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Intra-annual stable isotopes in the tree rings of *Hymenaea courbaril* as a proxy for hydroclimate variations in southern Amazonia

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Highlights

1. Tree rings of *Hymenaea courbaril* show a pronounced intra-annual variation in $\delta^{13}C$, and weaker variation in $\delta^{18}O$.
2. Intra-annual variation in $\delta^{13}C$ reflects carbon remobilization at the start of the growing season, while intra-annual $\delta^{18}O$ variation reflects seasonal variation in precipitation-$\delta^{18}O$ and climate.
3. Year to year variation in tree ring $\delta^{18}O$ is correlated with variation in regional river discharge and offers a good potential for seasonal climate reconstructions.

Abstract

The hydrology of Amazonia is changing due to climate and land-use changes, especially in the southern region, which has warmed and dried faster than other tropical regions. Yet there are no long-term hydrological records to put these changes in a historical perspective. Here we investigate the use of tree-ring carbon ($\delta^{13}C$) and oxygen isotopes ($\delta^{18}O$) to assess the seasonal variation in climate for the southern Amazonia basin. We analysed the intra-annual variation of $\delta^{13}C$ and $\delta^{18}O$ in 10 segments for each tree ring from 2013 to 2017 from individuals of *Hymenaea courbaril*, a long-lived and widespread neotropical tree species. We find strong seasonal patterns of tree-ring $\delta^{13}C$ supporting previous observations of annual growth rhythms for this species. The intra-annual variation in $\delta^{18}O$ shows point to the lowest values generally just after the middle of the rings that corresponds to the peak rainy season. We find strong correlations between the $\delta^{18}O$ in the middle of the growth ring and vapour pressure deficit ($r = 0.92$, $P = 0.02$) and precipitation ($r = -0.93$, $P = 0.02$). We further find associations between the oxygen isotopic series and the discharge of the Araguaia basin's main rivers during the rainy period. Our results show that these $\delta^{18}O$ records are sensitive to fluctuations in rainfall and humidity, and thus reflect river discharge in the region, and that longer reconstructions of $\delta^{18}O$ tree ring of *Hymenaea courbaril* could provide a novel proxy to assess past hydrological changes.

Keywords: jatobá-da-mata, oxygen isotopes, carbon isotopes, intra-annual isotopes, river discharge.

Introduction

Observations suggest that climate change and land-use change have resulted in increased amplitude of the hydrological cycle across the Amazonia, affecting the length of the dry period and the frequency of rainfall extremes during the wet season (Fu et al., 2013; Gloor et al., 2013; Barichivich et al., 2018; Espinoza et al., 2019). Climate models suggest these changes in precipitation regime may become more extreme in coming years (Nobre et al., 2009; Boisier et al., 2015; Alves et al., 2017). For instance, the historic drought of 2015-2016 serves as an example of this trend (Jiménez-Muñoz et al., 2016), which is likely to affect river discharge (Callède et al., 2004; Cavalcante et al., 2019; Heerspink et al., 2020) as well as tree growth, mortality, and other key aspects of forest dynamics (Aleixo et al., 2019; Esquivel-Muelbert et al., 2020; Reis et al., 2022). Most river discharge observations rely on long-term hydrological records from central Amazonia that go back as far as 1902 for the Rio Amazonas and 1903 for the Rio Negro (Richey et al., 1989; Espinoza et al., 2022). In contrast, reliable records in southern Amazonia tend to be more recent, dating back only to 1971 (Coe et al., 2011; Ho et al., 2016). This region, however, has seen an increase in deforestation (Gatti et al., 2021), experienced the highest rates of warming, and is drying faster than anywhere else in the basin (Malhi et al., 2008; Jiménez-Muñoz et al., 2013; Haghtalab et al., 2020) or indeed any other tropical site (Jiménez-Muñoz et al., 2013; Alves et al., 2017; IPCC, 2022). While climate change threatens to disrupt ecosystems and societies in these rapidly changing regions, we lack good, long-term climate records essential for understanding climate impacts. Thus, alternative proxies are urgently needed to help put these recent changes in a long-term perspective.

Pre-instrumental climate can be assessed through natural archives like tree-ring widths and stable isotopes (McCarroll and Loader, 2004). In the tropics, tree-ring stable oxygen isotope ($\delta^{18}O$) variation has been shown to reflect primarily the isotopic composition of source water (i.e., precipitation, Brienen et al., 2012, 2013; Baker et al., 2015, 2016). The main processes that affect this source water (or precipitation) $\delta^{18}O$ in the tropics are the rainout of heavy isotopes during transport to the site of condensation and the local precipitation processes (Dansgaard, 1964; Vuille and Werner, 2005). The first of these, the rainout of heavier water (H_2O^{18}) during water vapour transport trajectory is affected by the accumulated rainfall along the water vapour transport to the site of condensation (Vuille and Werner 2005). Longer travel distances and lower temperatures and higher precipitation will result in lower (more depleted) $\delta^{18}O$ values. On top of this, local precipitation intensity - often referred to as the “amount effect” - additionally affects precipitation. Usually in the tropics, there is an inverse relationship between the amount of precipitation and the $\delta^{18}O$ (Dansgaard, 1964; Risi et al. 2008). In inland sites in the Amazonia basin, the isotopic composition of precipitation is inversely related to the amount of precipitation during water transport from the Atlantic Ocean to the site of precipitation (Salati et al., 1979; Vuille et al., 2003). This is because water vapour gets gradually depleted in heavy isotopes (H_2O^{18}) due to Rayleigh rainout processes leading to lower $\delta^{18}O$ during years with heavy precipitation (Dansgaard, 1964; Vuille and Werner, 2005; Risi et al., 2008). Tree-ring $\delta^{18}O$ in humid sites in the Amazonia basin strongly reflects this variation in precipitation $\delta^{18}O$, with relatively minor influences of leaf water enrichment (Brienen et al., 2012; Cintra et al., 2019). These previous results also show that tree-ring $\delta^{18}O$ in the western parts of the Amazonia basin primarily reflect the large-scale rainout processes (i.e., rainout during water vapour transport over land; Brienen et al., 2012; Baker et al., 2016).

