


The competitive advantage of a constitutive CAM species over a C₄ grass species under drought and CO₂ enrichment

KAILIANG YU,^{1,†} PAOLO D'ODORICO,^{1,2} SCOTT L. COLLINS ,³ DAVID CARR,¹ AMILCARE PORPORATO,⁴ WILLIAM R. L. ANDEREGG,⁵ WILLIAM P. GILHOOLY III,⁶ LIXIN WANG,⁶ ABINASH BHATTACHAN,⁷ MARK BARTLETT,⁴ SAMANTHA HARTZELL,⁴ JUN YIN,⁴ YONGLI HE,^{1,8} WEI LI,^{1,9} MOKGANEDI TATLHEGO,^{1,2} AND JOSE D. FUENTES¹⁰

¹Department of Environmental Sciences, University of Virginia, Charlottesville, Virginia 22904 USA

²Department of Environmental Science, Policy and Management, University of California, Berkeley, California 94710 USA

³Department of Biology, University of New Mexico, Albuquerque, New Mexico 87131 USA

⁴Department of Civil and Environmental Engineering, Duke University, Durham, North Carolina 27708 USA

⁵Department of Biology, University of Utah, Salt Lake City, Utah 84112 USA

⁶Department of Earth Sciences, Indiana University Purdue University Indianapolis (IUPUI), Indianapolis, Indiana 46202 USA

⁷Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, North Carolina 27607 USA

⁸Key Laboratory for Semi-Arid Climate Change of the Ministry of Education, College of Atmospheric Sciences, Lanzhou University, Lanzhou 730000 China

⁹State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Northwest A&F University, Yangling 712100 China

¹⁰Department of Meteorology, Pennsylvania State University, University Park, Pennsylvania 16802 USA

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Abstract. Plants with crassulacean acid metabolism (CAM) are increasing in distribution and abundance in drylands worldwide, but the underlying drivers remain unknown. We investigate the impacts of extreme drought and CO₂ enrichment on the competitive relationships between seedlings of *Cylindropuntia imbricata* (CAM species) and *Bouteloua eriopoda* (C₄ grass), which coexist in semiarid ecosystems across the Southwestern United States. Our experiments under altered water and CO₂ water conditions show that *C. imbricata* positively responded to CO₂ enrichment under extreme drought conditions, while *B. eriopoda* declined from drought stress and did not recover after the drought ended. Conversely, in well-watered conditions *B. eriopoda* had a strong competitive advantage on *C. imbricata* such that the photosynthetic rate and biomass (per individual) of *C. imbricata* grown with *B. eriopoda* were lower relative to when growing alone. A meta-analysis examining multiple plant families across global drylands shows a positive response of CAM photosynthesis and productivity to CO₂ enrichment. Collectively, our results suggest that under drought and elevated CO₂ concentrations, projected with climate change, the competitive advantage of plant functional groups may shift and the dominance of CAM plants may increase in semiarid ecosystems.

Key words: *Bouteloua eriopoda* (C₄ grass); CO₂ enrichment; competition; Crassulacean acid metabolism (CAM species); *Cylindropuntia imbricata* (CAM species); drought; semiarid ecosystems.

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† E-mail: ky9hc@virginia.edu

INTRODUCTION

Over the last century, human activities associated with fossil fuel burning and land use change have dramatically increased the concentrations of CO₂ and other trace gases in the atmosphere, a trend that is expected to continue in the decades to come (Vitousek et al. 1997, IPCC 2014). At the same time, global climate models predict increased precipitation variability along with more frequent extreme rainfall events, and increased risk of prolonged drought, especially in dryland regions (Smith 2011, IPCC 2014). These changes in environmental conditions combined with human activities have led to significant changes in vegetation cover and plant community composition (Poorter and Navas 2003, D'Odorico et al. 2012, Anderegg et al. 2013), with important impacts on ecohydrological and geochemical processes, regional climate, and the provision of ecosystem services such as livestock grazing, sheltering of the soil surface, and carbon sequestration (Anderegg et al. 2013, Reichstein et al. 2013).

In particular, both empirical and modeling studies in the Southwestern United States have focused on the transition from grasslands to shrublands (D'Odorico et al. 2012, Caracciolo et al. 2016) or widespread tree mortality under global environmental change (Breshears et al. 2005, McDowell et al. 2008, Anderegg et al. 2013). Responses by plants with crassulacean acid metabolism (CAM) and their competitive relationship under global change, however, remain understudied. Globally, CAM plants appear to be expanding in abundance and distribution across dryland ecosystems (Borland et al. 2009, Reyes-García and Andrade 2009), but the underlying drivers remain poorly understood. Yu et al. (2017a, b) experimentally found that a model facultative CAM species (*Mesembryanthemum crystallinum*) had a competitive advantage over a co-occurring grass (*Bromus mollis*) in California under global change scenarios of increased drought, soil salinity, and nitrogen deposition. An empirical evaluation of the response of constitutive CAM plants and their competitive relationships with other functional groups (i.e., C₄ plants) to CO₂ enrichment and changing climate, however, is still missing (Poorter and Navas 2003).

