

The δD records of *n*-alkane and *n*-alkanoic acid of tropical trees reflect δD of precipitation during the early stages of the leaf growth

Amrita Saishree¹, Shreyas Managave^{1*}, M. G. Yadava², Salam Maheshwori Devi¹, Prasanta Sanyal³

¹Earth and Climate Sciences, Indian Institute of Science Education and Research Pune, Pune 411008, India

²Physical Research Laboratory, Navrangpura, Ahmedabad, Gujarat 380009, India

³Department of Earth Sciences, Indian Institute of Science Education and Research Kolkata, Mohanpur 741246, India

* Corresponding author: Shreyas Managave; Email: shreyas@iiserpune.ac.in

Plain Language Summary

The hydrogen isotopic compositions of leaf wax compounds have been used extensively for reconstructing centennial-scale climate variability. However, ambiguity exists regarding the climate of which season these records reflect. Here, our long-term experiment, wherein tropical evergreen and deciduous species were irrigated with isotopically-labelled water, revealed that the hydrogen isotope record of both *n*-alkanes and *n*-alkanoic acids represents the climatic conditions prevailing during the early stages of the leaf growth. The climate models aimed at reproducing the leaf-wax-based hydrogen isotopic composition of precipitation should consider this bias while evaluating their predictive skills.

Abstract

The hydrogen isotopic compositions of the leaf-wax *n*-alkanes (δD_{alk}) and *n*-alkanoic acids (δD_{acid}) are known to reflect ambient climatic conditions (including precipitation δD values, δD_{Precip}). However, the climatic conditions of exactly which period (i.e. early or entire period of the leaf's lifespan) these biomarkers represent, i.e. the seasonality in δD_{alk} and δD_{acid} records, is still evolving. The seasonality studies on the δD_{alk} and δD_{acid} values, done only in extra-tropical regions, mostly indicate the δD_{alk} values are biased towards the early growing season whereas δD_{acid} values are not biased towards any season. To decipher the seasonality in the δD_{alk} and δD_{acid} records from the tropics, we conducted a long-duration experiment wherein deciduous and evergreen species were grown using normal water ($\delta D = -2\text{‰}$) during the early stages of the leaf's growth and later using isotopically-labeled water ($\delta D = 1000\text{‰}$). Our experiment revealed (i) in deciduous and evergreen species, δD_{alk} and δD_{acid} values reflect δD_{Precip} during the early stages of the leaf's growth, (ii) synchronous synthesis of *n*-alkanes and *n*-alkanoic acids, and (iii) in deciduous species, minor incorporation of the previous year's photosynthates in the leaf wax pool of the current year's mature leaves. Our study suggests that the δD_{alk} and δD_{acid} records in the tropics are biased towards the climatic conditions prevailing during the early stages of the leaf's growth. This bias should be considered while comparing the δD_{Precip} values generated from the leaf wax proxy records and isotope-enabled atmospheric circulation models.

Keywords: seasonality, paleoclimate, leaf wax, tropics, hydrogen isotope, climate models

1. Introduction

The hydrogen isotope (δD) values of leaf wax *n*-alkanes (δD_{alk}) (Hren et al., 2010; Tipple and Pagani, 2010; Collins et al., 2013; Ghosh et al., 2020) and *n*-alkanoic acids (δD_{acid}) (Tierney et al., 2008; Konecky et al., 2011; Feakins et al., 2014; Feakins et al., 2019; Managave et al., 2023) have been extensively used for reconstruction of centennial to millennial-scale climate variability. The leaf wax-based investigations have also generated past variability in the δD values of precipitation (δD_{Precip}) (Konecky et al., 2016; Ghosh et al., 2020; McGrath et al., 2021; Managave et al., 2023). Various isotope-enabled Global Circulation Models (GCMs) can simulate water isotopic composition of the precipitation (Sturm et al., 2010). The leaf wax-based reconstructed δD_{Precip} values thus allow validation of the outputs of the isotope-enabled GCMs simulations (Knapp et al., 2022). However, the clarity on δD_{Precip} values of which season the δD records of these biomarkers represent is necessary for such model-proxy comparison.

The leaf wax compounds are known to reflect δD_{Precip} (Feakins et al., 2016; Tipple and Pagani, 2013; Sachse et al., 2012) and ambient climatic conditions (Smith and Freeman, 2006; Feakins and Sessions, 2010; Tipple et al., 2013) prevailing during their synthesis. Sachse et al., (2010) and Tipple et al., (2013) have reported the early growing season bias in δD_{alk} records i.e. the δD_{alk} records reveal the climatic conditions prevailing during the early growing season whereas Huang et al., (2018) and Sachse et al., (2009) suggested that the δD_{alk} records integrate the climate conditions of the entire growing season. Further, a few studies (Freimuth et al., 2017; Yang et al., 2021) have observed that the δD_{alk} records are biased toward the early growing season whereas, δD_{acid} records are not biased towards any particular season. Most of the studies

on seasonal variations in leaf wax δD records have been conducted in temperate (Sachse et al., 2009; Sachse et al., 2010; Tipple et al., 2013; Freimuth et al., 2017) and sub-tropical regions (Yang et al., 2021; Huang et al., 2018). The seasonality of δD_{alk} and δD_{acid} records in tropical regions remains unknown.

The effect of vegetation type (i.e. deciduous vs evergreen) on the seasonality in the leaf wax δD records is not clear. The bulk of research on the seasonality of leaf wax production has focused on deciduous species (Sachse et al., 2009; Tipple et al., 2013; Freimuth et al., 2017; Huang et al., 2018) which suggested early growing season bias in the δD_{alk} records. Deciduous plants utilize stored carbohydrate reserves, accumulated during the late growing season of the previous year, for the synthesis of leaf wax compounds in the new leaves of the current growing season (Sessions 2006; Tipple et al., 2013; Freimuth et al., 2017). This implies that the leaf wax δD values reflect the climatic conditions (and δD_{Precip} values prevailing during the late growing season of the previous year and the early growing season of the current year. The extent to which this affects the seasonality of the leaf wax δD records is not fully understood. Unlike the deciduous species, evergreen species have shown continuous production of *n*-alkanes and hence no seasonal bias in δD_{alk} records (Yang et al., 2021). More studies are required to verify whether this applies to evergreen species in general as well.

As the δD values of the leaf wax compounds reflect ambient conditions, the timing of *n*-alkanes and *n*-alkanoic acids synthesis in leaf wax determines δD_{Precip} values of which season their δD records preserve (Kahmen et al., 2011; Sachse et al., 2015; Huang et al., 2018). The seasonal variation in the abundance of leaf wax compounds and their isotopic compositions can be used to reveal the timing of leaf wax production (Tipple et al., 2013; Yang et al., 2021).

However, the utility of the former in this context is limited as is it affected by factors such as wind ablation (Freimuth et al., 2017), temperature stress, intense UV radiation, and insect attacks (Shepherd and Griffiths 2006; Jetter et al., 2006). Temporally varying δD values of the leaf wax compounds have been used to reveal seasonality in their production (Tipple et al., 2013; Freimuth et al., 2017; Huang et al., 2018; Yang et al., 2021). However, the leaf wax δD variability in the periodically sampled leaves that flushed together could stem from the inter-leaf δD variability (up to 38‰; Hou et al., 2007) and/or synthesis of the new wax (with different δD values). When the former is larger, the leaf wax δD variability in the periodically collected leaves may not unambiguously prove the production of new leaf wax compounds.