While oxygen isotopes can thus be used to reconstruct large-scale variation in precipitation, additional analysis of carbon isotopes ($\delta^{13}C$) in tree rings may provide insights into plant physiological responses to climate (Barbour et al., 2004; Kahmen et al., 2011; Cintra et al., 2019). The $\delta^{13}C$ is a measure of the discrimination against ^{13}C during CO_2 diffusion into the leaf and subsequent fractionation during carboxylation (photosynthesis). Greater stomatal limitation over photosynthesis (i.e. during dry conditions when stomata tend to close) results in lower discrimination and consequently higher $\delta^{13}C$ values, while wetter conditions result in lower $\delta^{13}C$ (Farquhar et al., 1982; Barbour et al., 2000). At an intra-annual scale, carbon isotope variation between early wood and late wood is additionally affected by the remobilization of stored carbon reserves for annual wood production (Gessler et al., 2014; Locosselli et al., 2020).

Long-term trends in hydroclimate are usually assessed through tree-ring isotopes on an inter-annual basis (e.g., ring by ring; Danis et al., 2006; Li et al., 2020; Pagotto et al., 2021). However, high-resolution, intra-ring analysis can provide a useful lens into details of the seasonal climate variability during the growing season when using oxygen isotopes (Li et al., 2011; Monson et al., 2018), or in the case of carbon isotopes, provide insights in the seasonality of wood formation and use of carbohydrates (Cintra et al., 2019; Locosselli et al., 2020). This can be done by dividing tree rings into sub-annual segments that are then separately analysed in terms of isotopic composition. This approach has been used for assessing intra-annual variation of precipitation (Managave et al., 2010; Schubert and Timmermann, 2015; Muangsong et al., 2020; Xu et al., 2020), to study the hydrological cycle (Alvarez et al., 2018) and to assess a metropolitan area water supply (Locosselli et al., 2020). We here test this intra-annual approach to provide insight into seasonal $\delta^{18}O$ and $\delta^{13}C$ patterns and test its potential for reconstruction of past climate variation.

We aim to evaluate the strength of the climate signal in the rings of trees from the southern fringes of the Amazonia, a global hotspot of warming and an ecologically critically climate-sensitive location. We analysed high-resolution stable isotopes in tree rings of *Hymenaea courbaril* L., (locally denominated jatobá-da-mata), a long-lived, widespread neotropical species that can exceed 300 years old (Locosselli et al., 2017). Although *H. courbaril* tree rings have been proven annual (Lucchi, 1998; Westbrook et al., 2006; Lisi et al., 2008) and widely used before (e.g., Locosselli et al., 2013, 2016), some recent carbon dating studies cast doubt on the annual nature of its rings under seemingly less seasonal climate conditions (Santos et al., 2021). We analysed carbon and oxygen stable isotopes in ten segments of each tree ring produced between 2013 and 2017 (two years prior to and two years following the drought of 2016) to address the following questions: 1) Is intra-annual variation in $\delta^{13}C$ and $\delta^{18}O$ for *H. courbaril* consistent with expected patterns due to tree physiology and seasonal variation in climate?; 2) Can oxygen isotopic signals be used to reconstruct seasonal flow of the main rivers in the region during the wet season?

Materials and methods

2.1. Study area and Species

Sampling took place in a large fragment of forest located in the legal reserve area of Fazenda Vera Cruz (14º49'32''S and 52º06'20''W), Nova Xavantina, Mato Grosso, Brazil (Fig. 1). It is located at the transition of Amazonia and *Cerrado* biomes (Ratter et al., 1973; Marimon et al., 2014; Marques et al., 2020), and considered a pre-Amazonian transitional forest (Mews et al., 2012; Marimon et al., 2014). It is a closed canopy forest with trees that can reach 25 m in height (Marimon et al., 2006, 2014; Mews et al., 2012). The forest fragment lies in the Araguaia River Basin which is more than 2,300 km long, has a drainage area of approximately 380,000 km^2, and its main tributary is the das Mortes River with length of 1,070 km and a drainage area of about 62,000 km^2 (Fig. 1; Latrubesse and Stevaux, 2002; Rosin et al., 2015).

The climate is seasonal Aw type according to the Köppen classification (Alvares et al., 2013), with an average annual precipitation of 1369 mm, and two well-defined seasons, a rainy from October to March and a dry season that lasts six months when monthly rainfall is lower than 50 mm $month^{-1}$ (April to September, Fig. 2). The middle of the dry season corresponds to the hottest months of the year when maximum air temperatures regularly exceed 40 °C (Tiwari et al., 2020; Araújo et al., 2021).

We sampled trees of *H. courbaril,* a brevi-deciduous species that gradually replaces all old leaves with new ones during the dry season, but rarely becomes completely leafless (Lisi et al., 2008). It is characterised by a cylindrical trunk and it reaches heights of up to 35 metres tall, often emerging above the forest canopy (Carvalho, 2003). The tree rings of *H. courbaril* are visually distinct and delimited by a marginal parenchyma band (Locosselli et al., 2013). Although its growth rings have previously been proven to be annual (Luchi, 1998; Westbrook, 2006), recent studies using radiocarbon dating raised doubts about their annual nature (e.g., Santos et al., 2021).

We collected 39 increment cores from 17 living *H. courbaril* trees between March and July 2018 using a gasoline-powered Stihl drill with a borer of 1.5 cm in diameter and 1 m in length coupled to a motor drill (Krottenthaler et al., 2015). We collected three to four perpendicular radii from each tree, at breast height, or approximately 1.3 m from the topsoil. After sample collection, we closed the holes in the tree trunks with natural cork (Locosselli et al., 2016). Data on tree diameter at breast height (DBH), height, overall conditions, presence of lianas, and geographical coordinates were recorded for each tree in the field.

2.2. Sample preparation and tree-ring analysis

We glued the increment cores on wooden supports and, after drying them at room temperature for a couple of weeks, we polished them using sandpaper with different grits (60, 120, 220, 300, 400, 600, 1200, and 2000). After polishing, we scanned the samples with the EPSON Expression 12000XL Scanner and analysed the images using the WinDendro software (Regent Instruments) to identify, count, and measure the width of the tree rings. We visually cross-dated the tree rings among the radii of the same individual and then between the radii of different individuals, looking for the pointer years that correspond to narrow rings common to most trees in a population (Schweingruber et al., 1990). From the sampled specimens were included in the chronology 17 trees with between 1 and 3 radii with clearly visible and demarcated rings (Fig. A1, Table A1).

