Combined effects of warming and elevated CO_2 on the impact of drought in grassland species

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Abstract

Aims Drought is a major growth limiting factor in the majority of terrestrial ecosystems and is expected to become more frequent in the future. Therefore, resolving the drought response of plants under changing climate conditions is crucial to our understanding of future ecosystem functioning. This study responds to the need for experimental research on the combined effects of warming, elevated CO_2 and drought, and aims to determine whether the response to drought is altered under future climate conditions.

Methods Two grassland species, *Lolium perenne* L. and *Plantago lanceolata* L., were grown in sunlit climate-controlled chambers. Four climates were simulated: (1) current climate, (2) current climate with drought, (3) a warmer climate with drought, and (4) a climate with combined warming, elevated CO_2 and drought.

Results Warming did not alter the drought response, neither directly through photosynthesis nor indirectly through changes in water consumption. Also for combined warming and elevated CO_2 there were no effects on the plant response to drought for any of the measured parameters. However, simultaneous warming and elevated CO_2 mitigated the biomass response to drought through a positive pre-drought effect on photosynthesis and biomass response.

Conclusions Our results indicate that a positive pre-drought effect of combined warming and elevated CO_2 has the potential to compensate for drought-induced biomass losses under future climate conditions.

Abbreviations

C	Climate treatment with current air temperature and current atmospheric $[CO_2]$
D	Climate treatment with current air temperature, current atmospheric $[CO_2]$ and a drought period
DOY	Day of year
DT	Climate treatment with future temperature, current atmospheric $[CO_2]$ and a drought period
$DTCO_2$	Climate treatment with future temperature, future atmospheric $[CO_2]$ and a drought period
T_{air}	Air temperature

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PAR	Photosynthetically active radiation
ET	Evapotranspiration
SWC	Soil water content
RGR	Relative growth rate
LAI	Leaf area index
RAQ	Resin adsorption quantity
g_s	Stomatal conductance
A_{sat}	Light-saturated net CO_2 assimilation rate
ANOVA	Analysis of variance
SD	Standard deviation
SE	Standard error

Introduction

The impact of single climatic changes like warming, elevated CO_2 and extreme drought events, on plant growth have been the subject of numerous studies and have previously been synthesized (e.g. Chaves et al. 2002; Hudson et al. 2011; Long et al. 2004; Rustad et al. 2001). However, experimental research on plant responses to simultaneously occurring climate change factors is still rare, despite some recent efforts (Bloor et al. 2010; Mikkelsen et al. 2008). Yet, these studies are crucial for our understanding of future ecosystem functioning since most multifactor experiments to date have shown interactive and often unpredicted effects of multiple climate change factors (Albert et al. 2011a; Larsen et al. 2010; Shaw et al. 2002).

Precipitation is the major controlling driver of above-ground biomass production in grasslands (Sala et al. 1988) and is expected to exhibit prolonged summer droughts and a higher frequency of extremes in the future (IPCC 2007). Plant responses to drought stress in the field have been intensively studied (Chaves et al. 2002). Low soil water contents limit above-ground biomass production mainly through decreases in stomatal conductance, down-regulation of the photosynthetic machinery and/or increased allocation to the roots (Chaves et al. 2002). Moreover, drought can impair nutrient uptake (Hsiao 1973; Viets 1972) and suppress nitrogen (N) cycling through changes in the balance between immobilization and mineralization of N (Andresen et al. 2010).

In the absence of photosynthetic acclimation, elevated temperature increases rates of photosynthesis as long as the plant's optimal temperature is not exceeded (Berry and Björkman 1980). In general, biomass production is increased by warming if water is not

limiting (Dukes et al. 2005; Penuelas et al. 2007; Rustad et al. 2001; Wu et al. 2011). As the transpiration of a stand is approximately proportional to its green mass, this warming-induced biomass increment could increase water consumption (Larcher 2003). Moreover, this biomass-dependent enhanced water consumption can be further exacerbated by increased evapotranspiration through a direct effect of warming (Allen et al. 2003). Therefore, warming is expected to deteriorate drought stress through enhanced soil water depletion.

Elevated CO_2 often decreases plant water consumption due to reduced stomatal conductance, which leads to increased water use efficiency (Ainsworth and Long 2005) and reduced soil water depletion (Leuzinger and Korner 2007; Morison and Gifford 1984; Robredo et al. 2007). This can enable maintained plant carbon uptake during drought periods in elevated CO_2 (Leuzinger and Korner 2007; Morison and Gifford 1984; Robredo et al. 2007), but may be insufficient to prevent harmful carbon starvation during intense, frequent or long drought periods (McDowell et al. 2008). Moreover, elevated CO_2 causes allocation of carbon to root growth, which can enhance the plants' capacity to acquire water (De Luis et al. 1999). Hence, under future climate, elevated CO_2 could compensate for the possible detrimental effect of warming during extreme drought periods through a stimulation of photosynthesis or through indirect effects on water consumption and uptake. Besides effects on the plant water status, elevated CO_2 and warming have been found to alter the availability of N through complex changes in the release of nutrients from soil organic matter turnover and mineralization (Andresen et al. 2010).