The efficacy of the experiments involving isotopically-labeled source water in studying seasonality in the production of *n*-alkanes in a tree (Kahmen et al., 2011), and *n*-alkanes and *n*-alkanoic acids in a grass (Gao et al., 2012) has been demonstrated. A short-duration (50 days) experiment on one temperate tree species, wherein a pulse of tracer water was applied for 7 days, had shown that the *n*-alkane pool, once formed, does not change subsequently in matured leaves (Kahmen et al., 2011). However, experiments of longer duration are necessary to assess the production of *n*-alkanes and *n*-alkanoic acids in mature leaves if the turnover rate of these compounds is slow. Further, the suggestion that *n*-alkanes and *n*-alkanoic acids reflect climatic conditions during the early and entire growing seasons, respectively (Freimuth et al., 2017; Yang et al., 2021) has not been verified experimentally.

This study was aimed at understanding the seasonality in the δD_{alk} and δD_{acid} values of tropical deciduous and evergreen species. Here, by irrigating tropical evergreen and deciduous angiosperm trees with isotopically-labeled water for a longer duration and periodically

measuring the δD_{alk} and δD_{acid} values, we demonstrate that the leaf wax *n*-alkane and *n*-alkanoic acid preserve δD_{Precip} values prevailing during the early stages of the leaf's growth.

2. Materials and Methods

2.1 Experiment details

To know the leaf wax production pattern, an outdoor experiment was conducted at the Indian Institute of Science Education and Research Pune, Pune, India (18°32'44.9"N 73°48'30.0"E). Pune experiences a monsoonal climate with most of the rain occurring from June to September (Fig. S1). The saplings (2 to 3 years of age) of three deciduous (*Tectona grandis*, *Haldina cordifolia*, *Sterculia urens*) and four evergreen (*Syzygium cumini*, *Callophyllum inophyllum*, *Memecylon umbellatum* and *Diospyros malabarica*) angiosperm trees were grown under similar climate condition and with the same source water (Fig. S2). The trees were grown outdoors where they were exposed to ambient climate conditions. The selected plant species are found in mixed forests in the region (Deshpande et al., 1993).

2.1.1. Experiment to test the synthesis of new leaf wax in the mature leaves

Two irrigation regimes were employed in the year 2019: the first (till the 15th of August) with normal tap water ($\delta D = -2\text{‰}$) and the second (from the 17th of August to the 5th of December) with deuterium-labeled tracer water ($\delta D = 1000\text{‰}$) (Fig. S3). The pots were properly sealed to avoid an influx of precipitation or groundwater (Fig. S4). The plants were watered every alternate day. The water in excess of the field capacity of the soil was applied every time. Mature leaves of two individuals of all the species were collected periodically during both irrigation regimes. The first set of leaves was collected before isotopically-labeled water was

applied (on 12th August); after the application of isotopically-labeled water, the leaves were collected on 3rd September, 22nd October and 23rd November. The maturity of the sampled leaves was ensured by their lower position on the stem and periodic measurement of the leaf length and leaf mass per unit area (LMA) (Text S1, Fig. S5, S6). Most of the leaves on the deciduous species were matured before the application of the isotopically-labeled water and only a few leaves flushed subsequently. Should there be a synthesis of new leaf wax compounds in the mature leaves, the δD values of the leaves sampled during the second regime would reflect the δD signature of deuterium-labeled water.

2.1.2. Experiment to test the effect of carryover of photosynthates

To assess the effect of carryover of photosynthates from the end of the growing season of a year to the next on the seasonality in the leaf wax δD records, we continued the experiment on deciduous species (see Fig. S3 for details). The leaves showed senescence during the last week of November and the plants were irrigated with the isotopically-labelled water till the 5th of December 2019. Therefore, it is likely that the photosynthates that were transferred to the next year were synthesized using the isotopically-labeled water. After 5th December, the plants were irrigated again with the normal water to flush out the isotopically-labeled water from the soil. The shedding of leaves for different species happened over a period of ~3-4 months (i.e. from December to March). The plants were not irrigated for 47 days (from 13th January 2020 to 29th February 2020). We resumed watering with the normal tap water (once in four/five days) during the bud break period (March for the majority of the plants). The new leaves emerging at the beginning of the next growing season (i.e. year 2020) were collected in April, May and June. The trace of isotopically-labeled water in the leaf wax pool of the new leaves would indicate that

their δD values carry a signal of the δD of precipitation during the end of the growing season of the previous year.

2.2 Compound-specific investigations of n -alkanes and n -alkanoic acids

The leaf wax n -alkanes and n -alkanoic acids were extracted, quantified and analyzed for δD values at the Stable Isotope Laboratory of the Indian Institute of Science Education and Research Kolkata (SILIKA) (Text S2, S3, S4). The δD measurements were carried out using the Trace GC Ultra (Thermo Fisher Scientific, Strada Rivoltana 20090 Rodano, Milan, Italy), coupled with a MAT-253 IRMS via a GC Isolink (pyrolysis interface) and Thermo Fisher Scientific Conflo IV interface. The reproducibility of the standards during sample analysis was found to be $\pm 2\text{‰}$ ($1-\sigma$). The δD values for both n -alkanes and n -alkanoic acids were reported with respect to the Vienna Standard Mean Ocean Water (VSMOW).

2.3 Modeling of the extent of synthesis of new n -alkanes and n -alkanoic acids in the mature leaves

2.3.1 Modeled monthly δD_{alk} and δD_{acid} values if synthesized using the isotopically-labeled water alone

A conservative estimate of δD values of the new leaf wax compounds synthesized using the isotopically-labeled water alone during September, October and November were calculated by (i) modeling δD values of the leaf water during various months, (ii) estimating biosynthetic fractionations for various plants from the δD values leaf water and the biomarkers in the leaves collected prior to the application of isotopically-labeled water (for n -alkane, ε_{bio}^{alk*} ; for n -alkanoic

181 acid, $\varepsilon_{bio}^{acid*}$), and (iii) calculating the expected monthly δD values of leaf wax compounds from
 182 the modeled monthly leaf water δD values and the estimated biosynthetic fractionations. Table 1
 183 gives the details of the steps involved and notations used.

	Description	Notations	
Step 1	Modeling of the δD values of the leaf water for various months	$\delta D_{LW}^{Aug*}, \delta D_{LW}^{Sept*}, \delta D_{LW}^{Oct*}, \delta D_{LW}^{Nov*}$ (Equation S1)	
		<i>n</i> -alkane	<i>n</i> -alkanoic acid
Step 2	The measured monthly δD values of leaf wax compounds	$\delta D_{alk}^{Aug}, \delta D_{alk}^{Sept}, \delta D_{alk}^{Oct}, \delta D_{alk}^{Nov}$	$\delta D_{acid}^{Aug}, \delta D_{acid}^{Sept}, \delta D_{acid}^{Oct}, \delta D_{acid}^{Nov}$
	Estimation of the biosynthetic fractionation between δD_{LW}^{Aug*} and δD_{alk}^{Aug} (and δD_{acid}^{Aug})	ε_{bio}^{alk*} (Equation 1)	$\varepsilon_{bio}^{acid*}$ (Equation 2)
Step 3	Modeling of the expected monthly δD values of leaf wax compounds synthesized using the isotopically-labeled water alone	$\delta D_{alk}^{Sept*}, \delta D_{alk}^{Oct*}, \delta D_{alk}^{Nov*}$ (Equation 3)	$\delta D_{acid}^{Sept*}, \delta D_{acid}^{Oct*}, \delta D_{acid}^{Nov*}$ (Equation 4)

