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Lolium perenne and ***Plantago lanceolata***

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1 Combined elevated CO₂ and climate-warming induce lagged effects of drought
2 in *Lolium perenne* and *Plantago lanceolata*

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21

22 **Abstract**

23 Future climate scenarios predict increases in elevated atmospheric CO₂, air temperature and drought,
24 but the impacts of multiple climate change factors on ecosystem functioning remain unclear. In this
25 study, we compared drought responses of plants under future vs. current climate conditions. In
26 addition to focusing on stress during the drought itself, we also examined post-drought lagged effects,
27 and whether warming and elevated CO₂ alter these. We grew monocultures and mixtures of two
28 grassland species (*Lolium perenne* L. and *Plantago lanceolata* L.) in four simulated climate scenarios:
29 (1) current climate, (2) current climate with drought, (3) warmer temperature with drought and (4)
30 combined warming, elevated CO₂ and drought. *L. perenne* and *P. lanceolata* were influenced by the
31 climate scenario but not differently enough to modify the competitive balance. Warming aggravated
32 drought impacts on *L. perenne* and elevated CO₂ only partly compensated for these effects. In a
33 warmer climate, with or without elevated CO₂, drought continued to enhance senescence and mortality
34 in *L. perenne* long after the water shortage, while such lag effects were not observed in current
35 climate. In *P. lanceolata* a similar stimulation of senescence and mortality was induced, but only
36 under combined warming and elevated CO₂. These lag effects induced by the future climate may
37 reduce resilience.

38 Keywords:

39 Biomass; climate change; fluorescence; plant-plant interactions

40

41 **Introduction**

42 The global mean air temperature is expected to increase by 2-7 °C by the end of this century as a result
43 of rising levels of atmospheric carbon dioxide (CO₂) and other greenhouse gases (Allison et al. 2009).
44 Increased mean air temperature will likely accompany changes in precipitation, such as prolonged
45 summer drought (Stocker et al. 2013). Although the effects of future climate on plant growth has been
46 widely explored, most studies have investigated single factor effects such as elevated CO₂, warming,
47 and extreme events, or two-factor combinations of these. Studies that combine all of these climate
48 change components are rare because numerous experimental treatments are usually involved.

49 Drought stress is one of the major limitations for global plant productivity, primarily through
50 decreased stomatal conductance and down-regulation of photosynthetic machinery (Chaves et al.
51 2002), including photosynthetic enzyme activity (Reddy et al. 2004) and pigments (Jaleel et al. 2009).
52 Warming can stimulate plant biomass production via higher photosynthesis and/or mineralization rates
53 (Rustad et al. 2001; Wu et al. 2011), but can retard productivity via associated drought stress and heat
54 (De Boeck et al. 2008; Sherry et al. 2008). These associated stresses result from initially enhanced
55 evapotranspiration and soil water depletion (Allen et al. 2003). Similarly, warming is expected to
56 aggravate drought stress. Elevated CO₂ can stimulate plant growth directly through enhanced
57 photosynthesis, or indirectly through reduced water use and higher water-use-efficiency (Morgan et al.
58 2004), thereby counteracting drought effects (Morgan et al. 2004).

59 Climate extremes such as drought can cause lag or carry-over effects (Niu et al. 2014). Grassland
60 studies show that dry periods induce reduced productivity which continues long after the dry period
61 (Lauenroth and Sala 1992; Dunnett et al. 1998; O'Connor et al. 2001). Lagged responses may arise
62 from increased mortality over time (Bigler et al. 2007) and are detectable by comparing plant
63 functioning at the end of the growing season with that just after the drought period. As drought
64 frequency is likely to rise (Stocker et al. 2013), understanding how fast plant communities recover
65 from drought under elevated CO₂ and higher temperatures is needed to determine if insufficient or
66 compromised recovery threatens plant community stability in future conditions. It is unclear whether
67 or how elevated CO₂ and warming alter lagged plant responses after a drought event.

68 To address this, we used two common grassland species, *Lolium perenne* L., a perennial grass that
69 forms dense tussocks (Beddows 1967), and *Plantago lanceolata* L., a rosette-forming perennial forb
70 (Sagar and Harper 1964). The former has been reported to display isohydric behaviour under drought
71 while *P. lanceolata* is anisohydric (Van den Berge et al. 2014). These differences may determine
72 species-specific biomass responses to changing environmental conditions (Morecroft et al. 2004; Van
73 den Berge et al. 2014). For example, Morgan et al. (2011) showed that elevated CO₂ and warming
74 stimulated total aboveground biomass, due to more proportional growth of C₄, but not C₃ grasses. The
75 impact of climate change on species-specific productivity will depend on how climate affects water
76 availability, resource-use-efficiency and availability associated growth-limiting resources (Field et al.
77 1992; De Valpine and Harte 2001). Ultimately, species-specific productivity responses can alter
78 competitive interactions within plant communities by differentially changing resource requirements
79 among species (Dunnett and Grime 1999). Consequently, climate change factors could alter plant-
80 plant interactions, but empirical studies so far are rare.

81 The factors involved in climate change can interact, but there are few studies that document plant
82 response to drought, warming and elevated CO₂ (e.g. Hamerlynck et al. 2000; Dukes et al. 2005;
83 Kongstad et al. 2012; Naudts et al. 2013). Multifactor experiments have shown that combined
84 responses can be smaller than expected from additive, single-factor effects (Wu et al. 2011),
85 consequently multi-factor experiments are needed. Here we investigate effects of a summer drought on
86 grassland monocultures and mixtures, and explore how these effects are modified by warming and
87 elevated CO₂. Apart from focusing on impacts during the water-free period, we also examined whether
88 drought triggers lagged effects over growing season after the event has past, and whether warming and
89 elevated CO₂ alter these. The experimental design consisted of four simulated climate scenarios: (i)
90 current climate, (ii) current climate with drought, (iii) warmer climate with drought and (iv) warmer
91 climate with elevated CO₂ and drought. We hypothesized that (1) warming exacerbates leaf-level
92 drought stress by decreasing the maximal photochemical efficiency and increasing dead biomass, (2)
93 elevated CO₂ mitigates negative warming effects on the leaf-level drought stress by increasing

94 photochemical efficiency and reducing biomass loss, (3) warming and elevated CO₂ alter the lagged
95 plant response after drought (4) future climate conditions would alter plant-plant interactions.

96

97 **Materials and methods**

98 *Experimental set-up*

99 The study was conducted at the Drie Eiken Campus, University of Antwerp, Wilrijk, Belgium (51° 09'
100 N, 04° 24'E). The climate experiments took place in 16 south-facing climate-controlled chambers.
101 Details regarding this experimental platform are in Naudts et al. (2011). Four climate scenarios (four
102 chambers per scenario) were simulated in an additive design: (1) current temperature and atmospheric
103 CO₂ concentration (current climate, C); (2) current climate including a drought period (D); (3) future
104 temperature and current atmospheric CO₂, including a drought period (DT); and (4) future temperature
105 and atmospheric CO₂, including a drought period (DTCO₂).

106 *Microclimate*

107 The current condition chambers (C and D) followed air temperature based on daily averages calculated
108 from the period 1996-2005. Future temperature chambers simulated a continuous 3 °C warming
109 compared simulated current climate. Climate scenarios with elevated CO₂ had a target CO₂
110 concentration of 620 μmol mol⁻¹. Climate manipulations were based on the IPCC-SRES B2-scenario
111 prediction of moderate change for the year 2100 (IPCC 2001). Air temperature and relative humidity
112 were measured every 0.5 h with a combined humidity–temperature sensor (Siemens QFA66, Erlangen,
113 Germany) and photosynthetically active radiation (PAR) with a quantum sensor (SDEC, type
114 JYP1000, France). CO₂ concentration was measured and regulated with a CO₂ control group with an
115 infrared analyser (WMA-4, PPSystems, Hitchin, UK). During the experiment (DOY 118 – 307, 2010),
116 monthly average air temperature in C and D chambers was 12.3, 16.6, 18.8, 14.7 and 15.5 °C in May,
117 June, July, August and September, respectively. DT and DTCO₂ chambers were 3.0 ± 0.8 °C (SD)
118 warmer than current temperature chambers. Average vapour pressure deficit was 0.35 ± 0.02 and 0.46
119 ± 0.02 kPa (SD) in the climate treatments with ambient and warmed air, respectively. The average

120 daily PAR was 23.1, 25.3, 34.6, 42.1, 39.7 mol m⁻² d⁻¹ in May, June, July, August and September,
121 respectively, and did not differ between chambers (maximum delta of 2.4 ± 0.5 mol m⁻² d⁻¹ (SD), all
122 chambers combined). In the climate scenarios with current CO₂ (C, D and DT chambers), the
123 concentration was 392 ± 42 μmol mol⁻¹ (SD), while it was 615 ± 81 μmol mol⁻¹ (SD) in DT-CO₂.