To assess the impact of drought under future climate conditions, monocultures and mixtures of two grassland species were grown in four simulated climates, i.e. (i) current climate, (ii) current climate with a drought period, (iii) a warmer climate with a drought period and (iv) a climate with combined warming and elevated CO_2, and a drought period. In an earlier experiment on grassland communities we found no effect of combined warming and elevated CO_2 on the biomass response to drought (Naudts et al. 2011). However, in that experiment we only studied the combined effect of warming and elevated CO_2, whereas the additive experimental design of the present study provides insight on the effects of the single factors and allows comparison between current climate conditions

without drought and future climate conditions, including drought, warming and elevated CO_2. We hypothesize that: (1) warming deteriorates the negative impact of drought on plant growth through enhanced water consumption, and (2) combined warming and elevated CO_2 mitigates the detrimental drought impact through stimulated photosynthesis, reduced water consumption, carbon allocation to the roots and/or enhanced nutrient availability.

Material and methods

Experimental set-up

The study was performed at the Drie Eiken Campus, University of Antwerp, Wilrijk, Belgium (51 ° 09′ N, 04 ° 24′ E), where average annual precipitation is 776 mm (evenly distributed throughout the year) with an average annual air temperature of 10.8 °C. The experimental set-up consisted of 16 sunlit, south facing climate-controlled chambers. The distances between the chambers were maximised to avoid mutual shading. The interior surface area was 1.5×1.5 m, the height at the north side was 1.5 m and at the south side 1.2 m. The top of the chambers consisted of a colourless polycarbonate plate (4 mm thick), whereas the sides were made of polyethylene film (200 μm thick), both UV transparent. In the chambers four climate treatments (four chambers per climate) were simulated in an additive design: (1) a climate treatment mimicking current temperature and atmospheric $[CO_2]$ (current climate, C); (2) a climate also mimicking current temperature and atmospheric $[CO_2]$, but including a drought period (D);(3) a climate mimicking future temperature and current atmospheric $[CO_2]$, including a drought period (DT); and (4) a climate treatment mimicking future temperature and atmospheric $[CO_2]$, including a drought period (DTCO$_2$). The chambers simulating current temperatures (C and D) followed fluctuating air temperatures mimicking an average daily air temperature course, calculated for the period 1996–2005. The chambers with future temperature simulated a 3 °C warming compared to the simulated current climate. The climate treatment with elevated CO_2 had a target CO_2 concentration of 620 ppm. The CO_2 concentration was measured and regulated with a CO_2 control group with an infrared analyser (WMA-4, PPSystems, Hitchin, UK). In C, D

and DT chambers the CO_2 concentration was 392 ± 42 ppm (SD), while it was 615 ± 81 ppm (SD) in DTCO$_2$. Relative humidity and air temperature (T_{air}) were monitored with a humidity-temperature sensor (Siemens, type QFA66, Germany) and photosynthetically active radiation (PAR) was measured with a quantum sensor (SDEC, type JYP1000, France). All microclimate parameters were automatically logged every 30 min. Monthly average air temperature in C and D chambers was 12.33, 16.60, 18.80, 14.69 and 15.53 °C in May, June, July, August and September, respectively. For DT and DTCO$_2$, chambers were on average 3.02 ± 0.82 °C (SD) warmer than chambers with current temperature. Average daily PAR sum inside the chambers was 23.1, 25.3, 34.6, 42.1, 39.7 $mol\,m^{-2}\,d^{-1}$ in May, June, July, August and September, respectively, and differed little between climate treatments (maximum delta of 2.4 ± 0.5 mol $m^{-2}\,d^{-1}$). Average vapour pressure deficit was 0.35 ± 0.02 and 0.46 ± 0.02 (SD) in the climate treatments with ambient and warmed air temperature, respectively. The experimental drought period was applied by withholding water for 20 days (DOY 197–217).

