Acta Botanica Brasilica - 33(3): 425-436. July-September 2019.
doi: 10.1590/0102-33062018abb0272

Elevated atmospheric CO_2 concentration improves water use efficiency and growth of a widespread Cerrado tree species even under soil water deficit

João Paulo Souza[1*], Nayara Magry Jesus Melo[2], Alessandro Dias Halfeld[1], Kamilla I. C. Vieira[1] and Bruno Luan Rosa[1]

Received: August 3, 2018
Accepted: March 11, 2019

ABSTRACT

Changes in atmospheric CO_2 levels are accompanied by ecological interactions involving other environmental factors, such as drought, which can severely alter the water balance of plants, thereby influencing sap flow, gas exchange, and plant growth. The objective of this study was to evaluate the water use economy, leaf gas exchange, and growth of young plants of the species *Lafoensia pacari* subjected to high concentrations of atmospheric CO_2 ($[CO_2]$) and soil water deficit. Increased vapor pressure deficit was observed from 1200 to 1800 hours. Plants under elevated $[CO_2]$ exhibited increased leaf photosynthesis, resulting in improved growth. Specific leaf area was lower in plants under high $[CO_2]$, mainly after soil water deficit treatment. Daily irrigated plants growing under ambient $[CO_2]$ had higher stem sap flow velocity (cm h^{-1}) and hourly sap flow (kg h^{-1}), mainly during the hottest hours of the day, than plants under high $[CO_2]$. Improved water use by plants growing under high $[CO_2]$ may result in increased availability of water in the soil, partially offsetting future drought events and extending the growth period.

Keywords: biomass allocation, leaf gas exchange, sap flow, specific leaf area, water economy

Introduction

In plant communities with a pronounced dry season, plant water economy is of great importance to ecosystem functionality (Franco 2005; Oliveira *et al*. 2005; Goldstein *et al*. 2008). The Brazilian Cerrado shows well-defined climatic seasonality, with a dry and cold winter and a hot and wet summer (Klink & Machado 2005). Owing to pronounced seasonal precipitation, woody species show decreased stomatal conductance (*gs*), leaf transpiration rates (*E*), and net photosynthesis during the dry season (Moraes & Prado 1998; Franco 2002; Bucci *et al*. 2004; Prado *et al*. 2004). Stomatal control of water loss results in low variations in leaf water potential between the dry and rainy seasons (Franco 1998). The stomatal control of transpiration in adult woody Cerrado species is more dependent on the vapor pressure deficit (VPD), which is twofold higher in the dry season compared to the rainy season (Bucci *et al*. 2004; 2008), than on soil water availability (Meinzer *et al*. 1999; Naves-Barbiero *et al*. 2000). However, for juvenile woody Cerrado species, soil water deficit plays an important role in leaf transpiration (Costa *et al*. 2015), since juvenile plants have shallow root systems and compete with herbaceous

---

1 Instituto de Biologia, Universidade Federal de Viçosa, 35690-000, Florestal, MG, Brazil
2 Departamento de Botânica, Universidade Federal de São Carlos, 13565-905, São Carlos, SP, Brazil

* Corresponding author: joaopaulobio@hotmail.com

species for water (Hoffmann *et al.* 2004a; Rossatto *et al.* 2012). In addition to ontogenetic differences for the control of transpiration, in the Cerrado, root depth varies among species with contrasting leaf habitat (deciduous versus evergreens) and life forms (trees, shrubs, and grass), resulting in distinct patterns of water relations (Rossatto *et al.* 2012).

To our knowledge, no studies have examined the influence of elevated atmospheric CO_2 concentration ([CO_2]) on stem sap flow in woody species and the consequences of water economy in the plants in Cerrado physiognomies. Shifts in CO_2 may result in great changes in crown functionality in plant species (Cramer *et al.* 2001). Because plant transpiration is the main regulator of mass and energy exchange between the atmosphere and vegetation (Baldocchi & Meyers 1998), understanding the influence of long-term rising [CO_2] on water use by woody species is important for the modeling of future atmospheric conditions (Uddling *et al.* 2008). Sap flow in plants grown under high [CO_2] decreases as *gs* decreases (Wullschleger & Norby 2001; Gunderson *et al.* 2002; Cech *et al.* 2003). These decreases will result in decreased leaf transpiration and increased water-use efficiency (WUE) by plants in the plant community (Battipaglia *et al.* 2013). The decreased plant transpiration due to elevated [CO_2] could result in a decrease in relative air humidity, even at a regional scale (Leuzinger & Korner 2007; Keenan *et al.* 2013).

The importance of the influence of rising [CO_2] on the plant transpiration is related to whether the vegetation is under anthropogenic pressure, as is observed in the Cerrado (Klink & Machado 2005). Cerrado deforestation contributes not only to the deregulation of the local climate but also to the length of the dry season in Amazonian sites (Costa & Pires 2010). In Cerrado *sensu lato*, the effects of rising [CO_2] are combined with increased soil water deficit, making plants exhibit more efficient carbon gain, even under soil water deficit (Souza *et al.* 2016). In addition, high [CO_2] could potentially change biomass partitioning towards aerial structures in woody species, which benefits them when competing with herbaceous plants (Melo *et al.* 2018). Thus, the synergism between CO_2 and water availability could intensify the responses of woody species under changes in regional climate (Keenan *et al.* 2013).

Lafoensia pacari (Lythraceae) is a widespread tree species in Cerrado areas, occurring in 74 % of 376 areas analyzed by Ratter *et al.* (2003) and in Atlantic forest remnants in São Paulo, Brazil (Kortz *et al.* 2014). Besides, *L. pacari* possesses ethnopharmacological importance, with the leaves being used for wound healing and treating cutaneous mycoses and gastritis (Pereira *et al.* 2018). In this study, we aimed to evaluate the growth, stem sap flow, leaf water potential, leaf gas exchange, chlorophyll content, and biomass partitioning in young *L. pacari* plants grown under ambient or high [CO_2]. We also investigated the influence of soil water deficit in plants growing under distinct [CO_2]. Owing to the rising [CO_2] in the atmosphere, we expected decreased stem sap flow and *gs* in *L. pacari* plants. Hence, plants grown under elevated [CO_2] were expected to become more efficient in water use, even with soil water deficit. Thus, the increase in [CO_2] may benefit *L. pacari* plants, making them more efficient in saving water and thereby increasing leaf gas exchange and growth.

Materials and methods

Plant materials, soil characteristics, and experimental design

The species utilized in the experiment was *Lafoensia pacari* A. St.-Hil, which exhibits arboreal characteristics, occurring in Cerrado *sensu stricto* areas and in forest vegetation in Cerrado *sensu lato* areas (Santos *et al.* 2009). The soil utilized for the growth of *L. pacari* plants was typical of Cerrado *sensu stricto* areas in Minas Gerais state, Brazil, and has been described by Souza *et al.* (2016).

L. pacari (approximately three months old) seedlings were acquired and transferred to the soil in 10 L plastic pots. The treatments with distinct [CO_2] were as follows: two open-top chambers (OTCs) with 40 plants under ambient [CO_2] (430 ppm), and two OTCs with 40 plants under high [CO_2] (700 ppm). The OTCs used in this experiment were as described by Melo *et al.* (2018). After all plants were 270 days old, with 245 days under an atmosphere enriched with CO_2, the plants were divided into four treatments: 20 plants under ambient [CO_2], watered daily; 20 plants under ambient [CO_2], with cyclical suspension of watering; 20 plants under high [CO_2], watered daily; and 20 plants under high [CO_2], with cyclical suspension of watering. Soil water deficit was introduced thrice during the experiment by halting irrigation one month before the fourth (360 days old), fifth (450 days old), and sixth (540 days old) morphophysiological surveys. After each survey, all seedlings were watered daily until the next cycle. Air temperature and humidity were measured throughout the experimental period to calculate VPD according to Jones (1992).

