



Elevated CO₂ concentration improves functional traits and early growth of the widespread species *Enterolobium contortisiliquum* (Vell.) Morong

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ABSTRACT. *Enterolobium contortisiliquum* is a neotropical widespread species that occurs in native Atlantic Forest, Cerrado and Caatinga vegetation in Brazil. Outside Brazil, it occurs in Argentina, Bolivia, Paraguay, and Uruguay. The objective of this study was to investigate the influence of high carbon dioxide concentration [CO₂] on leaf gas exchange, chlorophyll content, chlorophyll *a* fluorescence, growth, and biomass allocation among roots, stems, and leaves in young plants of *E. contortisiliquum*. After germination, twenty days old individuals of *E. contortisiliquum* were grown in open-top chambers (OTC) at two different [CO₂] (400 ppm and 700 ppm). Physiological measurements were made when the plants had been grown in OTC for 170, 200, 230, and 260 days. The higher [CO₂] favored the early growth of *E. contortisiliquum* by increasing maximum net photosynthesis (P_N) and water use efficiency. Under high [CO₂], root dry mass increased in *E. contortisiliquum*, which might be a trait that will benefit this species by enhancing water uptake, particularly in seasonally dry environments.

Keywords: biomass partitioning; chlorophyll *a*; global climate change; maximum net photosynthesis; total leaf area.

Elevada concentração de CO₂ melhora as características funcionais e o crescimento inicial da espécie generalista *Enterolobium contortisiliquum* (Vell.) Morong

RESUMO. *Enterolobium contortisiliquum* é uma espécie neotropical generalista, encontrada na vegetação nativa da Mata Atlântica do Brasil, no Cerrado e na Caatinga. Fora do país, também pode ser encontrada em países como Argentina, Bolívia, Paraguai e Uruguai. O objetivo deste estudo foi investigar a influência da alta concentração de dióxido de carbono [CO₂] nas trocas gasosas foliares, no teor de clorofila, na fluorescência da clorofila *a*, no crescimento e alocação de biomassa entre raízes, caules e folhas em plantas jovens de *E. contortisiliquum*. Após a germinação, indivíduos de *E. contortisiliquum* de 20 dias de idade foram cultivados em câmaras de topo aberto (CTA), em duas diferentes [CO₂] (400 ppm e 700 ppm). As medições fisiológicas foram realizadas no momento do cultivo das plantas em CTA por 170, 200, 230 e 260 dias. A alta [CO₂] favoreceu o crescimento inicial de *E. contortisiliquum* aumentando a fotossíntese líquida máxima (P_N) e a eficiência do uso da água. Sob alta [CO₂], a massa seca da raiz aumentou em *E. contortisiliquum*, o que pode ser uma característica que beneficiará esta espécie, aumentando a absorção de água, particularmente em ambientes sazonalmente secos.

Palavras-chave: partição de biomassa; clorofila *a*; mudança climática global; fotossíntese líquida máxima; área foliar total.

Introduction

Climate changes due to human activities are associated with the increased emission of greenhouse gas (GHG), fires, deforestation, the formation of urban heat islands, and changes in land use (McNulty & Boggs, 2010; Cornelissen, 2011). Hence, global climate change today is a primary global concern (Buckeridge, Mortari, & Machado, 2007; Araújo, Dos Santos Oliveira, & Moura, 2015). CO₂ is considered the main GHG responsible for the change in the chemical

composition of the atmosphere, owing to the amount released by human activities (Solomon et al., 2007; Bunce, 2014).

Plants with high phenotypic plasticity respond to a wider range of environmental conditions and may invade new habitats (Geng et al., 2006; Baythavong, 2011). If any species have a great ability to spread, it may potentially leave areas that have become uninhabitable and colonize surrounding suitable areas (Vale, Alves, & Lorini, 2009; Clavel, Julliard, & Devictor, 2011; Franks, Weber, & Aitken, 2013).

