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Influence of climate seasonality on the accumulation of carbohydrates and cell wall polymers in organs of three tree species of a restinga forest

Influência da sazonalidade climática sobre o acúmulo de carboidratos e polímeros de parede celular em órgãos de três espécies arbóreas de uma floresta de restinga

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Abstract

Due to the great variety of environments in which tropical forests are distributed, the occurrence of tree species is conditioned to places that present favorable environmental characteristics. If the species does not show considerable phenotypic plasticity, its distribution limits will be reduced. However, given the predictions of climate change, environments may undergo relatively abrupt variations and species will have to respond quickly to new conditions. To investigate how this can influence the dynamics of carbon compounds in plant tissues, studies on environmental gradients, combined with seasonal analysis of climate, are a powerful tool. The objective of this study was to evaluate the seasonal variation (dry period-rainy period) in the concentrations of non-structural carbohydrates and cell wall polymers in stems, branches and leaves of tree species distributed in zones of a restinga (sandbank) forest. For this, we carried out collections in the dry season (winter) and in the rainy season (summer) in populations of *Eugenia bahiensis*, *Guarea macrophylla* and *Calophyllum brasiliense*. Starch and hemicellulose concentrations were lower in the rainy season. However, the reduction in hemicellulose concentrations was the most uniform result. Probably, these molecules were used as energy source in the reproductive and growth demands of the species. Due to a long period of drought, the other non-structural carbohydrates (glucose, fructose and sucrose) did not show significant variations. Small variations in cellulose and lignin contents were attributed to fluctuations in the concentration of other molecules. In general, it was assumed that the low rainfall recorded during the study interfered with the expected seasonal responses. This phenomenon may have reduced the differences in soil moisture between forest zones.

Keywords: Non-structural carbohydrates; Cellulose; Environmental gradient; Hemicelluloses; Lignin.

Resumo

Devido à grande variedade de ambientes nos quais as florestas tropicais estão distribuídas, a ocorrência de espécies arbóreas fica condicionada a locais que apresentam características ambientais favoráveis. Se a espécie não apresentar uma considerável plasticidade fenotípica, seus limites de distribuição serão reduzidos. Porém, diante das previsões de mudanças climáticas, ambientes poderão sofrer variações relativamente abruptas e as espécies terão de responder rapidamente às novas condições. Para investigar

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como isso pode influenciar na dinâmica dos compostos de carbono nos tecidos vegetais, estudos em gradientes ambientais, aliados à análise sazonal do clima, são uma ferramenta poderosa. O objetivo desse estudo foi avaliar a variação sazonal (período seco-período chuvoso) nas concentrações de carboidratos não estruturais e polímeros de parede celular em caules, ramos e folhas de espécies arbóreas, distribuídas em zonas de uma floresta de restinga. Para isso, realizamos coletas na estação seca (inverno) e na estação chuvosa (verão) em populações de *Eugenia bahiensis*, *Guarea macrophylla* e *Calophyllum brasiliense*. As concentrações de amido e hemiceluloses foram menores na estação chuvosa. Contudo a redução nas concentrações de hemiceluloses foi o resultado mais uniforme. Provavelmente, essas moléculas foram utilizadas como fonte energética nas demandas reprodutivas e de crescimento das espécies. Devido a um grande período de seca, os demais carboidratos não estruturais (glicose, frutose e sacarose) não apresentaram variações significativas. Pequenas variações nos teores de celulose e lignina foram atribuídas à flutuação na concentração de outras moléculas. De forma geral, foi suposto que o baixo volume pluviométrico registrado durante o estudo, tenha interferido nas esperadas respostas sazonais. Fenômeno este que pode ter reduzido as diferenças em umidade do solo entre zonas florestais.

Palavras-chave: Carboidratos não estruturais; Celulose; Gradiente ambiental; Hemiceluloses; Lignina.

INTRODUCTION

Research carried out on environmental gradients helps to understand how environmental conditions determine the structure and functioning of ecosystems (Guittar et al., 2016), even at very small spatial intervals (Lourenço Junior et al., 2020). Due to these characteristics, studies in heterogeneous environments were intensified as predictions about the planet's climate pointed to worrying scenarios. Some regions may face severe droughts (Gudmundsson et al., 2017) and others suffer from floods of varying intensities (Alfieri et al., 2017). Such phenomena can cause the extinction of the most vulnerable species, due to relatively abrupt changes in temperature and rainfall. The survival of species in these scenarios is based on strategies of tolerance to environmental variations, mainly for sessile populations such as plants. Thus, the species that show greater phenotypic plasticity will have a greater chance of survival.

Among the plant strategies for colonization of heterogeneous and seasonal environments, there is flexibility in the partition and allocation of carbon. It allows plants to direct energy molecules to growing organs or for reproduction, in addition to storage. The dynamics of the flow and storage of carbohydrates and cell wall polymers may be important for the survival of tree species in the Atlantic Forest Biome, due to the great ecosystem diversity in these areas. In this Biome, the environments differ in structure, diversity, climate and geomorphology (Joly et al., 2014). The formations of forest vegetation can be found from the flatter areas close to the ocean, such as sandbank formations, to those at higher altitudes further away from the coast.

