Elevated atmospheric CO$_2$ concentration improves water use efficiency and growth of a widespread Cerrado tree species even under soil water deficit

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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 ($g_s$), 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
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 CO₂ concentration ([CO₂]) on stem sap flow in woody species and the consequences of water economy in the plants in Cerrado physiognomies. Shifts in CO₂ 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 [CO₂] 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 [CO₂] 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 [CO₂] 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 [CO₂] 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 [CO₂] 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 [CO₂] 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 CO₂ 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 [CO₂]. We also investigated the influence of soil water deficit in plants growing under distinct [CO₂]. Owing to the rising [CO₂] in the atmosphere, we expected decreased stem sap flow and gs in _L. pacari_ plants. Hence, plants grown under elevated [CO₂] were expected to become more efficient in water use, even with soil water deficit. Thus, the increase in [CO₂] 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 arbooreal 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 [CO₂] were as follows: two open-top chambers (OTCs) with 40 plants under ambient [CO₂] (430 ppm), and two OTCs with 40 plants under high [CO₂] (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 CO₂, the plants were divided into four treatments: 20 plants under ambient [CO₂], watered daily; 20 plants under ambient [CO₂], with cyclical suspension of watering; 20 plants under high [CO₂], watered daily; and 20 plants under high [CO₂], 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 [CO₂] for 60, 155, 245, 335, 425, and 515 days. The total chlorophyll was determined using clorofiLOG
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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

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.

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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 \([\text{CO}_2]\) 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 \([\text{CO}_2]\) than under ambient \([\text{CO}_2]\) during the experimental period, even under soil water deficit, being more evident at 515 DBE (\(p < 0.05\), interactions between \(\text{CO}_2\) and DBE, and between \(\text{CO}_2\), water, and DBE; Fig. 1). For the first 245 DBE, plants under ambient \([\text{CO}_2]\) showed higher \(g_s\) and \(E\) than plants under high \([\text{CO}_2]\) did (except for \(E\) values at 155 DBE, \(p < 0.05\), interactions between DBE and \(\text{CO}_2\); Fig. 1). Importantly, at 515 DBE, high values of \(g_s\) were found in plants under elevated \([\text{CO}_2]\) and soil water deficit (\(p < 0.05\), interactions between \(\text{CO}_2\), water, and DBE; Fig. 1).

Plants under elevated \([\text{CO}_2]\) showed higher WUE than that of plants under ambient \([\text{CO}_2]\) from 60 to 245 DBE. However, after 335 DBE, there was an inversion, and plants under ambient \([\text{CO}_2]\) showed higher WUE than that of plants under elevated \([\text{CO}_2]\) (except at 425 DBE, \(p < 0.05\), interactions between \(\text{CO}_2\) and DBE; Fig. 1).

Only after soil water deficit application, plants grown under high \([\text{CO}_2]\) showed high total chlorophyll content (\(p < 0.05\), simple effect of \(\text{CO}_2\) or water; Fig. 1). The \(\Psi_{\text{leaf}}\) values were not influenced by \([\text{CO}_2]\). After soil water deficit, plants irrigated daily showed less negative values of \(\Psi_{\text{leaf}}\) 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 \([\text{CO}_2]\) and daily irrigation showed higher (\(p < 0.05\)) hourly sap flow, mainly at noon (double interaction between \(\text{CO}_2\) and water, \(p < 0.05\); Fig. 2), than plants under elevated \([\text{CO}_2]\) did (double interaction between \(\text{CO}_2\) and water, \(p < 0.05\); Fig. 2). Regardless of \([\text{CO}_2]\), 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 \([\text{CO}_2]\) and daily irrigation than that of the other treatments (double interaction between \(\text{CO}_2\) and water, \(p < 0.05\); Fig. 2). Thus, *L. pacari* plants grown under elevated \([\text{CO}_2]\) 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 \([\text{CO}_2]\) 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 \([\text{CO}_2]\) (430 ppm, watered daily and cyclical suspension of watering) or high \([\text{CO}_2]\) (700 ppm, watered daily and cyclical suspension of watering). Bold values indicate \(p < 0.05\).

| \([\text{CO}_2]\)     | Water                  | \(r\) values          |
|----------------------|------------------------|------------------------|
|                      | \(\text{VPD} × \text{SFV}\) | \(\text{VPD} × \text{HSF}\) |
| 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 \([\text{CO}_2]\) showed a larger active xylem area than that of plants under ambient \([\text{CO}_2}\) (main effect of \(\text{CO}_2\), \(p < 0.05\); Tab. 2).

**Table 2.** Active xylem area (cm\(^2\)) in plants grown under ambient \([\text{CO}_2]\) (430 ppm, watered daily and cyclical suspension of watering) or high \([\text{CO}_2]\) (700 ppm, watered daily and cyclical suspension of watering). Asterisks indicate differences between \(\text{CO}_2\) treatments (\(p < 0.05\)).

| \([\text{CO}_2]\)     | 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 \(\text{CO}_2\) treatments until 245 DBE (Fig. 3). After soil water deficit application, plants under elevated \([\text{CO}_2]\), regardless of water treatment, showed higher leaf area values than those of plants under ambient \([\text{CO}_2}\) (\(p < 0.05\), interactions between \(\text{CO}_2\) 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 \([\text{CO}_2]\), mainly after soil water deficit application (interactions between...
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**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 ▼). $P_n = \text{maximum net photosynthesis}$ (A); $g_s = \text{stomatal conductance}$ (B); $E = \text{leaf transpiration rate}$ (C); WUE = water-use efficiency (D); $\Psi_{\text{leaf}} = \text{leaf water potential}$ (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.
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**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$_2$] (Fig. 4). 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).

**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
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Figure 5. Vegetative morphometry in \textit{L. pacari} plants grown under ambient [CO\textsubscript{2}] (○) or high [CO\textsubscript{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\textsubscript{2} or periods of measurement: *, main effect; **, double interaction. DBE, days after beginning the experiment.

deficit (Schulze 1993). This was more evident in \textit{L. pacari} plants grown under elevated [CO\textsubscript{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 \textit{et al.} 2015). However, in our study, the interaction between elevated [CO\textsubscript{2}] and soil water stress appeared to have made \textit{L. pacari} plants more dependent on VPD for leaf gas exchange.

Plant species grown under elevated [CO\textsubscript{2}] demonstrate improved WUE (Leakey \textit{et al.} 2009; Keenan \textit{et al.} 2013). Enhanced WUE is crucial because it diminishes water stress and increases the growing season (Sleen \textit{et al.} 2015) for plant species. According to Sleen \textit{et al.} (2015), increased WUE occurs at a pan-tropical scale, but this high WUE is not accompanied by growth stimulation. Battipaglia \textit{et al.} (2013) found that five species under high [CO\textsubscript{2}] distributed in three FACE experiments in Italy and the United States of America showed a 70\% increase in WUE. Several published papers (Cech \textit{et al.} 2003; Wullschleger & Norby 2001; Leuzinger & Korner 2007; Sleen \textit{et al.} 2015) have reported improvements in
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.

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