Croton blanchetianus modulates its morphophysiological responses to tolerate drought in a tropical dry forest

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Abstract. An understanding of variations in morphophysiological leaf traits of plant models in dry tropical forests is essential for quantifying C fluxes from forest ecosystems in response to climate changes. The present study evaluated the influences of seasonal rainfall and different light conditions on the gas exchange, nutrients, organic compounds and morphological traits in *Croton blanchetianus* Baill. trees within a fragment of Caatinga forest. Stomatal conductance (g_s) and net photosynthesis (P_N) demonstrated variations within the diurnal cycle, with maximum values at approximately midday and minimum values at predawn. The P_N and the diurnal integrated CO_2 assimilation were lower during the dry season than in the rainy season. Water use efficiency was positively correlated with P_N ($r = 0.73$) during the dry season only. However, the correlation between P_N and g_s was observed during the rainy season only ($r = 0.60$). Thus we demonstrated that *C. blanchetianus* has a remarkable ability to adapt to global climatic changes and could be considered a model in studies exploring water relationships in woody plants; consequently, this species may be important in future reforestation studies.

Additional keywords: diurnal variation, gas exchange, leaf nutrient, morphological traits, seasonal variations.

Introduction

Apprehension is emerging regarding how global changes caused by increased CO_2 concentrations in the atmosphere may alter the climate in semiarid regions and dry forests (Pereira *et al*. 2014; Santos *et al*. 2014; Murray-Tortarolo *et al*. 2016). Tropical dry forests have a prolonged dry season, and thus low water availability, high temperatures and high evapotranspiration potential are important factors that affect physiological processes and plant growth of the vegetation in these semiarid environments (Bullock *et al*. 1995).

The effects of drought in tropical dry forests are currently receiving more research attention. In Brazil, the primary tropical dry forest is the Caatinga, which occupies different vegetation types within the semiarid region of north-east Brazil (Pereira *et al*. 2014; Santos *et al*. 2014). Recent studies (Marengo *et al*. 2010; Pereira *et al*. 2014; Santos *et al*. 2014) have revealed that modifications in vegetation cover during the last century may have caused significant effects on local and global climates. Because vegetation may be considered a weather mirror with which climate interacts (Foley *et al*. 2000), modifications to the climate that affect plant physiology and the structure of vegetation are predictable.

Croton blanchetianus Baill., popularly known as black quince, is the species of interest in the present study. *C. blanchetianus* is very abundant, has a shrubby habit and forms dense populations. Additionally, *C. blanchetianus* is a pioneer species that easily propagates and grows and can be classified as a semideciduous plant because at least 50% of the leaves are lost each year but plants never become completely deciduous (Santos and Melo 2010).

The consensus is that water shortage is the most important limitation to gas exchange (Tezara *et al*. 1999), causing negative influences on growth, development and productivity, and the downregulation of photosynthesis. Water shortages also increase diffusive resistance to CO_2 at the stomata level, in mesophyll conductance or both (Flexas *et al*. 2006). Additionally, alterations in photosynthetic metabolism arising from water stress lead to a decrease in the activity of Rubisco and in the dynamics of ATP synthesis (Lawlor and Tezara 2009; Flexas *et al*. 2016). Moreover, plants experiencing water limitation show alterations in chlorophyll fluorescence quenching (Faraloni *et al*. 2011), damage to photosynthetic membranes (Krieger-Liszkay 2005), loss of pigments (Pompelli *et al*. 2010*a*), increases in the carotenoid pool (Corcuera *et al*. 2005) and alterations in soluble

carbohydrates (Silva *et al.* 2010). Furthermore, among the processes most affected by drought, photosynthesis is notable and may also be restricted by nutrient availability (Hikosaka 2004; Mendes and Marenco 2015).

Stomatal function and C uptake in response to seasonal rainfall in dry tropical forests have become more relevant, considering the current trends of atmospheric CO_2 enrichment and temperature increases on a global scale. Thus the study of drought-tolerant species is crucial, particularly native evergreen species. This work was conducted primarily to understand which morphophysiological variables contribute to the establishment of *C. blanchetianus* plants subjected to lower water availability. Therefore, the study investigated the effects of rainfall seasonality and light conditions on the gas exchange, nutrients (macro- and micronutrients), organic compounds, antioxidative metabolism and leaf morphological traits in *C. blanchetianus* trees. Thus the primary question is whether *C. blanchetianus* has morphophysiological and biochemical mechanisms for survival in the water deficit period followed by a return to high metabolic rates in the rainy season.

Materials and methods

Environmental conditions and plant species

The experiment was conducted in the semiarid region of Pernambuco State, north-eastern Brazil (8°52′32″S, 36°22′00″W; 716 m above sea level). The climate in this region is considered to have the 'BSh' type in the Köppen classification (Köppen 1948) with 598 mm of total annual rainfall distributed within a 5-month rainy season (April to September). Mean monthly temperatures range from 20°C in July to 26°C in December. Relative humidity during the day averages 57% during the dry season and 77% during the rainy season (Fig. 1). The native vegetation of the study region is a seasonally dry forest with deciduous vegetation (Dantas *et al.* 2006).

For our field measurements, we selected 10 *Croton blanchetianus* Baill. plants located within an environment protected by treetops (natural shade, irradiance less than 70% of solar transmittance) and 10 plants under full sunlight. All plants were 1–3 m tall. All data were collected during the rainy season (May–July) and the dry season (October–December) of 2011.

