## REGULATION OF LEAF WATER POTENTIAL IN TREE SPECIES OF THE CERRADO-AMAZONIA TRANSITION IN RESPONSE TO CLIMATE SEASONALITY

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#### Abstract

Leaf water potential is a good indicator of tree species responses to rainfall seasonality, allowing to better understand water stress effects on vegetation. We investigated daily and seasonal patterns on leaf water potentials of 21 dominant species in three distinct vegetation types from the Cerrado-Amazonia transition to assess how they respond to seasonal changes in water availability. We found that the variation on leaf water potential ( $\Psi$ l) across species was higher in the dry compared to the rainy season, for all vegetation types. Typical cerrado and cerradão species showed similar patterns, with higher  $\Psi$ l regulation under higher VPD and lower variation in predawn  $\Psi$ l ( $\Delta\Psi$ pd), indicating root access to soil water even during intense drought. In contrast, seasonal forest species showed lower regulation under lower VPD effects in both seasons, and higher  $\Delta\Psi$ pd, indicating lower access to deep water in the soil. These differences revealed two main groups of species: higher  $\Psi$ l regulators with a strategy isohydric occurring in both typical cerrado (savannah) and cerradão (woodland), and lower  $\Psi$ l regulators with anisohydric strategy, occurring in seasonal forest. Our results suggest that seasonal tropical forest species will become very vulnerable to predicted increases on drought severity in the region.

Regulation of leaf water potential in tree species of the Cerrado-Amazonia transition in response to climate seasonality

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#### ABSTRACT

Leaf water potential is a good indicator of tree species responses to rainfall seasonality, allowing to better understand water stress effects on vegetation. We investigated daily and seasonal patterns on leaf water potentials of 21 dominant species in three distinct vegetation types from the Cerrado-Amazonia transition to assess how they respond to seasonal changes in water availability. We found that the variation on leaf water potential ( $\Psi_1$ ) across species was higher in the dry compared to the rainy season, for all vegetation types. Typical cerrado and cerradão species showed similar patterns, with higher  $\Psi_1$  regulation under higher VPD and lower variation in predawn  $\Psi_1$  ( $\Delta \Psi_{pd}$ ), indicating root access to soil water even during intense drought. In contrast, seasonal forest species showed lower regulation under lower VPD effects in both seasons, and higher  $\Delta \Psi_{pd}$ , indicating lower access to deep water in the soil. These differences revealed two main groups of species: higher  $\Psi_1$  regulators with a strategy isohydric occurring in both typical cerrado (savanna) and cerradão (woodland), and lower  $\Psi_1$  regulators with anisohydric strategy, occurring in seasonal forest. Our results suggest that seasonal tropical forest species will become very vulnerable to predicted increases on drought severity in the region.

**Keywords:** savanna, Neotropics, tropical forest, isohydric, anisohydric, seasonality, plant hydraulics, vapour pressure deficit, climate change, plant traits.

#### Introduction

In the tropics, species distribution occurs along gradients of water availability, affected by their resistance to drought (Aguirre-Gutiérrez et al., 2019). Thus, a marked seasonality, with well-defined periods of drought and rain, will favour plants with traits and ecological strategies that enhance survival under such circumstances (Franco, Rossatto, Silva, & Ferreira, 2014). Hence, plants occurring in highly seasonal habitats will display specialized mechanisms for water acquisition, maintenance and use, to guarantee a continuous flow, with strategies that allow homeostasis in hydraulic functioning (Oliveira et al., 2014).

The most common explanation of how hydraulic functioning occurs in plants is the unidirectional movement (i.e., the continuous soil-plant-atmosphere), where water flow occurs from less to more negative water potentials, establishing a difference of potentials through a pressure gradient (Oliveira et al., 2014). The leaf water potential  $(\Psi_1)$  reflects the tension in which the xylem transports water, and it is a crucial variable to check the water status of the plant at a given time, being influenced by leaf transpiration processes, which is determined by the atmospheric evaporative demand (Oliveira, Dawson, Burgess, & Nepstad, 2005; Palhares, Franco, & Zaidan, 2010) and by the water availability in the soil (Jones, 1998). If there are severe changes in these mechanisms, such as limited water availability and high evaporative demand, hydraulic failure (i.e., cavitation of the xylem vessels) can occur interrupting water transport, and eventually could lead to the plant death (McDowell, 2011). Thus, a higher vapour pressure deficit (VPD) during the dry season expose plants to more stressful conditions (Prado, Wenhui, Cardoza Rojas, & Souza, 2004), which require effective mechanisms to avoid excessive water loss and regulate plant water transport, which reflect mainly on stomatal regulation process (Martínez-Vilalta, Poyatos, Aguade, Retana, & Mencuccini, 2014). Water availability in the soil directly influences this control; roots access water in the soil, and their hydraulic conductivity will be a function of the daily transpiration of the plant (Meinzer et al., 1999). Based on the mechanism of transpiration regulation over time, there is a continuum of variation between two extreme strategies: isohydric species that adjust their stomatal opening to avoid significant drops on  $\Psi_1$ , and anisohydric species with lower stomatal control and higher daily and annual variation in the  $\Psi_1$  (Jones, 1998; Martínez-Vilalta & Garcia-Forner, 2017; Tardieu & Simonneau, 1998).