Restinga formations are present in practically the entire Brazilian coast and are considered of high interest for several studies in the field of Plant Biology (Magnago et al., 2013; Mendes et al., 2017; Lourenço Junior et al., 2020). Despite their significant original distribution, these ecosystems are in an advanced stage of degradation, mainly due to real estate speculation, which threatens a set of unique plant physiognomies. These are coastal sandy plains of marine origin, including beaches, sandy ridges, and depressions between ridges, dunes and lagoon margins (Martin et al., 1997). Topographic variations, water table level and flooding time influence vegetation structure, diversity and floristic composition (Monteiro et al., 2014). In these environments, species deal, for example, with various stressful or variable conditions, such as high temperatures (Scarano, 2002), concentrations of organic matter and aluminum saturation (Almeida Filho et al., 2013), salinity (Lourenço Junior et al., 2013), soil texture and water table level (Lourenço Junior et al., 2021). In addition, they must respond to climatic variations in temperature and rainfall throughout the seasons. In areas of topographical depression, where the water table is shallow, periods of greater precipitation can cause periodic flooding (Magnago et al., 2013; Lourenço Junior et al., 2020). On the other hand, higher areas, such as sandy ridges, are subject to water limitation, due to the presence of well-draining soils (Menezes et al., 2010; Lourenço Junior et al., 2021).

The restinga at Paulo Cesar Vinha State Park (PEPCV), in southeastern Brazil, is part of the largest continuum of the restinga ecosystem in the State of Espírito Santo. PEPCV is considered a priority area for the conservation of Atlantic Forest biodiversity (Instituto de Meio Ambiente e Recursos Hídricos, 2020). In the region, the period of higher rainfall is accompanied by higher temperatures and solar radiation, and the lower rainfall is accompanied by lower temperatures and radiation (Instituto Nacional de Meteorologia, 2022). Due to the influence of this climatic seasonality, variable topography and plant diversity, the forest areas of the PEPCV can be divided into periodically flooded, non-flooded and transitional forest zones (Magnago et al., 2013). The species that occupy these different zones are influenced by contrasting and variable environmental conditions during the year. For this reason, there are species that probably specialized in the colonization of a certain forest zone. Among them are *Calophyllum brasiliense* Cambess. (Calophyllaceae), *Eugenia bahiensis* DC. (Myrtaceae) and *Guarea macrophylla* Vahl (Meliaceae), distributed and restricted to the zones: periodically flooded, transitional and non-floodable (Lourenço Junior et al., 2020).

The peculiarity of a contrasting environment, both spatially and temporally (seasonal), such as the restinga forest, offers excellent conditions for the development of eco-physiological studies. This is so, because it is a well-defined environmental gradient on a very small spatial range, rarely addressed by research. Considering that environmental factors can influence the carbon reserves of plants (Tenhaken, 2015; Pommerrenig et al., 2018), the quantification of non-structural carbohydrates (glucose, fructose, sucrose and starch) and structural cell wall polymers (cellulose, hemicelluloses and lignin) in a heterogeneous environment can contribute to the knowledge about the seasonal dynamics of carbon biomolecules. This happens because less than 15% of the studies on the dynamics of non-structural carbohydrates were carried out in tropical ecosystems; most of them being limited to seedlings and understory species (Martínez-Vilalta et al., 2016). However, it is known that seasonal conditions of temperature, precipitation and phenological patterns influence the concentrations of these biomolecules in plant tissues (Newell et al., 2002). Studies investigating the behavior of cell wall polymers under seasonal climatic conditions are even scarcer. Furthermore, the responses are very varied or antagonistic, and further studies are needed to reveal how forests respond to hyper-dynamic environmental conditions.

Warmer and wetter periods can increase carbon fixation rates, leading to an accumulation of non-structural carbohydrates in some plant organs (Ribeiro et al., 2012). On the other hand, these periods may coincide with the phases of plant growth and reproduction, with high photosynthetic rates being insufficient to meet energy demands (Schädel et al., 2009). Hemicelluloses, despite being considered for a long time as structural molecules, can be an energy source during periods of higher energy expenditure, also functioning as reserve molecules (Kaakinen et al., 2004; Schädel et al., 2009). The other polymers, cellulose and lignin, are not mentioned as playing an energy reserve role, and their variations in plant tissues are related to the dynamics of the concentrations of other molecules (Chapin III et al., 1986).

In the above context, the objective of this study was to evaluate the seasonal variation (dry period-rainy period) of the concentration of non-structural carbohydrates and cell wall polymers in stems, branches and leaves of tree species, distributed in areas of a restinga forest. From the results, it was possible to test the hypotheses that: (i) all species present lower concentrations of non-structural carbohydrates in the rainy season; (ii) the concentration of hemicelluloses will be lower in the rainy season; (iii) there is no variation in the concentrations of cellulose and lignin, as these polymers are not used as an energy source by plants.