Leaf gas exchange and chlorophyll content index

Leaf gas exchange and chlorophyll content index (CCI) values were determined in two leaves in five individuals (n = five individuals per treatment) in each treatment. All leaves used were totally expanded without signs of senescence or herbivory. The measurements were made when plants were 90, 180, 270, 360, 450, and 540 days old and were under high atmospheric [CO_2] for 60, 155, 245, 335, 425, and 515 days. The total chlorophyll was determined using clorofiLOG (model CFL 1030; FALKER Porto Alegre, Rio Grande do Sul, Brazil). Leaf gas exchange was measured with an infrared gas analyzer (model LCA-4; Analytical Development Company, Hodesdon, UK), working in open mode (atmospheric air is passed through a chamber containing the leaf, which is dispensed after the second analysis). To saturate leaf photosynthesis (photosynthetic photon flux density = 1500 μmol m^{-2} s^{-1}), a light source (PLU-002; ADC) attached to a narrow Parkinson Leaf Chamber (PLCN-4) was used. Leaf temperature (25–27 °C) inside the PLCN-4 was maintained using a Peltier system. Leaf gas exchange measurements were made between 0800 and 1100 hours, the optimal period for leaf gas exchange for Cerrado species (Prado & Moraes 1997). The following leaf gas exchange parameters were measured: maximum net photosynthesis (P_N, μmol m^{-2} s^{-1}), stomatal conductance (*gs*, mol m^{-2} s^{-1}), and leaf transpiration rate (E, mmol m^{-2} s^{-1}). From the values of P_N and E, we obtained WUE = P_N/E, μmol $mmol^{-1}$.

Leaf water potential

The same individuals used to determine leaf gas exchange parameters were used to obtain leaf water potential (Ψ_{leaf}) values in the same period and plant age. Two leaves per individual (n = five individuals per treatment) were used for each treatment. All leaves were detached from shoots for immediate determination of Ψ_{leaf} using a portable digital pression chamber (SKPM model 1400/80; Skye Instruments Ltd.).

Stem sap flow

Sap flow was determined in the stem base in five individuals for each treatment at the end of the experiment when the plants were 540 days old. Sap flow velocity was determined by multiplying raw heat pulse velocity by correction factors (thermal diffusivity, asymmetry of alignment, and wound coefficient). Sap flow (in kilograms) was calculated by multiplying sap velocity by sap wood area (Burgess 2014). Sap flow was monitored for three days in each plant using a sap flow meter (ICT, model SFM1) by the heat ratio method (Burgess *et al*. 2000). Before the stem sap flow measurements, the active xylem area was determined in five individuals per treatment. Each of the five individuals was cut at the stem base and then submerged in a beaker with 2 % methylene blue. The beaker was covered with plastic to avoid dye evaporation. The plants were maintained for two days with the stem base submerged in the dye solution, and the stem was then cut; the region dyed blue was considered the active xylem area. The outer and inner diameters of the stained area were measured, and the active xylem area was determined according to methods described by Naves-Barbiero *et al*. (2000).

Leaf area and plant biomass

A group of five individuals for each treatment was used for leaf, stem, and root dry mass and leaf area measurements. Data collection was performed when plants were 90, 180, 270, 360, 450, and 540 days old and were grown in elevated [CO_2] for 65, 155, 245, 335, 425, and 515 days. Stem dry mass was determined after all buds and leaves were removed, when the leaf area and mass were measured (the same leaves removed from the stems). The root dry mass measurement was performed together with stem dry mass determination. Leaf area was calculated with Image-Pro software, 5.0 (Media Cybernetics, Inc., Silver Spring, MD, USA). Leaves, stems, and roots were dried out in a stove with air circulation (TECNAL TE -394/3; Piracicaba, São Paulo, Brasil) at 60 °C to a constant weight (Pérez- Harguindeguy *et al*. 2013), and the masses were measured using an analytical balance (Series BL-320H; Shimadzu, Tokyo, Japan). All leaves on the stems were considered independently of size and age. The leaf area per stem was determined by the sum of all leaf areas in the same individual.

Shoot-foliage relationships

From the determination of morphological traits (mass and area of leaves and length and mass of stems and roots), the following biometric indices were calculated: specific leaf area (SLA; cm^2 g^{-1}), root/stem ratio (RSR; g g^{-1}), and leaf area ratio (LAR; cm^2 g^{-1}).

Vegetative morphometry

The lengths and diameters of stems and numbers of leaves were determined in 10 individuals per species in plants under high and ambient [CO_2]. Stem length was measured by using a millimeter ruler, and a caliper was used to determine the diameter at the stem base. Total leaf number was registered weekly, when diameters and lengths of stems were determined. These measurements were initiated after 90 days of beginning the experiment (DBE). Two leaves for each individual (n = 10 individuals per treatment) were marked, and development was observed to determine leaf life span (LLS) from emergence until senescence (more than 90 % yellowing) or abscission. The same leaves previously marked for LLS measurement were utilized to calculate the leaf expansion interval (LEI; days to full expansion) and leaf expansion rate (LER; mature leaf area divided by LEI cm^2 $days^{-1}$).

Statistical analyses

For the first 245 DBE, the experiment had randomized blocks in a 2 × 3 factorial design, with two [CO_2] levels (430 and 700 ppm) and three measurement dates. After 245 DBE, the experiment had randomized blocks in a 2 × 2 × 3 factorial design, with two $[CO_2]$ levels (430 and 700 ppm), two water levels (with and without soil water deficit), and three measurement dates. The average values and standard errors were calculated for all morphophysiological and phenological traits measured in *L. pacari* plants. Analysis of variance and post-hoc Tukey's tests were applied to evaluate differences between treatments. The R program was used to perform all statistical analyses (R Development Core Team 2014). To verify the degree of correlation between VPD and stem sap flow, we used Spearman's correlation coefficient (Rs) because the entire data set did not demonstrate normal distribution. These correlations were performed using the OriginPro program, version 8.0 (http://www.OriginLab.com/).

Results

Leaf gas exchange, leaf water potential, chlorophyll content, and WUE

P_N values were higher in plants grown under elevated $[CO_2]$ than under ambient $[CO_2]$ during the experimental period, even under soil water deficit, being more evident at 515 DBE ($p < 0.05$, interactions between CO_2 and DBE, and between CO_2, water, and DBE; Fig. 1). For the first 245 DBE, plants under ambient $[CO_2]$ showed higher *gs* and E than plants under high $[CO_2]$ did (except for E values at 155 DBE, $p < 0.05$, interactions between DBE and CO_2; Fig. 1). Importantly, at 515 DBE, high values of *gs* were found in plants under elevated $[CO_2]$ and soil water deficit ($p < 0.05$, interactions between CO_2, water, and DBE; Fig. 1).

Plants under elevated $[CO_2]$ showed higher WUE than that of plants under ambient $[CO_2]$ from 60 to 245 DBE. However, after 335 DBE, there was an inversion, and plants under ambient $[CO_2]$ showed higher WUE than that of plants under elevated $[CO_2]$ (except at 425 DBE, $p < 0.05$, interactions between CO_2 and DBE; Fig. 1).