Photosynthetic parameters

Gas exchange parameters were measured using a portable system (Li-6400; Li-Cor). For this measurement, we selected two leaves per plant and 20 saplings each season (10 full sun plants and 10 natural shade plants). Measurements were conducted *in situ* under a clear sky and the leaf was exposed to irradiance of saturation of 1000 µmol m⁻² s⁻¹ from an light-emitting diode source, ambient CO_2 concentration (390 parts per million), air flow of 400 µmol s⁻¹, and ambient temperature and relative humidity. Data were collected at different times (0600, 0800, 1000, 1200, 1400, 1600 and 1800 hours; solar time) to assess the effect of time on stomatal conductance (g_s)
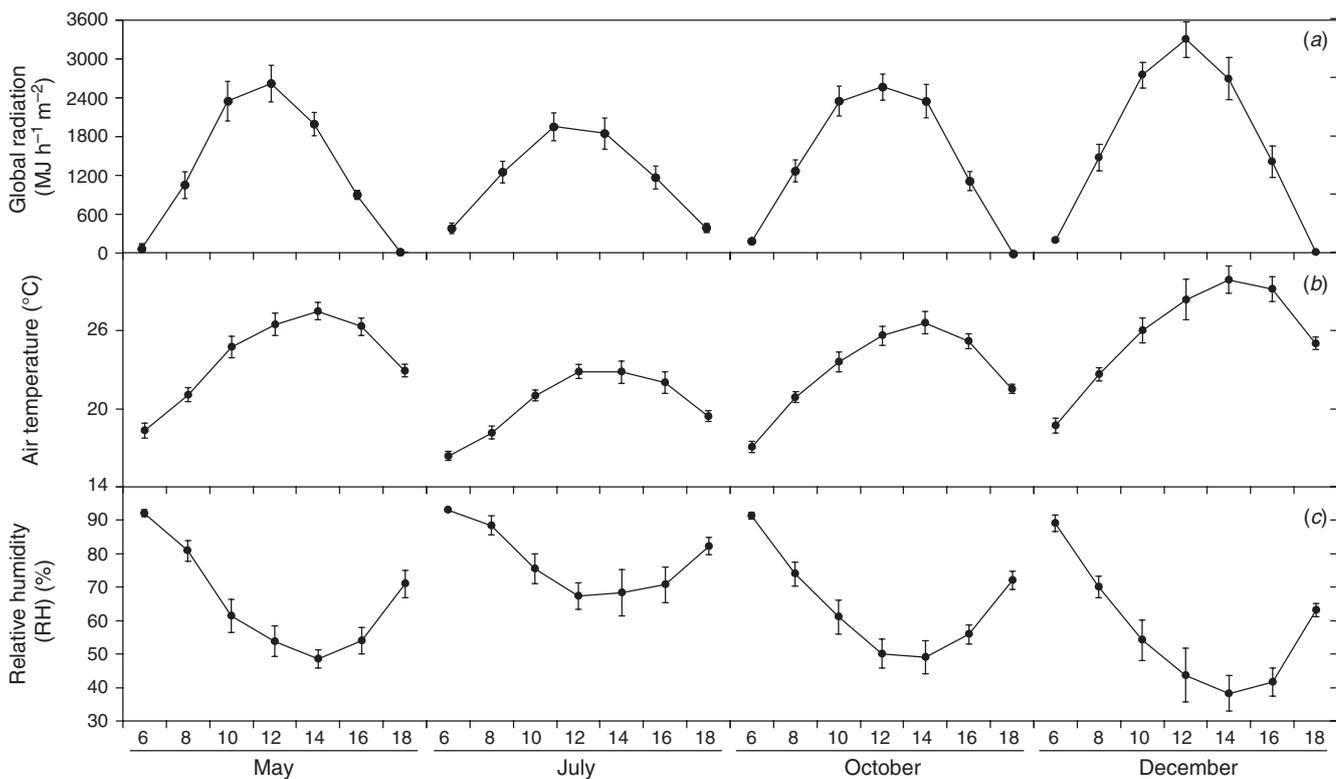

Fig. 1. Time course (hours) of (*a*) global radiation, (*b*) air temperature and (*c*) relative humidity observed in the rainy season (May–July) and the dry season (October–December) of 2011 in the semiarid region of the state of Pernambuco, north-eastern Brazil. The values represent the mean (± s.e.).

and net photosynthesis (P_N). Based on the ratio of P_N and transpiration, we calculated water use efficiency (WUE). Diurnal integrated CO_2 assimilation (integrated P_N) was obtained by integrating all measurements of P_N determined at different times of the day (Ribeiro *et al*. 2009).

Leaf area and specific leaf area

During the study period, to determine the phenology of the leaves, we were careful to collect only leaves that had completed their development in each season. Leaf area was measured for 200 healthy and expanded leaves collected in each season from both full sun and natural shade plants. The leaves were sampled randomly from different parts of the canopy of twenty trees. The leaves were then digitised using a scanner, and Image-Pro Plus software (ver. 4.5.0.29, Media Cybernetics) was used to analyse the images. From the identical plants used in the gas exchange measurements, 40 leaves were collected in each season to determine the specific leaf area (SLA), which was the ratio between leaf area and leaf mass.

Biochemical analyses

Leaf samples (discs 1.4 cm in diameter) were collected *in situ* under a clear sky between 0900 and 1000, immediately frozen in liquid N and then stored at $-20°C$ until analysis. Total soluble sugar content was determined by the phenol-sulfuric acid method (DuBois *et al*. 1956) and total free amino acids were measured using the ninhydrin reaction (Moore and Stein 1954). Chlorophyll *a+b* and total carotenoids were extracted with 80% (v/v) aqueous acetone and quantified spectrophotometrically (Pompelli *et al*. 2013). Amino acid and protein contents of crude extracts were determined using the Moore and Stein (1954) and Bradford (1976) methods, respectively.

Superoxide dismutase (SOD) was assayed as described by Pompelli *et al*. (2010*a*). Cellular damage was spectrophotometrically analysed through H_2O_2 accumulation after a reaction with KI. Malondialdehyde (MDA) accumulation was estimated as the total amount of 2-thiobarbituric acid-reactive compounds (Campos *et al*. 2012). The proline concentration was determined via the method of Bates *et al*. (1973).

Leaf nutrient contents

Concentrations of macro- and micronutrients in leaves were measured in leaf samples collected in both light conditions. Leaf dry mass was obtained after oven-drying at 60°C for 72 h. Leaves were then ground into a fine powder to pass a 40-mesh sieve, and nutrient contents (macro- and micronutrients) were determined according to Silva (2009).

Carbon isotope composition

From each tree, one of the youngest expanding leaves was collected between 0900 and 1000 hours under a clear sky and then oven-dried at 60°C. The leaves were ground to pass a 40-mesh sieve. Subsamples (1 mg) were then processed for C isotope analysis in a ThermoFinnigan Delta XL Plus Stable Isotope Ratio Mass Spectrometer (Thermo Scientific) coupled with an elemental analyser (Costech Analytical Technologies).

Data analyses

Each *C. blanchetianus* tree was one replicate. Five replicates were used to measure chlorophyll, carotenoids, nutrients and C isotope ratios, and 10 replicates were used for all other analyses. The data were analysed with a one-way ANOVA and means were compared using the Student–Newman–Keuls test at a significance level of 5%. The influence of concentration of the mineral nutrients on integrated P_N was evaluated using a canonical correlation analysis (Hardoon *et al*. 2004). The correlations among the parameters of gas exchange were also examined. The statistical analyses were performed using the software Sigma Plot ver. 11.0 (Systat Software, Inc.).

Results

Environmental conditions

The global radiation average varied throughout the year, with less intercepted global radiation during the rainiest months. In December, the greatest intercepted global radiation (11 654 MJ h^{-1} m^{-2}) was observed (Fig. 1). These values averaged 66.4% greater than the levels recorded in July (rainy season).

Precipitation extremes were observed in May (the wettest month, 120 mm) and in December (the driest, 0 mm) (Fig. 2*a*). Soil water storage ranged from 24.4 mm in the dry season to 49.3 mm in the rainy season (Fig. 2*b*). The evapotranspiration rate was at a maximum in December (4.8 mm) and a minimum in July (2.1 mm; Fig. 2*c*). The mean maximum PAR was ~297 µmol m^{-2} s^{-1} and 572 µmol m^{-2} s^{-1} for the rainy and dry seasons, respectively (Fig. 3*a*).

Seasonal and diurnal cycles in gas exchange parameters

Photosynthesis (P_N), stomatal conductance (g_s), internal-to-ambient CO_2 concentration (C_i : C_a ratio), water use efficiency (WUE), transpiration (E), leaf-to-air vapour pressure deficit (VPD) and leaf temperature (T_{leaf}) displayed pronounced seasonal and diurnal cycles. P_N and g_s were strongly affected by drought in December, declining dramatically with increasing soil water storage deficit (Fig. 3). Simultaneously, WUE dropped to values ranging below 6 mmol CO_2 mol^{-1} H_2O (Fig. 4). Comparing the months with extremes of precipitation (May and December), we observed a decline in P_N (60%), WUE (74%), g_s (34%) and transpiration (16%) in December.