Inside the chambers, plant communities with two common and co-occurring grassland species, *Lolium perenne* L. and *Plantago lanceolata* L. were assembled. Both species have a C3 photosynthetic pathway. The plants were sown at the end of March 2010 and the seedlings were transplanted at the end of April (day of year, DOY 116–118) into PVC containers (19 cm inner diameter, 40 cm height), filled with sandy soil (93.2 % sand, 4.6 % silt, 2.2 % clay; field capacity 0.13 $m^3\,m^{-3}$; pH 7.6; total Kjeldahl-N 0.42 g kg^{-1}; 1 % C in humus). Each community (and thus each container) contained six individuals planted in a hexagonal grid with a 5 cm interspace and one individual positioned at the centre of the grid. In each chamber 24 communities with four different plant compositions (further referred to as composition) were assembled: (1) six monocultures of *Lolium perenne* L.; (2) six monocultures of *Plantago lanceolata* L.; (3) six mixtures of both species with *Lolium perenne* L. as central plant; and (4) six mixtures of both species with *Plantago lanceolata* L. as central plant. All communities were fertilised with 10 $g\,m^{-2}$ NH$_4$NO$_3$, 5 $g\,m^{-2}$ P$_2$O$_5$, 10 $g\,m^{-2}$ K$_2$O and micro-elements (Fe, Mn, Zn, Cu, B, Mo). The fertiliser was given dissolved in water in two equal amounts at DOY 140 and 180. Irrigation was calculated from the monthly rainfall over the

period 1995–2005 and corrected for differences in evapotranspiration (ET) inside and outside the chambers. To this end, De Boeck et al. (2006) calculated ET inside the chambers from changes in soil water content (SWC) and the amount of administered water. The outside ET was calculated with Hamon's equation (Haith and Shoemaker 1987) based on day length, vapour pressure and air temperature. The containers were watered every 2 days according to the 10 year average of 14 to 15 rain days per month during the growing season. Total monthly irrigation matched 61.5, 64.4, 85.1, 80.2 and 80.9 mm in May, June, July, August and September, respectively. During the experimental drought period irrigation was interrupted in D, DT and DTCO$_2$. Water could freely drain from the containers while capillary rise of ground water towards the containers was prevented by a drainage system placed below the chambers. In each chamber, four containers (one in each composition) contained a profile probe tube for the PR2 soil moisture sensor (Delta-T Devices Ltd., UK). Soil water content (SWC) was measured once a week before the drought treatment (DOY 130–193) and twice a week during the experimental drought period.

Biomass harvest and resin bags

In each chamber above-ground biomass (shoot above 3.5 cm and stubble) of two communities per composition (eight communities per climate treatment) was harvested before drought, after drought and at the end of the growing season (DOY 197, 217 and 307). At the same time root biomass was also determined in one community per chamber (four replicates per climate treatment). Root samples were washed until they were free of soil.

Total community biomass included the sum of above-ground and root biomass and root/shoot ratio was calculated by dividing root biomass with above-ground biomass. Relative growth rate (RGR) during and after the drought period represents the increase in total biomass during the respective period, relative to the initial total biomass. All plant material was dried at 70 °C for 48 h and then weighed. Total leaf area was determined on four communities per climate treatment (one per chamber per composition) with a portable area meter (LI-3000A, Li-COR, NE, USA) before and after the drought period. Leaf area index (LAI) was calculated as the ratio of total leaf area (of the

community) to the ground area of a container. Ion exchange resin bags were used to estimate N availability in the presence of functioning plant roots (Binkley and Matson 1983). One resin bag was buried 5 cm below the soil surface in one community per composition in each chamber (four replicates per climate treatment) and collected during each harvest. Ammonium (NH$_4$) and nitrate (NO$_3$) were extracted from the resin bags in 50 mL 2 M HCl and measured colorimetrically. Resin adsorption quantity (RAQ) was calculated as cV/MA, where c is the concentration of nutrient in the HCl extract, V is the volume of the extract, M is the molar mass of the nutrient, and A is the capsule surface (11.4 cm^2).

Leaf gas exchange

Leaf stomatal conductance (g$_s$) was measured before and after the drought period (DOY 197 and 217), on the most recently matured leaf of the central plant, with an automatic porometer (AP4, Delta-T Devices Ltd., UK), taking into account abaxial and adaxial sides of the leaves. Also light-saturated net CO$_2$ assimilation rate (A$_{sat}$) was determined on the most recently matured leaf of the central plant with a portable gas exchange system (LI-6400, Li-COR, NE, USA) before and after drought (DOY 197 and 217). Leaf chamber conditions were controlled at 380 ppm CO$_2$ and 23.5 °C (block temperature) at saturating PAR (1,500 μmol m^{-2} s^{-1}) and ambient relative humidity for the current climate treatment. In DT the block temperature was 26.5 °C and for DTCO$_2$ the CO$_2$ concentration was controlled at 610 ppm. Measurements were performed on two communities per composition in each chamber, yielding eight measurements per composition and climate combination.

