Climate change compromises leaf units and lignin content in sun-tolerant *Paubrasilia echinata* plants

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Key message

RCP 8.5 climate change scenario increased sugar content and photosynthesizing leaf area production more intensely in *Paubrasilia echinata* plants of the shade-tolerant ecotype. Such climatic scenario inhibited the lignification of the sun-tolerant ecotype.

Abstract: It is of great global interest how shade-tolerant and sun-tolerant species will respond to the Representative Concentration Pathways (RCP) scenarios of climate change predicted by the end of the 21st century. To elucidate this question, we used young plants of the shade- and sun-tolerant ecotypes of *Paubrasilia echinata*, a tree native to the Brazilian Atlantic Forest. Plants were grown in three climate scenario simulations: current Atlantic Forest for the southeast region of Brazil (CAF); average temperature of CAF + 2.0 ºC and 580 ppm CO_2 (RCP 4.5); and average temperature of CAF + 3.5 °C and 936 ppm CO_2 (RCP 8.5). Two substrate moisture conditions were established for each scenario: 40 (water deficit) and 80% (control). After 90 days of cultivation in these microclimatic conditions, we carried out growth analyses (survival, number of folioles and foliolules of their compound leaves and increase in stem elongation), non-structural carbohydrate content (sugars and starch) and cell wall polymer content (cellulose, hemicelluloses and lignin). The pessimistic climate scenario (RCP 8.5 + water deficit) inhibited photosynthesizing leaf area production (number of folioles/foliolules) of the shade-tolerant ecotype more, but without affecting survival. In this climate scenario, the shade-tolerant ecotype showed a greater capacity to accumulate sugars than the other ecotype. Also in the pessimistic climate scenario, the shade-tolerant ecotype accumulated more of this polysaccharide in the stem, while in the sun-tolerant ecotype this occurred in the root. This scenario increased the cellulose content of both ecotypes to the detriment of the lignin content of the sun-tolerant ecotype. We conclude that the rise in CO_2, temperature and DPV along with low precipitation in RCP 8.5 predicted for the southeastern region of Brazil by the end of this century may compromise more the photosynthesizing leaf area production of the shade-tolerant ecotype and the wood quality of the sun-tolerant ecotype of *P. echinata*. However, this does not threat their survival.
Keywords: cellulose, CO_2, hemicelluloses, non-structural carbohydrates, RCP 8.5, water deficit

Introduction

The 5th report of the Intergovernmental Panel on Climate Change predicts a number of climate scenarios for the end of the 21st century called Representative Concentration Pathways (RCPs, IPCC 2014). RCPs are determined by their radiative forcing (W/m^2) defined by greenhouse gas (GHG) emissions. The main scenarios are RCP 2.6 (low emissions), RCP 4.5 (stabilization of emissions) and RCP 8.5 (high emissions). In RCP 4.5, W/m^2 should reach 6.0 by the middle of this century, stabilizing shortly after 2100 without exceeding the high level. RCP 8.5 is a pessimistic scenario characterized by very high GHG emissions with a forecast W/m^2 higher than 8.5, drought, a 1-4 ºC increase in the air and atmospheric CO_2 above 800 ppm by 2100 (IPCC 2014).

The impacts of the pessimistic climate scenario (RCP 8.5) on vegetation could be catastrophic and irreversible, with the possibility of affecting the survival and growth of tropical species (Amaral et al. 2022, 2023), including those of the Atlantic Forest distributed along the coast of Brazil, Uruguay and Argentina (Scarano and Ceotto 2015). This tropical biome is home to the second largest diversity of plant species in the world (Gagnon et al. 2016) categorized into pioneer (sun-tolerant or fast-growing) and non-pioneer (shade-tolerant or slow-growing) trees, which are recognized worldwide for their high biomass and strong drain on global C (Grace et al. 2014).

Climate change over the next few decades will require plants to adapt quickly (Dyderski et al. 2017). The survival and perpetuation of native vegetation in a climate change scenario will depend, in part, on the ability of its species to adjust the carbon pools between source and drain organs (Martínez-Vilalta et al. 2016). In the long term, plants will not survive if carbon pools do not meet the immediate demands of metabolism and growth (Dietze et al. 2014).

Carbon photoassimilated and not consumed in cellular respiration is accumulated in the plant cell as non-structural carbohydrates-NSC (sugars and starch) (Martínez-Vilalta et al. 2016) and cell wall polymers-CWP (cellulose, hemicelluloses and lignin) (Schädel et al. 2010). According to Würth et al. (2005), NSC in trees reflect the balance between photoassimilated carbon (source) and irreversible investment in structures or loss of carbon via cellular respiration from their drains. In addition to being a source of energy for the immediate demands of cell metabolism (Dietze et al. 2014), sugars (mono-, di-, tri- and oligosaccharides) are also environmental signaling (Würth et al. 2005, Hartmann and Trumbore 2016) and osmoregulating (Martínez-Vilalta et al. 2016) agents.

In tropical woody species, NSCs have been associated with defense mechanisms and/or adaptation to lower soil moisture (Würth et al. 2005, Zorzanelli 2023), cold (Macieira et al. 2023), waterlogging (Medina et al. 2009, Arenque et al. 2014), high irradiance (Mengarda et al. 2012) and increased atmospheric CO_2 (Grandis et al. 2021). CWPs comprise the largest stock of C in plant biomass and are considered key components of wood quality (Carpita and McCann 2000, Martínez-Vilalta et al. 2016). A positive association of hemicelluloses (Volponi et al. 2022a,b), cellulose

(Zorzanelli 2023) and lignin (Knupp et al. 2021, Volponi et al. 2022b) with low soil moisture has been found in the coastal vegetation of the Atlantic Forest. In general, these CWPs can decline in response to warming (Le Gall et al. 2015), and in some tropical species hemicellulose content can increase in winter (Macieira et al. 2023), and this polymer and lignin in greater demand for CO_2 (Le Gall et al. 2015, Grandis et al. 2021). In general, none of these studies considered the functional classes of sun-tolerant (pioneer or fast-growing) and shade-tolerant (non-pioneer or slow-growing) species studied.

These two functional classes differ in growth speed, photosynthetic rate, wood longevity and wood density, which are higher in sun-tolerant species (Swaine and Whitmore 1988, Whitmore 1990). As for NSC and CWP, the trunks of some sun-tolerant tropical tree species in the Atlantic Forest are richer in sugars (Macieira et al. 2020). However, Signori-Müller et al. (2022) found the opposite result in a sun-tolerant species (*Bixa arborea*) and a shade-tolerant species *(Eschweilera coriacea*) in the Amazon rainforest of Peru. As for starch, stems starch content did not differ between the two functional classes in the Atlantic Forest (Macieira et al. 2020) while in the Amazon Forest, the shade-tolerant species showed a higher value (Signori-Müller et al. 2022). The possible causes of contradiction in NSCs between these studies can be attributed to differences in climatic conditions at the time of collection of biological material, geographical location and number of species. Regarding CWP, trunks of shade-tolerant tropical species from the Atlantic Forest stand out for their greater richness in cellulose, hemicelluloses and lignin compared to sun-tolerant species from this biome (Macieira et al. 2020).

How the allocation of NSC and CWP of these two functional classes will respond to climate change scenarios is a question of great environmental and economic interest, but one that has been little explored. When exposed to water deficit, the sugar content in the vegetative organs (leaves, stem and root) of shade-tolerant tropical plants increased to the detriment of starch, and more intensely than sun-tolerant plants (Fonseca 2021). On the other hand, cold weather associated with low rainfall increased the sugar content to the detriment of starch in the aerial organs of the sun-tolerant species *Senna multijuga* (Macieira et al. 2023).

As for CWPs, sun-tolerant plants in the Atlantic Forest are more lignified than shade-tolerant plants in natural conditions (Macieira et al. 2020) or under water deficit (Fonseca 2021). Higher lignin deposition improves hydraulic conductivity in plants exposed to drought (Le Gall et al. 2015) and higher irradiance associated with heat (Martins et al. 2021, Gama et al. 2021). At low temperatures, the hemicellulose content of the sun-tolerant species *Senna multijuga* increased, but not that of the shade-tolerant species *Hymenaea aurea* (Macieira et al. 2023), both from tropical forests. According to Cuzzuol et al. (2022), there are indications that the hemicellulose values of sun-tolerant tropical plants may decrease in hotter and drier climates.