124 Water supplied to the chambers was calculated as in Naudts et al. (2011). Plants were watered every
125 two days according to the 10 year average of 14 to 15 raining days per month during the growing
126 season. Total monthly irrigation matched 61.5, 64.4, 85.1, 80.2, 80.9 and 69.7 mm in May, June, July,
127 August, September and October, respectively. Water freely drained while capillary rise was prevented
128 by a drainage system placed below the chambers. Profile probe tubes for the PR2 soil moisture sensor
129 (Delta-T Devices Ltd., UK) were installed in four containers, one of each composition (see below).
130 Experimental drought (in D, DT and DT-CO₂) was attained by withholding water for 20 days (DOY
131 197-217). The length of the imposed drought was severe but not extreme, based on previous
132 experimental work in the same chambers and soils (Naudts et al. 2011). Soil moisture was measured
133 once a week before the drought (DOY 130-193) and twice a week during the drought period.

134 *Plant communities*

135 Plant communities were established at the end of April (DOY 116-118) by transplanting six-week-old
136 seedlings in PVC containers (19 cm i.dia., 40 cm height), filled with sandy soil (93.2% sand, 4.6% silt,
137 2.2% clay; field capacity 0.13 m³ m⁻³; pH 7.6; Kjeldahl-N 0.42 g kg⁻¹; 1% C in humus). We used two
138 common co-occurring species, *L. perenne* and *P. lanceolata*, originating from wild populations in
139 England. Each of the 16 chambers contained six replicates of four plant community compositions:
140 monocultures of *L. perenne* and *P. lanceolata*, and mixtures of both species with either *L. perenne* or
141 *P. lanceolata* as the central target plant. Each community contained six individuals planted in a
142 hexagonal grid at 5 cm distance and one individual at the centre of the grid. Mixed communities with
143 *L. perenne* as a central plant contained four individuals of *L. perenne* and three individuals of *P.*
144 *lanceolata*, and vice versa for *P. lanceolata* central plants. All communities were fertilized with 10 g
145 m⁻² NH₄NO₃, 5 g m⁻² P₂O₅, 10 g m⁻² K₂O and micro-elements (Fe, Mn, Zn, Cu, B, Mo) dissolved in
146 water applied in two watering (DOY 140 and 180).

147 *Biomass harvest*

148 The aboveground biomass of one community per composition (so four communities per climate
149 treatment) was harvested in each chamber before and at the end of the drought (DOY 197 and 217),
150 and at the end of the growing season (DOY 307). For each harvest, live and dead biomass was
151 separated by species. All material was dried at 70 °C for 48 h, and then weighed. For statistical
152 analysis, the sum of aboveground biomass per species was divided by the number of that specie's
153 individuals in each community.

154 Before the drought, only age-related leaf senescence contributed the dead biomass. After the drought
155 dead biomass resulted from the combination of age-related leaf senescence with leaf and plant
156 mortality induced by drought. As we could not separate these, we will refer to the causes of dead
157 biomass as “senescence and mortality”. Furthermore, leaf senescence must be expressed relative to
158 total biomass (Jobbagy and Sala 2000; Benot et al. 2014). To verify if drought triggers lagged effects
159 on senescence and mortality, we compared the dead fractions of total aboveground biomass between
160 different treatments and times.

161 *Chlorophyll a fluorescence and analysis of photosynthetic pigments*

162 Chlorophyll a fluorescence, which can detect photosynthetic stress effects prior to visible leaf damage
163 (Lichtenthaler and Miehe 1997), was measured on the youngest fully expanded leaf of each species ×
164 composition × chamber combination (2 × 4 × 16). Measurements were taken in the morning (7-9 h) on
165 30-min dark-acclimated leaves with a Hansatech Plant Efficiency Analyzer (King's Lynn, Norfolk,
166 UK). Measurements were made on the same day for all treatments. From these the maximum quantum
167 yield of photosystem II was calculated as $F_v/F_m = (F_m - F_0)/F_m$ where F_v = variable fluorescence, F_m =
168 maximum fluorescence and F_0 = steady state fluorescence.

169 Tissue chlorophyll (Chl) a, Chl b and carotenoids concentrations of the youngest fully expanded leaf
170 of each species × composition × chamber combination (2 × 4 × 16) were determined. Samples of two
171 replicate communities per composition were taken in each chamber. For each measurement, three leaf
172 discs were punched from one leaf per community (base, centre and top) and immediately frozen in

173 liquid nitrogen. Pigments were determined after acetone extraction according to Porra et al. (1989)
174 (but see Online Resource 1).

175 These measurements were performed prior to and after drought (DOY 190 and 218), and at the end of
176 the growing season (DOY 298).

177 *Data analysis*

178 Parameters were analysed at the three timepoints during the experiment and separately for *L. perenne*
179 and *P. lanceolata*. To determine the overall effect of the climate scenario (C, D, DT and DT_{CO₂}),
180 composition (monoculture or mixture) or their interaction on the measured plant responses, a
181 Permutational Multivariate Analysis of Variance (PERMANOVA; with adonis function in R;
182 (Anderson 2001)) was performed. This analysis tests to which degree Euclidean distances among and
183 within treatments differ from random expectations. Because it is distribution-free, different measures
184 following different distributions can be integrated into one multivariate analysis. All measured
185 responses were scaled to the maximum in order to give equal weight in the permutational analysis.

186 General linear mixed models (GLM) in SAS (version 9.2, SAS Institute Inc., Cary, NC) (Littell et al.
187 1996) were applied to live and dead aboveground biomass, F_v/F_m , and pigment concentrations with
188 climate scenario and composition as fixed factors. All fluorescence, pigment ratios, and fractions of
189 dead aboveground biomass were arcsine transformed to meet data distribution assumptions. SWC was
190 analysed with repeated measures with DOY, composition and climate scenario as fixed factors.
191 Chamber was included as a random factor nested within climate scenario. Non-significant factors were
192 backwards-excluded from the model. In case of significant effects, *a posteriori* means comparisons
193 using Tukey test corrected for multiple comparisons were made. Effects were considered significant at
194 $P \leq 0.05$.

195

196

197

198 **Results**

199 1. Treatment effects

200 Prior to drought, warming and elevated CO₂ had no effect on the measured plant responses of either
201 species (Table 1). In contrast, after 20 days of drought, climate scenario altered the measured plant
202 responses (Table 1), and these effects were still present after 90 days of recovery. The plant
203 community context (monoculture versus mixture) had only affected *L. perenne* at three timepoints
204 during the experiment (Table 1). Moreover, the plant composition did not alter target plant responses
205 to any of the climate treatments (Table 1). We will therefore compare the climate treatment effects for
206 all community compositions combined.

207 2. Effect of warming and elevated CO₂ on the drought response

208 *Drought response under current climate*

209 The drought response under current climate conditions was determined by comparing plant
210 communities in C and D. SWC decreased considerably during the imposed drought from DOY 203
211 onwards (*a posteriori* comparison, $P < 0.0001$, Fig. 1) and still remained lower after resuming the pre-
212 treatment watering regime (*a posteriori* comparison, $P < 0.0001$, Fig. 1). Drought reduced live
213 aboveground biomass of *L. perenne* by 35% but not that of *P. lanceolata* (Fig. 2, Table 2). In contrast,
214 aboveground dead biomass of *P. lanceolata* was higher in D than in C at the end of the drought, which
215 was not the case for *L. perenne* (Fig. 3, Table 2). *P. lanceolata* dead aboveground biomass remained
216 higher in D than in C after recovery (Fig. 3). By the end of the study, *L. perenne* had nearly recovered
217 from the drought-induced growth reduction (Fig. 2, Table 2). F_v/F_m of both species was not affected by
218 drought, neither at the end of the drought period nor at the end of the season (Fig. 4, Table 2). Chl a+b
219 and carotenoids/chlorophyll ratio of *L. perenne* were not influenced by drought (Fig. 5, Table 2,
220 Online Resource 2), while total carotenoid levels increased (Fig. 6, Table 2). *P. lanceolata* leaves had
221 slightly higher Chl a+b, but similar carotenoid levels (Fig. 5, Fig. 6, Table 2), leading to decreased
222 carotenoid/chlorophyll ratios in D treatments (Online Resource 2). After recovery, Chl a+b and

223 carotenoids levels of *L. perenne* were similar in D as in C, while *P. lanceolata* leaves had slightly
224 increased carotenoids but similar levels of Chl a+b after recovery (Fig. 5, Fig. 6, Table 2).