MATERIAL AND METHODS

Characterization of the study area

Data collection took place in a restinga forest located in the Paulo César Vinha State Park (*PEPCV*) (UTM, 24K 351886 mE and 7721804 mS), Guarapari/ES, Brazil. According to the Köppen-Geiger climate classification system, the climate of the region is Aw, tropical hot and humid, with rainy summers and dry winters. The mean annual precipitation is 1,276 mm and the mean annual temperature is 24.2 °C (Instituto Nacional de Meteorologia, 2022). Figure 1 shows the monthly averages of precipitation and temperature between 1990 and 2021, according to records from the nearest meteorological station, located in Vitória/ES (Instituto Nacional de Meteorologia, 2022). The samples were distributed in the dry (September/2016) and rainy (March/2017) periods, according to the rainfall profile shown in Figure 1.

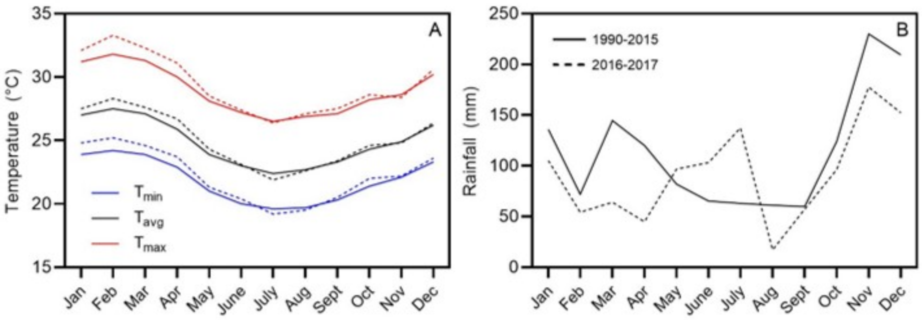

Figure 1: Average temperature (A) and precipitation (B) data from the historical series from 1990 to 2021 for the region (Instituto Nacional de Meteorologia, 2022). The solid line (—) represents the historical series between the years 1990-2015 and the dotted line (--) represents the years of study development (2016-2017).

The analyzed forest area covers approximately 11.2 hectares, 1.5 km (at low tide line) from the Atlantic Ocean (Figure 2). According to Lourenço Junior et al. (2020), in this area there are three forest zones: periodically flooded forest (PI), transitional forest (IT) and non-flooded forest (NI). The periodically flooded zone is located in areas of flat and depressed relief, with a shallow water table and subject to flooding in the rainy season; while the non-floodable zone corresponds to the higher altitude areas, with sandy soil, characteristic of a marine terrace (Martin et al., 1997). The transition zone, in turn, is located between the PI and NI zones, limited to a narrow strip of land, topographically sloping, with variable water table depth (Lourenço Junior et al., 2020).

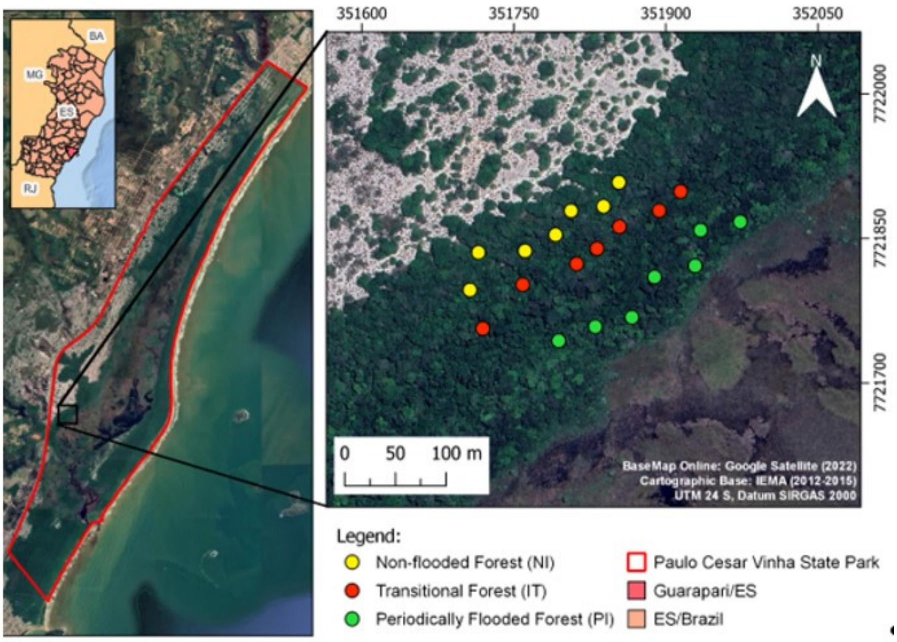

Figure 2: Location of the study area. On the left, the full scope of the Paulo César Vinha State Park. On the right, distribution of individuals sampled in the different zones of the arboreal restinga.