Considering that shade-tolerant species are well adapted to wetter and cooler forest environments (Poorter et al. 2010, Rossato and Kolb 2010) and accumulate more sugars to the detriment of starch and lignin under water deficit (Fonseca 2021), we hypothesize that *i*) plants of this functional class should accumulate more sugars to the detriment of starch and lignin in the pessimistic climate scenario (RCP 8.5 + water deficit) compared to sun-tolerant plants. Another hypothesis is that *ii*) in the pessimistic climate scenario, these physiological responses should compromise the survival and growth of shade-tolerant plants more than the sun-tolerant ecotype. To test these hypotheses, we used young plants of the small-leaf or "arruda" ecotype (shade-tolerant) and plants of the medium-leaf or "café" ecotype (sun-tolerant) of *Paubrasilia echinata* (Gama et al. 2021). (sun-tolerant), a tree native to the Brazilian Atlantic Forest biome. These ecotypes differ in the early growth phase (Gama et al. 2021). By eliminating taxonomic divergence, this taxon has proved to be an excellent biological model for investigating these two functional classes of tropical trees in contrasting environments (Cuzzuol et al. 2020, Gama et al. 2021, Cuzzuol et al. 2022, Fonseca et al. 2023). The aim of this study was to evaluate the effects of warming, CO_2 enrichment, higher DPV and water deficit in the pessimistic climate scenario (RCP 8.5) on the increase in photosynthesizing leaf area, stem elongation, survival and allocation of NSC and CWP in plants of the shade-tolerant ecotype and sun-tolerant ecotype of *P. echinata.*

Materials and methods

Plant material and experimental design

Seedlings of the small- (shade-tolerant) and medium leaf (sun-tolerant) ecotypes of *Paubrasilia echinata* (Lam.) Gagnon, HCLima, and GPLewis were produced in their seminal form in plastic bags (2 L) containing an organic substrate made by organic compost + soil + sand (2:1:1) at Reserva Natural Vale in Sooretama, Espírito Santo, Brazil (19°11’ 52” S and 40°05’29” W). The seeds were collected from adult matrices in a forest Atlantic fragment at Reserva Natural Vale. The germination and initial growth of plants occurred in a nursery (50% shade) under natural temperatures, precipitation, and photoperiods. Homogeneous and healthy plants aged one year, measuring 22±4 cm in height and containing 3–4 leaves were transferred to polyethylene pots (12 L) containing a commercial substrate (Vivatto Slim Plus 20) composed of bio-stabilized pine bark, vermiculite, charcoal mill, water, and phenolic foam. Fertilization was carried out by adding 4 g L^{-1} of controlled-release fertilizer with the following nutrients: nitrogen (15%), phosphorus (8%), potassium (12%), magnesium (1.2 %), sulfur (4%), iron (0.4%), boron (0.02%), zinc (0.02%), copper (0.05%), manganese (0.06%), and molybdenum (0.015%). The pots were transferred to a greenhouse (with controlled temperature and relative humidity) at the Department of Forest Science and Wood at the Federal University of Espírito Santo, in the municipality of Jerônimo Monteiro, Espírito Santo, Brazil

(20°47'25" S and 41°23'48" W), the climatic conditions of which were the same as those in Linhares, a municipality from northern Espírito Santo state in which *P. echinata* naturally occurs.

The plants remained in these conditions for 30 days so they could acclimatize themselves to the conditions in which they were set. They were irrigated every two days. Subsequently, the pots were randomly distributed and placed in open-top chambers (OTCs) measuring 1.80 m height × 1.0 m diameter inside three acclimatized greenhouses, totaling six OTC units at the greenhouse. Inside each OTC, six seedlings were randomly distributed — 18 of each ecotype, totaling thirty-six plants per greenhouse. OTCs have a ventilation and [CO_2] injection system at their bottom, which mixes [CO_2] with air and generates a flow of gases inside the chambers to maintain a constant concentration of [CO_2] and prevent heating.

The relative humidity and temperature (mean, maximum, and minimum) inside the greenhouses were monitored daily with a digital thermo-hygrometer (TH-02 Impac.com.br). Their values are shown in Table 1. The photon flux density (1200±50 μmol.m^{-2}.s^{-1}) inside the ovens was measured every 1 min for 10 min with a spectroradiometer (Spectrum Technologies, Inc., Illinois, USA), at 9:00 a.m. on a sunny and cloudless day.

Simulation of climate scenarios

In total, three scenarios were simulated based on the local summer period (December-February) as it better represents the region of Linhares. The first scenario (used as a control) was defined by the climate of the current Atlantic Forest (CAF) and represents the climatic conditions in which *P. echinata* ecotypes naturally occur (northern Espírito Santo), characterized by the climatological parameters provided by the National Institute of Meteorology (https://portal.inmet.gov.br/). The temperature and relative humidity totaled 26.1 ºC and 82%, respectively. From these values, curves of daily variation of the temperature and relative humidity of the air were projected, considering the current [CO_2] of 400 ppm.

Moreover, two other scenarios represent climate change projections: representative concentration pathway 4.5 (RCP 4.5) and representative concentration pathway 8.5 (RCP 8.5) for the 2081-2100 period, described in the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC 2013). The climate change scenarios RCP 4.5 and 8.5 received temperature increases of 2 and 3.5 ºC, respectively, with the average temperature of the current Atlantic Forest as a control. The temperature and relative humidity totaled 28.1 ºC and 80% in RCP 4.5; and 29.6 ºC and 74%, in RCP 8.5. Relative air humidity was calculated using the compensated water vapor partial pressure values of [CO_2] projections totaling 580 and 936 ppm, respectively.

In each climate scenario, two substrate moisture conditions were established: 80 (water balance) and 40% (water deficit) of the maximum water-retaining capacity (MWRC) of the substrate, controlled by daily weighing the pots containing the plants and, when necessary, replacing the water

lost by evapotranspiration following Amaral et al. (2020). The control sample was saturated with water and, after weighing, was taken to a forced circulation oven at a temperature of 105 °C for 24 hours to characterize its dry weight. Thus, the MWRC value was calculated using the equation below:

$$MWRC = Wps - Wp - [(Wsp*Wds)/Ws]$$

In which Wps is the weight of the pots with saturated substrate (g); Wp is the weight of the empty pots (g); Wsp is the weight of the substrate inserted in the pots (g); Wds is the weight of the dry control sample (g); and Ws is the weight of the saturated control sample (g).

The MWRC of all pots was determined using the equation above for the Wvs of each pot. Irrigation was controlled by daily weighing and the water lost by evapotranspiration was replaced when necessary. The water content of the substrate was evaluated daily to measure the tested water-retaining capacity (WRC).

In total, two automatic weather stations model CS500 (Campbell Scientific, Inc., Logan, UT, USA) with three combined sensors for air temperature and relative humidity were installed inside the greenhouse for microclimatic characterization of the scenarios reproduced separately in three greenhouses (Table 1). The data were stored in a data logger, model CR-10x (Campbell Scientific, Inc., Logan, UT, USA), with readings of the mean values at 10-minute intervals.

The air temperature in each simulated climate scenario was controlled by an evaporative cooling system (pad cooling), air conditioners, exhaust fans, and heaters activated by air temperature controllers (MT-543Ri plus, Full Gauge®). Air humidity controllers of the AHC-80 plus type (Full Gauge®) and a fogger-type misting system controlled the relative humidity of the air. The values of temperature and relative humidity of the air were programmed on Sistrad®. Moreover, photosynthetically active radiation was monitored by a Quantum model sensor (Li-Cor Inc, Lincoln, Nebraska, USA).

Microclimate characterization was performed throughout the 90 days of the experimental period. In this situation, the microclimate conditions were regulated by an evaporative cooling mechanism (pad cooling), air conditioning units, and heaters. Vapor pressure deficit (VPD) was calculated by subtracting the saturation vapor pressure from the partial water vapor pressure.

The daily temperature, VPD, RH, and [CO_2] of the three scenarios and their average values are shown in Table 1. In the current Atlantic Forest (CAF) the injection system supplied no [CO_2] to maintain environmental [CO_2]. In the RCP 4.5 and 8.5 scenarios, the established [CO_2] was maintained in the OTCs by the daily supply of CO_2. The supply of CO_2 by the gas pressure system performed the fertilization with CO_2 in the plants from 8 a.m. to 4 p.m., every day of the experiment. The daily monitoring of [CO_2] inside the greenhouses was carried out at noon by a portable analyzer model AG 535 (Testo, Lenzkirch, Baden-Württemberg, GER). After monitoring, the OTCs underwent [CO_2] adjustment to regulate the gas flow according to the established in each scenario.

After 90 days of exposure of plants to climatic treatments, we measure the length of the stems of plants and the number of leaves. Next, we collected samples of their vegetative organs to determine non-structural carbohydrates and cell wall polymers. Considering that the heartwood of *P. echinata* wood is used to make bows for musical instruments, the cell wall polymers were determined only in the stem.

Non-structural carbohydrates-NSC (soluble sugars and starch): ~ 10 g samples of fresh mass of vegetative organs (leaves, stems and roots) were microwaved for enzyme inactivation at 600w/90 seconds (Popp et al. 1996), dehydrated in a 65ºC forced air circulation oven for 5 days and pulverized in a ball mill (model TE-350, TECNAL, São Paulo, Brazil).