184 *Table 1. The steps and associated notations used to estimate the δD values of leaf wax*
 185 *compounds if synthesized using the isotopically-labeled water alone. Aug, Sept, Oct and Nov*
 186 *represent August, September, October and November, respectively. The terms with superscripted*
 187 *‘*’ indicate the modeled parameters.*

188 *Step 1:* The δD values of the leaf water during various months were calculated using the Craig-
 189 Gordon model, modified by Flanagan and Ehleringer (1991) (See Text S5 for details). The
 190 details of measurements of various atmospheric and plant physiological parameters are given in
 191 Text S5. *Step 2:* The ε_{bio}^{alk*} and $\varepsilon_{bio}^{acid*}$ for all plants were estimated using the following equations:

$$\varepsilon_{bio}^{alk*} = 1000 \times \left[\left(\frac{\delta D_{alk}^{Aug} + 1000}{\delta D_{LW}^{Aug*} + 1000} \right) - 1 \right] \quad (1)$$

$$\varepsilon_{bio}^{acid*} = 1000 \times \left[\left(\frac{\delta D_{acid}^{Aug} + 1000}{\delta D_{LW}^{Aug*} + 1000} \right) - 1 \right] \quad (2)$$

192 *Step 3:* The expected monthly δD_{alk} (for example, for November, δD_{alk}^{Nov*}) and δD_{acid} (for
 193 example, for November, δD_{acid}^{Nov*}) values if the leaf wax compounds were produced using the
 194 isotopically-labeled water alone were estimated as follows:

$$\delta D_{alk}^{Nov*} = \delta D_{LW}^{Nov*} + \varepsilon_{bio}^{alk*} \quad (3)$$

$$\delta D_{acid}^{Nov*} = \delta D_{LW}^{Nov*} + \varepsilon_{bio}^{acid*} \quad (4)$$

195 The expected δD_{alk} and δD_{acid} values were calculated for September and October as well.

196 2.3.2 Fraction of newly synthesized *n*-alkanes and *n*-alkanoic acids in mature leaves: a mass 197 balance approach

198 The fraction of newly synthesized leaf wax compounds was estimated using a mass balance
 199 approach. We considered (for example, for November) the δD_{alk}^{Aug} and δD_{alk}^{Nov*} as two end-
 200 members, and δD_{alk}^{Nov} as the mixture to estimate the fraction of newly synthesized *n*-alkanes
 201 (f_{new_alk}) during November, using the following equation:

$$f_{new_alk} = \frac{\delta D_{alk}^{Nov} - \delta D_{alk}^{Aug}}{\delta D_{alk}^{Nov*} - \delta D_{alk}^{Aug}} \quad (5)$$

202 Similarly, f_{new_acid} during November was estimated as:

$$f_{new_acid} = \frac{\delta D_{acid}^{Nov} - \delta D_{acid}^{Aug}}{\delta D_{acid}^{Nov*} - \delta D_{acid}^{Aug}} \quad (6)$$

The f_{new_alk} and f_{new_acid} values were also estimated for September and October using a similar approach.

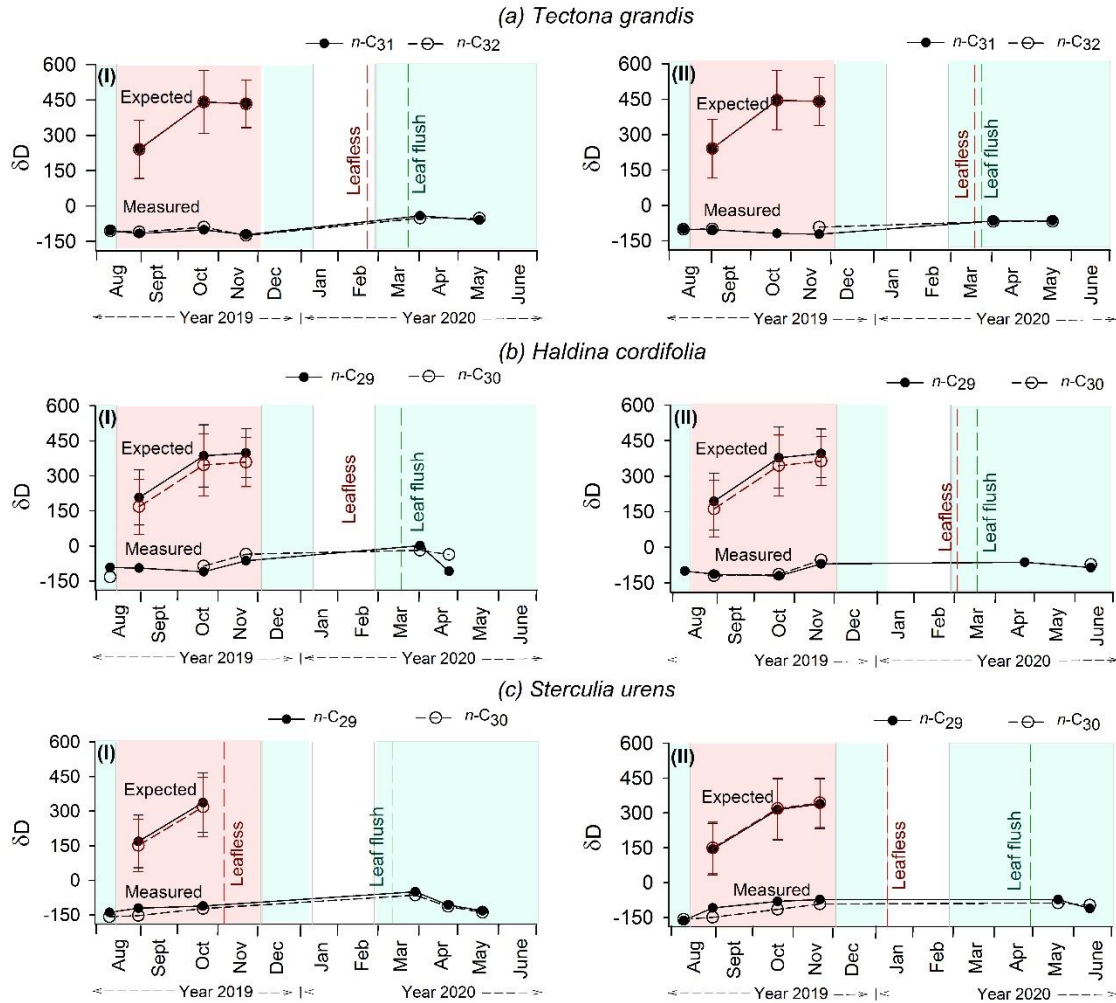
2.3.3 Uncertainty estimation

The uncertainty associated with the parameters used for modeling was estimated by employing Monte Carlo simulation. These parameters and associated 1-sigma uncertainty were derived from 1000 model runs with simultaneous and random 1-sigma perturbations with normal distribution of the input parameters given in Tables S1 and S2. The runs with the negative vapor pressure deficit values (i.e. when the water vapor pressure inside the leaf was lower than that of the atmosphere) were ignored.