Global climatic and geological disturbances often produce low-diversity biota dominated by a few widespread, broadly adapted species (Erwin, 1998; Isbell et al., 2015). Owing to environmental disturbances, in a global change, widespread species often replace specialist ones, resulting in a spatial biodiversity loss (McKinney & Lockwood, 1999; Clavel et al., 2011). This implies that ecological homogenization might also occur because many ecological specialists are replaced by common widespread and broadly adapted ecological generalists. Widespread species may adjust to climate change over time or have enough phenotypic plasticity to persist in the changed ecosystems through acclimatization. Similar responses were found by Aidar et al. (2002) studying *Hymenaea courbaril* L., a Brazilian species with wide distribution, that under elevated [CO₂], there were increases in leaf photosynthesis and water use efficiency (WUE) and no downregulation of photosynthesis. Under climatic changes, widespread species might establish faster in its surrounding environment (Aidar et al., 2002).

Enterolobium contortisiliquum (Vell.) Morong is considered a widespread species (Lima, Oliveira, & Rodrigues, 2009; Araújo & Paiva Sobrinho, 2011) and invasive with the potential to disrupt native plant communities (Cochrane, 1999). It occurs in native forest formations, in the Atlantic Forest domain (Lima et al., 2009; BFG, 2015) and in the Cerrado and Caatinga vegetation in northeastern Brazil (Souza, Melo, Halfeld, & Reis, 2017). At the southern limit of its distribution in Brazil, this species is found in seasonal forests, open fields, and shrub restinga habitat (Amorim, De Souza, Oliveira, Camacho, & De Melo, 2016). *Enterolobium contortisiliquum* also is found in Argentina, Bolivia, Paraguay, and Uruguay (Prado & Gibbs, 1993) and could expand its geographical distribution upon climatic changes, contributing to the fewer and simpler ecosystems in our newly homogenized biosphere.

The objective of this study was to investigate the responses of leaf gas exchange, chlorophyll *a* fluorescence, chlorophyll content index, growth, and pattern of biomass allocation among roots, stems, and leaves in *E. contortisiliquum* young plants grown in elevated and ambient [CO₂]. We hypothesized that in *E. contortisiliquum*, owing to its wide niche range, increased [CO₂] would stimulate leaf photosynthesis and WUE, and plants would adjust their morphology and biomass accumulation to acclimatize to the higher CO₂ environment. These ecophysiological adjustments would benefit *E. contortisiliquum* establishment even under predicted changes to climate conditions.

Material and methods

Open-top chambers, growth conditions and plant material

The experiment was conducted at the *Universidade Federal of Viçosa* (UFV), *Campus Florestal* (CAF), Minas Gerais (MG), Brazil (19° 52' 20" S and 44° 25' 12" W), using open-top chambers (OTC) as described by Silva, Ferreira, Pereira, Costa, & Miranda (2012). Plant exposure to CO₂ was done during the daytime period (06:00 to 18:00 hours). CO₂ concentration inside chambers was monitored daily with a CO₂ measuring device (Testo 535, Testo SE & Co. KGaA, Brazil).

Seeds of *E. contortisiliquum* were collected from trees in a forest fragment in Florestal, Minas Gerais State, Brazil. The seeds were washed with distilled water, treated with 2% sodium hypochlorite for 5 min. (Malavasi & Malavasi, 2004; Alexandre, Gonçalves, Rocha, Arruda, & Lemes, 2009), and washed again. To break physical dormancy (Malavasi & Malavasi, 2004; Alexandre et al., 2009), seeds were then mechanically scarified with sandpaper until the cotyledon was exposed. Scarified seeds were germinated on moist filter paper in a germination chamber (SL.225, SOLAB, Brazil) with a daily temperature cycle of 20-30°C and a photoperiod of 12 hours for 30 days (Malavasi & Malavasi, 2004). The seed germination occurred after 20 days from the beginning of the experiment. After 30 days, germinated seeds were planted in plastic pots with a capacity of 10 L. After an additional 10 days, young plants (20 days old) were transferred directly to the OTC with different [CO₂]. Twenty plants were grown under ambient [CO₂] (400 ppm) and 20 plants were grown under high [CO₂] (700 ppm). The plants were watered daily to field capacity of the soil. Seeds were sowed in an impoverished soil typical of Cerrado areas (Table 1). The soil analyses were performed at the Laboratory of Agricultural Chemistry at *Instituto Mineiro de Agropecuária* (IMA).