The soluble sugars were extracted from 10 mg of the powdered samples in hot 80% ethanol followed by centrifugation according to Pollock (1986). The supernatant was separated, and the operation repeated three more times. The supernatants were pooled and concentrated in a 50 °C oven for 24 hours and the precipitate dried under the same conditions mentioned. Sugars were resuspended in ultrapure water and glucose, fructose and sucrose were determined by high-performance liquid chromatography on a Shimadzu chromatograph (model SIL-10AF, Kyoto, Japan), Shim-Pack® SPR-Pb column (250 x 7.8mm), flow rate 0.6mL/min, temperature 80°C, refraction detector, eluted with ultrapure water, using Sigma® standards.

To the precipitate from the previous operation, we determined the starch content using the enzymatic method of Amaral et al. (2007), adding 0.5 mL (120 U $^{mL-1}$) of thermostable α-amylase from *Bacillus licheniformis* (cod. E-Anaam, Megazyme) diluted in 10 mM MOPS buffer at pH 6.5. The mixture was heated at 75ºC for 30 minutes and after cooling (50ºC) it received 0.5 mL of 30 U $^{mL-1}$ of amyloglucosidase from *Aspergillus niger* (cod. EAMGPU, Megazyme) in 100 mM sodium acetate buffer at pH 4.5. We transferred 50 µL of this extract to 250 µL of the Glucose PAP Liquiform reagent (Centerlab, Brazil) containing glucose oxidase (~11,000 U $^{mL-1}$) and peroxidase (~700 U $^{mL-1}$), 290 µmol $^{L-1}$ of 4-aminoantipyrine and 50 mM phenol pH 7.5. The samples were incubated at 50 ºC for 30 minutes twice, followed by reading in an ELISA reader (Thermo Scientific Express Multiskan FC Microplate Photometer, Massachusetts, USA) at 490 nm. Starch was estimated by quantifying the glucose released during starch hydrolysis. The absorbances were applied to the standard curve of glucose solution (Sigma®) of 0, 2.5, 5, 7.5 and 10 mg.mL^{-1}.

Cell wall polymers: Cellulose determination followed the protocol of Brendel et al. (2000). We added 80% acetic acid and 69% concentrated nitric acid to 100 mg of the powdered samples in screw-top glass tubes. The homogenate was boiled for 1 hour. After cooling, the mixture was transferred to a pre-weighed Falcon tube, where we added 99% ethanol. After stirring, the mixture was centrifuged at room temperature (25 °C) and the supernatant discarded. The precipitate was then washed sequentially: 99% ethanol, deionized water, 17% NaOH, deionized water and acetic acid. The washes

were interspersed by centrifugation and the supernatant discarded. The precipitate, consisting only of cellulose, was dried in an oven at 50 ºC for 48 hours, weighed and its value expressed in mg g^{-1} m.s.

For hemicellulose analysis we adopted the method of Schädel et al. (2010) with modifications (Macieira et al. 2020) where the enzymatic extraction of starch was replaced by dimethyl sulfoxide (DMSO). From 50 mg of the sample powder, we removed the soluble sugars in hot 80% ethanol followed by centrifugation (Pollock 1986). The precipitate was dried in a 50 ºC oven for 24 hours, weighed and mixed with DMSO under stirring for 24 hours. The homogenate was centrifuged, and the supernatant discarded. The precipitate was washed three times with distilled water, centrifuged, the supernatant discarded, and the precipitate dried in an oven at 50 ºC. The precipitate was weighed and mixed with neutral detergent (sodium tetraborate decahydrate 18 mmol, ethylenediaminetetraacetic acid 66 mmol, sodium dodecyl sulfate 10.4 mmol, dibasic sodium phosphate 32 mmol and distilled water) to extract water-soluble residues and pectins. After centrifuging and discarding the supernatant, we added sodium sulphite solution to the precipitate for hot protein extraction under continuous stirring. The homogenate was centrifuged, the supernatant discarded, and the precipitate (cellulose, hemicellulose and lignin) was washed sequentially: hot deionized water, 100% acetone and deionized water, each operation interspersed with centrifugation. The precipitate, composed of "cellulose and lignin", was dried and weighed. The hemicellulose content was determined by the gravimetric difference between the "total cell wall fraction" and the "cellulose and lignin fraction", with the value expressed in mg g^{-1} m.s.

The lignin content followed the method of Dos Santos et al. (2008). A 50 mM potassium sodium phosphate buffer solution at pH 7 was added to 150 mg of the samples, followed by centrifugation and the supernatant discarded. The precipitate was washed and stirred, and the supernatant discarded. After the last wash, the precipitate (protein-free cell wall fraction) was dried. 50 mg of the precipitate was removed and mixed with a solution of thioglycolic acid + 2 M HCl and incubated at 95 °C for 4 hours. The homogenate was centrifuged and washed three times with distilled water. NaOH 0.5 M was added to the precipitate and the mixture heated at 30 °C for 18 hours under constant stirring. After centrifugation, the supernatant was separated, and the precipitate washed with 0.5 M NaOH followed by centrifugation. The supernatant added to the previous one was acidified with HCl and left at 4 °C for 12 hours. The mixture was centrifuged, the supernatant discarded, and the precipitate washed with distilled water, which after drying was resuspended in 0.5 M NaOH followed by reading in a 280 nm spectrophotometer. Absorbance was applied to the equation of the lignin standard curve (Sigma®) in a gradient of 0-500 μg μL^{-1}. Values were expressed in mg g^{-1} m.s.

Statistical analysis: Statistical analyses were executed using the R software (R Core Team 2017). All data were subjected to a two-way analysis of variance (ANOVA) (3 climate scenarios and 2 substrate moisture) and the averages were compared by the Tukey test ($P \leq 0.05$) to evaluate the

interaction between climate scenarios and substrate moisture (Barros and Dias 2009) presented in Table 2. All of this respecting the assumptions of normality and homoscedasticity. To assess the variables in an integrated way, a multivariate analysis of principal components was performed.

Results

Morphological aspects: Water deficit inhibited stem elongation and the number of leaves, folioles and foliolules of the two ecotypes in the three climate scenarios (Fig. 1). However, this impact was smaller for RCP 8.5. These measures of plant growth in both ecotypes were higher in the RCP scenarios, especially in RCP 8.5, both in the control and under water deficit (Fig. 1). However, these growth measures reached higher values in this scenario under higher water availability (control). Although these variables were affected by the water deficit, it did not cause plant mortality of any ecotype in any climate scenario (Fig. 1). No type of anomaly was observed in the foliolules of any ecotype in any of the three climate scenarios, regardless of the water treatment. For more information on the growth measurements of the two *P. echinata* ecotypes in the CAF and RCP scenarios under control and water deficit, see the article by Venâncio et al. (2024).

Non-structural carbohydrates-NSC (soluble sugars and starch): The pessimistic climate scenario in general increased the sugar content of both ecotypes (Fig. 2a-f). In comparison, the shade-tolerant ecotype showed a greater capacity to accumulate sugars in leaves and stems in this climate scenario (Fig. 2a-f). In turn, the sun-tolerant ecotype accumulated more sugars in its roots.

The sugar concentrations of both ecotypes were influenced by substrate water levels and climate scenarios, with some similarities and intraspecific differences. Analyzing the effects of water deficit in each ecotype, we noticed that this treatment increased the leaf sugar content of both ecotypes in RCP 8.5 (Fig. 2a,b) and of the stem of the shade-tolerant ecotype (Fig. 2c) in this climate scenario. In the root, water deficit increased the sugar content of this ecotype in RCP 4.5 (Fig 2e) and of the other ecotype in the RCP scenarios (Fig 2e). In CAF, water deficit did not affect the leaf sugar content of any ecotype (Fig. 2a,b) but caused a decline in their roots (Fig. 2e,f). In the stem of the shade-tolerant ecotype, the values of these compounds did not differ from the water control (Fig 2.c) while in the other ecotype, they declined (Fig 2d).

Comparing the climate scenarios within each water level and ecotype, in water deficit the sugar content of the leaves and roots of the two ecotypes is higher (Fig. 2a,b) and lower (Fig. 2e,f), respectively, in RCP 8.5. Also under water deficit, the stem sugar content of the shade-tolerant ecotype was higher (Fig. 2b) and lower in the other ecotype (Fig. 2f) in this climate scenario. In the water control, the leaf sugar content of the shade-tolerant ecotype was higher in RCP 8.5 (Fig. 2b), lower in the stem (Fig. 2c) in this climate scenario and higher in the roots (Fig. 2e) of its plants in RCP. In the sun-tolerant ecotype of the water control, RCP 8.5 led to the lowest sugar levels in all its vegetative organs (Fig. 2b,d,f).