Stomatal conductance varied throughout the diurnal cycle under ambient temperature and humidity. However, the amplitude of the diurnal cycle was greater in the rainy season (Fig. 3): g_s increased in the morning with maximal values between 0800 and 1200 hours (a mean of 62 mmol m^{-2} s^{-1} in May and 44 mmol m^{-2} s^{-1} in December) and then declined and attained the lowest values in the late afternoon (Fig. 3). P_N followed a similar trend to that of g_s in the rainy season only, in which maximum values of 18 µmol CO_2 m^{-2} s^{-1} were recorded (Fig. 3*b*). In the dry season, P_N did not follow that trend and remained below 6 µmol CO_2 m^{-2} s^{-1} (Fig. 3*b*). The C_i : C_a ratio, VPD and T_{leaf} also followed the same trend as g_s in both seasons (Fig. 4*b*).

A strong and positive relationship was detected between WUE and P_N ($r = 0.73$; $P \le 0.0001$) during the dry season only, suggesting that WUE was fundamental to the decrease in
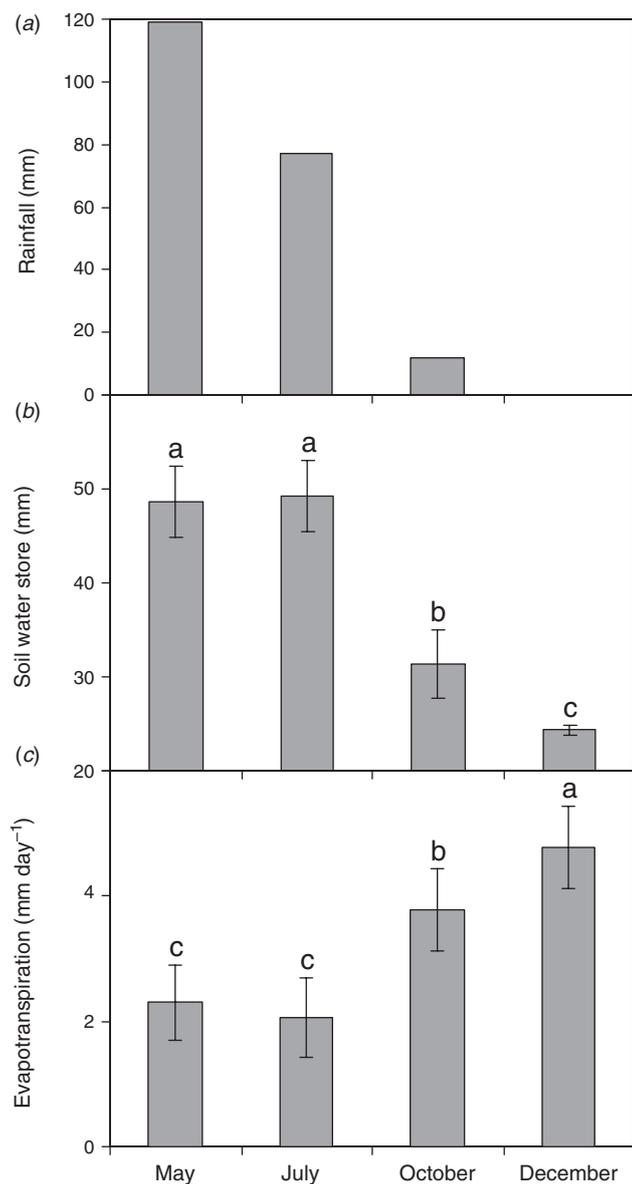
Fig. 2. (*a*) Rainfall, (*b*) soil water storage and (*c*) evapotranspiration (C) observed in the rainy season (May–July) and the dry season (October–December) of 2011 in the study area. The values represent the mean (± s.e.).

Biochemical analyses

Water deficit stress in December significantly increased concentrations of SOD, MDA and H_2O_2, but the increase was even more significant in proline levels. If we compare the two light conditions, only MDA and proline showed significant differences (Fig. 6). More relevant information was obtained when biochemical data were analysed via Pearson's correlations. We showed that although SOD activity showed a downward trend in full sun plants, SOD activity was not correlated with light, drought, or even with the elevation of H_2O_2, MDA or proline ($P \geq 0.05$). However, a negative correlation was detected between light and H_2O_2 ($r = -0.33$) (i.e. H_2O_2 decreased significantly in natural shade plants). Although we did not present the activity of catalase (CAT) and ascorbate peroxidase (APX) enzymes in this work, a strong correlation was observed between elevation of H_2O_2 and the accumulation of MDA ($r = 0.74$) and proline synthesis ($r = -0.70$). Furthermore, we showed that elevation of the MDA concentration was the key factor that culminated in proline synthesis ($r = 0.85$). Moreover, these data suggest that a decrease in CAT and APX activities must have been a strong cause of the elevation of H_2O_2 levels and, consequently, the production of MDA and the synthesis of proline as a way to dissipate the reducing power in NADPH in this reduced form.

Leaf area and SLA

Specific leaf area was higher during the rainy season ($23.5 \pm 2.1 \, m^2 \, kg^{-1}$ in natural shade plants; $20.3 \pm 1.3 \, m^2 \, kg^{-1}$ in full sun plants) than in the dry season. Leaf area was 9.3 ± 0.7 and $12.1 \pm 1.2 \, cm^2$ in full sun and natural shade plants, respectively, in the rainy season. However, leaf area and SLA showed no differences in the dry season ($4.0 \pm 0.4 \, cm^2$ and $7.7 \pm 0.7 \, m^2 \, kg^{-1}$ for natural shade plants and $3.8 \pm 0.3 \, cm^2$ and $7.3 \pm 0.2 \, m^2 \, kg^{-1}$ for full sun plants, respectively; Fig. 7).

Organic compounds and leaf nutrient contents

Carbohydrate, amino acid, soluble protein and total protein concentrations were higher in the rainy season than in the dry season for both light conditions. However, during the dry season, starch was 47% and 93% higher than that in the rainy season for natural shade and full sun plants, respectively (Table 1), suggesting that a reduction occurred in the level of export of the assimilates produced to the sink.

