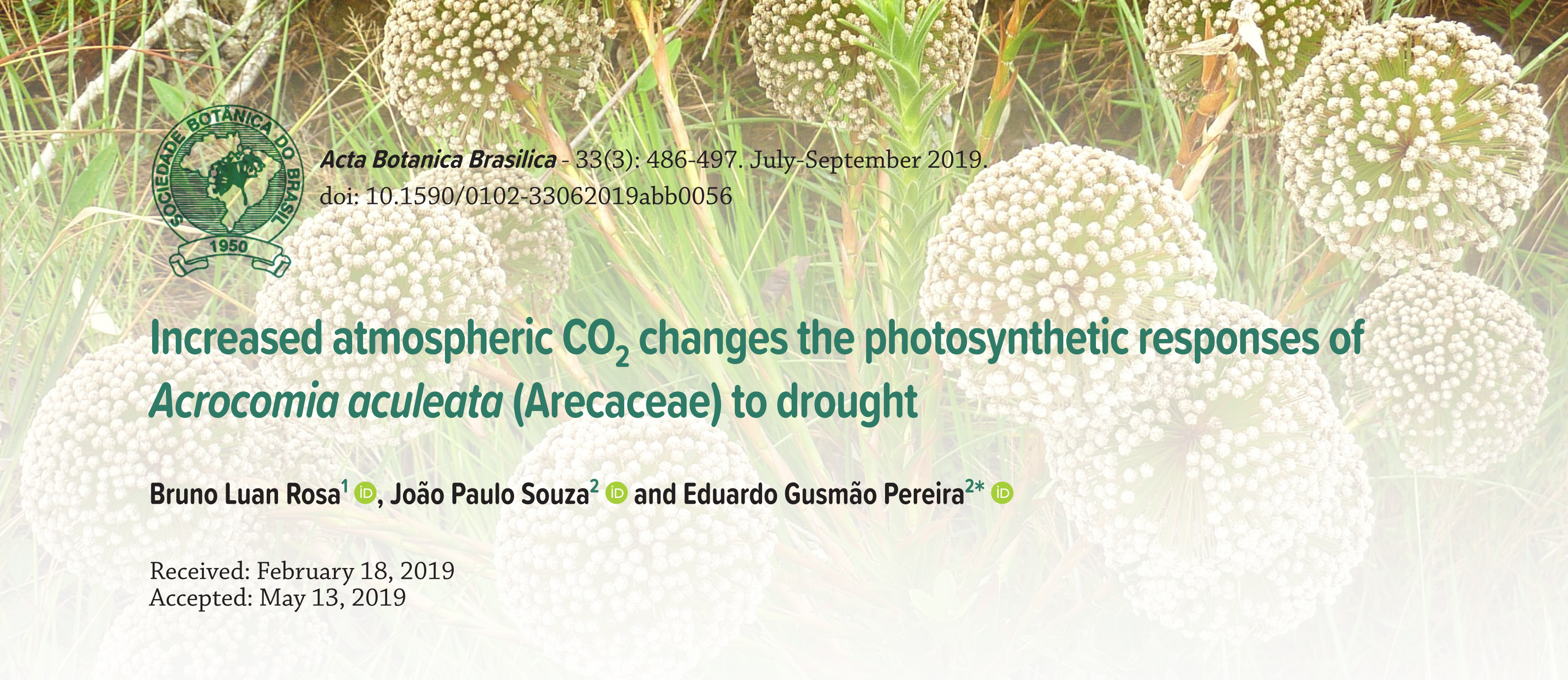

Acta Botanica Brasilica - 33(3): 486-497. July-September 2019.
doi: 10.1590/0102-33062019abb0056

Increased atmospheric CO_2 changes the photosynthetic responses of *Acrocomia aculeata* (Arecaceae) to drought

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Received: February 18, 2019
Accepted: May 13, 2019

ABSTRACT

Water availability is the main factor that explains current patterns of palm abundance. However, the interaction between water stress and increasing atmospheric CO_2 concentrations caused by climatic change and its effects on palm physiology remain poorly known. Macauba palm is a widespread Neotropical species commonly found in ecosystems subjected to seasonal drought and has potential use in oil production. The present work investigated the influence of increased CO_2 concentrations on photosynthetic responses to drought in macauba palm plants. Exposure to increased CO_2 concentrations led to up-regulation of photosynthesis through higher stomatal conductance and improved light and water use efficiency. Macauba palm plants under water stress, irrespective of CO_2 concentration, were able to maintain constant levels of proline and chlorophyll, while preventing oxidative damage. Plants grown at higher CO_2 concentrations were more capable of recovering from drought due to higher Rubisco carboxylation rate (Vc_{max}) and electron transport rate (J_{max}), which prevented a reduction in total dry mass at the end of the stress period. Stomatal control of photosynthesis, coupled with the prevention of severe damage under stress, would allow efficient biomass production by the macauba palm under future scenarios of climate change.

Keywords: climate change, photosynthesis, palm, water stress, water use efficiency

Introduction

The effects of climate change on photosynthesis in C3 plants are mainly related to the positive effect of increased atmospheric CO_2 concentration ($[CO_2]$) on the carboxylation capacity of Rubisco and to decreased transpiration rate (Long *et al.* 2004). Responses of leaf photosynthesis and transpiration to high $[CO_2]$ conditions are due to the suppression of photorespiration and increased supply of Rubisco substrate, accompanied by the partial closure of the stomata (Ainsworth & Long 2005; Leakey *et al.* 2009). Although some initial stimulus of photosynthesis can be observed immediately upon exposure to high $[CO_2]$, photosynthetic capacity is often reduced after a long-term growth at elevated CO_2 levels (Leakey *et al.* 2009; Córdoba *et al.* 2017). This photosynthetic acclimation in plants exposed to long-term high $[CO_2]$ is commonly defined as down-regulation of photosynthesis, a process triggered by cumulative reduction in the content of Rubisco in leaves and the concomitant reduction in the photosynthetic electron transport rate (ETR), along with source–sink imbalance (Ainsworth *et al.* 2004; Long *et al.* 2004; Gamage *et al.* 2018). Plant productivity with elevated $[CO_2]$ can also be limited by nutrient availability, since significant reductions in nitrogen concentration and increase in the C:N ratio are observed in plants exposed to high $[CO_2]$. However, the response of palm species to elevated $[CO_2]$

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remains poorly understood (Walther *et al*. 2007; Ibrahim *et al*. 2010; Renninger & Phillips 2016).