225 *Effect of warming on the drought response*

226 To determine whether warming altered drought responses, we compared D and DT. Warming did not
227 decrease soil water content during the drought period relative to current climate (*a posteriori*
228 comparison, $P = 0.886$, Fig. 1). Nevertheless, *L. perenne* dead aboveground biomass was 56% higher
229 in DT than in D at the end of the drought period and 60% higher after recovery (Fig. 3, Table 2).
230 Concurrently, F_v/F_m of *L. perenne* dropped in DT relative to D by the end of the drought, but increased
231 to pre-drought levels after recovery (Fig. 4, Table 2). Just after the drought decreased F_v/F_m in *L.*
232 *perenne* subjected to drought and warming was due to 53% decreased F_m rather than increased F_0 ($F_{3,42}$
233 $= 11.3$, $P = 0.0049$). Chl a+b and carotenoids of *L. perenne* were not affected by the warming at any
234 point during the experiment (Fig. 5, Fig. 6, Table 2). Contrary to *L. perenne*, warming modified none
235 of the responses of *P. lanceolata* to drought, neither at the end of the drought period nor at the end of
236 the growing season (Fig. 2, Fig. 3, Fig. 4, Fig. 5, Fig. 6, Table 2), except for carotenoid/chlorophyll
237 ratios which were significantly higher in DT at the end of the drought (Online Resource 2).

238 *Combined effect of warming and elevated CO₂ on the drought response*

239 To test for the effect of elevated CO₂ we compared DT with DT_{CO₂}. Ultimately we also compared
240 DT_{CO₂} with D, to know the total influence of a future climate on the drought impact. SWC decreased
241 significantly during the drought period in DT_{CO₂} (*a posteriori* comparison, $P < 0.0001$, Fig. 1), but
242 was not different from DT or D (*a posteriori* comparison, $P = 0.979$ and $P = 0.987$, respectively, Fig.
243 1). Also the live aboveground biomass of both species in DT_{CO₂} was not different from that of DT or
244 D, at any timepoint during the experiment (Fig. 2, Table 2). A similar response of dead aboveground
245 biomass of *L. perenne* and *P. lanceolata* was apparent between DT_{CO₂} and DT, except for *P.*
246 *lanceolata*, where dead biomass after recovery in DT_{CO₂} exceeded DT. Dead biomass was always
247 higher in DT_{CO₂} compared to D (Fig. 3, Table 2), except for *P. lanceolata* at the end of drought. For
248 both species, elevated CO₂ resulted in higher F_v/F_m (Fig. 4, Table 2), which equalized the stress levels

249 of DT CO_2 and D. After recovery, the F_v/F_m of both plant species in DT CO_2 was not different from that
250 of DT or D (Fig. 4, Table 2). Relative to DT and D treatments, DT CO_2 reduced the Chl a+b and
251 carotenoids levels in *L. perenne*, but not in *P. lanceolata* (Fig. 5, Fig. 6, Table 2). In the latter, pigment
252 levels in DT CO_2 were equal to those under drought in current climate. After recovery, the
253 carotenoid/chlorophyll ratio of both species in DT CO_2 did not differ from DT or D plants (Online
254 Resource 2).

255 3. Effect of warming and elevated CO_2 on lagged plant responses

256 To assess lag plant responses of drought we compared the fractions of dead biomass in each climate
257 scenario at the end of the season with those just after the drought. Comparing fractions of dead
258 biomass at the end of the season is not useful as differences between treatments can result from the
259 drought period itself (rather than from a lag effect on senescence) and may still be observable after 90
260 days of recovery.

261 In general, climate conditions altered the fraction of dead aboveground biomass in *L. perenne* and *P.*
262 *lanceolata* ($F_{3,12} = 23.14$, $P < 0.0001$, Fig. 7). Before drought, dead fractions were small and not
263 different between treatments (Fig. 7). By the end of the drought, the dead fraction in *L. perenne* had
264 risen to about 21% in DT and DT CO_2 , relative to 9% in C (Fig. 7). Without a lag effect of these
265 treatments, this increased dead biomass should reduce through new growth, as pre-drought differences
266 were absent. However, after 90 days of recovery, DT and DT CO_2 attained greater fractions of dead
267 biomass, (about 35% in DT and DT CO_2 as opposed to 22% in control, Fig. 7), indicating warm
268 temperatures in the drought period induced higher senescence and mortality throughout the remaining
269 growing season. Ambient temperature treatments (C and D) did not differ by the end of the season
270 (Fig. 7). In *P. lanceolata*, the pattern was only slightly different. In this species, drought increased
271 fractional dead aboveground biomass regardless of climate scenario, (about 47% in D and DT CO_2 and
272 50% in DT as opposed to 23% in C, Fig. 7, end of drought data). After recovery, DT CO_2 plants largely
273 maintained greater dead biomass fractions relative to controls (56% in DT CO_2 , 35% in C, Fig. 7),
274 while D and DT plants reached intermediate values (46%, Fig. 7). Fig. 3 showed pre-drought

275 differentiation between the future climates and control. This may imply warming accelerated
276 senescence and mortality. However, the ratio of dead to total biomass showed no difference between
277 future and current treatments (Fig.7, pre-drought data). Moreover, in *P. lanceolata*, the senescence and
278 mortality can be ascribed only to the drought treatment and not to warming because DT did not differ
279 from D just after the drought (Fig. 7). In *L. perenne* just after the drought the dead fraction in DT was
280 significantly higher than in D, so we cannot completely exclude warming itself as a mechanism for
281 enhanced senescence and mortality.

282 4. Effect of drought, warming and elevated CO₂ on plant-plant interactions

283 The aboveground biomass of *L. perenne* in a mixture with *P. lanceolata* as a central plant was always
284 higher than in the other plant compositions, whereas the aboveground biomass of *P. lanceolata* did not
285 differ between compositions (Online Resource 3). These plant-plant interaction patterns were not
286 altered by drought, warming or elevated CO₂ at any point over the experiment (Table 1).

287

288 **Discussion**

289 Experimental studies that simultaneously vary more than two climate change factors are still rare, and
290 observations are needed to qualify and validate conceptual and theoretical frameworks (Beier et al.
291 2012). Our results indicate that climate warming exacerbates drought effects on *L. perenne* and that
292 elevated CO₂ only partly compensates for this. Furthermore, drought in a warmer climate with or
293 without elevated CO₂ induced higher senescence and mortality in *L. perenne* long after drought ended,
294 while no such lag effects occurred under current climate. In *P. lanceolata* a similar stimulation of post-
295 drought senescence and mortality occurred with warming and elevated CO₂. Notwithstanding these
296 different responses, the imposed climate scenarios did not alter the competitive interactions between
297 these species.

298

299

300 1. Warming and elevated CO₂ as modifiers of the drought response

301 *L. perenne* responded to drought by producing less live biomass, while *P. lanceolata* accumulated
302 more necromass. This may originate from different capacities for water acquisition and transport
303 (Chaves et al. 2002). Van den Berge et al. (2014) showed that monocultures of *P. lanceolata* consume
304 more water under drought compared to those of *L. perenne*, owing to higher stomatal conductance at
305 the onset of drought and later stomatal closure. We also observed considerably drier soil conditions in
306 the monocultures of *P. lanceolata*, as opposed to the monocultures of *L. perenne*, irrespectively of the
307 climate scenario (data not shown). The anisohydric behaviour of *P. lanceolata* species matches the
308 absence of significant biomass loss we observed, since prolonged stomatal opening during drought
309 would facilitate CO₂ uptake, while the isohydric strategy of *L. perenne* might explain its reduced
310 growth.

311 Warming did not modify the live biomass response to drought, suggesting that the plants were equally
312 restrained by the water shortage under current and warmer climate conditions. Contrary to Zavalloni et
313 al. (2008), warming also did not enhance soil drying in our communities. This was unexpected
314 because evapotranspiration was anticipated to increase in a warmer climate due to a higher
315 atmospheric demand. However, warming-accelerated senescence might have contributed to the
316 unaltered soil water availability under warmer conditions (Zavaleta et al. 2003).

317 Warming did increase dead biomass of *L. perenne* by the end of the drought. At the same time the
318 F_v/F_m decreased in this species. In our study F_v/F_m of *L. perenne* mainly declined by a lower F_m while
319 F_0 remained constant, suggesting reliance on rapidly reversible photoprotection related to enhanced
320 non-photochemical quenching via the xanthophyll cycle (Long et al. 1994). The higher carotenoids
321 levels in DT relative to D also support this, as xanthophyll carotenoids protect plants from photo-
322 oxidative damage through thermal dissipation (DemmigAdams and Adams 1996). The complete
323 recovery of the aboveground biomass, F_v/F_m and carotenoids of *L. perenne* at the end of the season
324 indicates photoprotection of photosynthetic tissues, but it should be noted that after the drought period
325 plants developed new leaves, as shown in the increased live biomass at the end of the season.

