Title

Multiple climate change factors jointly increased the competitiveness of a C_4 grass against its C_3 competitor

Author names and affiliations

Gen-Chang Hsu[1,2], Po-Ju Ke[2], Chuan-Kai Ho*[2]

[1]Department of Life Science, National Taiwan University, Taipei, Taiwan

[2]Institute of Ecology and Evolutionary Biology, National Taiwan University, Taipei, Taiwan

***Correspondence:** ckho@ntu.edu.tw

Abstract

C_3 and C_4 plants are the two major terrestrial plant groups that respond differently to temperature, water, and CO_2. Although much research has focused on the individual effect of warming, water availability, and elevated CO_2 on biomass production and competitive interactions between C_3 and C_4 plants, the joint effects of these factors remains underexplored. We grew naturally co-occurring C_3 (*Oplismenus composites*) and C_4 (*Paspalum conjugatum*) grass under three temperature warming scenarios (control, +2°C, and +4°C), two water supply intervals (normal 2 days vs. prolonged 7 days [drought]), and two CO_2 concentrations (ambient 400 ppm vs. elevated 800 ppm) in growth chambers and measured their above- and below-ground dry biomass in monoculture and mixture to quantify their biomass performances and competitive responses. Warming and elevated CO_2 together enhanced the above- and/or below-ground biomass of both C_3 and C_4 grass. Moreover, temperature, water, and CO_2 interacted to increase the below-ground biomass of the C_4 grass. Surprisingly, the C_3 grass performed worse under interspecific competition (mixture biomass) relative to intraspecific competition (monoculture biomass) at elevated CO_2 compared to ambient CO_2, while the reverse is true for the C_4 grass. Furthermore, the C_4 grass was the most competitive against the C_3 grass under simultaneous 4°C warming, drought, and elevated CO_2. Taken together, our results suggest that the competitive balance could potentially shift in favor of C_4 plants under the projected temperature warming, increased drought frequencies, and rising atmospheric CO_2, with an increase in C_4 relative abundance in future plant communities.

Keywords

above- and below-ground biomass, C_3 and C_4 plants, competition, drought, elevated CO_2, global climate change, warming

Introduction

C_3 and C_4 plants are two major terrestrial plant groups that differ in their physiology and response to surrounding environments (Ehleringer and Monson 1993, Yamori et al. 2014). Their relative abundance in the community is closely related to various ecosystem functions and processes such as resource provisioning for herbivores, nutrient cycling, and carbon storage (Still et al. 2003, Warne et al. 2010, Pau et al. 2013). For example, C_3 plants generally have higher nitrogen-to-carbon ratios than C_4 plants (Barbehenn et al. 2004), and therefore their relative biomass production in the habitat may affect the nutritional availability for herbivores and even organisms at higher trophic levels (Warne et al. 2010). Importantly, because C_3 and C_4 plants evolved under different climates and have different adaptations to surrounding environments, understanding how they respond to climate change and the resulting dynamic shifts is crucial for the management of terrestrial ecosystems.

The performances of C_3 and C_4 plants under different environmental conditions can influence their relative competitive abilities (Pearcy et al. 1981, Edwards and Still 2008). Theoretical and empirical studies have suggested that C_3 plants generally perform better and are stronger competitors in cool and well-watered environments, whereas C_4 plants are more adapted to high temperatures and drought conditions and thus more competitive than in hot and arid environments (Teeri and Stowe 1976, Ehleringer and Monson 1993, Sage and Monson 1998, Edwards and Still 2008). In addition to temperature and water availability, C_3 and C_4 plants exhibit differential responses to CO_2 (Pearcy and Ehleringer 1984, Wand et al. 1999). The photosynthesis of C_3 plants are limited by current level of atmospheric CO_2, and increasing CO_2 concentration could stimulate their growth and therefore offer them competitive advantages over C_4 plants (Ward et al. 1999, Poorter and Navas 2003, Lee 2011). These shifts in C_3-C_4 competitive relationships in response to climate change may alter their relative abundance, which could generate profound consequences for community and

62 ecosystem dynamics (Ehleringer and Monson 1993, Still et al. 2003). Furthermore, different
63 climate change variables can have counteracting effects on their competitive relationships.
64 For example, while higher temperatures and drought conditions increase the competitive
65 ability of C_4 plants against C_3 plants, elevated CO_2 may enhance C_3 plant performance and
66 thus ameliorate the negative effects of warming and drought on their competitiveness,
67 highlighting the need of simultaneously considering multiple environmental stressors for
68 predicting C_3-C_4 plant dynamics under changing climate.

69 Different environmental factors can interact to exert a more strengthened (synergistic)
70 or weakened (antagonistic) effect on plants than the simple summation of their individual
71 effects (Wand et al. 1999). For example, an experiment showed a synergistic effect between
72 water and CO_2 treatment in C_3-C_4 mixture, where elevated CO_2 had a greater positive effect
73 on the C_4 biomass under drought compared to well-water conditions (Valerio et al. 2011). On
74 the other hand, another study found that the growth stimulation of elevated CO_2 on a C_3 crop
75 was dampened at higher temperatures, indicating an antagonistic effect between warming and
76 CO_2 (Alberto et al. 1996). While extensive research has focused on the individual effect of
77 warming, drought, and elevated CO_2 as well as two-factor interactions on C_3 and C_4 plant
78 performances and competition, studies examining all three factors simultaneously remain
79 scarce. Because of the concurrent changes in global temperature, precipitation patterns, and
80 atmospheric CO_2 concentration, investigating how these three factors jointly affect C_3-C_4
81 plant dynamics could help better predict future plant communities in the face of climate
82 change.