Sampling procedures

In each of the forest zones, seven individuals of *E. bahiensis* (abundant in the NI zone), *G. macrophylla* (abundant in the IT zone) and *C. brasiliense* (abundant in the PI zone) were sampled. We established that the individuals were evenly distributed throughout the study area, respecting a minimum distance of 5 meters between them. Only trees with a diameter at breast height (DBH) ≥ 5 cm were sampled (Lourenço Junior et al., 2020). Stem, branch and leaf samples were collected for analysis of the entire aerial part of the plants. The collection of branches and leaves was carried out with the aid of a pruner. The branch samples measured 3 cm in diameter by 25 cm in length. From the sampled branches, 10 fully expanded leaves were manually extracted, from the third to the fifth pair (from the apex), during the day, between 9:00 am and 3:00 pm. These organs were sampled in places in the canopy where the incidence of light was not permanent throughout the day due to difficulties in standardizing leaves of sun or shade in the different zones. Stems were sampled 1.5 m from the ground. We previously removed periderm and phloem to extract the fragmented xylem, using a battery-operated drill (BOSCH-GSB180-LI). The stem incision was limited to 5 cm in depth. The samples were immediately packed in thermal boxes containing ice and transported to the laboratory, where they were stored in an ultra-freezer at −80 °C until processing. The samples were lyophilized and pulverized in a ball mill (TE-350, TECNAL). AL).

Non-structural carbohydrates

Soluble sugar levels (glucose, fructose and sucrose) were determined from 150 mg per sample, following the method of Pollock (1986), with adaptations. After centrifugation, the liquid phase was concentrated by a lyophilizer. The samples were then diluted in 1 mL of solution containing ultrapure water and known concentrations of 1 gL^{-1} fructose, 1 gL^{-1} glucose and 1 gL^{-1} sucrose (using Sigma® standards). After being diluted, the samples were filtered through Millex gv pvdf syringe filters. Then, the concentrations of fructose, glucose and sucrose were determined by means of high performance anion exchange chromatography, with a refraction detector in Shimadzu RID-20A HPLC, 25 cm CLC - NH2 column, 80% acetonitrile mobile phase under flow rate of 1 $mLmin^{-1}$ at 40 °C, using Sigma® standards. The final result was determined by subtracting the amount of sugars used during the dilution of the lyophilized material, due to the fact that the sampled organs presented low concentrations of the analyzed molecules.

Starch content was determined by using the fast and sensitive enzymatic method of extraction and dosage (Amaral et al., 2007). The main steps of the method consist of: the extraction of soluble sugars, pigments, phenols and other substances, by alcoholic extraction; following enzymatic reactions with thermostable α-amylase (EC 3. 2. 1. 1) from *Bacillus licheniformis* (code E-ANAAM, MEGAZYME, Ireland) and amyloglucosidase (EC 3. 2. 1. 3) from *Aspergillus niger* (code E-AMGPU, MEGAZYME, Ireland); in the reaction with Glucose PAP Liquiform (CENTERLAB, Brazil); and in the reading, by colorimetric method, using an ELISA microplate reader at a wavelength of 490 nm.

Cell wall structural polymers

Cellulose quantification was performed following the procedures of the method proposed by Brendel et al. (2000). From 100 mg of a pulverized and dehydrated sample, sequences of additions of chemical reagents, washing and centrifugation were used, with the objective of removing non-cellulosic materials and isolating the material of interest. Finally, the cellulose concentration was determined by gravimetric difference. In order to determine total hemicelluloses, the method proposed by Schädel et al. (2010) was used. From 50 mg of powdered dry mass, cellulose, hemicellulose and lignin polymers were isolated through chemical reactions, washing and centrifugation. The last reaction of the process consisted of the total removal of hemicelluloses from the sample, and then quantification, which was determined by the gravimetric difference between the “total cell wall fraction” and the “cellulose and lignin fraction”. Lignin quantification was based on the protocol by Santos et al. (2008). 150 mg of each dried and powdered sample were used. From the portion of lignin isolated in the samples, it was possible to determine the concentration of the polymer, by reading the absorbance in a spectrophotometer at a wavelength of 280 nm. For the construction of the standard curve, lignin solutions (Sigma®) were used at concentrations from 0 to 50 μg μL^{-1} and from 100 to 500 μg μL^{-1}.

Statistical analysis

Data normality was assessed by the Shapiro-Wilk test (Shapiro & Wilk, 1965). Data sets that showed normal distribution were submitted to Student's t test for independent samples ($P \leq 0.05$). The means of variables that were not normally distributed were analyzed using the Mann-Whitney test for independent samples ($P \leq 0.05$).

RESULTS AND DISCUSSION

Non-structural carbohydrates

Including all species, leaves were the organs that registered the highest mean total glucose concentration (1.56%), followed by branches (0.35%), and stems (0.18%). However, there were no significant variations between the dry and rainy seasons. On the other hand, in the branches of *C. brasiliense* and *E. bahiensis,* higher concentrations of glucose were observed in the rainy season. Regarding the concentration of glucose in the stems, *G. macrophylla* and *E. bahiensis* showed significant variations between the periods sampled, with the highest concentrations of the former occurring in the dry season and in the latter in the rainy season (Figure 3A-3C).