2.3. Tree-ring isotope analyses

For isotopic analysis, we chose rings from the period from 2013 to 2017 to capture the 2015–2016 El Niño year, which was the hottest year recorded in recent times (Bennett et al., *in press*). The preparation of isotopic samples was done at the University of Leeds. We choose three trees with wide and distinct tree rings for the intra-annual isotope analysis (Fig. A2). We used a circular saw (Proxxon KS 230, Saw Blade 28 020, Locosselli et al., 2020) to cut transversal thin sections with a thickness of 2mm from the increment cores, and then isolated full segments from the thin sections containing the years from 2013 to 2017. These thin wood sections were then placed inside supports made from Teflon sheets to extract cellulose as per Kagawa et al. (2015). We treated the samples twice in a 5% NaOH solution for 2 hours in a water bath at 60 °C to remove resins, fatty acids, and tannins. We then washed the samples with deionized boiling water and treated the samples four times in 7.5% $NaClO_2$ solution at pH 4-5, also in a water bath at 60 °C, totaling 37 hours. We finally washed the samples with deionized boiling water and dried them in a lyophilizer (Kagawa et al., 2015; Schollaen et al., 2015).

To assess the intra-annual isotope patterns, we sampled 10 segments from each ring, totalizing 300 samples. Ring sections were separated according to weight rather than width which avoids problems with variation in growth rate throughout the growing season between trees and has been shown to result in a strong common signal observed among specimens (Locosselli et al., 2020). To do this, we first cut out a sample of cellulose of the entire tree ring equalling 10 times the target weight for stable isotope analyses (i.e. 10 * 0.5 mg for $\delta^{18}O$ + 10 * 1 mg for $\delta^{13}C$ = 15 mg). We then removed thin sections of cellulose, layer by layer, measuring their weight using a precision scale until it reached the total weight of 1.5 mg. This procedure was performed for each of the ten segments within a ring (and until all cellulose was used up). We packed 0.5 mg in silver cups for oxygen isotopes and we packed 1 mg of cellulose in tin cups for carbon isotopes analysis.

The isotope analysis was done at the University of Leicester. Oxygen isotope ratios were then measured using an Isotopes Ratio Monitoring Mass Spectrometer (Sercon 20-20 IRMS, Sercon IRMS, Crewe-UK) interfaced to a high temperature furnace, equipped with a glassy carbon reactor at 1400 degrees Cº. Laboratory cellulose standards were measured after every 10 samples and these were used to calculate the precision (precision = 0.15‰). Carbon isotopes were separately measured over an elemental analyzer coupled to the same mass spectrometer and precision was determined using cellulose laboratory standards (precision = 0.1‰). Isotopic ratios were expressed according to the delta notation. The isotopic ratios for the pure cellulose standards (sigma-aldrich) were $\delta^{13}C$ = -24.9‰ and $\delta^{18}O$ = 29.8‰.

$$\delta = \left[\frac{\left[\frac{^{13}C}{^{12}C} \right]_{sample}}{\left[\frac{^{13}C}{^{12}C} \right]_{standard}} - 1 \right] \times 1000$$

2.4. Intra-annual isotope series synchronisation

For each year, we synchronised the isotope series among different trees by shifting the entire individual $\delta^{18}O$ series one or two positions to the left or right (see example Fig. A3) to match the valleys and picks of all series in a year. This procedure aims at correcting eventual temporal offsets in the start and end of the growth season, or differences in the rate of cambial growth rate during the growing season among individuals (see Locosselli et al., 2020). We only shifted the full intra-annual series and did not merge the values of adjacent intra-annual positions, nor did we split any series for synchronisation.

The decision on the best relative position of the individual series was taken based on the calculated Gleichläufigkeit (GLK) values that is a measure of the common signal between series (Eckstein and Bauch, 1969). The GLK is a nonparametric method used in dendrochronology to evaluate the strength of the synchronization by calculating the percent common year-to-year growth changes between two tree ring series. For each year in which the growth rate of the series is in phase, in other words similarly increases or decreases in two tree-ring series, a value equal to one is summed, while if growth is in anti-phase, a value minus one is summed. In cases when one series changes the growth rate in a year and the other one remains constant, a value equal to zero is attributed. The average of these values allows one to assess the synchrony between the two analysed series, and how this synchrony changes when shifting the inter-annual series for the best dating position. We used the same principle here, but instead of looking for changes in the inter-annual variation of tree-ring width, we sought for the synchrony between intra-annual $\delta^{18}O$ values for each year between 2013 and 2017. We performed a pairwise comparison of the intra-annual series for each year, and averaged the calculated GLK values to choose the one that represented the best synchrony among the three series (Gleichläufigkeit, Bura and Wilmking, 2015; 'dplR', Bunn, 2008). The synchronisation resulted in substantial improvements in the GLK values for all years when compared to non-synchronized series, indicating a better match between the series' peaks and dips (Fig. 3). The non-synchronized series of oxygen and carbon presented a mean GLK of 0.51 and 0.63, respectively, while the synchronised series resulted in a GLK mean of 0.68 for both isotopes, which represents up to a 33% increase in GLK values. We then produced for both oxygen and carbon isotopes average series using the adjusted (synchronised) data (Fig. 3).

2.5. Hydroclimate correlation analysis

We calculated Pearson's correlation values between the average intra-annual oxygen isotopes of each tree-ring position (from one to ten) and monthly values of local precipitation, vapour pressure deficit (VPD), and river discharge of the main basins of the Mortes and Araguaia Rivers, and presented the result as heatmaps. We obtained local precipitation and evapotranspiration data from a weather station of the National Institute of Meteorology (INMET - www.inmet.gov.br, XAVANTINA - 83319) located in Nova Xavantina (14°69'79” S and 52°35'02” W), about 20 km from the sampling site, and river stage data from weather stations of the Brazilian National Water Resources Information System (SNIRH - www.snirh.gov.br/hidroweb). The average $\delta^{18}O$ series were then plotted against the climate data guided by using the intra-annual position and month with the highest correlation value for visual comparison.

We also performed Pearson's correlation between annual isotope values and annual climate data. For this, we chose the positions of oxygen isotopes within the tree ring that best correlated with monthly climate data (positions 3 to 7). We averaged these positions, resulting in annual values of oxygen isotopes between 2013 and 2017. Next, we performed Pearson correlations between annual isotopic values and mean values of local precipitation, VPD, and river discharges during the rainy season (October to March).