83 To examine the joint effects of the aforementioned three climate change factors on the
84 biomass performances and competitive responses of C_3 and C_4 plants, we grew naturally co-
85 occurring C_3 (*Oplismenus composites*) and C_4 (*Paspalum conjugatum*) grass in growth
86 chambers under three temperature warming scenarios, two water supply intervals, and two

87 CO_2 concentrations. We compared the above- and below-ground dry biomass in monoculture
88 and mixture to assess their competitive responses under different experimental treatments.
89 Based on the the ecophysiology of C_3 and C_4 plants, we predict a greater competitiveness of
90 the C_3 grass under lower temperatures, ample water supply, and elevated CO_2, whereas the C_4
91 grass will be more competitive under warming, drought, and ambient CO_2.
92

93 **Materials and Methods**

94 *Preparation of plant individuals*

95 Ramets of *O. composites* and *P. conjugatum* were collected from the campus of National
96 Taiwan University (N25° 00' 57", E121° 32' 19") in October, 2017. These ramets were
97 planted in seedling nursery pots using fully-mixed peat moss soil (Kekkila natural peat moss
98 soil) as the substrate. All plant individuals were first cultivated in greenhouse at ambient CO_2
99 concentration with 25/20°C day/night temperature for two weeks, allowing their recovery and
100 establishment prior to the start of experiment.

101

102 *Experimental design*

103 To examine the effects of major climate change factors on the performances and competitive
104 interactions of the C_3 and C_4 grass, we used a full-factorial design crossing three temperature
105 warming scenarios, two water supply intervals, and two CO_2 concentrations (see the
106 following paragraph for more details). Each treatment combination contained four replicate
107 pots of C_3 monoculture, C_4 monoculture, and C_3-C_4 mixture (each with four individuals; see
108 the following text), yielding a total of 144 pots (12 treatment combinations × 3 planting
109 schemes × 4 replicate pots (Fig. 1). To avoid the potential confounding effect of initial size,

110 similar-sized individuals of each two grasses (C_3 grass: 8.6 ± 4.3 cm; C_4 grass: 6.2 ± 4.5 cm;
111 mean ± SD) were selected from the greenhouse nursery for the experimental pots. Four
112 individuals were grown and equally-spaced in each pot (13 cm in height x 12 cm in diameter),
113 conforming to their densities at the site where the ramets were collected (personal
114 observations). In the monoculture pots, all four individuals belonged to the same species; in
115 the mixture pots, two individuals of each species were planted. Each replicate pot was
116 assigned a pot number prior to the start of the experiment; monoculture and mixture pots
117 were paired for analysis of competitive responses (Fig. 1). Pots were placed in growth
118 chambers receiving respective treatments and a constant relative humidity of 70%,
119 photoperiod of 12:12 light/dark, and photosynthetic photon flux density (PPFD) of 160–200
120 $\mu mol \cdot M^{-2} \cdot S^{-1}$.

121 Three warming scenarios were used in the temperature treatment: control (+0°C), 2°C
122 warming (+2°C), and 4°C warming (+4°C), based on the projection that mean global surface
123 temperature will be 1.8 ± 0.5°C and 3.7 ± 0.7°C above the 1986–2005 reference period by
124 2100 under intermediate (Representative Concentration Pathway [RCP] 4.5) and high carbon
125 emissions scenario (RCP 8.5), respectively (IPCC 2014). To reflect the daily temperature
126 fluctuations in the field, a control temperature curve was derived using hourly temperature
127 records in 2016 from the nearest weather station (Taipei weather station; mean: 23.3°C; range:
128 21.6–25.9°C). Temperature curves for the +2°C and +4°C treatment were obtained by
129 horizontally shifting the control temperature curve upward by 2°C and 4°C, respectively (Fig.
130 S1). Two watering regimes were used in the water treatment: 2 days and 7 days, representing
131 the normal and prolonged precipitation interval (drought). The normal interval of 2 days was
132 determined based on the mean annual number of precipitation days between 1981 and 2011
133 (165.5 days per year) using weather records from the Taipei weather station. The water
134 volume for a single watering event was 160 ml (derived by multiplying the average

135 precipitation on each rainy day [14.5 mm; mean annual precipitation of 2405 mm divided by
136 165.5 days] with the pot surface area [110 cm^2]). Two CO_2 concentrations were used in the
137 CO_2 treatment: 400 ppm (ambient) and 800 ppm (elevated), reflecting the current level of
138 atmospheric CO_2 and the projected atmospheric CO_2 between RCP 6.0 (670 ppm) and RCP
139 8.5 (936 ppm) (IPCC 2014).

140 Plants were harvested after six weeks of cultivation and separated into above- and
141 below-ground parts. The below-ground parts were carefully washed to remove the soil, with
142 as many fine roots retained as possible. All plant materials were oven-dried at 50°C for seven
143 days. Dry biomass was measured to the third decimal place in the unit of gram (g).

144

145 *Data analyses*

146 To examine the effects of our experimental treatments on the above- and below-ground dry
147 biomass of the C_3 and C_4 grass, we fitted linear mixed effects models (LMMs) with
148 temperature warming, water supply interval, CO_2 concentration, culture type (monoculture vs.
149 mixture), and their interactions as fixed effects, and pot as a random effect, using the "lmer"
150 function in the *lme4* R package (Bates et al. 2015). Models were fitted separately for the
151 above- and below-ground biomass of the two species (i.e., a total of four models). The
152 response (dry biomass of individual plant) was log-transformed; initial plant height was also
153 included as a covariate. The significance of predictors was assessed via *F*-tests (type III sum
154 of squares) using the "joint_tests" function in the *emmeans* R package (Lenth et al. 2018).
155 Tukey post-hoc comparisons of treatment levels were conducted using also the *emmeans* R
156 package.