Theory suggests that  $\Psi_1$  at predawn reflects the water potential of the soil (Tardieu & Simonneau, 1998) and

therefore, its variation during different periods of the year can be used as an estimate (proxy) of root depth, i.e., the range of water acquired by roots (Brum, Teodoro, Abrahão, & Oliveira, 2017; Scholz, Bucci, Arias, Meinzer, & Goldstein, 2012). Thus, plants with shallow roots may show more negative values of  $\Psi_1$  in the dry season as the dry season, while plants with deep roots would maintain less negative  $\Psi_1$  values (Scholz et al., 2012).

The Cerrado-Amazonia transition, the world's largest savanna-forest transition, has a strongly seasonal climate, with a wide variety of vegetation types, ranging from savannas (e.g., typical cerrado and rocky cerrado), characterized by open environments with high temperature and solar radiation, to closed-canopy environments (e.g., cerradão, gallery forest and semi-deciduous seasonal forest), with higher moisture and the presence of Amazonian species (Marimon, Lima, Duarte, Chieregatto, & Ratter, 2006; Marimon et al., 2014). Some studies suggested that, in Brazilian savannas (Cerrado), water availability in the soil is not a limiting factor (Franco & Lüttge, 2002) but the higher VPD imposes a strong limitation on transpiration (Franco & Lüttge, 2002; Meinzer et al., 1999). Some authors suggest that, although several woody species from Cerrado have deep roots and can access the water table throughout the year (Bucci et al., 2008; Oliveira et al., 2005), the simple access to underground water reserves does not ensure they extract enough water to compensate for the high evaporative demand during prolonged droughts (Franco, Matsubara, & Orthen, 2007). On the other hand, in forests formations, stomata tend to show high sensitivity with the increase in the atmospheric evaporative demand (Cunningham, 2004). However, forest plants have other hydraulic mechanisms to deal with high VPD, through continuous water absorption in the soil (Juárez, Hodnett, Fu, Goulden, & Von Randow, 2007; Oliveira et al., 2005). Still, we do not have enough information on how  $\Psi_1$ regulation occurs in trees along the Cerrado-Amazonia transition.

Hence, savanna and forest formations of the Cerrado-Amazonia transition provide a good opportunity to investigate how species from different vegetation types respond to limiting access to water. These species are under the influence of pronounced climate seasonality, high temperatures, and marked variations in humidity, which can trigger a water deficit gradient during the year (Marimon et al., 2020; Peixoto et al., 2018). In this highly seasonal region, where the rainy and dry seasons are well defined (INMET, 2018),  $\Psi_1$ regulation may be a distinct trait reflecting ecological strategies from species in different vegetation types. Based on the premise that physiological activity of the root system and leaf stomatal control depend on the balance between the atmospheric evaporative demand and the water availability in the soil (Oliveira et al., 2014; Oliveira et al., 2005; Palhares et al., 2010), we set out to test the following hypotheses: (i) Different vegetation types will show distinct patterns of  $\Psi_1$  regulation, which will depend on daily and seasonal VPD. Thus, we expect savanna environments (typical cerrado), which occur under higher variation in temperature, air humidity, and, consequently, VPD (Prado et al., 2004), species will be more isohydric, as a strategy to prevent water loss. In contrast, in forest environments, species will be anisohydric with higher tolerance to environmental changes; (ii) typical cerrado will show more and forest will show less interspecific variation in  $\Psi_1$  regulation. In addition, we expect that, in all vegetation types,  $\Psi_1$  variations will be associated with the minimum  $\Psi_1$  and the variation of predawn  $\Psi_1$  (root proxy;  $\Delta \Psi_{pd}$ ).

Material and methods

#### Study area

The studied areas are located in southern Amazonia, in the transition between Cerrado and Amazonia biomes, state of Mato Grosso, Nova Xavantina municipality (Fig. 1). The climate is Aw, according to the Köppen's classification (Kottek, Grieser, Beck, Rudolf, & Rubel, 2006), with well-defined wet (October to March) and dry seasons (April to September), annual rainfall around 1500 mm, and average annual temperature of 25  $^{\text{o}}$ C (Marimon et al., 2020). Between 2000 and 2017, according to data provided by the National Institute of Meteorology (conventional station of Nova Xavantina), the average annual rainfall was 1364 mm, with a minimum of 957.1 mm (2007) and the maximum of 1858 mm (2006). The average annual temperature recorded in this period was 26.3  $^{\text{o}}$ C, with the lowest average annual temperature recorded in 2003 and 2007 (18  $^{\text{o}}$ C) and the highest in 2007 (34.5  $^{\text{o}}$ C). Between 1997 and 2019 this region also presented a

general trend of markedly increasing temperature and declining precipitation, with more negative maximum cumulative water deficits (MCWD) values (Marimon et al., 2020).

Despite the relatively small spatial scale (25 km), there is a huge difference in vegetation types in the study area, with distinct species composition and vegetation structure comprising typical savanna and forest formations (Marimon et al., 2014). We assessed three vegetation types: savanna (typical cerrado - STC), transitional community (cerradão - SCF) and forest (semi-deciduous seasonal forest - SSF). According to Ribeiro and Walter (1998), the typical cerrado is a cerrado *stricto sensu* subtype with predominantly arboreal-shrubby vegetation, 20 to 50% of tree cover, and tree heights between 3 and 6 m. The cerradão is characterized by mostly continuous canopy with xeromorphic aspects, being considered an ecotonal community (Ratter, Richards, Argent, & Gifford, 1973), with species (e.g., *Hirtella glandulosa* and *Emmotum nitens*) that characterize the transition between forests and savannas on the southern Amazonian border (Marimon et al., 2006). At last, the semi-deciduous seasonal forest (hereafter forest) is characterized by tall and closed canopy vegetation and well-defined vertical strata (Askew, Moffatt, Montgomery, & Searl, 1970; Marimon et al., 2006). The typical cerrado and cerradão show Red-Yellow Latosols, dystrophic, acidic, and alic soils (Marimon-Junior & Haridasan, 2005), and the semi-deciduous seasonal forest shows Plintosols (Marimon et al., 2014).

