

Supporting Information for

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

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NA

Introduction

[This document consists of 6 figures, 6 texts and 7 tables.]

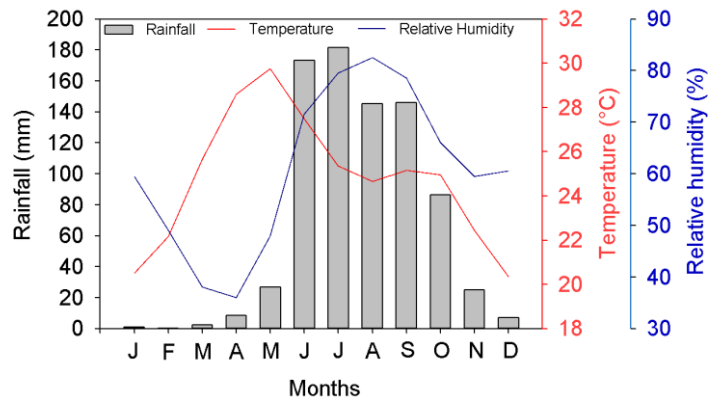


Fig S1. Monthly climatology of rainfall, temperature and relative humidity in the study area. [Data source: India Meteorological Department *Climatological Normals*: (1981 - 2010)]

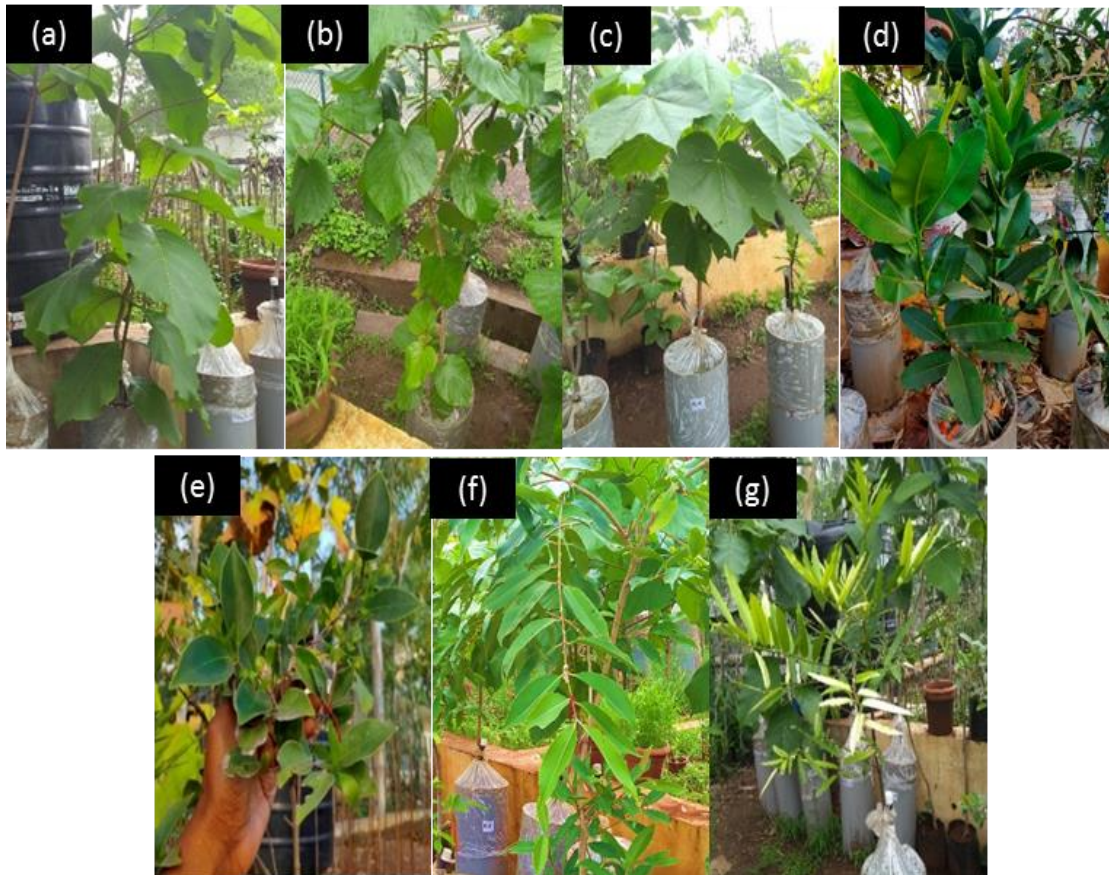


Fig S2. Deciduous [*Tectona grandis* (a), *Haldina cordifolia* (b), *Sterculia urens*(c)] and evergreen [*Callophylum inophyllum* (d), *Memecylon umbellatum* (e), *Syzygium cumini* (f), and *Diospyros malabarica* (g)] species considered in the present study.

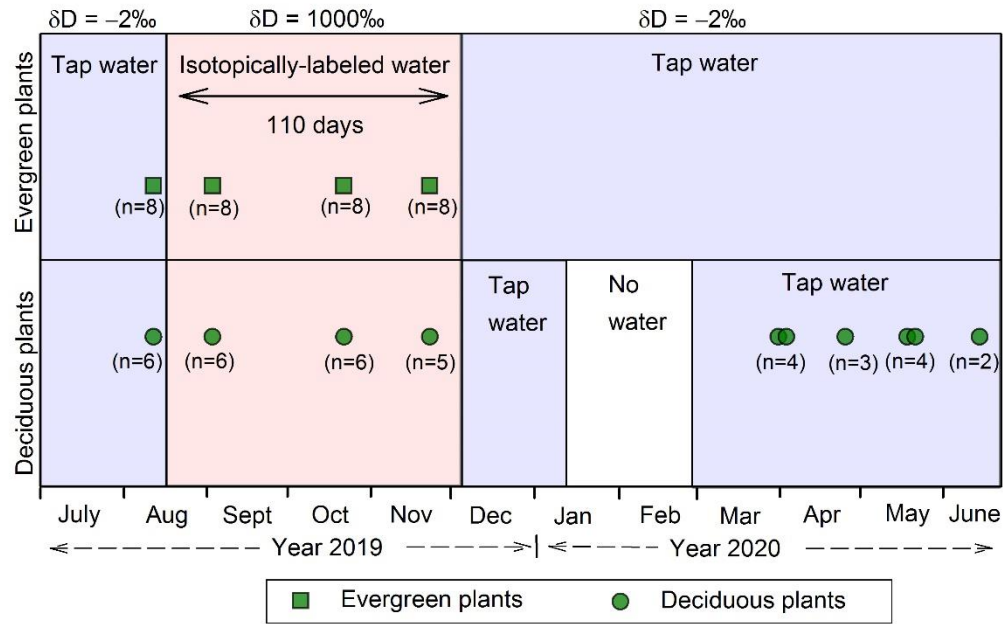


Fig S3. A schematic illustration of the irrigation regime employed and sampling carried out in this study. The purple and beige colors indicate the period of application of normal and tracer water, respectively. After the leafshed, the deciduous species were not irrigated for 47 days (from the 13th of January 2020 to the 29th of February 2020). The squares and circles indicate the day of collection of the leaves; the associated numbers in the parenthesis indicate the number of trees sampled on a given day. In the growing season of 2020, new leaves of only deciduous species were collected.



Fig S4. Pots sealed to prevent the influx of rain. To prevent access to groundwater the pots were kept on the concrete base.

Text S1: Leaf maturation period

To identify mature leaves in deciduous species, leaf growth measurement (length measured in one of the fixed dimensions) and leaf mass per unit area (LMA) analysis were carried out (Fig. S5, S6). The isotopically-labeled water was applied after stabilization of the leaf growth (10 to 64 days) and LMA (32 to 82 days). In the case of evergreen species, thicker leaves lying at a lower level on a branch were considered mature and were sampled.