3 Results and Discussion

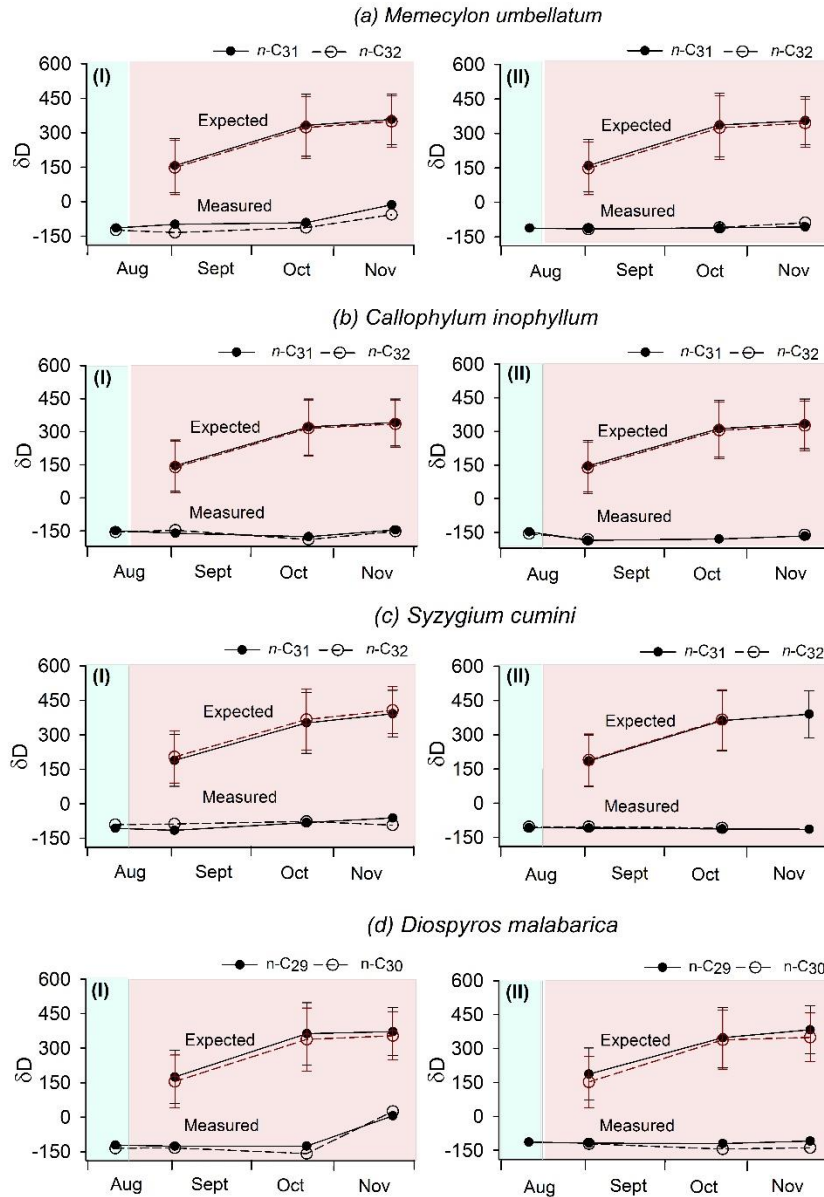
3.1 Temporal changes in the measured δD_{alk} and δD_{acid}

Figures 1 and 2 show temporal changes in δD_{alk} and δD_{acid} values in periodically collected leaves of various species (data in Table S3, S4). Except for *Sterculia urens* (Fig. 1c), variations in the δD_{alk} and δD_{acid} values after the application of isotopically-labeled water did not show a systematic increasing trend in other species. Further, two individuals of the same species did not always show a coherent evolution of δD_{alk} values (and δD_{acid} values) (e.g. Figure 2d).



219

220 Fig. 1. The measured and expected δD_{alk} (solid lines) and δD_{acid} (dashed lines) values of
 221 deciduous species when irrigated with normal ($\delta D = -2$ ‰) water (green region) and
 222 isotopically-labeled ($\delta D = 1000$ ‰) water (pink region). The period when the plants were not
 223 irrigated is shown by white color. The symbols with error bars represent expected δD values if
 224 the biomarkers were synthesized using the isotopically-labeled water alone. Two panels for each
 225 species present δD variability of two individuals.



226

227 Fig. 2. The measured and expected δD_{alk} (solid lines) and δD_{acid} (dashed lines) values of
 228 evergreen species when irrigated with normal ($\delta D = -2\text{‰}$) water (green region) and
 229 isotopically-labeled ($\delta D = 1000\text{‰}$) water (pink region). The symbols with error bars represent
 230 expected δD values if the biomarkers were synthesized using the isotopically-labeled water
 231 alone. The months are of the year 2019. Two panels for each species present δD variability of
 232 two individuals.

233

234 The δD variations observed in mature leaves in this study could reflect the inter-leaf δD
235 variability (i.e. δD variations among different leaves that flushed together) and/or incorporation
236 of new wax produced using the isotopically-labeled water. The inter-leaf variability in the δD_{alk}
237 and δD_{acid} values could vary up to 38‰ (Hou et al., 2007). Therefore, smaller variations in δD
238 values of leaves collected during the application of isotopically-labeled water could also
239 represent inter-leaf δD variability established prior to the application of the isotopically-labeled
240 water. This could also explain the lowering of the δD values in leaves of a few individuals
241 collected after application of the isotopically-labeled water (e.g. Fig. 1a). The larger increase in
242 δD values (e.g. >38‰) likely indicates incorporations of new wax (synthesized using
243 isotopically-labeled water) in the leaf wax pool.

244 *3.2 Comparison of the expected and measured leaf wax δD values in the mature leaves collected*
245 *after application of the isotopically-labeled water*

246 Figures 1 and 2 show the expected and measured δD_{alk} and δD_{acid} values after the
247 application of isotopically-labeled water (data in Table S3, S4, S5). The experiment ensured that
248 the plants had no access to ground or precipitation water; the source water δD value during the
249 second irrigation regime was 1002‰ higher than that during the first regime. However, because
250 of the isotopic exchange of the leaf water with the deuterium-depleted ambient water vapor
251 (Kahmen et al., 2011), a concomitant increase in δD values of leaf wax compounds (over the δD
252 values of August) was not expected. Nevertheless, the expected δD values of both compounds
253 were much higher than the measured δD values (by $253 \pm 111\%$ to $565 \pm 127\%$) for September,
254 October and November (Fig. 1, 2; Table S6). No systematic differences were observed between

the expected δD_{alk} and δD_{acid} values (Table S5). This indicated that *n*-alkanes and *n*-alkanoic acids in the leaf wax pool of the mature leaves either did not or partially included newly formed compounds during September to November.

3.3 Fraction of newly synthesized leaf wax during a growing season

The δD_{alk} and δD_{acid} values of the leaves collected in August (δD_{alk}^{Aug} , δD_{acid}^{Aug}), September (δD_{alk}^{Sept} , δD_{acid}^{Sept}) and October (δD_{alk}^{Oct} , δD_{acid}^{Oct}) were, in general, lower than for those collected in November (δD_{alk}^{Nov} , δD_{acid}^{Nov}) (Fig. 1, 2, Table S3). Thus δD_{alk}^{Nov} and δD_{acid}^{Nov} were likely to show the maximum inclusion of newly synthesized leaf wax compounds (i.e. synthesized using isotopically-labeled water) in the wax pool. A mass balance approach (Equation 5, 6) indicated no or variable degree of inclusion of newly formed wax compounds in the total leaf wax pools of the plants during November (Table S7). The maximum inclusion of $\pm 5\%$ and $33 \pm 7\%$ of *n*-alkanes and *n*-alkanoic acids, respectively was observed in only one of the two plants of *Diospyros malabarica*. *Sterculia urens* was the only species that showed $\sim 13\%$ inclusion in both plants (Table S7). If the negative values of inclusion (resulted due to the lower δD values in November than in August) were considered as no inclusion, the average inclusion of *n*-alkanes and *n*-alkanoic acids in all plants were 7% (s.e.m. = 2%) and 9% (s.e.m. = 3%), respectively. This implies that the bulk of the *n*-alkanes and *n*-alkanoic acids are synthesized during the early stages of the leaf's development in tropical angiosperm trees. For other months, the average inclusions were 2 to 3 % for both compounds (Table S7).

