

Interannual Variation in Temperature and Rainfall can Modulate the Physiological and Photoprotective Mechanisms of a Native Semiarid Plant Species

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Abstract

Climate change may affect ecosystems worldwide, but can be aggravated in arid environments, leading to decreased rainfall and a consequent increase in air temperature. **Objective:** The present investigation aimed to study the behavior of a native plant, adapted to seasonal dry conditions, when faced with different conditions in two years: one extremely dry and the other with rains within the expected range. **Methods/Analysis:** To study the effects of the seasonal water deficiency, we collected all data in two consecutive years: 2012 and 2013. **Results:** In both years, we obtained data in May (rainy season) and August (dry season). The predawn water potential (Ψ_w) ranged from -5.6 to -4.2 MPa in 2012 and from -2.16 to -2.98 MPa in 2013. These patterns show that even in the rainy season of the dry year, the plants were subjected to a very high water deficit when compared to the wet year; this was due to the sparse rains of 2012, the driest year of the preceding decade in the study area. All gas exchange and carbon uptake tended to zero in 2012; however, all the parameters recovered when the rains returned in 2013. Considering the entire data set, the water use efficiency (WUE) data presented a positive and significant correlation with all the photosynthetic parameters. However, when we excluded the 2012 data, the WUE was negatively correlated with transpiration (E), stomatal conductance (g_s) and leaf temperature (T_{leaf}). On average, the potential photosynthesis (P_{N-pot}), maximum carboxylation velocity of Rubisco (V_{c-max}) and the maximum electron transport rate (J_{max}) values were on average 62%, 50% and 63% lower in the dry season than in rainy season in 2013. **Discussion/Conclusions:** We argue that the increase of intercellular carbon caused the increase in photosynthesis to its limitation point of photosynthesis by J_{max} . Below the collimation point, the photosynthetic rates were limited by V_{c-max} . Thus, the prolongation of the dry season could have potential impacts on the electron transport rate and on Rubisco carboxylation efficiency, taking into account the current concentrations of atmospheric carbon dioxide. During a prolonged drought, the reduced Ψ_w , osmotic adjustment and efficient antioxidative system appear to be the main strategies that *C. pyramidale* uses to avoid fatal plant dehydration, maintaining basal rates of net photosynthesis. However, with the intensification of climate changes expected for the study area, the species may no longer be a symbol of the ecosystem but rather a symbol of environmental degradation.

Keywords: World Climatic Change, Electron Transport Rate, Carbon Uptake, Gas Exchange, Reactive Oxygen Species, Water Scarcity

1. Introduction

Climate-related risk levels often increase with increased temperature and are sometimes directly linked to other dimensions of climate change, such as a severe decline of rainfall together with isolated torrential rainfall in some areas¹. In addition, the fractions of the global population that will experience water scarcity and be affected by major river floods are projected to increase with the level of warming in the 21st century. Thus, floods and droughts will intensify, and the continued emission of greenhouse gases will cause further warming and long-lasting changes in all components of the climate system². Moreover, continued high emissions of CO₂ would not only lead to mostly negative impacts for biodiversity, ecosystem services and economic development but also amplify risks for livelihoods and food and human security, increasing the likelihood of severe, pervasive and irreversible impacts for people and ecosystems³. With the worsening of global climate changes, phenomena such as El Niño and La Niña can become more frequent and reach even larger areas⁴. This result may change precipitation, thereby affecting the quantity and quality of water resources. Predictions suggest that the global climate changes will, in particular, more negatively affect arid and semiarid regions, leading to prolonged dry periods and causing a series risk to biodiversity².

Water is the most limiting resource for the plant growth, and this fact can prompt much discussion regarding the ways in which the predicted water shortage can affect plant growth and development. In a similar manner, increases in atmosphere temperature can be affect plant metabolic profile. Due to the complexity of this and the uncertainties of forecasting climate evolution on a small scale, any prediction about the extent of plant physiology induced by the climate evolution remain largely unpredictable⁵. It is consensus that the plants existing in arid and semiarid regions have adapted to survive periods without the proper amount of water⁶⁻⁷ using satellite mapping, discussed the variation in rainwater use efficiency and vegetation resilience after prolonged drought periods in the Amazon region. To non-adapted plants, the dry period could cause not only a severe restriction of biomass production but even death. However, plants adapted to water shortages normally show a direct relation between hydraulic economy and the degree of protection against excess irradiance and UV radiation⁸. The first event that

prevents photosynthetic damage is stomatal closure, as shown in some cultivated^{9,10} and uncultivated¹¹⁻¹⁵ species. To avoid the damage caused by water deficit, linked or not to increase of atmosphere temperature, plants may respond to the most drastic shortage by losing their leaves to minimize the water lost to the atmosphere. However, by decreasing stomatal conductance (g_s), plants limit their CO₂ uptake, leading to a reduction in net photosynthesis (P_N). The decrease of P_N also affects plant growth and biomass production by limiting Rubisco activity¹⁶. A water shortage may also induce reactive oxygen species (ROS) production, which causes serious damage to plant metabolism. To avoid this damage and protect the cells, plants can produce certain molecules (e.g., proline, glycinebetaine and polyols) and change the enzymes kinetics to metabolize ROS into less dangerous molecules, such H₂O and O₂. Changes in the temperature dependence of photosynthesis may be ascribed to changes in the activity and amount of photosynthetic components and/or CO₂ concentration in the carboxylation site. However, the response of each factor to temperature seems to differ among species. The responses of native plants to extreme climatic events (e.g., increase of temperatures together with decrease of rainfall) are not well comprehended. Basher et al¹⁷ described that *Eucalyptus* spp. are well adapted to warmer temperatures and lower rainfall so would be expected to adapt readily to the expected future climate changes. Furthermore, plant physiological measurements are crucial to the calibration of dynamic global vegetation models^{18, 19} that are used to simulate the responses of vegetation in the face of increase of temperature and decrease of rainfall which is provided for IPCC report²⁰. Based on this background, the following two hypotheses were tested: 1) *Cenostigma pyramidale*, a Caatinga native species, has physiological and biochemical mechanisms that allow the species to survive a water deficit period and the resume high metabolic rates in the rainfall season and 2) That the maximum carboxylation velocity of Rubisco and maximum electron transport rate are involved with the possible changes in plant photosynthetic performance during the dry season. Thus, this work aimed to investigate the limits of adaptation to seasonal dry conditions by *C. pyramidale* in two highly contrasting climatologically situations: a very dry year in 2012 and a normally wet year in 2013. Finally, this is the first time a native Caatinga species is studied in two consecutive years, one with rains far below expected and another with rains within the average.

2. Material and Methods

2.1 Study Site, Plant Material and Climatologic Measures

The study was conducted during both 2012 and 2013 years in a preserved fragment of seasonally tropical dry forest in a semiarid region of the Caatinga, Serra Talhada, Pernambuco, Brazil (07°57'46"S, 38°23'02" W; 472 m a.s.l.), that encompasses one of the major Brazilian seasonal tropical dry forests²¹, because it is commonly subjected to a severe water shortage during the dry season (June to December). This region is densely populated, with approximately 27 million inhabitants distributed with sub regional averages of 20-30 inhabitants per km², so this forest is one of the most populated and biologically diverse semiarid regions in the world, with a high endemism rate²¹.