Breaking down NSCs (glucose-G, fructose-F, sucrose-S and starch-A), water deficit increased G (Fig. 3a,b), F (Fig. 3g,h) and S (Fig. 3m,n) contents in the aerial part organs of the shade-tolerant ecotype to the detriment of the root (Fig. 3c,i,o) at RCP 8.5. In RCP 4.5, the levels of these sugars also increased in the roots of this ecotype under water deficit (Fig. 3c,i,o) without showing a well-defined pattern of responses in its aerial organs (Fig. 3a,bg,h,m,n). In CAF, this water treatment reduced G content in all three organs (Fig. 3a-c) and root F (Fig. 3i), S (Fig. 3o) and A (Fig. 3u) in the shade-tolerant ecotype. These last three molecules did not differ in their water control in the aerial organs of this ecotype (Fig. 3g,h,m,n,s,t). For the sun-tolerant ecotype, water deficit generally increased the levels of G (Fig. 3d-f), F (Fig. 3j-l) and S (Fig. 3p-r). In CAF, water deficit increased the contents of root G (Fig. 3e) and leaf S (Fig. 3q), and the decline of these sugars (Fig. 3f,r) and root F (Fig. 3l) in this ecotype. The starch content did not show a well-defined pattern of responses to water deficit (Fig. 3s-x). In any case, they increased in the stems of the shade-tolerant ecotype in RCPs (Fig. 3t) and declined in its roots (Fig. 3u) in CAF in this water condition. In the sun-tolerant ecotype, the values of this polysaccharide increased in all the vegetative organs in CAF (Fig. 3v-x) and the stem in RCP 4.5 (Fig. 3w) and the root in RCP 8.5 (Fig. 3x).

Comparing the climatic treatments of each ecotype within the water factor, we noticed that in the case of water deficit, the highest leaf (Fig. 3a,g,m) and stem (Fig. 3b,h,m) G, F and S contents in the shade-tolerant ecotype occurred at RCP 8.5, and in the root (Fig. 3c,i,o) at RCP 4.5. The sun-tolerant ecotype also showed similar results to those described above, generally presenting higher levels of G, F and S in its leaves (Fig. 3d,j,p) and roots (Fig. 3f,l,r). In the water control, the two ecotypes generally showed lower levels of G, F and S in their three vegetative organs in RCP scenarios, especially in RCP 8.5 (Fig. 3a-x). As for starch, the shade-tolerant ecotype showed the same result in both water conditions (Fig. 3s-v), while the other ecotype showed this behavior only in the stem (Fig. 3w). The remaining organs of the sun-tolerant ecotype did not show a clear response.

In a qualitative analysis between the two ecotypes, we found higher G, F and S values in the aerial organs (Fig. 3a,b,g,h,m,n) and lower values in the roots (Fig. 3c,i,o,u) of the shade-tolerant ecotype compared to the other ecotype in RCP 8.5 under water deficit. In the water control, the shade-tolerant ecotype stands out for its higher root sugar content in this scenario compared to the sun-tolerant ecotype (Fig. 2c,f). And the higher value of these compounds in the latter ecotype in CAF (Fig. 2c,f).

Cell wall polymers-CWP (cellulose, hemicelluloses and lignin): The cellulose and stem lignin contents of the two ecotypes were influenced by the water levels in the substrate in the three climate scenarios (Fig. 4), presenting some similarities and intraspecific differences. In general, water deficit increased the cellulose content of both ecotypes in the three climate scenarios, except for the shade-tolerant ecotype in CAF for which the value of this polymer decreased (Fig. 4a,b). On the other hand,

water deficit reduced the lignin content of the shade-tolerant ecotype in CAF and the sun-tolerant ecotype in this scenario and in RCP 8.5 (Fig. 4e,f). In the other scenarios, the lignin values of the two ecotypes did not differ from the water control. Similarly, the hemicellulose content of both ecotypes did not differ between the two substrate water levels in the three climate scenarios (Fig. 4c,d).

Comparing the climate scenarios within each water level and ecotype, we found that water deficit increased the cellulose content of the shade-tolerant ecotype in the RCP scenarios (Fig. 5a) and the opposite in the sun-tolerant ecotype, which reached the lowest values in RCP 8.5 (Fig. 4b). Also under water deficit, the lignin content of the shade-tolerant ecotype did not differ between climate scenarios, while in the sun-tolerant ecotype the highest values of this polymer occurred in CAF and RCP 8.5 (Fig. 4f). In the water control, the RCP scenarios reduced cellulose content, especially in RCP 8.5 (Fig. 4a,b), and lignin (Fig. 4e,f) in both ecotypes. The hemicellulose content of these ecotypes did not differ between climate scenarios within each substrate water level (Fig. 4c,d).

In a qualitative analysis between the two ecotypes, the cellulose content of the shade-tolerant ecotype in the RCP 8.5 scenario was higher than that of the sun-tolerant ecotype, regardless of substrate water level (Fig. 4a,b). The opposite is true for the higher lignin content in the sun-tolerant ecotype under both water conditions (Fig. 4e,f). Hemicellulose content was similar between the two ecotypes in all climate scenarios (Fig. 4c,d).

Principal Components: The first two components represent 65.72% of the data for the shade-tolerant ecotype (Fig. 5a,b) and 61.80% for the sun-tolerant ecotype (Fig. 5c,d). In the shade-tolerant ecotype (first dimension), the grouping by a strong coordinate on the axis indicates the highest monosaccharide values (G and F) in the aerial part organs (leaves and stem) and leaf S (Fig. 5a,b). In the second dimension, the grouping in the negative coordinate on the axis is related to the higher hemicellulose (H) values. The grouping in the positive coordinates indicates higher values for all root NSC (Fig. 5a,b). Plants of the shade-tolerant ecotype showed higher values of monosaccharides in the aerial part organs and of stem S in the RCP 8.5 scenario under water deficit. Higher H values can be seen in RCP 4.5 and CAF in the control and water deficit, respectively. In the RCP 8.5 and CAF scenarios of water control and RCP 4.5 in water deficit, we found the highest root NSC values (Fig. 5a,b). In the sun-tolerant ecotype (first dimension), the grouping in the strong coordinate on the axis indicates high values of root sugars (F, G and S) and lower values of these in leaves (Fig. 5c,d). In the second dimension, the negative coordinates are related to the higher values of root A, leaf monosaccharides and low values of these in the root (Fig. 5c,d). Plants of the sun-tolerant ecotype showed the highest leaf G and lignin (L) values in the CAF and RCP 8.5 scenarios under water deficit. In RCP 4.5 and CAF under water deficit and control, respectively, there were higher values of root

sugars and H, with the first scenario in water control showing the highest values of stem sugars and cellulose (C) (Fig. 5c,d).

Discussion

The results allow to validate some of our hypotheses, including that shade-tolerant plants should accumulate more sugars (G, F and S) in the pessimistic climate scenario (RCP 8.5 + water deficit) compared to sun-tolerant plants, as seen in their leaves and stems. This greater need of the aerial organs of the shade-tolerant ecotype to accumulate more sugars in the pessimistic climate scenario can be interpreted as part of defense mechanisms to abiotic stress, including osmotic adjustment (Mengarda et al. 2012), to guarantee the vital metabolic activities of this functional class which is more sensitive to water deficit (Fonseca et al. 2023, Venâncio et al. 2024), heat (Cuzzuol et al. 2022) and the pessimistic climate scenario (Venâncio et al. 2024). Sun-tolerant plants, on the other hand, do not need this physiological adjustment as much (Cuzzuol et al. 2022, Venâncio et al. 2024) due to their ecophysiological features, which are better adapted to lower water availability (Fonseca et al. 2023, Venâncio et al. 2024).

This divergence in the sugar balance of aerial organs between the two functional classes in the pessimistic climate scenario can also be interpreted as due to their different metabolic characteristics. Some studies suggest that the metabolism of the shade-tolerant ecotype is slower than that of the sun-tolerant ecotype due to its slower growth rate and photosynthetic activity (Cuzzuol et al. 2020,2021, Gama et al. 2021, Venâncio et al. 2024). This ecophysiological features might be related to the surplus of sugars in its leaves and stems in the pessimistic climate scenario. This greater need to adjust sugars in the aerial part organs of the shade-tolerant ecotype in the pessimistic climate scenario can also be attributed to its lower antioxidant capacity compared to the sun-tolerant ecotype (Cuzzuol et al. 2020, Gama et al. 2021).

Unlike the responses of the aerial organs, the sugar content in the roots of the shade-tolerant ecotype declined in the pessimistic climate scenario, whereas in the sun-tolerant ecotype it accumulated significantly. These responses may be associated with the metabolic differences between the two ecotypes, as mentioned above. Thus, roots of the shade-tolerant ecotype (more vulnerable to water deficit) must have consumed more sugars or synthesized fewer of these molecules in RCP 8.5 + water deficit, consequently compromising root growth in this climate scenario (Venâncio et al. 2024). The opposite must have occurred in the roots of the sun-tolerant ecotype since it is better adapted to water deficiency (Fonseca et al. 2023, Venâncio et al. 2024).