The photosynthates formed using the isotopically-labeled water were likely used to form structural components (such as the latewood) of plants and/or stored for utilization in the next growing season. The utilization of photosynthates formed during the mid- and late-growing

seasons to form the latewood of the current year and the earlywood of the next year has been demonstrated (Kagawa et al., 2006).

3.4 Timing of *n*-alkane and *n*-alkanoic acid synthesis

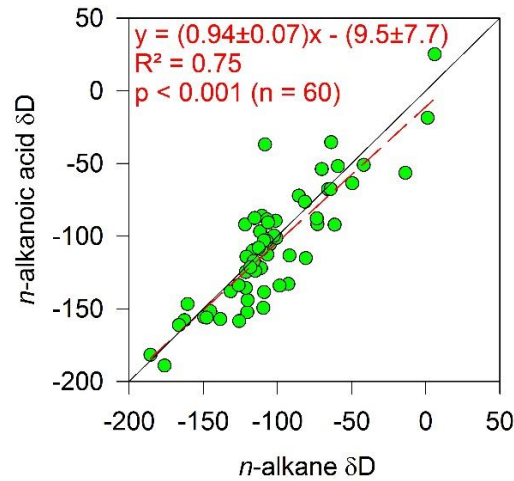


Fig. 3. Correlation between the observed δD_{alk} and δD_{acid} values. The black and red lines are 1:1 and linear best-fit lines, respectively.

The field-based investigations of temperate (Freimuth et al., 2017) and sub-tropical (Yang et al., 2021) angiosperms revealed that in deciduous species, *n*-alkanes are synthesized during the early growing season, whereas *n*-alkanoic acids are produced throughout the growing season. This implies that *n*-alkanes and *n*-alkanoic acids record δD of precipitation during the early and entire growing season, respectively. Had this been held for tropical species, the *n*-alkanoic acids alone would have reflected the δD values of the isotopically-labeled water in this study. However, we observed covariation (with a slope of 0.94 ± 0.07) in the temporal evolution of δD_{alk} and δD_{acid} values (Fig. 3) ($r^2 = 0.7$, $p < 0.05$, $n = 60$). Contrary to extra-tropical studies (Freimuth et al., 2017, Yang et al., 2021), our results suggested synchronous production of *n*-

alkanes and *n*-alkanoic acids, and their bias towards δD of precipitation during the early stages of the leaf's growth. Our results found no difference in the production pattern of *n*-alkanes and *n*-alkanoic acids of tropical evergreen and deciduous species. This is in contrast to the suggestion of continuous production of *n*-alkanes only in evergreen species in sub-tropics (Yang et al., 2021).

3.5 Effect of the transfer of photosynthates from a growing season to the next

It has been suggested that in deciduous species, stored carbohydrate reserves (formed during the previous year's late growing season) are utilized for the synthesis of leaf wax *n*-alkanes and *n*-alkanoic acids in new leaves of the current growing season (Tipple et al., 2013; Freimuth et al., 2017). The carryover of bud waxes produced in winter to the next growing season has also been indicated (Freimuth et al., 2017). This implies that the δD_{alk} and δD_{acid} values in the new leaves carry the δD_{Precip} signal of the end of the previous growing season. Our experiment allowed us to verify the extent to which the carryover of photosynthates affects δD_{alk} and δD_{acid} values in the leaves and its implication for the seasonality in the leaf wax δD records.

Two/three sets of young leaves were collected from deciduous species at the beginning of the year 2020 (Fig. S3). The leaves in the first set were not fully expanded; the rest were fully expanded. The δD values of *n*-alkanes and *n*-alkanoic acids of the first set of leaves (collected in April, 2020) were either equal to or more (by the maximum of 79‰) than δD_{alk}^{Nov} and δD_{acid}^{Nov} (Table S4, Fig. 1). It has been suggested that the metabolic shift from heterotrophic (i.e. derived from the photosynthates formed in the previous growing season) to autotrophic (i.e. formed by photosynthesis using ambient water) synthesis occurs when the leaf is expanded to 30 - 60% of its maximum size (Turgeon, 1989). As the size of the leaves from the first set falls within this

range, their δD_{alk} and δD_{acid} values likely reflect the mixing of the current and previous year's photosynthates. Had they been synthesized using the stored photosynthates (likely synthesized using the isotopically-labeled water) alone they would have shown much higher δD values, similar to δD_{alk}^{Nov*} and δD_{acid}^{Nov*} . The lowering of δD values in the leaves of the second and third sets (Fig. 1) suggested a higher contribution from the autotrophic than the heterotrophic leaf wax synthesis (Gamara and Kahmen, 2015). The higher values of δD_{alk} than δD_{acid} in the first set of leaves (the difference of $12 \pm 7\%$) and the converse for the second set (the difference of $-17 \pm 29\%$) further supports this conclusion as suggested by Freimuth et al., (2017).

The δD values of the second/third set of new leaves were lowered; in some plants, the lowered values were comparable to δD values of August, September and October of 2019 (Fig. 1). This suggested that by the time the leaf matures, the contribution of the previous year's photosynthates to the leaf wax pool of the current year is minimal; hence does not significantly lessen the early growing season bias in the leaf wax δD records of deciduous species.

4. Implications of this study

4.1 Implication for the leaf wax δD -based studies from the tropics

We observed that the majority of the deciduous and evergreen mature leaves did not synthesize significant amounts of leaf wax *n*-alkanes and *n*-alkanoic acids for ~ 4 months (i.e. during the application of isotopically-labeled water for 110 days) in a growing season. As this period is significant compared to the range of the length of rainy season observed in tropical biomes (~60 to ~240 days, Bombardi et al., 2019), our results have a bearing on interpreting leaf wax δD -based paleo- δD_{Precip} reconstructions from the tropics.

In tropical deciduous forests, the leaf emergence and fall are associated with the start and end of the rainy season, respectively (Van Schaik et al., 1993; Mediavilla and Escudero, 2003). The leaf wax-based δD records from a catchment with seasonally dry tropical forests, which constitutes about 42% of tropical forests (Van Bloem et al., 2004), are likely to be biased towards δD of precipitation during the early growing season.

Tropical rain forest covers about 25% of tropical ecological zone (FRA, 2000). Deciphering the seasonality in the leaf wax δD records from evergreen biomes may not be straightforward due to varying leaf production patterns. While the sunlight dominantly controls pantropical leaf phenology (van Schaik et al., 1993; Tang et al., 2017; Li et al., 2021), the effect of vapor pressure deficit and soil moisture stress has also been observed (Li et al., 2021). The leaf phenology in many evergreen species varies from twice a year (bimodal production) with peaks occurring during April-March and September-October at the equator (3°S to 3°N), whereas unimodal production occurs during July-August at latitudes beyond 5°S and 5°N (Li et al., 2021). Thus, the leaf wax δD records in evergreen catchments at the equator likely integrate δD of precipitation of months associated with bimodal leaf production whereas those at relatively higher latitudes will reflect the δD of precipitation during the single episode of leaf flushing. Further, region-specific leaf flushing patterns have also been observed. For example, in a monsoonal climate the major leaf flushing in evergreen plants occurs immediately after the rainy season i.e. during the early dry season, but much before the flushing in deciduous species (Chakrabarty et al., 2021). This suggests the need to consider the leaf phenological pattern in the catchment while interpreting the δD -based leaf wax records from the tropical evergreen biomes.