Some studies suggest that changes in climate (i.e., temperature or rainfall) or increasing atmospheric CO₂ concentrations could be major drivers of CAM expansion in drylands (Drennan and Nobel 2000, Borland et al. 2009, Reyes-García and Andrade 2009). CAM plants feature high water storage in their tissues, nocturnal CO₂ uptake, and high water use efficiency and thus a high drought resistance (Drennan and Nobel 2000, Lüttge 2004, Borland et al. 2009). In this sense, CAM plants could still maintain a low rate of photosynthesis and growth during drought and moreover could have a relatively rapid recovery after drought. Interestingly, the prediction that nocturnal CO₂ uptake (C₄ photosynthesis) by CAM plants should saturate under CO₂ enrichment was not supported by experiments (Drennan and Nobel 2000). It, however, remains unclear whether the response of CAM plant's photosynthesis and productivity to CO₂ enrichment is comparable to that of C₃ plants or C₄ plants (Poorter and Navas 2003). It also remains poorly understood whether CAM plants have a competitive advantage over C₄ plants under drought and CO₂ enrichment.

The ability of CAM plants to increase in abundance also depends on their competitive relationship with other functional groups (i.e., C₃ and C₄ plants; Poorter and Navas 2003, Yu and D'Odorico 2015), an aspect of CAM plant sensitivity to changes in climate and atmospheric CO₂ concentrations that has remained largely understudied. Most previous studies only investigated the response of monocultures of CAM plants to CO₂ enrichment (see the review in Drennan and Nobel 2000). The response of plants as monocultures, however, may be strikingly different from plant responses when grown in mixture (Morgan et al. 2004, Dijkstra et al. 2010). In ecosystems where C₃ and C₄ plants dominate, previous studies showed that the C₃ grass *Pascopyrum smithii* and the C₄ grasses *Bouteloua gracilis* had positive responses to CO₂ enrichment when grown in monocultures yet they showed little response to CO₂ enrichment when grown in natural (mixed) communities (Morgan et al. 1998, 2004). In addition, CO₂ enrichment favored a C₃ forb (*Gossypium hirsutum*) more than a C₄ grass (*Sorghum bicolor*), and thus, C₄ species were indirectly disfavored when grown in mixture (Derner et al. 2003). As

a consequence, global environmental change (i.e., drought intensification) could disfavor some functional groups while indirectly increasing the competitive advantage of CAM plants. Likewise, CO₂ enrichment may favor CAM plants more than C₄ species, in particular, in drought conditions, thus leading to the indirect disadvantage of C₄ plants.

We first conducted a thorough meta-analysis of the literature to examine the response of photosynthesis and productivity by CAM plants to CO₂ enrichment. We found the positive response of daily CO₂ uptake and biomass by CAM plants alone to CO₂ enrichment, which is comparable to the response of C₃ plants. It, however, remains empirically untested how the competition between CAM and grasses changes under global environmental change. To this end, we then designed a set of growth chamber experiments to investigate the potential shift in the competitive relationships between a CAM plant and a C₄ grass under drought and CO₂ enrichment. *Cylindropuntia imbricata* (CAM species) and *Bouteloua eriopoda* (C₄ grass) co-occur in desert grasslands in the Southwestern United States and northern Mexico (i.e., Chihuahuan Desert). In our experiments, these species were subjected to two levels of CO₂ concentrations under drought and well-watered conditions. Both *B. eriopoda* (Poaceae) and *C. imbricata* (Cactaceae) are long-lived (>20 yr) and shallow-rooted perennials (Peters and Yao 2012). Field observations in native and protected desert grasslands without human disturbance (i.e., fires and grazing) indicate that *C. imbricata* has been increasing in abundance and distribution in desert grasslands currently dominated by *B. eriopoda* (Appendix S2: Fig. S1). The underlying mechanisms, however, remain unclear. Given the high drought resistance of *C. imbricata*, its capability of rapid recovery after droughts, and its lack of saturation in photosynthesis and productivity in response to CO₂ enrichment, we hypothesized that *C. imbricata* would have a competitive advantage over *B. eriopoda* under extreme drought and CO₂ enrichment conditions.

MATERIALS AND METHODS

We performed multiple Google Scholar and Web of Science searches using keywords of crassulacean acid metabolism, CAM, cactus,

succulent, CO₂, photosynthesis, and productivity. To be included in the meta-analysis, the paper needed to report a long-term (≥2 months) response of photosynthesis and biomass (productivity) to CO₂ enrichment which spans a range of 650–1000 ppm under optimal environments (i.e., adequate light and nutrient availability). If the studies reported the response of growth rate instead of photosynthesis and biomass (productivity), the growth rate was used to represent the response of photosynthesis and biomass (productivity). We found that published studies were scarce and identified 21 studies spanning 19 constitutive CAM species among five families across the Southwestern United States, Israel, Panama, Singapore, and China. See Appendix S1 for species and references used in the meta-analysis.

Seeds of the CAM plant, *C. imbricata*, and the C₄ grass, *B. eriopoda*, were ordered from <http://www.cactusstore.com> and <http://www.seedsource.com>, respectively. Seeds of each species were germinated on 5 July 2015 in plastic trays covered with 1–2 mm substratum of mineral soil in the greenhouse facility at the University of Virginia. Seedlings (of similar size) of *C. imbricata* and *B. eriopoda* were transplanted into plastic pots (13 cm in diameter and 11.5 cm in height with a capacity of 1.5 L) on 20 August 2015. *C. imbricata* and *B. eriopoda* were planted in either monoculture (4 individuals) or mixture (2 individuals of *C. imbricata* and 2 individuals of *B. eriopoda*). A mixture of Canadian sphagnum peat moss and calcined clay (6:3) was used as soil (Kieft 1998); its hydraulic conductivity (78 ± 23 mm/s) measured using an infiltrometer in the greenhouse was within the range of values measured under field conditions where these species co-occur (Ravi et al. 2009).

