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Biomass allocation in *Ceiba pentandra* (Malvaceae) under water stress and high CO_2 concentration

Alocação de biomassa em *Ceiba pentandra* (Malvaceae) sob estresse hídrico e alta concentração de CO_2

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Abstract

Climate models predict prolonged droughts in parts of Amazonia; however, it is unclear how trees of the region will respond to these changes. The aim of this study was to evaluate how elevated CO_2 and water stress affect biomass allocation to plant organs and photosynthesis of *Ceiba pentandra* at the juvenile stage. The treatments were two CO_2 levels (400 and 800 ppm) and two water regimes (soil at 50 and 100% field capacity) applied for 138 days. We measured the amount of biomass allocated to organs of the plant, photosynthesis and plant water use efficiency during the experimental period. Light saturated photosynthesis expressed on a leaf mass basis decreased 34.6% at elevated CO_2 and 24.7% under water stress. Total biomass gain per plant was unresponsive to elevated CO_2, but declined 43% under water stress ($p < 0.001$). However, plant water use efficiency increased 50% at elevated CO_2 and also under water stress ($p < 0.001$), but in contrast, the shoot/root ratio was unaltered by water stress and elevated CO_2. These results improve our understanding of the effect of elevated CO_2 and water stress in *Ceiba pentandra*, a multipurpose tree with wide occurrence in the Amazonian region.

Keywords: Photosynthesis acclimation; Shoot/root ratio; Water stress; Leaf mass to area ratio.

Resumo

Modelos climáticos preveem secas prolongadas em partes da Amazônia; no entanto, não está claro como as árvores da região responderão a essas mudanças. O objetivo deste estudo foi avaliar como o CO_2 elevado e o estresse hídrico afetam a alocação de biomassa aos órgãos da planta e a fotossíntese de *Ceiba pentandra* na fase juvenil. Os tratamentos foram dois níveis de CO_2 (400 e 800 ppm) e dois regimes hídricos (solo a 50 e 100% da capacidade de campo) aplicados por 138 dias. Na pesquisa foram mensuradas a quantidade de biomassa alocada aos órgãos da planta, fotossíntese e eficiência de uso da água das plantas durante o período experimental. A fotossíntese saturada de luz expressa com base na massa foliar diminuiu 34,6% em CO_2 elevado e 24,7% sob estresse hídrico. O ganho total de biomassa por planta não respondeu a CO_2 elevado, mas diminuiu 43% sob estresse hídrico ($p < 0{,}001$). A eficiência do uso da água da planta aumentou 50% em CO_2 elevado e sob estresse hídrico ($p < 0{,}001$), mas em contraste, a razão parte aérea/raiz não foi alterada por estresse hídrico e CO_2 elevado. Esses resultados melhoram nossa compreensão do efeito do CO_2 elevado e do estresse hídrico de *Ceiba pentandra*, uma árvore de uso múltiplo e de ampla ocorrência na região amazônica.

Palavras-chave: Aclimatação da fotossíntese; Razão parte aérea/raiz; Estresse hídrico; Razão massa foliar/área.

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INTRODUCTION

The Amazonian rainforest is of paramount importance at the global scale not only by the large area it covers and amount of carbon stored in its vegetation (Saatchi et al., 2007), but also for recycling large quantities of water and carbon and by its remarkable biodiversity. Because of the effect of deforestation, there are large areas available for rehabilitation in the Amazon region, and in this respect, it has been estimated that about 120,000 km^2 could be restored or reforested by 2030 (Brasil, 2020). It is widely accepted that temperature and atmospheric CO_2 concentration – [CO_2] will continue to increase in coming years, and that such changes may have an effect on the pattern of rainfall distribution over the Amazon region (Marengo et al., 2018). This is important, as it has been shown that microclimatic variability can affect the performance of Amazonian trees (Camargo & Marenco, 2022; Dias et al., 2022; Souza & Marenco, 2022). Regarding the effect of plant exposure to elevated CO_2 concentration (hereafter referred to as elevated CO_2), it has been found an increase in photosynthetic rate, a decline in stomatal conductance and an increase in water use efficiency at elevated CO_2 (Ainsworth & Long, 2005; Wang & Wang, 2021). Furthermore, it has been reported that after prolonged exposure to elevated CO_2 [weeks to months, Makino & Mae (1999)] photosynthesis can acclimate (down-regulation of photosynthesis) to elevated CO_2 (Drake et al., 1997; Kitao et al., 2007). Besides its effect on photosynthesis, subjecting the plants to high CO_2 concentration can also affect biomass production (Makino & Mae, 1999; Dieleman et al., 2012). However, the concurrent effect of an increase in CO_2 concentration and water stress are still under investigation. This is particularly important because it seems that droughts are becoming more prolonged in parts of the Amazon region (Marengo et al., 2018).

The majority of growth parameters have been reported to respond to elevated CO_2. For instance, relative growth rate and net assimilation rate can increase under high CO_2, while specific leaf area (inverse of leaf mass to area ratio) may decrease (Poorter & Navas, 2003). Therefore, it could be expected that the leaf area of the whole plant would decrease when it is subjected to elevated CO_2 (Poorter & Nagel, 2000). It has been reported that the root/shoot ratio can remain either unaltered (e.g. *Pinus taeda, Gossypium hirsutum*) or increase (e.g. *Triticum aestivum, Brassica napus*) in plant grown at elevated CO_2 and limiting water supply (Stulen & Hertog, 1993). In fact, it seems that the climatic origin of a given species affects how biomass is allocated under elevated CO_2 and warmer temperatures. *Eucalyptus grandis* from a humid climate allocated more biomass to roots, whereas *Eucalyptus platyphylla* from savanna woodlands increased the biomass in the shoot (Apgaua et al., 2019).