Table 1 Chemical characteristics of the soil utilized in the open-top chambers. Aluminum=Al³⁺ (cmol_cdm⁻³), calcium=Ca²⁺ (cmol_cdm⁻³), magnesium=Mg²⁺ (cmol_cdm⁻³), phosphorus=P (mg dm⁻³), potassium=K (mg dm⁻³) sum of bases=SB (cmol_cdm⁻³), cation exchange capacity=CEC (cmol_cdm⁻³), index of aluminum saturation=m, and index of base saturation=V.

Al ³⁺	P	K	Ca ²⁺	Mg ²⁺	H ⁺ Al	pH	SB	CEC	m	V
1.83	0.8	8	0.33	0.07	3.54	6.1	0.42	3.96	81.2%	10.68%

Leaf gas exchange and chlorophyll content index (CCI)

Leaf gas exchange and CCI were determined in five *E. contortisiliquum* plants from each [CO₂],

in two fully mature and healthy leaves. Measurements were made when plants were 180, 210, 240 and 270 days old and had been grown in OTC for 170, 200, 230 and 260 days, respectively. These measurements were made between 08:00 and 10:00 hours. The leaf gas exchange parameters determined were as follows: maximum net photosynthesis (P_N , $\mu\text{mol m}^{-2} \text{s}^{-1}$), stomatal conductance (g_s , $\text{mol m}^{-2} \text{s}^{-1}$) and leaf transpiration rate (E , $\text{mmol m}^{-2} \text{s}^{-1}$). Water use efficiency (WUE) was calculated as P_N/E ($\mu\text{mol mmol}^{-1}$). The carboxylation efficiency of Rubisco was calculated as P_N/C_i ($\mu\text{mol m}^{-2} \text{s}^{-1}$). Leaf gas exchange was measured with an infrared gas analyzer (LCA-4, ADC BioScientific, Ltd., Hoddesdon, UK) set in open mode. Photosynthetic photon flux density (PPFD = $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$) was generated with the light source (PLU-002, ADC) attached to a narrow Parkinson leaf chamber (PLCN-4). The Peltier system (ADC) kept PLCN-4 between 25 and 27 °C. The leaf inside the PLCN-4 was kept at 40–50% relative humidity and an ambient $[\text{CO}_2]$ of 400 ppm. The CCI was measured with a chloroFILOG, (model CFL1030, FALKER, Porto Alegre, Rio Grande do Sul, Brazil), in the same leaves used for gas exchange evaluations.

Chlorophyll *a* fluorescence

Ten young plants from each $[\text{CO}_2]$ were used for chlorophyll *a* fluorescence measurement on the same days that leaf gas exchange and CCI were recorded. Fluorescence was measured with a portable Mini-PAM chlorophyll fluorometer (Heinz Walz, Effeltrich, Germany). One leaf per individual was clamped with a dark leaf clip in the central region of the leaf 30 minutes before the measurements were made. Fully mature and healthy leaves were selected. After 30 minutes of dark-acclimation, leaf tissues were exposed to a low pulse of red light to determine the initial fluorescence (F_0). A pulse of saturation light with duration of 0.8 s was used to determine the maximal fluorescence (F_m). The potential photochemical efficiency of photosystem II (F_v/F_m , Kitajima & Butler, 1975) was estimated from these data. After dark measurements, the leaf was then exposed to PPFD of $1200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ for 40 s to determine light-acclimated parameters. The following variables were determined: ΦPSII (effective quantum efficiency of photosystem II, Genty, Briantais, & Baker, 1989), NPQ (non-photochemical quenching,

Bilger & Björkman, 1990), and ETR (electron transport rate, $\mu\text{mol electrons m}^{-2} \text{s}^{-1}$, Melis, Spangfort, & Andersson, 1987).

