

ORIGINAL ARTICLE

The environmental gradient influences the concentrations of carbon compounds in the biomass of *Protium icicariba* (DC.) Marchand in restinga area

O gradiente ambiental influencia as concentrações de compostos de carbono na biomassa de *Protium icicariba* (DC.) Marchand em área de restinga

Fabiano Caprini Volponi¹ , Ramon Negrão Santos Junior¹ , Felipe Machado Barreto¹ ,
Fernanda Bonini dos Reis¹ , João Pedro Zanardo de Andrade¹ ,
Geraldo Rogério Faustini Cuzzuol¹ , Paulo Cezar Cavatte¹ 

¹Universidade Federal do Espírito Santo – UFES, Vitória, ES, Brasil

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Abstract

The objective of this study was to analyze the concentrations of non-structural carbohydrates and cell wall polymers in *Protium icicariba* individuals along an environmental gradient of a Coastal Brazilian Atlantic Forest (restinga). The concentrations of non-structural carbohydrates (glucose, fructose, sucrose and starch) and cell wall polymers (cellulose, hemicellulose and lignin) were quantified in samples of stems, branches and leaves of individuals distributed in contrasting zones regarding the occurrence of floods [periodically floodable (PI), transitional (IT) and non-floodable (NI)]. Individuals from the non-flooded zone had higher concentrations of starch and hemicelluloses in the branches and leaves. On the other hand, cellulose and lignin concentrations were higher in the leaves of individuals from the periodically flooded and transitional zones. The variations in the concentrations of non-structural carbohydrates and cell wall polymers were mainly associated with water availability and plasticity of the species, in addition to possible influences of soil nutrient supply and the occurrence of herbivory. The sensitivity in the dynamics of the carbon molecules of *P. icicariba* in a diverse and spatially short environmental gradient, requires considerable plasticity of the species that, in the imminence of climatic changes, must favor its survival in the face of the imposition of new environmental filters.

Keywords: Cellulose; Climate changes; Lignin; Starch; Sucrose.

Resumo

O objetivo deste estudo foi analisar as concentrações de carboidratos não estruturais e polímeros de parede celular em indivíduos de *Protium icicariba* (DC.) Marchand ao longo de um gradiente ambiental de uma floresta de restinga. As concentrações de carboidratos não estruturais (glicose, frutose, sacarose e amido) e polímeros de parede celular (celulose, hemicelulose e lignina) foram quantificados em amostras de caules, ramos e folhas de indivíduos distribuídos em zonas contrastantes quanto à ocorrência de inundações [periodicamente inundável (PI), de transição (IT) e não inundável (NI)]. Os indivíduos da zona não inundável apresentaram maiores concentrações de amido e hemiceluloses nos ramos e folhas. Por outro lado, as concentrações de celulose e lignina foram maiores nas folhas dos indivíduos da zona periodicamente inundável e da zona de transição. As variações nas concentrações de carboidratos não estruturais e polímeros de parede celular foram associadas, principalmente, à disponibilidade hídrica e plasticidade da espécie, além de possíveis influências da oferta de nutrientes do solo e ocorrência de herbivoria. A sensibilidade na dinâmica das moléculas de carbono de *P. icicariba* em um gradiente ambiental diverso e espacialmente curto, exige considerável plasticidade da espécie que, na eminência das mudanças climáticas, deve favorecer sua sobrevivência diante da imposição de novos filtros ambientais.

Palavras-chave: Celulose; Mudanças climáticas; Lignina; Amido; Sacarose.

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Corresponding author: capriniivolponi@gmail.com

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INTRODUCTION

Climate change predicted for the coming decades could have considerable effects both at individual and ecosystem levels (Brienen et al., 2016; Scarano & Ceotto, 2015). Among the ecosystems that can be impacted by climate change, are the *restingas*, mainly because of their peculiarities. These environments, which extend along practically the entire Brazilian coast, are composed of varied phytophysiognomies, mainly influenced by topographic differences that determine the depth of the water table and the occurrence of floods (Lourenço Júnior et al., 2021; Monteiro et al., 2014).

In these places, *Protium icariba* (DC.) Marchand is often identified as one of the species with the greatest influence on the plant community (Ferreira & Silva, 2014; Lourenço et al., 2020). Popularly known as *almécega* or *almesca*, *P. icariba* is a species of the Burseraceae family, arboreal, dioecious and endemic to Brazil, with confirmed occurrences in the state of Espírito Santo state (Daly, 2015). Its leaves are compound, petiolate and imparipinnate. The flowers are numerous and arranged in simple, axillary racemes, generally white, and the fruits are drupe-like, ovoid-oblique, leathery, containing 4 to 5 seeds (Siani et al., 2004). Individuals of this species have a high capacity to exude aromatic and volatile resins used in traditional medicine (Rüdiger et al., 2007). Their reproductive phase is related to the wetter periods of the year and can vary from September to March (Braz & Mattos, 2010; Ferreira et al., 2010; Zamith & Scarano, 2004). Populations of *P. icariba* can be found both in non-flooded and periodically flooded restinga forests (Lourenço Júnior et al., 2021; Magnago et al., 2013).