Sumaúma (also known as kapok tree in English) is a multipurpose tree native to Central and South America and with great socio-economic potential. It is a fast growing species, adapted to low fertility soil, and a wide range of annual rainfall regimes – from 800 to 2500 mm (Neves et al., 2003; Hending et al., 2021), which makes this tree a promising candidate for reforestation projects (Román-Dañobeytia et al., 2015; Vargas-Simón et al., 2022). The sumaúma tree grows relatively fast in the juvenile stage, up to 22.5 mm $year^{-1}$ (Keefe et al., 2009) and has a large distribution over tropical regions worldwide. The wood of sumaúma is used in the timber industry (e.g. plywood manufacturing), while the fiber that covers the seeds is commonly used as fillers for pillows and cushions (Gómez-Maqueo & Gamboa-deBuen, 2022). Oil can be extracted from their seeds, and recently several medicinal properties have been associated with the sumaúma tree (Neves et al., 2003; Gómez-Maqueo & Gamboa-deBuen, 2022). Thus, the aim of this study was to evaluate how elevated CO_2 and water stress affect biomass allocation to plant organs and photosynthesis in *Ceiba pentandra* (L.) Gaertn (sumaúma).

MATERIAL AND METHODS

Plant material and greenhouse conditions

The experiment was carried out at the Instituto Nacional de Pesquisas da Amazônia - INPA (03°05'30" S, 59°59'35" W), Manaus, in a greenhouse and a growth chamber with automatic control of CO_2 concentration. For this experiment, we selected *Ceiba pentandra* (L.) Gaertn. (Malvaceae), which is called *sumaúma* in Portuguese.

Seeds of sumaúma were germinated in vermiculite and 15 days after emergence the plants were transferred to pots (21 cm diameter and 17 cm deep), containing 4.5 kg of forest soil, which was amended with 5 g of a slow-release fertilizer per kilogram of soil. The fertilizer composition was: N, 15%; P_2O_5, 9%; K_2O, 12%; Mg, 1%, and micronutrients. After transplanting the plants were kept for 90 days in the greenhouse at ambient temperature (~27°C) and ambient CO_2 concentration (~410 ppm) under well-watered conditions. Then the plants (53 cm height and 7.0 mm stem diameter) were randomly assigned into four groups: two CO_2 levels (400 ppm and 800 ppm) and two water regimes within each CO_2 level. The water regimes were: soil at 50% and 100% of field capacity, FC. Thus, the four treatments were: T_1: 400/100, T_2: 400/50, T_3: 800/100, and T_4: 800 ppm/50% FC. The plants grew under these conditions for 138 days (hereafter referred to as the experimental period). The plants at ambient CO_2 (400 ppm) were kept in the greenhouse, while those at elevated CO_2 (800 ppm) were transferred to the growth chamber (TPC-1, Winnipeg, Canada). In the greenhouse, mean photosynthetically active radiation (PAR) was 200 µmol m^{-2} s^{-1} (8.6 mol m^{-2} day^{-1}), relative humidity (RH) was 70–80%, mean temperature of 27.5°C (ranging from 26°C at night to 29°C at midday), and day/night ambient CO_2 concentration of 400/420 ppm. Thus, we used the daytime CO_2 concentration measured in the greenhouse to set the growth chamber [CO_2] at twice this value.

Growth chamber conditions

Following the PAR value recorded in the greenhouse, the light intensity in the growth chamber was set to keep a constant value of 200 µmol m^{-2} s^{-1} with a photoperiod of 12 hours (06:00–18:00), while the [CO_2] was kept constant at 800 ppm. The day/night temperature was set at 27/25 °C (average of 26 °C), and relative humidity was 80% (daytime) and 90% (nighttime).

Soil water content and water regimes

Before subjecting the plants to the water regimes, we gravimetrically determined the amount of water (volume) the soil could hold at field capacity (100% FC). Half of that value was used in the soil to be kept at 50% FC. During the experiment, every other day, the pots were weighed (7:00–8:00) to record the amount of water consumed by the plants. Then, we replenished the water loss by the plant to keep the soil water content at its target value (50% or 100% FC); for this step we took into account the small change in plant fresh weight due to growth over time. Evaporation from the soil surface was prevented by covering the pot with a plastic bag, which was tied at the base of the plant. To estimate the contribution of daily plant growth to total mass (pot, soil, water and plant fresh weight) an allometric equation was used [Y (g) = 3.838exp0.2861D, R^2= 0.95, n = 17, where D is diameter in mm). This equation was previously generated using another set of plants similar in size to those plants used in the treatments.

The plants were subjected to the treatments (T_1–T_4) for 138 days – the experimental period (March–August 2019). This period was sufficient for the plant to produce new leaves under the treatment conditions. At the end of the experimental period, we determined the dry mass of the stem, roots and leaves, number of leaves, leaf size, leaf mass to area ratio (LMA), whole plant water use efficiency (WUE_p), and gas exchange parameters. The new leaves produced during the experimental periods were used for gas exchange measurements and laboratory analyses.

Gas-exchange and nitrogen measurements

Gas-exchange data were measured between 08:00 and 14:00 on two fully expanded leaves per plant from the upper third with a portable infrared gas analyzer (Li-6400XT, Li-Cor, Lincoln, NE, USA). We measured light saturated photosynthesis per unit leaf mass ($A_{sat\text{-}mass}$) at the CO_2 concentration of treatments (400 ppm CO_2 for ambient conditions and 800 ppm for elevated CO_2). The PAR value at light saturation (1000 µmol m^{-2} s^{-1}) was determined after constructing a light-response curve (500, 250, 100, 50, 25, 12, 0, 500 1000 and 2000 µmol m^{-2} s^{-1}). We also measured light and CO_2 saturated photosynthesis (2000 ppm CO_2) per unit leaf mass ($A_{max\text{-}mass}$), and leaf nitrogen (N_{Leaf}) content. The N_{Leaf} was determined by the classical Kjeldahl method.

