Contrasting controls on tree ring isotope variation for Amazon floodplain and terra firme trees

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Isotopes in tropical trees rings can improve our understanding of tree responses to climate. We assessed how climate and growing conditions affect tree-ring oxygen and carbon isotopes (\(\delta^{18}O_{\text{TR}}\) and \(\delta^{13}C_{\text{TR}}\)) in four Amazon trees. We analysed within-ring isotope variation for two terra firme (non-flooded) and two floodplain trees growing at sites with varying seasonality. We find distinct intra-annual patterns of \(\delta^{18}O_{\text{TR}}\) and \(\delta^{13}C_{\text{TR}}\) driven mostly by seasonal variation in weather and source water \(\delta^{18}O\). Seasonal variation in isotopes was lowest for the tree growing under the wettest conditions. Tree ring cellulose isotope models based on existing theory reproduced well observed within-ring variation with possible contributions of both stomatal and mesophyll conductance to variation in \(\delta^{13}C_{\text{TR}}\). Climate analysis reveal that terra firme \(\delta^{18}O_{\text{TR}}\) signals were related to basin-wide precipitation, indicating a source water \(\delta^{18}O\) influence, while floodplain trees recorded leaf enrichment effects related to local climate. Thus, intrinsically different processes (source water vs leaf enrichment) affect \(\delta^{18}O_{\text{TR}}\) in the two different species analysed. These differences are likely a result of both species-specific traits and of the contrasting growing conditions in the floodplains and terra firme environments. Simultaneous analysis of \(\delta^{13}C_{\text{TR}}\) and \(\delta^{18}O_{\text{TR}}\) supports this interpretation as it shows strongly similar intra-annual patterns for both isotopes in the floodplain trees arising from a common control by leaf stomatal conductance, while terra firme trees showed less covariation between the two isotopes. Our results are interesting from a plant physiological perspective and have implications for climate reconstructions as trees record intrinsically different processes.

Keywords: carbon isotopes, Cedrela odorata, dual isotope, Macrolobium acaciifolium, oxygen isotopes, tropical forests.

Introduction

Intra-annual, high-resolution oxygen and carbon isotopes are increasingly being used for a multitude of applications, including climate reconstructions (Barbour et al. 2002, Ohashi et al. 2009, Roden et al. 2009, Fichtler et al. 2010, Managave et al. 2011), age and growth rate determinations in ringless tropical trees (Poussart et al. 2004, Poussart and Schrag 2005, Pons and Helle 2011), and for studying seasonality in growth and use of carbohydrate reserves (Helle and Schleser 2004, Ohashi et al. 2009, Fichtler et al. 2010, Gulbranson and Ryberg 2013). A prerequisite for using tree-ring isotope records is an understanding of the underlying physiological processes affecting tree-ring isotope ratios.

Much progress in our understanding has been made over the past decades for temperate trees (McCarroll and Loader 2004). In comparison, isotope studies of tropical trees remain scarce, despite their great potential to improve our understanding of tree functioning and for climate reconstructions (van der Sleen et al. 2009, Fichtler et al. 2010, Gulbranson and Ryberg 2013).
Beer et al. 2010 and the importance of these vast forests for the global carbon cycle (Phillips et al. 2009, Beer et al. 2010, Brienen et al. 2015, Pan et al. 2015). There is little information about what processes dominate variation of tree ring oxygen and carbon isotopes ($\delta^{18}$O and $\delta^{13}$C) in tropical environments, and how this varies between different tropical tree species.

Oxygen isotope signals in tree rings are mostly the result of variation in source water $\delta^{18}$O and evaporative leaf enrichment (Dongmann et al. 1974, Roden and Ehleringer 1999, Farquhar et al. 2007, Cernusak et al. 2016). In tropical trees oxygen isotopes have been shown to reflect both processes (Miller et al. 2006, Brienen et al. 2011, Kahmen et al. 2011, Bowman et al. 2013, Schollaen et al. 2013), but which of these effects dominates and under which conditions remains poorly known. Specifically, the contribution of leaf water enrichment to the final $\delta^{18}$O may vary between species and environments, due to variation in leaf transpiration arising from specific differences in leaf traits (e.g., varying pathlengths, Kahmen et al. 2008, Holloway-Phillips et al. 2016) and/or site humidity levels (Barbour and Farquhar 2000, Barbour et al. 2002, Kahmen et al. 2011).

Carbon isotope ratios in tree rings are affected by the ratio between photosynthetic assimilation rate and conductance to CO$_2$. The conductance of CO$_2$ from outside the leaf to the sites of photosynthesis consists of stomatal conductance, $g_s$, and mesophyll conductance, $g_m$, which both affect $\delta^{13}$C (Farquhar et al. 1982, Seibt et al. 2008, Farquhar and Cernusak 2012). As $g_s$ is often sensitive to water availability, $\delta^{13}$C has been shown to reflect drought levels at relatively dry sites in the tropics (Gebrekirstos et al. 2009, Fichtler et al. 2010, Brienen et al. 2011). Mesophyll conductance, $g_m$, is temperature dependent in many tree species and therefore variations in leaf temperature may also affect $\delta^{13}$C (Seibt et al. 2008, Griffiths and Helliker 2013, von Caemmerer and Evans 2015). Other studies show that $\delta^{13}$C signals also vary as a result of post-photosynthetic processes, specifically usage of carbon reserves (Helle and Schleser 2004, Eglin et al. 2010, Gulbranson and Ryberg 2013, Gessler et al. 2014).

A useful approach to understand what processes are reflected in isotope signals is simultaneous analysis of variations of $\delta^{18}$O and $\delta^{13}$C in tree ring cellulose. This is because stomatal conductance response to low humidity may affect both leaf $^{13}$C discrimination and leaf water $^{18}$O enrichment, potentially leading to covariation of $\delta^{13}$C and $\delta^{18}$O (step 1 in Figure 1a and step 2 in Figure 1b). This approach has, for example, been used to assist in the interpretation of carbon isotope signals in leaves of temperate trees (Scheidegger et al. 2000), and allowed at least a partial separation of leaf level fractionation processes from the other fractionating effects on $\delta^{18}$O and $\delta^{13}$C (Barbour et al. 2002, Barnard et al. 2012, Roden and Farquhar 2012, Roden and Siegwolf 2012) (Figure 1).

Here we analyse which processes affect isotopic variation in tree rings of Amazonian trees, using a dual isotope approach with intra-ring resolution. This approach allows us to assess at a fine temporal scale how tree-ring isotopic compositions reflect the trees’ responses to varying weather conditions during the growing season. We chose two tree species growing under very different environmental conditions in the western and southwestern Amazon basin: the deciduous species Cedrela odorata L. (Meliaceae) from terra firme (non-flooded) forests that grow primarily during the wet season (Dünisch et al. 2003, Brienen et al. 2012, 2015, Costa et al. 2013, Baker et al. 2017), and the brevi-deciduous species Macrolobium acaciifolium (Benth.) Benth (Fabaceae) from floodplain ecosystems, which grows when river stage levels are low, i.e., during the dry season (Schöngart et al. 2002, 2005, Assahira et al. 2017). The original motivation for looking at these contrasting environments was that terra firme trees would record wet season and floodplain dry season climate variation. We chose these species because both are spatially widespread (ter Steege et al. 2013), grow in contrasting conditions and produce distinct annual rings (see Figure S1 available as Supplementary Data at Tree Physiology Online). For both species, we investigate two trees from two sites at a high intra-ring resolution (four trees in total). The sites were selected to differ in precipitation amount and seasonality (Figure 2).