The variation of environmental characteristics of a restinga, such as soil moisture, can influence the dynamics and stock of carbon biomolecules (Dong & Beckles, 2019). These molecules play an important role, for example, in the osmoregulation of plant tissues and as an energy reserve in plants. These are essential functions for tolerance of a species that experiences environments with variable supply of water and nutrients. In this way, a tree population, distributed in an edaphic humidity gradient, can contribute to the current ecophysiological knowledge about the dynamics of carbon compounds in tropical forests and help to predict how possible climate changes may influence their concentrations in plant organic tissues.

Changes in the concentrations of non-structural carbohydrates (glucose, fructose, sucrose and starch) may occur without a defined pattern in plants subjected to water stress (Hoch, 2015). Tree species with higher concentrations of non-structural carbohydrates show greater resistance and resilience when subjected to drought conditions and low temperatures (Fürtauer et al., 2019; O'Brien et al., 2014). However, if a species is more sensitive to water stress, these carbohydrates are consumed, mainly by the energy expenditure of maintenance respiration (Piper, 2011). Soluble carbohydrates (fructose and sucrose) are concentrated in some organs of plants subjected to water stress. They have the role of maintaining osmotic balance, that is, decreasing the water potential of cells or tissues maintaining their turgor, or optimizing water absorption (Warren et al., 2011). In relation to structural carbohydrates, it has been reported, for example, that hemicellulose synthesis is stimulated under water stress conditions (Yang et al., 2006). On the other hand, cellulose contents do not show a standardized response, as they present different results between species and organs (Piro et al., 2003; Zheng et al., 2012). Meanwhile, lignin is considered as a carbon polymer involved in drought resistance, with higher concentrations in the stem (Moura-Sobczak et al., 2011) in conditions of low soil moisture (Lee et al., 2007).

This study aims to determine the concentrations of non-structural carbohydrates and cell wall polymers in stems, branches and leaves of individuals of a population of *P. icariba* occurring in contrasting zones regarding the occurrence of flooding episodes [periodically flooded (PI), transition (IT) and non-floodable (NI)]. The research hypotheses were: (i) there is variation in the concentration of non-structural carbohydrates and cell wall polymers among individuals of a *P. icariba* population distributed along the environmental gradient; (ii) the differences between the study areas are associated with the contrasting conditions regarding the occurrence of flooding episodes.

MATERIAL AND METHODS

Study area

The study area is located in Paulo Cesar Vinha State Park (20°35'25" S; 40°25'24" W), Municipality of Guarapari, State of Espírito Santo, Brazil. According to the Köppen-Geiger climate classification system, the climate in the region is Aw, tropical hot and humid with a rainy season (summer) and a dry season (winter). The average annual rainfall for the Capital Vitória is 1,276 mm and the average annual temperature is 24.2 °C (Instituto Nacional de Meteorologia, 2022).

It is a delimited restinga forest area with approximately 11.2 hectares (ha), parallel to the ocean, with a distance of about 1.5 km from it (Figure 1). In that area, three sampling zones were established, these being determined as periodically flooded forest (PI), transitional forest (IT) and non-flooded forest (NI) (Magnago et al., 2013).