Results

3.1. Variation in $\delta^{13}C$

The intra-annual series of $\delta^{13}C$ shows a clear pattern with high values at the beginning of the ring, followed by consistent decreases to minimum values towards the end of the tree rings (Fig. 4). These patterns show sharp shifts in carbon isotopes values near the parenchyma bands that occur in the ring boundaries between years. Specifically, at the start of each tree ring, the average carbon isotope value for the individuals is approximately -26‰ VSMOW, while towards the end of the tree ring the values decline to approximately -27‰ VSMOW, indicating a carbon isotope shift within the ring from earlywood to latewood. We did not find significant correlations between the synchronized intra-annual mean $\delta^{13}C$ series and the climate data during the growing season (FIg. A3)

3.2. Variation in $\delta^{18}O$ and hydrological records correlation

The series of $\delta^{18}O$ presented a clear repeating pattern with lowest $\delta^{18}O$ values right after the middle of the ring and highest values usually at the tree rings borders (Fig. 4).

The synchronized mean intra-annual series for $\delta^{18}O$ showed a negative correlation with precipitation in January at position 5 (r = -0.93, P = 0.02; Fig. 5), and a positive correlation with VPD during the month of February at position 4 (r = 0.92, P = 0.02; Fig. 5). We found significant correlations between tree-ring $\delta^{18}O$ and river discharge from the two main rivers of the Araguaia basin (Fig. 5). We further observed a negative correlation between the $\delta^{18}O$ at positions 3 and 7 within the tree ring and Mortes streamflow of December (r = -0.93, P = 0.01) and January (r = -0.93, P = 0.02; Fig. 5), respectively. The same $\delta^{18}O$ positions within the tree ring were also correlated with the Araguaia streamflow of December (r = -0.96, P = 0.04) and January (r = -0.93, P = 0.006; Fig. 5). We also find that the annual $\delta^{18}O$ series show good correlations with average climate data during the rainy period, from October to March (Fig 6; Table A2). $\delta^{18}O$ is positively related to VPD (r = 0.71, P = 0.17), and negatively with precipitation (r = -0.90, P = 0.03), and with river flow for das Mortes (r = -0.74, P = 0.14), and Araguaia rivers (r = -0.75, P = 0.13; Fig. 6). For visual comparison, according with the highest Pearson correlations, we plotted their position 5 with January VPD and precipitation series. We also plotted position 5 with das Mortes and the Araguaia river flow of February. The alignment between oxygen and rivers series was placed in the following month (February) as we took into account the GAP between the beginning of the rainy season and the rise in the river's streamflow (Marengo, 1995).

Discussion

Evidence of annual ring formation from intra-annual isotopes

Hymenaea courbaril has previously been used for several dendrochronological studies (e.g., Locosselli et al., 2013, 2016; Andrade et al., 2019), but a recent study questioned the annual nature of this species' tree rings in the Southwestern Amazonia where the dry season is very short (Santos et al., 2021). In the highly seasonal hydroclimate of the Southeastern Amazonia, the common intra-annual patterns of $\delta^{13}C$ observed in all trees and all years gives additional support to the previous observations of annual rings in *H. courbaril* under seasonal sites (e.g., Westbrook et al., 2006; Locosselli et al., 2016). The annual nature of these tree rings is further supported by observed strong associations between intra-annual $\delta^{18}O$ and the hydroclimate in the study site. For other sites, there is mixed evidence regarding the formation of annual rings by this species. Annual ring formation has been found in a few cases (e.g., Westbrook et al., 2006; Locosselli et al., 2016), but others also find that annual ring dating may be unreliable due to the frequent occurrence of rings that are missing locally (i.e., wedging ring), or across the full cross-section (i.e., years with no growth, Santos et al., 2021). Though the exact reason for the occurrence of such missing rings is still unknown, it is possible that it may occur more frequently at sites where climate seasonality is less pronounced and subject to erratic weather fluctuations (Santos et al., 2021). It is thus possible that this species may form false rings, or missing rings in regions with more erratic climate seasonality, but still present annual rings at locations with under pronounced climate seasonality (e.g., Westbrook et al., 2006; Locosselli et al., 2016; this study). This would indeed result in wide variation in the reliability of annual ring dating in *H. courbaril*, which has a widespread distribution in the Amazon covering a large range of climates, including less seasonal climates (Steege et al., 2013).

Synchronisation of isotope series

The comparison of the intra-annual isotope series indicated that growth rhythms were not 100% synchronised between trees. This issue became clear from the mismatches between peaks and valleys in the intra-annual $\delta^{18}O$ and $\delta^{13}C$ series among individuals (Fig. 3), probably due to differences between trees in the timing of onset of cambium activity at the beginning of the rainy season and cambium dormancy at the end of the rainy season. As illustrated in the example we used to demonstrate synchronisation for the rings of 2013 (Fig. A4), trees varied in the position of peak $\delta^{18}O$ or $\delta^{13}C$. For example, in 2013 peak $\delta^{18}O$ and $\delta^{13}C$ occurred in one tree at position 6 and in the other two trees at later positions 7 and 8. These differences are likely due to variations in the start of the cambial activity. Aligning the highest isotope values of each specimen to the same position helped to correct for these temporal offsets yielding better average GLK results (Fig. 3). These differences in cambium activity, as observed in our study, are expected in the tropics where low phenological synchrony has been widely reported (e.g., Vogado et al., 2016; Vasconcellos et al., 2017) mostly because of inter tree differences in microclimate conditions and genetic variability within the population (De Micco et al., 2016; Jiménez-Noriega et al., 2021). The fact that these series could be synchronised indicates that *H. courbaril* tree rings are highly sensitive to seasonal fluctuations in climate conditions.

Intra-annual variation in carbon isotopes

The recurring pattern of decreasing $\delta^{13}C$ from the beginning to the end of the tree ring (Fig. 3 and 4) is consistent with the expected pattern of tree ring $\delta^{13}C$ observed in other temperate (Helle and Schleser, 2004; Cernusak et al., 2009; Eglin et al., 2010) and tropical deciduous species (Poussart et al., 2004; Fichtler et al., 2010; Cintra et al., 2019). In the initial stage of the growing season, deciduous trees depend on stored reserves of starch produced during the previous growing season to support leaf flush and woody growth. As starch is enriched in ^{13}C (higher $\delta^{13}C$) relative to sugars produced during photosynthesis, the early part of the tree rings will have a high $\delta^{13}C$ (Eglin et al., 2010). As the growing season proceeds, more and more assimilates from current photosynthesis are used, resulting in a gradual decrease of wood $\delta^{13}C$ towards the end of the rings. This framework has been proposed as the basic expected mechanism behind the effect of the metabolism of stored reserves on $\delta^{13}C$ of wood (Helle and Schleser, 2004), with possible variations from different uses of stored reserves from year to year (Eglin et al., 2010). The fractionation effects associated with the use of stored carbohydrate reserves are consistent with the intra-annual patterns of other tropical ring-forming species (Cintra et al., 2019; Locosselli et al., 2020). The consistency of this pattern across each analysed ring in this study provides support for previous observations that those rings are likely of annual nature. The large influence of carbon remobilization effects that have been observed in the intra-annual carbon isotope series may explain the absence of correlation we observed between $\delta^{13}C$ and climate variables, and the absence of reflection in seasonal hydrology. Despite we found a correlation specifically between the Rio das Mortes riverflow in January and February and the isotope series, it's important to note that these series don't reflect in the river level when observed at seasonal basis.

