primary production remained stable throughout all 5 years of the experiment, despite recurrent severe drought events and despite different pre-experimental soil water status between years. In temperate grasslands, experimental drought events tend to reduce biomass productivity (Sternberg *et al.* 1999; Grime *et al.* 2000; Kahmen, Perner & Buchmann 2005). Fay *et al.* (2003), however, showed that the magnitude of reduction in ANPP is the same if rainfall quantity is reduced by 30% or if inter-rainfall-intervals are increased by 50% without a change in the annual amount of precipitation. Presumably, complementary responses in species interactions contributed to buffering primary production at the community level without changing community composition in our experiment (Wang, Yu & Wang 2007; Kreyling *et al.* 2008a,b,c,d). For example, the competitive effect of neighbouring plants on *L. corniculatus* was increased by drought as well as the facilitative effect of neighbouring plants on *A. elatius*. This is in accordance with a long-term study of 207 grassland plots, which demonstrated that biodiversity stabilizes community and eco-

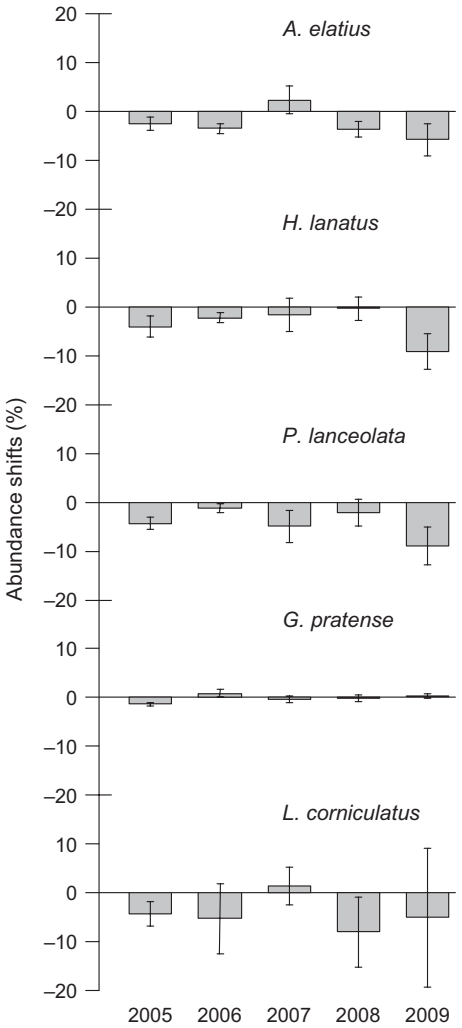

Fig. 4. Abundance shift (%) of the grassland species *Arrhenatherum elatius, Holcus lanatus, Plantago lanceolata, Geranium pratense* and *Lotus corniculatus* for the years 2005–2009 (mean ± SE of the absolute deviation of species cover under drought from the mean of control, $n = 15$ for *A. elatius* & *H. lanatus*, $n = 10$ for *P. lanceolata*, $n = 5$ for *G. pratense* & *L. corniculatus* per data point). No significant drought effects on species cover (ANOVA for each year and Linear Mixed-Effect Model for long-term trends: $P < 0.05$).

system processes, but not population processes (Tilman 1996). Here, primary production was one of the key parameters studied. The persistence of this general ecosystem function was stronger than expected. Concerning below-ground production, several studies (Trillo & Fernandez 2005; Newman, Arthur & Muller 2006) report increased root biomass in response to chronically decreased water supply, while a complete water withdrawal over defined periods of time result in stable or decreased below-ground biomass (Kreyling *et al.* 2008a).

CARBON FIXATION

Results from ecosystem CO_2 measurements showed a 36% increase in GPP during drought in the grassland, but a reduction in the assimilatory capacity of the leaves (Fig. 1). During water stress, there was an increase in tillering, leading to increased photosynthetic area of particular species, yet not an increase in absolute cover or green cover of the community. Thus, even though CO_2 assimilation was reduced at leaf level as a result of water stress, the overall effect of the large LA presented by the tillers lead to an increase in the contribution of particular species to ecosystem productivity, compensating for reduced photosynthetic rates at leaf level. Declining stomatal conductance as a result of stomatal closure was responsible for the observed low leaf-level CO_2 assimilation rates during stress. Zavalloni *et al.* (2009) reported a reduction in leaf assimilation, but increased biomass production in grassland subjected to extreme drought. In contrast, Stitt & Schulze (1994) point out that changes in photosynthesis not necessarily lead to changes in growth or biomass.