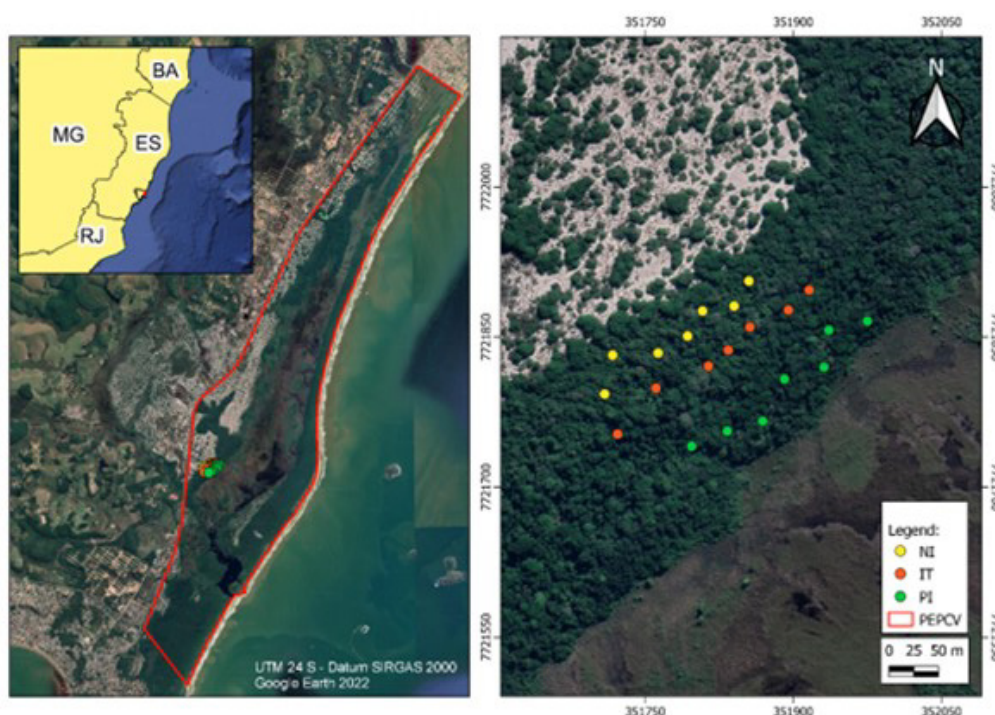


Figure 1: Study area. Distribution of the sample population of *Protium icariba*. Yellow circles represent individuals from the non-flooded forest zone (NI), red circles represent individuals from the transition zone (TI) and green circles represent individuals from the periodically flooded zone (PI). PEPCV = Paulo Cesar Vinha State Park.

With a characteristic periodic flooding in the summer, the periodically flooded zone has an area of approximately 5.8 ha and is located in an area of relief depression, between a flooded herbaceous formation and the transition zone. This forest area has a flat topography, visually dark soil with the presence of decomposing organic matter. Compared to the other zones, this one has a greater accumulation of organic matter, acidity, aluminum and salt concentrations, poor drainage, in addition to a shallow water table and periods of flooding (Lourengo Júnior et al., 2021).

The transition zone was delimited in an area of 0.75 ha, it is a narrow strip of land, approximately 15.0 m wide. It has a slight slope and is located between the periodically flooded and non-floodable zones. Finally, the non-floodable zone has an area of approximately 4.65 ha. It is located between an open, non-floodable shrub formation and the transition zone. With a flat topography, it is located in a higher area, typical of a marine terrace formed by the accumulation of sand during the Quaternary period (Suguio, 2017). The soil has characteristics of sandy texture, low water retention, thin leaf litter layer, lower proportion of

organic matter, low fertility compared to the periodically flooded zone (Lourenço Júnior et al., 2021).

Sampling Procedure

For consistency of the sampling effort, we selected seven individuals of *P. icariba* ($n=7$) in each forest zone, totaling a sample population of 21 individuals (Figure 1). For the choice of individuals, we considered those that were best distributed throughout the study area, obeying the minimum spacing of 5.0 meters between them and with stem diameter at breast height (DBH; determined at 1.3 m above the soil) ≥ 5 cm (Lourenço et al., 2020).

We carried out sampling of stems, branches and leaves in September 2016. The collection of plant material was carried out with the aid of a long-handled pruner, allowing the removal of samples from branches located up to 12 m in height. The branches were standardized with approximately 3.0 cm in diameter and 25.0 cm in length. The fully expanded leaves from the third to the fifth pair (from the apex) were manually collected from the same sampled branches. The stems were sampled as per DBH. For this, the periderm and phloem were previously removed by scraping so that fragmented samples were extracted using a battery-powered drill (BOSCH-GSB180-LI). The stem incision was standardized with a depth of 5.0 cm.

All samples were immediately placed in thermal boxes containing ice and transported to the laboratory, where they were placed in an ultra freezer at -80°C . Then, all samples were freeze-dried (lyophilized) and pulverized in a ball mill (TE-350, TECNAL, São Paulo, Brazil) and later stored in plastic bottles at room temperature until analyzed.

Biochemical Analyses

Soluble carbohydrates

In order to determine the concentration of soluble sugars (glucose, fructose and sucrose), 150 mg of each sample was treated with 80% ethanol at 60°C for one hour (Pollock, 1986). After centrifuging, the liquid phase was concentrated in a lyophilizer. The samples were then diluted in 1 mL of solution containing ultrapure water and known concentrations of 1 gL-fructose, 1 gL- glucose and 1 gL- sucrose (using Sigma® standards). After being diluted, the samples were filtered through syringe filters (Millex gv pvdf diameter 13 mm and pore size $0.22\text{ }\mu\text{m}$ – millipore). Concentrations were determined by high performance anion exchange chromatography with a refraction detector in Shimadzu RID-20A HPLC, 25 cm CLC column – NH₂, 80% acetonitrile mobile phase under 1 mL per minute flow at 40°C , using Sigma® standards.

Starch

Starch content was determined using the fast and sensitive enzymatic method of extraction and dosage (Amaral et al., 2007). From 10 mg of each sample, soluble sugars, pigments, phenols and other substances were extracted by alcoholic extraction. From the resulting dry precipitate, 0.5 mL (120 U mL^{-1}) of thermostable α -amylase (EC 3.2.1.1) from *Bacillus licheniformis* (code E-ANAAM, MEGAZYME, Ireland) was added in sequence and 0.5 mL of a solution containing 30 U mL^{-1} of amyloglucosidase (EC 3. 2. 1. 3) of *Aspergillus niger* (code E-AMGPU, MEGAZYME, Ireland) to hydrolyze the bonds of starch molecules, releasing the monomers of glucose. The reading of the starch concentration was performed from the quantification of glucose released after reaction with Glucose PAP Liquiform (CENTERLAB, Brazil), which upon reacting with glucose generates a color intensity. The determination by colorimetric method was then performed by an ELISA microplate reader, at a wavelength of 490 nm. The standard curve was constructed with concentrations 0.0; 2.5; 5.0; 7.5; 10.0; 12.5; and 15.0 g L-starch Sigma® standard.