Typical cerrado and cerradão occur in the Bacaba Municipal Park (14°41' S; 52°20' W), and the forest is located at Vera Cruz Farm (14°49'27.1" S; 52°10'2.9" W) 25 km away from the other two (Fig. 1). We carried out the study in 1-ha permanent plots subdivided into 25 subplots (20 x 20 m each), which comprise the PELD/CNPq project (*Transição Cerrado-Amazônia: bases ecológicas e socioambientais para a conservação*, Proc. # 403725/2012-7 and 441244/2016-5), which are also part of the Amazon Network of Forest Inventories (RAINFOR; typical cerrado: NXV-01; cerradão: NXV-02 and forest: VCR-02).

To assess the hydraulic traits we selected the seven most representative species in each area, which showed the largest basal area contribution in the community, comprising a total of 21 species. In the typical cerrado, we choose adult trees with at least 5 cm in diameter at breast height (DBH), and in cerradão and forest, we selected trees with [?]10 cm of DBH.

## Measurements of leaf water potential $(\Psi_{\lambda})$

In August 2016 (peak of the dry season) and January, February and March 2017 (rainy season), we measured the  $\Psi_1$  in two leaves of five individuals from each species, using a pressure chamber (PMS Instruments Co., Albany, USA; model: 1505D- EXP; Scholander, Bradstreet, Hemmingsen, and Hammel (1965)). Measurements were taken at four different times during the day: predawn (0400-0600), early-morning (0700-0900), late-morning (1000-1130), and at midday (1200-1400 hours) in all vegetation types. We selected healthy and mature leaves, exposed to the sun. We also measured the temperature and relative humidity with a portable weather station (Kestrel 3500) in the vicinity of trees and where the leaves were collected. With these data, we calculated the Vapour Pressure Deficit (VPD) in each vegetation type (Abtew & Melesse, 2013).

We determined the relationship between predawn ( $\Psi_{\rm pd}$ ), and midday ( $\Psi_{\rm md}$ ) leaf water potential, for all species and vegetation types, to access  $\Psi_{\rm l}$  regulation. We used the linear model proposed by (Martínez-Vilalta et al., 2014),  $\Psi_{\rm md} = \Delta + ? * \Psi_{\rm s}$ , where  $\Psi_{\rm s}$  is the soil water potential, which we considered to be similar to  $\Psi_{\rm pd}$  (Tardieu & Simonneau, 1998); ? represents the slope angle and is a measure of the transpiration sensitivity to changes in water availability. For each species, we analysed this relationship using the model  $\Psi_{\rm md} ~ \Psi_{\rm pd} + (\Psi_{\pi\delta} | \text{ individuals})$ .

Individuals of each species were included as random and fixed effects in the model to estimate the values of slopes (?) and intercepts ( $\Lambda$ ) of the specific relationships between  $\Psi_{md}$  and  $\Psi_{pd}$  for each species. We measured each set of individuals of a given species under the same environmental conditions. Next, we classified species as strict isohydric if the ? value and its confidence interval (CI; defined as ?  $\pm$  1) included zero (? = 0), strict anisohydric if the CI of ? included 1 (? = 1), partially isohydric if CI did not include 0 and 1 (0 < ? <1), and extreme anisohydric if CI did not include 1 (? > 1) (Martinez-Vilalta et al., 2014).

For each species and communities, we also inferred the control of water potential through  $\Delta \Psi_{\rm md}$ , which is the difference between the minimum  $\Psi_{\rm l}$  at midday in the rainy season and the minimum  $\Psi_{\rm l}$  in the dry season (Martínez-Vilalta & Garcia-Forner, 2017). We also used the variation in  $\Psi_{\rm pd}$  (root proxy;  $\Delta \Psi_{\rm pd}$ ), calculated by the difference between  $\Psi_{\rm pd}$  in the rainy season and dry season, to estimate the root depth of species in relation to the water availability in the soil (Scholz et al., 2012). For this latter step, we used the modular values of the deltas.

#### Data analysis

We analysed leaf water potential regulation at two levels: species and vegetation types. We adopted the terminology of maximum water potential value ( $\Psi_{max}$ ) for the least negative potentials and the minimum water potential value ( $\Psi_{min}$ ) for the most negative potentials of the day. We performed all analyses in the R 4.0.1 environment (R Core Team 2020) and considered *p*-value<0.05.

We tested whether the dependent variable  $\Psi_1$  varied between species and among the four different periods of the day assessed in each season (dry and rainy). We also investigated whether  $\Psi_1$  and other hydraulic traits  $(\Psi_{\min}, \Delta \Psi_{pd}, \text{ and } \Delta \Psi_{md})$  varied among the vegetation types (typical cerrado, cerradão, and semi-deciduous seasonal forest) in different seasons (dry and rainy). For the analyses, we used Kruskal-Wallis non-parametric test, followed by the Dunn test as apost-hoc analysis, since data did not follow normality assumptions for parametric tests. We used *plyr*, *dunn.test*, and *FSA* packages (Dunn, 1964; Wickham, 2011). The weighted average for each attribute ( $\Psi_{\min}, \Delta \Psi_{pd}, \Delta \Psi_{md}$  and ?) in the vegetation types was calculated through weight the relative dominance in the basal area of the species, i.e., we used the relative dominance - proportion basal area specific of the species by the sum of the basal areas of all species in the community (Shepherd, 2010). We used the *weights*, *quantreg*, and *Hmisc* packages (R Core Team 2020).