Data analysis

Analyses of variance (ANOVA) were performed on above-ground, root and total community biomass, A$_{sat}$, g$_s$, LAI, RAQ and RGR with climate and composition as fixed factors. For A$_{sat}$ and g$_s$ also species was included in the model. Soil water content was analysed with repeated measures ANOVA with DOY, composition and climate as fixed factors. Chamber was always included as a random factor nested within climate. Non-significant factors were excluded from the model. In case of significant effects, the means

were compared a posteriori with Tukey corrections for multiple comparisons. Effects of drought were examined by comparing C and D, effects of warming on drought by comparing D and DT and effects of combined warming and elevated CO_2 on drought by comparing D and $DTCO_2$. Analyses were performed in SAS (version 9.1, SAS Institute Inc., Cary, NC) using the mixed procedure (Littell et al. 1996).

Parameters were analysed at three moments in the experiment: 'before drought' (DOY 197), '(immediately) after drought' (DOY 217) and at the end of the growing season 'after recovery' (DOY 307). The study focuses on the effects of warming and elevated CO_2 on the drought response and to clearly represent these results the intrinsic differences between plant compositions were not extensively discussed in the results, but briefly summarized here. Overall differences between monocultures and mixtures were not significant, however, there was an intrinsic difference between monocultures of *Lolium perenne* L. and *Plantago lanceolata* L. (the latter had a slightly lower biomass production). Differences in the drought response between plant compositions (climate x composition) were not significant for any of the parameters in none of the climate treatments ($P > 0.1$ in all cases) and therefore the climate x composition interaction could be excluded from the model. For reasons of clarity, and because the drought response did not differ between compositions for any of the climate treatments, we only show the results of the mixtures in the figures.

Results

Drought response under current climate conditions

The drought response under current climate conditions was determined by comparing plant communities in C and D. Soil water content decreased during the imposed drought from DOY 203 onwards and remained lower after re-watering until DOY 235 (Fig. 1; Table 1). Drought reduced above-ground, root and total biomass production respectively with 15.3 %, 21.5 % and 22.1 %. Also g_s, LAI, A_{sat} and RGR were lower in D than in C (data not shown, Figs. 2 and 3, respectively; Table 1). Except for root biomass production, plant communities were fully recovered from the drought-induced growth reduction by the end of the growing season (Fig. 3; Table 1). Root/shoot ratio was not altered by drought, neither immediately after drought nor at the end of the season (data not shown; Table 1). Also N availability in the plant communities was not changed by drought (Fig. 4; Table 1).
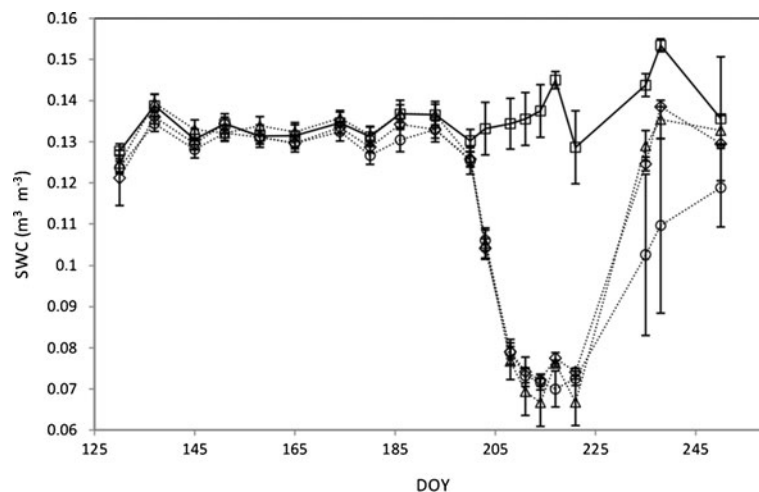
Fig. 1 Time course of soil water content (SWC) in communities with mixtures of *Lolium perenne* L. and *Plantago lanceolata* L. in current climate conditions (*white square*), current climate conditions with a drought period (*white circle*), warmer climate conditions with a drought period (*white triangle*) and future climate conditions with combined warming, elevated CO_2 and a drought period (*white diamond*). The drought period was initiated at day of year (DOY) 197 and re-watering started at DOY 217. Means±SE are indicated