On 9 September 2015, seedlings (6–7 cm in height for *C. imbricata* and 35–38 cm in height for *B. eriopoda*) in both monoculture and mixture were transported to Duke University's Phytotron Facility. These seedlings were then subjected to two levels of CO₂ concentrations and two water treatments: 400 ppm (ambient carbon, LC) and 800 ppm (high carbon, HC) under drought (low water, LW) and well-watered (high water, HW) conditions. Watering intensity and frequency were based on the mean value (≈120 mm; ≈1.3 mm/d) of growing season rainfall (July,

August, and September) from 1989 to 2015 appropriate for the Sevilleta National Wildlife Refuge (Petrie et al. 2014), an arid grassland where *C. imbricata* and *B. eriopoda* coexist and interact (Miller et al. 2009). To investigate the effects of extreme drought on plant growth under future climate (Smith 2011, IPCC 2014), for the first 63 d (the first stage until 13 November 2015) plants were watered every nine days with an intensity of 4.5 mm per event for the drought treatment (low water treatment, LW). Thus, the averaged watering intensity (0.5 mm/d) in the drought treatment is about 35% of normal conditions (1.33 mm/d) in the field and the drought lasted for 2/3 of the growing season duration. The plant water stress in the drought treatment was further enhanced in the growth chamber experiment because plants were grown in small pots with a high rate of water loss. Collectively, this resulted in extreme drought conditions in this experiment. To investigate the ability of plants to recover from extreme drought events (Rivero et al. 2007), starting on day 64 all plants from the LW treatment were watered every three days with an intensity of 4.5 mm per event for 39 more days (the second stage until 23 December 2015). By comparison, in well-watered conditions (high water treatment, HW) plants were watered every three days with an intensity of 4.5 mm per event over 102 d. The experiment was a split-plot design in which 6 CO₂ chambers were used for treatments of two CO₂ concentration gradients (each CO₂ level had three replicates), and in each CO₂ chamber, seedlings were randomly assigned to treatments of drought and well-watered conditions (each treatment had 10 replicates). Among the 10 replicates, 3 replicates were used for measuring photosynthesis/biomass while the rest were used for harvesting plants and measuring titratable acidity (TA). Plants in chambers were subjected to controlled light conditions (i.e., 12 h of light with photosynthetic photon flux density of 700 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ from 07:00 to 19:00 hours) and temperature (i.e., 25°C during the day and 20°C during the night) conditions.

Gas exchange between plants and surrounding air was measured at the end of the second stage of the experiment. The very small leaves of *B. eriopoda* limited our capability of measuring its gas exchange (Appendix S2: Fig. S2). The shoots of *C. imbricata* are cylindrical (Appendix S2:

Fig. S2). Thus, we constructed a customized Plexiglas cylindrical chamber (7 cm in diameter and 12.5 cm in height) closed on one end and with a hole (1.5 cm in diameter) on the other to measure gas exchange of *C. imbricata*. The chamber (model Li-6400-19 Custom Chamber Kit; LI-COR, Lincoln, Nebraska, USA) was then used to interface the customized cylinder chamber to the portable photosynthesis unit (model LI-6400XT; LI-COR). Measurements of gas exchange were conducted for each individual of *C. imbricata* using LI-6400XT unit with the leaf area (a) of $2 \times 3 \text{ cm}^2$ in a closed system mode. The net photosynthetic rate (A , $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) of *C. imbricata* was determined as $A = A_r \times a/S$, where A_r is the recorded value of the net photosynthetic rate by LI-6400XT and S is the total photosynthetic surface area (cm^2) of *C. imbricata*. After harvesting, the surface area of each plant stem was calculated as follows: Depending on the shape of *C. imbricata*, each *C. imbricata* stem was cut into 4–5 sections, each of which represents a more regular cylinder shape and has the surface area of $\pi \times d \times h$ (d is the stem diameter, and h is the lengths of each stem segment); S is the sum of the surface areas of all sections. Diameter and height were measured by a caliper with a resolution of 0.001 mm. Diurnal change of gas exchange for *C. imbricata* was measured at a constant leaf temperature of 20°C using three replicates in each treatment in each chamber. The CO₂ mixing ratio was set to 400 ppm at ambient CO₂ conditions, while the CO₂ mixing ratio was set to 800 ppm at high CO₂ conditions. The light intensity was the same as in the environment inside each chamber.

Titrateable acidity was measured at the end of the second stage of the experiment. Stems of *C. imbricata* were sampled at 3-h intervals and stored at –20°C. Each stem was cut into 3–4 disks; one disk with a regular cylindrical shape was selected to measure TA. Diurnal change in TA (mmol/m^2) was measured using the acid–base titration method (Caemmerer and Griffiths 2009), in which freshly made 10 mmol/L NaOH was added into a *C. imbricata* solution with 20 μL of a 1/5 dilution of phenolphthalein as indicator after boiling for 10 min.

Plants were harvested on 13 November (the end of the first stage) and 23 December 2015 (the end of the second stage), respectively, for

measuring soil moisture (gravimetric water content), plant biomass, and productivity. Gravimetric water content was calculated as the mass of water divided by mass of dry soil. When grown in mixture, *C. imbricata* and *B. eriopoda* were separated; a small amount of root fragments of *C. imbricata* or *B. eriopoda* remained in the soil after plant separation and were identified based on color, diameter, and shape. Shoots (leaves and/or stems) and roots were separated. Roots were washed free of soil through 0.1-mm mesh sieves. Fresh shoots were weighed on a scale; both shoots and roots were dried at 60°C for 72 h and weighed. Total biomass and shoot-to-root biomass ratios were calculated. Relative aboveground plant water content was determined as (fresh weight-dry weight)/(fresh weight, %).