Increased air temperature and droughts can occur synergistically with increased [CO_2], which may be even more compromising to the survival of palm species (Renninger & Phillips 2016; Safronov *et al*. 2017; Dusenge *et al*. 2019). Therefore, the capacity to withstand or avoid water stress can dictate the resistance of palms to drought and their biogeographic distribution (Pinheiro & Chaves 2011; Renninger & Phillips 2016; Esquivel-Muelbert *et al*. 2017). Palm species richness is generally centered in tropical and subtropical ecosystems characterized by constant water availability and a warm climate (Bjorholm *et al*. 2005; Eiserhardt *et al*. 2011). Palms occurring in savanna environments generally have higher stomatal conductance (g_s) and lower water use efficiency than woody and herbaceous species (Rossatto & Franco 2017). However, some palm species are described as resistant to drought, including the macauba palm (*Acrocomia aculeata*), which is also distributed in dry regions throughout the American tropics (Oliveira *et al*. 2002; Gomes *et al*. 2008; Pires *et al*. 2013; Medeiros *et al*. 2015; Mota & Cano 2016). The macauba palm grows in large populations either in degraded or natural areas with relatively fertile soils and annual rainfall of less than 1,500 mm (Motta *et al*. 2002; Ratter *et al*. 2003). Besides having efficient response mechanisms for water deficit, such as efficient stomatal control, increased water use efficiency and rapid recovery after rehydration (Mota & Cano 2016; Oliveira *et al*. 2016), *A. aculeata* also exhibits considerable resilience to fire (Bicalho *et al*. 2016) and photosynthetic adjustments that allow its establishment in various light conditions (Dias *et al*. 2018).

While the effects of water scarcity on *A. aculeata* have been addressed by some works (Mota & Cano 2016; Oliveira *et al*. 2016), its interaction with increasing [CO_2] caused by climate change remain to be elucidated. This study tests the hypothesis that the response of macauba palm to reduced soil water content is attenuated by long-term increase in [CO_2]. With higher [CO_2], macauba palm plants under water deficit should sustain a satisfactory photosynthetic rate while preventing excessive water loss through partial stomatal closure and conservation of *RWC* in leaves. However, photosynthetic acclimation to increased [CO_2] (Ainsworth *et al*. 2004), along with reduced Rubisco carboxylation rate and impaired light utilization accentuated by drought, should prevent fast recovery of photosynthesis after rewatering in successive drought cycles. This study also tested the hypothesis that these physiological effects will impair vegetative growth of macauba palm resulting in less biomass accumulation, primarily in water-stressed plants. Thus, the aim of this work was to investigate the influence of increased [CO_2] on the responses of photosynthesis and biomass production under drought in macauba palm plants. We evaluated whether the capacity of stress response and recovery of photosynthesis after successive water stress cycles are influenced by rising [CO_2] due to adjustments in plant growth, carboxylation capacity, stomatal responses, water status, chlorophyll content, and light use efficiency of macauba palm plants.

Materials and methods

Experimental conditions

The experiment was conducted in open-top chambers installed outside a greenhouse, as described by Silva *et al*. (Silva *et al*. 2012), located at the Federal University of Viçosa, *Campus* Florestal, Minas Gerais State, Brazil (575 m a.s.l., 19°53'84"S 44°25'68"E). Individual three-month-old saplings of *Acrocomia aculeata* (Jacq.) Lodd. Ex Mart were transplanted into 8-liter plastic pots filled with a mixture of soil, sand, and cattle manure (2:1:1) fertilized with 8 kg m^{-3} simple superphosphate, 1 kg m^{-3} ammonium sulfate and 0.3 kg m^{-3} potassium chloride. Topdressing-fertilization was carried out during cultivation at 3-month intervals using 0.5 g urea, 0.5 g potassium chloride and 0.25 g magnesium sulfate per plant. The soil characteristics were the same as reported by Souza *et al*. (2016). Fifty-seven days after transplanting, when all 20 plants used in the experiment resumed growth as confirmed by the development of new leaves, 10 plants were exposed to treatments with increased CO_2 concentration [CO_2]700 (700 μmol mol^{-1}), while the other 10 plants remained in chambers with current CO_2 concentration [CO_2]400 (400 μmol mol^{-1}). Both groups were irrigated daily and the CO_2 concentration inside the chambers was monitored daily with a Testo 535 CO_2 measuring device (Testo SE & Co. KGaA, Campinas, Brazil).

Mean photosynthetically active radiation (PAR) measured inside and outside the open-top chambers was 1690 and 2070 μmol photons m^{-2} s^{-1}, respectively, both measured at midday with the aid of a radiometer (model HD 2102.2, Delta OHM, Brazil). Mean ambient temperature (24.7 ± 0.77 °C), relative humidity (50.1 ± 4.8 %) and vapor pressure deficit (1.59 ± 0.18 kPa) were measured using an infrared gas analyzer (IRGA; model LI-6400XT, Li-Cor Inc., Lincoln, NE, USA). After 320 days, five plants in each CO_2 treatment were exposed to water deficit by suppressing irrigation until net photosynthetic rate approximated 0 μmol m^{-2} s^{-1}, at which point the plants were rehydrated to full recovery. The plants were exposed to three cycles of water withholding and rehydration. All measurements described below were made on the first fully-expanded pinnate leaf (using leaflets of the middle-leaf portion). The entire period of exposure to [CO_2] lasted 393 days.

Evaluation of photosynthetic responses to drought and CO_2 concentrations

Leaf gas exchange during the entire period (74 days) of water deficit cycles was measured using a portable

IRGA (LI-6400xt; LI-COR Inc., Lincoln, NE, USA). All treatments were measured from 8:00 to 11:00 at current CO_2 concentrations (ranging from 392 to 403 µmol CO_2 mol^{-1}) with a light intensity of 1,200 µmol photons m^{-2} s^{-1} provided by the LI-6400xt internal light-emitting diode light source (6400-02B Red-Blue; Li-Cor Inc., USA). Net photosynthetic rate (A, µmol m^{-2} s^{-1}), stomatal conductance (g_s, mol m^{-2} s^{-1}), transpiration rate (E, mmol m^{-2} s^{-1}), the ratio between intercellular and atmospheric CO_2 concentration (Ci/Ca), and instantaneous water use efficiency ($W_t = A/E$, µmol CO_2 mmol H_2O m^{-2} s^{-1}) were evaluated. Simultaneously with gas exchange evaluation, chlorophyll *a* fluorescence was measured in light-acclimated leaves (1,200 µmol photons m^{-2} s^{-1} for 120 s) using an integrated fluorescence chamber (LI-6400-40; Li-Cor Inc., USA). Steady-state fluorescence yield (F) was measured following a saturating light pulse that was applied to achieve light-acclimated maximum fluorescence (F_m'). The actinic light was then turned off and far-red illumination was applied (2 µmol m^{-2} s^{-1}) to measure light-acclimated minimum fluorescence (F_o'). The effective quantum yield of PSII in the illuminated leaf was determined according to Genty *et al*. (1989) using the formula: $\phi_{II} = (F_m' - F/F_m')$. Apparent electron transport rate (ETR) was calculated as: $ETR = \phi_{II} \times PAR \times I_A \times 0.5$; where 0.5 is the fraction of the excitation energy distribution in PSII (Melis *et al*. 1987), I_A is the leaflet absorptivity coefficient, and PAR is the photosynthetically active radiation.