326 Elevated CO₂ did not alter the live and dead biomass of either species at the end of the drought, so
327 productivity was equally restrained by water deficit under DT and DTCO₂. Kongstad et al. (2012)
328 found that elevated CO₂ did not counterbalance the drought effect on plant growth. Nevertheless, in *L.*
329 *perenne*, adding CO₂ increased in F_v/F_m and also lowered the concentration of carotenoids compared
330 with DT, indicating that stress levels were alleviated. Likewise, Hamerlynck et al. (2000) showed that
331 elevated CO₂ reduced the impact of drought and heat stress on photosynthesis. In our study the direct
332 compensatory effects of elevated CO₂ were too weak to mitigate biomass loss ensuing from drought.

333 In agreement with our first hypothesis, our results show warming aggravates negative impact of
334 drought in *L. perenne* by reducing PSII photochemical efficiency and inflicting leaf mortality and
335 senescence. Elevated CO₂ seems to compensate for the detrimental effect of warming on drought
336 through increased photochemical protection but not by decreasing the necromass, partly confirming
337 our second hypothesis. Contrary to *L. perenne*, warming or elevated CO₂ did not alter the drought
338 response of *P. lanceolata*.

339 2. Effect of warming and elevated CO₂ on lagged plant responses

340 In *L. perenne* grown in a future climate (DT and DTCO₂), the fraction of dead biomass lasted after the
341 drought had ended, while no such lag effect was apparent in current climate conditions by the end of
342 the season. *P. lanceolata* also exhibited post-drought lag effects on the fraction of dead biomass,
343 especially under combined warming and elevated CO₂. Consequently, drought in the current climate
344 did not trigger lagged effects but a future climate induced it, partly confirming our third hypothesis.
345 The persistence of increased dead biomass fractions after drought until the end of the season indicates
346 higher senescence and mortality in a future climate conditions. This cannot be ascribed to incomplete
347 recovery since this would result in lower fractions of dead biomass through new growth. Irrespective
348 of climate scenarios, senescence and mortality became fairly high by the end of the season. Probably,
349 dead biomass accumulation was stimulated by the 90-days recovery period without mowing and
350 greater competition for light during the shortening days in autumn.

351 Dry years can reduce net primary productivity in following years, relative to predictions based on
352 climate-productivity relationships alone (Lauenroth and Sala 1992; O'Connor et al. 2001; Wiegand et
353 al. 2004). These lag effects of drought are attributed to various mechanisms. First, carbohydrate
354 reserves under long drought are not replenished, causing mortality (Dunnett et al. 1998). Meristem
355 limitation can also follow after plant, root or tiller mortality after drought (Benson et al. 2004).
356 Changes in stored soil water (Wiegand et al. 2004) and lower nutrient mineralization and organic
357 matter decomposition under drought can drive drought lag effects (Schimel and Parton 1986).
358 However, in our study, the drought lag effects were observed only under warming treatments. It is
359 noteworthy that adding elevated CO₂ did not alter the fraction of dead leaves compared to DT. The
360 higher senescence and mortality in a future climate (DT and DT-CO₂) can therefore be ascribed mainly
361 to warming, and elevated CO₂ did not compensate negative warming effects.

362 In the current study, warming did not enhance soil drying, and SWC of all climate treatments
363 recovered after drought treatment ended, reaching more than 84% of the pre-drought values 10 days
364 after rewatering. Therefore, the observed lag effect on the fraction of dead biomass in DT is not due to
365 differences in soil moisture. We propose that other mechanisms than those measured, must be at the
366 basis of the observed lag effects. For instance, heat stress may cause cellular damage and secondary
367 stresses, such as osmotic and oxidative stresses (Vinocur and Altman 2005). Leaf senescence, on the
368 other hand, is controlled by a combination of environmental factors, such as temperature and drought,
369 and endogenous factors including age, reproductive maturity and hormone levels (Munne-Bosch and
370 Alegre 2004). Environmental factors may affect endogenous factors, accelerating leaf senescence
371 (Munne-Bosch and Alegre 2004). These mechanisms might explain our finding that the combination
372 of warming and elevated CO₂ maintained drought-induced senescence and mortality long after the
373 drought period.

374 Theory predicts that abiotic stresses such as drought events reduce the resilience of ecosystems
375 (Scheffer et al. 2001), but experimental studies show that grasslands can recover rapidly (Zavalloni et
376 al. 2008; Walter et al. 2011). While this was generally the case in our study, the lag effects on the
377 fraction of dead biomass induced by drought suggest future climate may reduce resilience. This is

378 especially important as drought is predicted to become more frequent in decades to come (Stocker et
379 al. 2013), and recurrent extremes have been shown to weaken the resistance of plant assemblages
380 already in current climate, owing to memory effects of previous events (Dreesen et al. 2014).

381 3. Effect of drought, warming and elevated CO₂ on plant-plant interactions

382 The interactions between *L. perenne* and *P. lanceolata* were not influenced by the climate scenario;
383 consequently, we reject our fourth hypothesis. The similar rooting depth of these species (Weeve
384 1975) suggests they would compete significantly during drought. However, *L. perenne* is able to
385 suppress the root production of herbaceous species, especially in the top soil (Wardle and Peltzer
386 2003), leading to divergent root exploitation zones. Limited interaction between the species owing to
387 root partitioning can therefore not be excluded, and would be in agreement with the observed limited
388 influence of the climatic factors on the neighbour effects. Possibly, more severe droughts are needed
389 for interspecific differences in response to climate to be expressed, as found in grassland (Grant et al.
390 2014). We propose further experimental research focussing on the influence of different neighbour
391 species is needed to understand whether and under which circumstances climate change can alter
392 plant-plant interactions.

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397

398 **References**

399 Allen LH, Pan DY, Boote KJ, Pickering NB, Jones JW (2003) Carbon dioxide and temperature effects
400 on evapotranspiration and water use efficiency of soybean. *Agron J* 95:1071-1081.

401 Allison I, Bindoff N, Bindschadler R, Cox P, de Noblet N et al (2009) The Copenhagen Diagnosis:
402 Updating the world on the latest climate science. The University of New South Wales Climate Change
403 Research Centre (CCRC), Sydney.

404 Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. *Austral Ecol*
405 26:32-46.

406 Beddows AR (1967) *Lolium Perenne* L. *J Ecol* 55:567-587.

407 Beier C, Beierkuhnlein C, Wohlgemuth T, Penuelas J, Emmett B et al (2012) Precipitation
408 manipulation experiments - challenges and recommendations for the future. *Ecol Lett* 15:899-911.

409 Benot ML, Saccone P, Pautrat E, Vicente R, Colace MP et al (2014) Stronger short-term effects of
410 mowing than extreme summer weather on a subalpine grassland. *Ecosystems* 17:458-472.

411 Benson EJ, Hartnett DC, Mann KH (2004) Belowground bud banks and meristem limitation in
412 tallgrass prairie plant populations. *Am J Bot* 91:416-421.

413 Bigler C, Gavin DG, Gunning C, Veblen TT (2007) Drought induces lagged tree mortality in a
414 subalpine forest in the Rocky Mountains. *Oikos* 116:1983-1994.

415 Chaves MM, Pereira JS, Maroco J, Rodrigues ML, Ricardo CPP et al (2002) How plants cope with
416 water stress in the field. Photosynthesis and growth. *Ann Bot* 89:907-916.

417 De Boeck HJ, Lemmens C, Zavalloni C, Gielen B, Malchair S et al (2008) Biomass production in
418 experimental grasslands of different species richness during three years of climate warming.
419 *Biogeosciences* 5:585-594.

420 De Valpine P, Harte J (2001) Plant responses to experimental warming in a montane meadow.
421 *Ecology* 82:637-648.

422 DemmigAdams B, Adams WW (1996) The role of xanthophyll cycle carotenoids in the protection of
423 photosynthesis. *Trends Plant Sci* 1:21-26.

424 Dreesen FE, De Boeck HJ, Janssens IA, Nijs I (2014) Do successive climate extremes weaken the
425 resistance of plant communities? An experimental study using plant assemblages. *Biogeosciences*
426 11:109-121.

427 Dukes JS, Chiariello NR, Cleland EE, Moore LA, Shaw MR et al (2005) Responses of grassland
428 production to single and multiple global environmental changes. *PLoS Biol* 3:1829-1837.

429 Dunnett NP, Grime JP (1999) Competition as an amplifier of short-term vegetation responses to
430 climate: an experimental test. *Funct Ecol* 13:388-395.