Only after soil water deficit application, plants grown under high $[CO_2]$ showed high total chlorophyll content ($p < 0.05$, simple effect of CO_2 or water; Fig. 1). The Ψ_{leaf} values were not influenced by $[CO_2]$. After soil water deficit, plants irrigated daily showed less negative values of Ψ_{leaf} compared to water-stressed plants ($p < 0.05$, interactions between DBE and water; Fig. 1).

Stem sap flow and active xylem area

Significantly higher ($p < 0.05$) values of stem sap flow velocity (cm h^{-1}) and hourly sap flow (kg h^{-1}) were observed between 1000 and 1300 hours (Fig. 2). However, after 1300 hours, there was a decrease in stem sap flow during the hottest period of the day (high VPD values). Plants under ambient $[CO_2]$ and daily irrigation showed higher ($p < 0.05$) hourly sap flow, mainly at noon (double interaction between CO_2 and water, $p < 0.05$; Fig. 2), than plants under elevated $[CO_2]$ did (double interaction between CO_2 and water, $p < 0.05$; Fig. 2). Regardless of $[CO_2]$, plants irrigated daily showed higher sap flow velocity, mainly in the hottest period of the day (double interaction between water and hour of the day, $p < 0.05$; Fig. 2).

However, stem sap flow was significantly higher ($p < 0.05$) in plants under ambient $[CO_2]$ and daily irrigation than that of the other treatments (double interaction between CO_2 and water, $p < 0.05$; Fig. 2). Thus, *L. pacari* plants grown under elevated $[CO_2]$ showed improved water use even under soil water deficit.

Correlation ($p < 0.05$, Tab. 1) was found only between stem sap flow velocity and VPD in *L. pacari* plants grown under elevated $[CO_2]$ and soil water deficit (Tab. 1).

Table 1. Spearman's correlation coefficient between water vapor pressure deficit (VPD), stem sap flow velocity (SFV), and hourly sap flow (HSF) in plants grown under ambient $[CO_2]$ (430 ppm, watered daily and cyclical suspension of watering) or high $[CO_2]$ (700 ppm, watered daily and cyclical suspension of watering). Bold values indicate $p < 0.05$.

$[CO_2]$	Water	r_s values	
		VPD × SFV	VPD × HSF
430 ppm	Watered daily	0.35	0.23
430 ppm	Cyclical suspension of watering	0.34	0.28
700 ppm	Watered daily	0.33	0.24
700 ppm	Cyclical suspension of watering	**0.51**	0.40

L. pacari plants grown under elevated $[CO_2]$ showed a larger active xylem area than that of plants under ambient $[CO_2]$ (main effect of CO_2, $p < 0.05$; Tab. 2).

Table 2. Active xylem area (cm^2) in plants grown under ambient $[CO_2]$ (430 ppm, watered daily and cyclical suspension of watering) or high $[CO_2]$ (700 ppm, watered daily and cyclical suspension of watering). Asterisks indicate differences between CO_2 treatments ($p < 0.05$).

$[CO_2]$	Water	Active xylem area
430 ppm	Watered daily	0.15 ± 0.04*
430 ppm	Cyclical suspension of watering	0.13 ± 0.01*
700 ppm	Watered daily	0.24 ± 0.04*
700 ppm	Cyclical suspension of watering	0.23 ± 0.04*

Leaf area and biomass allocation

There were no differences ($p > 0.05$) in average and total leaf areas among CO_2 treatments until 245 DBE (Fig. 3). After soil water deficit application, plants under elevated $[CO_2]$, regardless of water treatment, showed higher leaf area values than those of plants under ambient $[CO_2]$ ($p < 0.05$, interactions between CO_2 and DBE and water; Fig. 3).

From 245 DBE, leaf, root, stem, and total dry masses were higher ($p < 0.05$) in plants under elevated $[CO_2]$, mainly after soil water deficit application (interactions between

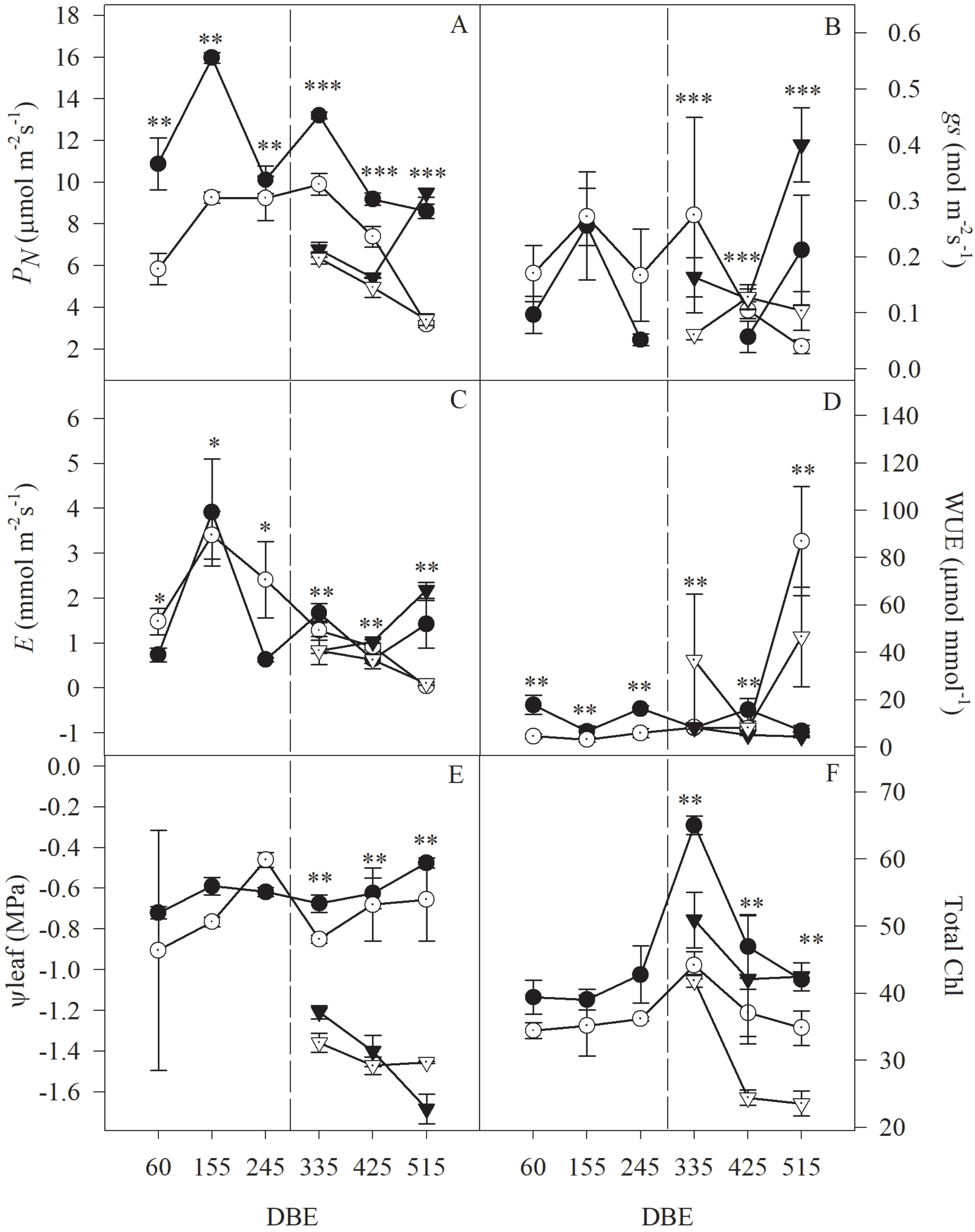

Figure 1. Leaf gas exchange in *Lafoensia pacari* plants grown under ambient [CO_2] (watered daily ○, and cyclical suspension of watering ▽) or high [CO_2] (watered daily ●, and cyclical suspension of watering ▼). P_N = maximum net photosynthesis (**A**); *gs* = stomatal conductance (**B**); *E* = leaf transpiration rate (**C**); WUE = water-use efficiency (**D**); Ψ_{leaf} = leaf water potential (**E**); Total chl = index of total chlorophyll (**F**). Symbols represent averages (n = 5), and bars represent ± standard error values. Asterisks indicate differences among CO_2, water, or periods of measurement: *, main effect; **, double interaction; ***, triple interaction. DBE, days after beginning the experiment. Dashed line indicates the beginning of water deficit treatment.

