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Photosynthesis and biomass accumulation in *Carapa surinamensis* (Meliaceae) in response to water stress at ambient and elevated CO_2

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Abstract

Climate models predict an increase in atmospheric CO_2 concentration and prolonged *droughts* in some parts of the Amazon, but the effect of elevated CO_2 is still unknown. Two experiments (ambient CO_2 – 400 ppm and elevated CO_2 – 700 ppm) were conducted to assess the effect of drought (soil at 50% field capacity) on physiological parameters of *Carapa*. At ambient CO_2 concentration, light-saturated net photosynthetic rate (P_{Nsat}) was reduced by 33.5% and stomatal conductance (g_s) by 46.4% under drought, but the effect of drought on P_{Nsat} and g_s was nullified at elevated CO_2. Total plant biomass and leaf area production were also reduced (42–47%) by drought. By changing leaf traits, *Carapa* is able to endure drought, as the consumptive use of water was reduced under drought (32–40%). The improvement of P_{Nsat} under elevated CO_2 and water stress and the leaf plasticity of *Carapa* broaden our understanding of the physiology of Amazonian trees.

Additional key words: chlorophyll fluorescence; leaf water potential; nonphotochemical quenching; water-use efficiency.

Introduction

The Amazon rainforest stores about 86 Pg of carbon in total biomass and it is estimated that about 50% of incident annual rainfall on the region is recycled by transpiration (Salati 1987, Saatchi *et al*. 2007). At a global scale, climate models predict an increase in temperature and atmospheric CO_2 concentration, which can reach up to about 900 ppm by 2100 (Way *et al*. 2015). Although the current length of the dry season seems to have little effect on tree growth rates in the central Amazon (Dias and Marenco 2016, Camargo and Marenco 2017), climate models predict expansion of areas affected by droughts in some parts of the Amazon *(*Cox *et al*. 2004, Duffy *et al*. 2015*)*. In the short term, exposure to elevated CO_2 enhances photosynthetic rates of C_3 plants (Kirschbaum 1994, Ainsworth and Rogers 2007, Way *et al*. 2015) by increasing the maximum carboxylation rate of Rubisco – V_{cmax} (Rubisco is substrate-limited at current CO_2 concentrations) and reducing photorespiration. Most of the time, plants under elevated CO_2 reduce g_s (Ainsworth and Rogers 2007, Leakey *et al*. 2012), which can improve water-use efficiency. On the other hand, long-term exposure to elevated CO_2 can lead to photosynthetic acclimation – a downward regulation of photosynthetic enhancement under elevated CO_2 (Gunderson and Wullschleger 1994, Way *et al*. 2015). Acclimation can be the result of a lesser amount of mineral nutrients (*e.g*., nitrogen) allocated to enzymes of the Calvin cycle and more assimilates partitioned to plant tissues not directly involved in carbon assimilation (Leakey *et al*. 2012). Thus, photosynthetic acclimation can occur in response to a reduced sink size (source–sink imbalance), changes

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Abbreviations: A_L – total leaf area; Chl – chlorophyll; C_i – intercellular CO_2 concentration; CUW – consumptive use of water; ETR – electron transport rate; FC – field capacity; F_m – maximal fluorescence yield of the dark-adapted state; F_m' – maximal fluorescence yield of the light-adapted state; F_s – steady-state fluorescence yield; F_v/F_m – maximal quantum yield of PSII photochemistry; F_v – variable fluorescence; g_s – stomatal conductance; J_{max} – maximum electron transport rate; F_0 – minimal fluorescence yield of the dark-adapted state; J_{max25} – J_{max} at 25°C; LMA – leaf mass per area ratio; NPQ – nonphotochemical quenching; T – temperature in Kelvin; P_N – net photosynthetic rate; P_{Nsat} – light-saturated P_N; P_{Nmax} – light- and CO_2-saturated P_N; RH – relative humidity; TNC – total non-structural carbohydrates; V_{cmax} – maximum carboxylation rate of Rubisco; V_{cmax25} – V_{cmax} at 25°C; VPD_L – leaf-to-air vapor pressure difference; W_T – total dry matter (biomass); WUE_i – intrinsic water-use efficiency; Γ – CO_2-compensation point; Γ* – Γ in absence of mitochondrial respiration; Ψ_L – leaf water potential; Φ_{PSII} – effective quantum yield of PSII photochemistry.
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in leaf carbohydrate signaling, root growth restriction, or low availability of mineral nutrients (Rogers *et al*. 1998, Moore *et al*. 1999).

Besides the increase in CO_2 concentration, global models also predict that climate change can lead to an increase of the length of the dry season in some parts of the Amazon region (Cox *et al*. 2004, Duffy *et al*. 2015), which can lead to lowering net photosynthetic rate (P_N), as under water stress P_N can be substantially decreased. It has been postulated, however, that the progressive increase in atmospheric CO_2 concentration can result in a greater crop yield and greater primary productivity of tropical forests (Lloyd and Farquhar 2008, Leakey *et al*. 2012).

Light not used in photochemical reactions (excess of light) may induce overexcitation of chlorophyll (Chl) *a*, and lead to the formation of highly reactive Chl molecules – triplet state Chl, ^{3}Chl (Papageorgiou and Govindjee 2014). The ^{3}Chl can interact with molecular oxygen and promote the production of strong oxidants (*e.g*., O^{2-}, H_2O_2, singlet oxygen – $^1O_2$) and peroxidation of membrane lipids, which lowers the photochemical efficiency of the leaf (Papageorgiou and Govindjee 2014). Nonphotochemical quenching (NPQ), an indicator of the thylakoid transmembrane pH gradient, triggers enzymatic and nonenzymatic (physicochemical) reactions that lead to the dissipation of excess excitation energy as heat (Maxwell and Johnson 2000, Papageorgiou and Govindjee 2014). Therefore, NPQ is a mechanism to avoid photo-oxidative damage and it increases with progressive stress (Tezara *et al*. 1999, Liu *et al*. 2017).