Total leaf chlorophyll content was measured periodically in the same leaves used for gas exchange analysis using a ClorofiLOG portable chlorophyll meter (Falker, Porto Alegre, Brazil). Each leaflet was evaluated based on the mean of three measurements taken from the leaflet lamina, while avoiding the midrib region.

After the third water deficit cycle, the response of A to Ci (A *vs* Ci) was measured in all plants (n=5 in each treatment) changing the reference CO_2 concentration (400, 300, 200, 100, 50, 400, 500, 600, 700, 800, 1000, 1200, and 1300 µmol CO_2 mol^{-1}) under ambient temperature (23–25 °C) and irradiance of 1,200 µmol photons m^{-2} s^{-1} using the CO_2 injector device of the LI-6400xt (6400-01 CO_2 injector; Li-Cor Inc., USA) equipped with 12-g high-pressure CO_2 cartridges. The maximum Rubisco (RuBP) carboxylation velocity (Vc_{max}), the electron transport rate associated with RuBP regeneration (J_{max}), and triose phosphate utilization (TPU) were calculated according to Sharkey *et al*. (2007). To avoid excessive stomatal effect on CO_2 diffusion, the *A vs Ci* curves were created one day after irrigation was resumed.

Measurements of leaflet water status in response to drought and rising CO_2

The leaf water potential (Ψw) of the plants was measured at the end of each water deficit cycle (when photosynthesis reached 0 µmol m^{-2} s^{-1}) using a pressure chamber (3005 Series, Soilmoisture Equipment Corp., Santa Barbara, USA) pressurized with nitrogen gas.

The determination of Ψw was done early in the morning (6:00 am) on the five plants of each treatment using leaflets opposite those used for gas exchange and fluorescence analysis. Leaflets were sectioned at the base and the central rib exposed with the aid of a sharp blade. The leaflets were then rolled and inserted into the rubber support in the pressure chamber. After measuring Ψw, the same leaflets were used to determine relative water content (*RWC)*.

Relative water content was evaluated in leaf discs (4.5 cm^2) cut in duplicate. After determining fresh weight (FW), the leaflets were placed in Petri dishes containing deionized water at room temperature for 20 h, the time required for macauba leaflets to reach maximum turgidity. Turgid weight (TW) was then determined and the leaflets placed to dry at 65 °C for 48 h to determine dry weight (DW). These data were used to calculate *RWC* using the following formula:

$$RWC = \frac{(\mathrm{FW} - \mathrm{DW})}{(\mathrm{TW} - \mathrm{DW})} \times 100$$

Analysis of lipid peroxidation and proline concentration in macauba palm leaves under drought at different CO_2 concentration

The concentration of thiobarbituric acid-reacting substances (TBARS), as final products of lipid peroxidation, was measured at the end of each drought cycle in leaflets from the same leaves used for gas exchange analysis. We used the method described by Du & Bramlage (1992), which accounts for interferences conferred by carbohydrates as described by Pinto *et al*. (2016). The results were expressed in ng of TBARS per mg of dry weight (ng mg^{-1}).

Proline concentration of leaves was evaluated in dried leaflets collected from the same leaves used for TBARS analysis at the period of maximum stress during the third water stress cycle. The leaflets were extracted in 80 % ethanol following the ninhydrin method for proline quantification (Bates *et al.* 1973).

Evaluation of morphological analysis and dry mass accumulation

At the end of the experiment, total leaf number, stem height (cm), total root length (cm), and mean diameter of stem base (cm) were measured in all plants. Stem dry mass (g), total leaf dry mass (g), total root dry mass (g) and plant total dry mass were also destructively measured at the end of the experiment in plants of both treatments, $[CO_2]400$ and $[CO_2]700$. It is worth mentioning that the stem described here corresponds to the subterranean bulb or pseudocaule characteristic of young palms.

Experimental design and statistical analysis

A randomized block design was implemented following a 2 x 2 factorial scheme with two atmospheric CO_2 concentrations (400 and 700 µmol mol^{-1}) and two conditions of water supply (well-watered plants and water-stressed plants). Each treatment was comprised five replications. The data were subjected to two-way repeated-measures analysis of variance (ANOVA), and the means compared using Tukey's test at 5 % significance level using SAEG 9.1 (Fundação Arthur Bernardes, UFV, 2007). All data were evaluated for homogeneity of variance and normality prior to the ANOVA (Cochran's Q and Lilliefors tests, respectively).

Results

Photosynthetic responses of macauba palm to increased atmospheric CO_2 concentration and cyclic drought

The treatment with $[CO_2]$700 resulted in significantly ($p < 0.05$) higher values for net photosynthesis (A), stomatal conductance (g_s) and transpiration (E), when the isolated effect of $[CO_2]$ was evaluated. The net photosynthetic rate approximated zero (maximum stress), regardless of the CO_2 concentration, after 25 days of exposure to drought during the first cycle of water stress (Fig. 1A and B).Recovery after rehydration in the first stress cycle was reached after two days for plants grown at $[CO_2]$700, but was delayed by five days for plants grown at $[CO_2]$400. The time when plants reached maximum stress levels in the second and third cycle was reduced to 14 days in both CO_2 treatments, but the photosynthetic rate was significantly lower in plants grown at $[CO_2]$400. The recovery period after rehydration in the second cycle of water stress lasted four days, and was the same in both CO_2 treatments (Fig. 1).

Despite the observed differences between the successive cycles of water withholding and rehydration, no significant interaction was observed between drought and CO_2 treatment for A, g_s and E (Tab. 1). In contrast, a significant statistical interaction for Ci/Ca and W_t responses to water limitation was dependent on current CO_2 concentration during plant growth (Tab. 1). The plants exposed to $[CO_2]$400 had a higher Ci/Ca ratio when challenged by water deficit. In addition, only the plants at $[CO_2]$700 were able to maintain high W_t under drought conditions (Tab. 1).