Biomass accumulation and leaf attributes

Plant biomass was estimated at the beginning (time = t_1) and gravimetrically measured at the end of the experimental period (time = t_2, 138 days). Plant diameter (*D*, at 6 cm from the base of the plant), plant height (*H*, from the base of the plant to apical bud), leaf and leaflet number, leaf size, total leaf area per plant, and plant water content (PWC, on a fresh basis, expressed as percentage of water) were also measured. The PWC was computed as (Equation 1):

$$\text{PWC (\%)} = \frac{100\ \times \text{TFM} - \text{TDM}}{TFM} \quad (1)$$

where, TFM and TDM denote the total plant fresh mass and total dry mass (in grams), respectively. The gain (Δ) of biomass during the experimental period was calculated as the difference between DM_2 and DM_1. Where, DM_1 indicates the dry mass of plant organs at the beginning of the experimental period (t_1) and DM_2 the dry mass at end of the experimental periods, t_2 (after 138 days under treatment conditions). The dry matter of plant organs at t_2 (DM_2) was obtained after oven-drying the plant material (72 °C to constant mass). Whereas, the total dry matter at t_1 (TDM_1) was estimated using plants from which an allometric equation based on stem diameter (*D*, mm) was obtained (Equation 2). Likewise, we also estimated the biomass of leaves (LDM_1), roots (RDM_1) and stems (SDM_1) at t_1, as follows (Equations 3-5):

$$TDM_1\ (g) = 1.1476exp0.2706D,\ n = 17,\ R^2 = 0.95 \quad (2)$$

$$LDM_1\ (g) = 0.3586exp0.2804D,\ n = 17,\ R^2 = 0.93 \quad (3)$$

$$SDM_1\ (g) = 0.3714exp0.2823D,\ n = 17,\ R^2 = 0.96 \quad (4)$$

$$RDM_1\ (g) = TDM_1 - (SDM_1 + LDM_1) \quad (5)$$

The biomass gain (ΔB) of leaves (ΔLDM), stems (ΔSDM), and roots (ΔRDM) and leaf area gain (ΔLA) during the experimental period was calculated as the difference between the values recorded at t_2 and t_1. Furthermore, at t_2 we also measured leaf area (LA), leaf mass to area ratio (LMA), and leaflet size. Leaf area (LA) was obtained using an area meter (LI-3050, Li-Cor, USA). The mean leaflet size (*S*L) was determined as the total leaf area (of the whole plant) divided by the number of leaves and leaflets per leaf. To obtain the leaflet size, only the leaves produced during the experimental period were used. Total dry mass (TDM) was obtained as the sum of leaf mass (LDM), stem mass (SDM) and root mass (RDM) after oven-drying the plant material as previously described. The water use efficiency of the whole plant (WUE_p) over the experimental period was calculated as the ratio between the increase in TDM and total amount of water used to irrigate the plant (IW, hereinafter referred to as irrigation water). That is, WUE_p = ΔTDM/IW, where, ΔTDM denotes the difference between TDM_2 and TDM_1. The IW was obtained by measuring (every other day) the amount of water consumed by the plant over the experimental period. From IW and leaf area data, the daily water consumption per unit leaf area (CW_{LA}) was estimated (CW_{LA}= IW per day divided by whole plant leaf area). While, the RGR (in mg g^{-1} day^{-1}) was calculated as (Hunt et al., 2002):

$$\text{RGR}\ (\text{mg g}^{-1}\text{day}^{-1}) = 1000 \times \frac{\ln \text{TDM}_2 - \ln \text{TDM}_1}{t_2 - t_1} \quad (6)$$

where, TDM_1 and TDM_2 denote the total plant dry mass (in grams) at t_1 and t_2, respectively; ln, the natural logarithm and "$t_2 - t_1$" the time interval (days) between the beginning (t_1) and end of the experimental periods, t_2 (i.e. 138 days).

Experimental design and statistical analysis

The experimental design used was a traditional split-plot, with CO_2 concentrations (400 and 800 ppm) as the main plot and the water regimes (50% and 100% FC) as the split-plot, with eight replications. Prior to statistical analyzes the data were tested for normality (Shapiro–Wilks, $\alpha = 0.05$), log transformed [log (x+1)] when necessary and then submitted to ANOVA. The means were compared using the Fisher-LSD test, at a probability level of $p = 0.05$. Statistica 7.0 (StatSoft, Inc. Tulsa OK, USA) was used for data analyzes.

RESULTS AND DISCUSSION

Photosynthesis and leaf nitrogen

The light saturated photosynthesis ($A_{sat\text{-}mass}$) decreased by 34.6% at elevated CO_2 (128.5 *versus* 84.0 nmol g^{-1} s^{-1}), and it also decreased (24.7%) under water stress (Figure 1A). Likewise, $A_{max\text{-}mass}$ decreased (25.6%) at elevated CO_2, being negatively affected by water stress ($p < 0.001$, Figure 1B, Table 1A, 2A in Appendix A).

A decline in $A_{sat\text{-}mass}$ can occur due to the effect of photosynthesis acclimation [down-regulation of photosynthesis, Makino & Mae (1999)], and also because of an increase in leaf density (increase in leaf mass to leaf area ratio under elevated CO_2, Table 3A in Appendix A). In fact, photosynthesis acclimation is a phenomenon that often occurs is response to plant exposure to elevated CO_2 (Drake et al., 1997; Kitao et al., 2007), and may be related to reallocation of nitrogen as discussed in next section.