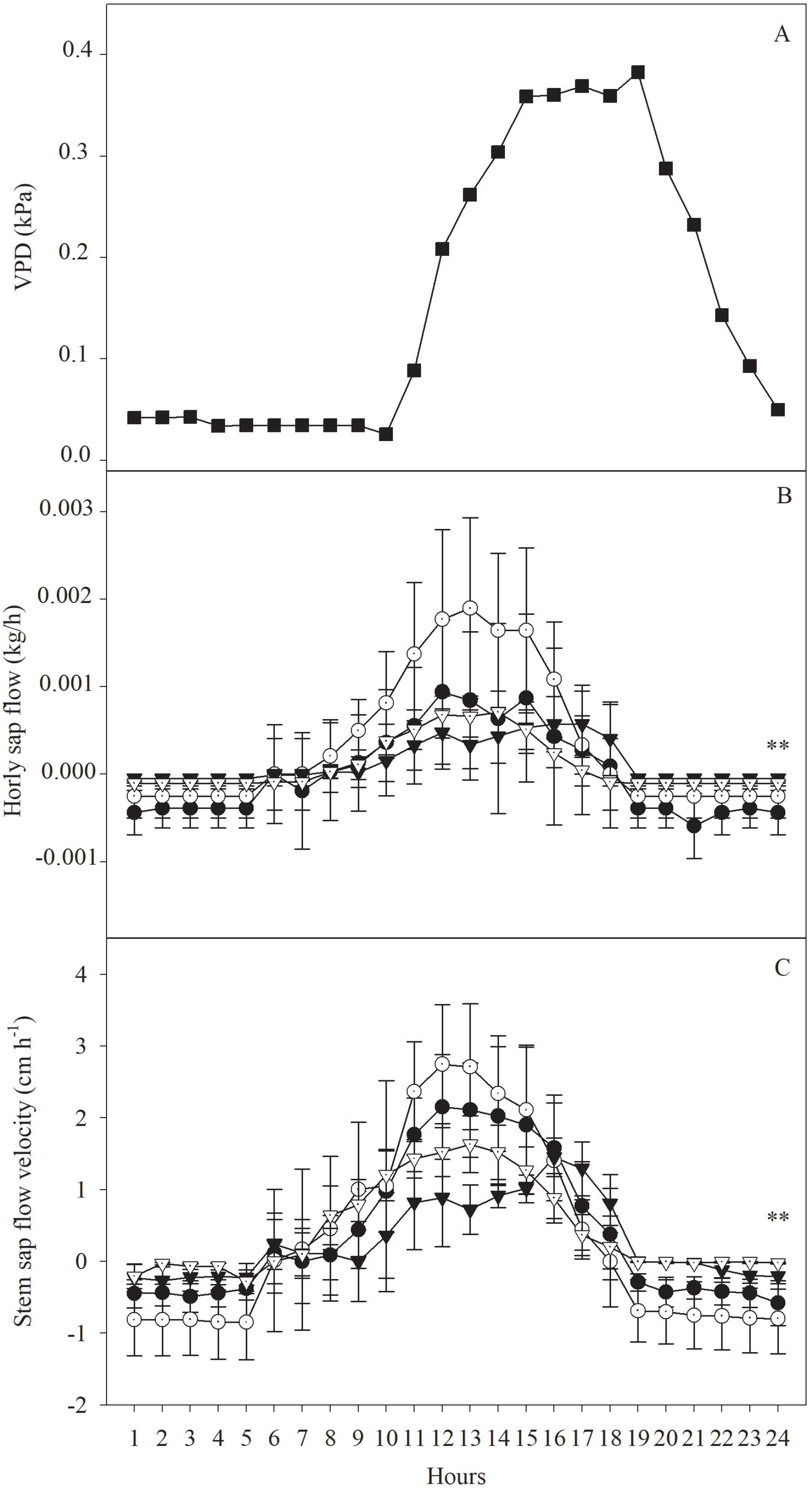

Figure 2. Vapor pressure deficit (VPD) (**A**), hourly sap flow (**B**), and stem sap flow velocity (**C**) in *L. pacari* plants grown under ambient [CO_2] (watered daily ○, and cyclical suspension of watering ▽) or high [CO_2] (watered daily ●, and cyclical suspension of watering ▼). Symbols represent averages (n = 5), and bars represent ± standard error values. Asterisks indicate differences among CO_2, water, or hours: *, main effect; **, double interaction.