Cenostigma pyramidale (Tul.) Gagnon & G.P. Lewis (Fabaceae) is the symbol species of the Caatinga environment and predominates in all landscapes of the ecosystem. This tree is deciduous and very important as a pioneer species during the ecosystem succession. In this study, we utilized *C. pyramidale* as a plant model to evaluate the effects of climatic changes on other plant species of the Caatinga. For this purpose, we selected six adult trees (approximately 15 years of age) in the reproductive phase and ranging from 2.0 to 3.0 meters in height. All the plants were identified by a taxonomist and deposited in a plant collection at Agronomic Institute of Pernambuco Herbarium (registered by IPA-88.494).

Temperature, rainfall and relative humidity (RH) data were collected daily by a pluviometer (mod.TE525, Campbell Scientific, Logan, Utah, EUA) and by a temperature sensor (mod.108, Campbell Scientific, Logan, Utah, EUA) on a meteorological tower located next to the research site. Note that in 2012, northeastern Brazil experienced its strongest drought (Figure 1²²). We used data collected in this year to understand how plants behavior during an extreme drought event and to compared this behavior with that occurring during a year with average rainfall.

2.2 Predawn and Noon Leaf Water Potential (Ψ_w)

To assessed leaf water status, we selected and collected one fully expanded leaf (*i.e.*, from the third node from the apex) per tree. We measured the leaf water potential (Ψ_w) at predawn (5:00 a.m) and noon (12:00 p.m) with a Scholander type pressure chamber²³.

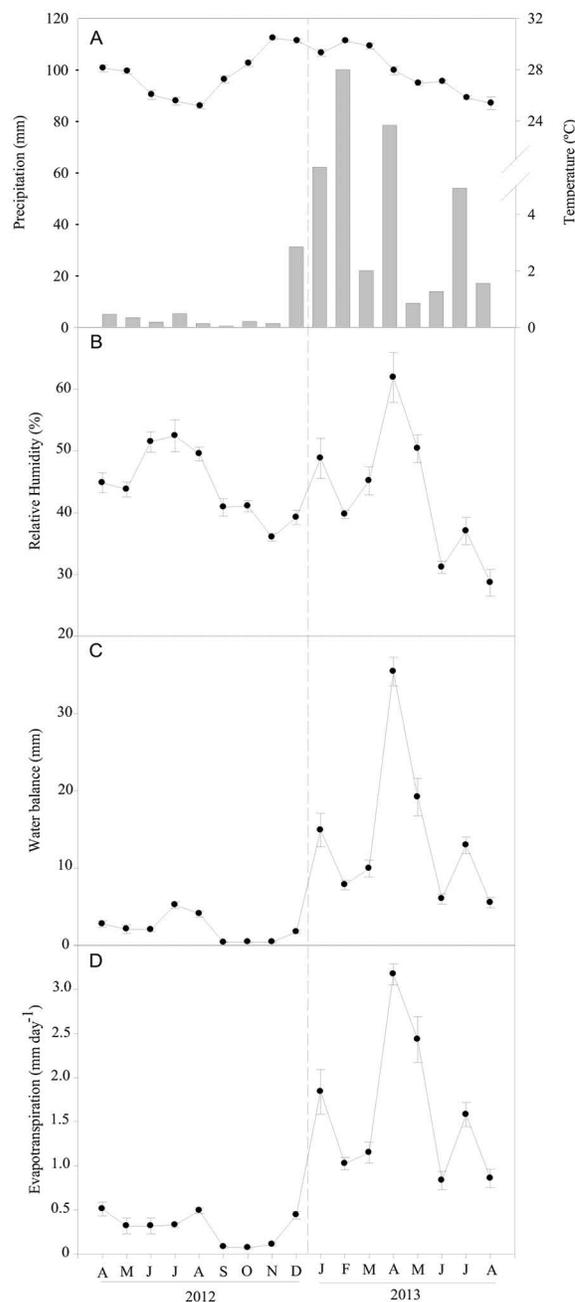


Figure 1. Climatic characteristics: (A) total monthly rainfall (bars) and mean temperatures (lines); (B) relative humidity, (C) soil water balance (store) and (D) evapotranspiration from April of 2012 to August of 2013 in the study area. The dotted line divides the 2012 and 2013 years. The values represent the average (\pm SE).

2.3 Seasonal and Diurnal Cycles in Gas Exchange Parameters

Gas exchange parameters (net photosynthesis, P_N ; stomatal conductance, g_s ; transpiration, E ; leaf

temperature, T_{leaf} ; and leaf-to-air vapor pressure deficit (VPD)) were measured on six healthy trees. Two healthy leaves per tree were used to assess the diurnal variation (08:00 - 18:00 h; solar time). These parameters were measured *in situ* under clear-sky conditions, and we used an infrared gas analyzer (Li-6400; Li-Cor, NE, USA), as described in details in²⁴. Water use efficiency (WUE) was determined by the ratio between P_N and E . Diurnal integrated CO_2 assimilation (integrated P_N) was obtained by integrating all measurements of P_N measured throughout the day, as previously described²⁴.

The response of P_N to the intercellular CO_2 concentration ($P_N:C_i$ response curves) was determined at saturating light of $1,200 \mu\text{mol m}^{-2} \text{s}^{-1}$ (this value was previously determined by light curves). The maximum carboxylation velocity of Rubisco ($V_{c\text{-max}}$) and the maximum electron transport rate (J_{max}) were calculated as recommended by²⁵. To facilitate comparisons with data reported in the literature, the values $V_{c\text{-max}}$ and J_{max} were standardized to 25°C using the equations described previously²⁶. In this study, the rate of photosynthesis measured in saturated $[\text{CO}_2]$ ($2,000 \mu\text{mol mol}^{-1}$) and saturating light ($1,200 \mu\text{mol m}^{-2}\text{s}^{-1}$) is referred to as potential photosynthesis ($P_{N\text{-pot}}$). These measures were taken only in 2013, a year with standard rainfall.

2.4 Leaf Area (LA) and Specific Leaf Area (SLA)

C. pyramidale presents bipinnate leaves. Therefore, to measure the leaf area, we collected two hundred and fifty fully expanded folioles randomly in the population and promptly digitalized them with a digital scanner (HP Photosmart 3110; 1200 x 1200 dpi, Hewlett-Packard, Palo Alto, CA). After that, the images were processed using Image-Pro Plus (version 4.5, Media Cybernetics, Silver Spring, USA) as described by²⁷. Then, the folioles were dried at 70°C for 72 hours to determinate their dry biomass. The specific leaf area (SLA) of folioles was calculated through the relation between dry mass (kg) and foliole area (m^2).

2.5 Biochemical Analysis

To analyze the effect of drought on plant metabolism, we collected leaves *in situ* between 09:00 a.m. and 10:00 a.m. under clear-sky conditions. The leaves were flash-frozen in liquid nitrogen and then stored at -20°C until analysis. Soluble sugars²⁸, total soluble free amino acids²⁹, *a* and *b*

and total chlorophylls and carotenoids³⁰ were analyzed with a *dual beam* spectrophotometer adjusted to the specific wavelength for each organic compound tested. To assess the effectiveness of the ant oxidative system, we measured hydrogen peroxide (H_2O_2), malonic dialdehyde (MDA) and proline accumulation. Proline was determined as reported by³¹, whereas MDA and H_2O_2 were determined according to³².

2.6 Statistical Analysis

Primarily, all data were tested the normal distribution using Shapiro-Wilk test and Kolmogorov-Smirnov test. Statistical analysis and the generation of graphs were performed using the ggplot2 R package³³. The analysis of variance (ANOVA) was performed to evaluate the differences between the factors, and the means compared using the Student-Newman-Keuls test ($p < 0.05$). For the multivariate analysis, correlation analysis was performed. Unless otherwise indicated, samplings and measurements were conducted using 6 replicates per treatment combination. The experiments were repeated in time giving similar results.