4.2 δD_{alk} and δD_{acid} records from regions with seasonally varying moisture sources

Many regions in the tropics exhibit seasonally varying moisture sources each having distinct isotopic characteristics during a growing season (Araguás-Araguás et al., 1998; Yadava et al., 2007; Levin et al., 2009; Sánchez-Murillo et al., 2016). For example, the southern part of India receives the southwest (from June to September) and the northeast (from October to December) monsoons, each having a distinct isotopic signature (e.g. Yadava et al., 2007). The regions near the transition between tropic and temperate zones often experience isotopically distinct tropical and extra-tropical air masses during summer and winter, respectively (Araguás-Araguás et al., 1998). Even though the seasonal vegetation from such regions receives both moisture sources, due to its production mainly during the early stages of leaf growth, *n*-alkanes and *n*-alkanoic acids might not record the δD_{Precip} values received during the latter part of the leaf growth. Therefore, the seasonality issue in leaf wax δD -based records is likely to be critical in regions that receive two or more moisture sources during the growing season.

5. Conclusion

Our seasonality study from the tropics, in conjunction with those from temperate (Tippie et al., 2013; Freimuth et al., 2017) and subtropical (Yang et al., 2021) regions, indicate that δD_{alk} records are biased towards the δD_{Precip} values prevailing during the early stages of the leaf's growth. This study indicates δD_{acid} records from the tropics are also biased towards the same. The δD_{Precip} during the early stages of the leaf's growth is preserved in the leaf wax δD records and should be considered during proxy-model comparison. In the case of catchments dominated by deciduous species, this period coincides with the early growing season. An examination of community-scale leaf production patterns is required to decipher the seasonality in δD_{alk} and δD_{acid} records from the evergreen biomes. Therefore, we recommend the inclusion of an

ecosystem-level assessment of the leaf maturation period within the catchment area in leaf wax-based paleo- δD_{Precip} studies.

Acknowledgments, Samples, and Data

Vijayananda Sarangi and Mahesh Ghosh are acknowledged for their help in the laboratory. Thanks to Vivek Kumar and Anil Sutar from IISER Pune for helping out with the field experiment. The help extended by Dr. Deepak Barua in executing the experiment and helping with the stomatal conductance and leaf temperature measurements is appreciated. Help extended by Prof. Ansgar Kahmen while preparing the isotopically-labeled water is acknowledged. We thank Prof. Sarah Feakins and Prof. Yongsong Huang for their input on an earlier version of the manuscript. We gratefully acknowledge Twenty-Twenty research grant for partly funding visits to SILIKA lab, IISER Kolkata. Funding by DST-SERB's SRG (SRG/2019/001349) is acknowledged.

Competing financial interest

The authors declare no competing interests.

Open Research

The data are given in the Supplementary Information file. The data presented in this paper are available in the Zenodo repository (Saishree et al., 2024). Link: Amrita Saishree, & Shreyas Managave. (2024). The δD records of n-alkane and n-alkanoic acid of tropical trees reflect δD of precipitation during the early stages of the leaf growth. <https://doi.org/10.5281/zenodo.10801254>

References

402 Araguás-Araguás, L., Froehlich, K., & Rozanski, K. (1998). Stable isotope composition of
 403 precipitation over southeast Asia. *Journal of Geophysical Research: Atmospheres*, 103(D22),
 404 28721-28742. <https://doi.org/10.1029/98JD02582>

405 Baer, D., Paul, J., Gupta, M., & O’Keefe, A. (2002). Sensitive absorption measurements in the
 406 near-infrared region using off-axis integrated-cavity-output spectroscopy. *Applied Physics B*,
 407 75(2):261–265, [https://doi: 10.1007/s00340-002-0971-z](https://doi.org/10.1007/s00340-002-0971-z).

408 Ball, J. T., Woodrow, I. E., & Berry, J. A. (1987). A model predicting stomatal conductance and
 409 its contribution to the control of photosynthesis under different environmental conditions. In
 410 *Progress in photosynthesis research: volume 4 proceedings of the VIIth international congress*
 411 *on photosynthesis providence, Rhode Island, USA, august 10–15, 1986* (pp. 221-224). Springer
 412 Netherlands. DOI:10.1007/978-94-017-0519-6_48

413 Bombardi, R. J., Kinter, J. L., & Frauenfeld, O. W. (2019). A global gridded dataset of the
 414 characteristics of the rainy and dry seasons. *Bulletin of the American Meteorological Society*,
 415 100(7), 1315-1328. doi:10.1175/BAMS-D-18-0177.1

416 Cernusak, L. A., Barbour, M. M., Arndt, S. K., Cheesman, A. W., English, N. B., Feild, T. S., et
 417 al. (2016). Stable isotopes in leaf water of terrestrial plants. *Plant, Cell & Environment*, 39(5),
 418 1087-1102. <https://doi.org/10.1111/pce.12703>

419 Chakrabarty, S., Sharma, S., Ganguly, S., Jezeera, A., Mohanbabu, N., & Barua, D. (2021).
 420 Quantitative estimates of deciduousness in woody species from a seasonally dry tropical forest
 421 are related to leaf functional traits and the timing of leaf flush. *BioRxiv*, 2021-03.
 422 <https://doi.org/10.1101/2021.03.03.433407>

423 Collins, J. A., Schefuß, E., Mulitza, S., Prange, M., Werner, M., Tharammal, T., et al. (2013).
 424 Estimating the hydrogen isotopic composition of past precipitation using leaf-waxes from
 425 western Africa. *Quaternary Science Reviews*, 65, 88-101.
 426 <https://doi.org/10.1016/j.quascirev.2013.01.007>

427 Deshpande S., Sharma B.D., & Nayar M.P. (1993). Flora adjoining areas of Mahabaleshwar 1.
 428 Botanical Survey of India.

429 Deshpande, R. D., Maurya, A. S., Kumar, B., Sarkar, A., & Gupta, S. K. (2013). Kinetic
 430 fractionation of water isotopes during liquid condensation under super-saturated condition.
 431 *Geochimica et Cosmochimica Acta*, 100, 60-72. <https://doi.org/10.1016/j.gca.2012.10.009>

432 Feakins, S. J., & Sessions, A. L. (2010). Controls on the D/H ratios of plant leaf waxes in an arid
 433 ecosystem. *Geochimica et Cosmochimica Acta*, 74(7), 2128-2141.
 434 <https://doi.org/10.1016/j.gca.2010.01.016>

435 Feakins, S. J., Kirby, M. E., Cheetham, M. I., Ibarra, Y., & Zimmerman, S. R. (2014).
 436 Fluctuation in leaf wax D/H ratio from a southern California lake records significant variability
 437 in isotopes in precipitation during the late Holocene. *Organic Geochemistry*, 66, 48-59.
 438 <https://doi.org/10.1016/j.orggeochem.2013.10.015>

439 Feakins, S. J., Peters, T., Wu, M. S., Shenkin, A., Salinas, N., Girardin, C. A., et al. (2016).
 440 Production of leaf wax *n*-alkanes across a tropical forest elevation transect. *Organic*
 441 *Geochemistry*, 100, 89-100. <https://doi.org/10.1016/j.orggeochem.2016.07.004>