We calculated and compared the coefficient of variance of the  $\Psi_1$  and other traits (?,  $\Psi_{\min}$ ,  $\Delta \Psi_{pd}$ , and  $\Delta \Psi_{md}$ ) between vegetation types in the dry and rainy seasons. We applied the Mood test (non-parametric data) to compare the  $\Psi_1$  variance between vegetation types and used F-test, from the *stats* package (R Core Team 2020), for other attributes. We applied a linear regression analysis to relate the  $\Psi_1$  values of species to the vapour pressure deficit (VPD) in each vegetation type in both seasons. We also compared linear regression curves for the bi-variate relationship of  $\Psi_1$  and VPD between different vegetation types (*Standardised Major Axis* - SMA), using the *smatr* package (Warton, Duursma, Falster, & Taskinen, 2012), for log<sub>10</sub> transformed variables.

#### Results

## Eqqeet of seasonality on $\Psi_{\lambda}$

We found a broad variation in  $\Psi_1$  among species and vegetation types (Table 1). The  $\Psi_{\min}$  varied from -2.8 MPa (*Qualea parvilfora*) to -0.9 MPa (*Eriotheca gracilipes*) in the dry season. In contrast,  $\Psi_{\max}$  varied from -0.24 MPa (*Guapira graciliflora*) to -0.1 MPa (*Eriotheca gracilipes*), in the rainy season. Regarding communities, on average, forest vegetation showed the lowest values, both for  $\Psi_{\max}$  (-0.3 MPa, rainy season) and  $\Psi_{\min}$ (-2.8 MPa, dry season).

The  $\Psi_1$  of the 21 species varied according to the dry ( $Q^2 = 179.1$ , df = 19, p < 0.001) and rainy seasons ( $Q^2 = 39.2$ , df = 19, p < 0.001) and also among the different times of the day (dry season:  $Q^2 = 82.3$ , df = 3, p < 0.001; rainy season:  $Q^2 = 250.7$ , df = 3, p < 0.001). In the dry season, we recorded a significant interspecific difference in  $\Psi_1$  values for all vegetation types (typical cerrado:  $Q^2 = 51.7$ , df = 6, p < 0.001; cerradão:  $Q^2 = 56.4$ , df = 6, p < 0.001; and forest:  $Q^2 = 3.5$ , df = 6, p < 0.001). On the other hand, in the rainy season, only cerradão species differed from each other in relation to  $\Psi_1$  ( $Q^2 = 14.15$ , df: 6; p = 0.02).

In both dry and rainy seasons,  $\Psi_1$  values of the typical cerrado and cerradão species were similar and differed from the forest (p < 0.05 for all tests, Fig. 2). In the dry season, the forest species showed the lowest mean values of  $\Psi_1$  at all times of the day, with a continuous decrease from predawn to midday (Figs. 2, 3). In contrast, in the rainy season,  $\Psi_1$  values in the forest species remained higher compared to those of other vegetation types (Fig. 2; forest and typical cerrado - dry season: Z = 4.48; p = 0.00 and rainy season: Z = -3.94; p = 0.00; and forest and cerradão - dry season: Z = 1.97; p = 0.00 and rainy season: Z = 3.89; p = 0.00). Regarding the  $\Psi_1$  variability, we noticed that vegetation types were more similar in the dry season, whereas, in the rainy season, we recorded a higher variability for the typical cerrado and cerradão (Table 2).

We observed a distinct regulation of  $\Psi_1$  in the forest species compared to other vegetation types, and species from cerradão and typical cerrado had relatively similar hydraulic strategies (Table 2 and Figs. 2, 3). The  $\Psi_{\rm min}$  also differed among vegetation types (Q<sup>2</sup> = 11.42; df: 2, p = 0.003) and the lowest values were recorded for the forest species. A similar pattern was recorded for the  $\Delta \Psi_{\rm pd}$ , which also differed among vegetation types (Q<sup>2</sup> = 16.92, df = 2, p < 0.001). Considering  $\Delta \Psi_{\rm pd}$  as an estimate of root depth (proxy), we found that forest species showed higher  $\Delta \Psi_{\rm pd}$  than those in the typical cerrado (Q<sup>2</sup> = 3.35; p = 0.00) and cerradão (Q<sup>2</sup> = 3.72; p = 0.00) according to the Dunn test (Fig. 3).

## Ρεγυλατιον οφ $Ψ_{\lambda}$

We observed that 47% of the species are partially isohydric, and 43% are extreme anisohydric (Fig. 4). Eriotheca gracilipes was the only strict isohydric species (10%), which showed the same pattern in both typical cerrado and cerradão. When we separated by vegetation types, we observed that 57% of typical cerrado and cerradão species are partially isohydric, whereas most forest species are extreme anisohydric (71%). We also found that all deciduous species are partially isohydric, while evergreen and brevideciduous species vary among vegetation types (Table 1). The forest species, *Cheiloclinium cognatum*, and the cerrado and cerradão species, *Eriotheca gracilipes*, represent the extremes in  $\Psi_1$  regulation (Fig. 4).

The  $\Delta \Psi_{\rm md}$  differed in all vegetation types (Q<sup>2</sup> = 26.26; p < 0.001), being the forest with less regulation (Table 3). Therefore, almost all species in the forest showed the same pattern of  $\Psi_1$  regulation for ? and  $\Delta \Psi_{\rm md}$ , as well as the highest variation values, indicating a lower control of water use and lower variability in the  $\Delta \Psi_{\rm md}$ .