Our objectives are: (i) to compare observed and expected intra-annual patterns of carbon and oxygen isotope within their tree rings based on existing mechanistic understanding; (ii) to assess the role of local climate and hydrological conditions and species differences on the intra-annual cycles of $\delta^{18}$O and $\delta^{13}$C; and (iii) to assess to what degree the dual-isotope approach may indicate what the main climatic and physiological drivers of variation in both isotopes at the intra-annual level are.

### Isotopes in tree rings: theory and modelling

**Carbon isotopes** Atmospheric CO$_2$ is the source of carbon for terrestrial trees. During CO$_2$ uptake by a leaf, four fractionating processes are of importance: CO$_2$ diffusion through the stomata and through the leaf mesophyll (i.e., cell membranes and cytoplasm), photorespiration and isotopic fractionation during the carboxylation reaction due to higher chemical affinity of the enzyme RuBisCO for $^{12}$CO$_2$ compared with $^{13}$CO$_2$ (step 1 in Figure 1a). These processes result in lower average plant organic $^{13}$C compared with the atmosphere, or a positive atmosphere–plant isotope discrimination ($\Delta$)

$$\Delta^{3}C = \frac{R_{\text{atm}} - R_{\text{plant}}}{R_{\text{plant}}} \cdot 10^{3} = \frac{\delta^{13}C_{\text{atm}} - \delta^{13}C_{\text{plant}}}{1 + \delta^{13}C_{\text{atm}}/1000}. \quad (1)$$

Here $R = \frac{N_{\text{rare}}}{N_{\text{abundant}}}$ with $N_{\text{rare}}$ the number of molecules of the rare isotope compound and $N_{\text{abundant}}$ the number of molecules of the abundant isotope compound in a sample, and with
**δ(permille) = (R_{std}/R_{atm} - 1) \cdot 10^{3}, where \( R_{std} \) is the isotope ratio of an internationally recognized standard.**

Farquhar et al. (1982) formulated a model to predict this discrimination that considers fractionation during diffusion through stomata and through the mesophyll, during carboxylation and fractionation due to photorespiration:

\[
\Delta^{3}C = a \left( \frac{c_{s} - c_{a}}{c_{s}} \right) - a_{m} \left( \frac{c_{i} - c_{c}}{c_{i}} \right) + b \left( \frac{c_{c}}{c_{s}} \right) - f \Gamma^{*} \left/ c_{a} \right.
\]

where \( c_{s} \) is CO\(_{2} \) partial pressure inside leaf intercellular space, \( c_{a} \) is CO\(_{2} \) partial pressure in air, \( c_{c} \) is the CO\(_{2} \) partial pressure in the chloroplast, \( a \approx 4.4 \% \) is the fractionation caused by slower diffusion of \(^{13}\)CO\(_{2} \) compared with \(^{12}\)CO\(_{2} \) through stomata, \( a_{m} \approx 1.8 \% \) is the fractionation during CO\(_{2} \) diffusion through the mesophyll, \( b \approx 30 \% \) is fractionation during carboxylation caused by discrimination of RuBisCO against \(^{13}\)CO\(_{2} \) inside the leaf, \( f \approx 12 \% \) is the discrimination due to photorespiration and \( \Gamma^{*} \) is the CO\(_{2} \) compensation point in the absence of day respiration (see also Seibt et al. 2008).

The model predicts that if \( c_{c} \) is close to \( c_{a} \), then discrimination is primarily due to non-equilibrium fractionation associated with carboxylation \(( \approx \% \)\). If, on the other hand, CO\(_{2} \) in the leaf is being drawn down by assimilation and \( c_{c} \) drops, then the carboxylation reaction causes an increase of the \(^{13}\)CO\(_{2} \) to \(^{12}\)CO\(_{2} \) ratio inside...
Figure 2. Map with the sampling sites (a) and annual cycles of climatic variables (b–e, h–k, n–q, t–w) and predicted intra-annual tree ring isotopes (f, g, l, m, r, s, x, y) for the two floodplain (FP) and the two terra firme (TF) sites. Annual cycle of monthly precipitation (grey bars) and river flood level (blue, showing levels above tree base) (top row), monthly VPD (second row), monthly cloud cover (third row), monthly δ¹⁸O in precipitation (fourth row), predicted δ¹⁸O in tree rings (solid line for effects of only source water δ¹⁸O and stippled line for added effects of leaf water enrichment) (fifth row) and predicted δ¹³C in tree rings, ignoring post-photosynthetic processes (lowermost row). The vertical dashed lines indicate the assumed growing seasons for each site—see details in section Data analysis. Climatic data shown are from CRU TS 4.00. Rainfall δ¹⁸O data are from the GNIP database. River level data are from the Brazilian Hidroweb-SNIRH database and from the ORE-HYBAM database for the Peruvian site.
the leaf (a Rayleigh distillation, step 1 in Figure 1a). The consequent enrichment of CO₂ inside the leaf offsets the effects of fractionation by carboxylation and lowers the net discrimination slightly towards the value for fractionation by diffusion (Δs). For plants, the magnitude of fractionation thus depends on the CO₂ partial pressure difference c₄ – c₅ between the outside and inside of the leaf. This difference is controlled by the ratio between carbon assimilation rate (A) and CO₂ flux in the leaf via

$$A = g_{sc} \left( \frac{c_s - c_i}{P} \right) = g_m \left( \frac{c_s - c_i}{P} \right),$$

which expresses that at steady state, assimilation rate A is equal to diffusive CO₂ flow though stomata and into the chloroplast. Here P is atmospheric air pressure, gₘ is the stomatal conductance to CO₂ and gₘ is the mesophyll conductance to CO₂. Equation (2) may then be expressed as

$$\Delta^{3}C = a + (b - a) \frac{c_i}{c_a} - (b - a_m) \left( \frac{A}{c_o g_m} \right) - f_\Gamma^{18}/c_o. \quad (3)$$

Isotope ratios of sugars produced in the leaf may undergo alterations before being incorporated in wood tissue by processes such as respiration, re-fixation of respired CO₂, and production and remobilization of carbon reserves, primarily starch (e.g., Cernusak et al. 2009, Treydte et al. 2014). In particular, for some deciduous trees wood formation before leaf flush requires the mobilization of non-structural carbohydrate reserves (NSC, step 2 in Figure 1a). Non-structural carbohydrate reserves that have accumulated by the end of the previous growing season are usually enriched in $^{13}$C in comparison with new photosynthesis assimilates (Brugnoli et al. 1988, Damesin and Lelarge 2003). Thus the use of stored NSC during the growing season may lead to higher $\delta^{13}$C in initial tree ring sections (Helle and Schleser 2004, Skomarkova et al. 2006, Ohashi et al. 2009, Gulbranson and Ryberg 2013, Gessler and Treydte 2016), and possibly in other ring sections as well (Eglint al. 2010). These post-photosynthetic processes may partially decouple the $\delta^{13}$CTR signal from current years’ leaf fractionation processes, potentially dampening the climatic signal in $\delta^{13}$CTR.