The exposure of macauba palm to $[CO_2]$700 resulted in higher effective quantum yield (ϕ_{II}) of PSII and ETR (Fig. 2). The soil water deficit in the three consecutive stress cycles reduced ϕ_{II}, ETR and photochemical quenching (q_L) (Fig. 2). The effect of water deficit on the photochemical capacity of the plants was more severe in the second and third cycle, reaching lower values of ϕ_{II}, ETR, and q_L mainly in plants grown at $[CO_2]$400 (Fig. 2). Despite changes in chlorophyll fluorescence response, drought did not impair total chlorophyll content of the leaflets, but a significant increase in chlorophyll was found in plants grown at $[CO_2]$700 (Fig. S1 in supplementary material).

The CO_2 response curves evaluated after the third water deficit cycle showed significantly higher Rubisco carboxylation velocity (Vc_{max}) and electron transport rate associated with RuBP regeneration (J_{max}) in plants grown at $[CO_2]$700 (Fig. 3). However, when plants were subjected to water stress at both CO_2 concentrations, the Vcmax was reduced significantly (Fig. 3A, B), while the reduction of J_{max} was not significant (Fig. 3C, D). The *TPU* in photosynthesis was not affected by drought or CO_2 concentration (Fig. 3E, F).

Table 1. The ratio between internal and external CO_2 concentration (Ci/Ca), instantaneous water use efficiency (W_t; µmolCO_2^{-1} mmolH_2O^{-1} m^{-2} s^{-1}) and proline concentration (µmol gDW^{-1}) in macauba palm plants after exposure to increased CO_2 level (700 µmol mol^{-1}) or ambient CO_2 concentration (400 µmol mol^{-1}). The plants were grown in open-top chambers and exposed to cyclic drought (ws) or irrigated daily (ww).

		C_i/C_a	W_t	Proline
700	ws	0.57 ± 0.27 Ab	5.34 ± 3.24 Aa	44.36 ± 6.63 Aa
	ww	0.65 ± 0.09 Aa	4.58 ± 1.57 Aa	50.02 ± 5.25 Aa
400	ws	0.76 ± 0.27 Aa	2.61 ± 2.52 Bb	40.18 ± 6.85 Aa
	ww	0.66 ± 0.19 Aa	4.32 ± 1.04 Aa	38.45 ± 6.96 Aa

Means followed by the same letter in columns do not differ according to Tukey's test at 5 % significance level. Capital letters compare treatments with different CO_2 concentrations and lower case letters compare treatments with different water levels. Means ± SE (n = 5).

Changes in leaflet water status in response to drought and rising CO_2 levels

The *RWC* was increased significantly in leaflets of plants grown at $[CO_2]$700 (Fig. 4A, B). In addition, isolated effects of drought on *RWC* were observed in the three water stress cycles. At the end of the third cycle, the reduction of *RWC* in response to drought was more severe in plants grown at $[CO_2]$400 than in plants grown at $[CO_2]$700 (Fig. 4 B). In contrast, leaflet water potential (Ψw) was reduced by the cyclic drought but not by the CO_2 treatments (Fig. 4C, D). The plants grown in open-top chambers at $[CO_2]$400 and exposed to water stress showed significantly lower values of Ψw in the second and third cycle of stress compared to plants grown at $[CO_2]$700 (Fig. 4C, D).

Water deficit and increased CO_2 concentration did not lead to oxidative damage or proline accumulation

No significant differences were found in lipid peroxidation (Fig. S2 in supplementary material) or proline concentration (Tab. 1) in tissues of macauba palm leaflets in response to cyclic drought or increased CO_2 atmospheric concentration.