157 To quantify the competitive responses of the C_3 and C_4 grass in each treatment
158 combination, we first averaged the above-/below-ground dry biomass of the four individuals
159 in each monoculture pot as well as the biomass of the two individuals of the same species in
160 each mixture pot. We then calculated the log response ratio (lnRR) of the pot-level mean
161 biomass in paired monoculture and mixture pots as log(mixture mean biomass / monoculture
162 mean biomass). This lnRR measures the relative effects of interspecific versus intraspecific
163 competition received by the focal individual, effectively representing the relative "sensitivity"
164 of a species to interspecific and intraspecific competition (Bennett et al. 2016). A positive
165 lnRR indicates a better performance under interspecific competition compared to intraspecific
166 competition (i.e., less suppression by interspecific competition) and vice versa for a negative
167 lnRR. The lnRRs were analyzed using linear models with temperature warming, water supply
168 interval, CO_2 concentration, plant species, and their interactions as predictors. The
169 significance of predictors was assessed via *F*-tests (type III sum of squares) as above; the
170 estimated marginal means (EMMs) of lnRRs of the two grass species derived from the linear
171 models were compared within each treatment combination using the *emmeans* R package. All
172 analyses were performed in R version 4.1.2 (R Core Team 2021).

173

174 **Results**

175 *Biomass performances of the C_3 and C_4 grass*

176 (1) C_3 above- and below-ground dry biomass

177 The above-ground dry biomass of the C_3 grass (*O. composites*) was affected by temperature
178 warming, water supply interval, and CO_2 concentration (Table 1). The above-ground biomass
179 was higher under 4°C warming for all but normal water supply interval × ambient CO_2

180 treatment (Fig. 2), indicating an interaction between temperature and water as well as
181 between temperature and CO_2 (Table 1). Prolonged water supply interval reduced the
182 biomass, whereas elevated CO_2 increased the above-ground biomass (Table S1).

183 The below-ground dry biomass of *O. composites* was affected by temperature
184 warming and water supply interval but not CO_2 concentration (Table S2). The below-ground
185 biomass did not differ among temperature treatments at ambient CO_2 but was higher under
186 4°C warming at elevated CO_2 (Fig. 2), indicating an interaction between temperature and
187 CO_2 (Table S2). Prolonged water supply interval reduced the below-ground biomass (Table
188 S1).

189

190 (2) C_4 above- and below-ground dry biomass

191 The above-ground dry biomass of the C_4 grass (*P. conjugatum*) was affected by temperature
192 warming and CO_2 concentration but not water supply interval (Table 1). The above-ground
193 biomass was higher under 4°C warming across all water × CO_2 treatments (Fig. 3). Elevated
194 CO_2 enhanced the above-ground biomass production (Table S1).

195 Similar to the response of above-ground dry biomass to the experimental treatments,
196 the below-ground dry biomass of *P. conjugatum* was affected by temperature warming and
197 CO_2 concentration but not water supply interval (Table S2). The effect of temperature
198 warming on below-ground biomass varied across water and CO_2 treatments (i.e., Temperature
199 × Water × CO_2 interaction; Fig. 3; Table S2). Specifically, at normal water supply interval,
200 the below-ground biomass was lower under 2°C warming compared to control temperature at
201 ambient CO_2, while there was no difference in biomass among temperature treatments at
202 elevated CO_2. At prolonged water supply interval, the below-ground biomass was the lowest

203 under 2°C warming, although the difference between 2°C and 4°C warming was not
204 statistically significant at ambient CO_2. Elevated CO_2 enhanced the below-ground biomass
205 production (Table S1).

206 When examining the effect of culture type, we found a significant Temperature ×
207 Culture type interaction for the C_4 grass (Table 1; Table S2). In particular, the C_4 above-
208 ground biomass was considerably higher in mixture than in monoculture (Fig. S2), suggesting
209 that warming may increase the competitive ability of C_4 grass when grown with C_3 grass.

210

211 *Competitive responses of the C_3 and C_4 grass*

212 The lnRRs of above- and below-ground dry biomass were not affected by temperature
213 warming, water supply interval, or CO_2 concentration; however, the competitive responses of
214 the two grasses depended on the CO_2 concentration (Species × CO_2 interaction; Table 2; Fig.
215 4). At ambient CO_2, the C_3 grass had similar above-ground biomass under interspecific
216 (mixture) and intraspecific (monoculture) competition (lnRR ≈ 0) and higher below-ground
217 biomass under interspecific competition compared to intraspecific competition (lnRR > 0),
218 whereas the C_4 grass had lower above- and below-ground biomass under interspecific
219 competition compared to intraspecific competition (lnRR < 0). In contrast, the competitive
220 responses reversed at elevated CO_2, with the C_3 grass having lower biomass (lnRR < 0) and
221 the C_4 grass having higher biomass when grown in mixtures (lnRR > 0) (Fig. 4). These
222 results suggest that increasing CO_2 concentration could benefit C_4 grass when in competition
223 with C_3 grass.

224 Overall, the above- and below-ground competitive responses of the two grass species
225 in each treatment combination were generally comparable, despite some variations in their
226 signs and magnitudes (Fig. S3). However, the lnRRs of both above- and below-ground dry

227 biomass did differ between the two species under a combination of 4°C warming, prolonged
228 water supply interval, and elevated CO_2, where the C_3 grass performed worse (lnRR < 0) and
229 the C_4 grass performed better (lnRR > 0) in mixtures compared to their corresponding
230 monocultures (Fig. 5a; Fig. S3). Indeed, the positive lnRRs of the C_4 grass under multiple
231 environmental stressors were largely due to an increase in mixture biomass rather than a
232 reduction in monoculture biomass in comparison to the controlled baseline treatment (Fig.
233 5b). Together, these results indicate a greater competitive ability of the C_4 grass under higher
234 temperature, drought condition, and elevated CO_2.

235

236 **Discussion**

237 We examined the joint effects of three major climate change factors—temperature, water, and
238 CO_2—on the biomass performances and competitive responses of a C_3 and a C_4 grass in
239 growth chambers. Warming and elevated CO_2 generally enhanced the above- and/or below-
240 ground biomass of both grasses, with a synergism between these two factors. In contrast to
241 theoretical predictions and experimental results (Carter and Peterson 1983, Pearcy and
242 Ehleringer 1984), the C_3 grass performed worse under interspecific competition (mixture
243 biomass) relative to intraspecific competition (monoculture biomass) at elevated CO_2, while
244 the reverse is true for C_4 grass. Furthermore, the C_4 grass outperformed the C_3 grass most
245 under simultaneous 4°C warming, drought conditions, and elevated CO_2, suggesting that the
246 competitive balance could potentially shift in favor of C_4 grass under the projected
247 temperature warming, increased drought frequencies, and rising atmospheric CO_2. Taken
248 together, our results provide empirical evidence for C_3 and C_4 biomass performances and
249 competition in response to multiple environment stressors, underlining the importance of

250 considering multi-variable effects and offering useful insights into C_3-C_4 plant dynamics
251 under future climate change.