In summary, two processes contribute to intra- and interannual variation in $\delta^{13}$CTR: (i) $^{13}$C discrimination during leaf carbon uptake and photosynthesis; and (ii) carbon remobilization from non-structural carbon reserves. $\delta^{13}$CTR derived from reserves is enriched with $^{13}$C and for deciduous species tends to be used primarily during the initial phase of tree ring formation. $^{13}$C discrimination at the leaf level is controlled by the ratio of CO₂ inside the leaf to CO₂ in air. If this ratio is low—either because of low stomatal/mesophyll conductance to CO₂ associated with high vapour pressure deficit (VPD) or low temperatures, or due to high assimilation rates—discrimination will be small and vice versa.

Oxygen isotopes More processes contribute to $\delta^{18}$O variation in tree ring cellulose ($\delta^{18}$O_TR) compared with $\delta^{13}$C_TR. First, $\delta^{18}$O_TR is related to the isotopic composition of source water ($\delta^{18}$Oₜₘ), which may originate from rainfall and/or from groundwater water (step 1 in Figure 1b). $\delta^{18}$Oₜₘ may change in the soil by fractionation during evaporation. Water is taken up from the soil by roots without fractionation (Ehleringer and Dawson 1992). Xylem water entering the leaf has thus the same $\delta^{18}$O as soil water. In the leaf, water will get enriched in H₂$^{18}$O compared with stem water due to preferential evaporation of light water, H₂$^{16}$O (Craig and Gordon 1965, Dongmann et al. 1974). Average leaf water $\delta^{18}$O ($\delta^{18}$Oₚₚ) depends on the extent of $^{18}$O enrichment of water at the sites of evaporation within the leaves ($\delta^{18}$Oₑₑ), and on how much H₂$^{18}$O diffuses from the sites of evaporation through the leaf lamina, which depends on transpiration (Farquhar and Lloyd 1993, Farquhar et al. 2007, Cernusak and Kahmen 2013). Transpiration is driven by leaf to air vapour pressure difference (VPD) modulated by stomatal conductance (which itself may depend on VPD) (step 2 in Figure 1b). Sugars produced in the leaf carry with them the $^{18}$O-enriched leaf water signal ($\delta^{18}$O(wp) until they are broken down during cellulose synthesis, when they exchange oxygen isotopes with water in the stem (step 3 in Figure 1b).

The roles of the above-mentioned processes have been incorporated into models. The earliest model for $\delta^{18}$O at the leaf sites of evaporation is from Dongmann et al. (1974) based on a model of Craig and Gordon (1965) for fractionation during the process of evaporation from a water surface. The Dongmann model considers a water flow from roots to the stomata to the atmosphere (Craig and Gordon 1965), equilibrium fractionation $\varepsilon^e$ during evaporation from tissue in the stomata and kinetic fractionation $\varepsilon_k$ during diffusion of molecules from the leaf to the atmosphere through stomata. The resulting model for the isotopic signature at the site of evaporation $\delta^{18}$Oₑₑ is (same as Eq. (1) of Stemberg 2009, but algebraically rearranged):

$$\delta^{18}O_{ew} = (\delta^{18}O_{sw} + \varepsilon_k) \left( \frac{\varepsilon_i - \varepsilon_i}{\varepsilon_j} \right) + \varepsilon^e + \delta^{18}O_{sw} \left( \frac{\varepsilon_j}{\varepsilon_i} \right). \quad (4)$$

$\varepsilon_i$ and $\varepsilon_a$ are the intracellular and ambient vapour pressure, respectively, $\varepsilon_i = \frac{32g_s^{18} + 21g_s^{16}}{g_s^{18} + g_s^{16}} - 26.5\%$ is the kinetic isotopic fractionation of diffusion through stomata and boundary layer, gₙ is the leaf boundary layer conductance (Farquhar et al. 1989):

$$\varepsilon^e = 2.644 - 3.206 \left( \frac{10^P}{T} \right) + 1.534 \left( \frac{10^P}{T} \right)^2 = 9.57\% \text{ (at } 20 \text{ °C})$$

is the temperature-dependent isotopic equilibrium fractionation between vapour and water at the evaporation site inside the stomata, where T is the leaf temperature in Kelvin. At higher temperatures $\varepsilon^e$ tends to decrease, but this effect is relatively small (Bottinga and Craig 1969).

To interpret the model it is helpful to express leaf transpiration through stomata as $E = g_s \times \frac{e_i - e_a}{P} = g_s \times \frac{VPD}{P}$ where VPD $\equiv e_i - e_a$ is leaf to air vapour pressure difference (or ‘deficit’) and P is the atmospheric pressure. Thus, if VPD $= 0$, there is no leaf.
to air water flow and thus no flow from the stem into the leaves. In this case, $\delta^{18}$Oes is just the sum of atmospheric $\delta^{18}$Oa ($e_i/e_a = 1$) and the equilibrium fractionation $\varepsilon^+$ of evaporation inside the stomata. If in contrast there is a flow of water from the leaf via stomata to the atmosphere (i.e., when VPD > 0) and thus also a flow of stem (source) water to the stomata, then there is also a contribution to $\delta^{18}$Oes from source water $\delta^{18}$Osw, and from kinetic fraction $e_k$ during diffusion of water molecules through the stomatal opening (see Figure 3a).

The Dongmann et al. (1974) model described above tends to overestimate leaf water $\delta^{18}$O. Farquhar and Lloyd (1993) suggest that this is because average leaf water $\delta^{18}$O is a mixture of $\delta^{18}$O at the evaporative site ($\delta^{18}$Oes) and in the source water ($\delta^{18}$Osw). The relative contribution of $\delta^{18}$Oes and $\delta^{18}$Osw to average leaf water $\delta^{18}$O ($\delta^{18}$Osw) depends on the degree of back-diffusion of isotopes from the evaporation site along the water-stream from veins to stomata. The lower the water flow, the more important is the effect of back-diffusion and vice versa. The importance of back-diffusion can be measured by the Péclet number

$$\phi \equiv \frac{\text{Advection}}{\text{Diffusion}} = \frac{u}{D},$$

the ratio of advective water transport (velocity $u$, which can be expressed using transpiration as $u = \frac{E}{c}$ where $c$ is concentration of water, e.g., in mol m$^{-3}$) by the water stream from soil to air via stomata and the counteracting diffusive transport ($D$ molecular