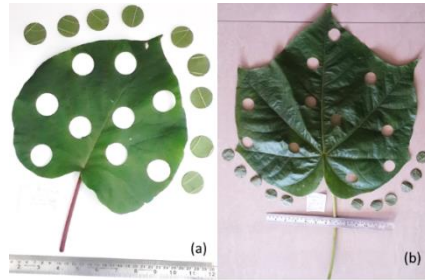


Fig S5. Different parts of leaves were sampled for LMA measurements. For example, (a) *Haldina cordifolia*, and (b) *Sterculia urens*.

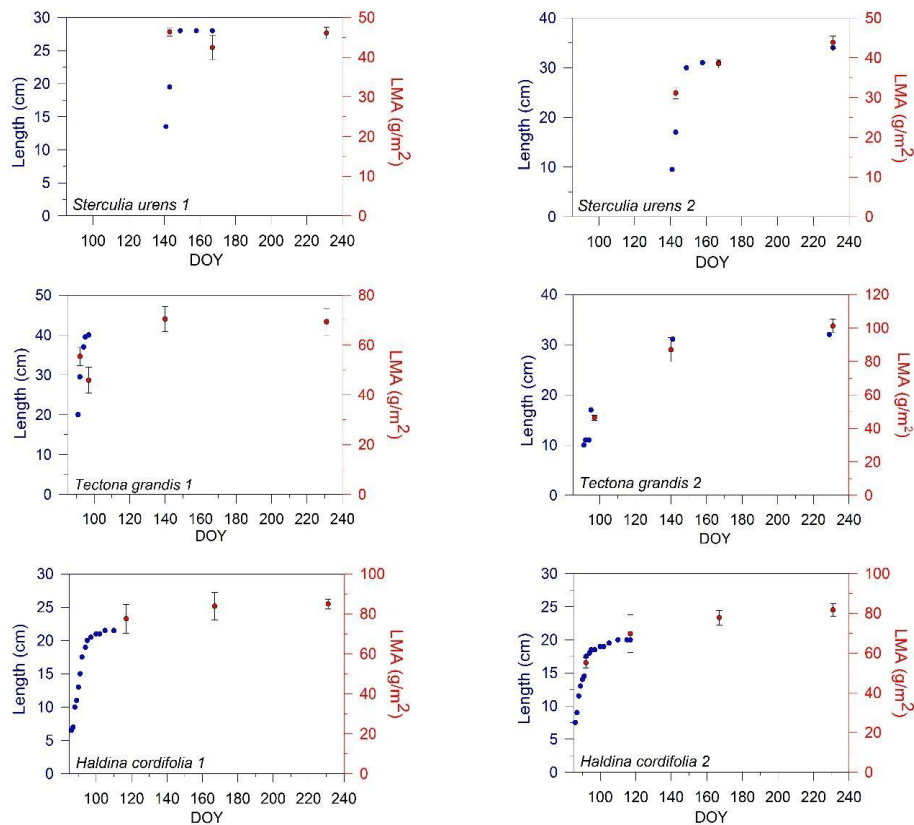


Fig S6. Leaf lengths (blue circles) and LMA (red circles) variation in deciduous leaves.

Text S2: *n*-alkane and *n*-alkanoic acid extraction

To obtain the total lipid fraction of the leaf samples, the dried leaves were powdered and ultrasonication technique was followed as described by McInerney et al. (2011). The samples were mixed with a reaction solvent composed of 93:7 v/v dichloromethane/methanol (HPLC grade) and subjected to sonication for 20 minutes at room temperature (25-30°C). The extracted lipid was concentrated using a Rotavapor (R-210; Buchi Labortechnik AG, Flawil, Switzerland). Short-column silica gel chromatography was used to extract *n*-alkane from the total lipid fraction. The column was prepared by using a pre-ashed pipette that was filled with activated silica gel (100-200 mesh), and the non-polar fraction (*n*-alkanes) was separated using hexane as the eluent. Subsequently, a mixture of methanol and dichloromethane in a ratio of 2:1 was utilized to elute the silica gel columns, to obtain the acid fraction. The acids fractions were subjected to saponification using 1 M KOH in methanol at 70 °C for 2 hours. After incubation, the vials were allowed to cool and then 5 % NaCl solution in DCM-extracted HPLC-grade water was added to each vial. The pH of the mixture was lowered (<2) using HCl and the “acids” fractions were extracted using hexane. The “acids” fractions were then extracted using hexane. To perform analysis of *n*-alkanoic acid, the acid fraction was methylated using BF₃-methanol and converted into fatty acid methyl esters. Finally, the fatty acid methyl esters were passed through an anhydrous Na₂SO₄ column to eliminate any moisture present.