Although the Amazon is of great importance in the global scenario, little is known about how Amazonian tree species would respond to the combined effect of elevated CO_2 and water stress. In this work we hypothesized that plants subjected to elevated CO_2 accumulate more biomass, increase photosynthetic rates, and improve intrinsic water-use efficiency (WUE_i). Another premise was that the photochemical efficiency of the leaf is enhanced in plants subjected to CO_2 enrichment. Thus, the aim of this study was to assess photosynthetic rates and the photochemical efficiency and growth of young trees of *Carapa* in response to water stress under two growth conditions, ambient and elevated CO_2.

Materials and methods

Plant material and growth environments: Two experiments were carried out at the National Institute for Research in the Amazon – INPA (03°05′29”S, 59°59′35” W), Manaus, AM. In Manaus, annual mean rainfall, temperature, and solar radiation are 2,300 mm, 26.5°C, and 18.03 MJ day^{-1}, respectively (http://www.inmet.gov.br/portal/, period of 1961–1990). One experiment was conducted at ambient CO_2 conditions (about 400 ppm) under a greenhouse conditions (hereinafter referred to Experiment 1) and the second in a growth chamber at elevated CO_2 – 700 ppm (Experiment 2). In both experiments, seeds of *Carapa surinamensis* Miq (Meliaceae, hereinafter *Carapa*) were germinated in vermiculite and 15 d after emergence, the plants were transferred to pots containing 7 kg of substrate (a mixture of soil of the first 20 cm of the soil forest, amended with mulched material and NPK (5 g kg^{-1}, 10:10:10). Six months later, the 0.6-m tall plants were randomly sorted into two groups of ten plants, one group for Experiment 1 (under greenhouse) and the second for Experiment 2, the latter to be conducted under growth chamber conditions. Both experiments lasted 163 d (hereinafter referred to as the experimental period, 14 July–23 December, 2015).

Experiment 1 – under greenhouse conditions: Before initiating the experiment and for several days, we measured the light conditions (PAR) inside the greenhouse, which turned out to be about 8.6 mol m^{-2} d^{-1}, and because its latitudinal location (–3.091°) incoming irradiance remained rather constant throughout the year. We used this data to serve as a reference for setting the light intensity in the growth chamber (Experiment 2).

The treatments in this experiment were two water regimes (five plants per treatment): moderate drought (induced by keeping the soil at 50% field capacity – FC) and soil at 100% FC (well-watered plants). In the greenhouse, PAR, temperature, and relative humidity (RH) were measured using specific sensors (*Li-190 SA, Li-Cor*, Lincoln, US and *Humitter 50Y*, *Vaisala*, Oyj, Finland) connected to a datalogger (*Li-1400*, *Li-Cor*, Lincoln, US), which was set to record data at 15-min intervals. Also, the CO_2 concentration was measured on randomly selected days using an infrared gas analyzer (*Li-6400XT*, *Li-Cor,* Lincoln , US) with empty chamber.

Before submitting the plants to the water regimes, we determined the 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. Every morning (7:00–8:00) during the whole experimental period, we determined the mass of each potted plant (accuracy of 1 g) and restored the volume of water required to keep the soil at its target water content (50 or 100% FC). For further information, we also measured soil moisture with an electronic device (*MPM-160B, ICT International*, Armidale, Australia). The experimental period of 163 d was long enough for the plant to flush new leaves, which were used for gas-exchange measurements and laboratory analyses.

Experiment 2 – under growth chamber conditions: In this experiment, we also assessed the effect of two water regimes (soil at 50% FC and soil at 100% FC), which followed the same protocol as described for Experiment 1. The growth chamber (*TPC-19*, *Biochambers*, Winnipeg, Canada) has a working area of 1.72 m^2 and 1.52 m height,

and hence it provides enough room for the plants (five per treatment) to grow for 163 d. Electronic devices were used to keep constant the ambient conditions in the growth chamber. CO_2 concentration was set at 700 ppm, day/night temperature at 27/25°C. The RH inside the growth chamber was 80–90%. We set the light intensity at 200 µmol(photon) m^{-2} s^{-1} (*i.e.*, 8.6 mol m^{-2} d^{-1} over a 12-h photoperiod). We used this light intensity to emulate that of the greenhouse, so that some comparisons could be made at the end of the study.

In both experiments and at the end of the experimental period (163 d), we measured gas exchange, Chl fluorescence, total dry matter (W_T), leaflet number, and size and total leaf area (A_L), leaf mass per area ratio (LMA), proline, and total nonstructural carbohydrate (TNC) content of leaves.

Gas exchange was measured with a portable gas-exchange system (*Li-6400XT*, *Li-Cor*, Lincoln, US). The measurements were carried out between 08:00 and 14:00 h in two fully expanded leaves per plant, which had been produced during the experimental period. Gas exchange was measured after a stabilization period of about 10 min at [CO_2] of 400 ppm in the leaf chamber (about 240 ppm of internal CO_2 concentration – C_i) and 250–500 µmol m^{-2} s^{-1}. P_N/C_i response curves were generated at light saturation [1,000 µmol(photon) m^{-2} s^{-1}, this PAR value was determined after constructing a light-response curve], ambient temperature (27°C), relative humidity of 70 ± 5%, and air flow of 500 µmol s^{-1}. CO_2 concentration in the leaf chamber was changed step by step as previously described (Nascimento and Marenco 2013). Light-saturated net photosynthetic rate (P_{Nsat}) and stomatal conductance (g_s) were measured at a light intensity of 1,000 µmol(photon) m^{-2} s^{-1} and CO_2 concentration of 400 ppm (Experiment 1) and 700 ppm (Experiment 2). The last CO_2 point of the P_N/C_i curve (2,000 ppm in the leaf chamber) corresponded to the light and CO_2-saturated net photosynthetic rate (P_{Nmax}). Intrinsic water-use efficiency (WUE_i) was determined as the P_{Nsat}/g_s ratio, and the consumptive use of water (CUW, on a leaf area basis) was determined by every morning recording the amount of water daily added to plants (to keep the soil at 50% or 100% FC). The maximum carboxylation rate of Rubisco (V_{cmax}) and maximum electron transport rate (J_{max}) were calculated using the classic Faquhar´s model (Farquhar *et al*. 1980), and the parameters described by von Caemmerer (2000):