Total chlorophyll was significantly affected by drought (Table 1). Additionally, water shortages increased the chlorophyll *a/b* ratio, carotenoid : chlorophyll ratio and total carotenoids (Table 1). Significant differences were detected between seasons in full sun and natural shade leaves for N, N : P ratio, Mg and S (Table 2). However, P was significantly different between seasons in full sun leaves only, whereas K was significantly different between seasons in natural shade leaves. However, Ca did not show significant variations between treatments (Table 2). In both light conditions, C isotope composition ($\delta^{13}C$) increased with the increase in drought intensity (Table 2); namely, in the rainy season, the $\delta^{13}C$ values were more negative (–28.3‰ for full sun and –30.9‰ for natural shade; Table 2). Based on the canonical correlation analysis, a robust correlation between P_N and leaf nutrients

P_N under soil water storage deficit. By contrast, P_N was tightly coupled with g_s in the rainy season only ($r = 0.60$; $P \leq 0.001$), confirming that P_N was strongly dependent on g_s in the period of greater water availability. Stomatal conductance was negatively correlated with VPD ($r = -0.61$; $P \leq 0.001$) in the dry season only. In the afternoons of the rainy season, g_s decreased in conjunction with decreases in T_{leaf} ($r = 0.83$; $P \leq 0.0001$), whereas during the dry season, the correlation between g_s and T_{leaf} became less significant ($r = 0.59$; $P \leq 0.05$). Independent of light conditions, the factor that controlled g_s was the relative humidity ($r = 0.42$; $P \leq 0.05$) and not the T_{leaf} ($P = 0.69$). In the dry season, the integrated P_N values of natural shade and full sun plants averaged 66% and 64% lower than the rainy season values, respectively (Fig. 5).
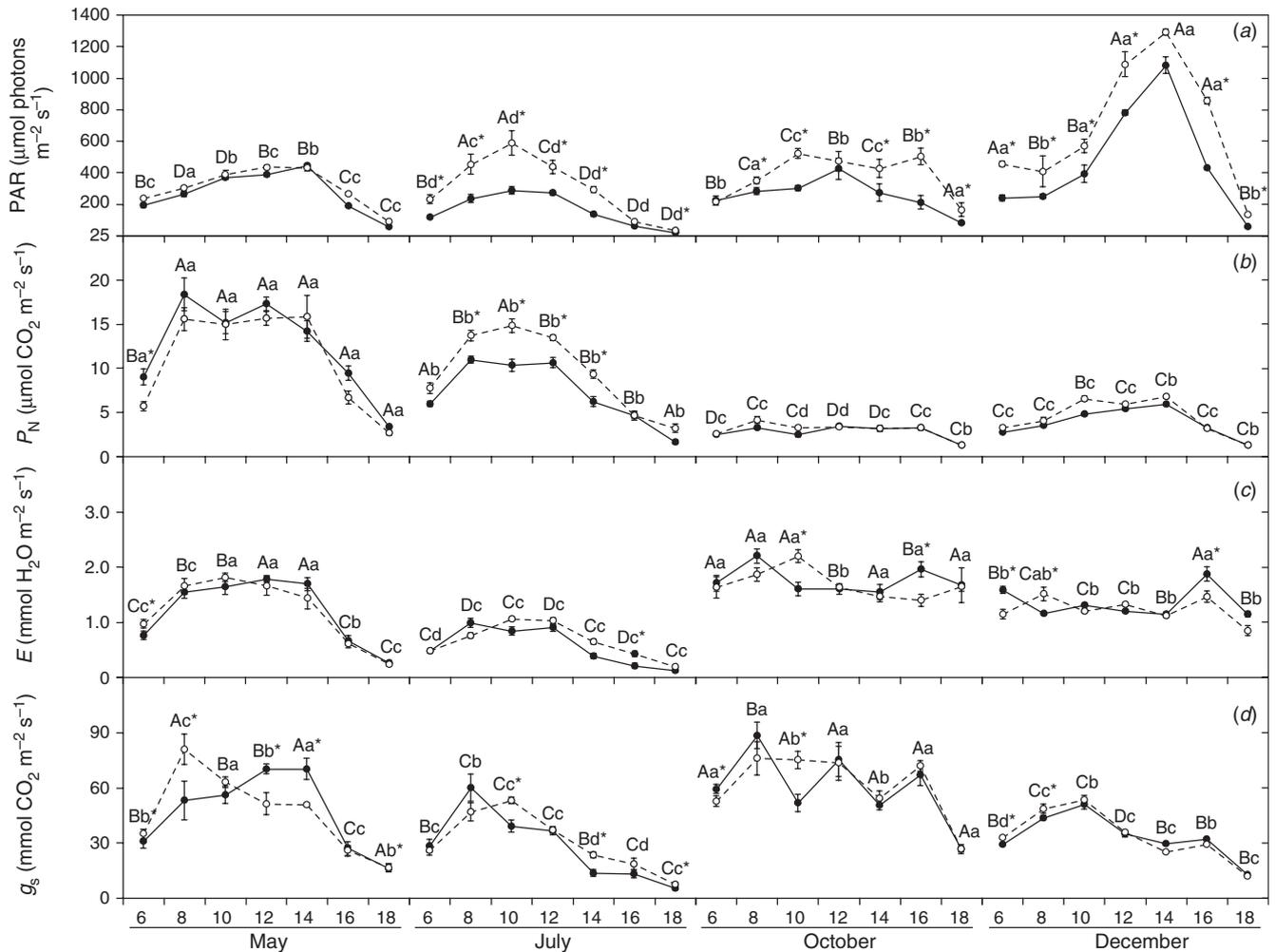
Fig. 3. Time course (hours) of (*a*) PAR, (*b*) net CO_2 assimilation rate (P_N), (*c*) transpiration (E) and (*d*) stomatal conductance (g_s) measured for full sun (open symbols) and natural shade (black symbols) *Croton blanchetianus* plants. Data were collected during the rainy season (May–July) and the dry season (October–December) of 2011. Significant seasonal differences among the means are represented by different capital (full sun plants) or lowercase letters (natural shade plants; Newman–Keuls test at $P \leq 0.01$). Asterisks denote significant differences between the means of the full sun and natural shade leaves within the same month and time (*t*-test, at $P \leq 0.05$). Each point represents the mean ($\pm$ s.e.), $n = 10$.

($r = 0.88$, $P < 0.0001$) was observed (Table 3). According to the canonical correlation for leaf nutrients and P_N, the element with the greatest influence on leaf photosynthetic rates was N (canonical coefficient = –0.515; Table 3).

Discussion

In the present study, we provide a seasonal profile of the diurnal photosynthetic performance of *C. blanchetianus* trees grown under two light conditions (full sun and natural shade). Additionally, we show that water deficit stress led to significant alterations of morphophysiological traits. We observed that photosynthesis in *C. blanchetianus* was greatly influenced by the effects of environmental factors, primarily the decline in water availability. Limitation of photosynthetic activity under severe water deficit in December was attributed to stomatal function, because g_s values were ~34% lower than those in the rainy season; thus, a decline of ~60% in P_N was