Leaf area and leaf, stem, and root biomass

The leaf area was determined from all leaves of five individuals in each $[\text{CO}_2]$ (the same plants used for leaf gas exchange). The leaf area was measured nondestructively with a portable leaf area meter (LI-3000C, Li-Cor Inc., USA), on the same dates as leaf gas exchange measurements. A different set of five individuals from each $[\text{CO}_2]$ was destructively sampled when the plants were 330 days old (grown in OTC for 320 days) to determine root length (RL, cm), stem dry mass (SDM, g), leaf dry mass (LDM, g), root dry mass (RDM, g), and total dry mass (TDM, g). Root length was measured with a ruler. Stem dry mass was determined after all buds and leaves were removed to calculate leaf area and mass. The leaves, stems, and roots were dried in an oven with air circulation (TECNAL TE-394/3, Piracicaba, São Paulo, Brazil) at 60°C to a constant weight (Perez-Harguindeguy et al., 2013). The masses were measured with an analytical balance (SHIMADZU–Series BL–320H, Tokyo, Japan). The total dry weight was calculated from the sum of the leaf, stem, and root dry masses from the same individual.

Shoot-foilage relationships

The following biometric indices were calculated after all measurements were taken: leaf area ratio (LAR, total leaf area/total dry mass, $\text{cm}^2 \text{g}^{-1}$), specific leaf area (SLA, leaf area/leaf dry mass, $\text{cm}^2 \text{g}^{-1}$), and root/shoot ratio (RSR, root dry mass/shoot dry mass).

Photosynthetic nitrogen-use efficiency

The 10 individuals that were destructively sampled were also measured for leaf nitrogen (N) content (g kg^{-1}). Foliar N analyses were performed according to the methodology proposed by Malavolta, Vitti, and Oliveira (1997) and photosynthetic N-use efficiency (PNUE, P_N/N content, $\mu\text{mol} (\text{CO}_2) \text{mol}^{-1} (\text{N}) \text{s}^{-1}$) was calculated.

Vegetative morphometry

The leaf number, stem length (cm), and diameter (cm), were measured weekly in the entire experimental period from 20 individuals under ambient or high $[\text{CO}_2]$. Two leaves were followed from their emergence to determine their leaf expansion interval (LEI, days to full expansion), leaf expansion rate (LER, mature leaf area divided by LEI, $\text{cm}^2 \text{days}^{-1}$), and leaf life span (LLS, days). The

leaf life span was noted weekly from emergence until senescence (more than 90% yellowing) or leaf abscission.

Statistical analyses

The experiment followed a randomized block design with factorial arrangement (2×4) consisting of two $[\text{CO}_2]$ (400 ppm and 700 ppm) and four dates of measurements (170, 200, 230, and 260 days). Only leaf number, diameter and length of stem were measured until 320 days. We calculated the mean and standard error for all physiological, morphological, and morphometrical variables. An analysis of variance was used to test differences between CO_2 treatments and also its interaction with sampling dates. *A posteriori* Tukey's test was applied a 5% significance level. The program R was utilized to perform all statistical analyses (R Core Team, 2014).

Results

Leaf gas exchange and CCI

Enterolobium contortisiliquum plants grown under high $[\text{CO}_2]$ showed higher ($p < 0.05$) P_N and WUE (Figure 1A, D) than plants under ambient $[\text{CO}_2]$ from 170 days after beginning the experiment (DBE) onward (interaction between CO_2 and plant age). The E and g_s did not show differences ($p > 0.05$) between plants under ambient and high $[\text{CO}_2]$ (Figure 1B, C); however, there is an increment along time. There were no differences ($p > 0.05$) in chlorophyll values between plants under ambient and elevated $[\text{CO}_2]$. There was no difference ($p > 0.05$) in PNUE between plants grown under ambient $[\text{CO}_2]$ ($37.55 \mu\text{mol} (\text{CO}_2) \text{mol}^{-1} (\text{N}) \text{s}^{-1}$) and high $[\text{CO}_2]$ ($56.67 \mu\text{mol} (\text{CO}_2) \text{mol}^{-1} (\text{N}) \text{s}^{-1}$). *Enterolobium contortisiliquum* plants grown under elevated $[\text{CO}_2]$ showed higher ($p < 0.05$) carboxylation efficiency of Rubisco along whole experimental period than plants under ambient $[\text{CO}_2]$ (interaction between CO_2 and plant age, Table 2).