Fructose content varied little between collection periods. In the branches of *C. brasiliense*, the highest concentration was recorded in the rainy season, while in the stems of *E. bahiensis* the highest levels were observed in the dry season. No variation was observed in the leaves, which had the highest average concentration of fructose (0.89%), followed by branches (0.28%) and stem (0.13%). The results for fructose concentrations are very close to those observed for glucose (Figure 3D-3F).

The only variations observed in sucrose concentrations occurred in the leaves of *G. macrophylla* and *E. bahiensis*, which presented higher levels in the rainy season (Figure 3G-3I). As in the other soluble carbohydrates, the highest concentrations of sucrose were found in leaves (1.05%), followed by branches (0.75%) and stem (0.30%). No significant variations in stem starch were observed. However, the population of *C. brasiliense* presented a higher concentration of starch in the branches during the dry season. A similar result was observed in the leaves of the species *C. brasiliense* and *E. bahiensis*, which also presented higher levels of starch in the dry season (Figure 3J-3L). Finally, similar average concentrations of the polysaccharide were observed between the organs: stem (2.25%), branches (2.59%) and leaves (2.15%).

The entire sampling period coincided with one of the worst droughts ever recorded in the state of Espírito Santo. The accumulated rainfall from April to September 2016, according to Instituto Nacional de Meteorologia (2022), was 229.7 mm. That number represents almost half of the average recorded for the interval in the historical record from 1990-2015, with 451.4 mm. The accumulated rainfall in the months of October to March 2017 was 588.5 mm. The average historical accumulated rainfall for the same period (1990-2015) is 916.3 mm. In addition, the accumulated precipitation in the 30 days prior to the dry and rainy season collections were, respectively, 33.1 and 38.3 mm.

The inconsistency in the seasonal variation of starch, especially in the stem and branches, is possibly related to the low rainfall accumulated in the months preceding the collections. During this period, the plant may have prioritized the maintenance of starch stocks (Hartmann & Trumbore, 2016), since the environment did not offer favorable climatic conditions for growth, even during the rainy season. Water stress is capable of inhibiting metabolic processes, including growth and reproduction (Abdelraheem et al., 2019). Thus, the prolonged drought may have affected the processes of starch hydrolysis and the mobilization of carbohydrates by the phloem (Sala et al., 2010). Therefore, it is understood that the branches are a potential source of energy reserve molecules and assume a strategic position, being physically closer to the new drain tissues (Taiz et al., 2017). For this reason, reductions in starch concentrations in branches were expected, in order to sustain the energy demand of the growth of new leaves. On the other hand, the small variation observed mainly in the leaves, is probably due to the use of starch as an energy source (Martínez-Vilalta et al., 2016). This fact is supported by the occurrence of flowering and fruiting in the populations studied during the rainy season. *G. macrophylla* was the only one not to present individuals in the reproductive phase in the same period. This feature corroborates the empirical results of Ramírez-Briones et al. (2017), who recorded lower starch concentrations during the flowering and fruiting period of *Diospyros digyna*. In cases of reproductive phenophases, starch would probably be mobilized for these processes.

As for sucrose, unlike Ramírez-Briones et al. (2017), the results indicated an increase in leaf concentrations in the rainy season (*G. macrophylla* and *E. bahiensis*), precisely during the phase of greatest energy demand for growth and reproduction. This result may be related to the need to reduce water potential and maintain leaf cell turgor. There may also have been an increase in sucrose production in order to supply the greater energy demand of the draining organs during water stress in the rainy season. This production could be intensified, for example, to supply root growth, as sucrose is the main sugar translocated by the phloem (Taiz et al., 2017). This was not reflected in lower concentrations of fructose and foliar glucose, since they are the monosaccharides necessary for the assembly of the sucrose molecule. This indicates that there was no mobilization in fructose and glucose concentrations to feed sucrose synthesis.

In the literature, carbohydrates fructose, glucose, sucrose and starch, can also be presented as a single variable – non-structural carbohydrates. Temperature and rainfall, together with the phenological stage of the species, are the main factors that influence the levels of non-structural carbohydrates in tropical forest tree species (Würth et al., 2005). In a semi-deciduous forest in Central America, variations in non-structural carbohydrate concentrations were observed between the dry and wet seasons, with some species showing lower levels during the rainy season (Würth et al., 2005). In the same forest, Newell et al. (2002) worked with the deciduous species *Urera caracasana* and *Cecropia longipes*, finding up to four times more non-structural carbohydrates at the end of the dry season, possibly used as an energy reserve. According to Schädel et al. (2009), photosynthesis may not provide enough carbon during sprouting, even in perennial species, with carbon stocks being an alternative. However, compared to other biomes; tropical ecosystems are known to have low seasonal variability of non-structural carbohydrates (Martínez-Vilalta et al., 2016).