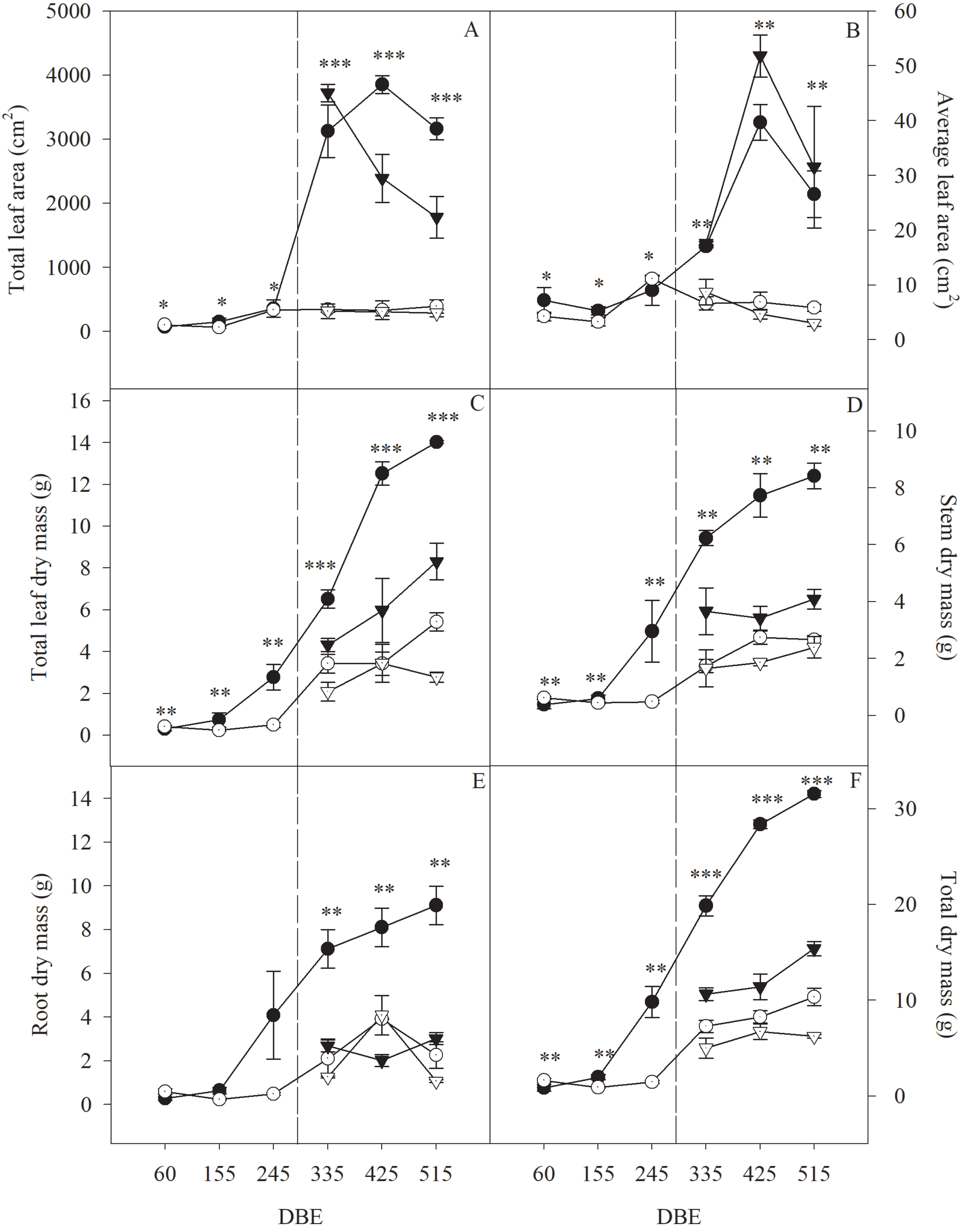

Figure 3. Morphological traits of *L. pacari* plants grown under ambient [CO_2] (watered daily ○, and cyclical suspension of watering ∇) or high [CO_2] (watered daily ●, and cyclical suspension of watering ▼). Total leaf area (**A**); average leaf area (**B**); total leaf dry mass (**C**); stem dry mass (**D**); root dry mass (**E**); total dry mass (**F**). Symbols represent averages (n = 5), and bars represent ± standard error values. Asterisks indicate differences among CO_2, water, or hours: *, main effect; **, double interaction. DBE, days after beginning the experiment. Dashed line indicates the beginning of water deficit treatment.

CO_2 and water; Fig. 3). The total dry mass was significantly higher ($p < 0.05$) in plants under elevated [CO_2] after 155 DBE (interactions between CO_2 and DBE), even after soil water deficit application (interactions between CO_2 and DBE and water; Fig. 3).

Biometric indices

There were no differences ($p > 0.05$) in RSRs between plants under elevated and ambient [CO_2] (Fig. 4). Moreover, SLA and LAR were higher ($p < 0.05$) in plants under ambient [CO_2] than under elevated [CO_2] at 245 DBE (interactions between CO_2 and DBE; Fig. 4). However, after soil water deficit application, plants under elevated [CO_2] showed higher ($p < 0.05$) LAR than that of plants under ambient [CO_2] (interactions between CO_2, DBE, and water; Fig. 4). Plants under ambient [CO_2] showed higher ($p < 0.05$) SLA, mainly after soil water deficit application (simple effect of CO_2; Fig. 4).

Vegetative morphometry

There were no differences ($p > 0.05$) in LERs and LLSs between plants under elevated and ambient [CO_2] (Tab. 3). However, LEI was higher ($p < 0.05$) in plants under elevated [CO_2] than under ambient [CO_2].

Table 3. Leaf development in *Lafoensia pacari* plants grown in ambient (430 ppm) [CO_2] or high (700 ppm) [CO_2].

[CO_2]	LEI (days)	LER ($cm^2/days^{-1}$)	LLS (days)
430 ppm	134.05 ± 67.18 *	0.07 ± 0.04	214.55 ± 64.66
700 ppm	171.85 ± 54.55 *	0.06 ± 0.02	238.35 ± 54.46

LEI = leaf expansion interval; LER = leaf expansion rate; LLS = leaf life span. Asterisks indicate differences between CO_2 treatments ($p < 0.05$).

Plants under elevated [CO_2] showed higher ($p < 0.05$) stem diameters and lengths from 120 DBE (interactions between CO_2 and DBE, Fig. 5). However, plants under elevated [CO_2] showed higher ($p < 0.05$) leaf numbers only after 330 DBE (interactions between CO_2 and DBE; Fig. 5).

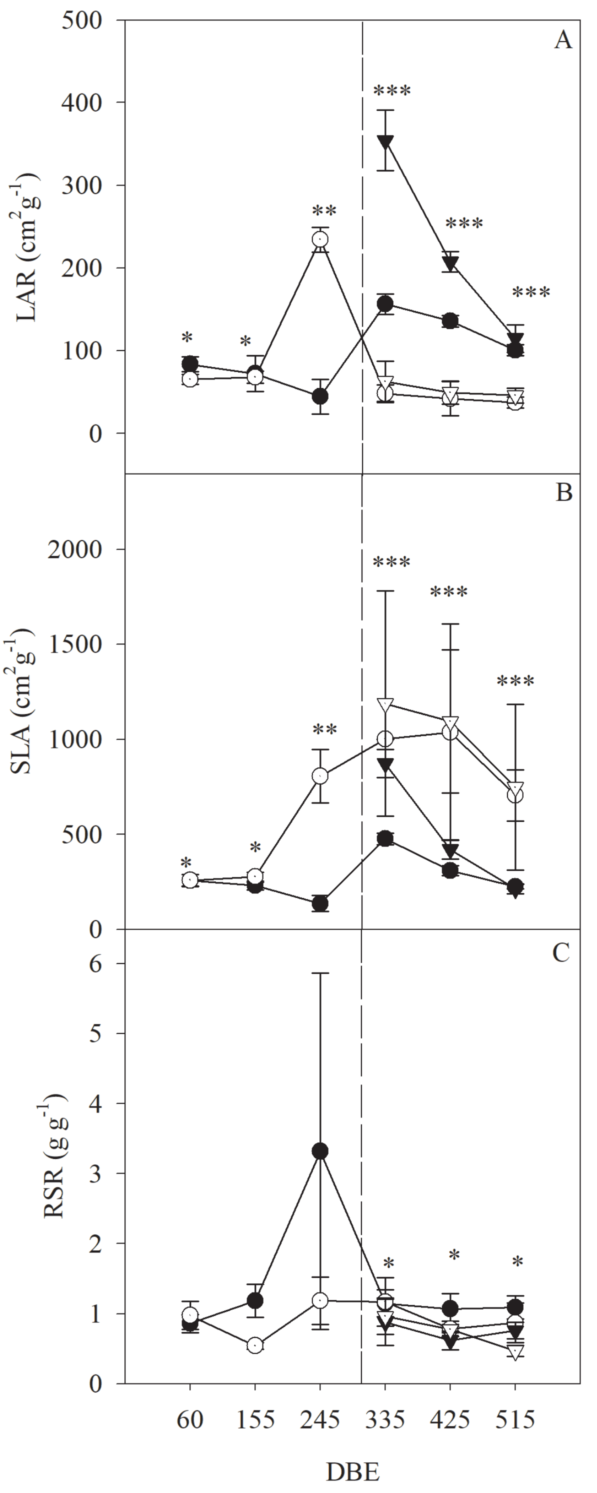

Figure 4. Biometrical index in *L. pacari* plants grown under ambient [CO_2] (watered daily ○, and cyclical suspension of watering ▽) or high atmospheric [CO_2] (watered daily ●, and cyclical suspension of watering ▼). LAR = leaf area ratio (**A**); SLA = specific leaf area (**B**); RSR = root/stem ratio (**C**). Symbols represent averages (n = 5), and bars represent ± standard error values. Asterisks indicate differences among CO_2, water, or hours: *, main effect; **, double interaction. DBE, days after beginning the experiment. Dashed line indicates the beginning of water deficit treatment.