431 Dunnett NP, Willis AJ, Hunt R, Grime JP (1998) A 38-year study of relations between weather and
432 vegetation dynamics in road verges near Bibury, Gloucestershire. *J Ecol* 86:610-623.

433 Field CB, Chapin FS, Matson PA, Mooney HA (1992) Responses of terrestrial ecosystems to the
434 changing atmosphere - a resource-based approach. *Annu Rev Ecol Syst* 23:201-235.

435 Grant K, Kreyling J, Heilmeyer H, Beierkuhnlein C, Jentsch A (2014) Extreme weather events and
436 plant plant interactions: shifts between competition and facilitation among grassland species in the
437 face of drought and heavy rainfall. *Ecol Res* 29:991-1001.

438 Hamerlynck EP, Huxman TE, Loik ME, Smith SD (2000) Effects of extreme high temperature,
439 drought and elevated CO₂ on photosynthesis of the Mojave Desert evergreen shrub, *Larrea tridentata*.
440 *Plant Ecol* 148:183-193.

441 IPCC (2001) *Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the*
442 *Third Assessment Report of the Intergovernmental Panel on Climate Change.* Cambridge University
443 Press, Cambridge, United Kingdom and New York, NY, USA.

444 Jaleel CA, Manivannan P, Wahid A, Farooq M, Al-Juburi HJ et al (2009) Drought stress in plants: a
445 review on morphological characteristics and pigments composition. *Int J Agric Biol* 11:100-105.

446 Jobbagy EG, Sala OE (2000) Controls of grass and shrub aboveground production in the Patagonian
447 steppe. *Ecol Appl* 10:541-549.

448 Kongstad J, Schmidt IK, Riis-Nielsen T, Arndal MF, Mikkelsen TN et al (2012) High resilience in
449 heathland plants to changes in temperature, drought, and CO₂ in combination: results from the
450 CLIMAITE experiment. *Ecosystems* 15:269-283.

451 Lauenroth WK, Sala OE (1992) Long-term forage production of north-american shortgrass steppe.
452 *Ecol Appl* 2:397-403.

453 Littell RC, Milliken GA, Stroup WW, Wolfinger RD (1996) *SAS system for mixed models.* SAS
454 institute Inc., Cary, North Carolina.

455 Long SP, Humphries S, Falkowski PG (1994) Photoinhibition of photosynthesis in nature. *Annu Rev*
456 *Plant Physiol Plant Molec Biol* 45:633-662.

457 Morecroft MD, Masters GJ, Brown VK, Clarke IP, Taylor ME et al (2004) Changing precipitation
458 patterns alter plant community dynamics and succession in an ex-arable grassland. *Funct Ecol* 18:648-
459 655.

460 Morgan JA, LeCain DR, Pendall E, Blumenthal DM, Kimball BA et al (2011) C₄ grasses prosper as
461 carbon dioxide eliminates desiccation in warmed semi-arid grassland. *Nature* 476:202-206.

462 Morgan JA, Pataki DE, Korner C, Clark H, Del Grosso SJ et al (2004) Water relations in grassland
463 and desert ecosystems exposed to elevated atmospheric CO₂. *Oecologia* 140:11-25.

464 Munne-Bosch S, Alegre L (2004) Die and let live: leaf senescence contributes to plant survival under
465 drought stress. *Funct Plant Biol* 31:203-216.

466 Naudts K, Van den Berge J, Janssens IA, Nijs I, Ceulemans R (2011) Does an extreme drought event
467 alter the response of grassland communities to a changing climate? *Environ Exp Bot* 70:151-157.

468 Naudts K, Van den Berge J, Janssens IA, Nijs I, Ceulemans R (2013) Combined effects of warming
469 and elevated CO₂ on the impact of drought in grassland species. *Plant Soil* 369:497-507.

470 Niu S, Luo Y, Li D, Cao S, Xia J et al (2014) Plant growth and mortality under climatic extremes: An
471 overview. *Environ Exp Bot* 98:13-19.

472 O'Connor TG, Haines LM, Snyman HA (2001) Influence of precipitation and species composition on
473 phytomass of a semi-arid African grassland. *J Ecol* 89:850-860.

474 Porra R, Thompson W, Kriedemann P (1989) Determination of accurate extinction coefficients and
475 simultaneous equations for assaying chlorophylls a and b extracted with four different solvents:
476 verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochim*
477 *Bioacta* 975:384-394.

478 Reddy AR, Chaitanya KV, Vivekanandan M (2004) Drought-induced responses of photosynthesis and
479 antioxidant metabolism in higher plants. *J Plant Physiol* 161:1189-1202.

480 Rustad LE, Campbell JL, Marion GM, Norby RJ, Mitchell MJ et al (2001) A meta-analysis of the
481 response of soil respiration, net nitrogen mineralization, and aboveground plant growth to
482 experimental ecosystem warming. *Oecologia* 126:543-562.

483 Sagar GR, Harper JL (1964) *Plantago major* L., *P. media* L. and *P. lanceolata* L. *J Ecol* 52:189-221.

484 Scheffer M, Carpenter S, Foley JA, Folke C, Walker B (2001) Catastrophic shifts in ecosystems.
485 Nature 413:591-596.

486 Schimel DS, Parton WJ (1986) Microclimatic controls of nitrogen mineralization and nitrification in
487 shortgrass soils. Plant Soil 93:347-357.

488 Sherry RA, Weng ES, Arnone JA, Johnson DW, Schimel DS et al (2008) Lagged effects of
489 experimental warming and doubled precipitation on annual and seasonal aboveground biomass
490 production in a tallgrass prairie. Glob Change Biol 14:2923-2936.

491 Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK et al (2013) IPCC: Climate Change 2013: The
492 Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the
493 Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge and New York.

494 Van den Berge J, Naudts K, De Boeck HJ, Ceulemans R, Nijs I (2014) Do interactions with
495 neighbours modify the above-ground productivity response to drought? A test with two grassland
496 species. Environ Exp Bot 105:18-24.

497 Vinocur B, Altman A (2005) Recent advances in engineering plant tolerance to abiotic stress:
498 achievements and limitations. Curr Opin Biotechnol 16:123-132.

499 Walter J, Nagy L, Hein R, Rascher U, Beierkuhnlein C et al (2011) Do plants remember drought?
500 Hints towards a drought-memory in grasses. Environ Exp Bot 71:34-40.

501 Wardle DA, Peltzer DA (2003) Interspecific interactions and biomass allocation among grassland
502 plant species. Oikos 100:497-506.

503 Weeve T (1975) Flora Neerlandica. Koninklijke Botanische Vereniging, Amsterdam.

504 Wiegand T, Snyman HA, Kellner K, Paruelo JM (2004) Do grasslands have a memory: Modeling
505 phytomass production of a semiarid South African grassland. Ecosystems 7:243-258.

506 Wu ZT, Dijkstra P, Koch GW, Penuelas J, Hungate BA (2011) Responses of terrestrial ecosystems to
507 temperature and precipitation change: a meta-analysis of experimental manipulation. Glob Change
508 Biol 17:927-942.

509 Zavaleta ES, Thomas BD, Chiariello NR, Asner GP, Shaw MR et al (2003) Plants reverse warming
510 effect on ecosystem water balance. Proc Natl Acad Sci US 100:9892-9893.

511 Zavalloni C, Gielen B, Lemmens CMHM, Boeck HJ, Blasi S et al (2008) Does a warmer climate with
512 frequent mild water shortages protect grassland communities against a prolonged drought? *Plant Soil*
513 308:119-130.

514

515 **Table and figure legends**

516 **Table 1** PERMOVA results testing effect of climate and plant composition on plant responses. Plant
 517 communities consist of monocultures and mixtures of *Lolium perenne* and *Plantago lanceolata*.
 518 Parameters were analysed before the drought (DOY 197), at the end of the drought (DOY 217) and
 519 after recovery, at the end of the growing season (DOY 307). P-values are presented in bold when
 520 significant (<0.05)

	<i>Lolium perenne</i>			<i>Plantago lanceolata</i>		
	Before drought	Drought	Recovery	Before drought	Drought	Recovery
Climate scenario	0.066	0.001	0.006	0.108	0.007	0.004
Composition	0.037	0.044	0.005	0.998	0.065	0.638
Climate scenario × composition	0.170	0.585	0.410	0.665	0.817	0.964

521

522

523

524 **Table 2** Significance levels (p-values) from *a posteriori* comparisons of plant responses under current climate, current climate with drought, warmer climate
525 with drought and warmer climate with elevated CO₂ and drought. Drought effect was determined by comparing current climate conditions with and without
526 drought, the warming effect by comparing of current climate and warmer climate conditions with drought and the combined warming and elevated CO₂ effect
527 on drought-treated plants by comparing of current climate with drought with those with combined warming, elevated CO₂ and drought. The plant communities
528 consist of monocultures and mixtures of *Lolium perenne* and *Plantago lanceolata*. P-values were corrected for multiple comparisons with Tukey honest
529 significant difference tests, bold results are significant (<0.05). Results obtained before drought (DOY 197), end of the drought (DOY 217) and end of
530 growing season data (DOY 307).