Table 1 Significance levels (p-values) from a posteriori comparisons of measured parameters in monocultures and mixtures of *Lolium perenne L.* and *Plantago lanceolata L.* in current climate conditions (C), current climate conditions with a drought period (D), warmer climate conditions with a drought period (DT) and future climate conditions with combined warming, elevated CO_2 and a drought period ($DTCO_2$). The drought effect was determined by comparison of C with D, the warming effect by comparison of D with DT and the combined warming and elevated CO_2 effect by comparison of D with $DTCO_2$. P-values were corrected for multiple comparisons with Tukey. P-values are presented in bold when significant (<0.05). Parameters were analysed at three moments in the experiment: 'before drought' (DOY 197), '(immediately) after drought' (DOY 217) and at the end of the growing season 'after recovery' (DOY 307)

	Drought effect		Effect of warming			Combined effects of warming and elevated CO_2		
	After drought	After recovery	Before drought	After drought	After recovery	Before drought	After drought	After recovery
SWC	**<0.001**	>0.100	>0.100	1.000	1.000	>0.100	>0.100	>0.100
g_s	**<0.001**	NA	1.000	1.000	NA	0.900	0.973	NA
LAI	**<0.001**	NA	0.194	**0.048**	NA	0.960	**0.031**	NA
A_{sat}	**0.024**	NA	0.685	0.771	NA	**0.012**	0.325	NA
AG	**0.050**	0.604	0.158	0.893	0.932	**0.001**	**0.049**	0.829
Roots	**0.006**	**0.006**	0.728	0.514	0.426	0.318	0.482	0.998
Total	**0.006**	0.098	0.215	0.692	0.990	**0.004**	**0.050**	0.965
Root/shoot	0.991	0.089	0.710	0.965	**0.018**	0.112	0.892	0.341
RGR	**0.020**	0.997	0.383	0.086	0.965	**0.011**	0.764	0.859
N availability (NO_3)	0.901	0.234	0.688	0.765	0.518	0.117	0.834	0.999
N availability (NH_4)	1.000	0.567	1.000	0.531	0.506	0.960	0.740	0.751

Effect of warming on the drought response

The effect of warming on the drought response was determined by comparing D and DT. Warming did not enhance soil water depletion compared to the drought period under current climate conditions (Fig. 1; Table 1). Moreover, except for LAI, which was slightly lower in DT than D during drought, warming did not alter any of the observed responses to drought, neither immediately after drought nor at the end of the growing season (Figs. 1, 2, 3, 4; Table 1). Because the overall absence of a warming effect on the drought response could originate from a possible beneficial effect of warming before the drought, we also considered the pre-drought period. However, also before the drought, warming did not affect SWC, g_s, A_{sat}, LAI, N availability, RGR and biomass production (Figs. 1, 2, 3, 4; Table 1).

Combined effect of warming and elevated CO_2 on the drought response

The combined effect of warming and elevated CO_2 on the drought response was assessed by comparing D and $DTCO_2$. Immediately after drought, above-ground and total biomass production was significantly higher in $DTCO_2$ than in D, suggesting that the drought response was alleviated by combined warming and elevated CO_2 (Fig. 2; Table 1). However, this mitigating effect on the drought response was not supported by any of the other parameters: LAI, g_s, RGR, A_{sat}, N availability did not differ between D and $DTCO_2$ (data not shown, Figs. 2 and 4, respectively; Table 1). This can be explained because the mitigating effect of combined warming and elevated CO_2 originated in a positive pre-drought effect of combined warming and elevated CO_2: stimulation of A_{sat} resulted in a higher above-ground and total biomass production before drought (Fig. 3; Table 1).

Discussion

Drought inhibited photosynthesis and biomass production, but did not alter root/shoot ratio or N availability. Further, LAI and g_s, which are the main plant traits affecting evapotranspiration, where decreased. We did not observe any changes in root/shoot ratio or N availability, which could be explained by the short duration of the experiment. Decreased photosynthesis, biomass
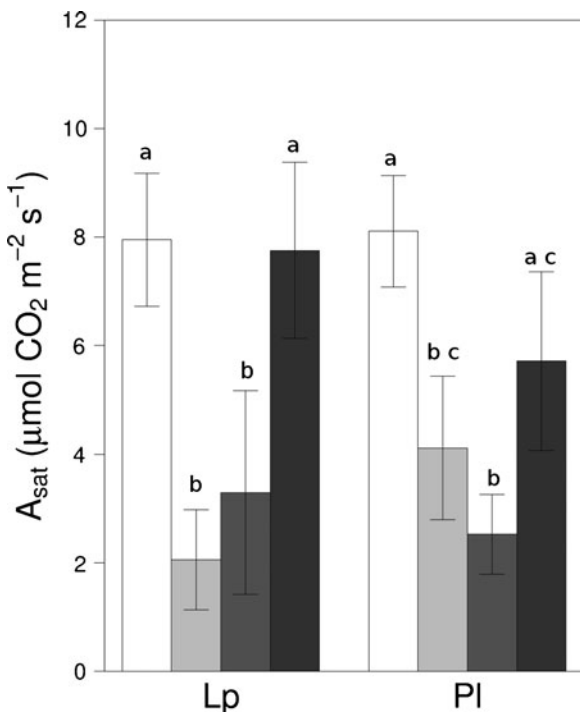
Fig. 2 Light-saturated net assimilation rate (A_{sat}) of *Lolium perenne* (Lp) and *Plantago lanceolata* (Pl) at the end of a drought period in communities with mixtures of these species. Measurements were performed on the most recently matured leaf of the central individual in the plant community. Plants were grown in current climate conditions (*white bars*), current climate conditions with drought (*light grey bars*), warmer climate conditions with drought (*dark grey bars*) or future climate conditions (warming and elevated CO_2) with drought (*black bars*). In climate conditions with a drought period, irrigation was stopped for 20 days (DOY 197–217 in 2010). Means±SE are indicated. Letters indicate differences for a posterior comparisons between climate treatments, separately tested for three moments in the experiment: 'before drought' (DOY 197), immediately 'after drought' (DOY 217) and 'at the end of the growing season' (DOY 307)