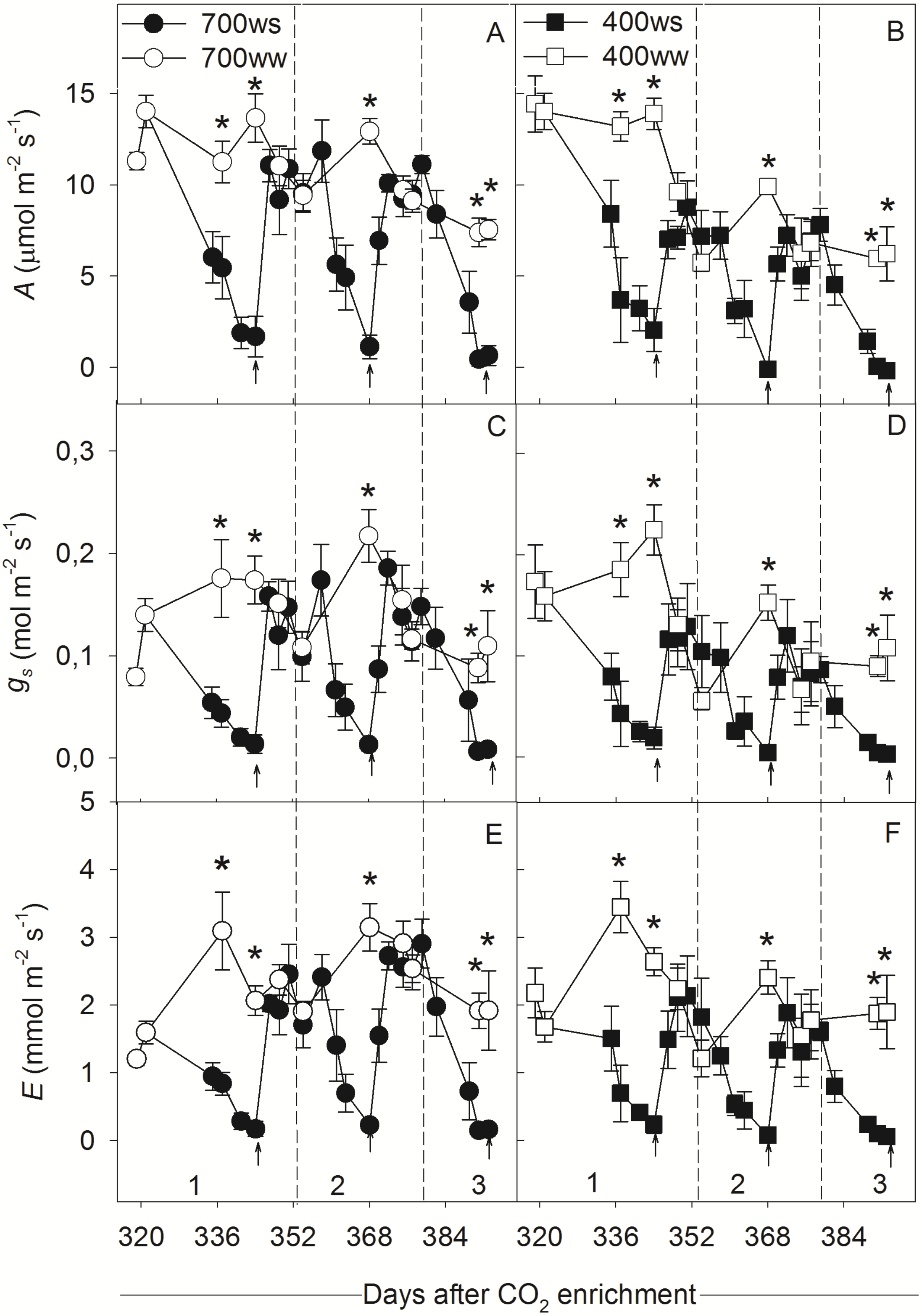

Figure 1. Values of: **A, B.** net photosynthetic rate (*A*); **C, D.** stomatal conductance (g_s); **E, F.** transpiration rate (*E*) in macauba palm plants after long-term exposure to $[CO_2]700$ (700 µmol mol^{-1}) (left panels) and $[CO_2]400$ (400 µmol mol^{-1}) (right panels). Filled symbols represent plants exposed to cyclic drought and open symbols represent well-watered plants, both grown in open-top chambers. The dotted lines separate the three cycles of water deficit, which are listed at the bottom of the graphs (E and F). Asterisks indicate significant differences between well-watered and water stress treatments at each CO_2 concentration, according to Tukey's test ($p < 0.05$). Means ± SE (n = 5).

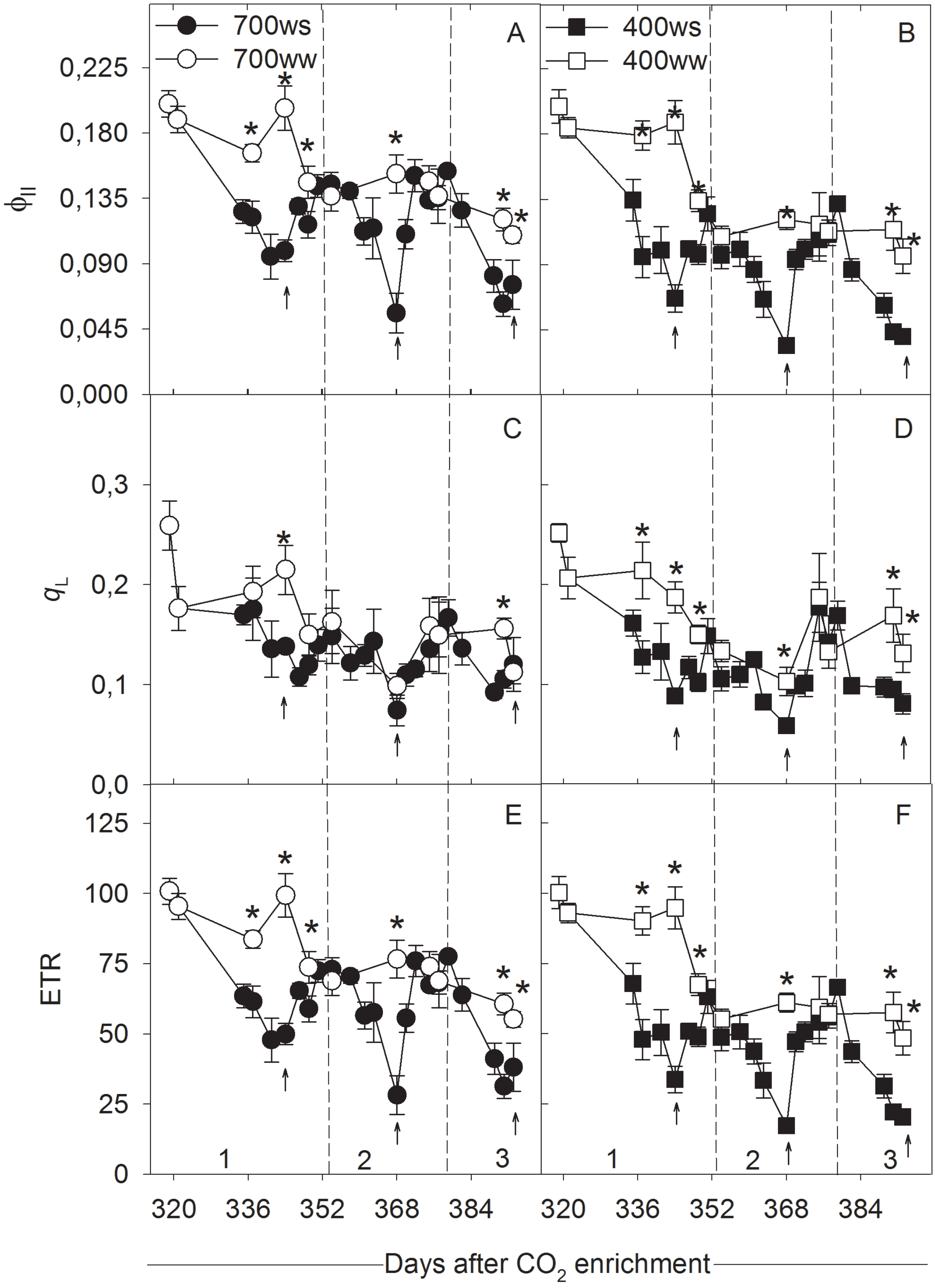

Figure 2. A, B. Effective quantum yield of PSII (ϕ_{II}); **C, D.** photochemical quenching (q_L); **E, F.** electron transport rate (ETR) in macauba palm plants after long-term exposure to $[CO_2]700$ (700 µmol mol^{-1}) (left panels) and $[CO_2]400$ (400 µmol mol^{-1}) (right panels). Filled symbols represent plants exposed to cyclic drought and open symbols represent well-watered plants, both grown in open-top chambers. The dotted lines separate the three cycles of water deficit, which are listed at the bottom of the graphs (E and F). Asterisks indicate significant differences between well-watered and water stress treatments at each CO_2 concentration, according to Tukey's test ($p < 0.05$). Means ± SE (n = 5).