Figure 3. Predicted effects of stomatal conductance ($g_s$) on leaf water $\delta^{18}$O at four different levels of relative humidity (RH). Modelled relationship between $g_s$ and $\delta^{18}$O of water at the sites of evaporation ($\delta^{18}$Oes) (a), $g_s$ and leaf transpiration ($E$) (b), $g_s$ and ‘mixture’ of source water with water from the sites of evaporation, alpha = $\left(\frac{(1-e^{-g_s})}{\rho}\right)$, (c), and $g_s$ and mean leaf water $\delta^{18}$O ($\delta^{18}$Osw) (d). Panels (e)–(h) illustrate predicted $\delta^{18}$O gradients between incoming leaf water ($\delta^{18}$Osw) and $\delta^{18}$O at sites of evaporation ($\delta^{18}$Oes) for low relative humidity (top panels) and high relative humidity (lower panels), and for low (left panels) and high stomatal conductance (right panels).
diffusivity of water and L the length of the path from stomata into leaf veins) (see also Cernusak and Kahmen 2013). Farquhar and Lloyd (1993) formulated a model of this effect that predicts δ\(^{18}\)O in the leaf within a distance L along veins from the evaporative site:

\[
\delta^{18}\text{O}_{sw} = \delta^{18}\text{O}_{sw} + (\delta^{18}\text{O}_{es} - \delta^{18}\text{O}_{sw}) + \frac{(1-e^{-\rho L})}{\rho} \tag{6}
\]

The term \(\alpha = \frac{(1-e^{-\rho L})}{\rho}\) determines the contribution of \(\delta^{18}\text{O}_{es}\) to \(\delta^{18}\text{O}_{sw}\), resulting from back diffusion. According to this model, if transport via advection of stem water to the evaporation site is much faster than the counteracting diffusive transport— i.e., when \(\rho\) is large— \(\delta^{18}\text{O}\) of leaf water is close to \(\delta^{18}\text{O}\) of source (stem) water. If, in contrast, transport by advection of stem water is less than by back-diffusion, then \(\delta^{18}\text{O}\) of leaf water will be close to \(\delta^{18}\text{O}_{es}\) (since \((\frac{1-e^{-\rho L}}{\rho} = 1 \text{ for } \rho \to 0)\) (Figure 3b and d). Thus, because the advective flux is given by transpiration, \(E\), the contribution of \(\delta^{18}\text{O}_{es}\) to \(\delta^{18}\text{O}_{sw}\) is controlled by both stomatal conductance to water, \(g_{sw}\) and VPD (Figure 3b-d). Since transpiration is linearly proportional to VPD, small changes in \(g_{sw}\) have a large effect on net transpiration (and thus on \(\delta^{18}\text{O}_{sw}\)) when VPD is large (see Figure 3).

Finally, tree ring cellulose \(\delta^{18}\text{O}\) (\(\delta^{18}\text{O}_{TR}\)) depends on post-photosynthetic fractionation, which occurs when sugars exchange oxygen with stem water during cellulose synthesis (step 3 in Figure 1b). According to Sternberg (2009) this fractionation can be parameterized as:

\[
\delta^{18}\text{O}_{\text{TR}} = \phi(\delta^{18}\text{O}_{sw} + \Delta) + ((1 - \phi) \ast (\delta^{18}\text{O}_{\text{sub}})) \tag{7}
\]

Here \(\phi \approx -0.4\) is the proportion of oxygen from sugars that exchanged with stem water during this process, \(\Delta\) is the average fractionation of the oxygen that exchanged with stem water and \(\delta^{18}\text{O}_{\text{sub}}\) is the \(\delta^{18}\text{O}\) of sugars that did not exchange with water during cellulose synthesis. Therefore, \(\phi\) tends to reinforce the source water \(\delta^{18}\text{O}\) signal in tree ring cellulose, without completely erasing the leaf water enrichment signal. Experiments have shown that ~40\% of the sugars exchange oxygen with stem water before incorporation into cellulose, with variations between different tree species (DeNiro and Cooper 1989, Luo and Sternberg 1992, Cernusak et al. 2005, Sternberg et al. 2006).

In summary, three processes control \(\delta^{18}\text{O}_{TR}\): (i) source water \(\delta^{18}\text{O}\); (ii) enrichment of source water in the leaf during evaporation, which depends on VPD and leaf transpiration rates (see Figure 3); and (iii) the degree of exchange of oxygen in exported sugars with stem water during cellulose synthesis. The degree of leaf enrichment increases linearly with increasing VPD, but also depends on transpiration rate (due to back-diffusion), and is thus related to \(g_{sw}\) and VPD. The sensitivity of leaf enrichment to \(g_{sw}\) is predicted to be highest under high VPD or low relative humidity (see Figure 3).

We thus expect that intra-annual variation of \(\delta^{18}\text{O}_{TR}\) will be primarily influenced by the seasonal cycle of source water or precipitation \(\delta^{18}\text{O}\), which varies quite strongly over trees’ growing season (Figure 2e, k, q and v). Leaf level enrichment processes will add to this ‘background’ variation by causing enrichment, which is expected to be greater under higher VPD and expected to be more strongly modulated by \(g_{sw}\) under drier conditions (see Figure 3).

Materials and methods

\(\delta^{18}\text{O}_{TR}\) and \(\delta^{13}\text{C}_{TR}\) predictions

To make our expectations more quantitative we have used the tree ring-isotope models described in the section Isotopes in tree rings: theory and modelling to predict the sensitivity of intra-annual variation in both isotopes to weather conditions during the trees’ growing seasons. Climatic variables that influence these predictions were VPD, temperature and source water \(\delta^{18}\text{O}\). Our predictions also depend on estimated responses to \(g_{sw}\) and \(g_{m}\) to the climatic variables. For both isotopes, stomatal conductance to water and CO2 were calculated as a function of VPD via:

\[
g_{sw} = 1.6g_{ic} = g_{m}\max\left(\frac{1}{1 + \frac{\text{VPD}}{\text{VPD}_{\text{mean}}}}\right) \tag{8}
\]

Here \(g_{m}\max\approx 0.5 \text{ mol m}^{-2} \text{ s}^{-1}\) is an assumed value for maximum \(g_{sw}\) and \(\text{VPD}_{\text{mean}}\) is the long-term mean of VPD during the growing season of each tree. For \(\delta^{13}\text{C}_{TR}\), \(g_{m}\) was estimated as a linear function of temperature via:

\[
g_{m} = g_{m25}(0.44 + 0.0587) \tag{9}
\]

(Evans and von Caemmerer 2013), where \(g_{m25}\) is the \(g_{m}\) at 25°C and \(T\) is temperature in Celsius. As \(g_{m}\) is highly variable between species (von Caemmerer and Evans 2015) and we have no information about \(g_{m}\) for either of the species in this study, we considered an assumed of \(g_{m25} = 0.19 \text{ mol m}^{-2} \text{ s}^{-1}\) for both species. For \(\delta^{13}\text{C}_{TR}\) predictions, we ignored carbon remobilization, as we lacked sufficient insight to quantify these processes. We also did not consider any seasonal variations in the growth rates of the trees, as we lack information on growth rhythm during the growing season for the two tree species at the study sites. Further details of the models and parameters used for predictions of \(\delta^{18}\text{O}_{TR}\) and \(\delta^{13}\text{C}_{TR}\) can be found in Table S2 available as Supplementary Data at Tree Physiology Online.