$$P_{Nc} = [V_{cmax}(C_i - \Gamma^*)]/[C_i + K_c(1 + O/K_o)] \quad (1)$$

$$P_{Nj} = [J_{max}(C_i - \Gamma^*)]/([4C_i + 8\Gamma^*)] \quad (2)$$

where P_{Nc} and P_{Nj} denote P_N limited by either Rubisco activity or RuBP concentration, respectively; Γ* represents the CO_2-compensation point in the absence of mitochondrial respiration (37.0 ppm, at 25°C); *O*, the intercellular oxygen concentration (0.210 mol mol^{-1}, at 25°C); K_c (404 µmol mol^{-1}, at 25°C) and K_o (248 mmol mol^{-1}, at 25°C) represent the Michaelis constants of Rubisco for carboxylation and oxygenation, respectively. V_{cmax} and J_{max} data were standardized to 25ºC (V_{cmax25}, J_{max25}) as follows (von Caemmerer 2000):

$$J_{max} = J_{max25} \exp\left(\frac{E_{aj}(T-298)}{(298RT)}\right) \frac{\left[1+\exp\left(\frac{298S-H}{(298R)}\right)\right]}{\left[1+\exp\left(\frac{ST-H}{RT}\right)\right]} \quad (3)$$

$$V_{cmax} = V_{cmax25} \exp\left(\frac{E_{av}(T-298)}{(298RT)}\right) \quad (4)$$

where, *T* is leaf temperature (in Kelvin), *R* is the gas constant (8.314 J K^{-1} mol^{-1}); E_{aj} is activation energy for electron transport (37.0 kJ mol^{-1}); *S* is entropy of activation (0.71 kJ K^{-1} mol^{-1}); *H* is enthalpy of activation (220 kJ mol^{-1}); E_{av} is activation energy for carboxylation (59.36 kJ mol^{-1}).

Chl fluorescence was measured under ambient conditions (CO_2 of about 400 ppm and 27°C) with a portable modulated fluorometer (*PAM-2500, Walz GmbH*, Effeltrich, Germany). For these measurements, we used the same leaves we had used to measure gas exchange. Early in the morning (06:00 h) on a 12-h dark-adapted leaf, maximal fluorescence yield of the dark-adapted leaf (F_m) was determined by applying a saturating light pulse of 6,000 µmol(photon) m^{-2} s^{-1}, 1.0-s duration. At midday (11:00–12:00 h) and under actinic light [230 µmol(photon) m^{-2} s^{-1}] we also determined the effective quantum yield of PSII photochemistry (Φ_{PSII}), electron transport rate (ETR), and nonphotochemical quenching (NPQ), as follows (Maxwell and Johnson 2000):

$$F_v/F_m = (F_m - F_0)/F_m \quad (5)$$

$$\Phi_{PSII} = (F_m' - F_s)/F_m' \quad (6)$$

$$ETR = 0.5\,(I_e \times \Phi_{PSII}) \quad (7)$$

$$NPQ = (F_m - F_m')/F_m' \quad (8)$$

where F_0 and F_m denote the minimal and maximal fluorescence yield of the dark-adapted state; F_v/F_m stands for the maximal quantum yield of PSII photochemistry; F_s and F_m' represent steady-state and maximal fluorescence yield of the light-adapted state, respectively; I_e indicates the PAR absorbed by the leaf, 0.5 is the fraction of quanta absorbed by PSII relative to PSI.

Proline content was determined in fresh leaf samples. A leaf sample was placed in a test tube containing 2 ml of ethanol and boiled in water bath until ethanol evaporation. In sequence, 2 ml of water was added, the tube was agitated and centrifuged (5 min, 5,000 × *g*), and a 100-µl extract was added to 1 ml of 1% ninhydrin (w/v, aqueous solution in 60% acetic acid). After reaction at 95°C

(20 min), the sample was cooled down to room temperature and then toluene was added (3 ml) and the sample left to stand for phase separation. The absorbance was read at 520 nm (Gibon *et al*. 2000). Total nonstructural carbohydrates (TNC) of leaves were assessed by hydrolyzing the starch in the leaf sample with 0.5 M NAOH and the precipitate removed by centrifugation (15 min at 1,000 × *g*). After reaction with phenol-sulfuric acid, the absorbance of the sample was recorded at 490 nm. LMA was calculated as the leaf mass to leaf area ratio, we also determined leaflet size (leaf area) with an area meter (*Li-3000*, *Li-Cor*, Lincoln, US). Total dry matter of plants (W_T) was obtained by oven-drying at 72°C to constant mass. Leaf water potential (Ψ_L) was determined at 06:00 and 12:00 h in one leaf per plant with a pressure chamber (*1505 D*, *PMS Instrument Company*, Albany, USA).