Chlorophyll a fluorescence

Only at 200 DBE, *E. contortisiliquum* plants grown under high $[\text{CO}_2]$ showed higher ($p < 0.05$) F_v/F_m values (Figure 2A) than plants under ambient $[\text{CO}_2]$ (interaction between CO_2 and plant age). Over the whole experiment, plants grown under high $[\text{CO}_2]$ showed significantly lower ($p < 0.05$) values of NPQ (Figure 2C) than plants under ambient $[\text{CO}_2]$ (interaction between CO_2 and plant age). However, ETR and ΦPSII did not show differences ($p > 0.05$) between CO_2 treatments along experiment period (Figure 2B, D).

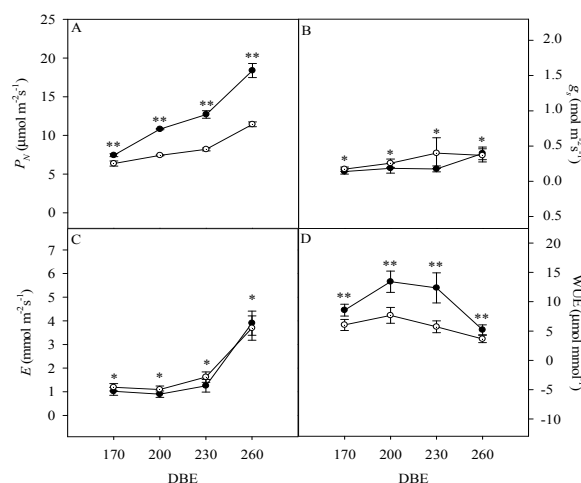


Figure 1. Leaf gas exchange traits in *E. contortisiliquum* plants grown under ambient (\circ) or high $[\text{CO}_2]$ (\bullet). P_N = maximum net photosynthesis (A); g_s = stomatal conductance (B); E = leaf transpiration rate (C); WUE = water use efficiency (D); DBE = days after beginning the experiment. Symbols represent averages ($n = 5$) and bars indicate the standard error. Differences between CO_2 treatments (ambient and high) or among measurement periods are indicated: *, main effect; **, double interaction.

Table 2 Carboxylation efficiency of Rubisco. Values represent the mean and standard error. Differences between CO_2 treatments (ambient and high) or among measurement periods are indicated: *, main effect; **, double interaction.

DBE	$[\text{CO}_2]$	P_N/Ci
170	400 ppm	0.010 ± 0.001 **
	700 ppm	0.014 ± 0.002 **
200	400 ppm	0.013 ± 0.001 **
	700 ppm	0.023 ± 0.002 **
230	400 ppm	0.015 ± 0.001 **
	700 ppm	0.033 ± 0.003 **
260	400 ppm	0.024 ± 0.003 **
	700 ppm	0.041 ± 0.003 **

Vegetative characteristics

During the entire experiment, *E. contortisiliquum* plants under high $[\text{CO}_2]$ had a greater ($p < 0.05$) leaf area (Figure 3A) than plants under ambient $[\text{CO}_2]$ (simple effect of CO_2).

Enterolobium contortisiliquum individuals grown under high $[\text{CO}_2]$ had greater ($p < 0.05$) RDM (Table 3) than plants under ambient $[\text{CO}_2]$ (interaction between CO_2 and plant age).

However, *E. contortisiliquum* plants both grown under ambient and high $[\text{CO}_2]$ did not differ ($p > 0.05$) significantly in biometrical index and leaf development variables (LAR, SLA, RSR, LEI, LER, and LLS (Table 4).