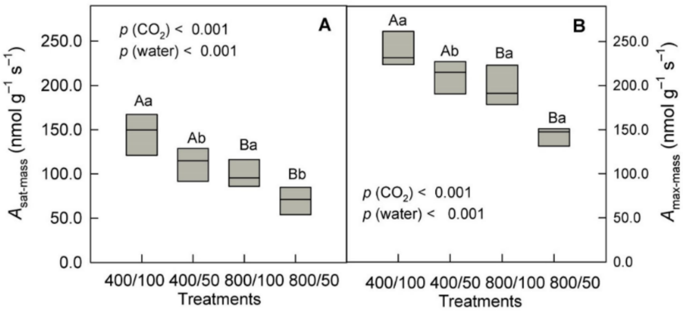

Figure 1. Light saturated photosynthesis on a mass basis ($A_{sat\text{-}mass}$, A) and light and CO_2 saturated photosynthesis per unit mass ($A_{max\text{-}mass}$, B) in *Ceiba pentandra* subjected to two CO_2 levels (400 and 800 ppm) and two water regimes –water (50 and 100% field capacity, FC). In the boxes, different uppercase letters indicate differences between CO_2 levels and different lowercase differences between water regimes (Fisher LSD test, $p = 0.05$, $n = 8$). The p and F values are shown in Table 1A in the Appendix A.

In comparison with ambient CO_2 grown plants, leaf nitrogen concentration (N_{Leaf}) decreased under elevated CO_2 (26.5 *versus* 23.6 mg g^{-1}, Tables 1-2), but the effect of water stress on N_{Leaf} was not significant ($p = 0.891$, Table 1A in Appendix A). The decline in N_{Leaf} at elevated CO_2 may mirror the increase in leaf density –dilution effect associated with an increase in mass per unit leaf area (Ceulemans & Mousseau, 1994; Curtis & Wang, 1998). Also, it may reflect the reallocation of nitrogen from leaves to other plant organs, and thereby associated with down-regulation of photosynthesis at elevated CO_2 (Ceulemans & Mousseau, 1994; Makino & Mae, 1999; Wang & Wang, 2021). Therefore, it is plausible to conclude that in this experiment, both an increase in LMA (Figure 2) and nitrogen reallocation from leaves to other organs (inferred from a decline in $A_{sat\text{-}mass}$, Table 3A in Appendix A) may be associated with decline in N_{Leaf} at elevated CO_2.

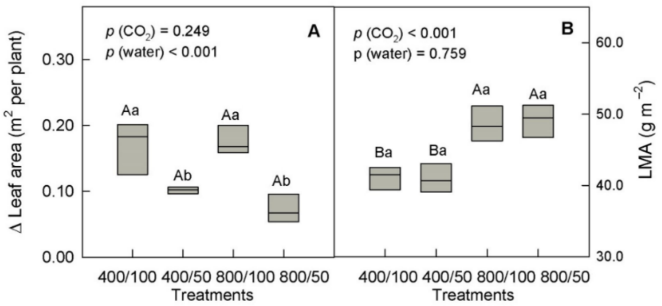

Figure 2. Increase (Δ) of leaf area during the experimental period (A) and the leaf mass to area ratio (LMA, B) in *Ceiba pentandra* subjected to two CO_2 levels (400 and 800 ppm) and two water regimes –water (50% and 100% FC). Further information as described in **Figure 1.**

Biomass allocation to plant organs

The amount of biomass allocated to stems was greatly decreased under water stress, particularly at ambient CO_2 level (Figure 3A). Therefore, on average the effect of elevated CO_2 on ΔSDM was not significant (p = 0.113), and due to the large decline in stem biomass at ambient CO_2 under water stress, the interaction between water regime and CO_2 level was significant (p = 0.017, Table 1A in Appendix A). Similarly, plants kept in well-irrigated soil accumulated more biomass in leaves and roots in comparison with plants under water stress (Figure 3B-3C), but over water regimes, the effect of elevated CO_2 on ΔLDM and ΔRDM was not significant (p > 0.07, Table 1A in Appendix A). The same trend was observed for leaf production (Figure 2), as leaf area (ΔLA) increased 47.7% in well-irrigated plants, when compared to water stress grown plants (0.172 *versus* 0.09 m^2 per plant, Table 3A in Appendix A), but with no effect of elevated CO_2 on ΔLA (p = 0.249, Table 1A in Appendix A). Due to the effect of water stress on biomass allocation to stems, leaves and roots, the gain of total dry matter (ΔTDM) was reduced by 43% under water stress (23.7 *versus* 13.5 g per plant, Figure 3D, Table 3A in Appendix A), but without an effect of elevated CO_2 on ΔTDM (p = 0.633, Table 1A in Appendix A). Following the trend observed for biomass allocation to roots, the volume of roots was greatly reduced under water stress, but the effect of CO_2 levels was neutral (Table 2).

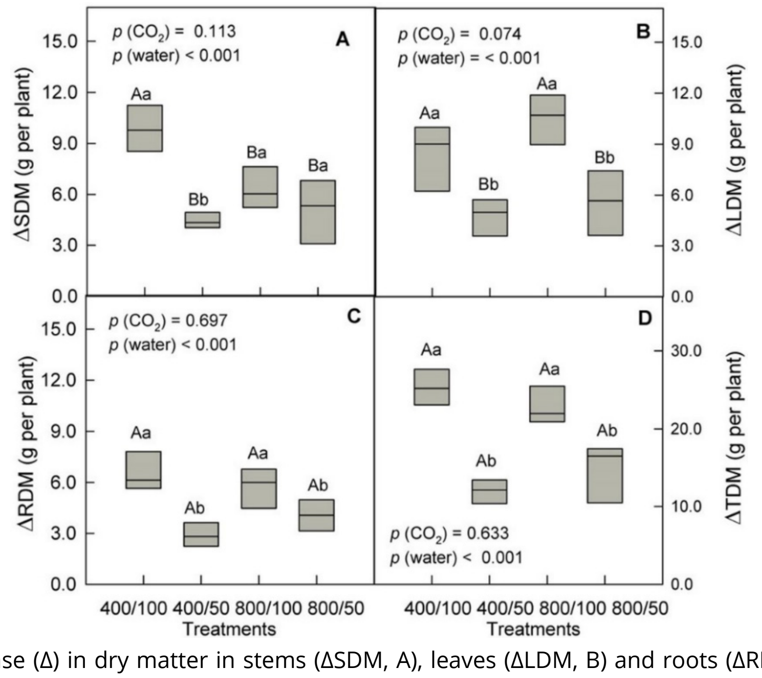

Figure 3. Increase (Δ) in dry matter in stems (ΔSDM, A), leaves (ΔLDM, B) and roots (ΔRDM, C), and the increase of total dry matter (ΔTDM, D) during the experimental period in *Ceiba pentandra* subjected to two CO_2 levels (400 and 800 ppm) and two water regimes –water (50% and 100% FC). Further information as described in **Figure 1**.