In the pessimistic climate scenario, the most representative sugar in the aerial organs of the shade-tolerant ecotype and in the root of the sun-tolerant ecotype was S, which accounted for 2.5% of the m.s. of the stem of the first ecotype and 0.6% of the m.s. of the root of the second ecotype. Monosaccharides (G and F), on the other hand, were below 0.3% of m.s. Given the greater sensitivity

of the shade-tolerant ecotype to water deficit (Fonseca et al. 2023, Venâncio et al. 2024), its aerial organs accumulated more S than monosaccharides. These data confirm that, in addition to its importance for cell metabolism (Dietze et al. 2014), S also functions as an environmental signaling (Hartmann and Trumbore 2016) and osmoregulating (Martínez-Vilalta et al. 2016) agent in plants of the shade-tolerant ecotype of *P. echinata* under growth-limiting conditions (Mengarda et al. 2012, Fonseca 2021). On the other hand, the higher concentration of S in the root of the sun-tolerant ecotype in the pessimistic climate scenario may be due to the greater strength of this drain indicated by its greater increase than that of the other ecotype when treated with water deficiency (Venâncio et al. 2024). In this condition, S must have acted strongly on root osmoregulation and root energy demand of the sun-tolerant ecotype in the pessimistic climate scenario (Venâncio et al. 2024).

The sugar results indicate that the aerial organs of shade-tolerant plants and the roots of sun-tolerant plants may express a buffering effect in the CO_2-enriched atmosphere combined with higher temperatures, higher evaporative demand (EVD) and water deficit of the RCP 8.5 climate scenario predicted for the Atlantic Forest of southeastern Brazil by the end of this century.

The hypothesis that the starch content of the shade-tolerant ecotype would decline more sharply in the pessimistic climate scenario compared to the sun-tolerant ecotype was also rejected for all three vegetative organs. In contrast, the concentration of starch increased in the stem of the shade-tolerant ecotype at RCP 8.5 + water deficit. This leads us to assume that plants of this functional class store root transient energy (starch) as part of their survival strategies in RCP 8.5 + water deficit, being able to mobilize it in periods of rehydration (Fonseca 2021, Knupp et al. 2021). On the other hand, the accumulation of starch concomitantly with sugars in the root of the sun-tolerant ecotype in the pessimistic climate scenario indicates an efficient NSC metabolism strategy of this ecotype, which is less vulnerable to water deficit (Fonseca et al. 2023, Venâncio et al. 2024). As a result, the surplus of root NSC in the sun-tolerant ecotype in the pessimistic climate scenario may have acted more intensely on the osmotic adjustment and metabolic demand of the increase in this organ compared to the other ecotype (Fonseca 2021), as discussed above.

Although starch is more rapidly depleted than sugars under severe stress (Garcia-Forner et al. 2016, Martínez-Vilalta et al. 2016), in the present study we did not identify this occurrence in the plants of any ecotype in the pessimistic climate scenario. Except for shade-tolerant roots, for reasons explained above. This allows us to assume that the water deficit applied (40% substrate humidity) was not as severe and capable of mobilizing this polysaccharide in the two functional classes of *P. echinata* in RCP 8.5 + water deficit.

Contrary to expectations, the pessimistic climate scenario did not increase the stem lignin content of the shade-tolerant ecotype. A similar result was also observed for the shade-tolerant ecotype in other environmental conditions that inhibited its growth (Cuzzuol et al. 2020, Gama et al. 2021). For

these authors, this may have been due to the inhibitory effect of RCP 8.5 + water deficit on the photosynthesis of the shade-tolerant ecotype (Venâncio et al. 2024), culminating in a lower availability of hexoses for lignin synthesis (Novaes et al. 2010). Although there was a surplus of glucose and fructose in both ecotypes in that scenario, part of these molecules may have been preserved for the shade-tolerant ecotype's defense mechanism against water deficit in the form of osmotic adjustments (O'Brien et al. 2015), which prevented greater lignification of its stem in the pessimistic climate scenario. In the case of the sun-tolerant ecotype, its oxidative stress defense metabolism, being more efficient than the shade-tolerant ecotype (Cuzzuol et al. 2020, Gama et al. 2021), seems to have consumed more sugars in RCP 8.5 + water deficit. This may have compromised the availability of substrate for lignin synthesis, culminating in a decrease in lignin synthesis, as seen in some species under water stress (Le Gall et al. 2015). The lower lignification of the sun-tolerant ecotype in the pessimistic climate scenario is worrying because this climate change scenario predicted for the southeastern region of Brazil by the end of this century could compromise the increase in photosynthesizing leaf area of the two *P. echinata* ecotypes (Venâncio et al. 2014) and the wood quality of the sun-tolerant ecotype. And, consequently, the world's musical activity. This species provides the best and incomparable raw material for making stringed instrument bows (Angyalossy et al. 2005). The world's great orchestras depend on the heartwood of this tree.

The lower lignification of the sun-tolerant ecotype in the pessimistic climate scenario may also be related to cellulose content. The higher values of this CWP in the two ecotypes in RCPs combined with water deficit can be attributed to the accumulation of sugars, especially sucrose (S), in their aerial organs. Considering that the hydrolysis of S provides precursor hexoses for cellulose synthesis (Novaes et al., 2010), we assume that the greater reserve of this disaccharide in the aerial organs of the two ecotypes in RCPs under water deficit has enhanced cellulose synthesis in these environmental conditions. However, less significantly for the sun-tolerant ecotype in the pessimistic climate scenario, where S content and stem cellulose declined compared to the current Atlantic Forest scenario (CAF) combined with water deficit. The lower stem S values of the sun-tolerant ecotype in RCPs under water deficit, especially in RCP 8.5, probably reflected its lower capacity for cellulose synthesis compared to the shade-tolerant ecotype.

Considering that the hemicelluloses of some species are involved in drought protection (Le Gall et al. 2015), we expected the content of this CWP to increase in the climate scenarios combined with water deficit. Especially in the shade-tolerant ecotype, which is more sensitive to the pessimistic climate scenario (Venâncio et al. 2024). However, the hemicelluloses of the two ecotypes in this study were not responsive to water deficit in the three climate scenarios. This indifference of hemicelluloses to climatic scenarios was also found in some organs of the two ecotypes of *P. echinata* (Cuzzuol et al. 2020, Gama et al. 2021, Martins et al. 2021, Cuzzuol et al. 2022) and other shade-tolerant tropical

species (Macieira et al. 2023) exposed to contrasting environmental conditions. For these authors, this is due to the resilience of tropical tree hemicelluloses to environmental changes (Le Gall et al. 2015). Another possibility for the lack of variation in hemicelluloses in this study in relation to climate treatments is that the water deficit applied (40% substrate humidity) was not severe enough to express intraspecific differences in hemicelluloses between the two ecotypes.

Also, in the qualitative comparison between the two ecotypes, the higher cellulose content of the shade-tolerant ecotype in RCP 8.5 (with and without water deficit) in relation to the sun-tolerant ecotype and the opposite in lignin content may be a reflection of their ecophysiological differences in the initial growth phase (Gama et al. 2021). While the sun-tolerant ecotype is richer in lignin than the shade-tolerant ecotype, the opposite is true for cellulose (Gama et al. 2021, Martins et al. 2021, Cuzzuol et al. 2022).

The hypothesis that the growth of the shade-tolerant ecotype would suffer a greater impact in the pessimistic climate scenario in relation to the sun-tolerant ecotype was validated when the number of folioles and foliolules declined by 42 and 51%, respectively, in relation to its water control, while in the shade-tolerant ecotype these values were lower (26 and 40%). A similar behavior also occurred in the other two climate scenarios. These results indicate that the production of folioles and foliolules of the shade-tolerant ecotype is more susceptible to water deficit compared to the sun-tolerant ecotype. The number of leaves of the two ecotypes did not show this intraspecific difference, representing a 48% reduction for both in the water deficit compared to the water control.

On the other hand, stem elongation of the two ecotypes showed the opposite behavior. While in the shade-tolerant ecotype this measure decreased by 27% in RCP 8.5 under water deficit, in the sun-tolerant ecotype this value was higher (35%). Thus, while on the one hand the number of folioles and foliolules of the shade-tolerant ecotype showed greater vulnerability to the pessimistic climate scenario (RCP 8.5 + water deficit) compared to the other ecotype, on the other hand, the stem elongation of that ecotype suffered less impact compared to the sun-tolerant ecotype in this climate scenario. This strategy of the sun-tolerant ecotype to ensure a leaf area capable of guaranteeing superior photosynthetic performance compared to the other ecotype in the pessimistic climate scenario (Venâncio et al. 2024) may be related to the larger size of its root NSC reservoir in this environmental condition. By exerting osmotic adjustment in the root of the sun-tolerant ecotype in RCP 8.5 + water deficit, the sugars must have contributed to greater water absorption and root hydrostatic pressure, culminating in more efficient water transportation to the leaves, as suggested by the greater water use efficiency of this ecotype in the pessimistic climate scenario (Venâncio et al. 2024). As a result, the better hydration of the stem and leaf tissues of the sun-tolerant ecotype must have contributed to the satisfactory growth and development of its folioles and foliolules compared to the other ecotype in the pessimistic climate scenario.