The tanks of CO₂ supplied a stable source of CO₂ in the growth chamber. CO₂ was sampled in the middle of the first stage and the second stage of our experiments from tanks using gas-tight vials. The carbon isotope composition of CO₂ gas samples (¹³C_g) was analyzed at Indiana University—Purdue University Indianapolis on a stable isotope ratio mass spectrometer (IRMS) (Model Delta V Plus; Thermo Fisher Scientific, Waltham, Massachusetts, USA) connected under continuous flow to a headspace gas analyzer (Thermo, GasBench II). The analysis showed minimal difference of ¹³C_g between these two stages, and we thus used the average values.

Aboveground plant samples were dried at 60°C for 72 h and then ground and homogenized for isotope and elemental analysis. Aboveground plant samples were combusted in an elemental analyzer (Costech Analytical, Valencia, California, USA; ECS 4010), and the carbon isotope composition of aboveground plant samples (¹³C_p) was analyzed under continuous flow on the same IRMS. The isotope discrimination of carbon isotope composition between CO₂ gas samples and aboveground plant samples was thus calculated as $d = ^{13}\text{C}_g - ^{13}\text{C}_p$ (Hoefs 1997, Cernusak et al. 2013).

Data analysis

The effects of CO₂, water treatment, species, competition, and time as well as their interactions on total biomass, the rate of total biomass change, shoot-to-root biomass ratio, isotope discrimination, and relative aboveground plant

water content were analyzed by a five-way ANOVA with each chamber as a whole-plot random factor. The effects of CO₂, water, competition, and time as well as their interactions on soil moisture were analyzed by a four-way ANOVA with each chamber as a whole-plot random factor. Total biomass measurements were natural log-transformed prior to ANOVA. In general, the most interesting effects were found in multiway interactions. To explore these interactions, we constructed pairwise orthogonal contrasts to detect differences between individual pairs of means. The effects of CO₂, water, competition, time, and their interactions on TA in *C. imbricata* were analyzed by repeated measures ANOVA. Titratable acidity was log-transformed prior to ANOVA. All statistical analyses were performed in SAS 9.4 (2013).

The meta-analysis of CAM plants' response to CO₂ enrichment was conducted using the metafor package in R. Due to the difference in species and experimental conditions among different studies or within the same study, individual observations were treated as the random effect. The meta-analysis requires the input of mean, standard deviation and/or standard error, and sample size. For those studies that did not report standard deviation and/or standard error, standard deviation and/or standard error were determined as the mean values of the CAM species in each family.

RESULTS

Meta-analysis

The meta-analysis on CO₂ enrichment including 19 constitutive CAM species across global drylands showed an average increase of 51% in daily CO₂ uptake and 33% in biomass (Fig. 1; Appendix S1), which is comparable to the biomass response of C₃ plants (+45%, 300 species) and greater than the biomass response of C₄ plants (+12%, 40 species) to CO₂ enrichment (Poorter and Navas 2003).

Competitive advantage of *C. imbricata* under drought

In our growth chamber experiment, CO₂ enrichment significantly increased the biomass of *C. imbricata* and *B. eriopoda* alone in both drought and well-watered conditions (increases ranged

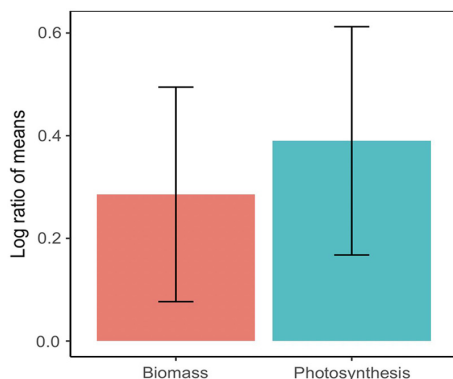


Fig. 1. Meta-analysis of log ratio of daily CO_2 uptake and biomass under elevated CO_2 concentrations to ambient CO_2 concentrations for 19 constitutive CAM species as monocultures across global drylands. Values are log ratio of means \pm 95% confidence intervals of published results. $P = 0.0071$ for biomass and $P = 0.0003$ for photosynthesis. The details of meta-analysis are presented in Appendix S1.

from 41% to 68%; all $P \leq 0.0184$ for *C. imbricata*, all $P \leq 0.0372$ for *B. eriopoda*, Fig. 2). CO_2 enrichment increased CO_2 uptake by *C. imbricata* both at night through CAM (C_4) photosynthesis (~20:00 to 08:00 hours) and during the late afternoon with C_3 photosynthesis (~15:00 to 18:00 hours; Fig. 3a, c). The increase in CO_2 uptake at night in response to CO_2 enrichment increased the accumulation of malic acid in the vacuole and thus TA ($P = 0.0355$, Fig. 3b, d). The response of mixed CAM- C_4 communities to CO_2 enrichment depended crucially on water conditions ($\text{CO}_2 \times \text{water} \times \text{competition}$ $P < 0.0001$; Appendix S2: Table S1). While CO_2 enrichment significantly increased the biomass of both *B. eriopoda* (48%) and *C. imbricata* (89%) in mixture in well-watered conditions in both stages (both $P \leq 0.0169$ for *B. eriopoda*, both $P \leq 0.0002$ for *C. imbricata*), CO_2 enrichment only favored *C. imbricata* in mixture in drought conditions (69% increase; $P \leq 0.0016$ for *C. imbricata*, $P \geq 0.131$ for *B. eriopoda*, Fig. 2). Moreover, in ambient CO_2 and drought conditions (LCLW) the biomass of *B. eriopoda* in mixture was significantly higher (74%) than that in grass alone (for both stages, $P < 0.0001$, Fig. 2), in contrast to the case of HCLW (for both stages, $P \geq 0.0832$, Fig. 2). Overall, these results suggest that *C. imbricata* had the competitive