Statistical analysis: Light intensity and temperature were similar in both experiments, but the relative humidity inside the growth chamber was a little higher than that in the greenhouse. Although temperature and light intensity were similar in both environments [mean PAR of about 8.6 mol(photon) m^{-2} d^{-1}], light quality could be different, so instead of analyzing the whole study as a factorial experiment (2 × 2) we analyzed the data as two separate experiments. Each experiment was a completely randomized design with two treatments (50 and 100% FC) and five replications (plants) per treatment. Data were subjected to analysis of variance (*ANOVA*). Statistical analyzes were performed using *SigmaPlot 11.0* (*Systat Software*, Richmond, USA).

Results

Experiment 1

The physical environment: In the greenhouse, RH was 70–80% and mean temperature of 27.5°C (ranging from 26°C at night to 29°C at midday), and mean ambient CO_2 concentration was 410 ± 17 ppm (day/night of 400/420 ppm). Confirming previous PAR data, PAR ranged from 7.99 to 9.28 mol(photon) m^{-2} d^{-1}. During the experimental periods, soil moisture (measured in the morning) remained at 21% (50% FC) and 31%, in the soil at 100% FC.

Physiological parameters: In Experiment 1 (ambient CO_2), P_{Nsat}, P_{Nmax}, and g_s decreased by 33.5, 21.1, and 46.4% under water stress, whereas V_{cmax25} and J_{max25} decreased by 19.1 and 16.4%, respectively. WUE_i increased by 24% under drought (Table 1), consistent with a reduction of CUW (32.2%) under water stress (Fig. 1, *P* values in Table 2). There was no effect of drought on the CO_2-compensation point (Γ), VPD_L (Table 1), the F_v/F_m ratio and NPQ, but Φ_{PSII} and ETR decreased about 28% under drought (Fig. 2), whereas and LMA increased by 8.3% under water stress (Fig. 3*A*). Although, the proline content (Table 1) and TNC and were not affected by drought, W_T and A_L decreased by 42 and 48%, respectively, under water stress (Fig. 3). Regarding plant allometry, leaf number, leaflet number, and leaf size were reduced by 30–40% under drought (Table 1). Leaf water potential (Ψ_L) ranged from –0.21 MPa (in well-watered plants at 06:00 h) to –0.78 MPa at midday under water stress (Table 1).

Experiment 2

Physiological parameters: Under elevated CO_2, there was no effect of drought on P_{Nsat}, P_{Nmax}, g_s, V_{cmax25}, and J_{max25} (Fig. 1, Table 2). The same was true for VPD_L, Γ, and WUE_i (Table 1), the F_v/F_m ratio and NPQ (Fig. 2*A,D*), and LMA (Fig. 3*A*), which were not affected by water stress. On the other hand, CUW was reduced by 40% under drought (Fig.1*D*), and ETR and Φ_{PSII} were 32% lower in water-stressed plants (Fig. 2*B,C*). Similar to that what we found under ambient CO_2 conditions, proline (Table 1) and TNC contents of leaves were not affected by water stress (Fig. 3*B*). Total leaf area (A_L) and W_T were reduced by 41% under water stress (Fig. 3*C,D*), and with respect to plant allometry, leaf and leaflet number were not affected by drought, but in agreement with the results of Experiment 1, the leaflet size was reduced (40.6%) under water stress (Tables 1, 2). Leaf water potential (Ψ_L) measured early in the morning and at midday were slightly higher than those recorded in Experiment 1. The Ψ_L ranged from –0.19 MPa in well-water plants (at 06:00 h) to –0.53 MPa at midday under water stress (Table 1), and during the experimental periods soil moisture (on a percentage basis) was as described for Experiment 1.

Comparison between experiments: In comparison to plants grown under ambient CO_2, P_{Nsat} increased by 64% (6.21 to 10.21 μmol m^{-2} s^{-1}) under elevated CO_2 in well-watered plants, and 153% (4.13–10.43 μmol m^{-2} s^{-1}) in plants subjected to water stress (Fig. 1*A*). In well-watered plants, g_s was lower under elevated CO_2, with a reduction of 24% (0.084 to 0.064 mol m^{-2} s^{-1}). Compared to ambient CO_2, g_s increased by 49% under elevated CO_2 in water-stressed plants. Therefore, g_s was similar between water regimes under elevated CO_2 (mean of 0.066 mol m^{-2} s^{-1}, Fig. 1*B*). It is important to note that VPD_L was similar in both experiments, with a mean of 1.58 kPa (Table 1). Over water regimes, mean P_{Nmax} values were similar in both CO_2 experiments (Fig. 1*C*). It is also worth noting that drought had a lesser effect on J_{max25} and V_{cmax25} at elevated CO_2 (Fig. 1*E,F*), and across experiments, the J_{max25}/V_{cmax25} ratio was about 1.80, with a high correlation ($r = 0.80$, $P<0.001$). The CO_2-compensation point (Γ) showed little variation over experiments (Table 1), with a general mean of 58 ppm. In comparison with plants grown in ambient CO_2, WUE_i improved by 86% under elevated CO_2 (Table 1). Although WUE_i only increased under water stress at ambient CO_2 (Table 1), in both experiments, the CUW was lower under water stress (32–40%). Neither elevated CO_2 nor drought affected the F_v/F_m ratio, but in both experiments, Φ_{PSII} and ETR were about 30% lower in the plants subjected to water stress. However, within a water regime, Φ_{PSII} and ETR were similar in both experiments (Fig. 2*B,C*). Contrary to expectation, there was no effect of water regimes on NPQ, but on average, plants grown under enriched CO_2

Table 1. Leaf water potential (Ψ_L), leaf proline content, leaf and leaflets number, leaflet size, CO_2-compensation point (Γ), leaf-to-air vapor pressure difference (VPD_L), intrinsic water-use efficiency (WUE_i) in *Carapa surinamensis* at two water regimes (soil at 50% FC and soil at 100% FC) under two grown conditions (Experiment 1 at ambient CO_2) and Experiment 2 (at elevated CO_2). Means followed by *the same letter* do not differ significantly (*F* test at $P = 0.05$). Each value stands for the mean (± SD) of five plants ($n = 5$).