Discussion

In the present study, *L. pacari* plants showed stomatal control of leaf transpiration, primarily during the hottest hours of the day, even in plants grown under elevated [CO_2]. At noon, Cerrado plants show decreased leaf transpiration and stem sap flow (Naves-Barbiero *et al.* 2000; Franco & Lutgge 2002), even with elevated solar radiation and atmospheric evaporative demand. In this way, the stomata confer strong control of transpiratory flow throughout the day, and this response is linked to increased vapor pressure deficit (Schulze 1993). This was more evident in *L. pacari* plants grown under elevated [CO_2] and soil water deficit (positive correlation between VPD and sap flow velocity). For young Cerrado plants, soil water availability has a great effect over leaf gas exchange (Costa *et al.* 2015). However, in our study, the interaction between elevated [CO_2] and soil water stress appeared to have made *L. pacari* plants more dependent on VPD for leaf gas exchange.

Plant species grown under elevated [CO_2] demonstrate improved WUE (Leakey *et al.* 2009; Keenan *et al.* 2013). Enhanced WUE is crucial because it diminishes water stress and increases the growing season (Sleen *et al.* 2015) for plant species. According to Sleen *et al.* (2015), increased WUE occurs at a pan-tropical scale, but this high WUE is not accompanied by growth stimulation. Battipaglia *et al.* (2013) found that five species under high [CO_2] distributed in three FACE experiments in Italy and the United States of America showed a 70 % increase in WUE. Several published papers (Cech *et al.* 2003; Wullschleger & Norby 2001; Leuzinger & Korner 2007; Sleen *et al.* 2015) have reported improvements in

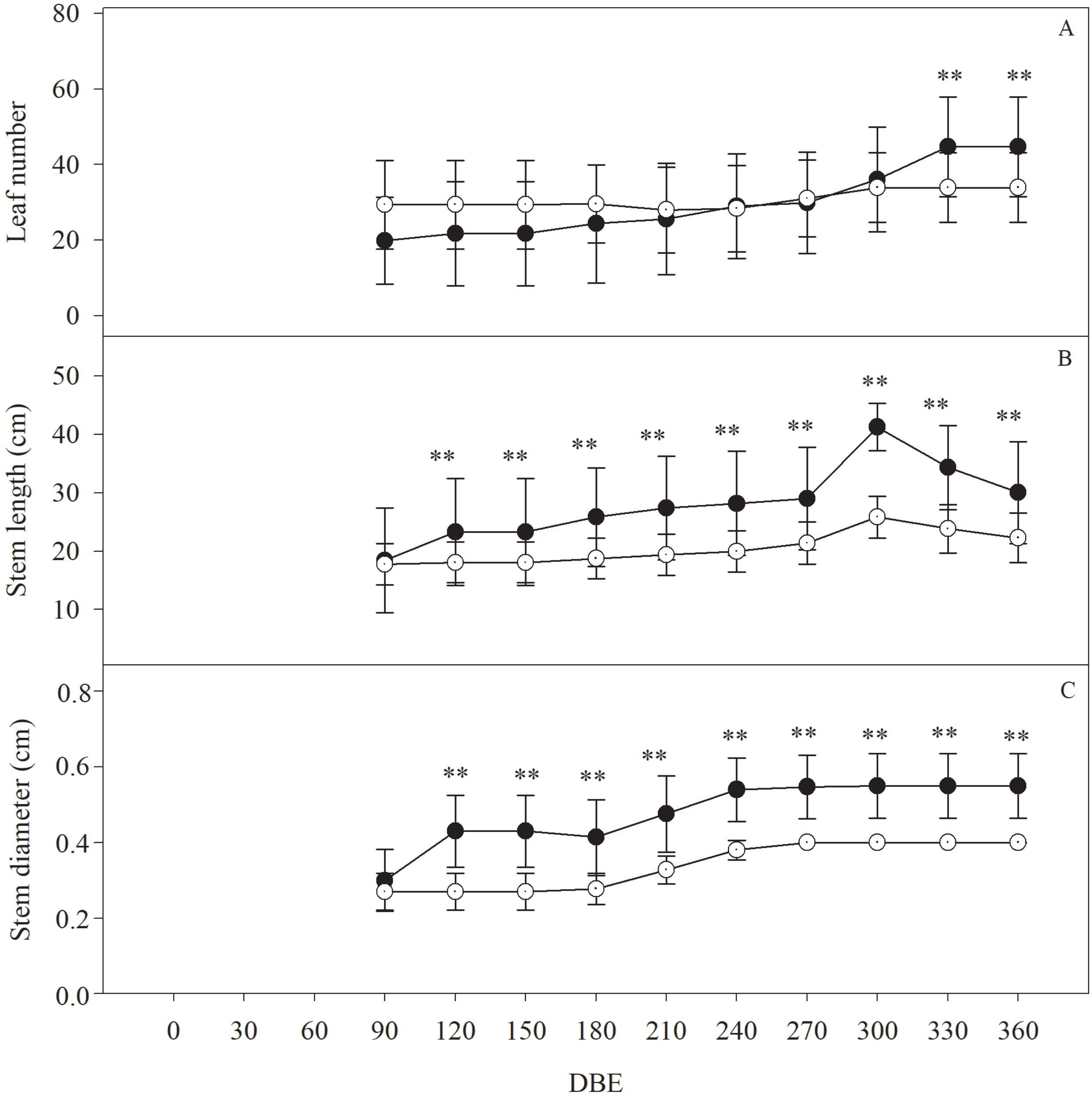

Figure 5. Vegetative morphometry in *L. pacari* plants grown under ambient [CO_2] (○) or high [CO_2] (●). Leaf number (**A**); stem length (**B**); stem diameter (**C**). Symbols represent averages (n = 5), and bars represent ± standard error values. Asterisks indicate differences between CO_2 or periods of measurement: *, main effect; **, double interaction. DBE, days after beginning the experiment.

WUE for forest species; however, there are few data (Souza *et al*. 2016) for savanna species grown under elevated [CO_2] and for the consequences of WUE on water economy by plant species.

In our study, *L. pacari* plants grown under high [CO_2] did not show decreased leaf transpiration but did exhibit higher WUE, except for 335 and 515 DBE. In these measurement periods (335 and 515 DBE), plants under elevated [CO_2] showed higher *gs*, resulting in elevated leaf transpiration and thus, lower WUE. However, in plants under elevated [CO_2], the increase in WUE was likely caused by increased leaf photosynthesis. The amount of water passing through the xylem in plants grown under high [CO_2] was lower than that of plants under ambient [CO_2], even producing large active xylem area. The low stem sap flow in *L. pacari* under elevated [CO_2] might have resulted from the greater control of stomatal conductance mainly under soil water stress; however, xylem cavitation might have occurred in individuals under elevated [CO_2], because under high [CO_2], *L. pacari* produces a larger active xylem area, being more susceptible to cavitation (Bobich *et al*. 2010).