These results suggest that in RCP 8.5 + water deficit, plants of the sun-tolerant ecotype invest more in leaf area to the detriment of stem elongation than the other ecotype. The end result is to achieve higher photosynthetic yields in this growth-limiting condition. This behavior seems to be a reflection of its heliophilous habit in the initial growth phase when its photosynthetic activity is higher than that of the shade-tolerant ecotype (Gama et al. 2021, Venâncio et al. 2024).

Finally, we reject the hypothesis that the strategies of increasing sugars to the detriment of starch and lignin could compromise the survival of plants of the shade-tolerant ecotype in the pessimistic climate scenario because the mortality of both ecotypes was zero in this and the other two climate scenarios. The high survival of other tropical species in this same climate scenario (Amaral et al. 2020,2023), as well as the ecotypes in the present study, suggest that 40% humidity of the substrate applied in these studies was not enough to impose a condition of severe water stress. Perhaps the lower moisture content could express possible intraspecific differences in survival between the two ecotypes in the climatic scenarios tested. Although the pessimistic climate scenario predicted for the end of this century should not jeopardize the survival of *P. echinata* ecotypes, it could affect the growth of its plants, especially the shade-tolerant ecotype, which had proportionally less photosynthesizing leaf area in the pessimistic climate scenario compared to the sun-tolerant ecotype.

Conclusions

Water deficit inhibited stem elongation and the number of folioles/foliolules of both ecotypes in the three climate scenarios, especially in the pessimistic climate scenario (RCP 8.5 + water deficit). However, this was more pronounced in the leaf organs of the shade-tolerant ecotype and in the stem of the sun-tolerant ecotype. In RCP 8.5 with greater water availability, plants of both ecotypes grew more than in CAF and RCP 4.5. The pessimistic climate scenario increased the sugar content (G, F and S) of the aerial organs of both ecotypes. However, this ability to accumulate sugars was higher in the shade-tolerant ecotype as a reflection of its greater sensitivity to water deficit (Venâncio et al. 2024) and other contrasting environmental conditions (Cuzzuol et al. 2020, Gama et al. 2021, Martins et al. 2021, Cuzzuol et al. 2022). The ecotypes also differed in starch concentration. In the pessimistic climate scenario, the shade-tolerant ecotype accumulated more of this polysaccharide in the stem, while in the sun-tolerant ecotype this occurred in the root. Water deficit increased the cellulose content of both ecotypes in the RCP scenarios, to the detriment of the lignin content of the shade-tolerant ecotype in CAF and the sun-tolerant ecotype in this scenario and in RCP 8.5 + water deficit. Considering that better quality tropical tree wood is more lignified (Maceira et al. 2021), we hypothesize that the rise in CO_2, temperature and DPV along with low precipitation in RCP 8.5 predicted for the southeastern region of Brazil by the end of this century may compromise more the photosynthesizing leaf area production of the shade-tolerant ecotype and the wood quality of the sun-tolerant ecotype of *P. echinata*. However, this does not threat their survival.

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Table 01. Microclimatic characterization of the simulated scenarios in the air-conditioned greenhouses from May 15, 2019 to Aug 15, 2019 in which T is the air temperature, VPD is vapor-pressure deficit, RH is air relative humidity, [CO_2] is the air atmospheric concentration of this gas, $_{med}$ is the average, $_{min}$ is the minimum, and $_{max}$ is the maximum.

climatic variables	CAF	RCP 4.5	RCP 8.5
	air temperature (ºC)		
T_{min}	24.3	25.4	26.7
T_{med}	25.8	27.7	29.4
T_{max}	26.4	28.5	29.7
	relative humidity (%)		
RH_{min}	72	48	44
RH_{med}	83	62	61
RH_{max}	95	82	77
	vapor pressure deficit (kPa)		
VPD_{min}	0.16	0.60	0.80
VPD_{med}	0.55	1.42	1.59
VPD_{max}	0.92	1.92	2.26
	atmospheric concentration of CO_2 (ppm)		
$[CO_2]_{min}$	374	532	912
$[CO_2]_{med}$	399	566	930
$[CO_2]_{max}$	451	602	965

Table 02. Variance analyses of cell wall polymers (cellulose, hemicelluloses and lignin) and non-structural carbohydrates (sugars and starch) in shade-tolerant and sun-tolerant ecotypes plants of *P. echinata* at 90 days of exposure in three climate scenarios (CAF, RCP 4.5, and RCP 8.5) and two substrate moisture (control hydric-80% and deficit hydric-40% of the maximum water retention capacity of the substrate). All variables were analyzed by two-way ANOVA, considering scenarios, hydric and scenarios x hydric as fixed factors. The *F* value corresponds to the ANOVA analysis and significant differences are indicated by * ($P \leq 0.05$).

Shade-tolerant ecotype				Sun-tolerant ecotype			
variable	scenarios	hydric	Interactions	variable	scenarios	hydric	Interactions
cellulose				cellulose			
F	0.21	0.00	6.17	*F*	47.8	2450	5.12
P	0.80	0.97	0.00*	*P*	0.000	0.000	0.013*
hemicelluloses				hemicelluloses			
F	0.40	4.05	0.74	*F*	7.83	70.8	4.02
P	0.67	0.05	0.48*	*P*	0.002	0.000	0.031*
lignin				lignin			
F	4.42	1.72	1.46	*F*	675	1398	155
P	0.02	0.20	0.25*	*P*	0.000	0.000	0.000*
glucose leaf				glucose leaf			
F	109	288	302	*F*	16.8	87.6	2.51
P	0.00	0.00	0.00*	*P*	0.000	0.000	0.102*
glucose stem				glucose stem			
F	162	10.6	140	*F*	8.13	86.8	4.11
P	0.00	0.06	0.00*	*P*	0.002	0.000	0.029*
glucose root				glucose root			
F	7.16	43.5	237	*F*	6.42	54.2	2.97
P	0.00	0.00	0.00*	*P*	0.005	0.000	0.070*
fructose leaf				fructose leaf			
F	992	792	1050	*F*	30.4	69.9	7.12
P	0.00	0.00	0.00*	*P*	0.000	0.000	0.003*
fructose stem				fructose stem			
F	224	238	456	*F*	5.9	1.25	8.46
P	0.00	0.00	0.00*	*P*	0.007	0.274	0.001*
fructose root				fructose root			
F	102	455	795	*F*	150	21.4	9.63
P	0.00	0.00	0.00*	*P*	0.000	0.001	0.008*
sucrose leaf				sucrose leaf			
F	78.1	330	86.3	*F*	67.8	136	26.3
P	0.01	0.00	0.00*	*P*	0.000	0.000	0.008*
sucrose stem				sucrose stem			
F	220	115	398	*F*	73.4	86.8	0.405
P	0.00	0.01	0.00*	*P*	0.000	0.000	0.671*
sucrose root				sucrose root			
F	6.87	49.2	48.1	*F*	62.4	110	0.737
P	0.01	0.00	0.00*	*P*	0.000	0.000	0.488*
starch leaf				starch leaf			
F	2.50	0.34	0.95	*F*	1.81	0.95	1.21
P	0.12	0.56	0.41	*P*	0.186	0.337	0.314*
starch stem				starch stem			
F	27.8	37.9	12.0	*F*	1.02	0.87	2.18
P	0.03	0.04	0.01*	*P*	0.375	0.358	0.134*
starch root				starch root			
F	17.6	5.84	24.2	*F*	4.88	3.07	2.02
P	0.00	0.03	0.00*	*P*	0.016	0.092	0.154*