In regions with marked seasonality, it is suggested that temperature plays an important role in signaling changes in climate (Tixier et al., 2019). Its influence on carbohydrate mobilization must be related to the combination with other environmental factors, such as water stress (Tixier et al., 2017). Nevertheless, the available data are insufficient to explain how subtle differences in temperature in tropical regions can influence the dynamics of non-structural carbohydrates. In seedlings of a cultivated species, *Citrus sinensis*, the moderate temperature variation from 20-25 °C to 25-30 °C stimulated photosynthesis, growth and non-structural carbohydrate contents in leaves, branches and stems, mainly starch (Ribeiro et al., 2012). In the same sense, Dickman et al. (2019) found a positive relationship between higher leaf temperatures and increased concentrations of non-structural carbohydrates in branches of drought-affected tropical tree species. However, the increase in maximum and minimum temperatures between the collection periods for the restinga region (maximum 28-33°C and minimum 19-22°C) was not accompanied by an increase in non-structural carbohydrate contents. The lower concentrations of these carbohydrates during the period of higher average temperature indicate similarity with the results of Zha et al. (2001). They found low concentrations of non-structural carbohydrates in leaves of adult trees (*Pinus sylvestris*) exposed to moderate and high temperatures for long periods. The reduction of carbohydrates in these trees was associated with an increase in maintenance of respiration that consumed carbon reserves, stimulated by the increase in temperature. This condition was also observed in the model species *Arabidopsis thaliana*. In association with different light intensities, the increase in temperature caused reductions in the concentrations of starch, glucose, fructose and sucrose, especially under low light conditions (Vasseur et al., 2011). However, the concomitance of a prolonged drought period, small average temperature variation and the reproductive stage of *C. brasiliense* and *E. bahiensis* leads us to suppose that the main factor for temporal variations in the concentrations of non-structural carbohydrates was the reproductive phase of the species. This hypothesis can be supported by the differentiated response of *G. macrophylla*, the only species not to present significant variation in the concentration of foliar starch and also the only one to not present reproductive phases during the collections during the rainy season. This fact contributes to other studies that point to starch as the main energy reserve molecule among non-structural carbohydrates (Schädel et al., 2010; Martínez-Vilalta et al., 2016).

Structural polymers

The highest average concentrations of cellulose were found in branches (16.85%), followed by stems (16.11%), and leaves (9.34%). No seasonal difference was evidenced in the stems. However, in the branches, the concentration of cellulose was higher in the rainy season for all species. Similarly, the leaves of *C. brasiliense* and *G. macrophylla* showed higher concentrations of this polymer at the end of the rainy season (Figure 4 A-4C).

Hemicellulose concentrations varied uniformly in all species and organs sampled. The highest concentration was always observed in the dry season (Figure 4D-4F). The highest average contents of hemicelluloses were observed in the stems (12.86%), followed by branches (10.10%) and leaves (4.60%), respectively.

Finally, there were subtle variations recorded for lignin concentrations. Only the branches of *C. brasiliense* and *E. bahiensis* showed the highest concentration of the phenolic compound in the rainy season, while for the leaves of *G. macrophylla*, the highest concentration was observed in the dry season (Figure 4G-4I). In the comparison between organs, the highest average lignin content was found in the stem (13.99%), followed by branches (11.95%) and leaves (6.12%).

Cellulose molecules are considered stable in plant tissues, that is, there is no great evidence related to fluctuation in their concentrations. According to Buckeridge et al. (2008), the cellulose polymer is exclusively mobilized during the degradation of reserve cell walls found in seeds or even in the formation of aerenchyma during flooding conditions. But, in general, cellulose concentrations tended to increase in the rainy season in branches and leaves. Chapin III et al. (1986) attributed the term “dilution” to explain the variations in cellulose contents in plant organs, but not due to the occurrence of structural carbohydrate remobilization. The reason, according to the authors, is the variation in the concentrations of reserve carbohydrates and proteins, which could explain the behavior of cellulose in restinga species, since the increase in the polymer was accompanied by reductions in the concentrations of starch and hemicelluloses.

Hemicellulose concentrations showed a standardized reduction in all sampled organs. The data confirm the dual function hypothesis of this structural polymer and energy reserve (Kaakinen et al., 2004). Because, when analyzing *Picea abies* seedlings in different seasons and under different nitrogen levels, Kaakinen et al. (2004), evidenced reductions in the concentrations of hemicelluloses in the stem, between the beginning and the end of the growing season. Those results were similar to those reported by Schädel et al. (2009), who analyzed hemicellulose concentrations in adult tree species from temperate climates. They identified that a deciduous species had reduced hemicellulose concentrations in branches during the vegetative growth phase. On the other hand, some deciduous and perennial species showed no variation in polymer concentrations. The authors suggested that the use of hemicelluloses as a carbon source during high demands, may be an intrinsic characteristic of each species. This is in addition to the possibility that the molecule replaces other reserve carbohydrates, such as starch, when present in lower concentrations in the tissues of certain species.