Parameter/Treatment	Experiment 1		Experiment 2		Mean	Mean
	100% FC	50% FC	100% FC	50% FC	Exp. 1	Exp. 2
Ψ_L, at 06:00 [MPa]	–0.21 ± 0.02^a	–0.35 ± 0.00^b	–0.19 ± 0.01^a	–0.32 ± 0.02^b	–0.28 ± 0.07	–0.25 ± 0.07
Ψ_L at 12:00 [MPa]	–0.66 ± 0.03^a	–0.78 ± 0.02^b	–0.47 ± 0.02^a	–0.53 ± 0.03^b	–0.72 ± 0.06	–0.50 ± 0.04
Proline [nmol cm^{-2}]	0.12 ± 0.06^a	0.13 ± 0.04^a	0.23 ± 0.15^a	0.18 ± 0.05^a	0.12 ± 0.05	0.21 ± 0.11
Leaf number	21.6 ± 1.1^a	14.4 ± 1.8^b	11.8 ± 1.9^a	11.2 ± 1.5^a	18.0 ± 4.1	11.5 ± 1.6
Leaflet number	114.0 ± 24.4^a	68.0 ± 8.2^b	77.6 ± 16.5^a	58.6 ± 14.8^a	91.0 ± 29.7	68.1 ± 17.8
Leaflet size [cm^2]	107.8 ± 16.0^a	74.0 ± 15.4^b	170.7 ± 29.6^a	101.4 ± 27.1^b	90.9 ± 23.2	136.0 ± 45.3
Γ [ppm]	54.2 ± 4.4^a	59.8 ± 6.2^a	59.5 ± 1.2^a	57.6 ± 7.6^a	57.0 ± 5.8	58.6 ± 5.2
VPD_L [kPa]	1.51 ± 0.05^a	1.60 ± 0.11^a	1.58 ± 0.11^a	1.65 ± 0.13^a	1.55 ± 0.09	1.61 ± 0.12
WUE_i [µmol mol^{-1}]	77.4 ± 9.9^b	96.1 ± 10.1^a	164.0 ± 16.5^a	159.5 ± 14.8^a	86.8 ± 13.6	161.8 ± 15.0

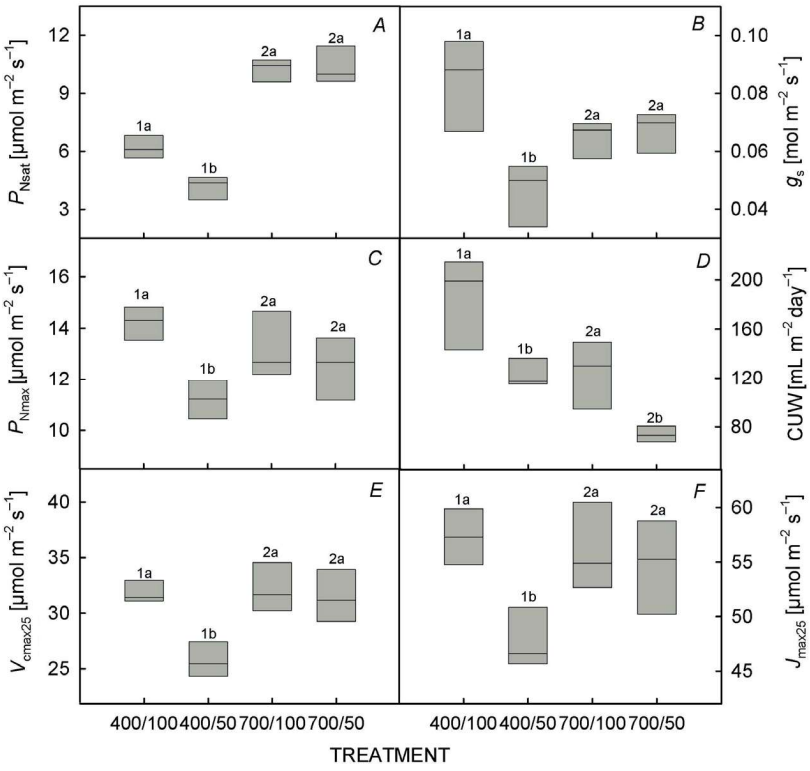

Fig. 1. Physiological parameters of *Carapa surinamensis* in response to two water regimes (soil at 50% FC and 100% FC), under two growth conditions: Experiment 1 (at ambient CO_2, about 400 ppm) and Experiment 2 (at elevated CO_2, 700 ppm). Light-saturated net photosynthetic rate (P_{Nsat}, *A*), stomatal conductance (g_s) at light saturation (*B*), light and CO_2-saturated net photosynthetic rate (P_{Nmax}, *C*), consumptive use of water (CUW, *D*), maximum carboxylation rate of Rubisco at 25°C (V_{cmax25}, *E*), and maximum electron transport rate at 25°C (J_{max25}, *F*). The numbers (1 or 2) above the box indicate the experiment; boxes (within experiment) with *the same letter* above are not significantly different (*F* test $P \leq 0.05$).

dissipated less energy as heat (Fig. 2*D*), which contributed for P_{Nsat} to increase under elevated CO_2.

With respect to plant allometry, we found a large difference in the leaflet size between experiments, which increased by 37% under water stress and by 58% in well-watered plants at elevated CO_2 (*i.e.*, increase of 50% over water regimes, Table 1). Because there was a reduction in leaf number and leaflet number in Experiment 2 (ambient *vs*. elevated CO_2, Table 1), A_L was similar in both experiments within a water regime (Fig. 3*D*). W_T was greater under elevated CO_2 in well-watered plants, but in both experiments, it declined by 42% under water stress (Fig. 3*C*).