Plants grown under high $[\text{CO}_2]$ had significantly more leaves ($p < 0.05$) (from 200 DBE onward), and longer and thicker stems (from 170 DBE onward), than plants under ambient $[\text{CO}_2]$ (interaction between CO_2 and plant age, Figure 4A, B e C).

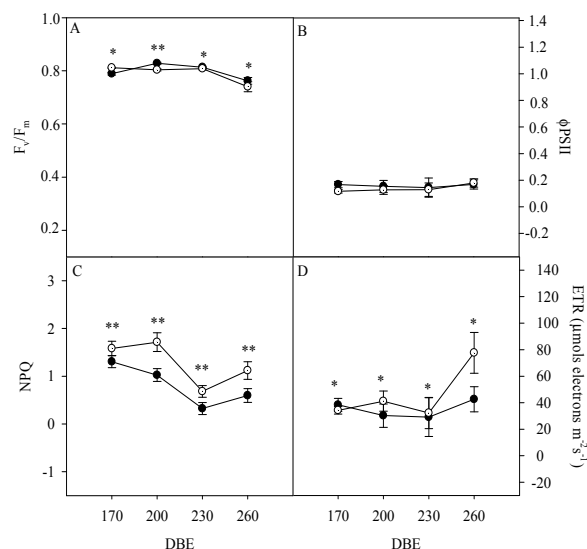


Figure 2. Chlorophyll *a* fluorescence traits in *E. contortisiliquum* plants grown under ambient (○) or high $[CO_2]$ (●). F_v/F_m = potential photochemical efficiency of photosystem II (A); Φ_{PSII} = effective quantum efficiency of photosystem II (B); NPQ = non-photochemical quenching (C); ETR = electron transport rate (D); DBE = days after beginning the experiment. Symbols represent averages ($n = 5$) and bars indicate standard error. Differences between CO_2 treatments (ambient and high) or among measurement periods are indicated: *, main effect; **, double interaction.

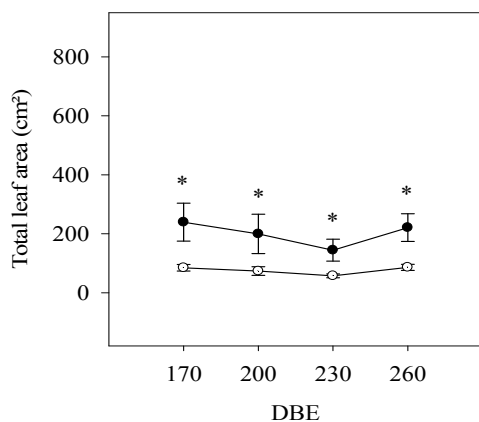


Figure 3. Total leaf area in *E. contortisiliquum* plants grown under ambient (○) or high $[CO_2]$ (●). DBE = days after beginning the experiment. Symbols represent averages ($n = 5$) and bars indicate standard error. Differences between CO_2 treatments (ambient and high) or among measurement periods are indicated: *, main effect; **, double interaction.

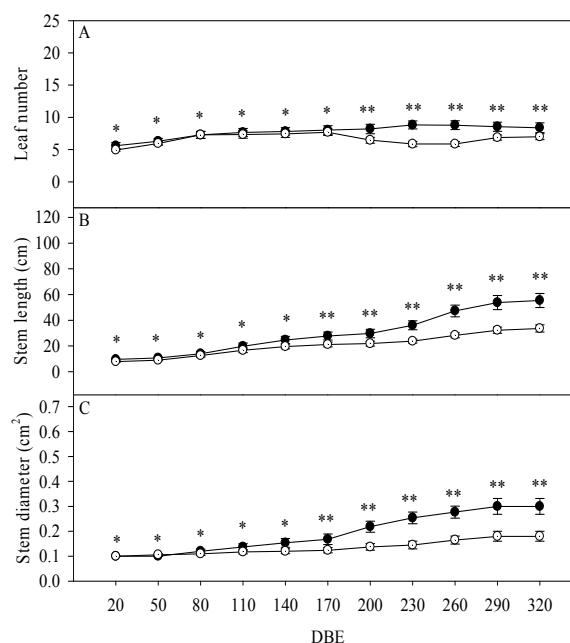


Figure 4. Morphometrical traits in *E. contortisiliquum* plants grown under ambient (○) or high $[CO_2]$ (●). Leaf number (A); stem length (B); stem diameter (C); DBE = days after beginning the experiment. Symbols represent averages ($n = 5$) and bars indicate standard error. Differences between CO_2 treatments (ambient and high) or among measurement periods are indicated: *, main effect; **, double interaction.