Although there are pioneering data reporting that hemicelluloses can be remobilized, the data from the present work are significant. Thus, they are uniform for all species sampled and occur in populations of a tropical forest. Hemicelluloses seem to be more representative in carbon reserves compared to non-structural carbohydrates for tree species from the restinga forest, under the conditions investigated.

In relation to lignin, its role seems to be restricted to structural functions, confirming the hypothesis. The results that revealed changes in lignin concentrations were restricted to branches and leaves. Most showed an increase in lignin concentration at the end of the rainy season, possibly attributed to lower concentrations of other molecules, such as hemicelluloses and starch. With the decrease in the concentration of other molecules, the same lignin contents become more representative in the dry mass of the tissues. This would be an adequate explanation, since lignin remobilization is unlikely, due to its strong interaction with hemicellulose and cellulose macromolecules (Canilha et al., 2010).

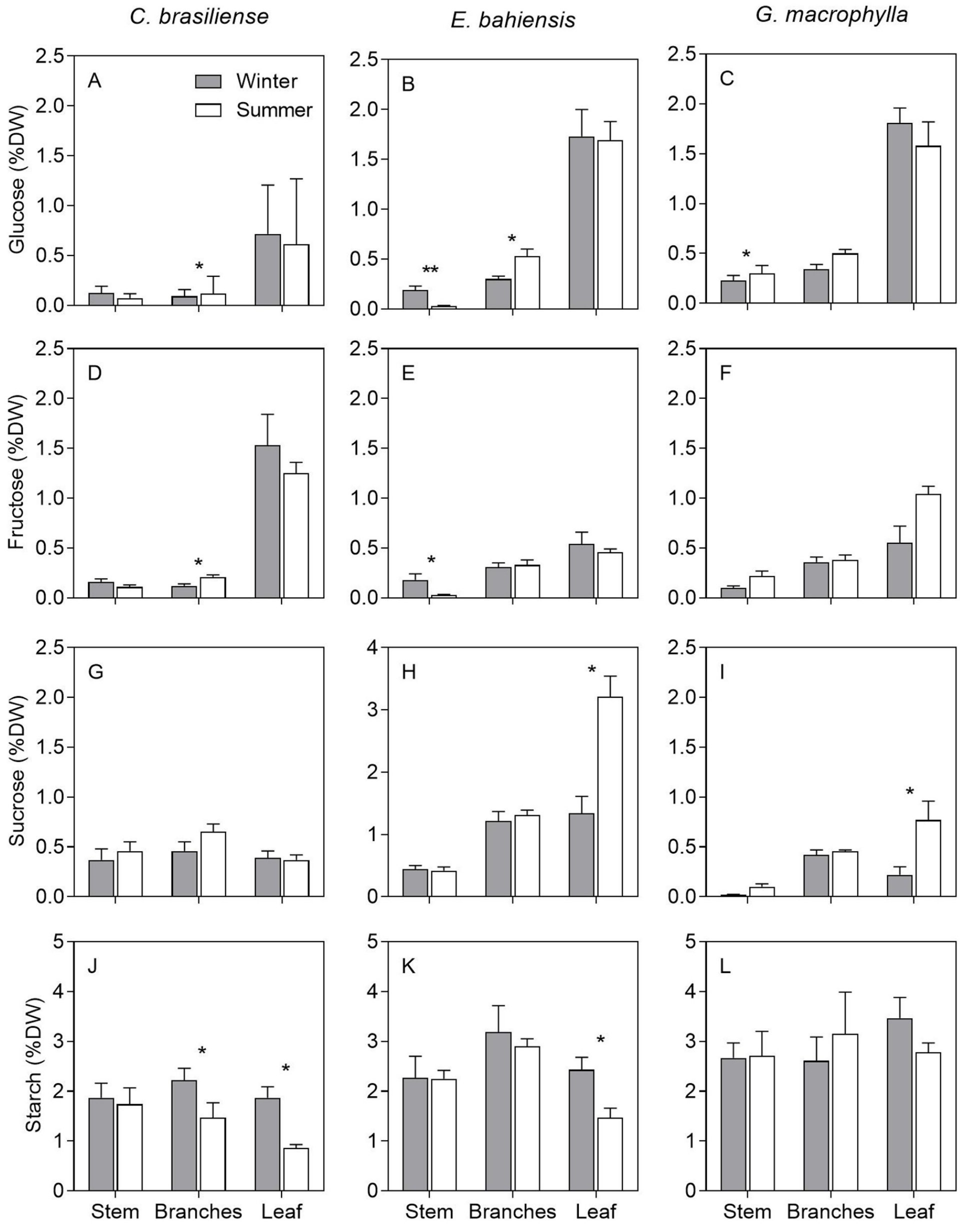

Figure 3: Glucose concentration (%DM) in the stem, branch and leaf of *Calophyllum brasiliense* (A), *Eugenia bahiensis* (B) and *Guarea macrophylla* (C). Fructose concentration (%DM) in the stem, branch and leaf of *Calophyllum brasiliense* (D), *Eugenia bahiensis* (E) and *Guarea macrophylla* (F). Sucrose concentration (%DM) in the stem, branch and leaf of *Calophyllum brasiliense* (G), *Eugenia bahiensis* (H) and *Guarea macrophylla* (I). Starch concentration (%DM) in the stem, branch and leaf of *Calophyllum brasiliense* (J), *Eugenia bahiensis* (K) and *Guarea macrophylla* (L). The bars represent the standard error of the mean (n=7). Asterisk (*) represents the difference between the evaluation seasons (dry-rainy), Student's t test or Mann-Whitney ($P \leq 0.05$).