3. Results

3.1 Environment Data

The climatic data show that 2012 was a very dry year, with cumulative precipitation of 72 mm recorded within the studied period, namely, 23.3% of the average expected precipitation (*i.e.*, the average precipitation of the previous 15 years = 308 ± 42.3 mm). The precipitation recorded in 2013 was 430 mm, within the expected range for an average period (573.1 ± 169.2). In 2013, the wettest month was May (100.1 mm), and the driest month was August (3.9 mm). As these months coincide with the historically rainiest and driest months, we chose them for our study. For the month of May, we observed that the accumulated rainfall was 96.2% lower in 2012 than in 2013. However, the air temperature showed no significant difference between the sampled years ($P = 0.89$), with values of 27.7 ± 1.8 mm and 27.8 ± 1.7 mm, in 2012 and 2013, respectively (Figure 1A). Relative humidity (RH) oscillated between 36% and 52% in 2012, and between 29% and 62% in 2013 (Figure 1B). Rainfall significantly influenced the soil water storage ($r = 0.85$), evapotranspiration ($r = 0.92$) but not RH ($P = 0.134$) or air temperature ($P = 0.956$); even so, the soil water and evapotranspiration

values were 6.6 and 5.4 times higher in 2013 than in 2012 (Figure 1C-D).

3.2 Leaf Water Potential (Ψ_w)

The predawn Ψ_w evaluated in 2012 showed clearly that the plants were very stressed, with values of -5.6 and -4.2 MPa for May and August, respectively. In 2013, the predawn Ψ_w values were 2.6 and 1.4 times higher than those registered for the same plants in 2012 for the same months. In both years, the amplitude of the midday Ψ_w values did not vary as much as that of the predawn values (Figure 2).

3.3 Seasonal and Diurnal Cycle in Gas Exchange Parameters

During the drier year (2012), we observed that all the gas exchange parameters were strongly affected by the water deficit in both seasons. The gas exchange, mostly the E value, was so low, that often the equipment could not register values, even when adjusted to high precision readings. As expected, the plants showed strong stomatal closure in response to the water deficit. As a consequence, P_N and E were close to zero at all times of the day, leading to a very low WUE. In 2013, the plants recovered their gas exchange activity for the major values of P_N . Even in the

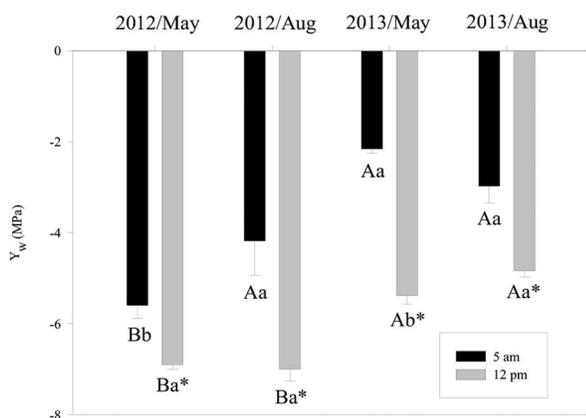


Figure 2. Mean water potential measured in predawn (05:00 am) or midday (12:00 pm) of *Cenostigma pyramidale* plants cultivated in natural environment from April of 2012 to August of 2013 in the study area. Different capital letters denote significant differences between the means for each year, different lower case letters denote significant differences for each evaluated months within the same year (SNK, $p \leq 0.05$) and asterisks denote significant differences between times of the day (t -test $p \leq 0.05$). The values represent the average (\pm SE) ($n = 6$).

dry season of 2013, these values were significantly higher ($P < 0.01$) compared to those of both 2012 seasons. In 2013, E was higher in May than in August. Consequently, water use efficiency (WUE) was seemingly higher in the dry period of 2013 (Figure 3). When comparing the integrated P_N values between seasons and years, the differences become still more evident because those values were 7.2 times higher in May of 2013 than of May of 2012. The same pattern was verified for August of 2012 and 2013, with the integrated P_N value was 6.3 times higher for 2013 than for 2012. Between seasons, within a year, we verified a significant effect only in 2013, when the integrated P_N values were 13.2% larger in May than in August (Supplementary, Figure S1).

An effect of the diurnal cycle on P_N , E , leaf-to-air vapor pressure deficit (VPD) and leaf temperature (T_{leaf}) was observed only in wetter year since the strong water deficit in the drier year lead to very low values for the gas exchange parameters. To understand this effect, we performed correlation analyses; these analyses show that P_N was strongly correlated with g_s ($r = 0.83$) and E ($r = 0.93$) but weakly correlated with WUE ($r = 0.24$) and VPD ($r = 0.22$) (Figure 4A). Very interesting data were verified when only the data for the drier (2012) or wetter (2013) year were evaluated (Figure 4B-C). The relationship between E and WUE that was weak ($r = 0.18$) when we analyzed all the data, became strong ($r = 0.57$) when only the 2012 data were analyzed. This apparent weak overall connection between these parameters was due to their strong negative correlation between these two factors recorded in 2013 ($r = -0.59$). The correlation between VPD and g_s was not significant when considering the entire dataset (Figure 4A) or only the 2012 dataset (Figure 4B). However, this correlation was moderately negative ($r = -0.43$) when examining only the 2013 data (Figure 4C).

3.4 Photosynthetic Curves

The CO_2 response curves of P_N were used to model the maximal rates of carboxylation velocity of Rubisco (V_{c-max}) and of maximum electron transport rate (J_{max}). Significant differences ($P < 0.05$) between the rainy and dry seasons existed for maximum photosynthesis (P_{N-max}), photosynthetic capacity (P_{N-pot}), stomatal conductance ($g_{s[2000]}$), maximum carboxylation velocity of Rubisco (V_{c-max}), maximum electron transport rate (J_{max}) and carboxylation efficiency (k_x). However, the CO_2 compensation point (Γ) values did not differ between seasons (Table 1). In the rainy season, the average values of P_{N-max} and V_{c-max}