Table 1. Growth and plant traits in response to exposure to two CO_2 concentrations (400 and 800 ppm) and two water regimes (50% field capacity – FC and 100% FC) for 138 days. Acronyms: H_G, daily growth in height; D_G, daily growth in diameter; RGR, relative growth rate; IW, total amount of water consumed per plant (irrigation water); CW_{LA}, consume of water per unit leaf area on a daily basis; TFW, total fresh weight; TDM/TFW, total dry matter to TFW ratio; PWC, plant water content; N_{Leaf}, leaf nitrogen concentration. Each value represents the mean (± SD) of eight plants (n = 8). The means over CO_2 levels and water regimes are shown in **Table 2.**

Variable	400 ppm		800 ppm	
	100% FC	50% FC	100% FC	50% FC
H_G (cm day^{-1})	0.21 ± 0.07	0.11 ± 0.03	0.20 ± 0.03	0.11 ± 0.02
D_G (mm day^{-1})	0.021 ± 0.008	0.024 ± 0.007	0.025 ± 0.003	0.030 ± 0.015
RGR (mg g^{-1} day^{-1})	9.92 ± 1.3	7.10 ± 1.5	10.64 ± 1.1	8.34 ± 1.6
Leaf number per plant	19.4 ± 3.0	15.3 ± 2.1	19.1 ± 1.8	14.5 ± 1.7
Leaflet number per plant	117.0 ± 23.0	84.5 ± 16.5	113.4 ± 12.8	77.8 ± 13.4
Leaflet size (cm^2)	21.4 ± 0.8	20.4 ± 1.1	21.1 ± 2.8	18.2 ± 3.4
Root volume (cm^3)	37.4 ± 6.0	26.3 ± 2.4	35.8 ± 4.8	26.9 ± 4.7
IW (kg per plant)	7.58 ± 1.1	2.01 ± 0.5	3.64 ± 0.6	1.85 ± 0.6
CW_{LA} [g(water) m^{-2} day^{-1}]	225.2 ± 19.6	90.8 ± 26.1	109.4 ± 14.2	92.4 ± 11.0
TFW (g plant^{-1})	134.9 ± 14.6	79.5 ± 7.2	125.9 ± 16.9	82.4 ± 20.1
TDM/TFW (unitless)	0.25 ± 0.010	0.24 ± 0.013	0.23 ± 0.010	0.26 ± 0.010
PWC (%)	75.3 ± 0.9	75.7 ± 1.3	76.7 ± 0.7	73.7 ± 1.0
N_{Leaf} (mg g^{-1})	26.1 ± 1.9	26.8 ± 1.9	23.9 ± 1.6	23.3 ± 0.8

Table 2. Means of growth and plant traits in response to exposure to two CO_2 levels (400 and 800 ppm) and two water regimes (50% field capacity – FC and 100% FC) for 138 days. Different uppercase letters and different lowercase letters indicate differences between CO_2 levels and water regimes, respectively (Fisher LSD test, p = 0.05). Each value represents the mean (± SD) of 16 plants (n=16). Values of p and F are shown in **Table 1A,** in the Appendix A and acronyms in **Table 1.**

Variable	CO_2		Water	
	400 ppm	800 ppm	100% FC	50% FC
H_G (cm day^{-1})	0.16 ± 0.07^A	0.16 ± 0.05^A	0.20 ± 0.05^a	0.11 ± 0.03^b
D_G (mm day^{-1})	0.023 ± 0.007^A	0.028 ± 0.011^A	0.023 ± 0.006^a	0.027 ± 0.012^a
RGR (mg g^{-1} day^{-1})	8.51 ± 2.0^B	9.49 ± 1.8^A	10.28 ± 1.2^a	7.72 ± 1.6^b
Leaf number per plant	17.3 ± 3.3^A	16.8 ± 2.9^A	19.3 ± 2.4^a	14.9 ± 1.9^b
Leaflet number per plant	100.8 ± 25.6^A	95.6 ± 22.3^A	115.2 ± 18.1^a	81.1 ± 14.9^b
Leaflet size (cm^2)	20.9 ± 1.1^A	19.7 ± 3.3^A	21.3 ± 2.0^a	19.3 ± 2.6^b
Root volume (cm^3)	31.9 ± 7.2^A	31.3 ± 6.5^A	36.6 ± 5.3^a	26.6 ± 3.6^b
IW (kg per plant)	4.79 ± 3.0^A	2.74 ± 1.1^B	5.61 ± 2.2^a	1.93 ± 0.5^b
CW_{LA} [g(water) m^{-2} day^{-1}]	158.0 ± 72.9^A	100.9 ± 15.1^B	167.3 ± 62.0^a	91.6 ± 19.4^b
TFW (g plant^{-1})	107.2 ± 30.7^A	104.2 ± 28.7^A	130.4 ± 16.0^a	80.9 ± 14.7^b
TDM/TFW (unitless)	0.245 ± 0.011^A	0.248 ± 0.018^A	0.240 ± 0.010^b	0.253 ± 0.015^a
PWC (%)	75.5 ± 1.1^A	75.2 ± 1.8^A	76.0 ± 1.0^a	74.7 ± 1.5^b
N_{Leaf} (mg g^{-1})	26.5 ± 1.9^A	23.6 ± 1.2^B	25.0 ± 2.0^a	25.1 ± 2.3^a

The height growth rate (H_G) was affected only by water stress, while the diameter growth rate (D_G) was neutral to the effect of both elevated CO_2 and water stress (Table 2). Thus, the increase in total biomass in well-watered plants was related to improved H_G rather than to an increase D_G. Although D_G tended to increase under elevated CO_2 (Table 2), that increase did not reach a significant level (p = 0.116, Table 1A in Appendix A). Notwithstanding the enhanced height growth in well irrigated plants, the shoot/root ratio was not altered by treatments – either CO_2 levels or water regimes (Figure 4, Table 3A in Appendix A).