Cellulose

For cellulose quantification, the method proposed by Brendel et al. (2000) was adopted with modifications. 100 mg were used for each sample. Initially, 2 mL of 80% acetic acid and 200 μ L of 65% nitric acid were added to break the bonds between the wall polymers and degradation of non-cellulosic material, which was then placed in a water bath at 100 °C for one hour. Then, 2.5 mL of 99% ethanol was added, which was homogenized and centrifuged at $4,324 \times g$ for 5 min, with the supernatant being later discarded. Next, the samples were washed sequentially as follows: (1) 5 mL of 99% ethanol to remove the degraded products in the extraction; (2) 5 mL of deionized water to remove traces of nitric acid; (3) 5 mL of 17% NaOH that was left to stand at room temperature for 10 min; (4) 5 mL of deionized water; (5) 2.2 mL of deionized water and 600 μ L of acetic acid. Another 2.2 mL of deionized water were added to remove non-cellulosic material that was still persistent and (6) 5 mL of deionized water. Between each wash (1 to 6), the samples were centrifuged at $4,324 \times g$ for 5 min at room temperature and the supernatants discarded. Finally, the samples were dried in an oven at 50 °C for 48 hours and the masses were measured to determine the percentage of cellulose per dry weight (%DW) by gravimetric method.

Hemicellulose

Total hemicelluloses was determined by the method proposed by Schädel et al. (2009) with modifications. From 50 mg of dry mass of each sample, the soluble sugars were removed. The precipitate was dried in an oven at 50 °C for 24 hours. With the samples free of soluble and dry sugars, 1.5 mL of dimethyl sulfoxide (DMSO) was added to extract the starch and kept under stirring for 24 hours. After this period, the samples were centrifuged at $18,227 \times g$ for 5 min at 7 °C and the supernatant discarded. After washing, the precipitate was transferred to an oven at 50 °C for 24 hours to later carry out the weighing of the completely dry material. Then, 1.5 mL of 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) was added to the previous precipitate to extract residues soluble in water and pectins, and 25 μ L of sodium sulfite solution was added for protein extraction. The samples were transferred to a water bath at 100 °C for 60 min under stirring on a magnetic plate. Then the samples were centrifuged and the supernatant discarded. The precipitate from this step (cellulose, hemicellulose and lignin) was washed sequentially as follows: (1) twice with 1.5 mL of hot deionized water; (2) once with 1.5 mL of 100% acetone and; (3) once with 1.5 mL of deionized water. The precipitate containing the "total cell wall fraction" was then transferred back to an oven at 50 °C for 24 hours and weighed. From the dried precipitate, 1.5 mL of acid detergent (H_2SO_4 1 N and 55 mmol of hexadecyltrimethylammonium bromide deoxycholic acid sodium salt) was added. The samples were submitted to a water bath at 100 °C for 60 min, which were then centrifuged and the supernatant containing hemicelluloses was discarded. The precipitate was washed in deionized water at least five times. The material was then dried and weighed later. The calculation to determine the concentration of hemicelluloses present in the samples was performed by the gravimetric difference between the "total cell wall fraction" and the "cellulose and lignin fraction". Hemicellulose concentrations were calculated as a percentage of dry weight (%DW).

Lignin

In order to determine the lignin concentrations, the protocol of Santos et al. (2008) was adopted with modifications. Initially, 150 mg of dry mass homogenized in 10 mL of 50 mM sodium potassium phosphate buffer at pH 7 were used. The material was centrifuged for 10 min at $4,324 \times g$ and the supernatant discarded. From this step, the precipitate was washed 12 times with different solutions. After the 12th wash, the precipitate was dried in an oven at 60 °C for 24 hours. The resulting material comprises the protein-free cell wall fraction. From the dried precipitate, 50 mg were weighed in 15 mL tubes, 1.2 mL of thioglycolic acid and 6 mL