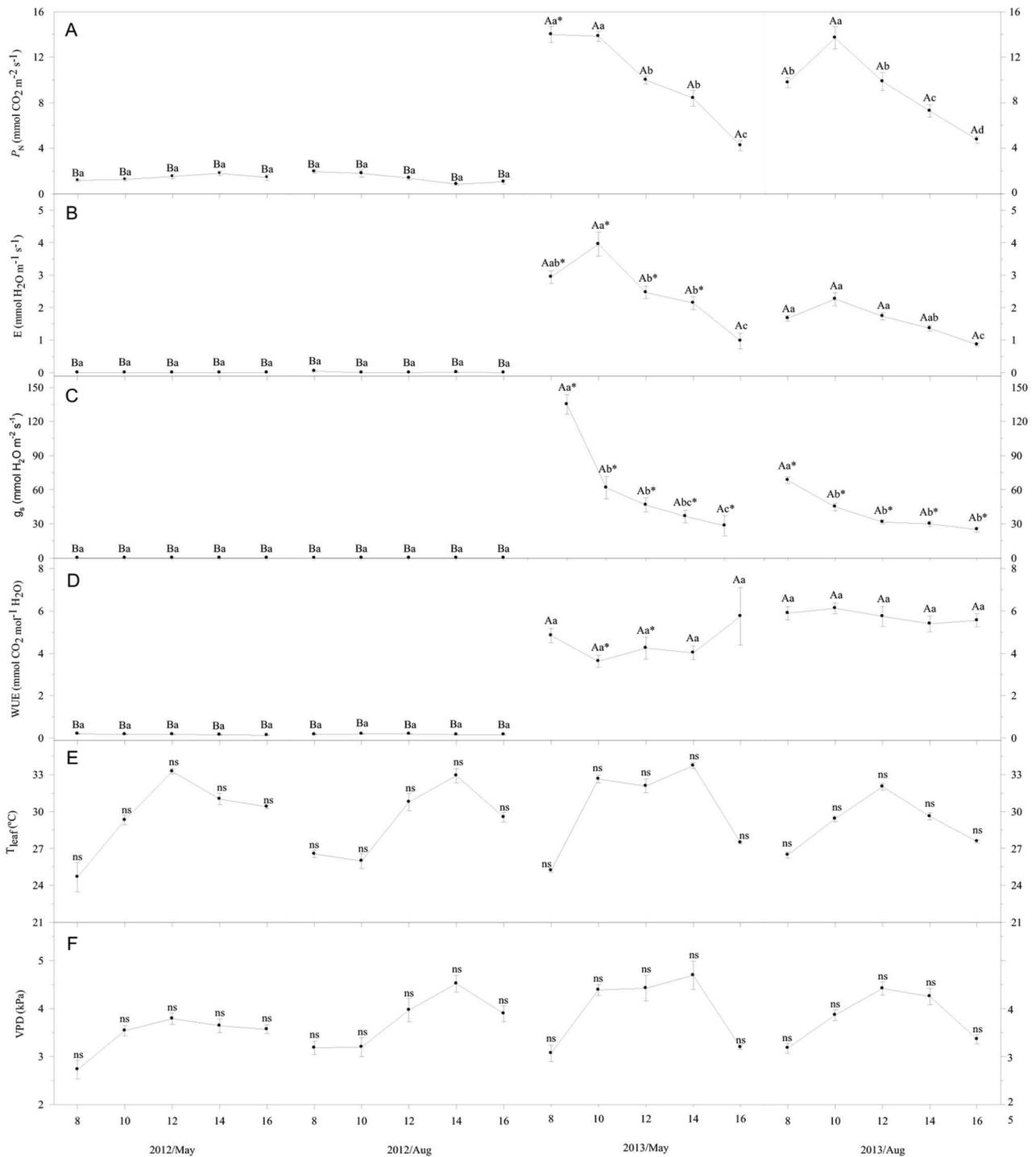


Figure 3. Net photosynthesis (P_N), transpiration rate (E), stomatal conductance (g_s), water use efficiency (WUE), leaf temperature (T_{leaf}) and vapor pressure deficit (VPD) of *Cenostigma pyramidale* plants cultivated in natural environment from April of 2012 to August of 2013 in the study area. Different capital letters denote significant differences between the means for each year within same time, different lower case letters denote significant differences for each times of the day within the same year and month (SNK, $p \leq 0.05$). Asterisks denote significant differences between evaluated months within the same year and time (t -test $p \leq 0.05$). The values represent the average (\pm SE) ($n = 6$).

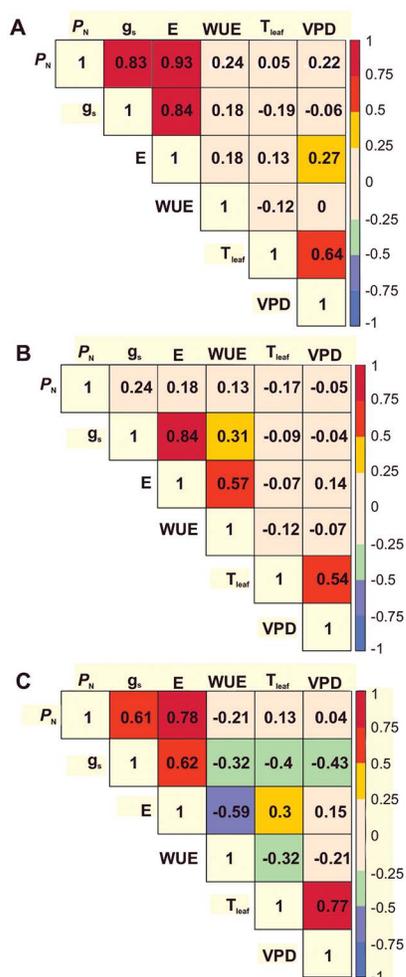


Figure 4. Correlation matrix of (A) all gas exchange data, (B) only 2012 data and (C) only 2013 data. The colour intensity indicates the intensity of the interaction between characteristics, at where, blue colours denotes negative correlations and red colours denotes positive correlations. All correlation coefficients were tested by Newman Keus test ($p \leq 0.01$).

were twice those observed in the dry season ($13.3 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $69.6 \mu\text{mol m}^{-2} \text{s}^{-1}$ compared to $6.7 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $35.4 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively). The effect of soil water balance was more pronounced on P_{N-pot} and J_{max} because in 2013, these respective values were 62% and 63% lower in the dry season than in the rainy season (Table 1).

In Figure 5, the initial portion of the curve shows the almost linear dependence of P_N and C_i , indicating limitation by the carboxylation of Rubisco. The intersection point of the continuous lines (representing the carboxylation rate, P_{N-C}) and dashed lines (representing the electron transport rate, P_{N-j}) signifies the collima-

Table 1. Maximum photosynthesis (P_{N-max}), potential photosynthesis (P_{N-pot}), stomatal conductance (g_s) in $[\text{CO}_2]$ saturated ($g_{s[2000]}$), maximum carboxylation velocity of Rubisco (V_{c-max}), maximum electron transport rate (J_{max}), CO_2 compensation point (Γ) and carboxylation efficiency (k_x) of *Cenostigma pyramidale* measured in May and August 2013 in northeastern Brazil. Different lower case letters denote significant differences between the means within each feature. The values represent the average (\pm SE) ($n = 6$).

Parameters	May 2013	August 2013
P_{N-max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	13.3 ± 2.0 a	6.7 ± 1.9 b
P_{N-pot} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	29.2 ± 1.6 a	11.2 ± 0.98 b
$g_{s[2000]}$ ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	0.12 ± 0.04 a	0.06 ± 0.02 b
V_{c-max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	69.6 ± 5.9 a	35.4 ± 4.2 b
J_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	173.6 ± 12.4 a	64.6 ± 7.4 b
Γ ($\mu\text{mol mol}^{-1}$)	89.9 ± 8.9 a	86.8 ± 8.4 a
k_x ($\text{mmol m}^{-2} \text{s}^{-1}$)	64.5 ± 5.2 a	40.6 ± 4.0 b

tion point (indicated by the vertical line) or equilibrium point, where photosynthesis is equally limited by both the carboxylation and the electron transport rate. In May of 2013, the collimation point of photosynthesis was near $438 \mu\text{mol mol}^{-1}$; however, in August of 2013, photosynthesis was collimated in approximately $178 \mu\text{mol mol}^{-1}$ of C_i . Independently of the conditions (May or August of 2013), an increase in C_i caused an increase in photosynthesis to the point that photosynthesis was limited by the rate of electron transport and triose phosphate utilization. By contrast, the carboxylation rate of Rubisco limited carbon assimilation at a low concentration of CO_2 (Figure 5).