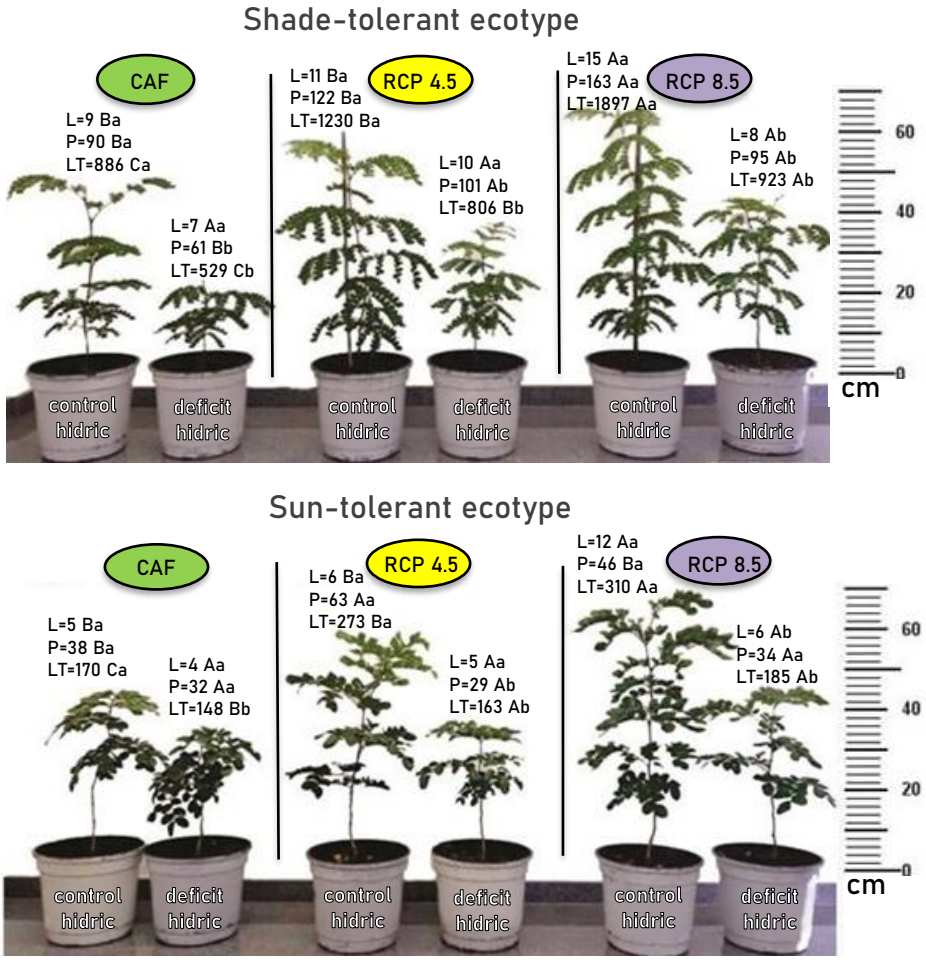


Figure 01: Morphological aspects of shade-tolerant and sun-tolerant *P. echinata* ecotypes plants at 90 days of exposure in three climate scenarios (CAF, RCP 4.5, and RCP 8.5) and two substrate moisture conditions (control hydric-80% and deficit hydric-40% of the maximum water retention capacity of the substrate). L = leaves, P = pinna, LT = leaflets. Capital letters compare the climatic scenarios within the same substrate humidity and lowercase letters compare the humidity substrate within the same climatic scenario, both according to Tukey's test ($P \leq 0.05$). The values represent means ± standard error (n = 5).

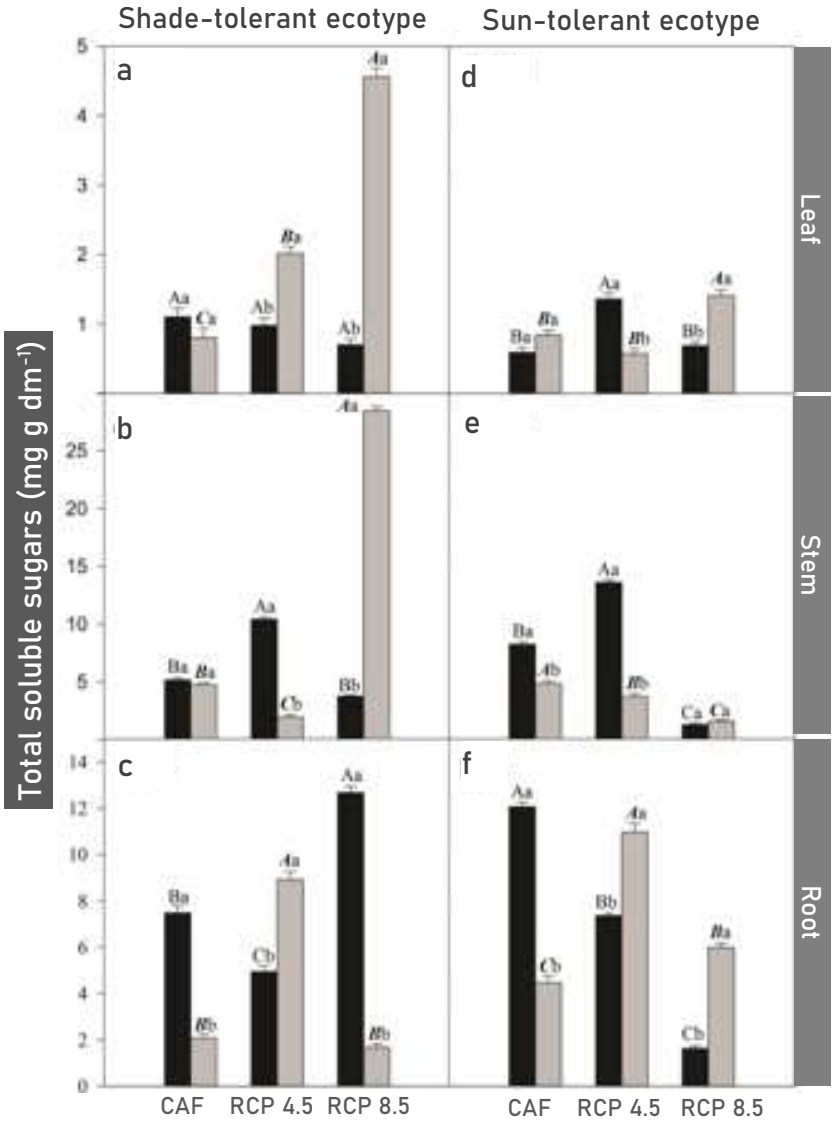


Figure 02: Total soluble sugars concentrations in vegetative organs of shade-tolerant and sun-tolerant *P. echinata* ecotypes plants at 90 days of exposure in three climate scenarios (CAF, RCP 4.5, and RCP 8.5) and two substrate moisture conditions (control hydric-80% and deficit hydric-40% of the maximum water retention capacity of the substrate). Capital letters compare the climatic scenarios within the same substrate humidity and lowercase letters compare the humidity substrate within the same climatic scenario, both according to Tukey's test ($P \leq 0.05$). The values represent means ± standard error (n = 5).

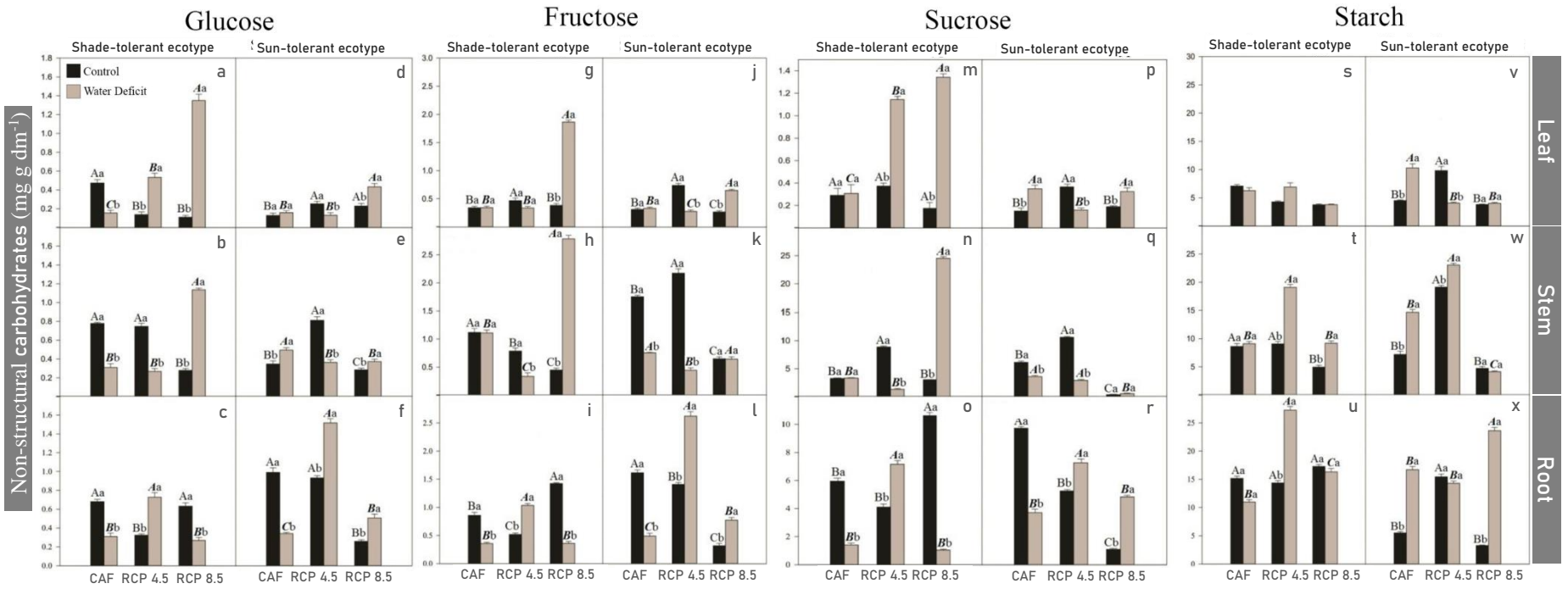


Figure 03. Non-structural carbohydrate concentrations (glucose, fructose, sucrose, and starch) in vegetative organs of shade-tolerant and sun-tolerant *P. echinata* ecotypes plants at 90 days of exposure in three climate scenarios (CAF, RCP 4.5 e RCP 8.5) and two substrate moisture conditions (control hydric-80% and deficit hydric-40% of the maximum water retention capacity of the substrate). Capital letters compare the climatic scenarios within the same substrate humidity and lowercase letters compare the humidity substrate within the same climatic scenario, both according to Tukey's test ($P \leq 0.05$). The values represent means ± standard error ($n = 5$).