Thus, *L. pacari* plants showed fine stomata control, primarily under elevated [CO_2], preventing water loss and increasing WUE. Several studies have shown that elevated [CO_2] influences water dynamics in the plant body, resulting in water conservation (Wullschleger & Norby 2001; Cech *et al*. 2003; Leakey *et al*. 2009; Keenan *et al*. 2013). In our study, *L. pacari* plants grown under elevated [CO_2] showed low water use, which could result in high water availability in the soil, partially offsetting drought events and extending the growth period (Sleen *et al*. 2015). Furthermore, the high water availability in the soil may result in changes in competitive interactions among plant species (Soliveres *et al*. 2015). In addition, the high biomass allocation to roots linked with low water use in *L. pacari* plants may result in water saving in the soil profile, which would make the water readily available to young woody and herbaceous plants of the Cerrado. All these modifications could be beneficial to native plants in natural areas. However, with the decrease in the amount of water transpired, air temperature might increase, and ambient humidity and recycling of continental precipitation might decrease (Keenan *et al*. 2013). Another important ecosystem process that would be changed by elevation in [CO_2] is decreased precipitation in areas that depend on water transpired in other regions (Keenan *et al*. 2013).

The lower SLA in *L. pacari* plants grown under elevated [CO_2] indicated that these plants allocated more biomass to leaves. At high [CO_2], the mesophyll resistance may increase due to increased biomass allocation to leaves (Medlyn *et al*. 2011; Temme *et al*. 2015), resulting in more resistance to leaf gas diffusion. Low SLA serves to take up more of the most limiting resource, carbon, required for optimal growth (Bloom *et al*. 1985). In this way, low SLA in *L. pacari* plants can reduce water vapor diffusion and contribute to water saving at the leaf level. The leaves of *L. pacari* also exhibit delayed development (high LEI). Souza *et al*. (2016) and Melo *et al*. (2018) found that for three woody Cerrado species, leaf expansion was faster when the species were grown under elevated [CO_2] than in plants under ambient [CO_2]. However, the woody Cerrado species studied by Souza *et al*. (2016) showed high SLA under high [CO_2]. Thus, *L. pacari* plants need more time to construct highly complex leaf cohorts under elevated [CO_2], showing higher LEIs than plants under ambient [CO_2]. These modifications in leaf structure (low SLA) could prevent water loss from leaves, making plants more efficient in water use.

Along with other physiological modifications, the elevation in [CO_2] highlighted the influence of CO_2 on the growth of Cerrado species. In a previous study, Souza *et al*. (2016) showed that woody Cerrado plants changed their normal ontogeny, speeding up aboveground growth, mainly after one year of age. After one year, *L. pacari* plants showed intense biomass accumulation, mainly in the aerial parts, and exhibited better physiological performance (leaf photosynthesis and chlorophyll content). Thus, even under soil water deficit, *L. pacari*, similar to other woody Cerrado species grown under elevated [CO_2] (Souza *et al*. 2016), would present better aerial growth but not at the expense of root production. This shift in the biomass allocation pattern to aerial parts could make savanna species taller and decrease incoming sunlight to beneath the strata (Bond *et al*. 2003). Because the cerrado *sensu stricto* (savanna) area is structured as a two-layered system (Walker & Noy-Meir 1982; Nardoto *et al*. 1998; Hoffmann *et al*. 2004b), the incoming light restriction beneath the strata can change competitive relationships among woody and herbaceous species (Melo *et al*. 2018).

This is the first study to demonstrate water saving by a savanna species grown under elevated [CO_2]. Although only one woody species was evaluated, our findings demonstrated that CO_2 elevation improved the water use, resulting in better growth capacity. Because Cerrado vegetation shows seasonal precipitation, which is important for the establishment and adaptation of Cerrado plants, the likely changes in water dynamics could alter functional processes in Cerrado areas, as well as ecological interactions (such as competition) between woody and herbaceous Cerrado species.

Acknowledgements

We thank FAPEMIG (FORTIS-TCT-10254/2014 and APQ 00281–11) for financial support.

References

Baldocchi D, Meyers T. 1998. On using eco-physiological, micrometeorological and biogeochemical theory to evaluate carbon dioxide, water vapor and trace gas fluxes over vegetation: a perspective. Agricultural and Forest Meteorology 90: 1-25.

Battipaglia G, Saurer M, Cherubini P, *et al*. 2013. Elevated CO_2 increases tree-level intrinsic water use efficiency: insights from carbon and oxygen isotope analyses in tree rings across three forest FACE sites. New Phytologist 197: 544-554.

Bloom AJ, Chapin III FS, Mooney HA. 1985. Resource limitation in plants-an economic analogy. Annual review of Ecology and Systematics 16: 363-392.

Bobich EG, Barron-Gafford GA, Rascher KG, Murthy R. 2010. Effects of drought and changes in vapour pressure deficit on water relations of *Populus deltoides* growing in ambient and elevated CO_2. Tree Physiology 30: 866-875.

Bond WJ, Midgley GF, Woodward FI. 2003. The importance of low atmospheric CO_2 and fire in promoting the spread of grasslands and savannas. Global Change Biology 9: 973-982.

Bucci SJ, Goldstein G, Meinzer FC, Scholz FG, Franco AC, Bustamante M. 2004. Functional convergence in hydraulic architecture and water relations of tropical savanna trees: from leaf to whole plant. Tree Physiology 24: 891-899.

Bucci SJ, Scholz FG, Goldstein G, *et al*. 2008. Water relations and hydraulic architecture in Cerrado trees: adjustments to seasonal changes in water availability and evaporative demand. Brazilian Journal of Plant Physiology 20: 233-245.

Burgess S. 2014. SFM1 Sap flow meter manual. Version 4.0. Armidale, ICT International.

Burgess SS, Adams MA, Bleby TM. 2000. Measurement of sap flow in roots of woody plants: a commentary. Tree Physiology 20: 909-913.

Cech PG, Pepin S, Körner C. 2003. Elevated CO_2 reduces sap flux in mature deciduous forest trees. Oecologia 137: 258-268.

Costa AC, Rezende-Silva SL, Megguer CA, Moura LMF, Rosa M, Silva AA. 2015. The effect of irradiance and water restriction on photosynthesis in young jatobá-do-cerrado (*Hymenaea stigonocarpa*) plants. Photosynthetica 53: 118-127.

Costa MH, Pires GF. 2010. Effects of Amazon and Central Brazil deforestation scenarios on the duration of the dry season in the arc of deforestation. International Journal of Climatology 30: 1970-1979.

Cramer W, Bondeau A, Woodward FI, *et al*. 2001. Global response of terrestrial ecosystem structure and function to CO_2 and climate change: results from six dynamic global vegetation models. Global Change Biology 7: 357-373.

Franco AC. 1998. Seasonal patterns of gas exchange, water relations and growth of it *Roupala montana*, an evergreen savanna species. Plant Ecology 136: 69-76.

Franco AC. 2002. Ecophysiology of woody plants. In: Oliveira PS, Marquis RJ. (eds.) The Cerrados of Brazil: ecology and natural history of a neotropical savanna. New York, Columbia University Press. p. 178-197.

Franco AC. 2005. Biodiversidade de forma e função: implicações ecofisiológicas das estratégias de utilização de água e luz em plantas lenhosas do Cerrado. In: Scariot A, Souza-Silva JC, Felfili JM. (eds.) Cerrado: ecologia, biodiversidade e conservação. Brasília, Ministério do Meio Ambiente. p. 179-196.