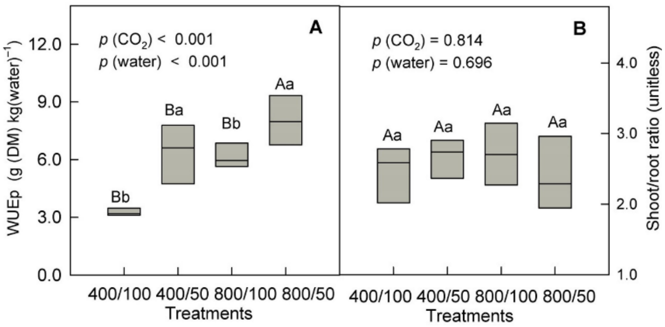

Figure 4. Plant water use efficiency (WUE_p) throughout the experimental period (A) and the shoot to root ratio (B) in *Ceiba pentandra* subjected to two CO_2 levels (400 and 800 ppm) and two water regimes –water (50% and 100% FC). Further information as described in **Figure 1.**

As we have shown, the gain of total dry matter during the experimental period (ΔTDM) was substantially reduced under water stress. This can be attributed to the strong reduction in leaf area and leaf biomass, and also to the direct effect of water stress on carbon assimilation, which altogether reduced photosynthesis at the whole plant level. The strong effect of water stress on leaf production may occur because cell division, leaf expansion and protein synthesis are greatly impaired by water stress (Bradford & Hsiao, 1982; Kozlowski & Pallardy, 1997). Indeed, these adjustments occur well before water stress induces stomatal closure (Bradford & Hsiao, 1982; Tardieu et al., 2015). Over CO_2 levels, ΔTDM increased in well-irrigated plants, which is consistent with the high photosynthetic rates of well-watered plants. The plant organs that most contributed to whole-plant biomass gain in well-irrigated soil were stems (particularly at ambient CO_2 concentration) and leaves. Under water stress, biomass allocated to roots and stems was more reduced at ambient CO_2 (~55%) than at elevated CO_2 (17-29%, Table 2A in Appendix A, Figure 3), but the shoot to root ratio was unresponsive to both water stress and elevated CO_2. Indeed, the shoot/ratio seems to be a robust parameter, as reported by Poorter & Nagel (2000), who found no effect of elevated CO_2 on biomass allocation to stems, roots and leaves. The lack of an effect of water stress and elevated CO_2 on the shoot/root ratio can be explained by observing that leaf biomass production tended to increase at elevated CO_2, whereas stem biomass tended to decrease. Meanwhile on average, the effect of elevated CO_2 on biomass allocated to roots was neutral.

The RGR was 24.9% higher in well-irrigated plants than in those under water stress (10.28 *versus* 7.72 mg g^{-1} day^{-1} (Table 2). It was also higher at elevated CO_2 than at ambient CO_2 concentration ($p = 0.038$, Table 1A in Appendix A). This increase, however, was not sufficient for the plant to increase biomass in response to elevated CO_2 (Figure 3D), as the slightly positive effect of elevated CO_2 on leaf dry matter ($p = 0.074$) was counterbalanced by a negative effect of elevated CO_2 on the amount of biomass allocated to stems ($p = 0.113$, Figure 3A-3B).

The number of leaves and leaflets, and leaf size were higher in well-irrigated plants, than in those subjected to water stress ($p < 0.05$), but over water regime the effect of CO_2 was not significant ($p > 0.05$, Table 1A in Appendix A). This can be explained by taking into account that cell expansion and leaf growth depend on water content, and thereby leaf growth may decrease under water stress (Bradford & Hsiao, 1982; Kozlowski & Pallardy, 1997).

We found that light saturated photosynthesis ($A_{sat\text{-}mass}$) declined at elevated CO_2 in well-watered plants. However, the decrease in $A_{sat\text{-}mass}$ at elevated CO_2 did not cause the same effect on ΔTDM ($p = 0.633$, Table 1A in Appendix A). At first sight, this result seems to be contradictory. Nevertheless, it can be explained by taking into account that biomass gain is a complex process that depends not only on photosynthesis, but also on carbon loss through respiration (Kozlowski & Pallardy, 1997). In this respect, it has been reported that respiration may decrease in plants grown at elevated CO_2 (Amthor et al., 1992; Curtis & Wang, 1998; Drake et al., 1997). Although there is not a general trend, in some woody species, such as *Castanea sativa* and *Quercus prinus* the whole plant respiration may decrease in response to elevated CO_2 (Ceulemans & Mousseau, 1994). The decline in respiration in plants subjected to elevated CO_2 may be due to direct effects on enzymes and to indirect effects brought about by changes in plant chemical composition (Gonzalez-Meler et al., 2004). Hence, a decline in photosynthesis may have a neutral effect on plant biomass if the loss of carbon through respiration is reduced.