The cerradão species showed higher coefficients of variance in all traits (?,  $\Psi_{\min}$ , and  $\Delta \Psi_{md}$ ), except for  $\Delta \Psi_{pd}$ , that was higher in the typical cerrado. However, there were no statistical differences in the coefficients of variation between all vegetation types (Tables 3 and S1).

# Eqgest og the apop pressure degisit ("PD) ov the leag water potential $(\Psi_{\lambda})$

The VPD differed among vegetation types but was higher in the dry season in all areas (Tables 4 and S2), with a significant negative correlation between VPD and  $\Psi_1$  (Fig. 5). The forest showed the lowest  $\Psi_1$  values and the lowest VPD amplitude in both seasons (Fig. 5 and Table 6).

In the dry season, typical cerrado VPD was 20% higher than that recorded in the cerradão, and approximately 30% higher than that of the forest (VPD<sub>typical cerrado</sub> > VPD<sub>cerradão</sub> > VPD<sub>forest</sub>), which confirms that species are subject to a higher atmospheric water demand during this season in the savanna vegetation (Table 4). We also observed a direct relationship between the decrease in  $\Psi_1$  and the increase in VPD; the cerradão showed environmental variations more similar to typical cerrado than to forest, which showed the lowest  $\Psi_1$  in the dry season (Fig. 5 and S2).

#### Discussion

Our results showed different strategies for water use and access among species and vegetation types in the Brazilian Cerrado-Amazonia transition. We observed differences between the hydraulic functioning of forest species in relation to typical cerrado and cerradão species, which tended to show stronger leaf water potential regulation (isohydric strategy) than forest species (anisohydric strategy). We also found that cerradão, the transitional community, which is considered to be floristically closer to forests than savannas (Morandi et al., 2016), showed hydraulic traits and environmental conditions more similar to the savanna (typical cerrado) than forest.

## Hydraulic functioning

We found that different intensities in water deficit across vegetation types determines different communitylevel patterns in the strategies of water use and access. We expected that main difference in hydraulic traits would occur between species from more contrasting vegetation types, typical cerrado and semi-deciduous seasonal forest, and that species from cerradão, which in our study area represents transitional vegetation between cerrado and forest, since ther are no marked differences in soil fertility (Marimon-Junior & Haridasan, 2005),would show intermediate strategies of water use. However, contrary to our expectations, we found that hydraulic traits of the cerradão species were generally more similar to typical cerrado than to semi-deciduous seasonal forest. We also observed that cerradão species showed a higher amplitude of variation for all hydraulic traits assessed, which reflects a great diversity of strategies within the species that comprise this transitional vegetation (Marimon et al., 2006; Marimon et al., 2014; Ratter, Ribeiro, & Bridgewater, 1997; Ratter et al., 1973).

Regarding community-level hydraulic traits, we suggest that typical cerrado in our study area is already in successional stage of thickening, turning to a dense cerrado (Morandi et al., 2016; Ribeiro & Walter, 1998). Both typical cerrado and cerradão are geographically close (900 m), showing similar soil parameters, topography (Marimon-Junior & Haridasan, 2005) and VPD, which reinforces the similar hydraulic functioning we reported here. Topography and soil physical characteristics are key factors in the plants hydraulic patterns as they are related to the level of groundwater and humidity regime that can affect plant water relations water absorption from the soil (Villalobos-Vega et al., 2014).

We could separate species into two very distinct groups: the first, composed of species from the typical cerrado and cerradao, with strategies of higher water potential regulation, and the second, composed of species from the forest, with lower water potential regulation. The typical cerrado and cerradao species, most of which characterized as partially isohydric, are exposed to higher VPD and also better regulate and have higher  $\Psi_1$  values. Besides, the lower variation in the  $\Psi_{pd}$  (values did not decrease much in the dry season) suggests that typical cerrado and cerradão species are growing with access to water in the soil (Palhares et al., 2010).

In addition, the stronger regulation of  $\Psi_1$  recorded for typical cerrado and cerradão species, which are subject to high atmospheric evaporative demand (VPD), may be related to more efficient regulation of stomatal opening, especially in response to changes in VPD, resulting in higher water use efficiency (Franco & Lüttge, 2002). In such conditions, deeper roots are critical to maintain water balance of Cerrado ecosystems (Oliveira et al., 2005), despite involving higher maintenance costs or strict control of plant water balance (Franco et al., 2005). For example, species such as *Eriotheca gracilipes* showed little seasonal variation in leaf water potential, suggesting access to deep water sources through root investment. Indeed, (Durigan, Melo, & Brewer, 2012) showed that this particular species invests in a main root, with approximately 2.5 m in length, without many fine roots.

Forest species showed a lower regulation of  $\Psi_1$ , with higher drop in  $\Psi_1$  and higher  $\Delta \Psi_{pd}$ , which might indicate shallower roots and less access to the water in the soil. Thus, these species might have higher transpiration rates, probably due to low stomatal control, both in dry and rainy seasons, which might help explaining the observed steep decrease in  $\Psi_1$  in this study, thus having a more acquisitive strategy in terms of carbon gain and less conservativism on safety strategies. These strategies might also indicate a mechanism for maintaining leaf temperature and reducing the negative effect of VPD (Tardieu & Simonneau, 1998). Regarding water availability in the soil, even though root depth estimates were considered shallow and with limited access to deep soil layers, they might compensate it through a more efficient water absorption capacity (Butler et al., 2013). According to these authors, the water absorption by surface root area increases with tree diameter in species that occurred in our study areas. They showed that typical cerrado species showed lower values of the root area absorption index  $(0.03 \text{ m}^2 \text{ m}^{-2})$  than cerradão  $(0.07 \text{ m}^2 \text{ m}^{-2})$  and forest  $(0.11 \text{ m}^2 \text{ m}^{-2})$ , which showed higher efficiency in the absorption capacity by surface root area.