Text S3: Identification and quantification of leaf wax *n*-alkanes and *n*-alkanoic acids

Gas chromatography (7890A GC System; Agilent Technologies, Santa Clara, CA, USA) was used to analyze the *n*-alkanes and *n*-alkanoic acids. The system was equipped with split/split-less injector, non-polar capillary column (HP5-MS; 30 m × 250 µm × 0.25 µm), and flame ionization detector (FID). The concentrated samples were injected in 1:1 split mode with an inlet temperature set to 320 °C. The oven temperature was ramped up from 60 °C to 320 °C at a rate of 8 °C minutes⁻¹ and held for 12 minutes. The characteristic retention time (RT) obtained from the calibration standards SUPELCO C₈-C₄₀ alkane and Fluka alkane mixture (C₁₀-C₄₀) was used to identify individual *n*-alkanes. The relative concentrations of the individual *n*-alkanes in the samples were also calculated using the same standards. To calibrate the system, the SUPELCO C₈-C₄₀ *n*-alkane standard and Fluka alkane mixture standard (C₁₀-C₄₀) were measured at different dilutions (1.0 ng, 1.5 ng, and 2.0 ng µl⁻¹) during the analysis of the samples. The peak areas of the individual *n*-alkanes (C₈-C₄₀ and C₁₀-C₄₀) were computed, and calibration graphs of peak areas against injected concentration were produced for the respective homologues (C₈-C₄₀ and C₁₀-C₄₀). The relative concentration of *n*-alkanes in the samples was then determined using the calibration equations that were obtained from regression analysis for the corresponding homologues. Similarly, the identification of individual *n*-alkanoic acids was achieved through the use of five

Sigma-Aldrich standards: Palmitic-C₁₆, Oleic-C₁₈, Behenic-C₂₂, Montanic-C₂₈, and Melissic-C₃₀ acid, each with known concentrations. During the analysis of *n*-alkanoic acids, the Fluka *n*-alkane mixture standard (C₁₀-C₄₀) and SUPELCO C₈-C₄₀ *n*-alkane standard were also analyzed. Equations for individual *n*-alkanoic acid homologues were derived using *n*-alkanoic acid and *n*-alkane standards. The calibrated equations (for respective homologues) were then used to calculate the relative concentrations of *n*-alkanoic acids in the samples. An uncertainty of $\pm 2\%$ was observed during the repeat measurements of *n*-alkanoic acid and *n*-alkane standards.

Text S4: the δD measurements

Leaf wax n-alkanes and n-alkanoic acids

The leaf wax *n*-alkanes and *n*-alkanoic acids 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. A non-polar capillary column HP5-MS was used for sample analysis. The samples were injected in splitless mode, and the inlet temperature was set to 280 °C, with helium used as the carrier gas at a flow rate of 1 ml minutes⁻¹. The temperature of the GC oven was set to increase at a rate of 10 °C per minute, starting from 40 °C to 320 °C, held isothermally for 12 minutes. To measure the δD , the hydrogen atoms in the samples underwent conversion to H₂ by a reduction interface in a pyrolysis furnace at 1420 °C. To standardize the hydrogen isotope values, H₂ reference gas was introduced into MAT-253 in a series of pulses at the beginning and end of each analysis. Before isotope analyses, the H₂ reference gas was calibrated against international standard mixtures A7 (C₁₆-C₃₀). To verify the performance of the instrument, a Fluka alkane mixture (C₁₀-C₄₀) at various dilutions (ranging from 30 to 100 ng μ l⁻¹) was routinely checked with known δD values. The reproducibility of the A7 and Fluka alkane mixture during sample analysis was found to be $\pm 2\%$ (1- σ). The H³⁺ factor was calculated using ISODAT NT 3.0 before measurements of hydrogen isotopes. The H³⁺ factor had a range of 7 to 10 ppm nA-1, indicating a contribution of <0.07-0.1% H³⁺ to HD⁺ (Sarangi et al., 2022). Pre-concentration and dilution procedure were carried out for the chain lengths of excessively low and high concentrations, respectively. Isotope fractionation associated with the addition of BF₃-methanol during *n*-alkanoic acid extraction was corrected using a mass balance equation:

$$\delta D_{acid} = \frac{[(2C_n + 2) * \delta D_{FAME}] - [3 * \delta D_{Me}]}{(2C_n - 1)}$$

where, δD_{acid} values are the corrected values for target *n*-alkanoic acid, C_n is the number of C-atom for each alkanoic acid chain length, δD_{FAME} values are uncorrected values measured from fatty acid methyl esters, and

δD_{Me} is the δD value of the methanol in BF_3 -methanol used to methylate the samples. The δD values of n -alkanes and n -alkanoic acids are reported with respect to Vienna Standard Mean Ocean Water (VSMOW).

Water and atmospheric vapor samples

The tap/tracer water and atmospheric vapor samples were analyzed for δD values at the Physical Research Laboratory (PRL) India, using a laser-based water isotope analyzer (ABB-LGR IWA-45P). The analyzer follows the off-axis integrated cavity output spectroscopy (OA-ICOS) method for the measurement of isotopic composition (Baer et al., 2002). The method introduces laser photons of the known line strength in an optical cavity filled with sample water in vapor form, the measured absorption spectra is recorded and processed by post-analysis software to estimate the isotopic composition. Three standards supplied by ABB-LGR having different δD compositions (std-1: $-154 \pm 0.5\text{‰}$, std-2: $-51.60 \pm 0.5\text{‰}$, std-3: $-9.20 \pm 0.5\text{‰}$) were used in sequence after each batch of 4 water samples during measurements. A protocol ‘Standard Natural range optimized for high precision spline type’ of measurement was followed. This required 1ml volume of each sample in a standard glass bottle. Using 1 μ L syringe, samples from these bottles were extracted by an auto-injector system that passed it into a miniature chamber heated at 85°C converting the liquid water fully in vapor form before introducing it into the water isotope analyzer. The δD values are reported with respect to Vienna Standard Mean Ocean Water (VSMOW).

Text S5: Modeling the δD values of the leaf water during various months

The Craig-Gordon model, modified by Flanagan and Ehleringer (1991), was used to determine the isotopic enrichment of the leaf water. The following equation was used

$$R_{LW} = \alpha * \left[\alpha_k R_{XW} \left(\frac{e_i - e_s}{e_i} \right) + \alpha_{kb} R_{XW} \left(\frac{e_s - e_a}{e_i} \right) + R_a \left(\frac{e_a}{e_i} \right) \right] \quad (S1)$$

In equation (1), R is the molar ratio of heavy to light isotope and the subscripts a , LW and XW refer to bulk air, leaf water, and xylem water, respectively. α^* refers to the liquid-vapor fractionation factor, α_k refers to the kinetic fractionation factor associated with diffusion in air and α_{kb} is the kinetic fractionation factor associated with diffusion at the boundary layer. The default values of α_k and α_{kb} in the model were 1.0164 and 1.011, respectively (Roden et al., 1999). α^* varies with leaf temperature (Majoube, 1971). e_a , e_s and e_i are the partial pressure of water vapor in bulk air, leaf surface and leaf intercellular air space, respectively. e_s is the only term

that considers leaf physiological characteristics and is calculated using an equation developed by Ball (1987). The values of e_i were estimated from the leaf temperature. Boundary layer conductance was considered as $1 \text{ mol m}^{-2} \text{ s}^{-1}$ (Roden et al., 1999; Managave et al., 2014). Tipple et al., (2015) showed the utility of the Craig-Gordon model in modeling δD values of n -alkanes. Due to a lack of leaf parameters such as effective path length, a sophisticated model involving the Péclet effect (Cernusak et al., 2016) was not used. The isotopic composition of the leaf water calculated using Equation 1 is sensitive mainly to (i) leaf temperature, (ii) relative humidity, (iii) isotopic composition of the xylem water (i.e. source water) and atmospheric water vapor (Sachse et al., 2009; Managave, 2014).