The water stress had a neutral effect on the leaf mass to area ratio (LMA, Table 3A in Appendix A). However, it increased under elevated CO_2, which can be associated with an increase in non-structural carbohydrates under elevated CO_2 (Curtis & Wang, 1998; Drake et al., 1997; Oliveira & Marenco, 2019). In fact, increased LMA seems to be a common response to elevated CO_2 (Poorter & Navas, 2003; Ainsworth & Long, 2005; Aspinwall et al., 2017).

Plant water content and water use efficiency

The amount of water consumed by plants (irrigation water, IW) was responsive to both water stress and elevated CO_2, with the interaction between water regime and CO_2 level being significant ($p < 0.001$, Table 1A in Appendix A). The largest difference in IW within a CO_2 level (between soil water content) was recorded at ambient CO_2 (7.58 kg *versus* 2.01 kg per plant, Table 1). Taking ambient CO_2 and well-irrigated plants as the base line, WUE_p was 50% higher in plants at elevated CO_2, and also under water stress (about 4.8 *versus* 7.2 kg per plant, Figure 4A, Table 3A in Appendix A). The amount of water consumed per unit leaf area (CW_{LA}) was higher in plants at ambient CO_2 than at elevated CO_2 (158.0 *versus* 100.9 g m^{-2} day^{-1}, Table 2). It was also higher in well-irrigated plant than under water stress ($p < 0.001$, Table 1A in Appendix A). Therefore, the lowest WUE_p value was observed in well-irrigated plants at ambient CO_2 (Figure 4A). Hence, both elevated CO_2 and water stress improved water use efficiency. This can be explained by considering that under these conditions (elevated CO_2 and water restriction) stomatal conductance (and hence transpiration) often decreases (Drake et al., 1997; Ainsworth & Long, 2005; Dusenge et al., 2019). In fact, a drop in transpiration is a classical response to water stress (Bradford & Hsiao, 1982; Kozlowski & Pallardy, 1997; Oliveira & Marenco, 2019). This is important, as it shows that the negative effect of droughts under elevated CO_2 can be mitigated by increasing water use efficiency.

Following the sharp decline in total biomass under water stress, TFW was also reduced under water stress, but the effect of elevated CO_2 in TFW was neutral (Table 2). Although significant, PWC and the TDM/TFW ratio were only slightly affected by water stress. For instance, PWC decreased from 76.0% to 74.7%, under water stress (Table 2). This indicates that sumaúma has a rather tight control of stomatal transpiration, which may help the tree to endure periods of low water availability, and hence to thrive over a large range of tropical regions (Gómez-Maqueo & Gamboa-deBuen, 2022). This trait contributes to make the sumaúma tree potentially useful for reforestation programs (Román-Dañobeytia et al., 2015; Vargas-Simón et al., 2022).

CONCLUSIONS

We found that subjecting the plants to elevated CO_2 led to a decrease in light saturated photosynthesis on a leaf mass basis. Notwithstanding, the gain of total biomass was unresponsive to elevated CO_2, being both photosynthesis and total biomass negatively affected by water stress. However, water use efficiency improved by 50% in response to both elevated CO_2 and water stress. Even when the height growth rate decreased under water stress, the shoot/root ratio and the radial growth rate were neutral to both water limitation and high CO_2 concentration. Altogether, the performance of this tree under elevated CO_2 and water stress (in particular, its sustained radial growth rate and slight changes in plant water content under water limitation) suggests it could be evaluated in reforestation experiments under limiting water supply. Overall, these findings contribute to enhance our current knowledge of the effect of elevated CO_2 and water stress on *Ceiba pentandra*, a species with several uses over tropical regions worldwide.

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APPENDIX A. SUMMARY OF ANOVA AND MEAN VALUES OF DATA PRESENTED IN FIGURES 1–4. APPENDIX MATERIAL TO: BIOMASS ALLOCATION IN *Ceiba Pentandra* (MALVACEAE) UNDER WATER STRESS AND HIGH CO_2 CONCENTRATION BY SILVEIRA ET AL. (2023, HTTPS://DOI.ORG/10.18671/SCIFOR.V51.10).

Table 1A. Summary of the analysis of variance (*F* and *p* values) for the effect of CO_2 concentrations (CO_2) and water regimes (water) on the evaluated parameters. When necessary, data were log-transformed prior to statistical analysis. Acronyms as described in Figures 1-4 and Table 1.

Variable	CO_2		Water		CO_2 × water	
	F	*p*	*F*	*p*	*F*	*p*
$A_{sat\text{-}mass}$ (nmol g^{-1} s^{-1})	25.0	< 0.001	28.3	< 0.001	0.01	0.92
$A_{max\text{-}mass}$ (nmol g^{-1} s^{-1})	51.0	< 0.001	22.0	< 0.001	2.63	0.13
H_G (cm day^{-1})	0.04	0.841	32.8	< 0.001	0.07	0.789
D_G (mm day^{-1})	2.80	0.116	0.997	0.335	0.047	0.832
RGR (mg $g^{-1}day^{-1}$)	5.27	0.038	19.51	< 0.001	0.40	0.537
ΔLDM (g $plant^{-1}$)	3.73	0.074	30.08	< 0.001	0.34	0.569
ΔSDM (g $plant^{-1}$)	2.86	0.113	24.01	< 0.001	7.41	0.017
ΔRDM (g $plant^{-1}$)	0.16	0.697	26.69	< 0.001	4.45	0.053
Root volume (cm^3 $plant^{-1}$)	0.07	0.791	42.87	< 0.001	0.29	0.602
ΔTDM (g $plant^{-1}$)	0.24	0.633	38.67	< 0.001	2.11	0.168
Shoot/root ratio (unitless)	0.057	0.814	0.159	0.696	1.096	0.313
TDM/FW (unitless)	0.85	0.371	13.1	0.003	21.1	< 0.001
ΔLA (m^2 $plant^{-1}$)	1.45	0.249	54.17	< 0.001	1.81	0.200
Leaf number	0.48	0.502	22.80	< 0.001	0.15	0.702
Leaflet number	0.96	0.345	24.05	< 0.001	0.18	0.674
leaflet size (cm^2)	4.58	0.051	4.92	0.044	1.18	0.296
WUE_p [g (DM) kg(water)$^{-1}$]	44.12	< 0.001	45.89	< 0.001	7.23	0.018
CW_{LA} [g (water) m^{-2} day^{-1}]	27.35	< 0.001	104.76	< 0.001	50.94	< 0.001
IW (kg(water) $plant^{-1}$)	33.19	< 0.001	182.20	< 0.001	23.73	< 0.001
Leaf Nitrogen (mg g^{-1})	31.55	< 0.001	0.02	0.891	1.19	0.293
LMA (g m^{-2})	47.57	< 0.001	0.10	0.759	0.16	0.697
TFW (g $plant^{-1}$)	0.33	0.573	58.9	< 0.001	0.43	0.524
PWC (%)	1.0	0.34	13.0	0.002	21.0	< 0.001