Intra-annual variation in oxygen isotopes

We observed that the middle of the tree ring has a negative association with local precipitation during January, the middle of the rainy season (Fig. 6), and with precipitation across a larger region covering the Southern fringes of the Amazonia Forest (Fig. A5). We noted the lowest oxygen isotope values in the ring correspond to the year 2015, which coincided with a historic drought recorded in the Amazon region (Fig. 5 and 6; Jiménez-Muñoz et al., 2016). Other studies in the Amazonia similarly report a negative relationship between $\delta^{18}O$ and regional precipitation because of the combined effects of rainout of heavy isotopes during moisture transport over long distances and the amount effect (Brienen et al., 2012; Baker et al., 2016; Cintra et al., 2021). Water vapour in the air that reaches the study region is transported from the Atlantic Ocean by the trade winds (Fig. A6), and slowly gets depleted in H_2O^{18} because of rainout of heavy water during precipitation events along the trajectory (Brienen et al., 2012; Baker et al., 2016). This results in the observed negative correlation between $\delta^{18}O$ and the amount of precipitation in the region upstream of the sampling site (Fig. A5).

While the correlation between the $\delta^{18}O$ and precipitation amounts indicates an influence of source water $\delta^{18}O$, we also find indications of an effect of evaporative leaf water enrichment, as revealed by the positive correlation between $\delta^{18}O$ and local VPD (Fig. 5). These results are in line with experiments that show that leaf water enrichment is greater under low relative humidity, or under high evaporative demand (Barbour et al., 2000; Roden et al., 2000; Liu et al., 2017). Field studies on leaf and wood cellulose $\delta^{18}O$ have shown varying effects of VPD, with strong effects of VPD on $\delta^{18}O$ under warm and/or dry environmental conditions (Kahmen et al., 2011; Cintra et al., 2019) and weaker or no effects in wetter sites (Anchukaitis and Evans, 2010; Brienen et al., 2012; Cintra et al., 2019). Thus, the relative effects of source water influences versus leaf water in tree rings may vary due to differences in leaf water enrichment due to variations in air relative humidity and species' leaf-level responses (Barbour et al., 2004; Cernusak et al., 2016). The relatively strong temperature increases in this region over recent years (Jiménez-Muñoz et al., 2013; Alves et al., 2017; IPCC, 2022) will lead to a decrease in the relative humidity of the atmosphere (Tiwari et al., 2020; Araújo et al., 2021), and may result in a stronger imprint of leaf transpiration on *H. courbaril* tree-ring $\delta^{18}O$.

Guided by the positions and months of highest correlations with precipitation and VPD, we plotted the $\delta^{18}O$ values againts the continuous climate series (Fig. 5). This approach aimed to visualize the relationship between tree ring oxygen isotope series and the climate data more clearly. Both precipitation and evapotranspiration define river basins' hydrology and river discharge (Coe et al., 2016, 2017; Maeda et al., 2017;

Heerspink et al., 2020). This is probably why the isotopic series of *H. courbaril* showed a better association with seasonal variation of the two main rivers of the Araguaia basin than with seasonal precipitation and/or VPD alone when compared at seasonal perspective (Fig. 5). This association was only clear after a small temporal shift in the river flow series taking into account the observed lag between the beginning of the rainy period and its effects on the streamflow (Marengo, 1995). Even though it is possible to observe associations between the isotopes and the monthly streamflow for both rivers, the signal recorded in the tree rings of *H. courbaril* is substantially higher for the Araguaia River, 150 km downstream of the sampling site than with the das Mortes River, 20 km from the sampling site. Such a large-scale hydrological signal in tree-ring $\delta^{18}O$ has been demonstrated before (Dinis et al., 2019), including in the Amazonia basin (Brienen et al., 2012; Cintra et al., 2022), but only a few studies addressed this issue at a sub-annual scale (Locosselli et al., 2020) and this is the first one in Southern Amazonia.

<u>Potential for climate reconstructions</u>

We found largely similar results when we analysed the data at an inter-annual scale. This analysis showed good correlations between averaged $\delta^{18}O$ from the middle of the tree ring of *H. courbaril* and precipitation, VPD, and river discharge (Fig. 6). These correlations are substantially greater for the rainy season averages from October-March than when compared to the whole year hydroclimatic averages (Table A2), demonstrating once again that this species indeed reflects the climate and hydrology of the southern portion of Amazonia during the rainy season. One caveat of our study is the short time span of the analyses that could potentially lead to artificial correlations. The interannual $\delta^{18}O$ data do however fit remarkably well with four different climate datasets (Fig. 6) and are in the right direction of theoretical expectations and in line with several recent oxygen isotope studies in the Amazon basin (Brienen et al., 2012; Baker et al., 2016; Cintra et al., 2021). These findings show that oxygen stable isotopes hold great promise to reconstruct / investigate hydrological rainfall and / or river discharge records at seasonal and annual variation, and improve our understanding of recent climatic changes in the Amazon basin. We hope that our study thus provides an incentive for the development of more tree ring studies from this region, leading to long, robust hydroclimate proxies for the southern Amazon.

Conclusion

Our results demonstrate that in regions with a well-defined dry season, *H. courbaril* presents tree rings that correspond to a hydrological year. The intra-annual variation in tree-ring $\delta^{18}O$ of *H. courbaril* presents a large-scale signal that can be used as a proxy for assessing seasonal river discharge and annual precipitation. It is thus a potential tool to reconstruct past high-frequency climate variability for a region that is experiencing strong warming in recent decades. Our results indicate that $\delta^{18}O$ tree ring records at sub-annual or annual resolution can provide an important proxy to understand how climate changes are affecting the basin's hydrological cycle at the southern border of the Amazonia. Given the constraints imposed by our limited dataset, we underscore the exploratory aspect and advocate for further investigation to solidify and expand upon our findings.