production, g_s and LAI are common plant responses to drought (Chaves et al. 2002), but the effects of changing climate on these responses have rarely been examined (Albert et al. 2011a; Bloor et al. 2010; Kongstad et al. 2012).

Warming did not affect the biomass or photosynthetic response to drought, i.e. biomass production and photosynthesis were equally restrained by drought under current and warmer climate conditions. Combined warming and elevated CO_2 alleviated the drought response, or in other words, biomass production and photosynthesis were higher in $DTCO_2$ than in D immediately after drought. However, relative growth rate during drought did not differ between D
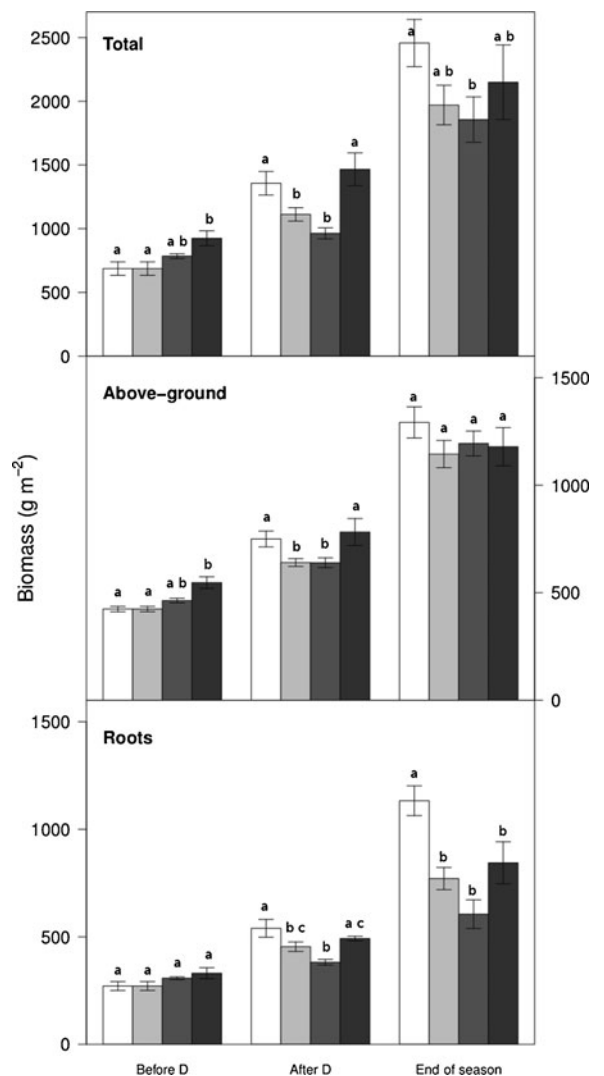
Fig. 3 Total (*top panel*), above-ground (*middle panel*) and root biomass (*bottom panel*) before drought, after drought and at the end of the growing season in communities with mixtures of *Lolium perenne* L. and *Plantago lanceolata* L. Plants were grown in current climate conditions (*white bars*), current climate conditions with drought (*light grey bars*), warmer climate conditions with drought (*dark grey bars*) and future climate conditions with combined warming, elevated CO_2 and drought (*black bars*). In climate treatments with a drought period irrigation was stopped for 20 days (DOY 197–217). Means±SE are indicated. Letters indicate differences for a posterior comparisons between climate treatments, separately tested for three moments in the experiment: 'before drought' (DOY 197), immediately 'after drought' (DOY 217) and 'at the end of the growing season' (DOY 307)