Table 2A. Growth and plant traits in response to exposure to two CO_2 concentrations (400 and 800 ppm) and two water regimes (50% field capacity –FC and 100% FC) for 138 days. Acronyms: Δ, increase in biomass or leaf area during the experimental period; ΔSDM, Δ of stem biomass; ΔLDM, Δ of leaf biomass; ΔRDM, Δ of root biomass; ΔTDM, Δ of total dry matter; ΔLA, Δ of leaf area; LMA, leaf mass to area ratio, shoot/root ratio (unitless); WUE_p, plant water use efficiency, $A_{sat\text{-}mass}$: Light saturated photosynthesis per unit mass; $A_{max\text{-}mass}$, Light and CO_2 saturated photosynthesis per unit mass. Each value represents the means (± SD) of eight plants. The means over CO_2 levels and water regimes are shown in Table 3A. Acronyms as described in Figures 1-4.

Variable	400 ppm		800 ppm	
	100% FC	50% FC	100% FC	50% FC
ΔTDM (g per plant)	24.81 ± 3.3	12.12 ± 2.8	22.57 ± 4.0	14.87 ± 4.6
ΔSDM (g per plant)	9.71 ± 1.9	4.3 ± 0.8	6.37 ± 1.5	5.26 ± 2.0
ΔLDM (g per plant)	8.48 ± 2.1	4.87 ± 1.5	10.64 ± 1.7	5.66 ± 2.2
ΔRDM (g per plant)	6.61 ± 1.1	2.96 ± 1.0	5.55 ± 1.5	3.95 ± 1.2
ΔLA (m^2 per plant)	0.170 ± 0.04	0.102 ± 0.01	0.175 ± 0.02	0.078 ± 0.03
LMA (g m^{-2})	41.1 ± 1.9	41.1 ± 3.1	48.4 ± 4.2	49.2 ± 3.1
Shoot/root (unitless)	2.51 ± 0.48	2.63 ± 0.37	2.69 ± 0.45	2.42 ± 0.55
WUE_p [g(DM) kg(water)$^{-1}$]	3.28 ± 0.24	6.37 ± 1.54	6.25 ± 0.86	8.09 ± 1.57
$A_{sat\text{-}mass}$ (nmol g^{-1} s^{-1})	143.2 ± 26.0	113.8 ± 23.8	99.3 ± 15.4	68.8 ± 17.0
$A_{max\text{-}mass}$ (nmol g^{-1} s^{-1})	239.7 ± 21.5	214.1 ± 25.2	195.2 ± 30.8	142.5 ± 11.1

Table 3A. Means of growth and plant traits in response to exposure to two CO_2 levels (400 and 800 ppm) and two water regimes (50% field capacity – FC and 100% FC) for 138 days. Different uppercase letters and different lowercase letters indicate differences between CO_2 levels and water regimes, respectively (Fisher LSD test, p = 0.05). Each value represents the mean (± SD) of 16 plants. Values of p and F are shown in Table 1A, in the appendix. Acronyms as described in Figures 1-4**.**

Variable	CO_2		Water	
	400 ppm	800 ppm	100% FC	50% FC
ΔTDM (g per plant)	18.5 ± 7.2^A	18.7 ± 5.8^A	23.7 ± 3.7^a	13.5 ± 4.0^b
ΔSDM (g per plant)	7.00 ± 3.1^A	5.82 ± 1.8^A	8.04 ± 2.4^a	4.78 ± 1.6^b
ΔLDM (g per plant)	6.68 ± 2.6^A	8.15 ± 3.2^A	9.56 ± 2.2^a	5.26 ± 1.8^b
ΔRDM (g per plant)	4.79 ± 2.1^A	4.75 ± 1.5^A	6.08 ± 1.4^a	3.45 ± 1.2^b
ΔLA (m^2 per plant)	0.136 ± 0.05^A	0.126 ± 0.06^A	0.172 ± 0.03^a	0.09 ± 0.03^b
LMA ($g\ m^{-2}$)	41.1 ± 2.5^B	48.8 ± 3.6^A	44.8 ± 4.9^a	45.1 ± 5.1^a
Shoot/root (unitless)	2.57 ± 0.42^A	2.55 ± 0.50^A	2.60 ± 0.46^a	2.52 ± 0.46^a
WUE_p [(g(DM) kg(water)$^{-1}$]	4.78 ± 1.9^B	7.17 ± 1.55^A	4.77 ± 1.65^b	7.18 ± 1.8^a
$A_{sat\text{-}mass}$ (nmol $g^{-1}\ s^{-1}$)	128.5 ± 28.5^A	84.0 ± 22.2^B	121.2 ± 30.7^a	91.3 ± 30.6^b
$A_{max\text{-}mass}$ (nmol $g^{-1}\ s^{-1}$)	226.9 ± 26.2^A	168.9 ± 35.2^B	217.5 ± 34.4^a	178.3 ± 41.4^b