531

	Drought effect		Effect of warming			Combined effects of warming and elevated CO ₂		
	Drought	Recovery	Before drought	Drought	Recovery	Before drought	Drought	Recovery
<i>Lolium perenne</i>								
Live aboveground biomass	0.006	0.219	0.983	0.976	0.923	0.099	0.883	0.999
Dead aboveground biomass	0.488	0.789	0.048	0.002	0.009	0.041	<0.001	0.004
F _v /F _m	0.958	0.665	0.968	<0.001	0.998	0.622	0.854	0.945
Chl a+b	0.134	0.997	0.917	0.822	0.910	0.988	0.025	0.912
Carotenoids	0.033	0.637	0.996	0.849	0.941	0.999	0.004	0.912
<i>Plantago lanceolata</i>								
Live aboveground biomass	0.999	0.998	0.940	0.985	0.999	0.916	0.899	0.989

Dead aboveground biomass	0.001	0.037	0.001	0.845	0.854	<0.001	0.740	0.015
F _v /F _m	0.610	0.566	0.961	0.080	0.876	0.320	0.999	0.917
Chl a+b	0.046	0.243	0.999	0.098	0.994	0.924	0.166	0.991
Carotenoids	0.723	0.035	0.903	0.899	0.900	0.947	0.402	0.828

532 **Fig. 1** Time course of soil water content (SWC) in current climate conditions (C, black circle), current
533 climate with drought (D, white circle), warmer climate with drought (DT, black triangle) and warmer
534 climate with elevated CO₂ and drought (DTCO₂, white triangle). The drought period was initiated at
535 day of year (DOY) 197 and re-watering started at DOY 217. Means ± SE are indicated (all community
536 compositions combined)

537

538 **Fig. 2** Live aboveground biomass of *Lolium perenne* (top panel) and *Plantago lanceolata* (bottom
539 panel) before the drought on DOY 197, at the end of the drought on DOY 217 and at the end of the
540 growing season on DOY 307, after recovery. Plants were grown in current climate conditions (C,
541 black bars), current climate with drought (D, dark grey bars), warmer climate with drought (DT, light
542 grey bars) and warmer climate with elevated CO₂ and drought (DTCO₂, white bars). The drought
543 period lasted 20 days (DOY 197-217). Means ± SE are indicated (all community compositions
544 combined). Letters indicate differences for posterior comparisons between climate treatments,
545 separately tested for each plant species

546

547 **Fig. 3** Dead aboveground biomass of *Lolium perenne* (top panel) and *Plantago lanceolata* (bottom
548 panel) for all community compositions before the drought (DOY 197), at the end of the drought (DOY
549 217) and end of the growing season (DOY 307). Climate scenarios are as in Fig. 2. Means ± SE are
550 indicated (all community compositions combined). Letters indicate differences for posterior
551 comparisons between climate treatments, separately tested for each plant species.

552

553 **Fig. 4** F_v/F_m of young fully expanded leaves of *Lolium perenne* (top panel) and *Plantago lanceolata*
554 (bottom panel) before drought (DOY 197), end of drought (DOY 217) and at the end of the growing
555 season (DOY 307). Each bar is the mean ± SE, of all community compositions combined. See Fig. 2
556 for climate scenarios. Letters indicate differences for posterior comparisons between climate
557 treatments.

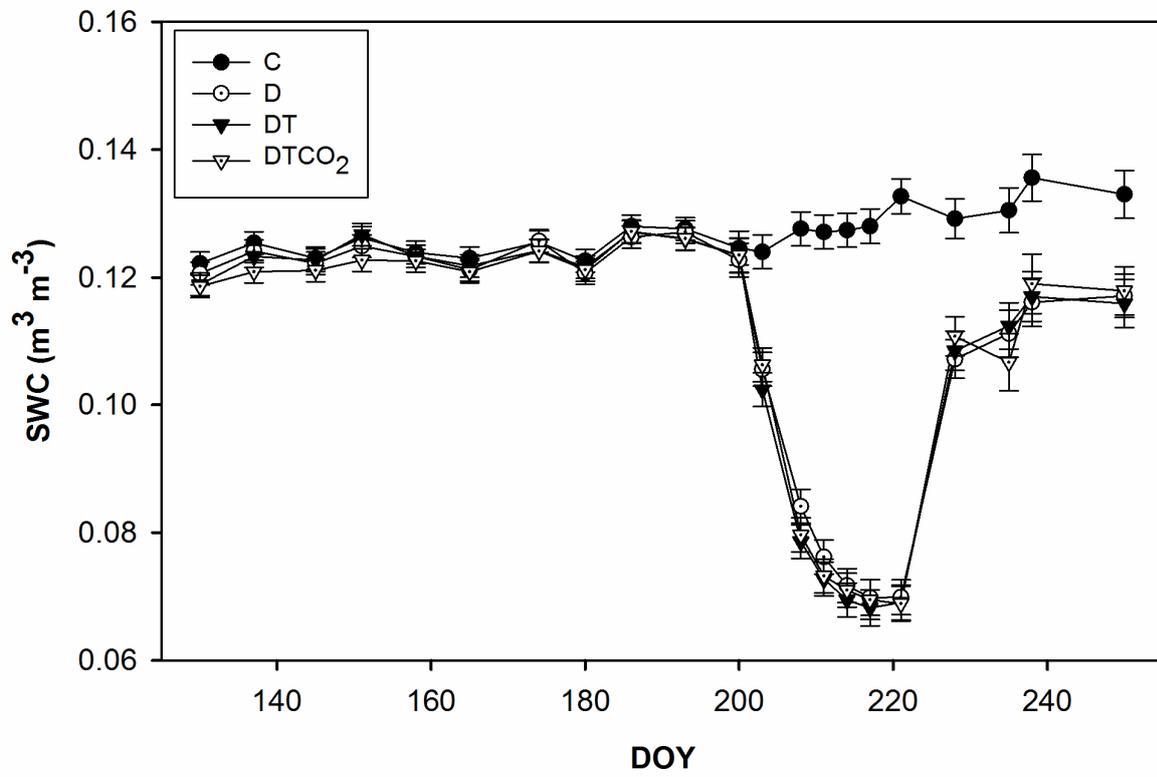
558 **Fig. 5** Leaf chlorophyll a+b of *Lolium perenne* (top panel) and *Plantago lanceolata* (bottom panel)
559 before the drought (DOY 197), end of drought (DOY 217) and at the end of the growing season (DOY
560 307). Bars are means \pm SE, pooled across all community compositions; see Fig. 2 for climate
561 scenarios. Letters indicate differences for posterior comparisons between climate treatments.

562

563 **Fig. 6** Leaf carotenoid concentrations of *Lolium perenne* (top panel) and *Plantago lanceolata* (bottom
564 panel) before the drought (DOY 197), end of drought (DOY 217) and at the end of the growing season
565 (DOY 307). Bars are means \pm SE, pooled across all community compositions; see Fig. 2 for climate
566 scenarios. Letters indicate differences for posterior comparisons between climate treatments.