Table 2. Summary of the analysis of variance (*F* values and *P* values in parenthesis) on the effect of water regimes on physiological parameters of *Carapa surinamensis* in Experiment 1 (at ambient CO_2) and Experiment 2 (at elevated CO_2). *Bold numerals* indicate significance ($P \leq 0.05$). Abbreviations and acronyms as described in the abbreviation´s section.

Parameter	Ambient CO_2	Elevated CO_2
P_{Nsat}	26.721 (**< 0.001**)	0.143 (0.715)
g_s	18.530 (**0.003**)	0.202 (0.665)
P_{Nmax}	36.518 (**< 0.001**)	0.722 (0.420)
CUW	9.084 (**0.017**)	15.213 (**0.005**)
V_{cmax25}	37.558 (**< 0.001**)	0.190 (0.674)
J_{max25}	21.976 (**0.002**)	0.240 (0.637)
F_v/F_m	3.828 (0.086)	0.0611 (0.811)
Φ_{PSII}	17.869 (**0.003**)	30.299 (**< 0.001**)
ETR	17.869 (**0.003**)	30.299 (**< 0.001**)
NPQ	1.421 (0.267)	0.329 (0.582)
LMA	6.816 (**0.031**)	0.0164 (0.901)
TNC	4.780 (0.060)	0.000402 (0.984)
W_T	6.855 (**0.031**)	8.911 (**0.017**)
A_L	10.067 (**0.013**)	21.779 (**0.002**)
Ψ_L (6 h)	162.390 (**< 0.001**)	273.067 (**< 0.001**)
Ψ_L (12 h)	56.277 (**< 0.001**)	12.536 (**0.008**)
Proline	0.189 (0.675)	0.225 (0.648)
Leaf number	56.348 (**< 0.001**)	0.305 (0.596)
Leaflet number	15.910 (**0.004**)	3.683 (0.091)
Leaflet size	11.616 (**0.009**)	14.940 (**0.005**)
Γ	2.668 (0.141)	0.284 (0.608)
VPD_L	3.168 (0.113)	0.891 (0.373)
WUE_i	8.702 (**0.018**)	0.202 (0.665)

Discussion

The RH in the greenhouse was a slightly lower (70–80% *vs*. 80–90% in the growth chamber) and also there could be some differences in light quality between the growth chamber and the greenhouse. Thus, we chose to analyze the data as two experiments instead of using a 2 × 2 factorial design. However, some comparison can be made taking into account that temperature and light intensity were similar in both environments, and that the RH gradient between them was small (≈10%). The direct effect of a small gradient in temperature seems to have little effect on photosynthetic rates (Lloyd and Farquhar 2008, Yamori *et al*. 2014). Furthermore, in a previous study we found that in the ranges of 25–30°C (temperature) and 72–77% (RH), g_s did not decline, actually it tended to increase with temperature (Mendes and Marenco 2017), so it seems unlikely that the small differences in RH and temperature had a significant effect on carbon uptake.

In well-watered plants, predawn Ψ_L was lower than that one expects from a soil at field capacity (about –0.01 MPa, Slatyer 1967). This shows that there was disequilibrium between pre-dawn Ψ_L and soil water potential, which seems to occurs most of the time (Donovan *et al*. 2001), and following the increase in transpiration Ψ_L decreased by midday, as expected. Disequilibrium between soil and plant water potential can indicate that the overnight equilibration period is not enough to eliminate internal gradients in water content (Donovan *et al*. 2001).

In well-watered plants, we found an increase of 64% in P_{Nsat} under elevated CO_2, but that increase was still higher (152%) in plants subjected to water stress. It is worth noting that the exposure to elevated CO_2 alleviated the negative effect caused by water stress on P_{Nsat}. The positive effect of the CO_2 enrichment on P_{Nsat} was greater than that reported by Ainsworth and Long (2005), who found that photosynthesis rose about 35% under elevated CO_2 across several C_3 species; whereas Nowak *et al*. (2004) reported that photosynthesis increased by 30–50% and net primary production about 20% under elevated CO_2.

In well-watered plants, g_s was 24% lower under elevated CO_2. Indeed, the most common response is a decrease of g_s under elevated CO_2 (Curtis and Wang 1998, Ainsworth and Long 2005, Leakey *et al*. 2012). Furthermore, we showed that subjecting the plants to elevated CO_2 negates the effect of water stress on g_s (*i.e*., there was no difference in g_s under elevated CO_2). Consequently, P_{Nsat} did not decline in water-stressed plants under CO_2 enrichment. The sharp drop of g_s at ambient CO_2 under water stress was attributed to the lower availability of water in the soil at 50% FC, rather than to difference in VPD_L, which remained rather constant across experiments (Table 1).

Under ambient CO_2 conditions, P_{Nmax} was lower under water stress (*P* value in Table 2), which is consistent with the strong relationship between g_s and P_N (Farquhar and Sharkey 1982, Lloyd and Farquhar 2008, Marenco *et al*. 2017). However, within a water regime, P_{Nmax} was similar in both experiments, particularly, in well-watered plants. This suggests absence of photosynthetic acclimation of plants subjected to elevated CO_2, which has also been found in other tree species (Ainsworth and Rogers 2007, Leakey *et al*. 2012). In well-watered plants, V_{cmax25} and J_{max25} were similar in both experiments, which is in agreement with the result of a meta-analysis carried out by Ainsworth and Long (2005) who found just a slight decline (6%) in V_{cmax} and no effect at all of elevated CO_2 on J_{max}. The decline in V_{cmax25} and J_{max25} found under water stress at ambient CO_2 (Fig. 1*E*) suggests that nonstomatal limitation to photosynthesis increased at ambient CO_2. In *Olea europaea*, for example, at ambient CO_2 conditions, up to 60% of photosynthesis limitation recorded under severe drought can be ascribed to a decline in mesophyll conductance (Perez-Martin *et al*. 2014). The J_{max25}/V_{cmax25} ratio was similar in both experiments (about 1.80) and