Franco AC, Lüttge U. 2002. Midday depression in savanna trees: coordinated adjustments in photochemical efficiency, photorespiration, CO_2 assimilation and water use efficiency. Oecologia 131: 356-365.

Goldstein G, Meinzer FC, Bucci SJ, Scholz FG, Franco AC, Hoffmann WA. 2008. Water economy of Neotropical savanna trees: six paradigms revisited. Tree physiology 28: 395-404.

Gunderson CA, Sholtis JD, Wullschleger SD, Tissue DT, Hanson PJ, Norby RJ. 2002. Environmental and stomatal control of photosynthetic enhancement in the canopy of a sweetgum (*Liquidambar styraciflua* L.) plantation during 3 years of CO_2 enrichment. Plant, Cell & Environment 25: 379-393.

Hoffmann WA, Lucatelli VM, Silva FJ, *et al*. 2004a. Impact of the invasive alien grass *Melinis minutiflora* at the savanna-forest ecotone in the Brazilian Cerrado. Diversity and Distributions 10: 99-103.

Hoffmann WA, Orthen B, Franco AC. 2004b. Constraints to seedlings success of savanna and forest tree across the savanna-forest boundary. Oecologia 140: 252-260.

Jones HG. 1992. Plants and microclimate: A quantitative approach to environmental plant physiology. 2nd. edn. Cambridge, Cambridge University Press.

Keenan TF, Hollinger DY, Bohrer G, *et al*. 2013. Increase in forest water-use efficiency as atmospheric carbon dioxide concentrations rise. Nature 499: 324-327.

Klink CA, Machado RB. 2005. Conservation of the Brazilian Cerrado. Conservation Biology 19: 707-713.

Kortz AR, Coelho S, Castello ACD, Corrêa LS, Leite EC, Koch I. 2014. Wood vegetation in Atlantic rain forest remnants in Sorocaba (São Paulo. Brazil). Check List 10: 344-354.

Leakey AD, Ainsworth EA, Bernacchi CJ, Rogers A, Long SP, Ort DR. 2009. Elevated CO_2 effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. Journal of Experimental Botany 60: 2859-2876.

Leuzinger S, Körner C. 2007. Water savings in mature deciduous forest trees under elevated CO_2. Global Change Biology 13: 2498-2508.

Medlyn BE, Duursma RA, Zeppel MJ. 2011. Forest productivity under climate change: a checklist for evaluating model studies. Wiley Interdisciplinary Reviews: Climate Change 2: 332-355.

Meinzer FC, Goldstein G, Franco AC, *et al*. 1999. Atmospheric and hydraulic limitations on transpiration in Brazilian cerrado woody species. Functional Ecology 13: 273-282.

Melo NMJ, Rosa RSEG, Pereira EG, Souza JP. 2018. Rising [CO_2] changes competition relationships between native woody and alien herbaceous Cerrado species. Functional Plant Biology 45: 854-864.

Moraes, JAPV, Prado, CHBA 1998. Photosynthesis and water relations in cerrado vegetation. In: Scarano FR, Franco AC. (eds.) Ecophysiological strategies of xerophytic and amphibious plants in the neotropics. Series Oecologia Brasiliensis. Rio de Janeiro, UFRJ. p. 45-63.

Nardoto GB, Souza MP, Franco AC. 1998. Estabelecimento e padrões sazonais de produtividade de *Kielmeyera coriacea* (Spr) Mart. nos cerrados do Planalto Central: efeitos do estresse hídrico e sombreamento. Revista Brasileira de Botânica 21 doi: 10.1590/S0100-84041998000300011.

Naves-Barbiero CC, Franco AC, Bucci SJ, Goldstein G. 2000. Fluxo de seiva e condutância estomática de duas espécies lenhosas sempre-verdes no campo sujo e cerradão. Revista Brasileira de Fisiologia Vegetal 12: 119-134.

Oliveira RS, Bezerra L, Davidson EA, *et al*. 2005. Deep root function in soil water dynamics in cerrado savannas of central Brazil. Functional Ecology 19: 574-581

Pereira LOM, Vilegas W, Tangerina MMP, *et al*. 2018. Lafoensia pacari A. St.-Hil.: Wound healing activity and mechanism of action of standardized hydroethanolic leaves extract. Journal of Ethnopharmacology 219: 337-350.

Pérez-Harguindeguy N, Díaz S, Garnier E, *et al*. 2013. New handbook for standardised measurement of plant functional traits worldwide. Australian Journal of Botany 61: 167-234.

Prado CHBA, Moraes JAPV. 1997. Photosynthetic capacity and specific leaf mass in twenty woody species of Cerrado vegetation under field conditions. Photosynthetica 33: 103-112.

Prado CHBA, Wenhui Z, Cardoza Rojas MH, Souza GM. 2004. Seasonal leaf gas exchange and water potential in a woody cerrado species community. Brazilian Journal of Plant Physiology 16: 7-16.

R Development Core Team. 2014. A language and environment for statistical computing. Vienna, R Foundation for Statistical Computing. http://www.R-project.org/. 19 Aug. 2016.

Ratter JA, Bridgewater S, Ribeiro JF. 2003. Analysis of the floristic composition of the Brazilian cerrado vegetation III: comparison of the woody vegetation of 376 areas. Edinburgh Journal of Botany 60: 57-109.

Rossatto DR, Sternberg LSL, Franco AC. 2012. The partitioning of water uptake between growth forms in a neotropical savanna: do herbs exploit a third water source niche? Plant Biology 15: 84-92.

Santos LW, Coelho MFB, Pirani FR. 2009. Phenology of *Lafoensia pacari* A. St.-Hil.(Lythraceae) in Barra do Garças, Mato Grosso State, Brazil. Revista Brasileira de Plantas Medicinais 11: 12-17.

Schulze ED. 1993. Soil water deficits and atmospheric humidity as environmental signals. In: Smith JAC, Griffiths H. (eds.) Water deficits: plant responses from cell to community. Oxford, Scientific Publisher. p. 129-145.

Sleen P, Groenendijk P, Vlam M, *et al*. 2015. No growth stimulation of tropical trees by 150 years of CO_2 fertilization but water-use efficiency increased. Nature Geoscience 8: 24-28.

Soliveres S, Maestre FT, Ulrich W, *et al*. 2015. Intransitive competition is widespread in plant communities and maintains their species richness. Ecology Letters 18: 790-798.

Souza JP, Melo NM, Pereira EG, Halfeld AD, Gomes IN, Prado CHB. 2016. Responses of woody Cerrado species to rising atmospheric CO_2 concentration and water stress: gains and losses. Functional Plant Biology 43: 1183-1193.

Temme AA, Liu JC, Cornwell WK, Cornelissen JH, Aerts R. 2015. Winners always win: growth of a wide range of plant species from low to future high CO_2. Ecology and Evolution 5: 4949-4961.

Uddling J, Teclaw RM, Kubiske ME, Pregitzer KS, Ellsworth DS. 2008. Sap flux in pure aspen and mixed aspen–birch forests exposed to elevated concentrations of carbon dioxide and ozone. Tree Physiology 28: 1231-1243.

Walker BH, Noy-Meir I. 1982. Aspects of the stability and resilience of savanna ecosystems. In: Huntley BJ, Walker BH. (eds.) Ecology of tropical savannas. Ecological studies (analysis and synthesis). Berlin/ Heidelberg, Springer. p. 556-590.

Wullschleger SD, Norby RJ. 2001. Sap velocity and canopy transpiration in a sweetgum stand exposed to free-air CO_2 enrichment (FACE). New Phytologist 150: 489-498.