primarily caused by stomatal limitation. Nevertheless, $C_i : C_a$ values of <0.5 denote stomatal level deficiency, since even with a low stomatal opening and influx of CO_2, photosynthesis can remain elevated for some time before falling. The drastic reduction of WUE (74%) in the dry season was consistent with this result and, coupled with the low g_s, C fixation could be limited. In compensation, the reduction in transpiration (16%) via greater stomatal closure (lower g_s) reduced excessive water loss, preventing leaf tissue dehydration and protecting the hydraulic architecture of the plant. Stomatal closure is often reported as a mechanism to reduce total transpiration (Singh 2004; Rosas-Anderson *et al*. 2014; Hsie *et al*. 2015). According to Warren and Adams (2004) and Hsie *et al*. (2015), a decrease in stomatal aperture causes a decrease in g_s, reducing leaf transpiration. As a consequence, this limits the uptake of CO_2 by the leaf and net photosynthesis decreases further. Hsie *et al*. (2015) describes that smaller stomata close more rapidly than larger ones, which may also have occurred in
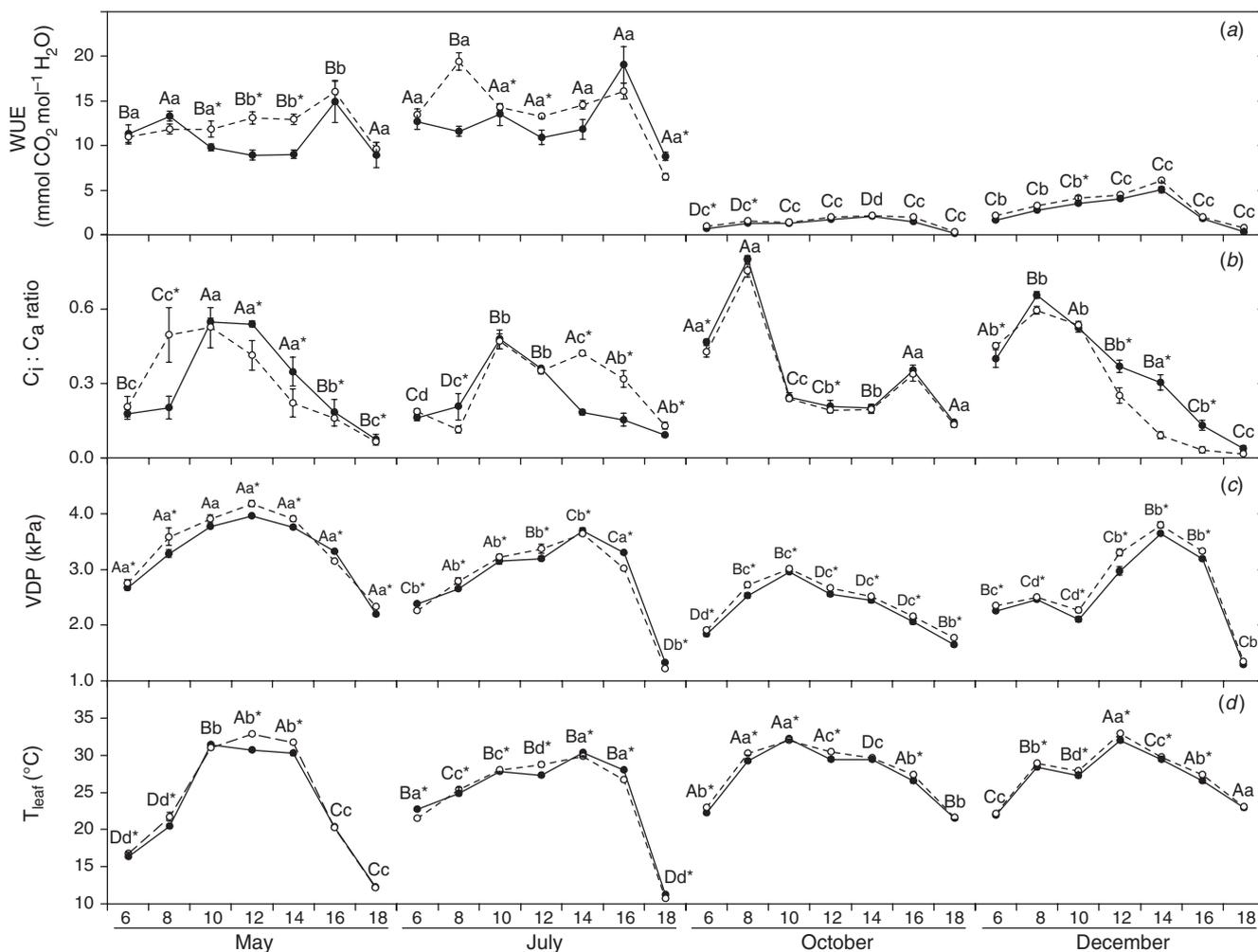
Fig. 4. Time course (hours) of (*a*) water use efficiency (WUE), (*b*) internal-to-ambient CO_2 concentration ($C_i : C_a$ ratio), (*c*) leaf-to-air vapour pressure deficit (VPD) and (*d*) leaf temperature (T_{leaf}) measured for full sun (open symbols) and natural shade (black symbols) *Croton blanchetianus* plants. Data were collected during the rainy season (May–July) and the dry season (October–December) of 2011. Significant seasonal differences among the means are represented by different capital (full sun plants) or lowercase letters (natural shade plants; Newman–Keuls test at $P \leq 0.01$). Asterisks denote significant differences between the means of the full sun and natural shade leaves within the same month and time (*t*-test at $P \leq 0.05$). Each point represents the mean ($\pm$s.e.), $n = 10$.

C. blanchetianus, since the leaves developed during the dry season were appreciably smaller than the leaves developed during the rainy season. It is possible that such leaves may have become thicker and more sclerophilic than leaves developed in the rainy season.

Because g_s is much higher than P_N during the dry season, a reduction in soil moisture content and WUE combined with high PAR might affect some photosynthetic traits in *C. blanchetianus*, such as mesophyll conductance, the Rubisco kinetics and electron transport (Flexas and Medrano 2002). Murata *et al.* (2012) suggest that the repair of PSII under environmental stress may be the critical step that determines the outcome of the photodamage–repair cycle. Mlinarić *et al.* (2017) verified that in high light, the D1 protein of the photosystem became gradually degraded, inactivated or both, but in healthy plants, this degradation was completely recovered at night, which shows that this protein is the key to understanding

the mechanisms that lead to the activation or deactivation of PSII under high light intensity and possibly also under conditions of water limitation. Bečková *et al.* (2017) support the conclusions of Mlinarić *et al.* (2017), who showed that the Psb27 accessory protein in these complexes suggests the involvement of PSI in PSII biogenesis, possibly by photoprotecting PSII through energy spillover. Additionally, a decrease in P_N under water deficit stress affects C delivery from source to sink tissues and its subsequent metabolism (Chakraborty *et al.* 2016). If global temperatures continue to increase and semiarid regions face prolonged drought, as most climate models predict (Marengo *et al.* 2012; Pereira *et al.* 2014), stomatal limitation may decrease carbon assimilation in *C. blanchetianus*, which is a landmark species of the Caatinga, primarily in the tropical dry forests in north-eastern Brazil (Santos *et al.* 2014).

Stomatal conductance and P_N demonstrated a diurnal cycle, with maximum values at approximately midday and minimum

values at predawn. Moreover, with increasing irradiance throughout the day, P_N and g_s tended to increase linearly, indicating that g_s and P_N could be influenced by the diurnal cycle (Ishida and Toma 1999; Mendes and Marenco 2014; Gitelson *et al.* 2017) in tropical dry forests, affected by T_{leaf} and VPD. The correlation between g_s and VPD was very pronounced during the dry season, as previously described in several other species (Pompelli *et al.* 2010a; Aasamaa and Sõber 2011; Arve *et al.* 2013; Giday *et al.* 2013; Hsie *et al.* 2015), and Hsie *et al.* (2015) clearly demonstrated that stomatal closure is very pronounced at high VPD.
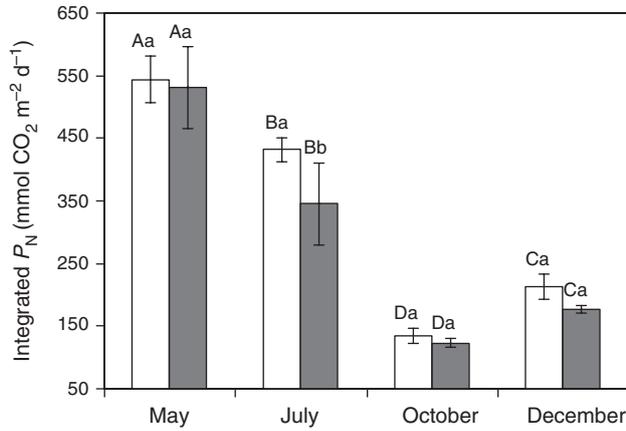

Fig. 5. Diurnal integrated CO_2 assimilation (integrated P_N) in full sun (open bars) and natural shade (black bars) *Croton blanchetianus* plants. Different capital letters denote significant differences among the means for each month under the same light conditions, and different lowercase letters denote significant differences between light conditions within the same month. Each value represents the mean ($\pm$ s.e.), $n = 10$.