442 Feakins, S. J., Wu, M. S., Ponton, C., & Tierney, J. E. (2019). Biomarkers reveal abrupt switches
 443 in hydroclimate during the last glacial in southern California. *Earth and Planetary Science*
 444 *Letters*, 515, 164-172. <https://doi.org/10.1016/j.epsl.2019.03.024>

445 Flanagan, L. B., & Ehleringer, J. R. (1991). Stable isotope composition of stem and leaf water:
 446 applications to the study of plant water use. *Functional Ecology*, 270-277.
 447 <https://doi.org/10.2307/2389264>

448 FRA (2000). Global Ecological Zoning for The Global Forest Resources Assessment 2000. Final
 449 report Rome, 2001. Forest Resources Assessment Programme, Working Paper 56, Rome 2001

450 Freimuth, E. J., Diefendorf, A. F., & Lowell, T. V. (2017). Hydrogen isotopes of *n*-alkanes and
 451 *n*-alkanoic acids as tracers of precipitation in a temperate forest and implications for
 452 paleorecords. *Geochimica et Cosmochimica Acta*, 206, 166-183.
 453 <https://doi.org/10.1016/j.gca.2017.02.027>

454 Gao, L., Burnier, A., & Huang, Y. (2012). Quantifying instantaneous regeneration rates of plant
 455 leaf waxes using stable hydrogen isotope labeling. *Rapid Communications in Mass*
 456 *Spectrometry*, 26(2), 115-122. <https://doi.org/10.1002/rcm.5313>

457 Gamarra, B., & Kahmen, A. (2015). Concentrations and $\delta^2\text{H}$ values of cuticular *n*-alkanes vary
 458 significantly among plant organs, species and habitats in grasses from an alpine and a temperate
 459 European grassland. *Oecologia*, 178, 981-998. <https://doi.org/10.1007/s00442-015-3278-6>

460 Ghosh, S., Sanyal, P., Roy, S., Bhushan, R., Sati, S. P., Philippe, A., & Juyal, N. (2020). Early
 461 Holocene Indian summer monsoon and its impact on vegetation in the Central Himalaya: Insight
 462 from δD and $\delta^{13}\text{C}$ values of leaf wax lipid. *The Holocene*, 30(7), 1063-1074.
 463 <https://doi.org/10.1177/0959683620908639>

464 Hou, J., D'Andrea, W. J., MacDonald, D., & Huang, Y. (2007). Hydrogen isotopic variability in
 465 leaf waxes among terrestrial and aquatic plants around Blood Pond, Massachusetts (USA).
 466 *Organic Geochemistry*, 38(6), 977-984. <https://doi.org/10.1016/j.orggeochem.2006.12.009>

467 Hren, M. T., Pagani, M., Erwin, D. M., & Brandon, M. (2010). Biomarker reconstruction of the
 468 early Eocene paleotopography and paleoclimate of the northern Sierra Nevada. *Geology*, 38(1),
 469 7-10. <https://doi.org/10.1130/G30215.1>

470 Huang, X., Zhao, B., Wang, K., Hu, Y., & Meyers, P. A. (2018). Seasonal variations of leaf wax
 471 *n*-alkane molecular composition and δD values in two subtropical deciduous tree species: Results
 472 from a three-year monitoring program in central China. *Organic Geochemistry*, 118, 15-26.
 473 <https://doi.org/10.1016/j.orggeochem.2018.01.009>

474 Jetter, R., Kunst, L., & Samuels, A. L. (2006). Composition of plant cuticular waxes. *Annual*
 475 *plant reviews volume 23: Biology of the plant cuticle*, 145-181.
 476 <https://doi.org/10.1002/9781119312994.apr0232>

477 Kagawa A., Sugimoto, A., Maximov T. C. (2006). $^{13}\text{CO}_2$ pulse-labelling of photoassimilates
 478 reveals carbon allocation within and between tree rings. *Plant, Cell and Environment*, 29, 1571–
 479 1584. <https://doi.org/10.1111/j.1365-3040.2006.01533.x>

480 Kahmen, A., Dawson, T. E., Vieth, A., & Sachse, D. (2011). Leaf wax *n*-alkane δD values are
 481 determined early in the ontogeny of *Populus trichocarpa* leaves when grown under controlled
 482 environmental conditions. *Plant, Cell & Environment*, 34(10), 1639-1651.
 483 <https://doi.org/10.1111/j.1365-3040.2011.02360.x>

484 Knapp, S., Burls, N. J., Dee, S., Feng, R., Feakins, S. J., & Bhattacharya, T. (2022). A Pliocene
 485 Precipitation Isotope Proxy-Model Comparison Assessing the Hydrological Fingerprints of Sea
 486 Surface Temperature Gradients. *Paleoceanography and Paleoclimatology*, 37(12),
 487 e2021PA004401. <https://doi.org/10.1029/2021PA004401>

488 Konecky, B. L., Russell, J. M., Johnson, T. C., Brown, E. T., Berke, M. A., Werne, J. P., et al.
 489 (2011). Atmospheric circulation patterns during late Pleistocene climate changes at Lake
 490 Malawi, Africa. *Earth and Planetary Science Letters*, 312(3-4), 318-326.
 491 <https://doi.org/10.1016/j.epsl.2011.10.020>

492 Konecky, B., Russell, J., & Bijaksana, S. (2016). Glacial aridity in central Indonesia coeval with
 493 intensified monsoon circulation. *Earth and Planetary Science Letters*, 437, 15-24.
 494 <https://doi.org/10.1016/j.epsl.2015.12.037>

495 Levin, N. E., Zipser, E. J., & Cerling, T. E. (2009). Isotopic composition of waters from Ethiopia
 496 and Kenya: Insights into moisture sources for eastern Africa. *Journal of Geophysical Research:*
 497 *Atmospheres*, 114(D23). <https://doi.org/10.1029/2009JD012166>

498 Li, Q., Chen, X., Yuan, W., Lu, H., Shen, R., Wu, S., et al. (2021). Remote sensing of seasonal
 499 climatic constraints on leaf phenology across pantropical evergreen forest biome. *Earth's Future*,
 500 9(9), e2021EF002160. <https://doi.org/10.1029/2021EF002160>

501 Majoube, M. (1971). Oxygen-18 and deuterium fractionation between water and steam (in
 502 French). *Journal de Chimie Physique et de Physico-Chimie Biologique*, 68, 1423-1436.

503 Managave, S., Huang, Y., Sutra, J.-P., Anupama, K., & Prasad, S. (2023). Holocene precipitation
 504 hydrogen isotopic values on Nilgiri Plateau (southern India) suggest a combined effect of
 505 precipitation amount and transport paths. *The Holocene*,
 506 <https://doi.org/10.1177/09596836231183110>.