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Figures

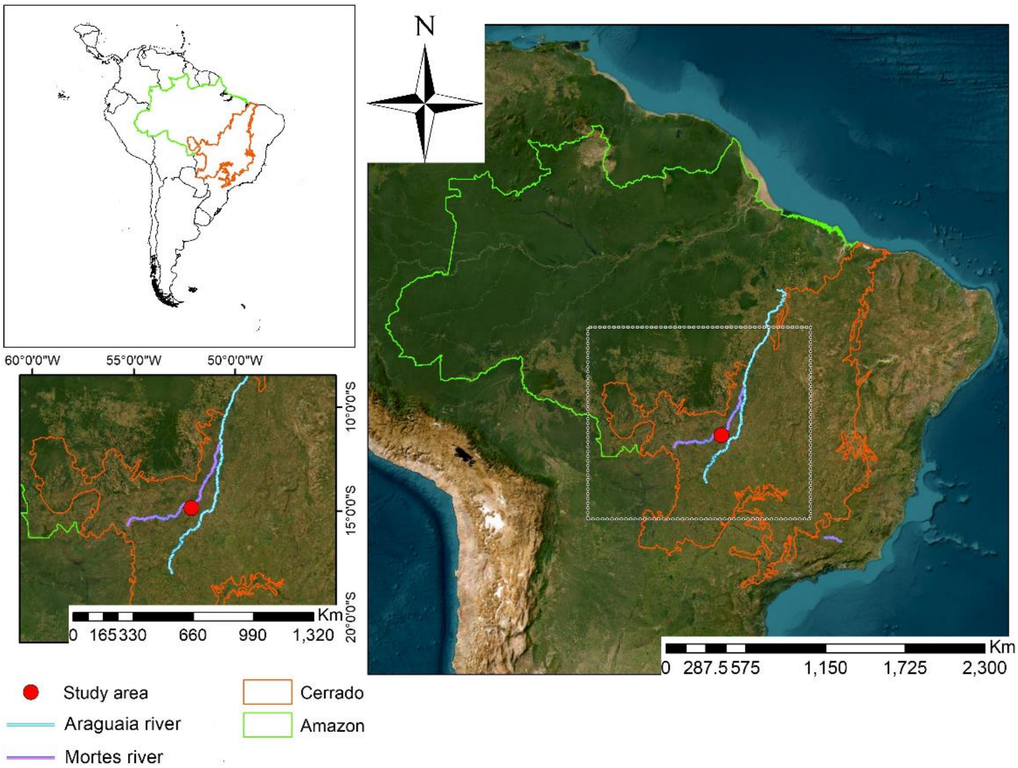

Figure 1: South America map with the Amazon biome (green line) and Cerrado biome (red line). The study area (circle) is located at the Amazon forest fingers, between the Amazonia-Cerrado transition. The main river that drainage the region is the Araguaia River (blue line) and its main tributary, the Mortes River (purple line).

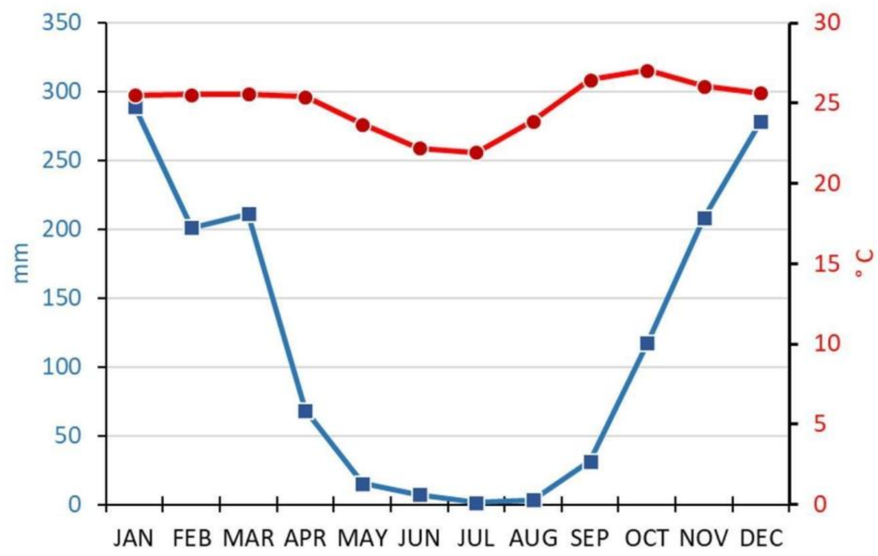

Figure 2: Climate diagram showing temperature and precipitation curves of INMET (National Institute of Meteorology) data from the Nova Xavantina region between 1998 and 2017. The red line indicates monthly temperature average (°C) and the blune line the monthly precipitation average (mm), showing a typical tropical climate, with a dry season between April and September.