Thus, forest species showed a less conservative stomatal regulation behaviour (anisohydric), characterized by open stomata and higher photosynthetic rates for long periods, even with a decrease in  $\Psi_1$  (Sade, Gebremedhin, & Moshelion, 2012). Therefore, we believe that the lowest VPD values recorded in the forest provide a mild to moderate environment under abiotic stress that directly influences plants and their capacity in the face of climate seasonality (Sade et al., 2012).

## Interspecific variability in hydraulic functioning

The differences in  $\Psi_1$  among species suggest a substantial interspecific variation in hydraulic functioning regulation of the different vegetation types. Species-level variations in hydraulic traits depend on the access to water in the soil and atmospheric evaporative demand, which are the main environmental drivers that influence plant hydraulic functioning, regulating gas exchange (Buckley & Mott, 2013). Thus, within the same vegetation type, we found species displaying different strategies; cerradão species with higher (*Eriotheca gracilipes*) or lower (*Tapirira guianensis*) regulation of leaf water potential; typical cerrado species with an estimate of high (*Euplassa inaequalis*) or limited (*Guapira graciliflora*) access to deeper layers of the soil; and forest species with support of higher (*Chaetocarpus echinocarpus ,Ephedranthus parviflorus*) or lower (*Brosimum pubescens*) tension in which the xylem transports water.

These different hydraulic strategies also seem to involve other structural characteristics (e.g., anatomical and morphological; Sperry, Meinzer, and McCulloh (2008)). In this case, the variability of morpho-functional traits in plant communities, at a local and global scale, provides evidence that species efficiently share resources (Brum et al., 2017; Meinzer et al., 1999). Thus, the variation in water availability is a dimension of the species niche, which represents a complex resource condition intrinsically connected to the availability of other resources (Araya et al., 2011). Our results also indicated that the differences in hydraulic functioning among species characterize responses that depend on climate seasonality, since all evaluated vegetation types are subject to similar seasonality effects, especially in the dry season, when the greatest VPD amplitude occurs. In this case, plant and environment water availability and the dynamics of soil-plant-atmosphere interaction affect the species responses to biotic and abiotic stress (Sade et al., 2012).

Although we have recorded different water use and acquisition strategies, forest species displayed a similar pattern for most hydraulic traits, showing lower  $\Psi_1$  values in both dry and rainy seasons. This strategy allows plants to function in a more negative  $\Psi_1$  range and regulate it according to the VPD, that is, a typically anisohydric strategy (McDowell et al., 2008). On the other hand, typical cerrado and cerradão species showed more variability in hydraulic traits, which are in agreement with theoretical models that predict that a limitation of water resources in arid or savanna environments would select different strategies for water use and conservation (Meinzer et al., 1999). Hence, these different hydraulic strategies may favor a higher species and functional diversity, especially where strong environmental filters are more evident, such is the case in the typical cerrado and cerradão (Neyret et al., 2016).

#### Hydraulic traits among vegetation types in the face of climate change

Hydraulic characteristics may reflect the evolutionary history of species that shape their performance over the years under the influence of environmental conditions (Reich & Cornelissen, 2014), which was evidenced in the present study through different responses given by savanna and forest species. Hence, in case extreme drought events become more intense and frequent in this zone of ecological tension/stress on the southern Amazonia,

as predicted (Marimon et al., 2014; Rifai et al., 2018), with deregulation in the mechanism of water demand and supply in the environment (increase in VPD and decrease in soil moisture), more resistant species and vegetation types, as well as species more vulnerable to drought events, can emerge (McDowell, 2011). In any case, we know that savanna vegetation is mainly composed by species with higher  $\Psi_1$  regulation that preserve water transport and help preventing hydraulic failures (e.g., *Guapira graciliflora*), even at the expense of reduction in the carbon balance and a possible overheating caused by photosynthetic damage (McDowell et al., 2008). On the other hand, forest species showed lower regulation of  $\Psi_1$  (e.g., *Cheiloclinium cognatum*) and might be susceptible to hydraulic failures (McDowell, 2011; McDowell et al., 2008), especially in extreme drought situations, although hydraulic failure will also depend on their resistance to xylem embolism. Thus, this may be an opportunistic and risky behaviour that under conditions of minimal or moderate stress can be beneficial. Still, it may be disadvantageous under intense or prolonged stress conditions (Sade et al., 2012).

Having high or low hydraulic regulation can bring severe implications for plants. Some studies have shown that, in isohydric species, xylem vessels embolism is more frequent, since the  $\Psi_{\text{lmin}}$  is close to P50 (i.e.,  $\Psi_{\text{l}}$ value in which the stem hydraulic conductivity is reduced by half), with a small safety margin (McDowell, 2011). On the other hand, these authors also noted that anisohydric species have higher safety margins because they maintain xylem tension above the water potential values that usually cause embolism. In this case, the safety margin can be an important characteristic with great interespecific variation. Safety margin variability at the community level might be favoured by natural selection in environments with seasonal water restriction, since the duration and intensity of the dry seasons provide different responses in the functioning of plant hydraulics and carbon assimilation (McDowell et al., 2008).