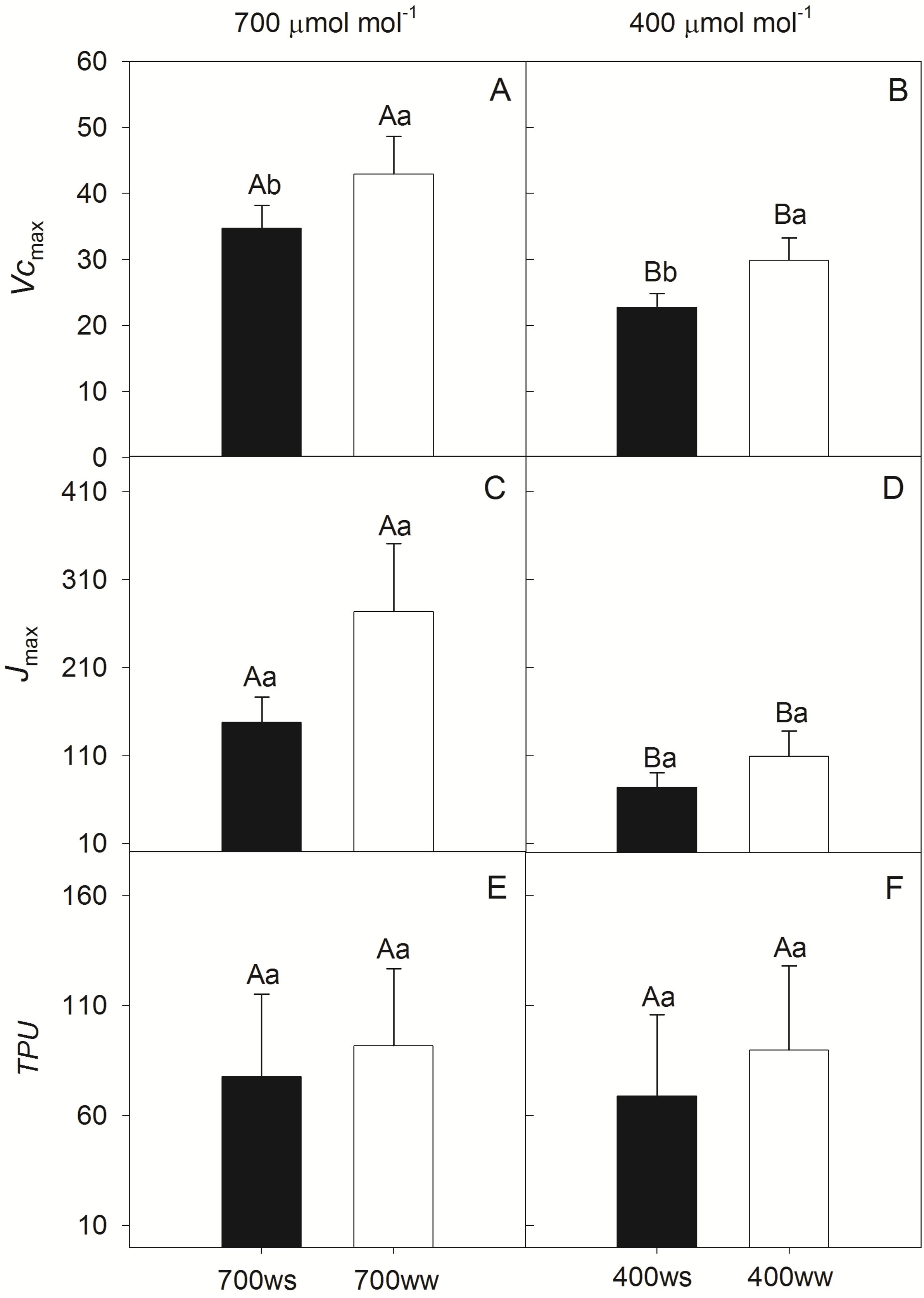

Figure 3. A, B. Maximum Rubisco carboxylation velocity (Vc_{max}); **C, D.** electron transport rate (J_{max}); **E, F.** triose phosphate utilization (*TPU*) in macauba palm plants after long-term exposure to [CO_2]700 (700 µmol mol^{-1}) (left panels) and [CO_2]400 (400 µmol mol^{-1}) (right panels). Filled columns represent plants exposed to cyclic drought and open columns represent well-watered plants, both grown in open-top chambers. Uppercase letters compare treatments with different CO_2 concentrations and lowercase letters compare treatments with different water levels according to Tukey's test ($p < 0.05$). Means ± SE (n = 5).

Morphological adjustments and dry mass accumulation in plants in response to CO_2 and cyclic drought

A significant interaction between drought exposure and CO_2 concentration was observed for stem length and total dry mass accumulation (Tab. 2). Only plants grown at $[CO_2]700$ did not exhibit reduced stem length and total biomass in response to drought. In addition, the stems of water-stressed plants at $[CO_2]700$ were longer compared to those of water-stressed plants at $[CO_2]400$ (Tab. 2). The $[CO_2]700$ increased significantly the leaf number and stem dry mass of *A. aculeata*. Nonetheless, water stress cycles caused a reduction in leaf and root dry mass, regardless of CO_2 concentration. No significant changes in root length were observed as a response to drought or CO_2 levels.

Table 2. Morphological traits and dry mass accumulation in macauba palm plants after exposure to increased CO_2 level (700 µmol mol^{-1}) or ambient CO_2 concentration (400 µmol mol^{-1}). The plants were grown in open-top chambers and exposed to cyclic drought (ws) or irrigated daily (ww).

		Length (cm^{-1})		Leaf number	Dry mass (g^{-1})			
		Stem	Root		Leaves	Stem	Root	Total
700	ws	39.0 ± 3.39 Aa	34.0 ± 4.49 Aa	5.8 ± 0.37 Aa	31.81 ± 3.47 Ab	43.33 ± 6.68 Aa	37.86 ± 2.34 Ab	113.00 ± 11.47 Aa
	ww	37.0 ± 3.70 Aa	34.4 ± 2.52 Aa	6.2 ± 0.20 Aa	40.31 ± 4.51 Aa	55.23 ± 6.65 Aa	44.79 ± 6.12 Aa	140.33 ± 12.68 Aa
400	ws	26.4 ± 2.23 Bb	33.6 ± 2.04 Aa	4.8 ± 0.20 Ba	31.58 ± 1.94 Ab	28.36 ± 2.64 Ba	37.65 ± 3.42 Ab	97.59 ± 4.93 Ab
	ww	33.10 ± 0.98 Aa	40.8 ± 4.55 Aa	5.6 ± 0.40 Ba	39.94 ± 3.08 Aa	37.83 ± 6.43 Ba	55.73 ± 3.45 Aa	133.50 ± 9.93 Aa

Means followed by the same letter in columns do not differ according to Tukey's test at 5 % significance level. Capital letters compare treatments with different CO_2 concentrations and lower case letters compare treatments with different levels of water. Means ± SE (n = 5).