Relative humidity data were obtained from a nearby (~1 km) Indian Meteorological Department (IMD) station records while temperature was measured in the field using a thermometer (Table S1). The stomatal conductance was measured using a leaf porometer (Decagon SC-1) (data Table S2). The correlations between the air and leaf temperature for various plants were established using thermistors (Ecomatik LAT-B2) and were used to estimate the leaf temperature and e_i . A cryogenic trap method (Deshpande et al., 2013) was used to get an idea about the monthly variability of the δD values of atmospheric water vapor. Table S1 gives the δD of source water and atmospheric water vapor values considered for various months. R_{LW} values are expressed in delta notation for various months (for example for August, δD_{LW}^{Aug*}).

Months	Barometric Pressure (KPa) [@]	Temperature (°C) [#]	Humidity (%) ^{\$}	$\delta\text{D}_{\text{atm vapor}}$ (‰) ^{&}	$\delta\text{D}_{\text{source water}}$ (‰)
August	94.4 ± 0.3	28.7 ± 2.0	82.0 ± 10	-61 ± 6	-2 ± 1
September	94.5 ± 0.3	27.1 ± 2.0	78.0 ± 10	-61 ± 6	1000 ± 2
October	94.8 ± 0.3	29.6 ± 1.9	64.0 ± 12	-76 ± 8	1000 ± 2
November	95.0 ± 0.3	28.1 ± 0.7	58.0 ± 9	-118 ± 12	1000 ± 2

Table S1. Climate parameters used as inputs for leaf water modeling.

[@] Monthly mean values from IMD station data

[#] Daily measurements from 9 to 12 pm

^{\$} Daily IMD measurements from 9 to 12 pm; for August it is climatological mean.

[&] Measured periodically. δD values of September were considered for August

Months	Stomatal conductance ($\text{mol m}^{-2} \text{ s}^{-1}$)						
	T_g	H_c	S_u	D_m	M_u	C_i	S_c
Aug	0.5±0.1	0.6±0.2	0.7±0.3	0.4±0.1	0.6±0.3	0.3±0.1	0.5±0.1
Sept	0.5±0.1	0.6±0.2	0.7±0.3	0.4±0.1	0.6±0.3	0.3±0.1	0.5±0.1
Oct	0.5±0.1	0.7±0.1	0.6±0.2	0.4±0.04	0.6±0.07	0.7±0.1	0.8±0.5
Nov	0.4±0.02	0.4±0.09	0.3±0.08	0.3±0.04	0.4±0.08	0.4±0.09	0.6±0.01

Table S2. Stomatal conductance used as inputs for the leaf water δD modeling.

Uncertainty estimation

The uncertainty associated with different parameters (Table 1) was estimated 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 the normal distribution of the input parameters given in Table S1 and S2. 10% uncertainty was considered for boundary layer conductance, barometric pressure and the δD value of atmospheric water vapor. Uncertainty in the leaf temperature was the standard error of estimation in the regression of air and leaf temperatures which ranged from 0.5 to 0.9 °C.