and $DTCO_2$, indicating that the mitigating effect of combined warming and elevated CO_2 mediated through a pre-drought effect rather than through the expected
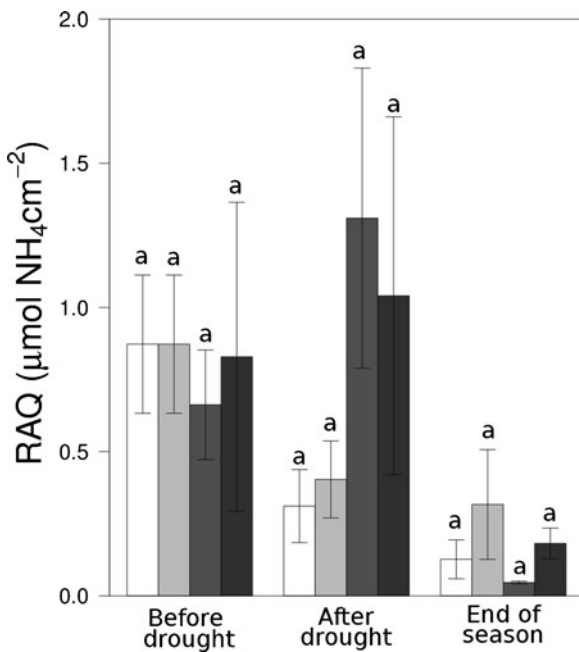
Fig. 4 Resin absorption quantity (RAQ) in communities with mixtures of *Lolium perenne* L. and *Plantago lanceolata* L. before and after a drought (D) period and at the end of the growing season. Communities were grown in current climate conditions (*white bars*), current climate conditions with drought (*light grey bars*), warmer climate conditions with drought (*dark grey bars*) and future climate conditions with combined warming, elevated CO_2 and drought (*black bars*). In climate treatments with a drought period irrigation was interrupted for 20 days (DOY 197–217). Means±SE are indicated. Letters indicate differences for a posterior comparisons between climate treatments, separately tested for three moments in the experiment: 'before drought' (DOY 197), immediately 'after drought' (DOY 217) and 'at the end of the growing season' (DOY 307).

changes during drought stress. Indeed, combined warming and elevated CO_2 enhanced photosynthesis and biomass production before drought, resulting in more biomass in $DTCO_2$ than in D immediately after the drought period. In a study that examined interactive effects between warming and precipitation change, overall above-ground biomass in drought stressed plots decreased along a warming gradient (Hoeppner and Dukes 2012). However, the biomass response to drought under current climate conditions was very small in that experiment, whereas in our study biomass was substantially decreased by single drought and did not decrease further by additional warming. Regarding the pre-drought mitigating effect of combined warming and elevated CO_2, our results are in agreement with an earlier study on grassland communities (Naudts et al. 2011). At the end of the growing season the overall

effects of changed climate conditions on biomass production were small to absent, confirming the high resilience to climate changes previously found in heath land plants (Kongstad et al. 2012).

Warming and/or elevated CO_2 can alter the biomass response to drought through direct changes on photosynthesis or indirect changes in evapotranspiration, root/shoot ratio and/or nutrient availability (Newman et al. 2011). The reduction in stomatal conductance during drought was not affected by warming and/or elevated CO_2 in our study. Similarly, g_s of temperate heath plants did not differ between D, DT and $DTCO_2$ (Albert et al. 2011b). Also the drought-induced inhibition of photosynthesis was not altered by single warming, however, combined warming and elevated CO_2 mitigated this photosynthetic inhibition. This is in contrast to an earlier study on grassland communities, in which stronger stomatal closure was found to cause a larger drought-induced reduction in A_{sat} in combined warming and elevated CO_2 (Naudts et al. 2011). The drought-induced decrease in LAI in our study was not altered by warming/or elevated CO_2. Effects of warming and elevated CO_2 on LAI have been found to be closely related to soil water availability: enhanced soil drying due to warming resulted in reduced LAI, while elevated CO_2 counteracted this effect by mitigating soil water depletion during drought (Dermody et al. 2007). Likewise, also in other studies, warming has been found to enhance soil drying in grassland communities (Zavalloni et al. 2008), while elevated CO_2 counteracted this effect (Naudts et al. 2011). The unaltered drought response of soil water availability and LAI under combined warming and elevated CO_2 in our study, confirm these results. However, the absence of a warming-induced enhanced soil drying was unexpected because evapotranspiration was anticipated to increase in a warmer climate due to a higher atmospheric demand. As additional warming did not further decrease g_s or LAI during drought, other warming-induced responses, like changes in phenology (e.g. accelerated senescence) might have contributed to the unaltered soil water availability under warmer environmental conditions (Zavaleta et al. 2003).