Sites and species selection

Terra firme sites and species

Two terra firme sites (i.e., non-flooded) differing in total annual rainfall were selected for this study (Figure 2a): a wet site in the Peruvian Amazon with total annual precipitation of 2500 mm (−4° 54' 00" N, −73° 47' 59.62"E), and a moist site in the Bolivian Amazon with 1700 mm annual precipitation (−10° 59' 60"N, −65° 00' 00"E). Precipitation in the moist site is highly seasonal, dropping below 100 mm per month for up to 5 months per year, while
mean monthly precipitation in the wet terra firme site rarely drops below 100 mm.

The species we choose for terra firme forests, *C. odorata*, grows during the wet season and stops growing at the onset of the dry season, when it sheds its leaves (Dünisch et al. 2003, Costa et al. 2013). New leaf flush occurs several weeks later at the end of the dry season (Dünisch et al. 2003, Brienen and Zuidema 2005). Previous studies on *C. odorata* from the south west of the Amazon basin have shown that $\delta^{18}$O$_{TR}$ reflects rain-out processes upwind of the growth site, and thus are a good proxy for basin-wide rainfall in the Amazon (Brienen et al. 2012, Baker et al. 2016).

**Seasonal floodplain sites and species** Várzea forests are one of the most representative seasonal floodplain forests, supporting annual flooding of up to 7 m (Wittmann et al. 2012). Two várzea floodplain sites were selected, a 'wet floodplain site' in the Colombian Amazon receiving 2600 mm of annual precipitation ($−4^\circ$–$30^\circ$ $00'O$N, $−70^\circ$–$00' 00'E$), and a 'moist site' in the Bolivian Amazon receiving 1700 mm of annual rainfall ($−11^\circ$–$33^\circ$ $30'O$N, $−67^\circ$–$18' 54'E$).

*Macrolobium acaciifolium*, the species chosen for this environment, renews its canopy during the flooded period, which lasts for ~6 months, after which growth restarts, often when trees are still flooded (Schöngart et al. 2002). Growth rates are highest in the beginning of the terrestrial phase just after the flooding recedes and stops once the trees get flooded due to anoxic conditions around the roots (Schöngart et al. 2005). The terrestrial phase starts at the peak of the dry season in the wet floodplain site and during wet-dry season transition at the moist floodplain site. This species forms annual rings that follow the annual cycle of the flood-pulse of the rivers (Schöngart et al. 2005, Assahira et al. 2017).

**Tree ring sampling and isotopes analysis**

For each C. *odorata* tree we cut a disc, and 10 mm cores were extracted from *M. acaciifolium* trees. One of the selected C. *odorata* trees is part of a published oxygen isotopes chronology that has been validated by radioncarbon dating (Baker et al. 2017). Tree rings were microscopically identified by wood anatomical features. For two samples of each species (one sample per site; see Figure 2), 9–11 rings were cut into thin segments of $0.02$–$3$ mm for the intra-annual high resolution analysis. Very thin segments of $0.02$ mm were cut using a core microtome (Gärtner and Nievergelt 2010). On average, rings were separated into $10$–$22$ sections parallel to ring boundaries, although for a few very narrow rings only five sections could be cut. For *M. acaciifolium*, six additional samples were cut into three even-sized segments for the intra-annual medium resolution analysis. This was done to assess the representativeness of the floodplain trees for the general patterns in these environments. Three of these six additional samples are from the moist floodplain site and the other three are from a wet floodplain site located 500 km upstream of the wet floodplain site shown in Figure 2.

Cellulose was extracted from the wood using the Brendell et al. (2000) method, except for C. *odorata* from moist terra firme site where cellulose was extracted following Wieloch et al. (2011). Only the carbon isotope series from the west Amazonian floodplain was based on wholewood. Samples were freeze-dried and weighed in a precision balance to pack $0.5 \pm 0.05$ mg of samples in silver capsules for $\delta^{18}$O analysis and $1 \pm 0.1$ mg in tin capsules for $\delta^{13}$C analysis. Isotope analysis was done at the University of Leicester using an Isotopes Mass Spectrometer (Sercon 20-20 IRMS, Sercon IRMS, Crewe-UK) with precision of $0.15\%$.

**Climate data**

Local monthly precipitation, vapour pressure, temperature and cloud cover data for all sites were obtained from Climate Research Unit (CRU TS 4.00 0.5° resolution). Daily river stage data from the sites in Colombia and Bolivia were obtained from the nearest river gauging stations through the Hidroweb portal (http://www.snirh.gov.br/hidroweb/) from the Brazilian National System of Hydric Resources Information (SNIRH). As there were no local station data available for the site in Peru, we used instead monthly river data from the virtual river gauging station data from The Environmental Research Observatory (ORE) Geodynamical, Hydrological and Biogeochemical control of erosion/alteration and material transport in the Amazon basin (HYBAM).

The $\delta^{18}$O-data for precipitation were obtained from the Global Network of Isotopes in Precipitation and in the Global Network of Isotopes in River (GNIP and GNIR), accessed through the Water Isotopes System for Data Analysis, Visualization and Electronic Review (WISER, http://nds121.iaea.org/wiser/index.php). For the Bolivia site, we complemented this with monthly precipitation $\delta^{18}$O data (M. Gloor and R.J. Brienen, unpublished data).

Seasonal changes in climate were calculated for the same calendar years of the analysed tree rings at each site. Figure 2 shows the seasonal changes in monthly precipitation, inundation (floodplain sites only), VPD, cloud cover and rainfall $\delta^{18}$O for all studied sites.

**Data analysis**

For each site, seasonal changes in intra-ring $\delta^{18}$O$_{TR}$ and $\delta^{13}$C$_{TR}$ were predicted using the available climatic data (see Climate data) from the site-specific growing seasons. Site-specific growing seasons were defined for each tree using the available information on growth rhythms for the *M. acaciifolium* (Schöngart et al. 2002) and C. *odorata* (Dünisch et al. 2003, Costa et al. 2013). These vary considerably between the terra firme (wet site: September–June; moist site: October–April) and floodplain sites (wet site: May–November; moist site: April–October). The model inputs used for the tree-ring isotopes predictions are...
presented in Table S2 available as Supplementary Data at Tree Physiology Online.

Covariation between observed isotope records was assessed using Pearson correlation coefficient. To assess the effect of inter-annual variation in climate on tree ring isotopes, we calculated mean isotope values for the complete ring. We then related the inter-annual variation in δ\(^{13}\)C and δ\(^{18}\)O for the full ring to local temperature, rainfall and cloud cover during the entire growing season of the trees. We did not consider VPD for these analyses, as the available data may not be accurate enough at the inter-annual level. In addition to local climate variables, we also considered Amazon basin-wide precipitation, which has been shown to influence local precipitation δ\(^{18}\)O (Baker et al. 2016). Amazon basin-wide precipitation was calculated as the spatially integrated mean precipitation for the hydrological basin (see Baker et al. 2016). These analyses were done for the four trees with high intra-annual resolution.