NUTRIENT CYCLING

Nutrient cycling was clearly affected by drought. The annually recurrent drought events increased leaf C:N and plant-available soil ammonium, whereas they decreased the decomposition rate and mycorrhization rate. Obviously, water stress has an impact on the activity and abundance of ammonium oxidizing prokaryotes, resulting in increased ammonium in the soil, which, however, can hardly be taken up by plants (Gleeson *et al.* 2010). The microbial community seems generally irresponsive to drought treatment where the only significant effect was an increase in microbial biomass, however the relative abundance of different microbial groups remained unchanged except for arbuscular mycorrhizal fungi. This is in accordance with other findings showing that drought changes community structure in arbuscular mycorrhizal fungi including their carbohydrate and nitrogen storage bodies, so that they take up less nitrogen (Shi *et al.* 2002). Our results suggested that composition of microbial groups in soils is generally resistant to drought treatment. This observation is in agreement with previous reports (Williams 2007, Williams & Xia 2009; Andresen *et al.* 2010). Both leaf C:N ratio(s?) and microbial data suggest that there was an increase in C:N ratio which may explain lower soil respiration under drought conditions. This may suggest lower activity of microbial communities which is reflected by the decreased rate of decomposition. In this study, leaf and microbial C:N ratio and litter decomposition responded to drought treatment, but biological and geochemical responses of climate treatment are complex (Andresen *et al.* 2010), and future work should include multi-factorial experiments taking into account environmental factors such as soil type, soil water and land use (Singh *et al.* 2010).

Additionally, our results show that climate extremes further affect the abundance of herbivores associated with the plant community. For instance, we suggest that the reduction of abundances of athropods by drought events may translate to changes in the top–down control of vegetation by herbivores and slowed decomposition dynamics due to a lower activity of decomposers.

COMMUNITY RESPONSES

Relative importance of each species in a community context was affected by the drought treatments as measured by the similarity of species abundances to the starting conditions for each plot. The effect size, however, was comparably small, presumably because species compositions were held constant over the course of the experiment by weeding out non-target species. Furthermore, competitive balance, based on species-specific biomass production, was altered and variability in flowering was affected. Particularly, averaged over all species, drought advanced the mid-flowering day within the season and expanded the length of the flowering period. On the other hand, no significant shifts in relative abundance of single species were observed (Fig. 4). Generally, however, shifts in species composition might require substantial lag phases (Grime *et al*. 2000; Buckland *et al*. 2001), especially as non-target species were not allowed to immigrate into our plots.

LIMITATIONS OF THE EVENT EXPERIMENT

All the results discussed above stem from one site, i.e. one particular climate, one soil type, one form of experimental manipulations and a limited set of species. Certainly, an array of factors such as the investigated ecosystem type, time scales, level of nutrient availability, water holding capacity of soils, level of biodiversity or particular design and execution of the experimental treatments will modify the effects of drought on ecosystem properties. Therefore, similar approaches from other sites and climatic conditions are clearly needed in order to test the generality of the observed findings. In particular, experiments with strongly controlled species compositions need to be compared to natural or semi-natural communities. Another important gap of knowledge that cannot be answered by our experiment is the importance of interactions between the climatic drivers, as there is clear evidence that effects of drivers such as warming, drought, N-deposition and CO_2-increase are not additive (Shaw *et al*. 2002; Andresen *et al*. 2010).

Generally, manipulation artefacts or hidden treatments are a concern for global change field experiments. Rain-out shelters are the usual device to simulate drought even though they are known to cause artefacts in the microclimate (Fay *et al*. 2000). Our artefact control treatment showed that the slight temperature increase and the alterations in irradiance or wind speed due to our shelters caused no effect on the measured response parameters, presumably because the shelters were active only during the short manipulation periods. Other artefacts, however, might be more important, yet less investigated, such as preferential site selection by animals due to the close proximity of different climatic conditions between the treatments blocks (Moise & Henry 2010). Such spatial patterns at small distances clearly differ strongly from drought effects at landscape levels.

We set the magnitude of the drought manipulation based on statistical recurrence of dry spells in the local climate data series (1961–2000). Recurrence of extreme events itself, however, is subject to climate change, leading to an amplification of precipitation extremes with ongoing climate change (Allan & Soden 2008). For the ambient conditions in our experiment, though, the statistical recurrence of the different manipulation years fell well within those of the long-term averages for air temperature, precipitation sum or length of rain-free periods (data not shown). This may be among the reasons, why we did not observe large effects on biomass production.