of 2M HCl were added. Then, the samples were incubated at 95 °C for 4 hours. After that time, they were centrifuged and washed with distilled water. Then 7 ml of 0.5 M NaOH were added, then incubated at 30 °C for 18 hours under constant stirring on a magnetic stirrer. The samples were centrifuged and the supernatant reserved. The precipitate was washed with 3 ml of 0.5 M NaOH, then centrifuged. The resulting supernatant was combined with the previous one, acidified with 1.8 ml of HCl and left at 4 °C for 12 hours without stirring for precipitation. After this period, the samples were centrifuged and washed twice with distilled water, centrifuged again at $4,324 \times g$ for 10 minutes at room temperature and the supernatant discarded. The precipitate obtained was dried at 60 °C for 24 hours and resuspended in 5 mL 0.5 M NaOH. The dilutions were standardized in 13 mL of 0.5 M NaOH. From this dilution, 300 μL were withdrawn, which was finally diluted in 2 ml of 0.5 M NaOH for reading. The determination of the lignin content was performed by reading the absorbance in a spectrophotometer at a wavelength of 280 nm. For this, lignin solution (Sigma®) was used to construct the standard curve, at concentrations of 0; 10; 20; 30; 40; 50; 100; 200; 300; 400 and 500 $\mu\text{g } \mu\text{L}^{-1}$.

Statistical analysis

Initially, data normality was assessed by the Shapiro-Wilk test. Variables with normal distribution were submitted to analysis of variance (ANOVA), and the difference between treatments was evaluated by Tukey's test ($p \leq 0.05$). The means of variables that did not show normal distribution were analyzed using the Kruskal-Wallis test ($p \leq 0.05$). Principal component analysis (PCA) was performed to demonstrate relationships between the independent variable (forest areas) and the dependent variables (non-structural carbohydrates and cell wall polymers). The analysis was performed only for leaves, as it was the organ with the most significant relationships. By the same principle, the soluble carbohydrates glucose and fructose were not included.

RESULTS AND DISCUSSION

Non-structural carbohydrates

In general, the concentration of total non-structural carbohydrates in relation to dry mass was different between the organs sampled, with higher values being found in leaves (8.38%), followed by branches (5.31%) and stems (2.58%), respectively. Among the non-structural carbohydrates, the starch concentration was the one that presented the greatest variations between forest zones. The leaves showed higher concentration of glucose in relation to the other organs (1.27%). However, no significant differences were observed in relation to the evaluated areas (Figure 2A). Likewise, leaves were the organs that presented the highest concentrations of fructose (1.19%), with no significant differences between forest zones. As for the branches, the individuals located in the non-flooded zone had higher fructose concentration than those in the periodically flooded zone (Figure 2B). There was no difference in sucrose concentration between forest zones, regardless of the organ (Figure 2C). The leaves showed the highest concentrations of this oligosaccharide (3.68%), followed by branches (1.64%) and stems (0.55%) respectively.

The starch contents in the leaves and branches of the individuals distributed in the non-floodable zone were higher than the values found in the PI zone (Figure 2D). Individuals located in the periodically flooded zone had the lowest starch concentrations, regardless of the organ evaluated. Individuals in the transition zone presented the most heterogeneous responses. In the stem, a higher concentration of starch was found in relation to individuals from the periodically flooded zone. There was a lower concentration in leaves compared to those in the non-floodable zone. In the branches, however, the starch concentration was not significantly different from the other zones. In general, the highest starch concentrations were found in branches (3.32%) and leaves (2.24%).

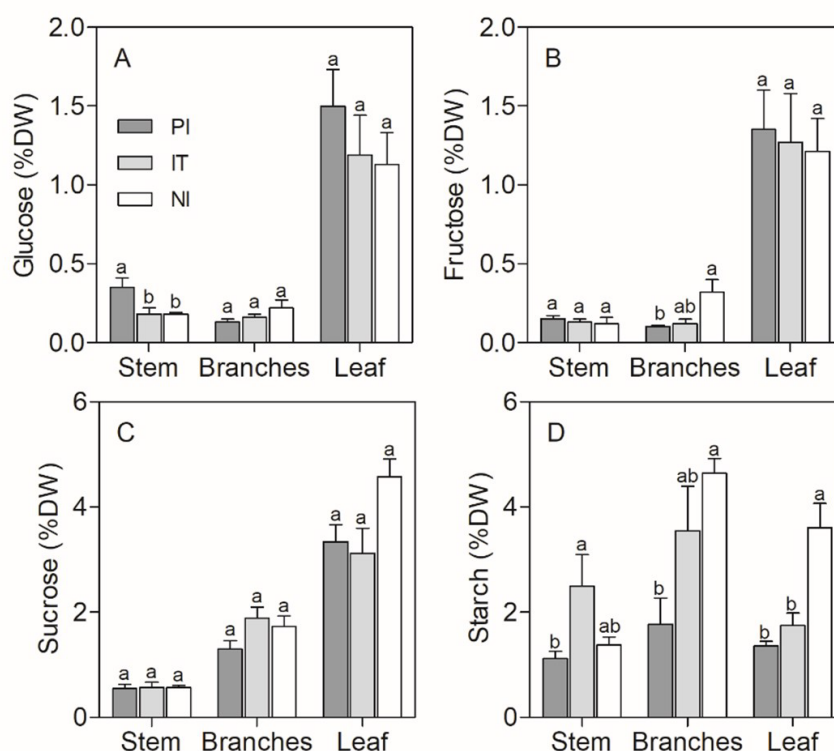


Figure 2: A: glucose concentration (%DW) in stem, branch and leaf; B: fructose concentration (%DW) in stem, branch and leaf; C: sucrose concentration (%DW) in stem, branch and leaf; and D: starch concentration (%DW) in stem, branch and leaf of *Protium icariba*. The columns represent the average of individuals sampled in the periodically flooded zone (PI), transition zone (IT) and non-flooded zone (NI). The bars represent the standard error of the mean (n=7). The letters compare the means of individuals from each zone for a given organ, Tukey or Kruskal-Wallis test (p≤0.05).