3.5 Foliole Leaf Area (LA) and Specific Leaf Area (SLA)

The leaf area data could show the plant photosynthetic surface and then demonstrate its alteration by climate change. The leaf area presented the same pattern in both periods, with the dry months showing a lower LA compared to the wet ones (Table 2). However, we argue that the leaves collected in 2012 did not change their pattern of leaf investment because the SLA showed no significant differences between the seasons. By contrast, the leaves collected in 2013 presented lower SLA compared to those collected in 2012, and those gathered in August of 2013 (dry season) showed the lowest averages among all the samples (Table 2).

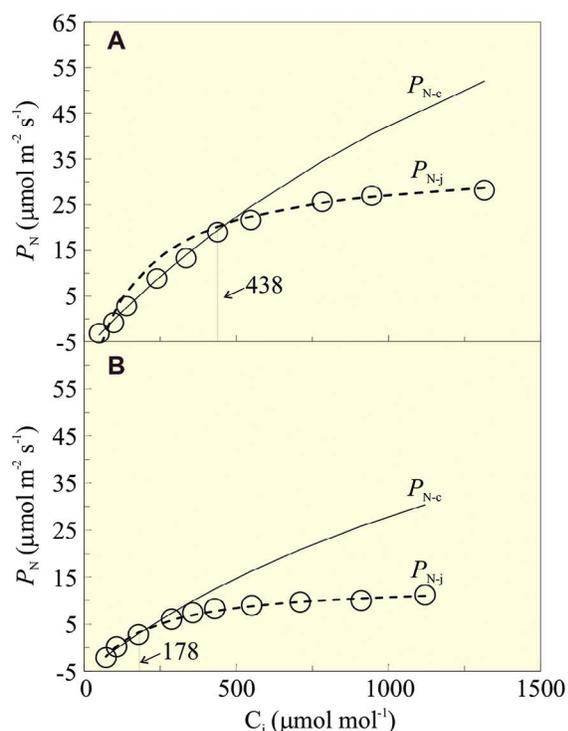


Figure 5. Photosynthetic rate as a function of the intercellular $[CO_2]$ concentration (C_i) of *Cenostigma pyramidale* plants cultivated in natural environment in May (A) and August 2013 (B) in the study area. The solid line shows the limitation of photosynthesis imposed by the carboxylation rate of Rubisco (P_{N-c}). The dashed line indicates the limitation the photosynthesis imposed by electron transport (P_{N-j}). The vertical solid line shows the value of C_i at which occurs the transition from photosynthesis limited by P_{N-j} to photosynthesis limited by P_{N-c} . The numerals (in the graphs) show the values for C_i in $\mu\text{mol mol}^{-1}$. The values represent the average (\pm SE) ($n = 6$).

3.6 Biochemical Analysis

The biochemical analysis showed that the soluble sugars increased in the dry month in both years ($P < 0.01$) (10.3% in 2012 and 22.6% in 2013). Independent of the season, the soluble sugars were higher ($p < 0.01$) when measured in 2012 compared to 2013 (Figure 6A). The soluble amino acids followed a different pattern; the concentration of amino acids decreased during the dry month in 2012 ($P < 0.01$) but increased in 2013. The mean amino acids value was $26.7 \pm 0.3 \text{ mmol kg}^{-1} \text{ DW}$ in May of 2012 but suddenly fell to $20.5 \pm 0.5 \text{ mmol kg}^{-1} \text{ DW}$ in August of 2012 (Figure 6B). In 2013, the amino acids showed an inverse pattern, with $16.9 \pm 0.4 \text{ mmol}$

Table 2. Leaf area (LA) and specific leaf area (SLA) of *Cenostigma pyramidale* measured in two distinct years in north-eastern Brazil. Different capital letters denote significant differences between the means for each year, and different lower case letters denote significant differences for each evaluated months within the same year. The values represent the average (\pm SE) ($n = 250$).

Evaluations	LA (cm^2)	SLA ($\text{m}^2 \text{kg}^{-1}$)
May 2012	2.50 ± 0.04 Aa	15.07 ± 0.24 Aa
August 2012	1.85 ± 0.03 Ab	14.92 ± 0.35 Aa
May 2013	2.66 ± 0.06 Aa	13.40 ± 0.52 Ba
August 2013	1.88 ± 0.04 Ab	11.31 ± 0.21 Bb

$\text{kg}^{-1} \text{ DW}$ in wet and $18.6 \pm 0.3 \text{ mmol kg}^{-1} \text{ DW}$ in the dry one. The soluble proteins varied in the same pattern as that described for amino acids. However, differently from the other compounds, the values reported in 2013 were higher than those observed in 2012 ($P < 0.01$) (Figure 6C). Proline is an amino acid related to water stress, signaling an excess of reductant power, such as that of NADPH. In 2012, the proline concentration ranged from $100.2 \pm 6.0 \text{ mmol kg}^{-1} \text{ DW}$ to $59.5 \pm 3.2 \text{ mmol kg}^{-1} \text{ DW}$ in the wet and dry months, respectively. In 2013, an inverse situation was verified, with the concentration in the dry month was 126 times higher than that in the wet month (Figure 6D). The hydrogen peroxide (H_2O_2) concentrations did not show any difference between the months within the same year; however, the respective concentration in the wet and dry months of 2013 was 13.2% and 16.9% lower compared with that of 2012 (Figure 6E). Malonic aldehyde (MDA) evidenced a pattern similar to that of the other two compounds, with May of 2013 showing a basal value and the other months showing values at least four times higher (Figure 6F). The correlation analyses revealed interesting data because the correlations coefficients of soluble proteins with amino acids, amino acids with proline, and soluble sugars with MDA were $r = -0.49$, $r = 0.52$ and $r = 0.57$, respectively.

The chlorophyll concentration showed significant variation ($p < 0.01$) between the two years, with 3.6 times less chlorophyll detected in 2012 than in 2013 (Figure 7A). When the analysis was only within 2013, we observed that the leaves contained 30.5% less chlorophyll in the dry month (August) than in the wet month (May). A similar pattern occurred in 2012, with August showing the lower values (Figure 7A). The chlorophyll a:b ratio fluctuated