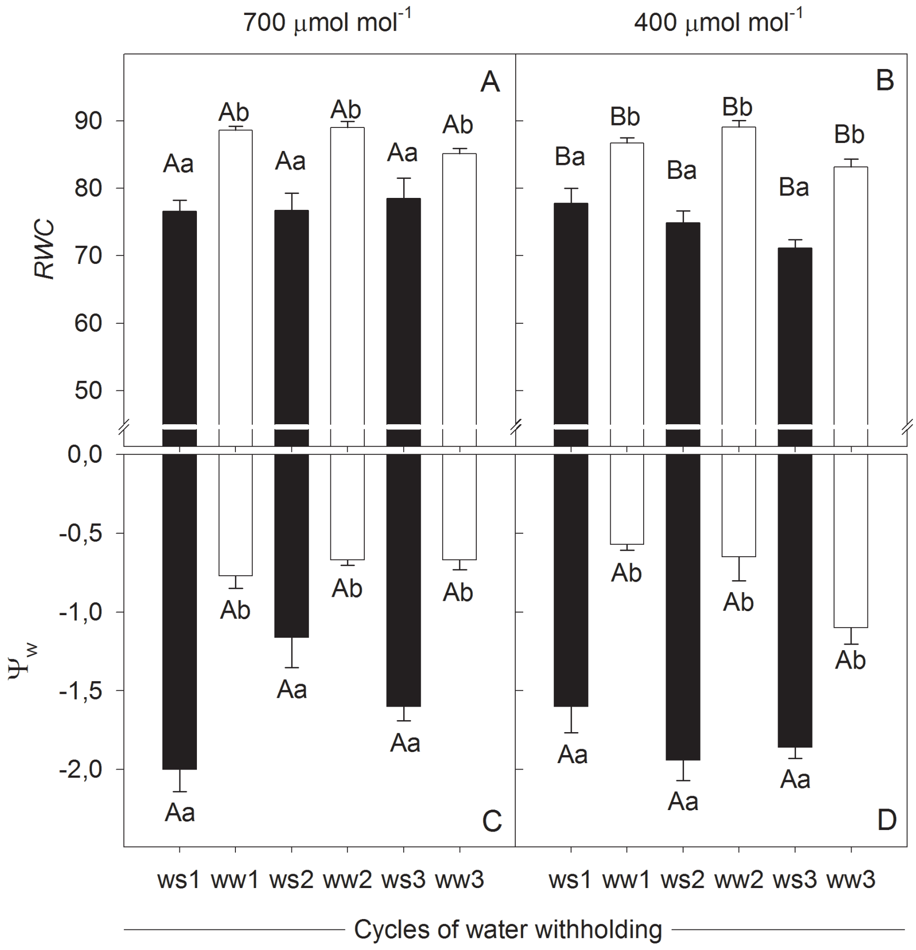

Figure 4. A, B. Relative leaf water content (*RWC*); **C, D.** leaf water potential (Ψw) in macauba palm plants after long-term exposure to $[CO_2]700$ (700 µmol mol^{-1}) (left panels) and $[CO_2]400$ (400 µmol mol^{-1}) (right panels). The plants were grown in open-top chambers and exposed to cyclical drought (filled columns) or irrigated daily (open columns). Uppercase letters compare treatments with different CO_2 concentrations and lowercase letters compare treatments with different water levels according to Tukey's test ($p < 0.05$). Means ± SE (n = 5).

Discussion

The higher photosynthetic responses linked to stomatal control in macauba palm leaves under water stress and $[CO_2]700$ indicate no down-regulation of photosynthesis as commonly reported (Ainsworth & Long 2005; Leakey *et al.* 2009; Córdoba *et al.* 2017). In general, the stimulation of photosynthesis may cease partially or totally due to negative feedback effects, but the implications of exposure to high $[CO_2]$ on trees are less clear compared to the responses of herbaceous species (Poorter & Navas 2003; Way *et al.* 2015; Ågren & Kattge 2017). In most plant species, stomatal apertures shorten after exposure to high $[CO_2]$, which, together with *TPU* limitation, might contribute to downward acclimation of photosynthesis after plants are exposed to high $[CO_2]$ (Ainsworth & Rogers 2007). Unlike for herbaceous plants, in tree species, due to their longer lifespan, this down-regulation of photosynthesis caused by stomatal conductance is not immediately evident at high $[CO_2]$ (Curtis & Wang 1998; Gunderson & Wullschleger 1994; Medlyn *et al.* 2001). Persistent stimulation of photosynthesis in trees experiencing increased $[CO_2]$ has been commonly reported (Way *et al.* 2015; Quentin *et al.* 2015; Gunderson & Wullschleger 1994), but only a few studies evaluated the photosynthetic responses of palm species to CO_2 enrichment (Ibrahim *et al.* 2010; Aranjuelo *et al.* 2009). Improved photosynthetic response, higher stomatal conductance, and greater light and water use efficiency by macauba palm indicated up-regulation of photosynthesis under long-term $[CO_2]700$. This observation contradicts our hypothesis, which posited that long-term increment of $[CO_2]$ would weaken the response of macauba palm to drought stress.

The up-regulation of macauba palm photosynthesis was also confirmed by the CO_2-response curves (*A vs Ci*) and the higher Vc_{max} and J_{max} in plants grown at $[CO_2]700$. Gunderson & Wullschleger (1994) compiled a series of *A vs Ci* curves from tree species grown either at current CO_2 or high $[CO_2]$ and estimated that Vc_{max} and J_{max} were somewhat lower in most plants grown at high $[CO_2]$; whereas photosynthesis in a few species was up-regulated, no palm species was evaluated. In contrast to macauba palm, which exhibits fast growth in a range of fertilization management, the slow-growing palm *Chamaerops humilis* exposed to long-term high $[CO_2]$ exhibited lower $Vc_{max}$and J_{max} in comparison with plants exposed to current CO_2 (Aranjuelo *et al.* 2009). The lower photosynthetic capacity of this palm species under high $[CO_2]$ was attributed to its inability to increase sink strength to balance the enhanced carbohydrate supply (Aranjuelo *et al.* 2009).

The higher W_t observed in macauba palm leaves at $[CO_2]700$ is due to increased photosynthetic capacity of the plants. In contrast, higher W_t in several tree species under high $[CO_2]$ has been commonly related to reduced stomatal conductance (Gessler *et al.* 2014; Werner *et al.* 2012). The increase in leaf photosynthesis simultaneously with W_t may stimulate biomass accumulation in palm species (Ibrahim *et al.* 2010). Moreover, under water stress conditions, the lower *Ci*/*Ca* that was detected only in plants grown at $[CO_2]700$ indicates that stomatal pore shortening restricted CO_2 diffusion into the mesophyll, compromising carbon assimilation. In contrast, increased *Ci*/*Ca* ratio under severe water stress, as observed in plants grown at $[CO_2]400$, confirms the absence of stomatal limitation to photosynthesis (Chaves & Oliveira 2004).