In order to further explore the effects of seasonal climate variation on tree ring isotopic composition, we also calculated the dry season length and climate means over moving periods of 2–8 months across the trees’ growing seasons. Dry season length was defined as in Marengo et al. (2001) and calculated using daily rainfall data from Tropical Rainfall Measuring Mission (TRMM 3B42 0.25° resolution). These climate means were correlated with mean isotope values for the whole ring and with the mean isotope values for three intra-ring segments. For these analyses, the six M. acaciifolium oxygen series with medium intra-ring resolution were also included.

All analyses were done using the data analysis tool R, version 3.2.3.

Results

Predicted δ\(^{13}\)C\(_{\text{TR}}\) and δ\(^{18}\)O\(_{\text{TR}}\) patterns for each site showed different contributions from seasonal changes in δ\(^{18}\)O\(_{\text{sw}}\), VPD and temperature, and from estimated responses of \(g_{\text{sw}}\) and \(g_{\text{m}}\). For δ\(^{18}\)O\(_{\text{TR}}\) most of the predicted variation comes from δ\(^{18}\)O\(_{\text{sw}}\), but leaf water enrichment caused by changes in VPD and \(g_{\text{sw}}\) responses also contribute significantly to predicted δ\(^{18}\)O\(_{\text{TR}}\) for the two trees growing at the drier sites (i.e., the moist floodplain and moist terra firme site; see Figure 2f, l, r and x and Figure S2 available as Supplementary Data at Tree Physiology Online).

Using two different assumptions for effective pathlength (L) we also note that pathlength significantly affects the Péclet effect, especially under low relative humidity (see Figure S3 available as Supplementary Data at Tree Physiology Online, see also Kahmen et al. 2008, Holloway-Phillips et al. 2016). For our predictions however, we did not vary pathlength, as we had no species-specific data on pathlengths, and predicted contributions of leaf water enrichment to δ\(^{18}\)O\(_{\text{TR}}\) thus reflects only site differences in VPD and \(g_{\text{sw}}\) (not pathlength difference).

For δ\(^{13}\)C\(_{\text{TR}}\) and \(g_{\text{sc}}\) responses to VPD contributed to most of the predicted δ\(^{13}\)C\(_{\text{TR}}\) variations in all trees, except for the C. odorata from the wet terra firme site, which showed weak δ\(^{13}\)C\(_{\text{TR}}\) variations (Figure 2g, m, s and y). Temperature effects over \(g_{\text{m}}\) also contributed significantly to the predicted δ\(^{13}\)C\(_{\text{TR}}\) patterns for M. acaciifolium the moist floodplain site—where seasonal temperature variations were biggest—but showed little contribution for the other trees (see Figure S4 available as Supplementary Data at Tree Physiology Online).

Observed within-ring δ\(^{18}\)O\(_{\text{TR}}\) variation matches the predicted patterns very well in all four trees with high intra-ring resolution (Figure 4—blue lines), although the within-ring amplitude was about two times lower for the observed patterns (4–5‰) compared with predictions (~10‰; see Table 1). The observed δ\(^{13}\)C\(_{\text{TR}}\) patterns matches predictions quite well in the two C. odorata trees from the terra firme sites and for the M. acaciifolium tree from the moist floodplain site (Figure 4b and c, and right panels—red lines), but less well for the M. acaciifolium tree from the wet floodplain sites (Figure 4a and d, and right panels—red lines). The observed average amplitude for δ\(^{13}\)C\(_{\text{TR}}\) is similar to predictions (~1‰). δ\(^{13}\)C\(_{\text{TR}}\) in the initial section of individual rings was frequently higher than predicted in the M. acaciifolium from the wet floodplain and in some years for the C. odorata from moist terra firme, both of which show pronounced δ\(^{13}\)C\(_{\text{TR}}\) increases of up to 2‰ across ring boundaries (Figure 4b and c—red lines).

Comparison of δ\(^{13}\)C\(_{\text{TR}}\) and δ\(^{18}\)O\(_{\text{TR}}\) time series showed strong common features for some trees but less so for others. Within-ring δ\(^{13}\)C and δ\(^{18}\)O cycles in the floodplain trees were correlated in several years, especially at the moist site (Figure 4a and Table 1). In contrast, the terra firme trees only showed significant correlations between δ\(^{18}\)O\(_{\text{TR}}\) and δ\(^{13}\)C\(_{\text{TR}}\) in those rings with exceptionally large within-ring variations (Figure 4c and Table 1).

We also note that the δ\(^{18}\)O time series of the two terra firme trees showed very similar patterns both on short and longer time scales (see Figure S5 available as Supplementary Data at Tree Physiology Online).

The regression analysis between climate variables and the tree-ring isotopes series revealed various significant correlations. For the two moist sites (terra firme and floodplain), we found that mean inter-annual ring δ\(^{13}\)C was negatively correlated with local precipitation during the driest period of their growing seasons (Figure 5). No climatic effects on tree ring δ\(^{13}\)C were found for the trees from the wet sites. Inter-annual mean ring δ\(^{18}\)O variation of the two M. acaciifolium trees from the floodplain sites was positively correlated with cloud cover during the growing season (\(P < 0.1\)), while δ\(^{18}\)O of the C. odorata trees from the terra firme sites was negatively correlated with Amazon-wide precipitation amounts (Figure 5).
Additional significant correlations with climate variables were found for specific ring segments and for shorter periods of 4–8 months within the growing season, showing the same overall pattern described above. Here we show correlations for sequentially moving windows of 4 and 6 months duration, as these summarize the patterns observed for both longer and shorter
time spans (see Figure S6 available as Supplementary Data at Tree Physiology Online). These analyses included three additional *M. acaciifolium* trees per site (see Figure S7 available as Supplementary Data at Tree Physiology Online). For the *M. acaciifolium* trees from the wet floodplain site, average tree ring δ¹⁸O was also higher when the dry season started earlier (corr. Coef = 0.59, \( P < 0.05 \)) or was longer (corr. Coef = 0.64, \( P < 0.05 \)). Finally, for all trees the isotopic composition of specific ring segments showed generally correlations with more climate variables compared with mean ring isotopic time-series.