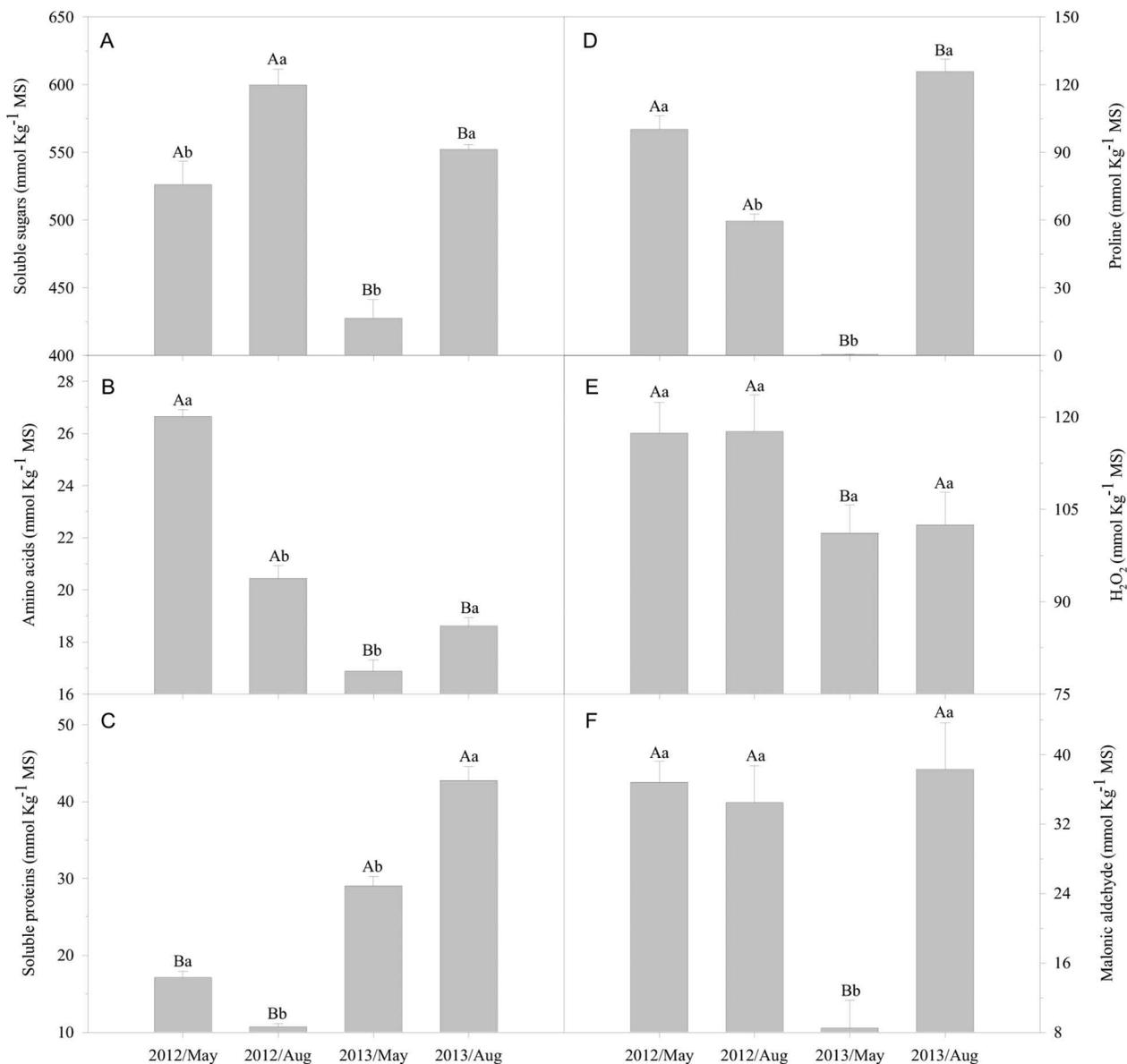


Figure 6. Soluble sugars (A), amino acids (B), soluble proteins (C), proline (D), hydrogen peroxide (H₂O₂) (E) and malonic aldehyde (F) of *Cenostigma pyramidale* plants cultivated in natural environment from April of 2012 to August of 2013 in the study area. Different capital letters denote significant differences between the means for each year and different lower case letters denote significant differences for each times of the day (SNK, $p \leq 0.05$). The values represent the average (\pm SE) ($n = 6$).

very little, with a slight difference in 2013 between May and August, but these values did not differ significantly from those in 2012 (Figure 7B). The carotenoid content also varied between the months and years ($P < 0.05$); however, the plants produced more carotenoids in 2013 than in 2012 (Figure 8C). With the strong increase in the chlorophyll and carotenoid levels, the Chl:Carotenoids ratio was not expected to be significant. However, this ratio was 61% and 86.5% higher in the plants during 2013.

By contrast, no significant difference existed between the rainy and dry seasons examined in the two years of the study. The degrees of both chlorophyll bleaching and the synthesis of carotenoids were significantly correlated with the decrease in proline, H₂O₂ and MDA. For example, total Chl was strongly negatively correlated with MDA ($r = -0.53$), proline ($r = -0.27$) and H₂O₂ ($r = -0.20$); and the synthesis of carotenoids presented a strongly negative correlation with MDA accumulation ($r = -0.44$). The

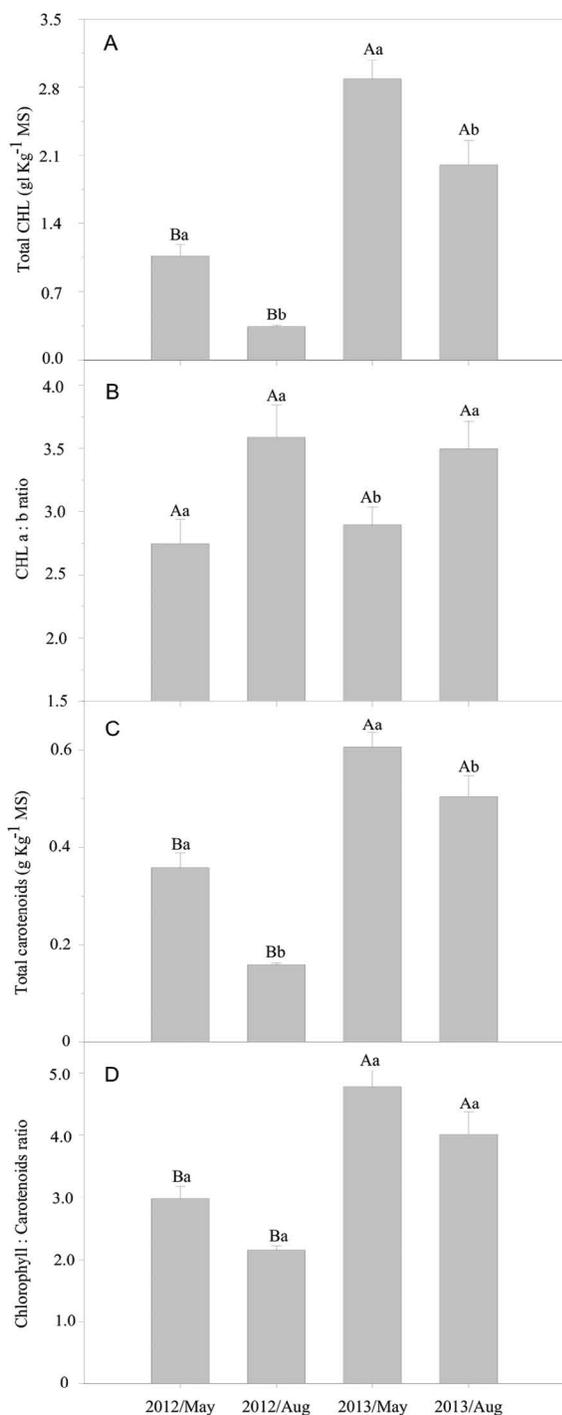


Figure 7. Total chlorophyll (Chl) (A), Chl a/b ratio (B), total carotenoids (C) and Chl: Carotenoids ratio (D) of *Cenostigma pyramidale* plants cultivated in natural environment from April of 2012 to August of 2013 in the study area. Different capital letters denote significant differences between the means for each year and different lower case letters denote significant differences for each times of the day (SNK, $p \leq 0.05$). The values represent the average (\pm SE) ($n = 6$).

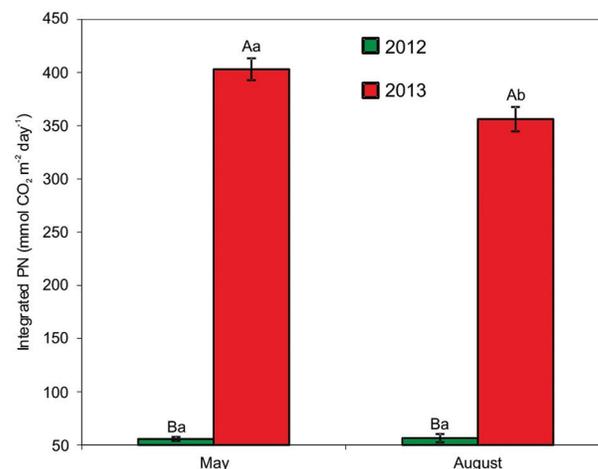


Figure 8. Diurnal integrated CO₂ assimilation (integrated P_N) in 2012 (green bars) and 2013 (red bars) *Cenostigma pyramidale* plants. Different capital letters denote significant differences between the means for each year under the same month, and different lowercase letters denote significant differences between the means for each month within the same year. Each value represents the mean (\pm SE) ($n = 6$).

same pattern was verified between the Chl:Carotenoids ratio and MDA ($r = -0.44$). Strong evidence that the decrease in chlorophyll led to increase in proline synthesis and higher lipid peroxidation is shown by the strong correlation between proline and MDA ($r = 0.81$).