We can consider that there was little variation in the concentrations of non-structural carbohydrates among the subpopulations of *P. icariba*. Only the starch concentration varied significantly. Therefore, we believe that this variation is associated with the contrasting conditions of edaphic humidity, since species resistant to water deficit may present accumulation of non-structural carbohydrates. However, if the species is susceptible to stress, a decline in the concentrations of these compounds can be observed (Piper, 2011). Taking into account that *P. icariba* occurs in the three forest zones and that there were differences in starch concentrations, mainly between the non-flooded and periodically flooded zones, it is possible to infer that individuals from the non-flooded zone, as they are more susceptible to the effects of water deficit, store more starch during limiting periods. Starch accumulation may be a plastic physiological and biochemical condition of *P. icariba*, essential for its generalist distribution characteristic. Another explanation may be linked to the fact that the entire region was subjected to a prolonged period of drought. Water deficit can affect starch hydrolysis and carbohydrate mobilization by the phloem (Sala et al., 2010), especially in individuals from the forest zone with a sandier and deeper soil.

Individuals in the transition zone are more related to those occurring in the non-floodable zone, mainly in relation to starch concentrations. However, in addition to the higher levels of starch, individuals from the non-floodable zone and, possibly, those from the transition zone, should have higher levels of soluble carbohydrates. This is because the intracellular accumulation of these molecules reduces the water potential of the cells, allowing the maintenance of cell turgidity. Through this mechanism, the stomata remain open, ensuring transpiration and photosynthesis under conditions of low soil water potential (Anjum et al., 2017; Funkhouser et al., 1994). However, no significant differences were observed between the zones. This may mean little participation of these

molecules in the osmotic regulation mechanism in *P. icariba*. Alternatively, the salinity conditions of restinga soils, mainly in the periodically flooded zone, can stimulate the accumulation of soluble carbohydrates, comparatively neutralizing the isolated effects of water stress for higher concentrations of soluble carbohydrates in the non-flooding zone. This is because both low water availability and salinity stimulate osmotic adjustment responses in plants. Even so, in purely comparative terms, the non-floodable zone is, according to principal component analysis (PCA), more related to higher sucrose concentrations, indirectly involved in osmotic regulation in leaves.

Given the complexity of the environments analyzed, another consideration for comparisons in starch contents is the differences in soil fertility. Nonstructural carbohydrates tend to be negatively related to nitrogen (N), phosphorus (P) and potassium (K) concentrations. This relationship cited by Chapin III et al. (1986) concerns tundra shrubs, where accumulation of non-structural carbohydrates occurred in regions of soils deficient for these minerals. Similar results were obtained by Kaakinen et al. (2004), who analyzed the concentration of starch and soluble carbohydrates in *Picea abies* seedlings subjected to different concentrations of nitrogen. In the experiment, this conifer's seedlings, submitted to the lowest nutrient supply, accumulated higher levels of non-structural carbohydrates than those submitted to the highest supply. The lower availability of the nutrient may have been insufficient to sustain growth, causing non-structural carbohydrate reserves to be unused (Ericsson et al., 1996).

We hypothesized that the higher levels of organic matter and litter in the periodically flooded zone can guarantee greater nutrient supplies compared to the non-flooded zone. Perhaps this is one of the characteristics that limit the growth of trees in the zone with lower nutrient availability, as individuals from the non-flooded zone are those with the smallest height and stem diameter (Lourenço et al., 2020). This characteristic allows the stocks of non-structural carbohydrates to be greater in the non-floodable zone, as their mobilization is less required during the slower phases of growth.

Cell wall polymers

The leaves were the only organs to show significant differences in cellulose contents between forest zones. Individuals from the non-flooded zone presented leaves with lower cellulose concentrations compared to individuals from the periodically flooded and transitional zones (Figure 3A). No significant differences were identified in hemicellulose concentrations between forest zones, regardless of the organ (Figure 3B). Individuals from the non-floodable zone showed higher concentration of lignin in the stems than those distributed in the transition zone. In relation to leaves, individuals from periodically flooded and transitional zones had higher lignin concentrations than individuals from the NI zone (Figure 3C).