252

253 *Effects of warming and elevated CO_2 on biomass performances*

254 We found that warming (in particular 4°C warming) increased the above- and below-ground
255 biomass of the C_3 grass, contradicting the common notion that C_3 grass performance should
256 not be enhanced, or even potentially be reduced, under higher temperatures (Sage and Kubien
257 2007). A possible explanation for this observation is that the control temperature used in the
258 study was below the thermal optimum of photosynthesis for the C_3 grass and therefore
259 warming indeed led to a further increase in carbon fixation and biomass production.
260 Additionally, the C_3 grass may shift its thermal optimum as the temperature rises, as research
261 has suggested that C_3 plants have broader thermal ranges and higher potential for thermal
262 acclimation (Sage and Kubien 2007, Yamori et al. 2014). Consequently, future climate
263 warming might not necessarily suppress C_3 plants as generally predicted if they are able to
264 exhibit plastic thermal acclimation to higher temperatures.

265 Elevated CO_2 enhanced the above-ground biomass of the C_3 grass as well as the
266 above- and below-ground biomass of the C_4 grass. The higher C_3 grass biomass at elevated
267 CO_2 agrees with the widely-documented positive response of C_3 plants to increasing CO_2
268 (Ainsworth and Long 2005, Wang et al. 2012). In contrast, biomass enhancement of the C_4
269 grass is surprising as theory predicts that increasing CO_2 concentration may not benefit the
270 performance of C_4 plants because of their lower CO_2 saturation points for photosynthesis
271 (Sage 1994, Ghannoum et al. 2000). Nonetheless, empirical evidence regarding the effect of
272 CO_2 on the performance of C_4 plants is mixed. In fact, some studies have reported biomass
273 stimulation by CO_2 in C_4 plants (e.g., Owensby et al. 1993, Wand et al. 1999). These findings

274 indicate that the response of C_4 plants to CO_2 could be species-specific and a future rise in
275 atmospheric CO_2 may potentially benefit certain C_4 plants.

276

277 *Interactions among treatment factors*

278 The interaction between temperature and CO_2 appeared to be the most conspicuous two-way
279 interaction among the three treatment factors across both grass species (Table 1; Table S2).
280 The synergistic effect of temperature warming and elevated CO_2 suggests that climate
281 warming together with increasing atmospheric CO_2 could further enhance the biomass
282 production of plants regardless of their photosynthetic pathways (Wang et al. 2012). For the
283 C_3 grass, such a synergism may arise from a greater ameliorating effect of CO_2 on
284 photorespiration with rising temperature (Long 1991, Dusenge et al. 2019). Furthermore,
285 elevated CO_2 could increase the thermal optimum of net carbon assimilation rate of the C_3
286 grass (Sage and Kubien 2007), leading to greater biomass production under simultaneous
287 warming and elevated CO_2. On the other hand, C_4 plants generally do not respond strongly to
288 rising CO_2 because of their CO_2 concentration mechanism. The potential mechanisms
289 underlying the observed positive temperature × CO_2 interaction in the C_4 grass warrant
290 further investigations.

291 Our results revealed a three-way interaction among temperature, water, and CO_2 for
292 the below-ground biomass of the C_4 grass. There are a few possible explanations for this.
293 First, warming-induced physiological changes (e.g., reduced stomatal conductance) could
294 provide cross tolerance to drought (Havaux 1992). Second, elevated CO_2 may increase water
295 use efficiency and thus help alleviate the impact of water stress (Xu et al. 2013). Third, more
296 resources may be allocated to below-ground growth to increase water uptake in response to
297 temperature warming and water limitation (Malik et al. 1979, Sharp and Davies 1989).

298 Research on how C_3 and C_4 plants respond to multiple stressors has been limited, and our
299 finding provides experimental evidence that future climate change could affect plant biomass
300 production in a complex way that might not be predicted from individual factors alone. More
301 studies on how multiple climate change factors jointly affect C_3 and C_4 plant performances
302 are needed to verify if such a three-way interaction is a common phenomenon.

303

304 *Competitive responses under experimental treatments*

305 Countering previous studies showing greater competitive advantages of C_3 plants at elevated
306 CO_2 (Marks and Strain 1989, Ziska 2001), our results show that higher CO_2 concentration
307 could potentially benefit the C_4 grass when in competition with the C_3 grass. Specifically, the
308 C_3 grass was suppressed more by interspecific competition relative to intraspecific
309 competition at elevated CO_2 compared to ambient CO_2, whereas the C_4 grass performed
310 better under interspecific competition relative to intraspecific competition at elevated CO_2
311 compared to ambient CO_2 (Fig. 4). Such patterns can be partly explained by a greater effect
312 of elevated CO_2 on the C_4 above-ground biomass in mixture compared to monoculture (CO_2
313 × Culture type interaction; Table 1). Moreover, elevated CO_2 combined with 4°C warming
314 and drought resulted in the largest difference in the competitive responses of the two grasses,
315 with an increased interspecific competitive ability of the C_4 grass against the C_3 grass (Fig. 5).
316 These results suggest that the competitive balance could potentially shift in favor of C_4 plants
317 under future climate scenarios, which may have potential impacts on both natural and
318 agricultural systems. For example, C_4 plants are generally less nutritious than C_3 plants, and
319 increasing C_4 relative abundance in the habitat could reduce the nutritional availability for
320 herbivores (Barbehenn et al. 2004). Furthermore, C_3 crops may suffer greater yield loss
321 because of increasing competition pressure from C_4 weeds (Korres et al. 2016).