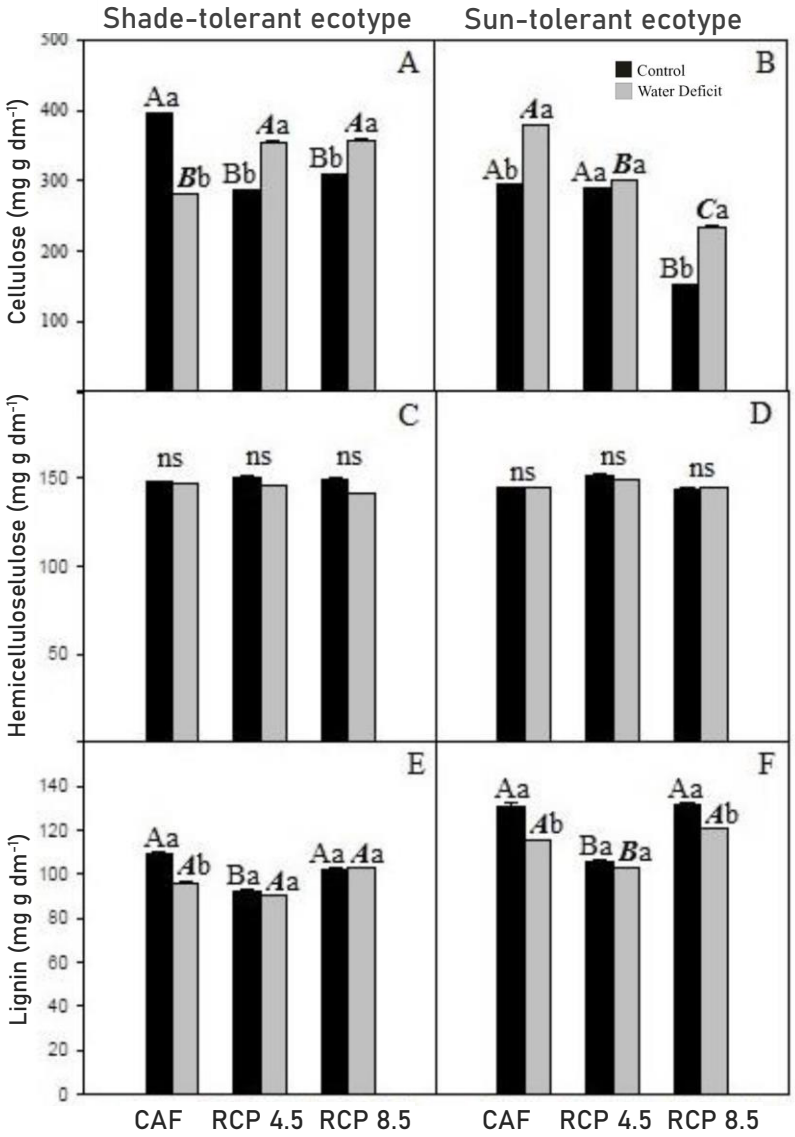


Figure 04. Cell wall polymer contents (cellulose, hemicellulose, and lignin) in the stems of plants of the shade-tolerant and sun-tolerant ecotypes of *P. echinata* at 90 days of exposure to three climate scenarios (CAF = current Atlantic Forest, Representative Concentration Pathway VCR CP 4.5 and 8.5) at 80% (water control) and 40% (water deficit) of the substrate's water retention capacity. Capital letters compare the means (n = 5) of each substrate water treatment between the scenarios and lowercase letters compare the substrate water treatments within each scenario and ecotype ($P \leq 0.05$).

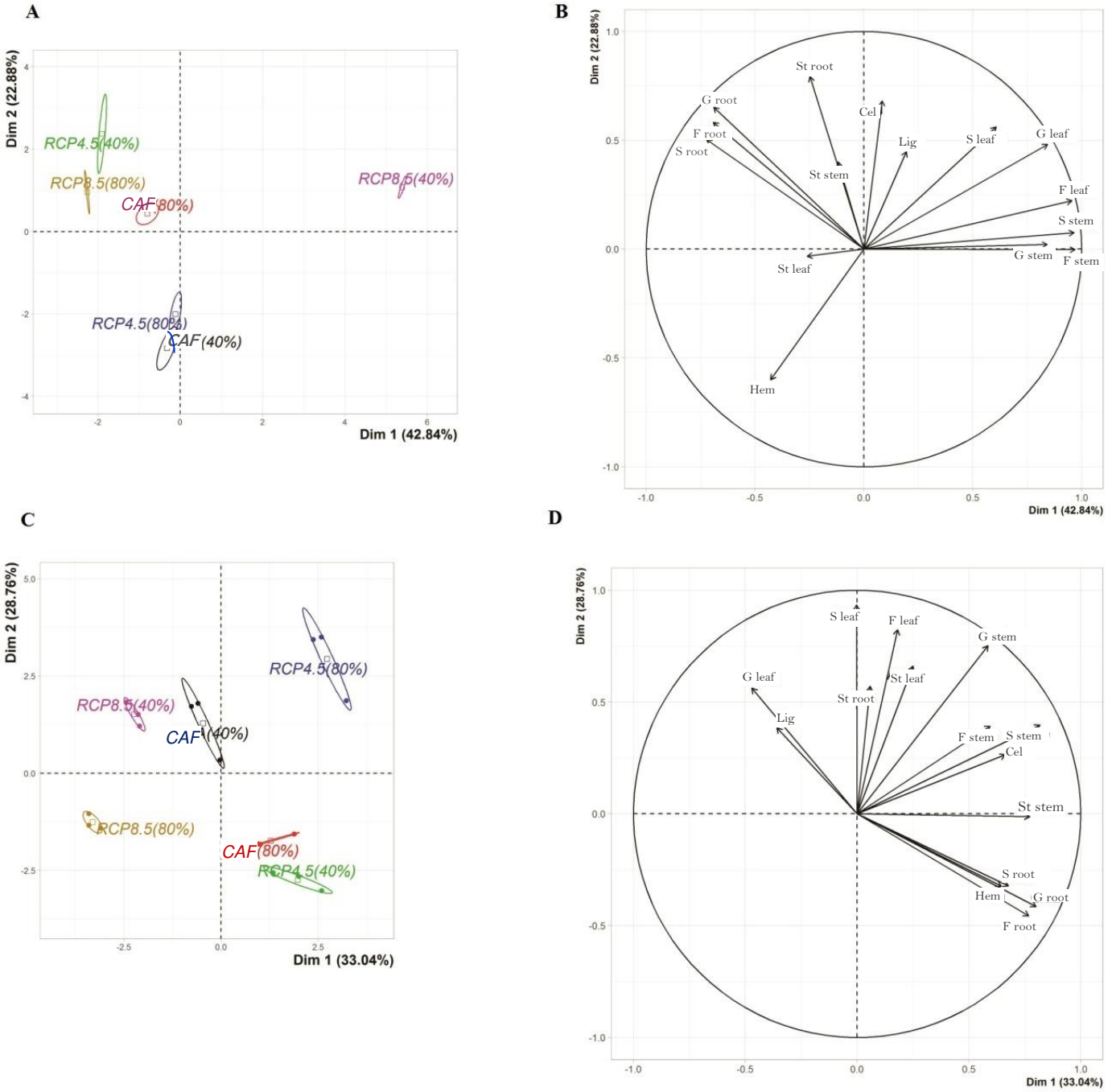


Figure 05: Principal component analysis of NSC and CWP in plants of the shade-tolerant and sun-tolerant ecotypes of *P. echinata* subjected to three climate scenarios (CAF = current Atlantic Forest, VCR CP 4.5 and 8.5) at 40% (water deficit) and 80% (control) of the substrate's water retention capacity. A,B) factor map of shade-tolerant ecotype plants; C.D) factor map of individuals of the sun-tolerant ecotype. Cel (cellulose), Hem (hemicelluloses), Lig (lignin), A (starch), G (glucose), F (fructose), and S (sucrose).