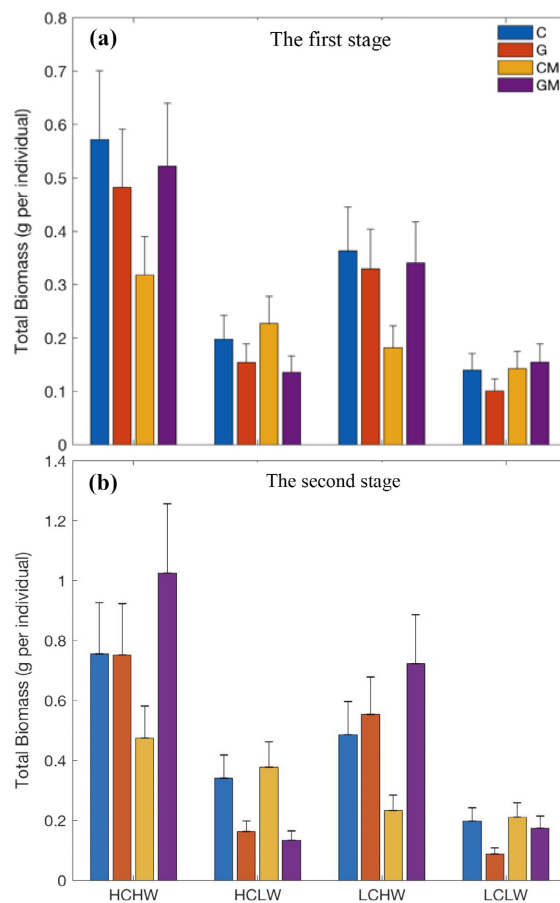


Fig. 2. Total biomass (TB, g) of *Cylindropuntia imbricata* (CAM species) and *Bouteloua eriopoda* (C_4 grass) in CAM alone (C), grass alone (G), and mixture (CM, GM) in the first stage (a, 13 November 2015) and second stage (b, 23 December 2015) in each treatment. HCHW refers to high CO_2 and well-watered conditions; HCLW refers to high CO_2 and drought conditions; LCHW refers to low CO_2 and well-watered conditions; and LCLW refers to low CO_2 and drought conditions. The error bars represent 95% confidence intervals ($n = 9$). The data were natural log-transformed before ANOVA.

advantage over *B. eriopoda* under the manipulated extreme drought and CO_2 enrichment conditions.

Competitive advantage of *B. eriopoda* under well-watered conditions

The increase in water availability significantly increased the biomass of both *C. imbricata* (87%)