322 Despite some variations in the signs and magnitudes, the competitive responses of the
323 two grass species were by and large similar in most treatment combinations, indicating
324 similar relative effects of interspecific versus intraspecific competition received by the two
325 grasses. Surprisingly, we did not find any main effect of temperature, water, or CO_2 treatment
326 on the above- and below-ground competitive responses of the two grasses, despite strong
327 effects of these factors on their biomass production. A lack of treatment effect on the
328 competitive responses may occur if the two grasses respond to the treatments in a similar
329 fashion (e.g., positive responses of both C_3 and C_4 grass to elevated CO_2), and consequently
330 there might be no competitive shift because neither species would gain a net advantage over
331 the other. This shows that performances of individual species alone may not necessarily
332 reflect their competitive relationships, which depend largely on the species' relative
333 performances when grown together.

334

335 *Conclusions*

336 Using an experimental approach, our study shows that warming and elevated CO_2 together
337 enhanced the biomass performances of both C_3 and C_4 grass, and temperature, water, and
338 CO_2 interacted to affect the C_4 biomass. Moreover, simultaneous warming, drought, and
339 particularly elevated CO_2, benefited the C_4 grass most when in competition with the C_3 grass,
340 suggesting potential shifts in their competitive balance along with an increase in C_4 relative
341 abundance under future climate scenarios. Changes in C_3-C_4 community composition can
342 have profound consequences for the functioning of natural and agricultural systems,
343 including primary productivity, nutrient cycling, herbivore nutritional balance, and crop
344 production. As the effects of climate change are multifaceted and often involve complex
345 interactions and feedbacks over time and space, long-term and large-scale field experiments

346 involving more species will represent a promising research avenue that scales up our
347 laboratory results to better predict C_3-C_4 plant dynamics under changing climate.

348

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353

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458 **Tables and Figures**

459 Table 1. Results of the linear mixed models (LMMs) examining the effects of temperature
460 warming, water supply interval, CO_2 concentration, and culture type (monoculture vs.
461 mixture) on the above-ground dry biomass of the C_3 and C_4 grass

Predictor	C_3 grass			C_4 grass		
	F	*df*	*P**	*F*	*df*	*P**
Temperature warming	57.42	2, 103	**< 0.001**	56.66	2, 99	**< 0.001**
Water supply interval	26.13	1, 99	**< 0.001**	1.60	1, 99	0.208
CO_2 concentration	48.7	1, 99	**< 0.001**	18.8	1, 100	**< 0.001**
Culture type	3.54	1, 99	0.063	2.30	1, 99	0.132
Temperature × Water	4.41	2, 99	**0.015**	2.82	2, 99	0.064
Temperature × CO_2	18.07	2, 101	**< 0.001**	3.10	2, 99	**0.049**
Temperature × Culture type	1.07	2, 99	0.346	4.89	2, 100	**0.009**
Water × CO_2	5.74	1, 99	**0.018**	0.73	1, 99	0.394
Water × Culture type	0.39	1, 99	0.534	0.25	1, 99	0.619
CO_2 × Culture type	0.26	1, 102	0.613	4.57	1, 99	**0.035**
Temperature × Water × CO_2	0.13	2, 99	0.881	1.84	2, 99	0.164
Temperature × Water × Culture type	1.97	2, 99	0.145	2.43	2, 100	0.093
Temperature × CO_2 × Culture type	0.90	2, 100	0.409	0.15	2, 99	0.861
Water × CO_2 × Culture type	3.08	1, 99	0.082	0.96	1, 99	0.331
Temperature × Water × CO_2 × Culture type	4.07	2, 99	**0.020**	0.26	2, 99	0.769

462 * Significant effects ($P < 0.05$) are highlighted in bold.

463

464 Table 2. Results of the linear models examining the effects of temperature warming, water
465 supply interval, CO_2 concentration, and species on the log response ratios (lnRRs) of above-
466 and below-ground dry biomass in mixture relative to monoculture (log[mixture
467 biomass/monoculture biomass])

Predictor	Above-ground			Below-ground		
	F	*df*	*P**	*F*	*df*	*P**
Temperature warming	0.91	2, 72	0.406	0.59	2, 71	0.556
Water supply interval	2.94	1, 72	0.091	0.40	1, 71	0.528
CO_2 concentration	0.22	1, 72	0.637	0.25	1, 71	0.621
Species	1.59	1, 72	0.211	0.12	1, 71	0.730
Temperature × Water	1.77	2, 72	0.177	1.29	2, 71	0.281
Temperature × CO_2	1.36	2, 72	0.262	1.56	2, 71	0.218
Temperature × Species	1.98	2, 72	0.146	1.61	2, 71	0.207
Water × CO_2	0.74	1, 72	0.392	2.67	1, 71	0.106
Water × Species	0.07	1, 72	0.792	4.97	1, 71	**0.029**
CO_2 × Species	5.66	1, 72	**0.020**	7.87	1, 71	**0.006**
Temperature × Water × CO_2	2.87	2, 72	0.063	2.98	2, 71	0.057
Temperature × Water × Species	0.44	2, 72	0.645	0.46	2, 71	0.631
Temperature × CO_2 × Species	0.31	2, 72	0.736	0.58	2, 71	0.561
Water × CO_2 × Species	1.72	1, 72	0.194	1.43	1, 71	0.235
Temperature × Water × CO_2 × Species	1.60	2, 72	0.210	3.38	2, 71	**0.039**

468 * Significant effects ($P < 0.05$) are highlighted in bold.

469

Figure 1. An illustration of the experimental design of this study. The experiment was conducted using a factorial design crossing three temperature warming scenarios (control, +2°C, and +4°C), two water supply intervals (normal 2 days vs. prolonged 7 days), and two CO_2 concentrations (ambient 400 ppm vs. elevated 800 ppm). Each treatment combination contained four replicate pots of C_3 (*Oplismenus composites*) and C_4 grass (*Paspalum conjugatum*) in monoculture as well as C_3-C_4 mixture. Four plant individuals (four C_3 or C_4 in monoculture and two C_3 + C_4 in mixture) were grown in each pot. Replicate monoculture and mixture pots in each treatment combination were paired *a priori* by assigning the same pot number for analysis of competitive responses.