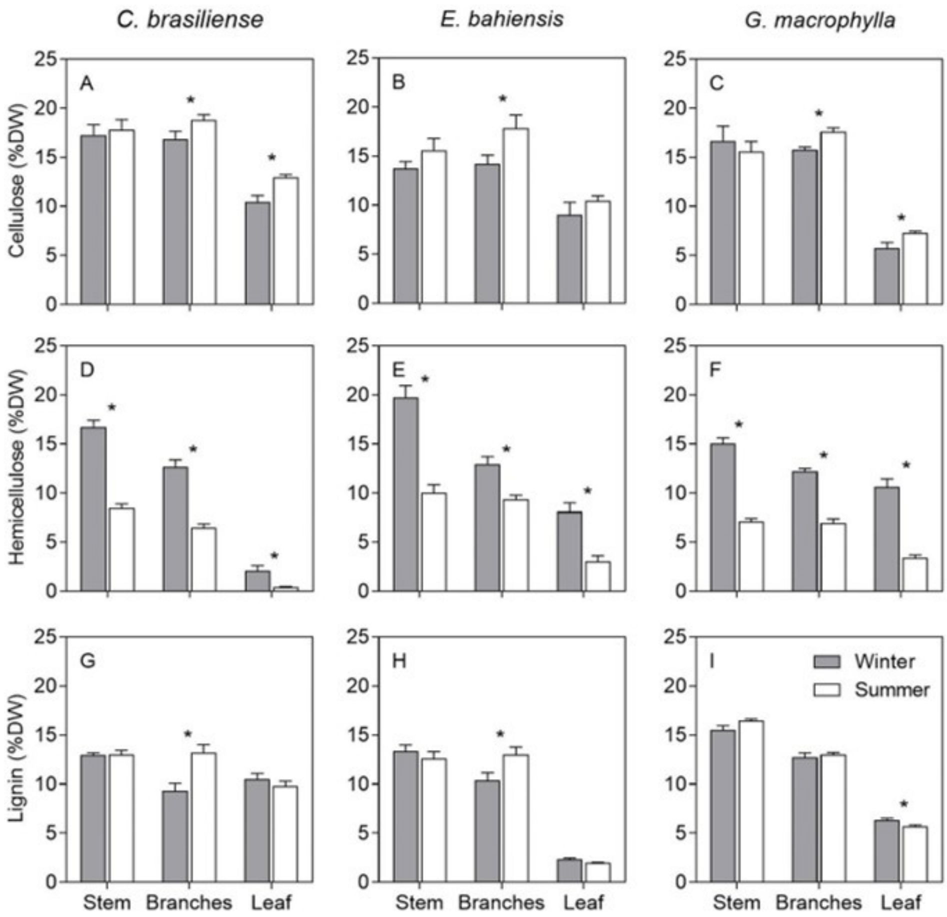

Figure 4: Cellulose concentration (%DM) in the stem, branch and leaf of *Calophyllum brasiliense* (A), *Eugenia bahiensis* (B) and *Guarea macrophylla* (C). Hemicellulose concentration (%DM) in the stem, branch and leaf of *Calophyllum brasiliense* (D), *Eugenia bahiensis* (E) and *Guarea macrophylla* (F). Lignin concentration (%DM) in the stem, branch and leaf of *Calophyllum brasiliense* (G), *Eugenia bahiensis* (H) and *Guarea macrophylla* (I). The bars represent the standard error of the mean (n=7). Asterisk represents the difference between evaluation seasons (dry-rainy), Student's t test or Mann-Whitney (P ≤ 0.05).

CONCLUSION

The average rainfall during the study period was extremely atypical. This fact reduced the significant differences in water availability between forest zones. The prolonged drought possibly influenced the low concentrations of non-structural carbohydrates, and certainly influenced the insignificant seasonal variation. Among the carbohydrates, starch was the most important energy reserve molecule among non-structural carbohydrates, although this result is not common to all organs and species sampled. It is possible that hemicellulose molecules are the main energy reserve molecules for trees in the restinga forest under prolonged drought conditions, requiring further analyses at the community level. This variation in the levels of energy molecules may be associated with the increase in energy demands in the reproductive phase and in the energy supply in a period of low primary production. The small and non-uniform variations in the concentration of cellulose and lignin could possibly be associated with the variation in the contents of other biomolecules.

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