4. Discussion

Forest disturbance, such as fire³⁴, or weather, such a water shortage³⁵, extremely hot and dry summer³⁶ and hail-storms characteristic of south-central North America are capable of severe damage to trees, including broken branches and felled trees. For example³⁷, shows that climate data of a climate scenario, including temperature change and precipitation change for the period 2000-2100 according to the IPCC A2 scenario lead to the smallest area of regeneration. The average age thus increases from 56 years in 2000 to 72 years in 2100. No doubt exist that the conscientious and protective use of forests must be practiced because the fixation of CO₂ and the decrease of the greenhouse effect depend on these forests. Within this perspective, studies on the ecophysiological and biochemical characteristics of the species inserted in vulnerable ecosystems are crucial to reaching this goal.

Forests are heavily affected by water stress due to low precipitation. A feature widely present in the dry forests

worldwide is the reduction of leaf water potential as a protective strategy against the lack of soil or atmospheric humidity. We described in this study that *C. pyramidale* showed a very negative Ψ_w in 2012, even in the *predawn* measurements. In their works³⁸, classified *C. pyramidale* as a species that already begins the day with a very negative Ψ_w during the dry season, which can become more negative as the day progresses. However, the water potential values reported for *C. pyramidale* by these authors most closely approximated those observed in the present study during 2013, the study year with the rainfall close to that expected for the region, with values ranging from -2 and -4MPa.

In 2012, a year with very little rainfall, the Ψ_w values were even more negative, reaching as low as -7.5 MPa during midday in August. Other studies on native tropical dry forest species show very negative water potentials, such as studies on *Calotropis procera* (M.F. Pompelli, manuscript in preparation) and also *C. pyramidale*³⁹. These findings demonstrate that these plants can support high pressures and recover when the water availability is restored, evidencing the high capacity of adaptation by this species to a dry environment. Only plant species that are fully adapted to semiarid conditions can withstand pressures that very negative. To do so, the walls of the plant veins must be highly lignified in a way that avoids collapse or cavitation. However, in present study, we did not investigate that issue in the present study. Nonetheless, these conducting vessels combined with a lack of turgor pressure for the cell elongation can lead to a high dry mass accumulation per area unit in the stressed plants⁴⁰.

With an increase in leaf dry biomass, one would expect that there would be a decrease in SLA, as already shown by others^{41,42}. However, it was not exactly what this work showed, since SLA was strongly elevated in the driest year in comparison to the driest year, although there was a moderate decrease in SLA when the comparison is done between the driest and the driest season within each year. As in 2012, it was not possible to distinguish a well defined dry or rainy season, the SLA comparison between May and August was not significant ($P = 0.767$), while in the year with rains within the expected range, SLA was decreased by ~ 16% ($P < 0.001$). This result corroborates our initial hypothesis of *C. pyramidale* shows higher tolerance if exposed to recurrent water deficit, since *C. pyramidale* is a native species and strongly adapted to the seasonal variations of the environment⁴³. Similar results were taken from⁴⁴ with *Cynophalla flexuosa*, an another

native species from semi arid Caatinga. Based on this, we argue that the higher SLA in the dry year indicated that the leaf biomass might have been compromised by the very low water potentials, which directly affect the turgor pressure needed for the cellular elongation. Additionally, a strong lignified cell wall can avoid the cavitation and cell collapse caused by such extremely negative pressures as previously reported by⁴⁵. By the way, it is worth noting that SLA depends not only on LA but also on leaf density and thickness. Nevertheless, plants with smaller gas exchange surfaces tend to lose less water by transpiration^{40,45}.

In general, stress can be caused by several factors; however, water stress is quite common. With water stress, plants must face high irradiance and high VPD, that can cause irreversible damage to gas exchange mechanisms and carbon metabolism. In severe water restriction, as we observed in 2012, the stomata could decrease the aperture size, independent of VPD, which can nevertheless compound the decrease^{12,15}. These strategies can help the plant water economy, avoiding great falls in P_N and increases in E ^{46,47}. Once that the water status was re-established in 2013, all the plant parameters strongly recovered. The lowest values of g_s in the 2013 dry season can be attributed mainly to a typical feed forward response - stomatal closure with VPD^{12,14}. When comparing 2013 (rainy year) to 2012 (dry year), we argue that the stomata evaluated in 2012 tended to remain less open during the dry season, reducing the total daily photosynthesis. These results suggest that in *C. pyramidale*, photosynthesis is limited more by stomatal conductance than by the others parameters. Certain authors have reported that stomatal closure or smaller stomatal size may drastically affect gas exchange^{9,12,15,24}. In this study, we showed the relation between stomatal closing and gas exchange by the correlation between these factors: $r = 0.830$ for P_N and g_s ; $r = 0.203$ for P_N and PAR; and 0.220 for P_N and VPD. The most striking differences, however, were found between years (2012 and 2013), confirming that the main environmental parameter limiting the photosynthetic performance of this species was water availability, diminished in 2012 by La Niña phenomena that drastically decreased the rains in northeastern Brazil in 2012⁴⁸. Apparently, the reduced water potential was the main strategy that was used by *C. pyramidale* to decrease desiccation and maintain basal photosynthetic rates. A reduction in transpiration, via increased stomatal closure, prevents excessive water loss in cases of low water availability and protects the hydraulic architecture of the plant, even under conditions of low

leaf water potential⁴⁷. In 2013, we verified a decrease in the transpiration rate that did not lead to an equal decrease in P_N during the same months and times, increasing WUE in May and August of 2013. This fact can be corroborated by the negative correlation between WUE and g_s as well as WUE and E ($r = -0.32$ and $r = -0.59$; respectively). Overall, the stomatal control over P_N decreased during the water privation, which is confirmed by a 21% WUE increase (mean value), according to the results by⁴⁹, and the narrow range of WUE variation. More significant effects on P_N and g_s suggests a co-regulation of the stomatal aperture and photosynthetic activity⁵⁰. By contrast, when the stomatal opening was minimal in the very dry year, a slight increase in g_s caused WUE to increase ($r = 0.31$), but a strong increase occurred in the strength of the correlation between E and WUE ($r = 0.84$). Several studies show that trees can increase their WUE when exposed to a water deficit¹¹⁻¹³. To do so, plants must show efficient stomatal control or even biochemistry^{16, 51}, as observed in the species studied.