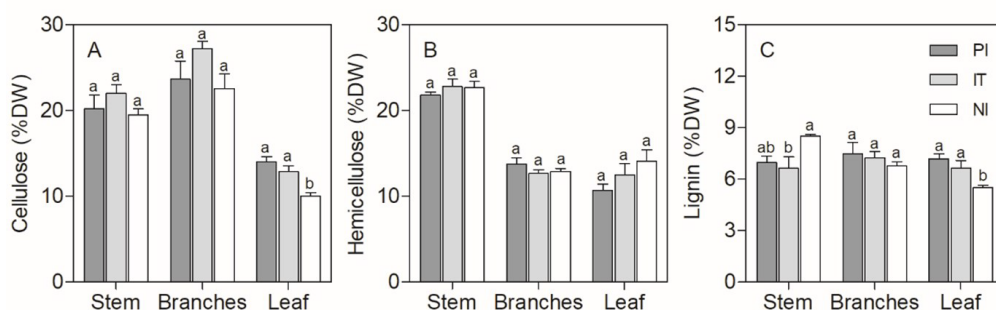


Figure 3: Cellulose concentration (%DW) in stem, branch and leaf; B: hemicellulose concentration (%DW) in stem, branch and leaf; C: lignin concentration (%DW) in stem, branch and leaf of *Protium icariba*. The columns represent the average of individuals sampled in the periodically flooded zone (PI), transition zone (IT) and non-flooded zone (NI). The bars represent the standard error of the mean (n=7). The letters compare the means of individuals from each zone for a given organ, Tukey or Kruskal-Wallis test (p ≤ 0.05).

The leaves were the most sensitive and responsive organs in this study. It is an organ considered to have a high level of plasticity and suitability for different environmental conditions (Gratani et al., 2006). However, the comparative result between the lignin concentrations of the leaves in the different humidity conditions did not corroborate our expectations. The data did not confirm higher concentrations of lignin in the leaves of individuals from the non-floodable zone, as in most studies, which relate higher levels of the phenolic compound to drought conditions (Gu et al., 2020; Li et al., 2013). Under these conditions, plants tend to produce more secondary compounds and develop thicker cuticles to protect against oxidative stress and transpiration (Yamasaki et al., 2007). However, the contradiction of higher concentrations of lignin in the periodically flooded zone, even in the dry season, may be explained by other factors, such as the availability of nutrients and herbivore.

Higher concentration of nitrogen (N), for example, can be a stimulating condition for lignin levels in plant tissues. Gramineae treated with higher N availability presented higher lignin contents (Pitre et al., 2007). The same result is shared by adult trees of *Picea abies*, which when subjected to higher concentrations of nutrients, especially nitrogen, presented 7% higher concentrations of lignin in the secondary xylem (Anttonen et al., 2002). Perhaps, due to the fact that the periodically flooded zone concentrates more nitrogen compounds (Lourenço Júnior et al., 2021), we can suggest a certain influence of soil fertility on higher lignin concentrations.

Another important observation is the fact that the leaves of individuals in the periodically flooded region visibly suffer more damage by herbivory. If the fact is confirmed, the higher lignin content in the leaves of the subpopulation of the periodically flooded zone may be related to the strategy of avoiding biological damage, since the lignification process was attributed as a line of defense, since this polymer is considered indigestible and non-degradable by most organisms (Fürstenberg-Hägg et al., 2013; Poorter & Bongers, 2006). Furthermore, the higher concentration of cellulose in the leaves of the subpopulation of the periodically flooded zone may, in the same way, have the same function. During studies on defense mechanisms against herbivory in Caatinga trees, Dourado et al. (2016) identified that the species that had the highest leaf hardness were the least affected. Therefore, greater investment in structural cell wall polymers, such as cellulose and lignin, may hinder herbivory in the wetter region of the forest, a location likely to have the highest concentration of herbivorous insects among the environmental conditions analyzed (Fernandes et al., 2004; Fernandes & Price, 1988). This observation is possible and suggested for investigation in future research.

We are certain that the environmental humidity gradient influences the cellulose concentrations, even though *P. icariba* is considered a generalist species and, for this reason, it is considerably plastic. For Kreuzwieser et al. (2009) water stress conditions can influence plants to invest more energy in maintenance metabolism and less in growth, until the stress is overcome. As a result, the synthesis of structural polymers for the formation of secondary cell walls is suppressed. In this way, molecule contents of such as cellulose in the tissues decrease. This principle is also equivalent to a mechanism for replacing molecules, where energy reserve molecules, such as starch, come to represent greater proportions in plant tissues. This principle is equivalent to the increase in hemicellulose concentrations that, some time ago, were indicated as having an extra structural function as an energy source in periods of greater demand (Kaakinen et al., 2004; Schädel et al., 2009). That is, part of the structural dry mass of individuals from the non-floodable zone may have been replaced by energy reserve molecules, starch and hemicelluloses. A strategy that would optimize survival in periods of lower photosynthetic activity and which require greater energy expenditure such as reproduction, since the non-floodable zone imposes greater water restriction than the others. Higher foliar concentrations of hemicelluloses were results shared by studies that indicated the participation of hemicelluloses on plant resistance to water stress. Under drought conditions, several genes encoding structural cell wall polymers are activated, including genes responsible for the production of hemicelluloses such as xylose isomerase (Yang et al., 2006). Although the study by Yang et al. (2006) focused on *Oryza sativa* roots, these genes are not specific to this tissue alone. The increase in these polymers, hours after submission to water stress, suggests that this is a water stress resistance strategy. Another

evidence was the participation of the transcription factor ASR1 in the increased expression of genes of cell wall polymer synthesis in leaves of *Solanum lycopersicum* (Ricardi et al., 2014). This would ensure changes in the elasticity and integrity of cell walls, providing greater tolerance in relation to water stress.