Discussion

The high P_N in *E. contortisiliquum* plants grown under high $[CO_2]$ may be associated with higher carboxylation rate of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) due to increased carbon availability for the leaves (Ceulemans, Janssens, & Jach, 1999; Polley, Johnson, & Derner, 2003; Ainsworth & Long 2005), as found in this study. Once CO_2 and O_2 compete for the same active site in Rubisco, with increased carboxylation rate, the oxygenation process decrease (Drake et al., 1999; Ainsworth & Rogers, 2007; Ramalho et al., 2013).

Stomatal apparatus in most plants tend to narrow with increasing $[CO_2]$ (Ainsworth & Rogers, 2007). However, not all plants respond to the increase in $[CO_2]$ with a decline in stomatal conductance. Curtis (1996) found no evidence of a significant decrease in g_s in 41 woody species in response to rising $[CO_2]$.

Table 3 Biomass partitioning in *E. contortisiliquum* plants grown under ambient or high $[CO_2]$. RDM = root dry mass; SDM = stem dry mass; LDM = leaf dry mass; TDM = total dry mass; RL = root length. Values represent the mean and standard error. Significant differences between CO_2 treatments are indicated: *, $p < 0.05$.

$[CO_2]$	RDM (g)	SDM (g)	LDM (g)	TDM (g)	RL (cm)
400 ppm	18.53 \pm 3.72 *	2.45 \pm 1.41	2.20 \pm 1.24	23.19 \pm 6.18	39.8 \pm 2.08
700 ppm	32.01 \pm 5.19 *	6.32 \pm 1.18	3.26 \pm 0.36	41.60 \pm 6.64	33.8 \pm 2.41

Table 4 Biometrical indices and leaf development in *E. contortisiliquum* plants grown under ambient or high [CO₂]. LAR = leaf area ratio; SLA = specific leaf area; RSR = root/shoot ratio; LEI = leaf expansion interval; LER = leaf expansion rate; LLS = leaf life span. Values represent the mean and standard error. No significant differences were found between CO₂ treatments ($p < 0.05$).

[CO ₂]	LAR (cm ² g ⁻¹)	SLA (cm ² g ⁻¹)	RSR (g g ⁻¹)	LEI (days)	LER (cm ² days ⁻¹)	LLS (days)
400 ppm	9.99 ± 2.40	178.33 ± 40.24	7.35 ± 1.45	32.84 ± 3.09	1.76 ± 0.42	230.34 ± 20.28
700 ppm	13.41 ± 2.30	154.04 ± 16.65	3.31 ± 0.11	30.25 ± 1.45	2.13 ± 0.74	208.42 ± 13.45

Even with no changes in E values, the higher P_N contributed to increased WUE in the whole experimental period in plants under high [CO₂]. The high WUE in *E. contortisiliquum* plants grown in elevated [CO₂] is a typical response in trees (Murray, 1995). Moreover, plants grown in soils with low N availability can increase WUE (Illenseer & Paulilo, 2002) and decrease PNUE (Cabrera-Bosquet, Molero, Bort, Nogués, & Arous, 2007). However, *E. contortisiliquum* plants under high [CO₂] maintained constant PNUE at high WUE, even without changing their g_s , decreasing their water loss as CO₂ was absorbed.