The values of V_{c-max} and J_{max} obtained in the present study are within the range recorded for other tropical forest species⁵². However, the values of V_{c-max} and J_{max} in the dry season are low when compared to those obtained in the rainy season. This indicates that in the dry season, *C. pyramidale* allocated a small amount of assimilates and nitrogen to proteins synthesis and Rubisco activity. The reduction in photosynthetic rates during the dry season can be attributed to diffusive factors, as well as to the fall in the electron transport rate and the RuBP-regeneration capacity. A water deficit markedly reduced carboxylation efficiency (k_x). The lower values of k_x and P_{N-pot} suggest a reduction in the amount and/or activity of Rubisco with decreasing water potential. Although P_N is limited more by g_s in the dry season, the proportion by which water stress increases the limitation of P_N caused by non-stomatal factors associated with photosynthetic metabolism becomes more evident in *C. pyramidale*. Under water stress conditions, reductions in both Rubisco activity and nitrate reductase activity have been observed⁵¹. In the rainy season, below the collimation point (C_i values of $450 \mu\text{mol mol}^{-1}$), photosynthesis is limited by the carboxylation rate of Rubisco; in turn, V_{c-max} consequently becomes strongly dependent on C_i . A more detailed analysis shows that the lines representing the carboxylation rate (P_{N-c}) coincide exactly with the observed photosynthesis values. This results indicates that below the collimation point, the photosynthetic rates were limited by V_{c-max} , signifying a

lower investment in proteins associated with Rubisco^{53, 54}. By contrast, in the dry season, both the carboxylation and the electron transport rate seem to limit the photosynthetic rates due to a deficiency in the regeneration of its substrate (ribulose biphosphate). Note that in high CO_2 levels, assimilation was limited specifically by J_{max} , a situation reflected in the photosynthetic capacity (P_{N-pot}). Disregarding the eventual acclimatization of photosynthesis to an atmosphere enriched with CO_2 , estimates made with modeling techniques predict that as the CO_2 concentration of the air increases, an increase in photosynthetic rates will also occur, at least until 2050⁵⁴. However, with the increase in the atmospheric concentration of CO_2 , photosynthesis can be restricted by electron transport and by triose phosphate utilization⁵⁵.

The elevation in the concentration of total soluble sugars and amino acids could be related to osmotic adjustment, important in the balance between the water lost and the biological functions. However, the increase of soluble sugars in the leaves can be related to possible damage to source-sink mechanism⁵⁶, once the leaves (especially in 2012) show a very low sap flow caused by the very low water potential. Additionally, soluble sugars such as sucrose have an important role in the electron-free capture and thus the formation of ROS⁵⁷. However the positive correlation between soluble sugars and MDA, an important indicator of cellular degradation by ROS, shows that such formation is not the case in the present study. The lowest concentration of soluble proteins in 2012 may have occurred as part of the leaf senescence process, during which part of their important leaf compounds are Translocated from the leaf back to the plant. With an aggravation of the water deficit, protein hydrolysis may occur⁵⁸ and affect even those proteins connected to the oxidative apparatus. Proteins may also be recycled to synthesize not only other proteins, including those that protect against water loss and especially those in the cell wall, but also amino acids related to protection against ROS, such as proline, since such recycling requires a comparative low energy investment. That conclusion can be corroborated by the negative correlation found between soluble proteins and amino acids, as well as the positive correlation between amino acids and proline. However, we showed that the protein content was higher in the drier month of 2013. This results can be associated with the production of proteins related to protection against ROS, possibly catalase and superoxide dismutase enzymes. Also⁴⁷, also suggest that plants subjected to water deficit cycles tend

to show more rapid and accurate responses when exposed to a second stress period, a circumstantial evidence that *C. pyramidale* can be more efficiently prepared for the dry season. For that purpose, plants improve their photosynthetic rate and consequently their reserves trying to minimize the effects of water restriction. These facts seem consistent with the decreased amount of cellular H₂O₂ in August of 2013, which suggests that certain of these proteins can contribute to the antioxidative metabolism against ROS. For a long time, we thought that proline was related to osmotic adjustment in plants exposed to water stress. However, more recent studies^{59, 60} show that proline may work more as a dissipater of reducing power (e.g., NADPH) than as actor affecting the mechanisms of osmotic adjustment. A high concentration of proline in the dry year (2012) and the dry month (August of 2013) can indicate that *C. pyramidale* uses this molecule as a defense against ROS. ROS can cause the production of MDA by the peroxidation of cell membranes. This evidence could be corroborated by the fall in the electron transport rate and the RuBP-regeneration capacity, as previously described above.

The total chlorophyll content as well as the chlorophyll a and b contents are indicators of overall plant health and directly influence a plant's ability to absorb light for photosynthesis⁶¹. This ability is crucial to maintaining vital processes of the plant system. Some plant protective mechanisms may be activated during a biotic stress, such as an increased production of pigments. Carotenoids, which include carotenes and xanthophylls, are pigments that are closely associated with chlorophylls and play a role in light absorption and photosynthesis⁶². The main role of carotenoids during photosynthesis involves their ability to sequester damaging oxygen radicals and triplet chlorophyll that are readily produced by photosynthetic complexes during light harvesting. A review by⁶³ highlighted the role of xanthophylls cycle carotenoids in photoprotective energy dissipation during photosynthesis, without which the photosynthetic process may be severely inhibited. The degradation of chlorophyll is important for plant protection against damage caused in the photosystems (PS), mostly in PSII, this degradation is considered a strategy for decreasing the production of reactive oxygen species (ROS)⁵⁸. Based on this information, we argue that the synthesis of carotenoids, perhaps violaxanthin and zeaxanthin, could protect the photosystems, promoting security by dissipating excess energy and protecting against the membrane peroxidation

caused by free radicals⁶¹. The PSII efficiency decrease is known to be regulatory, serving a photoprotective role; and an increased level of energy dissipation, which results in a lower quantum yield of PSII electron transport (F_{PSII}), may help to protect PSII from over excitation and photodamage⁶⁴. Thus, this result suggests that a decrease in chlorophyll and specific carotenoids at the PSII level which confers a greater capacity to face with water stress. Moreover, the plants evaluated in 2012 showed a lower total chlorophyll content associated with a lower Chl:Carotenoid ratio during water privation. This reduction contributes to survival under water stress conditions because on one hand the chlorophyll loss due to the reduced number of photons absorbed by leaves leads to an enhanced photoprotective and antioxidant capacity of leaves per amount of photons absorbed⁶¹.

5. Conclusions

Our results, obtained partly during one of the most severe drought events in the last 50 years, show that an extreme limitation in precipitation levels may lead to declines in soil water storage and the consequent suppression of plant growth. We demonstrated that in the 2012 drought year, *C. pyramidale* presented efficient stomatal control, decreasing the aperture degree of the stomatal pore, and concomitantly increased its WUE, exhibiting a wide capacity to regulate water use and avoid water stress. Therefore, reduced water potential, osmotic adjustment and an efficient antioxidative system were the main strategies that the plant used to decrease desiccation and maintain basal rates of photosynthesis. Thus, once the water status was re-established in 2013, the plants presented a strong recovery of all the examined parameters.

However, our results regarding the CO₂ response curves imply that a prolongation of the dry season could have potential impacts on the electron transport rate and on Rubisco carboxylation efficiency, taking into account the current rates of atmospheric CO₂. Most climate models predict an increase in CO₂ and temperature on a global scale. If atmospheric CO₂ continues to increase, the inference that carbon uptake by *C. pyramidale* in the Caatinga could be restricted by maximum electron transport rates seems plausible. Due to the significant territorial extent of the Caatinga and species diversity in the region, more studies are still needed to determine the mechanisms by which specific factors associated with water deficit influence Rubisco carboxylation and electron transport rates in

the various functional groups. In the context of mitigating climate change on a global scale, forest management entities should significantly lengthen cutting intervals in arid and semiarid regions.

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7. Conflict of Interest

All authors contributed substantially and approved the final submission.

All authors declare that they have no conflict of interest.

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