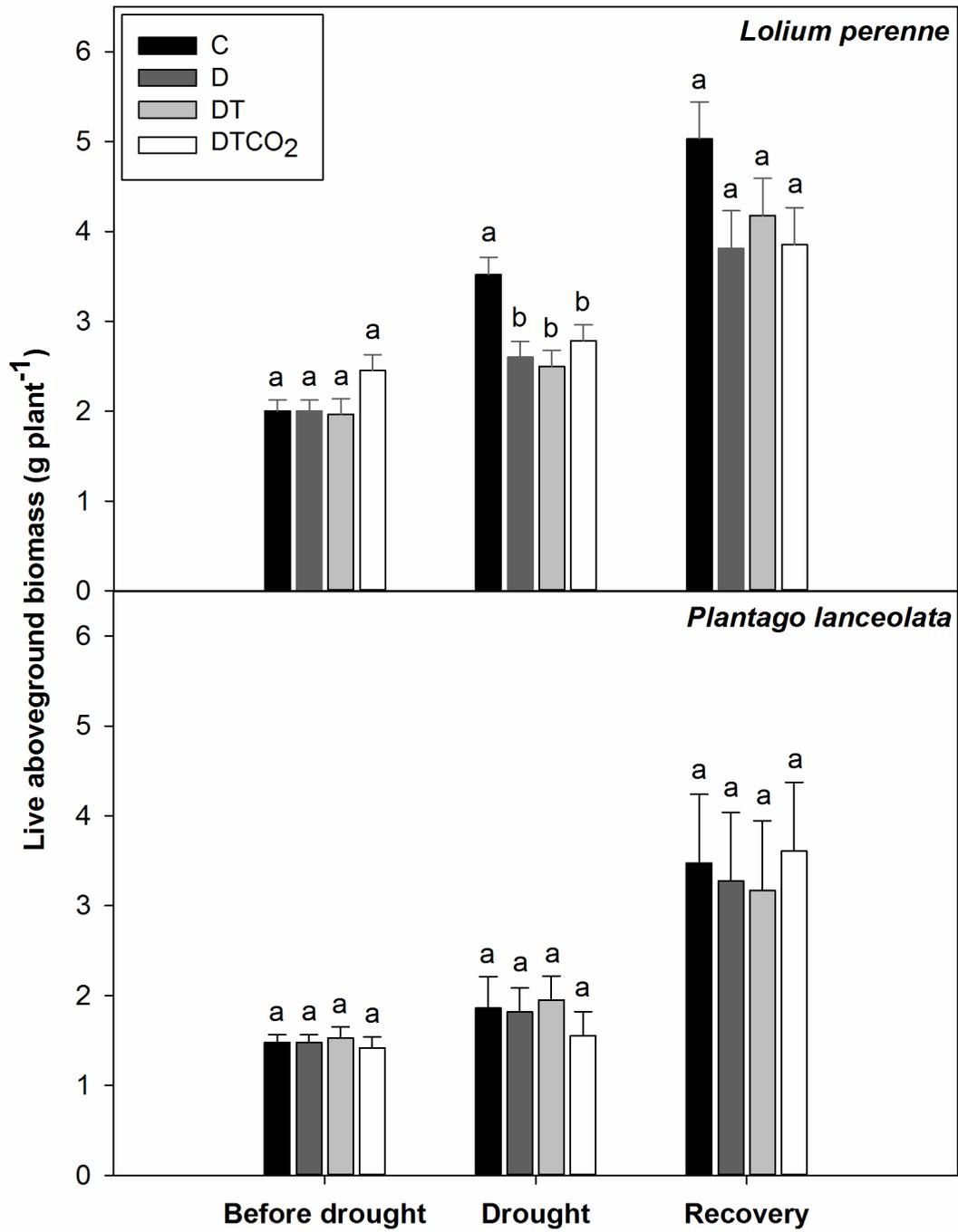
567

568 **Fig. 7** Dead fraction of total aboveground biomass of *Lolium perenne* (top panel) and *Plantago*
569 *lanceolata* (bottom panel) before the drought (DOY 197), end of drought (DOY 217) and at the end of
570 the growing season (DOY 307). Bars are means \pm SE, pooled across all community compositions; see
571 Fig. 2 for climate scenarios. Letters indicate differences for posterior comparisons between climate
572 treatments.



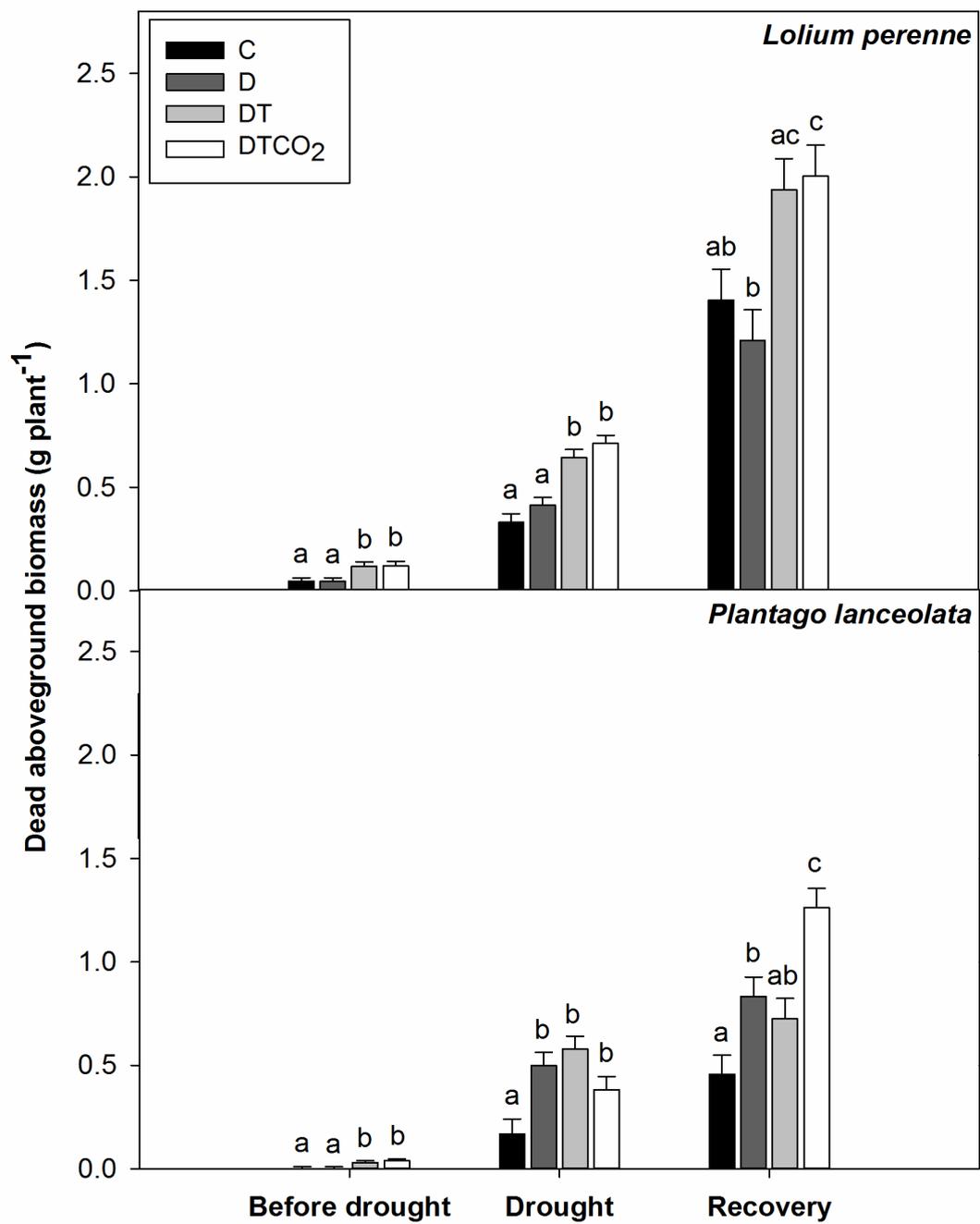
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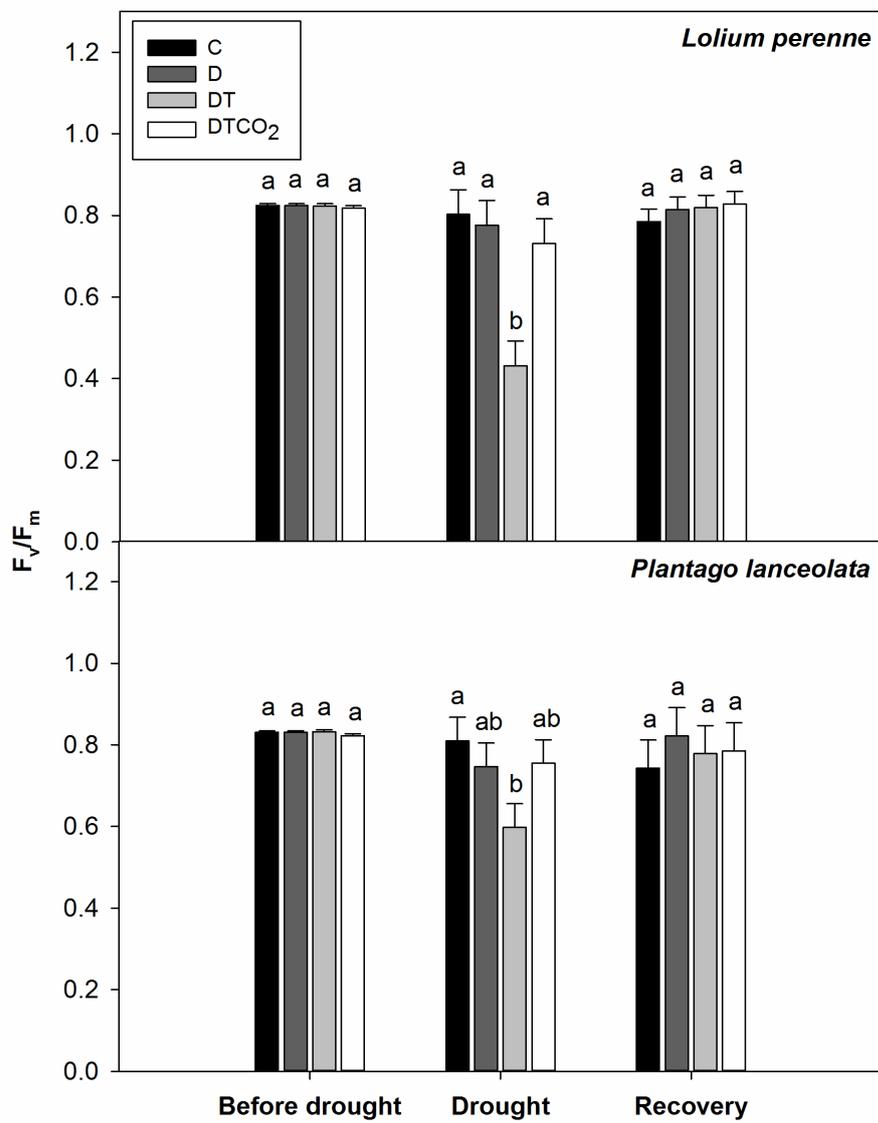
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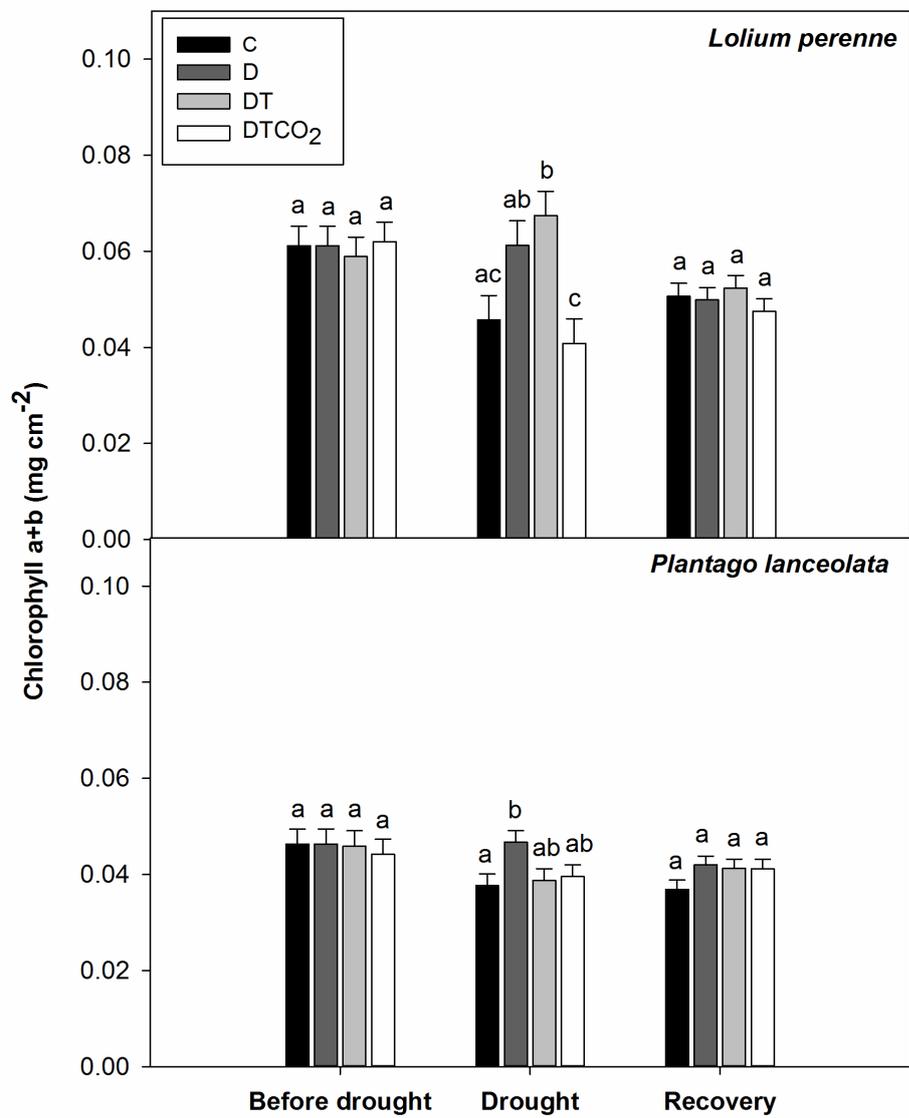
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582 Fig. 4