Some plant species respond to elevated [CO₂] with an increase in Fv/Fm (Li, Liu, Wu, & Zou, 2008). The punctual increase in Fv/Fm in *E. contortisiliquum* plants grown under high [CO₂] probably reflected greater light use efficiency (Ribeiro, Souza, Oliveira & Machado, 2005). The increase in light use efficiency associated with higher chlorophyll content is important for seedlings under climatic changes since it is expected a beneficial effect on the trees with an increase in [CO₂] (Bond, Midgley & Woodward, 2003; Melo, Rosa, Pereira, & Souza, 2018) and plant communities may be denser. The increased Fv/Fm values in *E. contortisiliquum* plants under elevated [CO₂] could reflect improved PSII repair capability (Allakhverdiev & Murata, 2004). In addition, the elevated [CO₂] may improve electron transport chain, since under ambient [CO₂] at 25°C the electron transport chain is down-regulated by around 40% (Ott, Clarke, Birks, & Johnson, 1999). The electron transport chain regulates the flow of electrons from PSII to PSI under high [CO₂] condition; thus *E. contortisiliquum* plants growing under high [CO₂] would present higher light use efficiency than plants under ambient [CO₂].

So, with less irradiance reached to beneath strata in plant communities, the ability of plants to convert light in the photochemical process will help the plants to use light to perform photosynthesis. To corroborate with this statement *E. contortisiliquum* plants grown under high [CO₂] produced larger leaves than plants under ambient [CO₂]. Larger leaves improve the surface area to intercept light but may shade leaves of small individuals or basal leaves on the same shoots (Ward & Strain, 1999). In addition, the high non-photochemical dissipation of

excess energy (NPQ) in plants under ambient [CO₂] could indicate gradual inactivation of photoprotective mechanisms common under light stress, as cyclic PSII electron transport (Allakhverdiev, Yruela, Picorel, & Klimov, 1997), which does not occur in plants under elevated [CO₂].

The higher root dry mass observed in plants grown under elevated [CO₂] when compared with those grown at ambient [CO₂], indicates the plasticity in biomass partitioning among plant organs. In general, high biomass allocation to roots in plants grown under high [CO₂] could indicate increased fine roots production, which are responsible for water and nutrients uptake from the soil (Norby & O'Neill 1991; Liberloo et al., 2006). According to Souza et al. (2016), the high root biomass in cerrado woody plants grown under elevated [CO₂] would make these plants more resistant to dry periods and more efficient for water uptake from the soil. In dry seasonally environments, the increase in the drought resistance may come at the cost of aerial growth, which may a competitive disadvantage for light interception. However, *E. contortisiliquum* grown under high [CO₂] did not show decreased aerial growth. In this way, *E. contortisiliquum* plants under elevated [CO₂] will be benefit in competition below as well as above ground. According to Ronquim, Prado, and Souza (2009) and Khurana and Singh (2001), a higher biomass allocation to the plant organs (such as the root system) that are responsible for acquiring limiting resources indicates that some factor, as [CO₂], is benefitting such growth. The ability to allocate more resources to the root system may be a strategy that explains the wide distribution of tree species in natural ecosystems (Ronquim et al., 2009), and our results indicated that it may be a factor that will benefit in the broad distribution of *E. contortisiliquum*. The high [CO₂] favored production of new leaves and thus an increase in leaf number in *E. contortisiliquum* plants. Besides the increase in leaf number can reflect greater retention of older leaves (a lower leaf drop rate) and not an increase in a new cohort of leaves (Melo et al., 2018). However, in our experiment, LLS was not shifted in *E. contortisiliquum*, so the greater leaf number was due to increased leaf production.

Conclusion

The long-term exposition of young *E. contortisiliquum* plants to high [CO₂] did not result in the increases in stem dry matter. However, root dry mass in *E. contortisiliquum* increases, which might be a trait that will benefit this species by enhancing water uptake, particularly in seasonally dry environments. Our study improved the understanding of how a widespread species adjust the ecophysiology due to climatic changes. Also, future studies need to be accomplished for the comparison between specialist and widespread species responses under rising [CO₂]. This might contribute to issues of biotic homogenization and loss of functional diversity in ecosystems.

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