One of the first physiological processes affected by water stress is stomatal conductance (Cornic 2000). Stomatal conductance in palms has been shown to be sensitive to increasing leaf-to-air vapor pressure deficit (VPD), leading to greater stomatal closure, increased temperature and potential limitation of carbon gain (Renninger & Phillips 2016). In addition, stomatal sensitivity to VPD does not change in plants grown in high $[CO_2]$ (Medlyn *et al.* 2001). Lower photosynthetic capacity under drought conditions has been reported for other palm species (Oliveira *et al.* 2002; Gomes *et al.* 2008; Suresh *et al.* 2010; Passos *et al.* 2009; Silva *et al.* 2017; Silvestre *et al.* 2017). However, unlike most palms, macauba palm is naturally found in environments characterized by seasonal drought and high temperatures (Pires *et al.* 2013) and therefore is able to adjust to, and recover from, water stress (Mota & Cano 2016). In several plant species exposed to harsher drought conditions, other processes not related to stomata are commonly impaired, such as Rubisco carboxylation activity, RuBP regeneration, ATP supply, and light use efficiency (Flexas & Medrano 2002; Lawlor 2002; Chaves & Oliveira 2004; Grassi & Magnani 2005). Thus, macauba palm plants under water stress, irrespective of CO_2 concentration, were able to maintain constant proline and chlorophyll contents and TBARS accumulation even after the slight reduction in Vc_{max}. It is noteworthy that plants grown at $[CO_2]700$ were more capable of recovering from drought due to higher Vc_{max} and J_{max} (Fig. 3) and thus avoid reduction in total dry mass at the end of the stress period (Tab. 2).

In addition, the better response to drought by macauba palm plants grown in $[CO_2]700$ was emphasized by the values of *RWC* and Ψw measured throughout the successive water deficit cycles and by the lower photochemical capacity of plants at $[CO_2]400$ and under water stress. The immediate change in *RWC* during the first water stress cycle was similar between water-stressed plants at both CO_2 concentrations; however, in the third water stress cycle, *RWC* increased slightly in plants grown at $[CO_2]700$, but decreased significantly in plants grown at $[CO_2]400$. The lower leaf water potential for plants grown at $[CO_2]700$ in the first water stress cycle might be related to the accumulation of osmotically active solutes, such as sucrose derived from greater photosynthesis. Carbohydrate accumulation and sink imbalance is often associated with down-regulation of photosynthesis in plants under increased $[CO_2]$ (Córdoba *et* *al.* 2017; Gamage *et al.* 2018), which has negative effects on electron transport capacity and photoinhibition (Pammenter *et al.* 1993; Adams *et al.* 2013). Nevertheless, it is remarkable that the long-term exposure of macauba palms to [CO$_2$]700 resulted in higher ϕ_{II} and ETR and prevented photoinhibition of photosynthesis and damage due to oxidative stress under severe water deprivation, as assessed by TBARS values (Fig. S2 in supplementary material). The effective prevention of oxidative damage might be the result of enzymatic and non-enzymatic antioxidant mechanisms activated by water stress coupled with regulatory processes of energy partitioning that allow fast recovery of the photochemical capacity of macauba palm photosynthesis.

This capacity of macauba palms for fast recovery of photosynthesis after resuming watering is an important trait that indicates drought tolerance (Pinheiro & Chaves 2011) and contributes to higher productivity in a changing environment. Moreover, macauba palm plants presented efficient mechanisms to avoid metabolic damage upon water stress as indicated by stable values of lipid peroxidation and chlorophyll content. Oxidative damage and chlorophyll degradation under water stress may impair fast recovery by plants and lead to higher mortality as drought severity increases (Chaves *et al.* 2009). The complete recovery of photosynthesis after drought in macauba palm indicates that photochemical and biochemical traits were not severely affected by water stress (Cornic 2000). Accumulation of abscisic acid in leaves suppresses photosynthetic recovery through lowering stomatal conductance after drought, as observed for the dwarf coconut (Gomes *et al.* 2006) and the oil palm (Silva *et al.* 2017). Despite the low leaflet water potential (Ψw = -2 MPa) for macauba palms in the first water stress cycle, the time for complete recovery of plants was short. This suggests the presence of an efficient mechanism of stomatal control of photosynthesis that is coupled with prevention of severe damage under stress. This mechanism may allow efficient biomass allocation in future scenarios of climate change, as confirmed by our results regarding morphological adjustments and dry mass accumulation.

The stimuli to photosynthesis due to [CO$_2$]700 resulted in more efficient biomass accumulation in water-stressed macauba palm plants. This response is not related to improved water uptake resulting from high biomass allocation in roots under water stress, which has been suggested as an adaptive strategy in some tree species (Khurana & Singh 2002). This is supported by the fact that the main morphological adjustments of macauba palms to CO$_2$ levels occurred in aboveground organs, as highlighted by the lower root-to-shoot ratio in plants at [CO$_2$]700 (data not shown). The effects of water stress on biomass accumulation may be attenuated due to high [CO$_2$], as reported for plants from seasonally dry environments (Souza *et al.* 2016). The palm species *Elaeis guineensis* and *Chamaerops humilis* grown under increased [CO$_2$] had higher plant biomass, leaf area and relative growth rate than individuals under current CO$_2$ (Ibrahim *et al.* 2010; Aranjuelo *et al.* 2009). The greater leaf number and biomass allocation in the stem of macauba palm in response to the high [CO$_2$] might be linked to improved light capture by the whole plant (Chazdon 1986; Takenaka *et al.* 2001), which is coupled to higher light use efficiency (higher ETR and ϕ_{II}). Improved carbon assimilation may contribute to competition and establishment of this species in a changing environment.

Conclusion

Long-term exposure to [CO$_2$]700 resulted in up-regulation of photosynthesis in macauba palm through some morphological and physiological adjustments including increased stomatal conductance, increased Rubisco carboxylation, increased light use efficiency, and increased above ground biomass production. The [CO$_2$]700 changed the responses of macauba palms to cyclic drought. Macauba palm plants under conditions of [CO$_2$]700 exhibited a faster recovery time after rehydration, maintained *RWC* during successive water stress cycles, and were able to prevent reduction of total dry mass. The effective responses of macauba palm to drought and [CO$_2$]700 reveal that this plant has the potential to adjust to future climate change encompassing increased atmospheric CO$_2$.

Acknowledgements

This work was supported by Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) (grant number APQ-01244-13 to EGP and APQ-00281–11 to JPS) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (grant number 470116/2013-7 to EGP). E. G. Pereira also thanks CNPq for a research productivity grant (311532/2017-9).

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