Single warming and warming combined with elevated CO_2 did not affect the root/shoot ratio from drought-stressed plants in this study. In literature, root/shoot ratio has generally been found to be increased by elevated CO_2 and/or drought (De Luis et

al. 1999; Luo et al. 2006), but also unaltered root/-shoot ratio has been reported in response to elevated CO_2, warming and/or drought (Lilley et al. 2001).

Hence, the drought response of photosynthesis, g_s, LAI, soil water availability and root/shoot ratio was not affected by changed climate conditions. Also N availability was not influenced by warming and/or elevated CO_2 during drought and across the entire growing season. Warming has been found to stimulate N mineralization, however, this effect can be counteracted when combined with elevated CO_2 and/or drought (Andresen et al. 2010; Hovenden et al. 2008). The lack of an effect of climate on the impact of drought on photosynthesis, water consumption, root/shoot ratio and N availability during drought confirmed the absence of an effect of warming and/or elevated CO_2 on the drought response at the biomass level.

Complementarity in the acquisition of water due to a combination of deep-rooted and shallow-rooted species can lead to more efficient water use in multi-species communities compared to monocultures (Berendse 1982; De Boeck et al. 2006). Therefore, one might expect an increased resistance against drought in mixtures. Nevertheless, our results show no difference in the biomass response to drought between monocultures and mixtures for any of the climate treatments. This is in agreement with several studies that show no effect of species number on the resistance of communities to abiotic stress (De Boeck et al. 2008; van Ruijven and Berendse 2010). However, these studies also report an increased pre-disturbance biomass and/or improved recovery in species rich communities (De Boeck et al. 2008; van Ruijven and Berendse 2010). The absence of such effects in our study can be explained by the low number of species and the rather short duration of the experiment. A study on the combined effects of species richness, drought, warming and elevated CO_2 has, to our knowledge, never been conducted. Although a much wider range of species numbers need to be tested and long-term experiments are required, our results suggest that also under future climate conditions, more diverse communities are not necessarily more resistant against drought.

To date, the main comparison of concern to semi-arid regions is the comparison between C and DT. Our results showed reduced stomatal conductance and, consequently, inhibited photosynthesis under dry,

warm climate conditions. The decreased photosynthetic rate resulted in a lower total biomass production at the end of the growing season, which was mainly explained by impaired root biomass production. The latter is in agreement with the warming-induced root death found in temperate grasslands (Edwards et al. 2004). As drought is predicted to become more frequent in the future (Meehl et al. 2000), the key comparison regarding the response of grasslands to future climate conditions, is the comparison between C and $DTCO_2$. Our results clearly indicate that biomass production did not differ between these climate conditions, because of a beneficial effect of combined warming and elevated CO_2 early in the season. These findings are in agreement with the second year of an experiment on an upland grassland in the French Central Massif, which also had an additive design, yielding T, DT and $DTCO_2$ treatments (Bloor et al. 2010). We cannot compare our findings on the effect of climate on the drought response with that study, because it did not include a single drought treatment. Yet, we can compare the results on the comparison between C and $DTCO_2$: similar to our findings, a positive effect on above-ground biomass in $DTCO_2$ during spring resulted in the lack of an effect between C and $DTCO_2$ later in the growing season. However, the beneficial pre-drought effect under future climate conditions was due to warming, whereas in our study it was mainly due to elevated CO_2. The larger responsiveness to warming in the upland grassland study can be explained by lower ambient temperatures than in our experiment, while the lack of an effect of elevated CO_2 can be explained by nutrient limitation. Our results contrasted with those of the third year of the experiment on upland grasslands (Bloor et al. 2010). In that third year, above-ground biomass in $DTCO_2$ was lower than in C, despite a positive effect of future climate conditions in spring. Further research on the long-term response of grasslands should clarify this inter-annual difference and determine whether the mitigating pre-drought effect of combined warming and elevated CO_2 will be sufficient to prevent drought-induced biomass loss in the future.

We conclude that, in contrast to our first hypothesis, single warming did not deteriorate the drought-induced inhibition of biomass production, neither directly on the photosynthetic level nor indirectly through changes in water consumption. In agreement with our second hypothesis, combined warming and

elevated CO_2 alleviated the drought response of above-ground and total biomass production. However, this mitigation mediated through a positive pre-drought effect and not through changes during drought, as was expected. Most importantly, our results indicate that the beneficial pre-drought effect of combined warming and elevated CO_2 has the potential to compensate for drought-induced biomass losses under future climate conditions.

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