Conclusion

Our experimental data demonstrate that climate extremes initiate ecosystem-regulating functions such as water and nutrient cycling, gas exchange and compositional dynamics while maintaining primary production. They indicate an important contribution of ecological complexity to the maintenance of productivity in the face of increased temporal climate variability and extraordinary weather events. However, single species reactions can not be translated directly to the community and ecosystem level. A potential reason for different drought impacts on various ecosystem properties may lie in the temporal hierarchy of fast versus slow response patterns. In our temperate grassland, we observed the following response dynamics within half a decade of recurrent drought events: very fast alteration of soil moisture status, subsequent fast change in nutrient cycling and gas exchange, slow species-specific response in primary production, inertia in community productivity.

Such data on multiple response parameters within climate change experiments foster the understanding of mechanisms of resilience, of synergisms or decoupling in biogeochemical processes, and of fundamental response dynamics to drought at the ecosystem level.

As it was the case with the open questions on the consequences of the crisis of biodiversity, we see this complexity in studying impacts of climate extremes as a new chance for a boost in ecological theory. Additionally, comprehensive studies on the complex responses will help developing coping strategies for the adapted management of these ecosystems.

Future challenges consist of analysing responses for multiple ecosystem functions and at multiple levels of organization with the goal of assessing how they interact to influence emergent ecosystem properties, such as ecosystem function and stability. The observed stability in primary production in the face of recurrent severe drought does not mean that the responses at the ecosystem level are null. On the contrary, the observed changes in ecosystem-regulating functions in terms of gas exchange, nutrient cycling, water regulation and community stability suggest a prominent role of climate extremes in ecosystem response to climate change. However, modelling the behaviour of ecosystems during and after climate extremes at larger spatial scales and over longer periods of time requires more in-depth knowledge on possible response mechanisms at the level of plant communities. Potential epigenetic, physiological or trophic responses need to be rigorously further explored experimentally. Laboratory studies on molecular mechanisms have to be related to stud-

ies with the same species in the field. Field studies must integrate various levels of functional diversity (Beierkuhnlein *et al.* in press). Phenotypical diversity of populations has to be considered. Life cycles of plant species and cohorts can be of crucial importance. Gradients in soil types have to be integrated. Then, we can reach a better understanding of the mechanisms that are initiated in plant communities by extreme events.

Future work is needed to elucidate the role of biodiversity and of biotic interactions in modulating ecosystem response to extreme weather events. Further, we need more data on impacts of climate extremes on multiple ecosystem properties from various ecosystems and biomes, in order to foster the search for generality across different categories of response. Here, a major challenge is to assess the speed of response across various parameters, including long-term feedbacks, i.e. caused by a nitrogen-dependent feedback on productivity (Haddad, Tilman & Knops 2002).

Generally, scientists are challenged by relating the ecosystem properties measured (here: net ecosystem exchange, biomass above- and below-ground, carbon fixation by photosynthesis, nutrient ratios) to ecosystem functions and services, such as productivity, carbon fixation, nutrient cycling, decomposition and water regulation. Measuring ecosystem services is a fast-developing research area with many debates on how to assess the services adequately.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Table S1. Search items for searching the ISI Web of Science® Data base for publications on weather events and climate extremes. Asterisks are place holders within the search string.

Table S2. Links for searching the ISI Web of Science® Data base for publications on weather events and climate extremes.

Table S3. Sampling years of all response parameters presented in Figure 1. Given are data from years with maximum drought effect.

Figure S1. Research on ecological effects of climate extremes and weather events based on publications found in the ISI Web of Science (for search details see Table 2). (a) Temporal development of the number of publications on climate extremes (n = 380) in the last two decade (shown is only the last decade); total yield 1134 peer-reviewed papers. (b) Studied extreme weather events (n = 464 including double or triple assignments) of the relevant peer-reviewed papers (n = 380) yielded by the literature study. Twenty four publications did not specify the event. (c) Research activity in the three main biomes by proportion of publications based on 380 peer-reviewed papers particularly studying effects of climate extremes on ecosystem functions. Grassland includes deserts, peat and wetlands. Shrubland includes tundra. Any one paper may have been assigned to multiple subject areas. (d) Studied effects of extreme weather events on ecosystem properties arranged by ecosystem services and functions based on 380 peer-reviewed papers particularly studying effects of climate extremes on ecosystem functions.

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