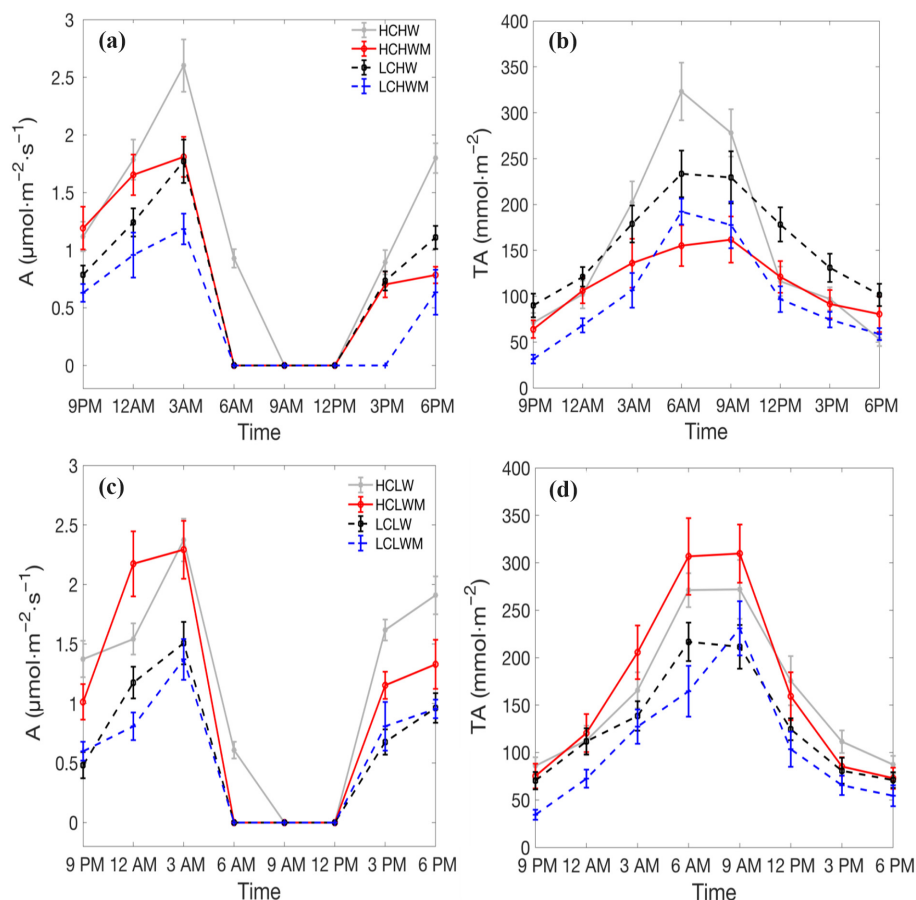


Fig. 3. Diurnal change of rates of carbon dioxide uptake (A , $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and titratable acidity (TA ; mmol/m^2) by *Cylindropuntia imbricata* in well-watered (a, b) and drought (c, d) conditions. *C. imbricata* performs C_4 photosynthesis at night and C_3 photosynthesis in the late afternoon, as shown by TA measurements. Symbols for each treatment are the same as Fig. 2. M refers to mixture. Values are mean \pm standard error ($n = 9$).

and *B. eriopoda* (309%) when grown alone and in mixture, except for *C. imbricata* in mixture under ambient CO_2 conditions in the second stage (LCS; $P = 0.3381$ for LCS; all $P \leq 0.0242$ for other conditions, Fig. 2). *C. imbricata* is able to store water, and its relative aboveground plant water content was 2.7 times higher than *B. eriopoda* (all $P < 0.0001$; Appendix S2: Fig. S3). Thus, its growth, which was limited by drought in the first stage, recovered quickly in the second stage of well-watered conditions (Figs. 2, 3). By comparison, all individuals of *B. eriopoda* suffered from high water stress in the first stage under drought conditions and did not recover even after the water treatment returned to well-watered conditions in the second stage of the

experiment, as observed by a non-significant increase of biomass by *B. eriopoda* in the second stage (Fig. 2). In well-watered conditions, there was a lower photosynthetic rate (Fig. 3a, c), lower nocturnal TA (92.4 vs. 133.6 mmol/m^2 , $P < 0.0001$; Fig. 3b, d), 45% smaller biomass (all $P < 0.0001$, Fig. 2), and an 18% smaller relative aboveground plant water content in *C. imbricata* in mixture with *B. eriopoda* than *C. imbricata* plants grown alone. *B. eriopoda* exhibited a high growth rate in well-watered conditions (Fig. 4).

Collectively, these results indicate that while *B. eriopoda* had a lower ability to recover from drought than *C. imbricata* and was disadvantaged relative to *C. imbricata* in drought conditions, *B. eriopoda* had a strong competitive

advantage over *C. imbricata* in well-watered conditions.

Isotope

The main effects of CO₂, water, and species but not competition on isotope discrimination were significant ($P < 0.0001$; Appendix S2: Table S1). Regardless of other conditions, CO₂ enrichment significantly reduced isotope discrimination of both *C. imbricata* (10.63–4.82‰) and *B. eriopoda* (7.67–4.50‰; CO₂ × Species $P < 0.0001$; Appendix S2: Table S1; all $P < 0.0001$, Fig. 5). The response of isotope discrimination to water depended on CO₂ (CO₂ × Water $P < 0.0001$; Appendix S2: Table S1). In particular, in the first stage of the experiment under ambient CO₂ conditions, water additions significantly reduced isotope discrimination (12.11–11.05‰) of *C. imbricata* ($P \leq 0.0108$, Fig. 5a), in contrast to the pattern observed in high CO₂ conditions where water addition increased isotope discrimination (2.81–6.60‰; $P < 0.0001$, Fig. 5a). Consistent with this pattern, water additions in the drought treatment in the second stage significantly increased isotope discrimination of both

C. imbricata in high CO₂ conditions as compared to the first stage (2.81‰ in November vs. 4.80‰ in December; $P \leq 0.0001$; Fig. 5a, b). Generally, *C. imbricata* had a higher isotope discrimination (7.73‰) than *B. eriopoda* (6.09‰; Fig. 5).

DISCUSSION

Studies of response of CAM plants and their competitive relationship with other functional groups under global change scenarios are scarce. Our study was motivated by the limited

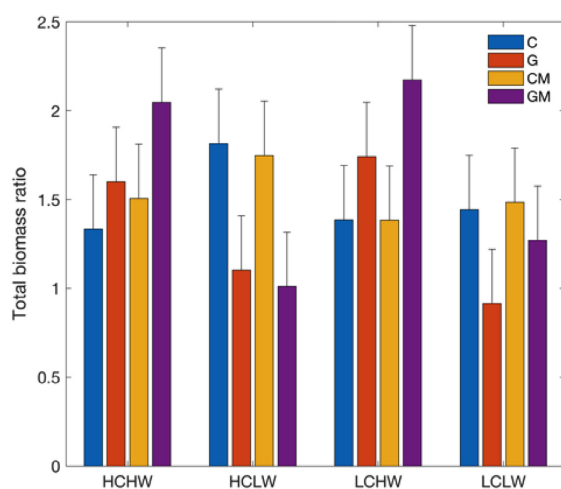


Fig. 4. The total biomass ratio between the first and the second stage of *Cyindropuntia imbricata* (CAM species) and *Bouteloua eriopoda* (C₄ grass) in CAM alone (C), grass alone (G), and mixture (CM, GM) in each treatment. Symbols for each treatment are the same as Fig. 2. The error bars represent 95% confidence intervals ($n = 9$).