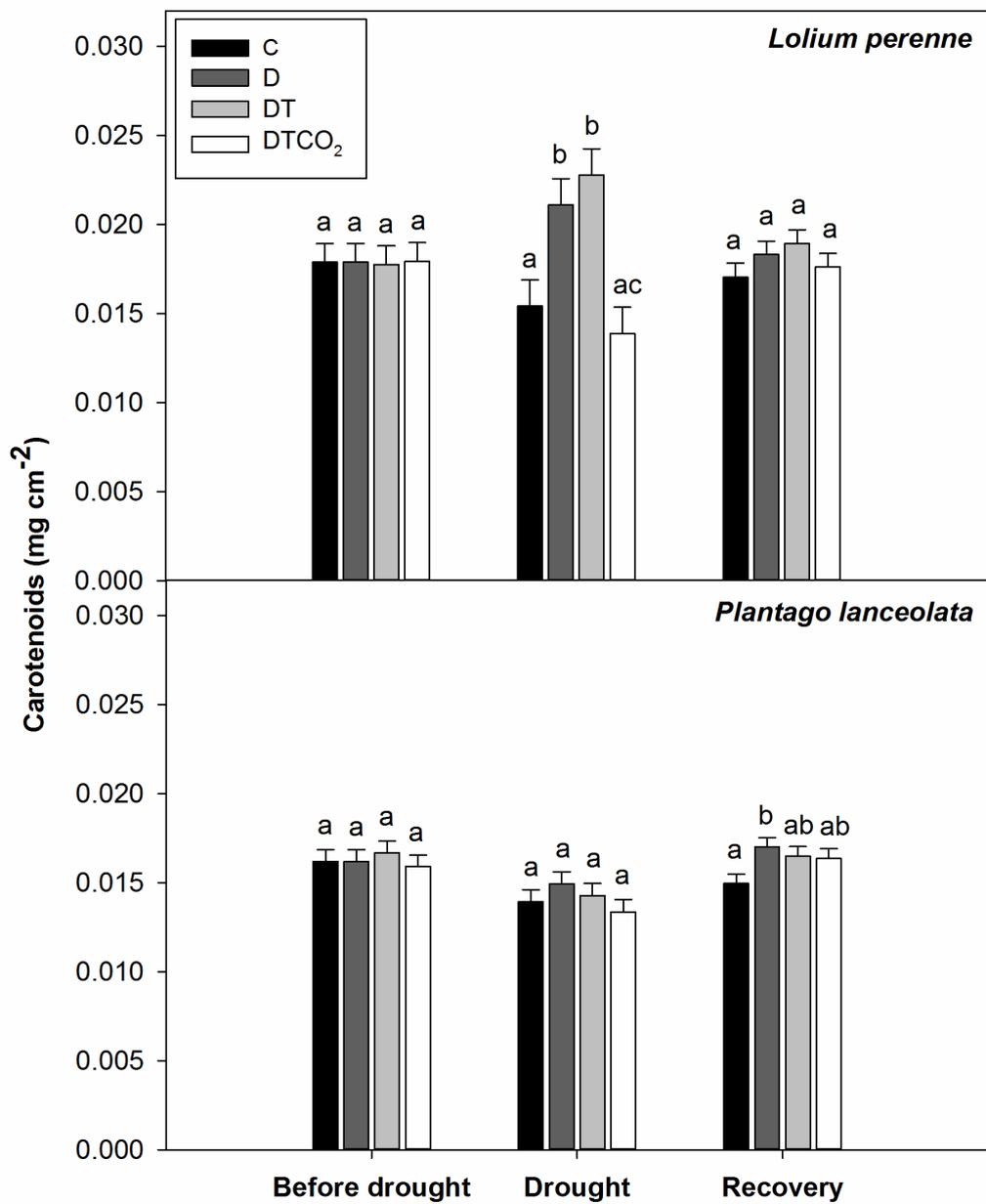
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