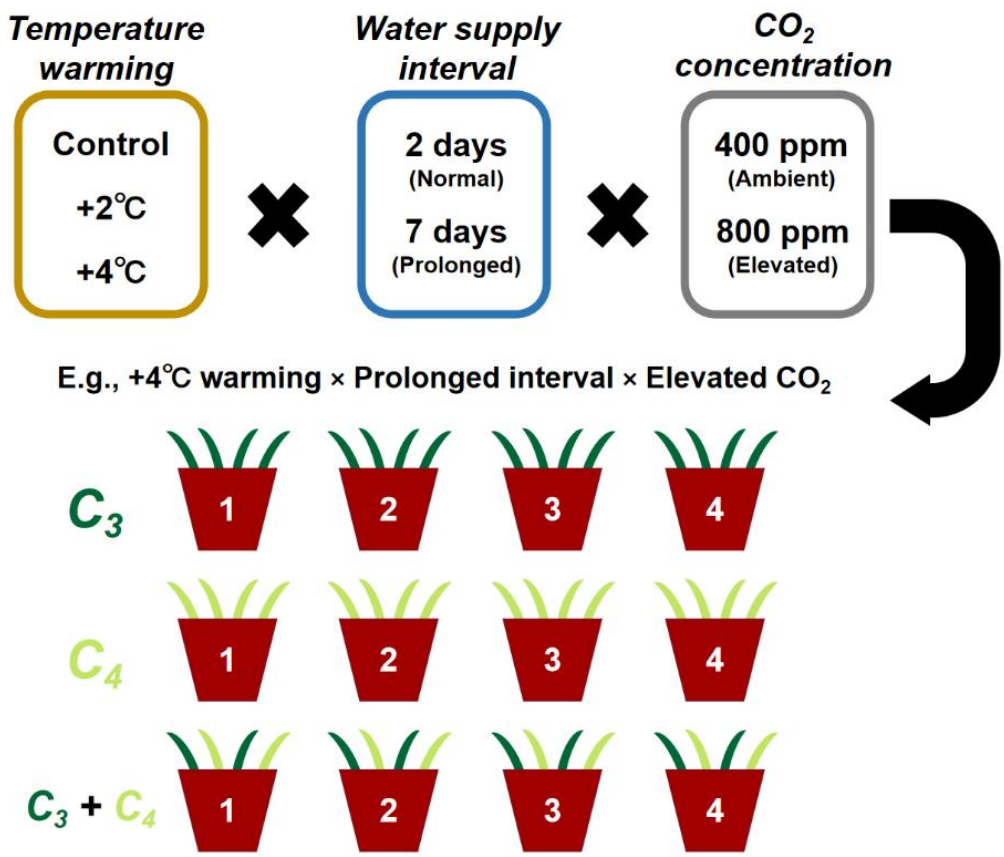

Figure 2. Above- and below-ground dry biomass of the C_3 grass under different temperature warming, water supply interval, and CO_2 concentration treatments. Tukey post-hoc comparisons were conducted for the three temperature scenarios within each water × CO_2 treatment combination using the estimated marginal means (EMMs) from the LMMs. Different letters indicate statistical significance ($P < 0.05$).