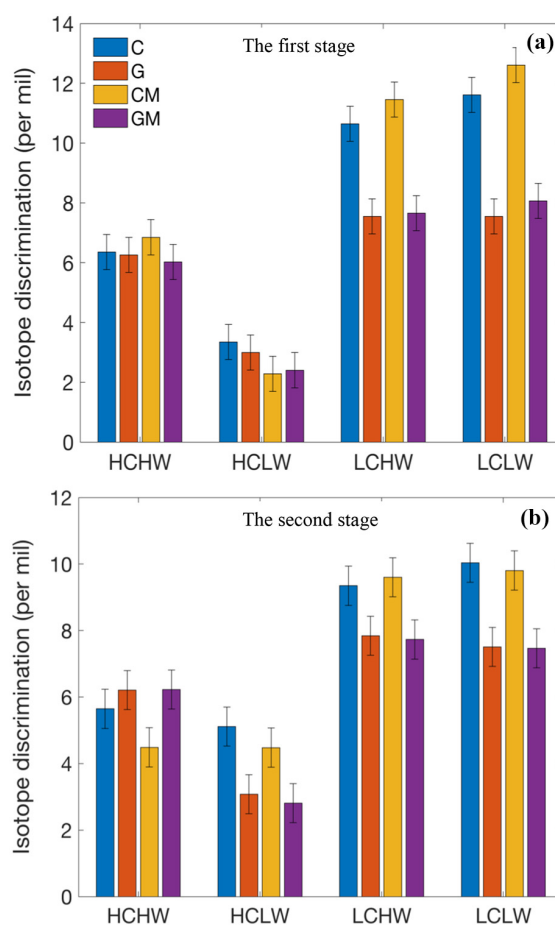


Fig. 5. Isotope discrimination of *Cyindropuntia imbricata* (CAM species) and *Bouteloua eriopoda* (C₄ grass) in CAM alone (C), grass alone (G), and mixture (CM, GM) in the first stage (a, 13 November 2015) and the second stage (b, 23 December 2015) in each treatment. Symbols for each treatment are the same as Fig. 2. The error bars represent 95% confidence intervals ($n = 9$).

understanding about the increase of *C. imbricata* in distribution and abundance observed in desert grasslands in the Southwestern United States and northern Mexico (i.e., Chihuahuan Desert). This study showed the competitive advantage of CAM plants (*C. imbricata*) over grasses (*B. eriopoda*) under extreme drought and CO₂ enrichment conditions (Fig. 2). A meta-analysis synthesized the evidence that photosynthesis and productivity responses by monocultures of CAM plants to CO₂ enrichment are comparable to C₃ plants, while data for CAM responses in competition with other functional groups are lacking under CO₂ enrichment conditions. These results collectively suggest that with projected increases in atmospheric CO₂ concentrations and drought, the abundance of CAM plants may increase in semiarid ecosystems with important implications for species composition and ecosystem carbon and water cycling.

Response of *B. eriopoda* and *C. imbricata* alone

As previously found for other C₄ grasses (Smith et al. 2000, Morgan et al. 2001), *B. eriopoda* showed a positive response to CO₂ enrichment under both drought and well-watered conditions (Fig. 2). This positive response may result from the direct or the indirect effect of CO₂ enrichment in reducing stomatal conductance (see isotope analysis below), which increases soil moisture availability (Appendix S2: Fig. S4) and water use efficiency of *B. eriopoda*. The indirect effect of CO₂ enrichment was consistent with the decreased isotope discrimination of *B. eriopoda* (Fig. 5), which likely corresponds to the decrease in stomatal conductance and intercellular CO₂ concentration. We note, however, that isotope discrimination in C₄ plants is also largely affected by the fraction of CO₂ leakiness (ϕ) out of sheath cells (Farquhar 1983, Farquhar and Cernusak 2012), and thus, interpreting isotope discrimination in C₄ plants is more complicated than C₃ plants. ϕ quantifies the extent to which Rubisco fractionation is expressed, and thus, isotope discrimination increases with ϕ (Murphy and Bowman 2009, Cernusak et al. 2013). A value of $\phi > 0.37$ would lead to increased isotope discrimination with intercellular CO₂ concentration, as found for C₃ plants (Cernusak et al. 2013). Consistent with other studies (Van de

Water et al. 2002, Liu et al. 2005), our study showed a higher isotope discrimination of *B. eriopoda* in high water conditions in high CO₂ conditions but not ambient conditions (Fig. 5), which suggested a higher value of $\phi > 0.37$ in high CO₂ conditions than ambient conditions. However, given that we did not directly measure the photosynthesis rate for *B. eriopoda* and ϕ , we suggest that further work is needed to quantitatively support our interpretation of the indirect effect of CO₂ enrichment.

The rate of CAM (C₄) photosynthesis performed at night by *C. imbricata*—which is typically thought to saturate quickly with increasing atmospheric CO₂ concentrations (Drennan and Nobel 2000, Poorter and Navas 2003, Borland et al. 2009)—still had a positive response to CO₂ enrichment (Fig. 3), consistent with the response of most CAM species (Fig. 1; Drennan and Nobel 2000). This likely occurs because CO₂ uptake at night in ambient CO₂ conditions is still restricted by a low mesophyll conductance limiting the supply of CO₂ to the photosynthetic tissue (Drennan and Nobel 2000, Nelson and Sage 2008, Ripley et al. 2013). *C. imbricata* performed a large fraction (>70–80%) of CAM (C₄) photosynthesis during the night (Fig. 3). Similar to the case of *B. eriopoda*, *C. imbricata* responded to CO₂ enrichment with a statistically significant increase in photosynthesis and productivity (Ainsworth and Long 2005, Fay et al. 2012). This response may be associated with a reduction in stomatal conductance and was evidenced by the higher soil moisture availability found in well-watered conditions (Appendix S2: Fig. S4) and the lower photosynthetic isotope discrimination in high CO₂ conditions (Fig. 5), but we note that interpreting isotope discrimination in CAM plants is more complicated than C₃ plants.