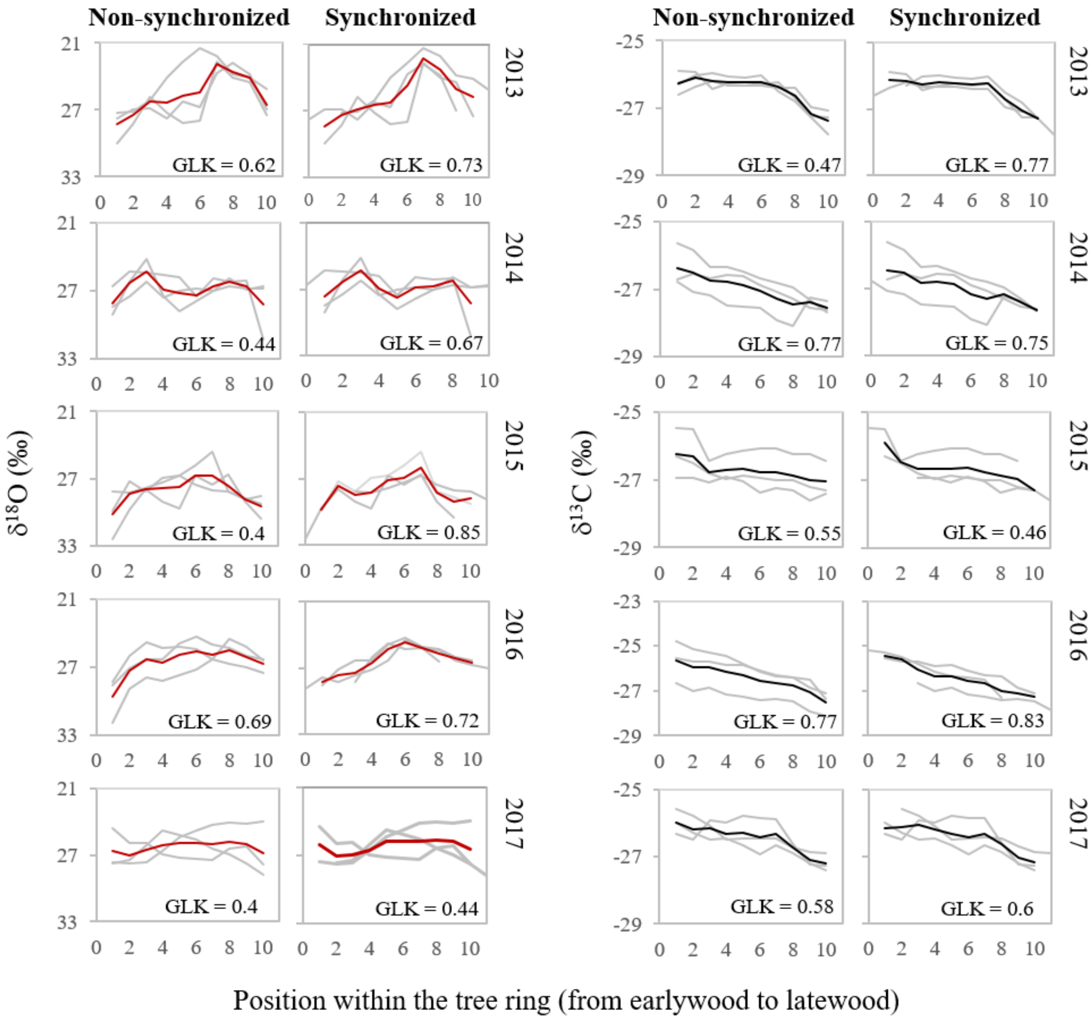

Figure 3: Comparisons between non-synchronized and synchronised series of $\delta^{18}O$ and $\delta^{13}C$ measured in each tree ring of *Hymenaea courbaril* trees samples from the southern Amazon. Series of individual trees are represented in grey, while the average series of $\delta^{18}O$ is represented in red and $\delta^{13}C$ in black. GLK averages were calculated for all positions. The y-axis for the $\delta^{18}O$ series are reversed.

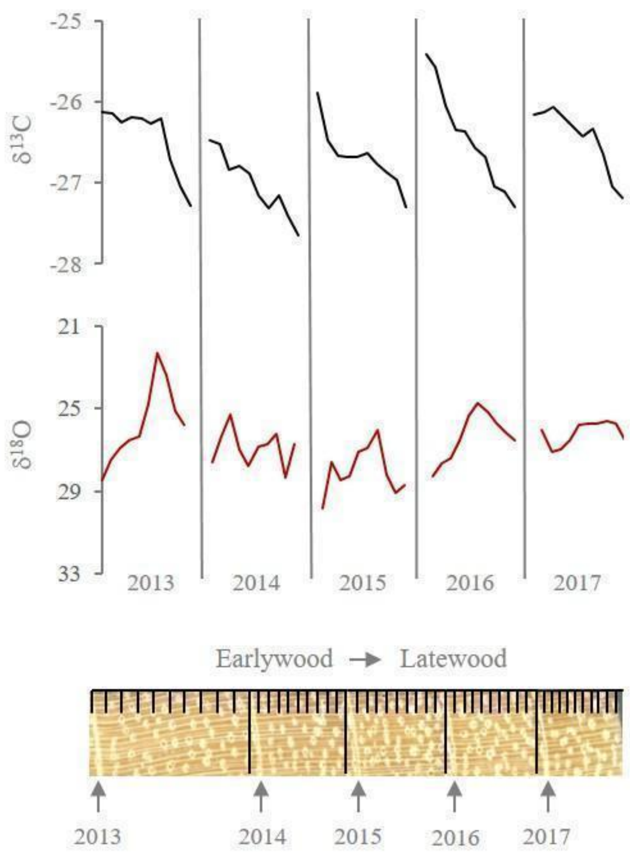

Figure 4: Average of annual $\delta^{13}C$ and $\delta^{18}O$ synchronised series between 2013 and 2017, positioned from earlywood (position 1) to latewood (position 10). The y-axis for the $\delta^{18}O$ are reversed. Above a sampled radius of *Hymenaea courbaril* as an example of the 10 divisions made in each tree ring. Separations were performed by dividing the weight of the tree ring into ten parts.