Taking into account that the zones with higher altitude in relief (non-flooded and transitional) are more sensitive to water stress, it is possible to infer that this is the main factor responsible for the differences in hemicellulose concentrations between forest zones. Thus, individuals from the non-floodable zone have higher hemicellulose contents in the cell wall as a resistance resource to stressful periods of low productivity.

With the Principal Component Analysis (PCA), it was possible to relate in the multivariate space the independent variable forest zone and the most significant dependent variables (sucrose, starch, cellulose, hemicelluloses and lignin) for leaf (organ that showed the best response). Individuals from the non-flooded zone are located mainly to the right of axis 1 and, therefore, are associated with higher concentrations of starch, sucrose and hemicelluloses (Figure 4). On the other hand, individuals in the periodically flooded zone are on the left and, therefore, are associated with higher concentrations of cellulose and lignin. Individuals in the transition zone do not show a defined pattern of distribution.

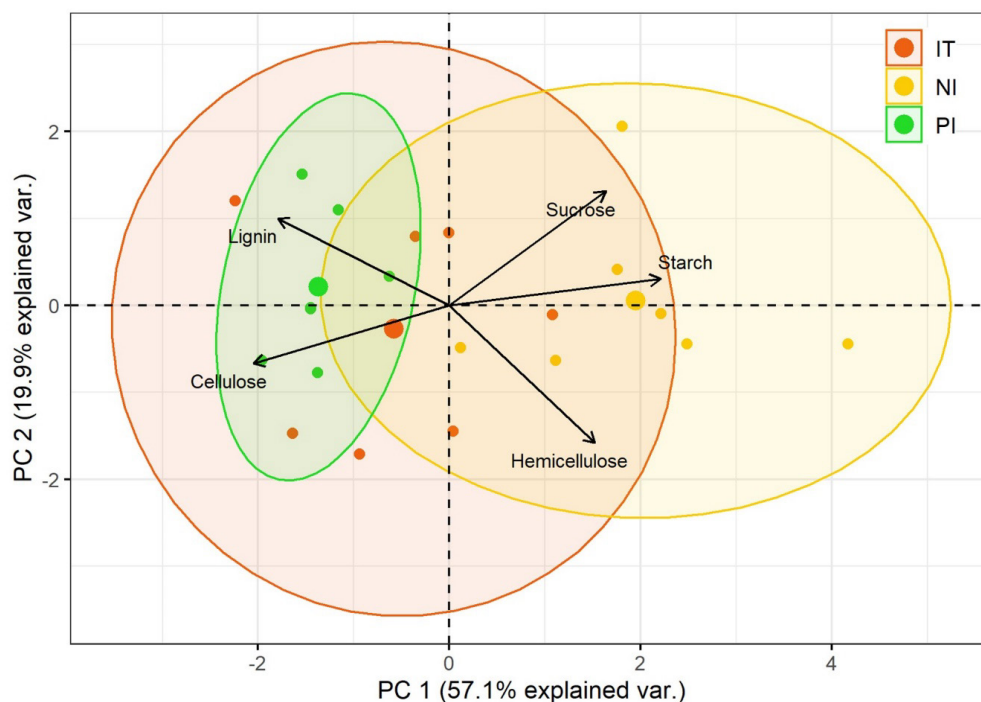


Figure 4: Principal component analysis (PCA) of individuals of *Protium icariba*. The concentrations of starch, cellulose, hemicelluloses, lignin and sucrose in leaves of individuals occurring in periodically flooded (PI), transition (IT) and non-flooded (NI) zones of a restinga forest were analyzed.

CONCLUSION

(1) The generalist habit of *P. icariba* provides the existence of differences in the contents of non-structural carbohydrates and structural polymers, mainly in leaves. (2) Individuals located in the non-floodable zone have higher levels of starch and hemicelluloses, especially in relation to individuals distributed in the periodically flooded zone. (3) Cellulose concentration was higher in the periodically flooded zone, mainly due to the influence of water stress in the non-flooded zone, which should cause reductions in the participation of structural polymers in leaf biomass. (4) The higher lignin content in individuals from the periodically flooded zone may be related to the greater availability of organic matter and possibly to

greater herbivory. (5) The plasticity shown by *P. icarica* may favor its survival in the face of climate change. (6) More research at the community level is critical to determine whether forests with higher moisture content are the main reservoirs of organic carbon in plants.

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