Additionally, g_s decreased with decreased T_{leaf} throughout the afternoon. Although g_s can decrease with decreases in T_{leaf} temperature (Peak and Mott 2011; Lloyd and Farqhar 2008), in *C. blanchetianus*, T_{leaf} increased within a moderate range ($28-35°C$), with a consequent increase of g_s, reflecting a positive effect on the rate of P_N. Moreover, the decline in g_s between 0800 and 1400 hours probably occurred because of increased VPD. Stomata are expected to close in response to low humidity, either through a feed-forward response (Buckley 2005) or as a result of faster water loss by guard cells (Mott and Peak 2010). Stomatal closure often leads to a decrease in intercellular CO_2 concentration and therefore a decrease in the $C_i : C_a$ ratio. In this study, we observed a similar trend in both water regimes; thus, we argue that reduced g_s can limit P_N by reducing the supply of CO_2 to Rubisco.

The $\delta^{13}C$ values were more negative during the rainy season and had higher WUE, whereas during the dry season, the ^{13}C composition of the leaves was higher (less negative), though WUE decreased. The $\delta^{13}C$ has been suggested as an indicator of water stress through stomatal closure and the apparent decrease in WUE (Ehleringer and Cooper 1988; Ehleringer 1991; Ehleringer *et al.* 1992). Our data are in agreement with Antunes *et al.* (2016), who observed a less negative isotopic signature under drought conditions in *Spondias tuberosa* Arruda. Consistent with this, Winter (1981), cited by Farquhar *et al.* (1982), 'found that leaves of *Cicer arietinum* [L.] plants exposed to drying cycles had lower C_i and greater (i.e. less negative) δ values than those of control plants'. Further, Winter *et al.* (1981) found that specimens of two C_3 halophytes from Western Australia had less negative δ values after 3 months of dry conditions. Although this is in contrast to a previously study by Martin and Ruiz-Torres (1992) in which WUE maintained a highly positive correlation with P_N and g_s, this
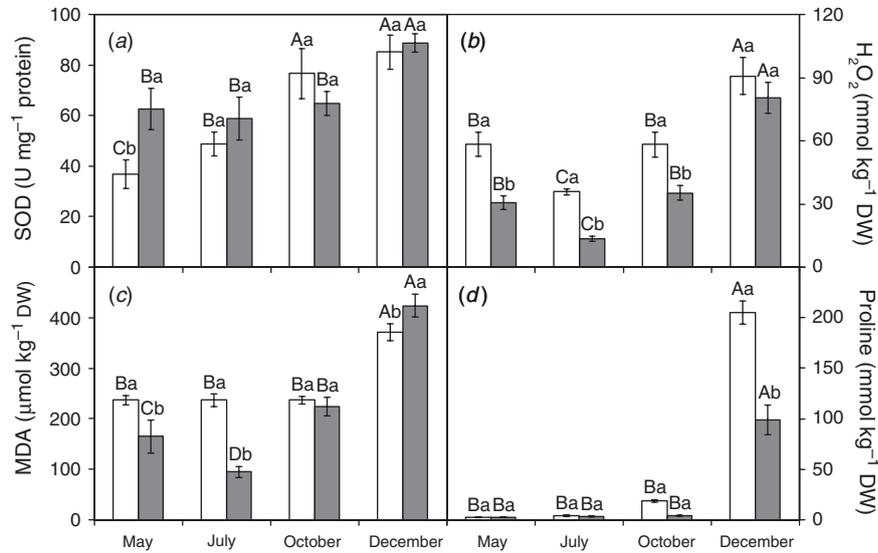
Fig. 6. (*a*) Superoxide dismutase (SOD), (*b*) H_2O_2, (*c*) malondialdehyde (MDA) and (*d*) proline in full sun (open bars) and natural shade (black bars) *Croton blanchetianus* plants. Different capital letters denote significant differences among the means for each month under the same light conditions, and different lowercase letters denote significant differences between light conditions within the same month. Each value represents the mean ($\pm$ s.e.), $n = 5$.

Fig. 7. Drawings of the leaf morphology of *Croton blanchetianus* plants collected during (*a*, *b*) the rainy season (May) and (*c*, *d*) the dry season (December) for (*a*, *c*) natural shade and (*b*, *d*) full sun plants. Note the differences in the leaf venation. Numbers below the drawings represent the leaf area (LA) and the specific leaf area (SLA). Each value represents the mean (± s.e.). Bar = 5 mm.

fact can be attributed to nonstomatal inhibition of photosynthesis under water deficit stress. Our data are in contrast to those described by Martin and Ruiz-Torres (1992), but strongly agrees with the classic work of Farquhar *et al.* (1982) and Ehleringer *et al.* (1992). It should be noted that in this study, we show that the $C_i : C_a$ values were always <0.5, which has already been agreed on by plant physiologists (Signarbieux and Feller 2011; Zhou *et al.* 2013) as a sign of stomatal limitation.

Light penetration in dry forests is relatively low during the rainy season compared with that in the dry season (Parker *et al.* 2005), which can be observed in Fig. 3. Significant differences in PAR at leaf level in full sun and natural shade plant leaves were registered in the rainy and dry seasons, but the differences in light penetration between these leaves were highly significant during the dry season. Indeed, in many species in arid environments,

Table 1. Concentration of soluble carbohydrates, starch, amino acids, proteins, chlorophyll, carotenoids and their ratios measured in full sun and natural shade plants of *Croton blanchetianus*

Different capital letters denote significant differences between months under the same light conditions (full sun or natural shade), and different lowercase letters denote significant differences between light conditions within the same month ($P \leq 0.001$, Newman–Keuls test). Each value represents the mean (± s.e.)