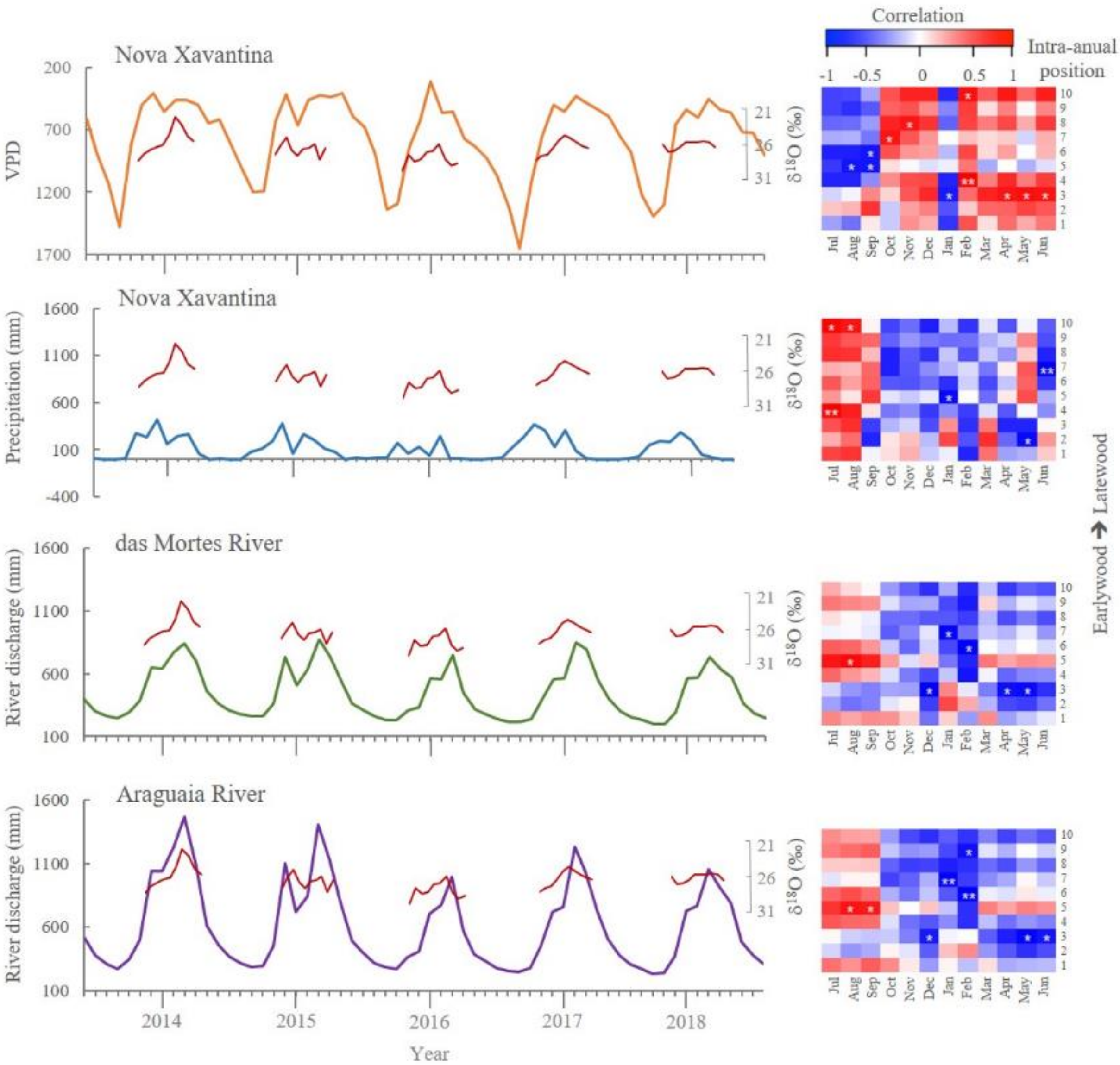

Figure 5: Vapour Pressure Deficit (orange line), Precipitation (blue line), das Mortes (green line), and Araguaia river discharge (purple line) between June 2013 and July 2018. The mean oxygen isotopic series (red lines) were positioned according to the best correlation found between monthly climate data and the isotopic positions within the tree rings (oxygen isotope at position 5 within the tree ring aligned in January). For the relationship with the rivers, we took into account the gap of approximately 1 month between the beginning of the rainy season and the increase in river flow (oxygen isotope at position 5 within the tree ring aligned in February). The y-axes for $\delta^{18}O$ and VPD have been reversed solely for visual comparison purposes and not for statistical analysis. Heatmaps show the correlation values between oxygen isotopic series and climate data. For the intra-annual position the sample sizes are n = 5. Correlation values are presented per month between vapour pressure deficit, precipitation, and river discharges. *

significant values for α = 0.05 and ** for α = 0.01. Climate data were obtained from climate stations of the cities of Nova Xavantina and Cocalinho, Mato Grosso, Brazil.

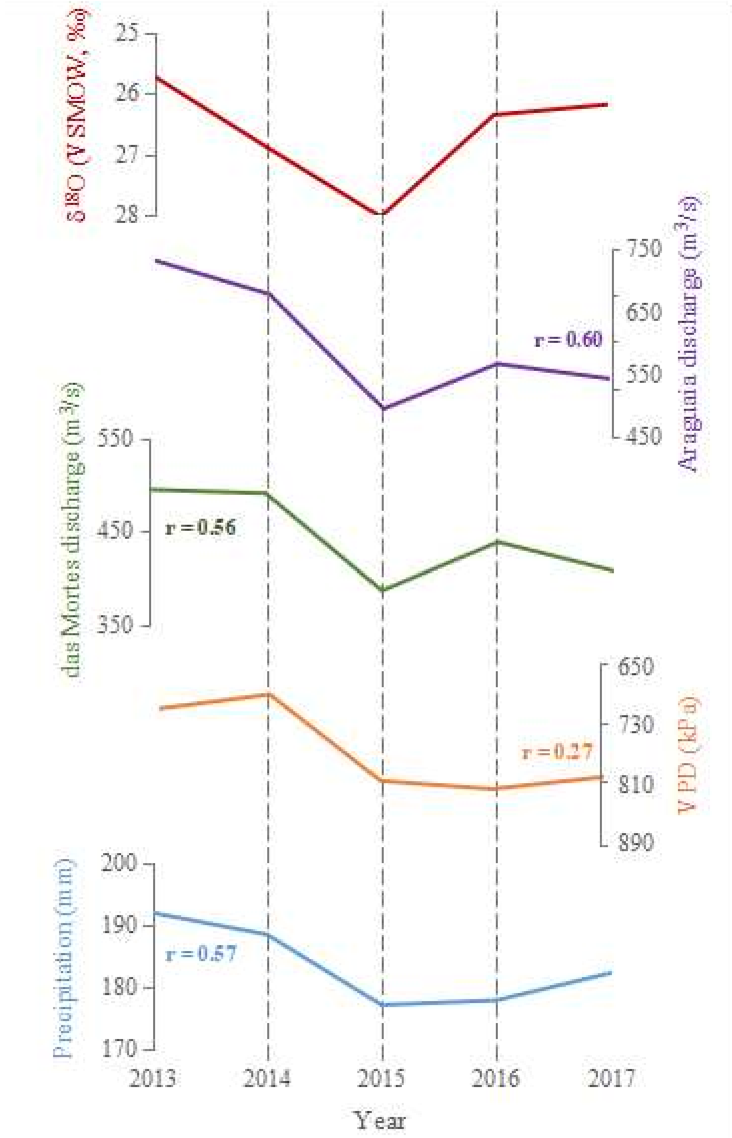

Figure 6: Annual oxygen isotope series of *Hymenaea courbaril* (red line), local precipitation (blue line), Araguaia river discharge (purple line), das Mortes river discharge (green line), and Vapour Pressure Deficit (orange line) between 2013 and 2017. The annual isotope series correspond to the average of the 3 to 7 (n = 5 samples for each position) isotope positions within the tree ring and the hydroclimatic data comprise the rainy season average, from October to March. Values indicate the Pearson correlation coefficients between the $\delta^{18}O$ and the records for the period shown (for all $P < 0.001$).

Note that the y-axis for the $\delta^{18}O$ and VPD are reversed for visual comparison purposes but not for statistical analysis.