**Discussion**

**High-resolution intra-ring variation in δ¹³C and δ¹⁸O**

Our results show that the observed intra-annual δ¹³C_tr patterns agree reasonably well with the predicted δ¹³C_tr patterns, and that δ¹³C_tr variations thus follow seasonal variation in VPD (since our model does not include NSC reserve remobilization). For the floodplain species *M. acaciifolium*, within-ring variation in δ¹³C_tr shows strong positive peaks in δ¹³C_tr in the initial or middle sections of the ring. These δ¹³C_tr peaks coincide with the drier conditions at the initial and middle periods of the growing season at the wet and moist site, respectively. In the moist terra firme tree, we find decreases in δ¹³C_tr from the initial to the final sections of the ring, which is consistent with steadily decreasing water stress as the rainy season progresses. As predicted, we find a rather constant mean δ¹³C_tr at the wet terra firme site due to generally humid conditions throughout the growing season of this tree (Figure 4). Thus, observed δ¹³C_tr patterns are largely consistent with expected plant stomatal responses to changes in VPD during the growing season of each tree. Our model also included temperature effect on \( g_m \). This
effect slightly improved the match between predicted and observed δ\(^{13}\)C\(_{\text{TR}}\) patterns for the M. acaciifolium trees from the moist floodplain site (Figure 4 and see Figure S4 available as Supplementary Data at Tree Physiology Online). For this tree, we also noted that seasonal temperature variations predict relatively large variation in δ\(^{13}\)C\(_{\text{TR}}\) due to mesophyll conductance without requiring any change in g\(_{\text{m}}\) (see Figure S4e available as Supplementary Data at Tree Physiology Online). While this effect is only predicted in one tree, it shows that the temperature effects on g\(_{\text{m}}\) could significantly influence seasonal variations in δ\(^{13}\)C\(_{\text{TR}}\) and may be more important to isotope discrimination than generally assumed (Griffiths and Helliker 2013, von Caemmerer and Evans 2015).

In addition to climate effects, we expected to observe effects of NSC remobilization on intra-annual δ\(^{13}\)C\(_{\text{TR}}\). Both investigated species completely change their leaves annually and remain leafless for several weeks (C. odorata) or some days (M. acaciifolium) (Schöngart et al. 2002, Dünisch et al. 2003). We thus expected sharp increases in δ\(^{13}\)C\(_{\text{TR}}\) values at tree ring boundaries in the records of all trees, related to starch-dependent stem growth before initial leaf flush. We only observe clear sharp increases in δ\(^{13}\)C\(_{\text{TR}}\) for M. acaciifolium from the wet floodplain site (Figure 4b). As these sharp increases occurred exactly across the ring boundaries, and as peak δ\(^{13}\)C\(_{\text{TR}}\) due to climate was predicted to occur later in the season (see Figure 4p), we suspect these patterns may be due to use of NSC at the start of the growing season. We also find smaller peaks early in the rings of C. odorata from the moist site. Cedrela odorata is indeed known to use starch from the previous year for stem growth at relatively dry sites (e.g., Gebrekirstos et al. 2009, Fichtler et al. 2010, Brienen et al. 2012). The only tree for which we do not find a relationship with precipitation in the expected direction is the C. odorata tree growing at the wet terra firme site. This is probably because the site is so wet (precipitation rarely drops below 100 mm per month). We have no explanation for the observed positive correlation between precipitation and δ\(^{13}\)C in this tree.

The relations between climate variables and tree-ring oxygen isotopes suggest that different dominant drivers control inter-annual variation in δ\(^{18}\)O in the two species; in the M. acaciifolium floodplain trees δ\(^{18}\)O covaries with temperature, cloud cover, local precipitation and dry season length, while for the two C. odorata terra firme trees δ\(^{18}\)O covaries with basin-wide precipitation amount (Figure 5 and see Figure S6 available as Supplementary Data at Tree Physiology Online). Temperature, cloud cover and precipitation may reach increasingly stressful levels when the dry season is longer, and probably affect δ\(^{18}\)O in floodplain trees primarily via their effects on evaporative leaf water enrichment above source water δ\(^{18}\)O. Leaf water enrichment depends on water vapour pressure of the atmosphere, air temperature, isotopic composition of atmospheric water, leaf temperature and stomatal conductance (Barbour et al. 2000, Barbour and Barbour 2007, Kahmen et al. 2008; see Figure 1). Surprisingly, tree-ring δ\(^{18}\)O for both floodplain trees correlates most strongly with cloud cover, and this pattern is still consistent in the analysis including the six trees with medium intra-ring resolution oxygen series (see Figure S6 available as Supplementary Data at Tree Physiology Online). This may be because reduced cloud cover during the dry season may lead to higher leaf temperatures (Doughty and Goulden 2009), lower air humidity (Quaas 2012) and thus raised leaf-to-air vapour pressure difference (VPD), and consequently reductions in stomatal conductance (Lloyd and Farquhar 2008).
Overall, these results are consistent with theory and experimental studies of the effects of local moisture conditions over leaf water enrichment and $\delta^{18}O_{TR}$ variations (Barbour and Farquhar 2000, Barbour et al. 2000, Barbour 2007, Cernusak et al. 2016).

For the terra firme trees, which grow during the wet season, tree ring $\delta^{18}O$ is mainly influenced by Amazon basin-wide precipitation (Figure 5), and less by local climate (see Figure S6 available as Supplementary Data at Tree Physiology Online). This suggests that the tree ring $\delta^{18}O_{TR}$ signal is a precipitation $\delta^{18}O_{TR}$ signal. Precipitation $\delta^{18}O_{TR}$ in turn is the result of the cumulative effects of all precipitation events upwind from the sites. This is because heavy water isotopes are gradually removed at each precipitation event during moisture transport from the tropical Atlantic to the study sites, so precipitation will be more depleted in $\delta^{18}O$ during years with more rain over the Amazon, assuming incoming $\delta^{18}O_{TR}$ does not vary from year to year (Salati and Vose 1984, Vimeux et al. 2005, Villacís et al. 2008). These results are consistent with the known precipitation $\delta^{18}O$ influence on $\delta^{18}O_{TR}$ in C. odorata tree rings (Brienen et al. 2012, Baker et al. 2015, 2016). In line with this is the coherence in $\delta^{18}O_{TR}$ patterns within rings (intra-annually) observed for the two terra firme sites, C. odorata trees from Bolivia and Peru, which are ~1000 km apart (see Figure S5 available as Supplementary Data at Tree Physiology Online). These results demonstrate that source water is the dominant influence on tree ring $\delta^{18}O$ for these C. odorata trees. We note, however, that $\delta^{18}O$ for the C. odorata tree at the moist site showed some (weak) correlations with local precipitation during the start of the growing season (see Figure S6 available as Supplementary Data at Tree Physiology Online), consistent with weak local effects observed in longer tree-ring $\delta^{18}O_{TR}$ series from the same site in Bolivia (Brienen et al. 2012). Also consistent with this is the predicted contribution of leaf enrichment to $\delta^{18}O_{TR}$ in initial ring sections of this tree, caused by relatively dry conditions during the start of the growing season (Figure 4a). In all, for these C. odorata trees, source water is the dominant signal, with possible weak influences of local precipitation at the start of the growing season for the moist trees. Although we do not have replications for these trees, these results are consistent with previous studies which show that mean inter-annual tree-ring $\delta^{18}O$ variations of C. odorata trees from different sites (including our same moist terra firme site) are driven by source water $\delta^{18}O$ (Brienen et al. 2012, Baker et al. 2015, 2016).