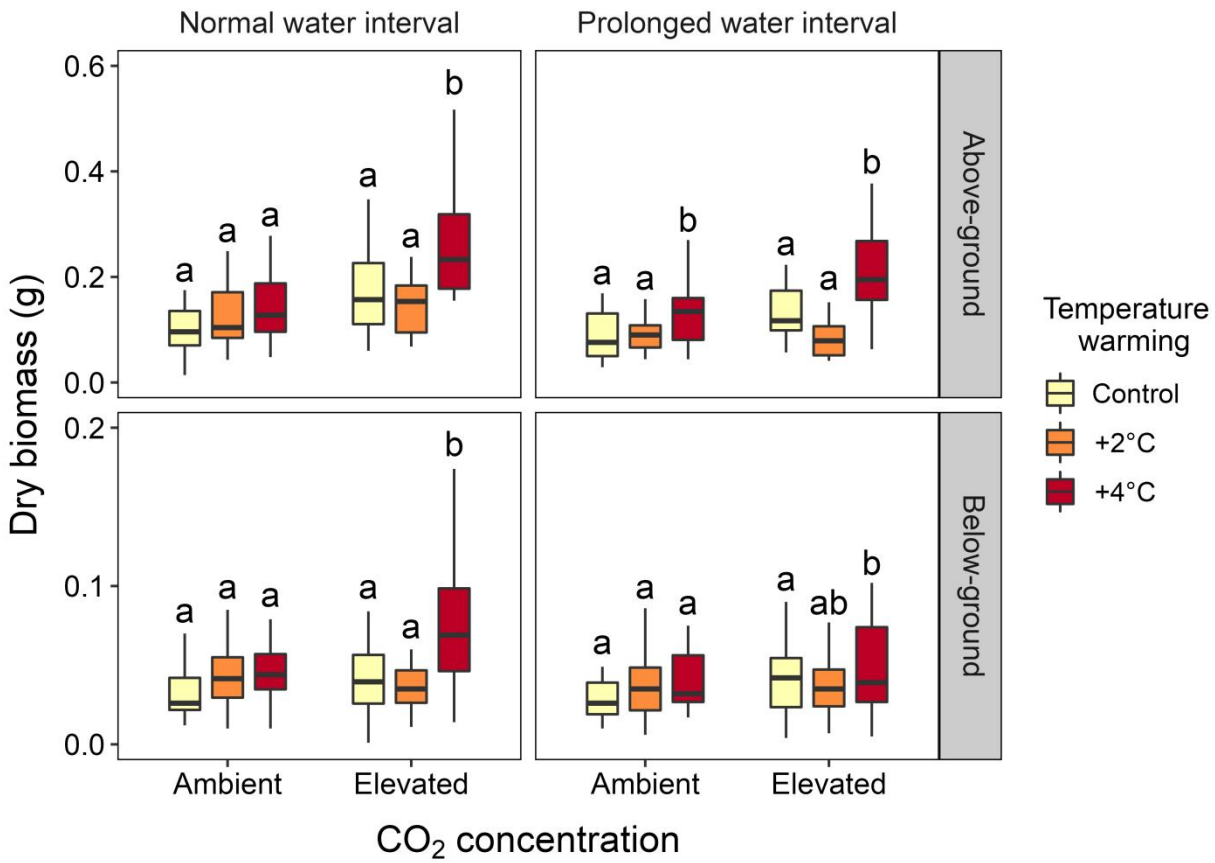

Figure 3. Above- and below-ground dry biomass of the C_4 grass under different temperature warming, water supply interval, and CO_2 concentration treatments. Tukey post-hoc comparisons were conducted for the three temperature scenarios within each water × CO_2 treatment combination using the estimated marginal means (EMMs) from the LMMs. Different letters indicate statistical significance ($P < 0.05$).

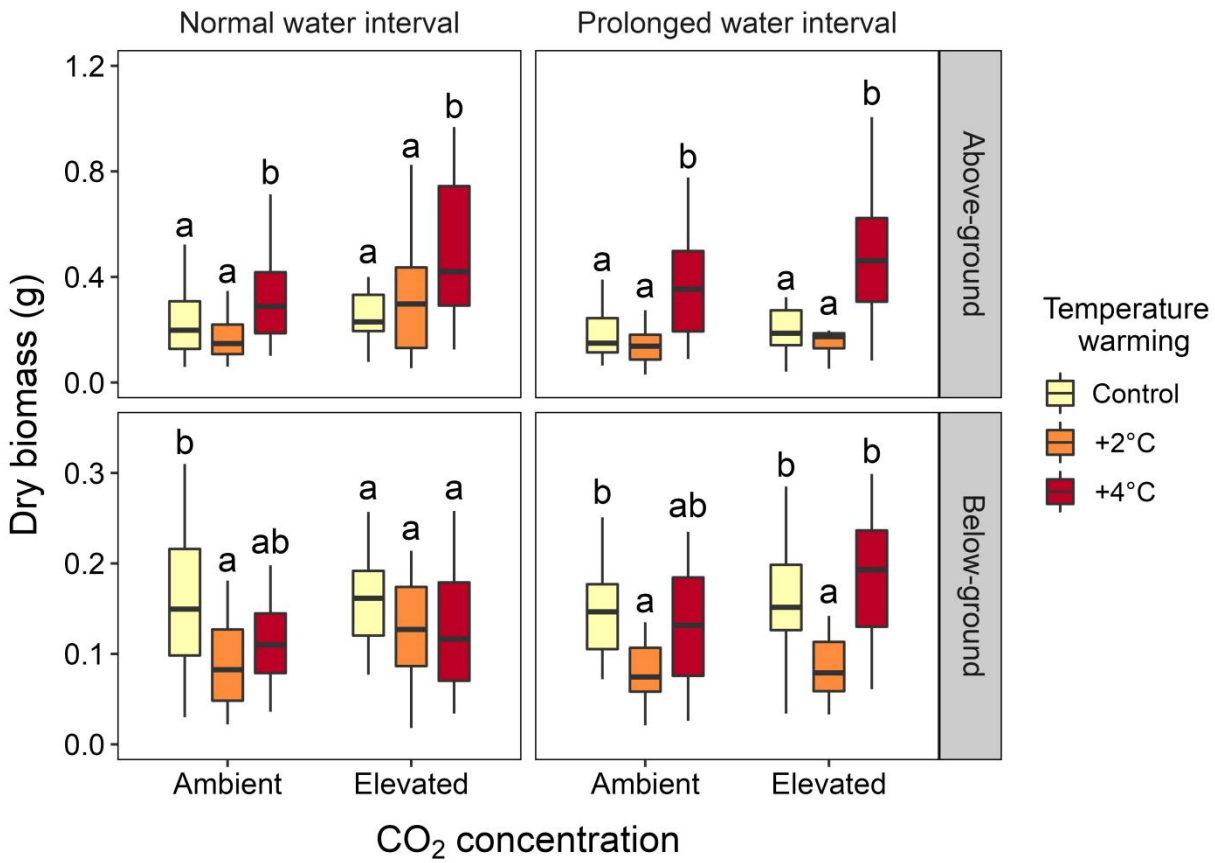

Figure 4. Interactions between CO_2 concentration and species for the lnRRs of above- and below-ground dry biomass. Points and error bars represent the EMMs and standard errors derived from the linear models. Grey dashed lines (lnRR = 0) indicate equal effects of interspecific and intraspecific competition; a positive lnRR indicates a better performance under interspecific competition compared to intraspecific competition (i.e., weaker suppression by interspecific competition) and vice versa for a negative lnRR.