Parameters	Full sun				Natural shade			
	May	July	October	December	May	July	October	December
Carbohydrates (mmol kg⁻¹ DW)	400.4 ± 11.6Aa	483.6 ± 50.2Aa	239.6 ± 20.0Ca	320.7 ± 15.4Ba	316.7 ± 26.4Ba	410.1 ± 8.6Aa	320.5 ± 38.2Ba	332.1 ± 19.1Ba
Starch (mmol kg⁻¹ DW)	158.2 ± 15.7Bb	146.4 ± 16.5Ba	161.9 ± 7.3Ba	425.7 ± 38.2Aa	232.1 ± 15.2Ba	162.5 ± 26.9Ba	161.5 ± 21.5Ba	419.2 ± 47.4Aa
Amino acids (mmol kg⁻¹ DW)	90.2 ± 5.5Aa	77.0 ± 7.0Aa	28.0 ± 2.1Ba	41.0 ± 3.6Ba	50.9 ± 3.0Bb	74.6 ± 12.8Aa	43.4 ± 4.7Ba	43.9 ± 3.7Ba
Soluble proteins (g kg⁻¹ DW)	33.9 ± 2.5Aa	28.4 ± 1.8Aa	19.3 ± 0.8Ba	9.9 ± 0.5Ca	24.6 ± 4.1Ab	18.1 ± 2.3Ab	11.8 ± 0.9Ba	14.5 ± 0.4Bb
Total proteins (g kg⁻¹ DW)	177.4 ± 9.8Ba	243.4 ± 14.9Aa	134.4 ± 7.1Ca	132.7 ± 15.5Cb	197.4 ± 11.7Aa	267.1 ± 6.3Aa	169.6 ± 11.0Ba	180.7 ± 7.9Ba
Total chlorophyll (g kg⁻¹ DW)	2.2 ± 0.1Ba	2.8 ± 0.1Ab	1.3 ± 0.1Bb	1.5 ± 0.2Ba	2.3 ± 0.3Ba	3.5 ± 0.2Aa	2.0 ± 0.2Ba	1.9 ± 0.1Ba
Total carotenoids (mg kg⁻¹ DW)	386.1 ± 65.4Ba	425.9 ± 59.4Ba	301.2 ± 14.8Bb	585.5 ± 79.5Aa	405.4 ± 62.7Bb	389.5 ± 17.5Ba	460.3 ± 46.0Ba	663.5 ± 42.9Aa
Chlorophyll *a/b* ratio	2.4 ± 0.1Ba	2.5 ± 0.1Ba	2.6 ± 0.1Aa	2.9 ± 0.1Aa	2.1 ± 0.1Ba	2.0 ± 0.1Bb	2.7 ± 0.1Aa	2.7 ± 0.2Aa
Carotenoids : chlorophyll ratio	211.4 ± 15.6Ba	157.6 ± 13.3Ca	225.5 ± 6.8Ba	296.6 ± 15.4Aa	219.2 ± 7.9Ba	135.5 ± 7.7Ca	210.7 ± 7.1Ba	321.3 ± 22.7Aa

Table 2. The concentration of mineral nutrients and their ratios measured in full sun and natural shade plants of *Croton blanchetianus*

Different capital letters denote significant differences among months under the same light conditions (full sun or natural shade), and different lowercase letters denote significant differences between light conditions within the same month ($P \leq 0.001$, Newman–Keuls test). Each value represents the mean (± s.e.). $\delta^{13}C$, C isotope composition

	Full sun				Natural shade			
	May	July	October	December	May	July	October	December
Macronutrients (g kg^{-1})								
N	38.4±1.6Aa	39.0±2.4Aa	21.5±1.1Ba	21.2±2.6Bb	41.6±1.9Aa	42.7±1.0Aa	27.1±1.8Ba	28.9±1.3Ba
N : P ratio	13.7±2.2Aa	13±1.9Aa	13.4±1.3Aa	11.2±2.3Ba	9.2±1.8Ab	10.7±1.1Ab	8.2±2.1Bb	8.3±1.9Bb
P	2.8±0.1Ab	3.0±1.0Aa	1.6±0.9Bb	1.9±0.1Bb	4.5±1.0Aa	4.0±0.5Aa	3.3±0.7Aa	3.5±0.5Aa
K	24.6±4.0Aa	29.8±4.7Aa	21.9±0.9Aa	27.6±1.1Aa	25.6±2.9Aa	23.4±4.3Aa	22.2±0.7Aa	18.5±1.1Bb
Ca	6.6±0.6Aa	7.2±0.6Aa	5.6±0.4Aa	6.7±0.7Aa	5.9±0.5Aa	6.7±0.9Aa	5.1±1.3Aa	5.7±0.6Aa
Mg	2.9±0.5Ba	2.8±0.5Ba	2.2±0.3Ba	3.4±0.2Aa	2.4 0.5Ba	2.7±0.5Ba	2.6±0.4Ba	3.7±0.3Aa
S	2.7±0.2Bb	3.3±0.2Aa	2.6±0.3Ba	2.3±0.2Bb	4.0±0.3Aa	3.0±0.2Ba	2.5±0.1Ba	3.5±0.4Aa
Micronutrients (mg kg^{-1})								
B	69.4±6.7Ba	69.5±4.5Ba	65.6±4.7Ba	81.9±7.9Aa	65.7±2.6Ba	62.4±3.4Ba	61.7±7.7Ba	77.5±8.3Aa
Cu	28.2±6.5Aa	11.0±1.5Ba	8.6±0.4Ba	8.0±0.7Ba	22.7±4.5Aa	6.5±0.2Bb	6.4±0.6Ba	6.5±0.6Ba
Fe	230.7±11.7Ab	142.8±10.6Ca	147.2±10.1Ca	213.1±15.1Ba	318.7±43.5Aa	133.1±14.8Ca	166.7±8.0Ca	245.1±44.2Ba
Mn	1433.2±290.8Ba	1602.3±150.4Aa	1359.2±159.3Ba	1590.4±298.9Aa	711.0±70.3Ab	730.1±99.4Ab	692.1±112.8Ab	729.3±19.6Ab
Zn	25.2±2.1Aa	20.1±2.6Ba	15.1±1.1Ca	18.0±0.8Ba	25.9±1.7Aa	17.2±3.0Ba	10.3±2.8Cb	15.5±0.2Bb
Na	8120.5±279.1Aa	8691.8±1083.1Aa	5825.0±445.6Ba	6441.4±615.7Ba	6707.1±342.3Ab	6907.6±966.5Ab	5530.9±418.7Ba	5130.0±582.6Bb
Other								
$\delta^{13}C$ (‰)	−28.6±0.1Aa	−28.3±0.4Ab	−26.8±0.1Ba	−26.2±0.3Ba	−27.9±0.1Ba	−30.9±0.1Aa	−27.2±0.2Ca	−26.0±0.1Da

Table 3. The coefficients of canonical correlations (CC) between diurnal integrated CO_2 assimilation (integrated P_N) and the concentrations of mineral nutrients measured in full sun and natural shade plants of *Croton blanchetianus*

Variables	CC1
Integrated P_N	−1.00
Leaf nutrients contents	
N	−0.515
P	−0.016
Ca	−0.184
Cu	−0.404
Fe	−0.259
Mn	0.406
Zi	0.017
K	−0.210
Mg	0.169
S	0.106
Na	−0.197
R canonical	0.8788
P-value	0.000015

the response of leaf traits and gas exchange may be more closely related to drought tolerance than to shade tolerance (Markesteijn *et al*. 2007).

Water deficit stress significantly reduced the integrated P_N values in both light environments. However, these results must be considered with caution (Amthor 2000) because it is important to understand that measuring photosynthesis during the day, not punctual photosynthesis, is highly coupled with manual measurements and user errors. Nevertheless, although they are only indicative because of the uneven measuring intervals over the dry and wet seasons, these values are very valid in scenarios of global climate change.