Dual-isotope analysis

A striking property of the isotope records is the strong intra- and inter-annual covariation between carbon and oxygen isotopes in the floodplain M. accaciifolium trees (see Figure 4, Table 1 and see Figure S8 available as Supplementary Data at Tree Physiology Online). This covariation is particularly strong for the M. accaciifolium tree from the moist floodplain site with highly similar features in the intra-annual patterns (Figure 4a), and also good correlations between mean annual $\delta^{13}C_{TR}$ and $\delta^{18}O_{TR}$ (see Figure S8 available as Supplementary Data at Tree Physiology Online). Such strongly covarying patterns suggest a common driver. The one common process for $\delta^{13}C_{TR}$ and $\delta^{18}O_{TR}$ is the response of stomatal conductance to water status of the soil–plant continuum and VPD. These results thus strongly support the climate-$\delta^{18}O_{TR}$ analysis for this species, which suggests that variation in $\delta^{18}O_{TR}$ is primarily controlled by leaf $^{18}O$ enrichment, and that the initial source water signal is dampened in the final tree ring $\delta^{18}O$ signal.

In contrast, in the two terra firme trees, the $\delta^{18}O_{TR}$ and $\delta^{13}C_{TR}$ records of each tree are generally uncorrelated (Figure 4c and d), indicating that they are not both primarily influenced by stomatal conductance effects on leaf $^{13}C$-discrimination and/or on leaf $^{18}O$ enrichment. This decoupling of variation in $\delta^{13}C_{TR}$ and $\delta^{18}O_{TR}$ in this species could be due to a lack of control of stomatal conductance on $\delta^{13}C_{TR}$, $\delta^{18}O_{TR}$ or both. As we observe a weak negative relation between $\delta^{13}C_{TR}$ and precipitation only at the moist site and an opposite relation at the wet site, control of stomatal conductance on leaf $^{13}C$-discrimination seems to be weaker in C. odorata. While this is one possible explanation, a more plausible reason for the lack of covariation between $\delta^{18}O_{TR}$ and $\delta^{13}C_{TR}$ is that $\delta^{18}O_{TR}$ in this species mainly records variation in source water $\delta^{18}O$ and only weak local climate effects, as we showed here (see Figure S6a available as Supplementary Data at Tree Physiology Online) and also in Brienen et al. (2012). This lack of $^{18}O$ leaf enrichment signals in $\delta^{18}O_{TR}$ for C. odorata may be due to either low levels of leaf enrichment above the source water in the leaf, or because any occurring leaf isotope enrichment is not transferred to the final tree ring $\delta^{18}O$ in this species because of extensive exchange of leaf exported sugar with stem water during cellulose synthesis (Sternberg 2008). The former explanation, a lack of leaf enrichment, could be due to species-specific leaf traits, such as higher leaf transpiration rates and/or longer effective pathlengths reducing strongly the effect of back diffusion on average leaf $\delta^{18}O$ (Kahmen et al. 2008, Cernusak and Kahmen 2013; see also Figure S3 available as Supplementary Data at Tree Physiology Online).

Apart from purely species-specific effects, differences in the leaf enrichment contributions to $\delta^{18}O_{TR}$ for the four trees could also be influenced by variation in trees’ growing season humidity. Predictions from isotope theory, confirmed by lab experiments (Rodén and Ehleringer 1999, Roden and Farquhar 2012), are that leaf water enrichment above the plant source water is small for trees growing in humid conditions, and increases with increasing VPD (Barbour et al. 2002, 2004, Roden and Siegwolf 2012). Interestingly, for each species, the tree growing at the drier sites (moist floodplain and moist terra firme) showed stronger correlations between intra-annual...
δ\^{18}O_{TR} and δ\^{13}C_{TR} variations than the tree growing at the wetter site (wet floodplain and wet terra firme). This provides some indication that growing season water availability/relative humidity may control the strength of the covariation between both stable isotopes in tree rings of tropical trees. More research is needed to assess how environmental conditions, specifically relative humidity, affect the strength of source water vs leaf enrichment signals in tree rings.

Conclusions

We investigated δ\^{13}C and δ\^{18}O in cellulose of two Amazon terra firma and two floodplain trees located along a precipitation gradient. We show here that intra-annual variation in isotopes (δ\^{13}C_{TR} and δ\^{18}O_{TR}) in four Amazon trees growing in different environments follow predictions based on isotopic theory. Observed intra-annual variation in δ\^{13}C_{TR} agreed well with Farquhar’s model of leaf level 13C discrimination considering stomatal responses to seasonal variation in VPD and temperature effects on mesophyll conductance, and suggest a direct transfer of climate signals from leaf to tree ring. We do also find some signatures of post-photosynthetic carbon remobilization effects on δ\^{13}C_{TR}, which are especially clear in the wet floodplain tree and to a lesser degree in the moist terra firme tree. Intra-annual variation in δ\^{18}O_{TR} closely matched seasonal variation in source water and the predicted effects of leaf water enrichment due to variation in VPD.

The inter-annual variation in δ\^{13}C_{TR} was controlled by local precipitation for trees at the drier growing conditions, but not at the wettest site. Inter-annual variation in δ\^{18}O_{TR} showed different controls in the two species; the floodplain species M. acacii revealed variation in leaf water enrichment in response to local climate (cloud cover), while the terra firme species C. odorata recorded source water δ\^{18}O variation, which is controlled by large-scale rainout signals (i.e., basin-wide precipitation).

The four trees showed differences in the degree of covariation between δ\^{13}C_{TR} and δ\^{18}O_{TR} with the strongest covariation in the floodplain tree experiencing the driest growing conditions and lowest covariation for the wet terra firme tree. Higher covariation in the drier sites are most likely the result of stomatal responses to δ\^{18}O_{TR} signal (in the wettest site) to primarily leaf level process dominated δ\^{18}O_{TR} signal (in the driest site). Our data cannot reveal whether variation in control of δ\^{18}O_{TR} signals is caused by species-specific differences in physiology (C. odorata vs M. acacii), or truly reflects a dominant influence of VPD gradients.

Our results provide some clear insights, but also raise new questions. Firstly, we showed that across the four trees, δ\^{13}C_{TR} reflected primarily photosynthetic carbon-discrimination responses to humidity and temperature. We also found signatures of carbon remobilization effects, but surprisingly, these were not linked to species phenology. More research under what circumstances carbon remobilization occurs and how it affects δ\^{13}C_{TR} will help interpreting tree ring isotope signals. Secondly, our results suggest that δ\^{18}O_{TR} can be controlled by very different processes, source water δ\^{18}O variation vs leaf water enrichment, but it remains unclear which process dominates when and under what circumstances. Difference in the controls on δ\^{18}O_{TR} have profound implications for the interpretation of δ\^{18}O_{TR} in palaeo-climatic and plant physiological studies. For example, source water δ\^{18}O signals may record large-scale rainout information over continents (Brienen et al. 2012, Baker et al. 2016), or hurricane influences on coastal sites (Miller et al. 2006), while leaf water enrichment signals are expected to reflect climate variation of a much more local nature via VPD (i.e., Kahmen et al. 2008).

Supplementary Data

Supplementary Data for this article are available at Tree Physiology Online.

Conflict of interest

None declared.

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