Response of *B. eriopoda* and *C. imbricata* in mixture

Our experiment provided experimental evidence that *C. imbricata* has a competitive advantage over *B. eriopoda* in drought and enriched CO₂ conditions (Fig. 2). *C. imbricata* coped with drought conditions by efficiently exploiting rainfall pulses and then drawing maximum benefit from storing the absorbed water (Lüttge 2004, Borland et al. 2009). This led to higher relative aboveground plant water content (Appendix S2:

Fig. S3) and thus more favorable tissue water potential, lower water stress, and higher resistance to droughts than *B. eriopoda*. By contrast, *B. eriopoda* had a low drought resistance and did not recover after droughts, as seen also in field experiments (Báez et al. 2013) likely because of the damage of plant water-transport system caused by cavitation in the xylem (Holloway-Phillips and Brodribb 2011, Cao et al. 2012). Under drought conditions, a more positive response to CO₂ enrichment by *C. imbricata* indirectly disfavored *B. eriopoda* (Fig. 2), which indicated that interspecific competition between this CAM plant and C₄ grass can counteract the favorable direct effect of CO₂ enrichment. Similarly, other studies showed that the positive effects of C₃ (*P. smithii*) and C₄ grasses (*Bouteloua gracilis*) as monocultures under CO₂ enrichment were not observed in natural (mixed) communities (Morgan et al. 1998, 2004). The competitive advantage of C₄ species (*S. bicolor*) under ambient CO₂ concentration was flipped into a competitive disadvantage under CO₂ enrichment, which favors C₃ species (*G. hirsutum*) more than C₄ species (Derner et al. 2003).

Bouteloua eriopoda had a strong competitive advantage over *C. imbricata* in well-watered conditions (Fig. 2). As previously found for grasses in mesic systems (Collins et al. 2012), *B. eriopoda* exhibited a high growth rate in well-watered conditions (Fig. 4) and an extensive root system with a high root/shoot ratio (Appendix S2: Fig. S5). These traits allowed this grass to take advantage of the well-watered conditions under ambient CO₂ levels, thereby indirectly limiting the response of *C. imbricata* to the increase in water availability (Figs. 3, 4). The efficient water use strategies of grasses accounted for its competitive advantage over woody plants in savannas in response to increased interannual rainfall variability (Yu et al. 2017c). This efficient water usage by grasses even promoted its transitions to a stable state of grass dominance initiated by stochastic rainfall variability (Chen et al. 2018).

Broad implications

Previous studies showed that high drought resistance accounted for the competitive advantage of a model facultative CAM species (*M. crystallinum*) over its co-occurring grass species (*B. mollis*) found in California grasslands

under global change scenarios (Yu et al. 2017a, b). This study investigated whether water stress and concurrent CO₂ enrichment—which are known to affect the interactions between C₃ and C₄ plants (Smith et al. 2000, Poorter and Navas 2003, Ainsworth and Long 2005, Fay et al. 2012)—could allow constitutive CAM plants (*C. imbricata*) to increase in abundance in C₄-dominated (*B. eriopoda*) desert grasslands in the United States. We showed the experimental evidence of the competitive advantage of a CAM species (*C. imbricata*) over a C₄ (*B. eriopoda*) grass species under drought and CO₂ enrichment conditions. While not investigated in this study, warming trends may further benefit CAM plants because these plants exhibit maximum photosynthetic rates at higher temperatures (Borland et al. 2009, Reyes-García and Andrade 2009) and evidence shows that the climate is becoming more arid in this region (Rudgers et al. 2018). The ability to store water and tolerate drought is a well-known characteristic of most CAM species (Lüttge 2004, Borland et al. 2009). The positive response of most CAM species to CO₂ enrichment, comparable to that of C₃ species, and higher than that of C₄ species, has also been widely reported (Drennan and Nobel 2000, Poorter and Navas 2003, Borland et al. 2009) and was further documented in our meta-analysis (Fig. 1). We suggest that more CAM species may further expand their abundance under future global change scenarios in drylands, and thus, experiments using more CAM species are greatly needed in future studies. Moreover, we note that compared to field conditions where plant roots are less constrained, plants growing in small pots in growth chambers experience much higher water stress, as shown here by low plant water content (Appendix S2: Fig. S3) and lack of recovery of grasses after returning to well-watered conditions. Thus, future research needs to better quantify the competitive relationship between CAM plants and grasses in field conditions under global environmental change.

CONCLUSIONS

Our study provides the experimental evidence that the extreme water stress and concurrent atmospheric CO₂ enrichment allow for the competitive advantage of CAM plants (*C. imbricata*)

over co-occurring C_4 grasses (*B. eriopoda*) found in desert grasslands in the Southwestern United States. The physiological mechanisms underlying the competitive advantage of *C. imbricata* are likely to be associated with its high drought resistance, rapid recovery after droughts, and lack of saturation in the response to CO_2 enrichment. We suggest that understanding the potential shift of the competitive relationships between CAM plants and C_4 grasses is crucial for evaluations of possible shifts in dryland vegetation composition, the related changes in ecosystem resilience and productivity, and the provision of ecosystem services under global environmental change.

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