507 Managave, S. R. (2014). Model evaluation of the coherence of a common source water oxygen
 508 isotopic signal recorded by tree-ring cellulose and speleothem calcite. *Geochemistry,*
 509 *Geophysics, Geosystems*, 15(4), 905-922. <https://doi.org/10.1002/2013GC004983>

510 McGrath, S. M., Clemens, S. C., Huang, Y., & Yamamoto, M. (2021). Greenhouse gas and ice
 511 volume drive Pleistocene Indian summer monsoon precipitation isotope variability. *Geophysical*
 512 *Research Letters*, 48(4), e2020GL092249. <https://doi.org/10.1029/2020GL092249>

513 McInerney, F. A., Helliker, B. R., & Freeman, K. H. (2011). Hydrogen isotope ratios of leaf wax
 514 *n*-alkanes in grasses are insensitive to transpiration. *Geochimica et Cosmochimica Acta*, 75(2),
 515 541-554. <https://doi.org/10.1016/j.gca.2010.10.022>

516 Mediavilla, S., & Escudero, A. (2003). Stomatal responses to drought at a Mediterranean site: a
 517 comparative study of co-occurring woody species differing in leaf longevity. *Tree physiology*,
 518 23(14), 987-996. <https://doi.org/10.1093/treephys/23.14.987>

519 Roden, J. S., & Ehleringer, J. R. (1999). Observations of hydrogen and oxygen isotopes in leaf
 520 water confirm the Craig-Gordon model under wide-ranging environmental conditions. *Plant*
 521 *physiology*, 120(4), 1165-1174. <https://doi.org/10.1104/pp.120.4.1165>

522 Sachse, D., Billault, I., Bowen, G. J., Chikaraishi, Y., Dawson, T. E., Feakins, S. J., et al. (2012).
 523 Molecular paleohydrology: interpreting the hydrogen-isotopic composition of lipid biomarkers
 524 from photosynthesizing organisms. *Annual Review of Earth and Planetary Sciences*, 40, 221-
 525 249. <https://doi.org/10.1146/annurev-earth-042711-105535>

526 Sachse, D., Gleixner, G., Wilkes, H., & Kahmen, A. (2010). Leaf wax *n*-alkane δD values of
 527 field-grown barley reflect leaf water δD values at the time of leaf formation. *Geochimica et*
 528 *Cosmochimica Acta*, 74(23), 6741-6750. <https://doi.org/10.1016/j.gca.2010.08.033>

529 Sachse, D., Kahmen, A., & Gleixner, G. (2009). Significant seasonal variation in the hydrogen
 530 isotopic composition of leaf-wax lipids for two deciduous tree ecosystems (*Fagus sylvatica* and
 531 *Acer pseudoplatanus*). *Organic Geochemistry*, 40(6), 732-742.
 532 <https://doi.org/10.1016/j.orggeochem.2009.02.008>

533 Sachse, D., Dawson, T. E., & Kahmen, A. (2015). Seasonal variation of leaf wax *n*-alkane
 534 production and $\delta^2\text{H}$ values from the evergreen oak tree, *Quercus agrifolia*. *Isotopes in*
 535 *environmental and health studies*, 51(1), 124-142.
 536 <https://doi.org/10.1080/10256016.2015.1011636>

537 Saishree A., Managave S. (2024). The δD records of *n*-alkane and *n*-alkanoic acid of tropical
 538 trees reflect δD of precipitation during the early stages of the leaf
 539 growth. <https://doi.org/10.5281/zenodo.10801254>

540 Sánchez-Murillo, R., Birkel, C., Welsh, K., Esquivel-Hernández, G., Corrales-Salazar, J., Boll,
 541 J., et al. (2016). Key drivers controlling stable isotope variations in daily precipitation of Costa
 542 Rica: Caribbean Sea versus Eastern Pacific Ocean moisture sources. *Quaternary Science*
 543 *Reviews*, 131, 250-261. <https://doi.org/10.1016/j.quascirev.2015.08.028>

544 Sessions, A. L. (2006). Seasonal changes in D/H fractionation accompanying lipid biosynthesis
 545 in *Spartina alterniflora*. *Geochimica et Cosmochimica Acta*, 70, 2153-2162.

546 Shepherd, T., & Wynne Griffiths, D. (2006). The effects of stress on plant cuticular waxes. *New*
 547 *Phytologist*, 171(3), 469-499. <https://doi.org/10.1111/j.1469-8137.2006.01826.x>

548 Smith, F. A., & Freeman, K. H. (2006). Influence of physiology and climate on δD of leaf wax
 549 *n*-alkanes from C_3 and C_4 grasses. *Geochimica et Cosmochimica Acta*, 70(5), 1172-1187.
 550 <https://doi.org/10.1016/j.gca.2005.11.006>

551 Sturm, C., Zhang, Q., & Noone, D. (2010). An introduction to stable water isotopes in climate
 552 models: benefits of forward proxy modelling for paleoclimatology. *Climate of the Past*, 6(1),
 553 115-129. <https://doi.org/10.5194/cp-6-115-2010>

554 Tang, H., & Dubayah, R. (2017). Light-driven growth in Amazon evergreen forests explained by
 555 seasonal variations of vertical canopy structure. *Proceedings of the National Academy of*
 556 *Sciences*, 114(10), 2640-2644. <https://doi.org/10.1073/pnas.1616943114>

557 Tierney JE, Russell JM, Huang Y et al. (2008) Northern Hemisphere controls on tropical
 558 southeast African climate during the past 60,000 years. *Science* 322:252– 255.
 559 <https://doi.org/10.1126/science.1160485>

560 Tipple, B. J., & Pagani, M. (2010). A 35 Myr North American leaf-wax compound-specific
 561 carbon and hydrogen isotope record: Implications for C₄ grasslands and hydrologic cycle
 562 dynamics. *Earth and Planetary Science Letters*, 299(1-2), 250-262.
 563 <https://doi.org/10.1016/j.epsl.2010.09.006>

564 Tipple, B. J., & Pagani, M. (2013). Environmental control on eastern broadleaf forest species'
 565 leaf wax distributions and D/H ratios. *Geochimica et Cosmochimica Acta*, 111, 64-77.
 566 <https://doi.org/10.1016/j.gca.2012.10.042>

567 Tipple, B. J., Berke, M. A., Doman, C. E., Khachatryan, S., & Ehleringer, J. R. (2013). Leaf-
 568 wax *n*-alkanes record the plant–water environment at leaf flush. *Proceedings of the National*
 569 *Academy of Sciences*, 110(7), 2659-2664. <https://doi.org/10.1073/pnas.1213875110>

570 Tipple, B. J., Berke, M. A., Hambach, B., Roden, J. S., & Ehleringer, J. R. (2015). Predicting
 571 leaf wax *n*-alkane ²H/¹H ratios: controlled water source and humidity experiments with
 572 hydroponically grown trees confirm predictions of Craig–Gordon model. *Plant, Cell &*
 573 *Environment*, 38(6), 1035-1047. <https://doi.org/10.1111/pce.12457>

574 Turgeon, R. (1989). The sink-source transition in leaves. *Annual review of plant biology*, 40(1),
 575 119-138. <https://doi.org/10.1146/annurev.pp.40.060189.001003>

576 Van Bloem, S., Lugo, A. E., & Murphy, P. G. (2004). Regional Forest Types-Tropical Dry
 577 Forests. https://tigerprints.clemson.edu/forestry_env_pub/20

578 Van Schaik, C. P., Terborgh, J. W., & Wright, S. J. (1993). The phenology of tropical forests:
 579 adaptive significance and consequences for primary consumers. *Annual Review of Ecology and*
 580 *Systematics*, 24(1), 353-377. <https://doi.org/10.1146/annurev.es.24.110193.002033>.

581 Yadava M. G., Ramesh R., & Pandarinath K. (2007) A positive 'amount effect' in the Sahyadri
 582 (Western Ghats) rainfall. *Current Science*, 93(2):560–564.
 583 <https://www.jstor.org/stable/24099225>

584 Yang, Y., Zhang, Y., & Huang, X. (2021). Comparison of $\delta^2\text{H}$ values of leaf wax *n*-alkanes and
585 *n*-alkanoic acids in subtropical angiosperms. *Palaeogeography, Palaeoclimatology,*
586 *Palaeoecology*, 577, 110537. <https://doi.org/10.1016/j.palaeo.2021.110537>