#### Conclusion

The hydraulic functioning of the species within the three studied vegetation types differed in response to the marked climate seasonality of the region. Species from the typical cerrado and cerradão showed relatively similar hydraulic strategies, with higher  $\Psi_1$  regulation under more intense VPD effects. On the other hand, forest species showed lower  $\Psi_1$  regulation, suggesting less access to deep water, and under lower effects of VPD. These characteristics allowed us to propose two different species groups: one with higher regulation and isohydric behavior (typical cerrado and cerradão species) and the other with lower regulation and anisohydric behavior (semi-deciduous seasonal forest species), both under current climate conditions.

In case drought events become more intense and frequent in this region, forest species, which have lower hydraulic regulation, may be more vulnerable. However, to better understand drought resistance conditions of species we need to consider more drought-resistance traits, such as the critical xylem water potential and hydraulic safety margins, both integrated with the regulation attributes of  $\Psi_1$ . Our study described key hydraulic traits and how they vary throughout the year for the major vegetation types that occur in the transition of the two largest South American biomes. It also advanced the knowledge of the hydraulic functioning patterns of this unique and vulnerable region, to better understand species and community-level hydraulic responses in a future scenario of more frequent and more extreme drought events.

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#### Author contributions

IO, HJ, RSO, MS, BSM, BHMJ designed idea, IO secured funding and BSM, BHMJ provided logistical support, HS, EC & MS collected data, HS analysed data with support from IO, FB, MS and RSO, HS wrote the first draft of the manuscript with IO, MS and BSM data and all authors provided comments in the final draft of the manuscript.

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#### Tables

**Table 1.** Mean values of hydraulic traits for each species sampled in typical cerrado (STC), cerradao (SCF), and semi-deciduous seasonal forest (SSF). Leaf phenology (LP) classified as brevideciduous (BD), deciduous (DE) or evergreen (EG), and species relative dominance (RD) in each community. Hydraulic traits: slope angle (?) between the ratio of the leaf water potential ( $\Psi_1$ , MPa) at predawn ( $\Psi_{pd}$ ) and midday ( $\Psi_{md}$ ), minimum  $\Psi_1$  ( $\Psi_{min}$ ) variation of predawn  $\Psi_1$  ( $\Delta \Psi_{pd}$ ), and variation of  $\Psi_1$  at midday ( $\Delta \Psi_{md}$ ).

Species (Families)	LP	RD
Typical Cerrado	Typical Cerrado	Typical Cerrado
Davilla elliptica A.StHil. (Dilleniaceae)	BD	4.6
Eriotheca gracilipes (K.Schum.) A.Robyns (Malvaceae)	BD	3.5
Euplassa inaequalis (Pohl) Engl. (Proteaceae)	BD	4.2
Guapira graciliflora (Mart. ex Shum.) Lundell (Nyctaginaceae)	DE	3.7
Qualea parviflora Mart. (Vochysiaceae)	DE	9.6
Qualea grandiflora Mart. (Vochysiaceae)	DE	3.4
Roupala montana (Klotzsch) K.S.Edwards (Proteaceae)	EG	5.7
Cerradão	Cerradão	Cerradão
Eriotheca gracilipes (K.Schum.) A.Robyns (Malvaceae)	EG	4.3
Emmotum nitens (Benth.) Miers. (Metteniusaceae)	EG	7.5
Hirtella glandulosa Spreng. (Chrysobalanaceae)	EG	20.5
Myrcia splendens (Sw.) DC (Myrtaceae)	EG	4.4
Tachigali vulgaris L.G.Silva & H.C.Lima (Fabaceae)	EG	17.4
Tapirira guianensis Aubl. (Anacardiaceae)	BD	5.2
Xylopia aromatica (Lam.) Mart. (Annonaceae)	EG	4.8
Semi-deciduous Seasonal Forest	Semi-deciduous Seasonal Forest	Semi-deciduous
Amaioua guianensis Aubl. (Rubiaceae)	EG	7.7
Brosimum rubescens Taub. (Moraceae)	EG	3.3
Chaetocarpus echinocarpus (Baill.) Ducke (Peraceae)	EG	9.4
Cheiloclinium cognatum (Miers.) A.C.Sm. (Celastraceae)	BD	3.9
Ephedranthus parviflorus S. Moore (Annonaceae)	EG	10
Mabea fistulifera Mart. (Euphorbiaceae)	BD	6.4
Protium altissimum (Aubl.) Marchand (Burseraceae)	EG	8.6

Table 2. Mean  $\pm$  standard deviation ( $\mu \pm$  sd), and coefficient of variation (CV) for leaf water potential ( $\Psi_1$ ), considering all periods of the day and species sampled in typical cerrado (STC), cerradão (SCF), and semi-deciduous seasonal forest (SSF). Trait variances were compared using the Mood test. *P* -values are shown in bold, when significant (p < 0.05).