Low P_N during the dry season could also be attributed to reductions in leaf area and SLA, reflecting lower dry matter accumulation in plant leaves, which indicates that leaf structure is an important determinant of leaf assimilation capacities, as previously described (Oguchi *et al*. 2005; Nouvellon *et al*. 2010). According to Eamus (1999), semideciduous species show strong reductions in leaf area, reflecting lower rates of photosynthesis. Photosynthesis on a leaf area basis depends not only on the Calvin cycle but also on the mesophyll structure of leaves (Keenan *et al*. 2010; Scoffoni *et al*. 2015). The reduction of the leaf blade area and the senescence of older leaves are adjustments that, as emphasised by Dias-Filho and Dawson (1995), lead to water saving, allowing the species to better withstand water stress. This water saving occurs because plants with smaller gas exchange surfaces tend to lose less water through the transpiration process (Shao *et al*. 2008).

As the drought progressed, SOD activity increased significantly. The partial stomatal closure and suppression of CO_2 fixation that occurred with the progression of drought might have caused an increase in photorespiration rates and exacerbated the production of free radicals (reactive oxygen species), which would be metabolised by enzymes in the antioxidative system. Nishiyama *et al*. (2006) found that reactive oxygen species suppress the synthesis of proteins and,

in particular, that of the D1 protein, which is required for the repair of PSII. However, mending of PSII also depends on the intracellular level of ATP (Murata *et al*. 2007). Both catalase and ascorbate peroxidase activity were not significantly altered by the treatments (data not shown). It is important to emphasise that the CAT and peroxidases (APX) protect PSII from photoinhibition because these enzymes are efficient scavengers of H_2O_2 (Murata *et al*. 2007). This interruption of the expected relationship between SOD and APX or CAT was probably crucial to the increased accumulation of H_2O_2 in *C. blanchetianus* plants under drought, particularly in unprotected leaves (full sun). The H_2O_2 accumulation then led to elevated lipid peroxidation and MDA accumulation. Therefore, we argue that the efficiency of the antioxidant system of *C. blanchetianus* is weak, and that therefore, other factors must control survival during the dry season, such as osmotic adjustment.

Soluble carbohydrates and free amino acids are very important compounds in plant osmoregulation during water stress (HongBo *et al*. 2006). The reductions in leaf soluble carbohydrates under stress suggested a drought avoidance mechanism in *C. blanchetianus*. In the case of photosystem overload, transducing signals may occur between the chloroplast and nucleus to synthesise proline from glutamate. This synthesis consumes two NADPHs, which alleviates the photosystems (Delauney and Verma 1993). Therefore, proline accumulation may be considered evidence that photosystems are overloaded, because proline synthesis has the effect of dissipating reducing power by continuing the flow of electrons. More recently, other compounds have gained importance, such as compatible osmolytes, and we can highlight the roles of betaine glycine (Gupta *et al*. 2014) and sugar alcohols (e.g. pinitol, sorbitol and mannitol) (Arbona *et al*. 2013; Chakraborty *et al*. 2016). A possible explanation for the increase of starch in the dry season is that N deficiency has a greater effect on reducing growth than on reducing the photosynthetic rate; therefore, assimilated and nonexported C was directed to the formation of transient starch (DaMatta *et al*. 2008). The reduced formation of amino acids and proteins might also have contributed to the accumulation of starch in leaves.

The decrease in total chlorophyll content during the dry period could be attributed to photoprotection of PSII (Pompelli *et al*. 2010*a*), because a reduction in the concentration of chlorophyll decreases the pressure on photosystems, which is a very important pattern, particularly in drought conditions and under high irradiance (Pompelli *et al*. 2010*b*). Based on our observations, the reduction in P_N could also be associated with damage to leaf pigments or photosystems. The total chlorophyll per leaf area unit can be partially overwhelmed in both full sun and shaded leaves (Lichtenthaler *et al*. 2007). However, compared with full sun leaf matter, the dry matter of shaded leaves has more total chlorophyll. Irradiance remarkably affected chlorophyll catabolism, which is consistent with results reported by others (Brand 1997; Martínez and Guiamet 2004). The increase in the chlorophyll *a/b* ratio, carotenoid : chlorophyll ratio and the total carotenoids during the dry season might have been the result of leaf abscission by neighbouring plants, which exposed the individuals of *C. blanchetianus* grown under shade to increased irradiance. Because a high of carotenoid content or a high carotenoid : chlorophyll ratio are associated with the dissipation of excess energy as heat, an increase in this ratio would be expected in dry-season leaves, as reported in this study.

We recorded low N levels in the dry season, which indicated that large quantities of N were translocated from the deciduous leaves to perennial organs before leaf abscission. Indeed, a reduction in total chlorophyll content was observed as a response to water deficit stress, which could be attributed to the leaves progressing towards senescence. Thus, our data indicate that the decrease in P_N might be caused by a reallocation of N, possibly as a contribution to photoprotection. These results are consistent with those of Shangguan *et al*. (2000), who reported that drought reduced leaf N content. Additionally, the positive effect of leaf N on integrated P_N (canonical correlation analysis; –0.515) was also observed by Pompelli *et al*. (2010*c*) and Mendes and Marenco (2015); thus the photosynthetic rates of the species studied were affected because N is involved in Rubisco activity and subsequently in the stimulation of CO_2 uptake. The strong positive correlation between P_N and leaf N content in combination with declining N concentrations and a decrease in leaf area and SLA during the dry season might explain the reduced integrated P_N in leaves from *C. blanchetianus* plants in full sun and natural shade during the dry season. Earlier studies have also shown that soil water deficits reduce N uptake and the mobilisation of N to leaves (Durand *et al*. 2010; Gonzalez-Dugo *et al*. 2012). Moreover, because of the low N concentrations we found in leaves, the N : P ratio was much smaller than the value reported by Fyllas *et al*. (2009). Therefore, a low N : P ratio suggests that N was the more limiting element in *C. blanchetianus* plants, primarily in natural-shade plants, as previously reported in many terrestrial plants (Güsewell 2004).

In summary, we investigated the seasonal cycle of C assimilation and the resilience of *C. blanchetianus* to drought to better understand the mechanisms controlling leaf function throughout the drought season in the Caatinga (dry tropical forest). We conclude that *C. blanchetianus* plants have a high capacity to acclimate to environmental changes, mainly by stomatal closure. Additionally, the morphophysiological responses of *C. blanchetianus* are modulated primarily because of water restrictions. Production of new leaves with morphophysiological traits adapted to a new environment is apparently the most relevant acclimation strategy of this species to seasonal rainfall variation. For example, in the dry season, osmotic adjustment, decreased g_s and smaller leaf area avoided desiccation and reduced transpirational demand. Thus, *C. blanchetianus* may be considered a model plant for studies of water relationships in woody plants subjected to water deficits in the Caatinga ecosystem, because the species demonstrates great resilience to the seasonal changes in rainfall. Consequently, *C. blanchetianus* may also be used in the regeneration of degraded areas in semiarid Brazil and around the world. Furthermore, the data generated in this study are important for calibrating and parameterising climatic and environmental models to predict the effects of drought and identify the vulnerability of this biome.

Conflicts of interest

Acknowledgements

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