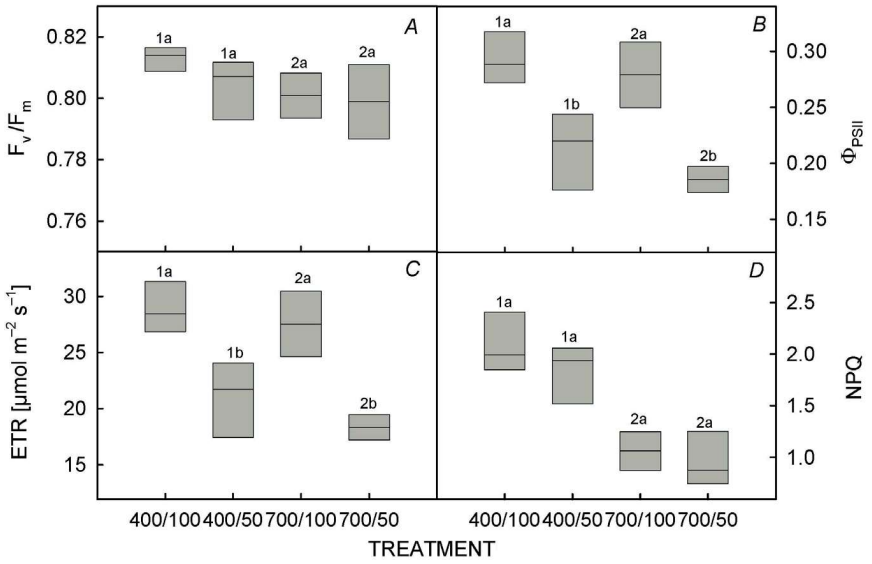

Fig. 2. Fluorescence parameters of *Carapa surinamensis* in response to two water regimes (soil at 50% FC and 100% FC), under two growth conditions: Experiment 1 (at ambient CO_2, about 400 ppm) and Experiment 2 (at elevated CO_2, 700 ppm). Maximal quantum yield of PSII photochemistry (F_v/F_m, *A*); effective quantum yield of PSII photochemistry (Φ_{PSII}, *B*), electron transport rate (ETR, *C*), and nonphotochemical quenching (NPQ, *D*). The numbers (1 or 2) above the box indicate the experiment; boxes (within experiment) with *the same letter* above are not significantly different (*F* test $P \leq 0.05$).

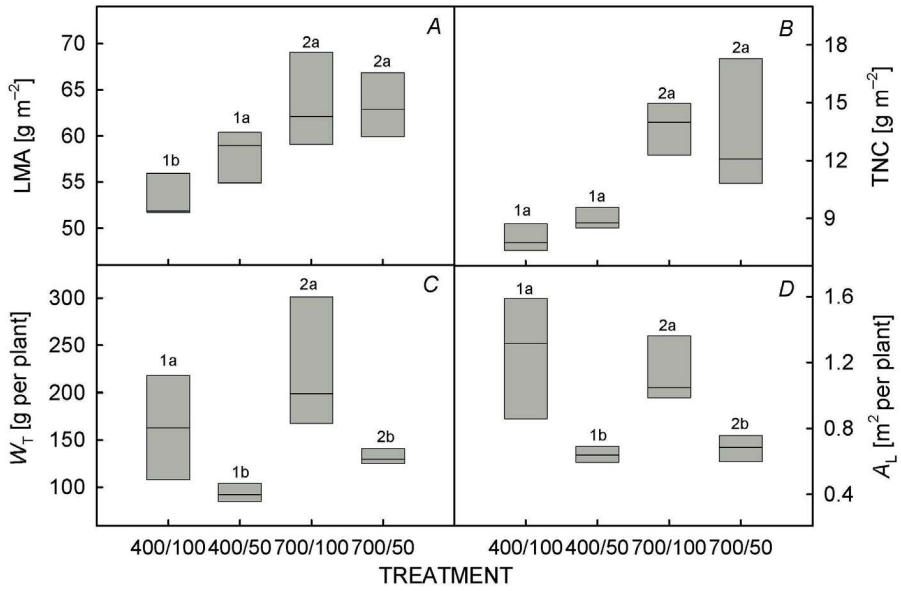

Fig. 3. Leaf mass per area ratio (LMA, *A*), total nonstructural carbohydrate (TNC) of leaves (*B*), total dry matter (W_T, *C*), and total leaf area (A_L, *D*) of *Carapa surinamensis* in response to two water regimes (soil at 50% FC and 100% FC), under two growth conditions: Experiment 1 (at ambient CO_2, about 400 ppm) and Experiment 2 (at elevated CO_2, 700 ppm). The numbers (1 or 2) above the box indicate the experiment; boxes (within experiment) with *the same letter* above are not significantly different (*F* test $P \leq 0.05$).

close to the value of 1.67 reported by Medlyn *et al*. (2002). However, it is lower than that of 2.52 (V_{cmax} of 42.2 µmol m^{-2} s^{-1}) reported by Manter and Kerrigan (2004) or the value of 2.1 (V_{cmax} of 51.0 µmol m^{-2} s^{-1}, for tropical forest species) found by Wullschleger (1993). Our lower V_{cmax} (and J_{max}) can be attributed to the fact our plants were grown at mild irradiance. In fact, compared to shade leaves, P_{Nmax} can double in sun leaves (Marenco *et al*. 2017). The high correlation between J_{max} and V_{cmax} indicates that carbon assimilation is tightly regulated by the amount of resources allocated to the components of the photosynthetic process (Farquhar *et al*. 1980, Wullschleger 1993).