Vegetation	$\Psi_{ m l}$	$\Psi_{ m l}$	$\Psi_{ m l}$	
	Seasons	$\mu \pm \sigma \delta$	CV	
Typical cerrado	Rainy	$-0.87 \pm 0.61$	-70.1	
	Dry	$-1.77 \pm 0.99$	-55.6	
Cerradão	Rainy	$-0.84 \pm 0.58$	-68.4	
	Dry	$-1.60 \pm 0.87$	-54.4	
Semi-deciduous seasonal forest	Rainy	$-0.56 \pm 0.33$	-54.3	
	Dry	$-2.23 \pm 1.16$	-52.3	
Seasons	Mood Test STC x SCF	Mood Test STC x SSF	Mood Test SCF x SSF	

Vegetation	$\Psi_{l}$	$\Psi_{l}$	$\Psi_{l}$
Rainy	$Z = 0.83 \ p = 0.40$	$Z = 2.91 \ p = 0.00$	$Z = 1.94 \ p = 0.05$
Dry	$Z = 0.69 \ p = 0.48$	$Z = -0.39 \ p = 0.68$	$Z = -1.60 \ p = 0.10$

Table 3. Weighted mean  $\pm$  standard deviation ( $\mu \pm$  sd) and coefficient of variation (CV) of the hydraulic traits analyzed in species from typical cerrado (STC), cerradão (SCF), and semi-deciduous seasonal forest (SSF). Weighted means were determined through the dominance in basal area of the species in each vegetation type. We compared the variances of the attributes using F-test. Significant *p* values are shown in bold (*p* <0.05). Hydraulic traits: ? = slope angle between the ratio of leaf water potential at midday and predawn (MPa),  $\Psi_{\rm min}$  = minimum potential,  $\Delta \Psi_{\rm pd}$  = variation in  $\Psi_{\rm pd}$  at predawn, and  $\Delta \Psi_{\rm md}$  = variation in  $\Psi_{\rm md}$  at midday.

Traits	STC	STC	SCF	SCF	$\mathbf{SSF}$	$\mathbf{SSF}$	STC/SCF	STC/SSF	S
	$\mu \pm sd$	CV	$\mu \pm sd$	CV	$\mu \pm sd$	CV	F test	F test	F
?	$0.80 \pm$	56.4	$0.83 \pm$	70.2	$1.35 \pm$	63.3	0.60 P =	$0.28 \ p =$	2.1
	0.45		0.60		0.85		0.84	0.62	0.7
$\Psi_{\mu u}$	-2.00 $\pm$	-32.4	-1.86	-36.0	-2.08 $\pm$	-24.3	$0.92 \ p =$	$1.68 \ p =$	1.8
•	0.64		$\pm 0.67$		0.50		0.97	0.83	0.8
$\Delta \Psi_{\pi\delta}$	-0.76 $\pm$	93.3	-0.66	70.8	-1.55 $\pm$	-38.3	$2.31 \ p =$	$1.45 \ p =$	0.0
	0.72		$\pm 0.47$		0.59		0.74	0.88	0.8
$\Delta \Psi_{\mu \delta}$	-0.99 $\pm$	56.8	-0.86 $\pm$	-82.1	-2.32 $\pm$	-22.8	$0.63 \ p =$	$1.13 \ p =$	1.'
·	0.56		0.71		0.53		0.85	0.95	0.8

**Table 4.** Characterization of the vapor pressure deficit (KPa) of the vegetation types (STC = typical cerrado, SCF = cerradão, and SSF = semi-deciduous seasonal forest) in dry ( $r^2 = 0.14$ ; p < 0.001) and rainy seasons ( $r^2 = 0.32$ ; p < 0.001).

Seasons	STC	SCF	SSF	
Dry				
Maximum	4.95	4.73	3.77	
Minimum	0.43	0.18	0.3	
Mean $\pm$ sd	$2.43\pm1.63$	$1.94\pm1.59$	$1.69 \pm 1.64$	
Rainy				
Maximum	2.80	3.13	1.13	
Minimum	0	0	0	
Mean $\pm$ sd	$0.92\pm1.52$	$0.74\pm1.53$	$0.2\pm1.63$	

#### **Figure legends**

Figure 1. Location of study areas (Cerrado: typical cerrado; Cerradão; Forest: semi-deciduous seasonal forest). Database: MMA (Ministry of the Environment; site: mapas.mma.gov.br).

Figure 2. Comparisons of  $\Psi_1$  between different seasons (rainy and dry) and across different periods of the day in the typical cerrado (orange bars), cerradão (light green), and forest (dark green). Asterisks indicates significant differences (p < 0.05).

Figure 3. Comparison between cerrado (orange bars), cerradão (light green), and forest (dark green) for the variation in  $\Psi_1$  predawn ( $\Delta \Psi_{pd}$ , MPa) and the variation in  $\Psi_1$  at midday ( $\Delta \Psi_{md}$ , MPa). Asterisks indicates

significant differences (p < 0.05).

**Figure 4**. Leaf water potential regulation of 21 species divided into two groups: forest (comprising cerradão and semi-deciduous seasonal forest) and savanna (typical cerrado). The slope angle (? +- CI - confidence interval) estimates the value that allows classifying: square - the species as strict isohydric (SI), triangle - partially isohydric (PI), and circle - extreme anisohydric (EA). The line shows the distance of confidence interval that to differ the types of leaf water potential regulation. No species was identified as strict anisohydric. Complete list of species names is shown in Table 1.

Figure 5. Linear regression between the leaf water potential ( $\Psi_1$ , MPa) and the Vapor Pressure Deficit (VPD, KPa) of the typical cerrado (orange), cerradão (light green), and forest (dark green), in the Cerrado-Amazonia Transition, Brazil. Circles represent individuals sampled in each vegetation type. Intercepts between regression lines differed in both rainy (p < 0.001), and dry seasons (p < 0.001) (*Standardised Major Axis*- SMA).

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Jancoski et al\_figures.docx available at https://authorea.com/users/470926/articles/562734regulation-of-leaf-water-potential-in-tree-species-of-the-cerrado-amazonia-transitionin-response-to-climate-seasonality