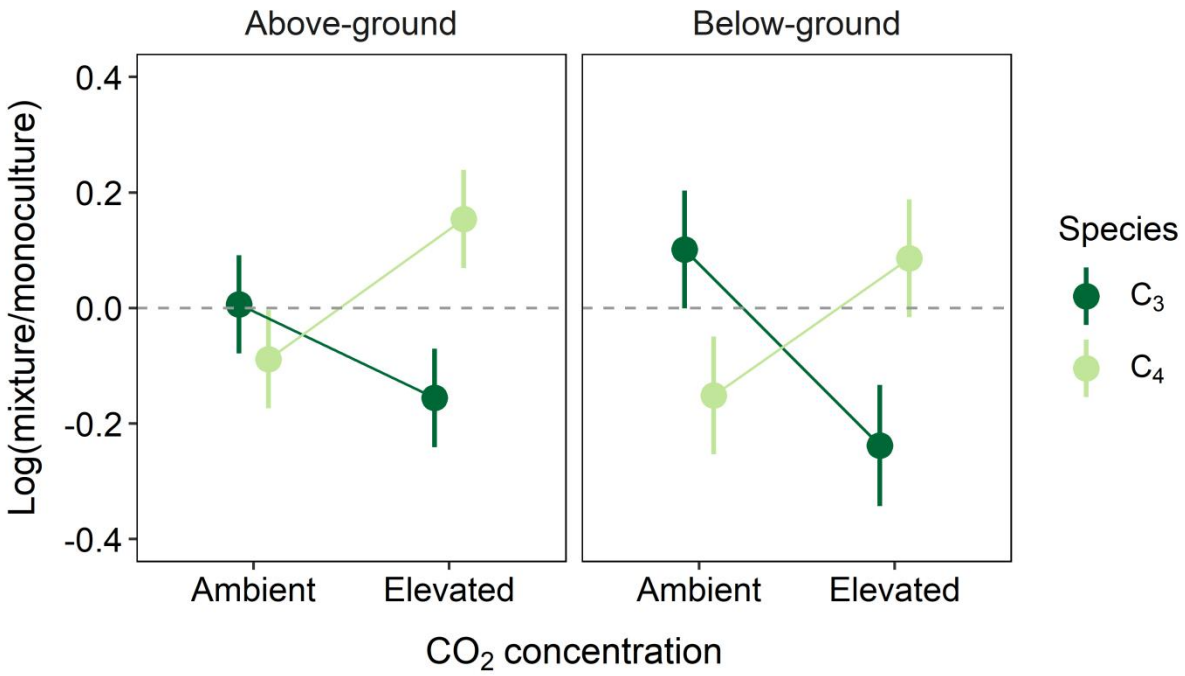
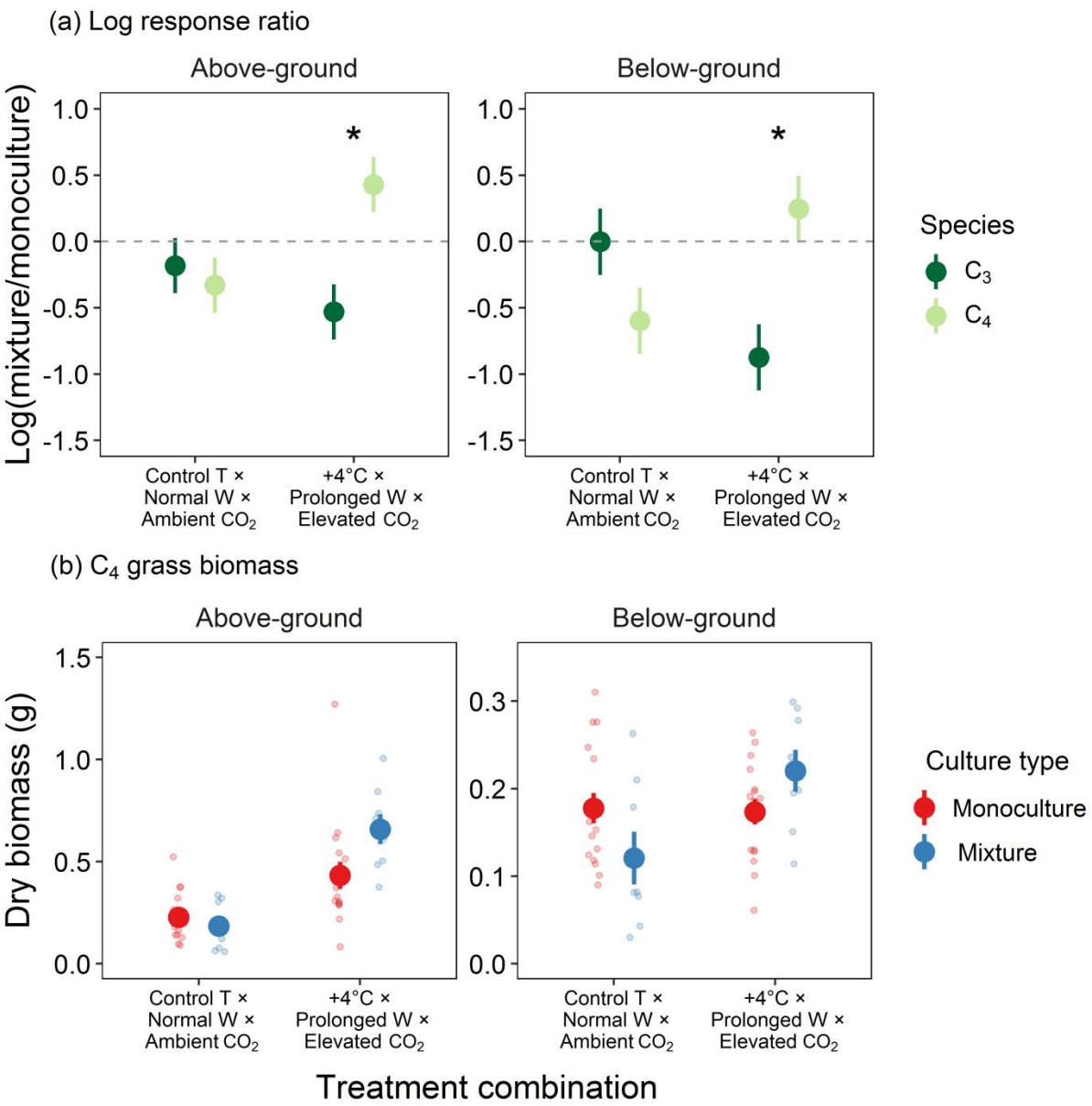
Figure 5. LnRRs of above- and below-ground dry biomass of the two grass species (a) and above- and below-ground dry biomass of the C_4 grass (b) under two opposite treatment combinations: (1) control temperature × normal water supply interval × ambient CO_2, and (2) 4°C warming × prolonged water supply interval × elevated CO_2. Points and error bars in (a) represent the EMMs and standard errors derived from the linear models; points and error bars in (b) represent original means and standard errors. Asterisks denote a significant difference in lnRRs between the two grass species ($P < 0.05$).