WUE_i improved at ambient CO_2 because the decline of g_s was steeper than that of P_{Nsat} (46% *vs.* 33%) under water stress. On the other hand, WUE_i did not increase in water-stressed plants at elevated CO_2 because there was no

effect of water stress on g_s or P_{Nsat} under that condition. In comparison with the ambient conditions, the enhancement of WUE_i in Experiment 2 (86%) can be explained by the increase of P_{Nsat} at elevated CO_2. This improvement is within the range of 76–86% reported by Drake *et al*. (1997). The CUW drastically dropped under water stress, particularly under elevated CO_2 (Fig. 1*D*). This suggests that improvement of WUE_i and reduction of CUW under elevated CO_2 would help the plant to endure prolonged droughts.

Irrespective of CO_2 conditions, similar F_v/F_m values under drought indicate that exposure to water stress did not impair PSII efficiency, as F_v/F_m values close to 0.80 are typically found in nonstressed leaves (Björkman and Demmig 1987). The decline in ETR and Φ_{PSII} under water stress was associated with a drop in P_{Nsat} but that association was not observed when P_N was measured at saturated CO_2 conditions (P_{Nmax}), which indicates that the reduction in Φ_{PSII} and ETR is overcome by the increase in CO_2 concentration. This occurs because at saturated CO_2, photorespiration decreases substantially (Wingler *et al*. 2000). In rice, severe water stress leads to production of superoxide and malondialdehyde (an indicator of lipid peroxidation) and a drop in Φ_{PSII} and F_v/F_m (Yang *et al*. 2014). In this study, it seems unlikely that such severe damage could have occurred as the F_v/F_m ratio did not decrease under drought (Table 2). Instead the decline in ETR and Φ_{PSII} under water stress can be attributed to an increase in the reduced plastoquinone pool, which can lead to a decrease in photochemical quenching (Maxwell and Johnson 2000). Although it has been found that NPQ can rise under water stress (Tezara *et al*. 1999, Liu *et al*. 2017), that effect was not found in this study, which suggests that the water stress applied was severe enough as to induce partial stomatal closure, but at the same time not too strong to increase significantly the loss of energy as heat, perhaps because the plants were grown at a mild light intensity [200 μmol(photon) m^{-2} s^{-1}].

In both experiments, W_T was substantially decreased under water stress (Fig. 3), which can be largely ascribed to the strong reduction in leaf area experienced by water-stressed plants. Under ambient CO_2 conditions, W_T also declined in response to a significant reduction in P_{Nsat} (Table 2). The strong effect of water stress on leaf area production (A_L) can occur because cell division, leaf expansion, and protein synthesis are impaired by water stress; actually these adjustments occur well before drought could induce stomatal closing (Bradford and Hsiao 1982, Tardieu *et al*. 2015). Irrespective of the water regimes, W_T increases by 40% in plants subjected to elevated CO_2 which is in tandem with the increase of P_N with CO_2 enrichment. It has been found that across several species above-ground biomass increases by about 20–30% in plants subjected to elevated CO_2 (Curtis and Wang 1998, Ainsworth and Long 2005), whereas starch content increased by 60–80% under elevated CO_2 (Nowak *et al*. (2004). LMA was greater under elevated CO_2, which is consistent with the results reported by others (Eamus *et al*. 1993, Ainsworth and Long 2005, Aspinwall *et al*. 2017), and that can be attributed at least in part to the greater content of TNC under elevated CO_2 (Fig. 3). One can see in Fig. 3 that within a water regime, A_L was similar in both experiments, but with a large difference in leaflet size (increase of 50% at elevated CO_2) and leaflet number (decrease of 25% in Experiment 2), which shows that at elevated CO_2 a drop in leaf number was offset by an increase in leaf size.

Leaf water potential (Ψ_L) was lower under drought at ambient CO_2, even thought there was no difference in proline content between water regimes, which was unexpected as proline is an osmolyte of common occurrence in plants (Yoshiba *et al*. 1997). This suggests that (*1*) the water stress was not too strong to increase proline synthesis or (*2*) that in *Carapa* other compatible solutes, such as sugar alcohols or quaternary ammonium compounds (*e.g*., glycine betaine), can be accumulated instead of proline. For example, in several species (*e.g*., *Pisum sativum* and *Ricinus communis*) sugars are the major osmolytes (Blum 2017). We attributed the higher proline content (per unit area) under elevated CO_2 to the greater LMA observed in plants grown in that environment, as LMA is dependent on leaf thickness (Niinemets 1999).

We hypothesized that plants grown at elevated CO_2 would respond to CO_2 enrichment by improving their photosynthetic performance and their biomass gain, which was confirmed, but in contrast to our hypothesis, WUE_i did not improve at elevated CO_2. WUE_i improved under water stress at ambient CO_2 because water stress led to a stronger decline in g_s than in P_{Nsat}, but that effect was not observed at elevated CO_2 because in the latter condition there was no effect of water stress on P_{Nsat} or g_s. Although total leaf area remained rather constant within a water regime, it was observed that the drop in leaflet number under elevated CO_2 can be offset by increasing leaflet size, and that subjecting the plants to elevated CO_2 entirely negates the effect of water stress of P_{Nsat} and g_s. This study is important because it shows the potential of *Carapa* to adjust its morphology and physiology either to endure drought or to improve carbon uptake at elevated CO_2 conditions. It is shown that water stress leads to a substantial reduction in the CUW by reducing leaf area production and at ambient CO_2 also by lowering g_s. This adjustment, however, also leads to a strong reduction in total plant biomass. The elevated CO_2 conditions negated the effect of water stress on P_N, but it only mitigated the effect of water stress on biomass accumulation because of the strong reduction of leaf area in water-stressed plants. These findings widen our understanding to the effect of elevated CO_2 on the physiology of Amazonian species.

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