<i>n</i> -alkanes					<i>n</i> -alkanoic acids				
Plants	$\delta D_{\text{alk}}^{\text{Aug}}$	$\delta D_{\text{alk}}^{\text{Sept}}$	$\delta D_{\text{alk}}^{\text{Oct}}$	$\delta D_{\text{alk}}^{\text{Nov}}$	$\delta D_{\text{acid}}^{\text{Aug}}$	$\delta D_{\text{acid}}^{\text{Sept}}$	$\delta D_{\text{acid}}^{\text{Oct}}$	$\delta D_{\text{acid}}^{\text{Nov}}$	
Deciduous	<i>Tg1</i>	−105	−117	−101	−121	−106	−110	−89	−125
	<i>Tg2</i>	−101	−102	−119	−122	−101	−100	-	−92
	<i>Hc1</i>	−93	−95	−110	−64	−133	-	−86	−35
	<i>Hc2</i>	−100	−114	−121	−70	-	−120	−114	−54
	<i>Su1</i>	−139	−120	−111	-	−157	−152	−122	-
	<i>Su2</i>	−163	−109	−81	−73	−158	−149	−115	−92
Evergreen	<i>Mu1</i>	−115	−99	−92	−14	−124	−134	−113	−56
	<i>Mu2</i>	−112	−116	−111	−107	-	−117	−109	−89
	<i>Sc1</i>	−106	−115	−81	−61	−91	−88	−76	−92
	<i>Sc2</i>	−107	−109	−113	−115	−103	−103	−108	-
	<i>Ci1</i>	−149	−161	−176	−145	−156	−147	−189	−152
	<i>Ci2</i>	−148	−186	−180	−166	−156	−182	-	−161
	<i>Dm1</i>	−121	−126	−126	6	−136	−134	−159	25
	<i>Dm2</i>	−113	−118	−120	−109	-	−122	−144	−139

Table S3. Measured δD values of *n*-alkanes and *n*-alkanoic acids in mature leaves of each plant for August, September, October and November. Species abbreviations: *Tg*- *Tectona grandis*, *Hc*- *Haldina cordifolia*, *Su*- *Sterculia urens*, *Mu*- *Memecylon umbellatum*, *Sc*- *Syzygium cumini*, *Ci*- *Callophylum inophyllum*, and *Dm*- *Diospyros malabarica*.

Plants	<i>n</i> -alkanes						<i>n</i> -alkanoic acids					
	δD_{alk}^{Apr}			δD_{alk}^{May}		δD_{alk}^{June}	δD_{acid}^{Apr}			δD_{acid}^{May}		δD_{acid}^{June}
	1 st	4 th	26 th	19 th	22 nd	15 th	1 st	4 th	26 th	19 th	22 nd	15 th
<i>Tg1</i>	-	-42	-	-59	-	-	-	-51	-	-52	-	-
<i>Tg2</i>	-	-66	-	-64	-	-	-	-68	-	-68	-	-
<i>Hc1</i>	1	-	-108	-	-	-	-19	-	-37	-	-	-
<i>Hc2</i>	-	-	-63	-	-	-86	-	-	-	-	-	-72
<i>Su1</i>	-50	-	-107	-	-132	-	-64	-	-113	-	-138	-
<i>Su2</i>	-	-	-	-	-73	-112	-	-	-	-	-88	-97

Table S4. Measured δD values of *n*-alkanes and *n*-alkanoic acids in young leaves of each plant during April, May and June. Species abbreviations: *Tg*- *Tectona grandis*, *Hc*- *Haldina cordifoli*, *Su*- *Sterculia urens*.

Plants		<i>n</i> -alkanes			<i>n</i> -alkanoic acids		
		δD _{alk} ^{Sept*}	δD _{alk} ^{Oct*}	δD _{alk} ^{Nov*}	δD _{acid} ^{Sept*}	δD _{acid} ^{Oct*}	δD _{acid} ^{Nov*}
Deciduous	<i>Tg1</i>	241±124	441±133	434±101	240±124	441±133	433±101
	<i>Tg2</i>	241±124	446±127	441±102	241±124	446±127	441±102
	<i>Hc1</i>	207±118	386±133	398±105	168±118	347±133	359±105
	<i>Hc2</i>	194±120	378±129	396±103	162±120	345±129	364±103
	<i>Su1</i>	169±114	337±129	363±103	152±114	319±129	345±103
	<i>Su2</i>	144±111	314±131	339±107	149±111	319±131	344±107
Evergreen	<i>Mu1</i>	157±118	332±135	358±111	148±118	323±135	349±111
	<i>Mu2</i>	160±115	337±138	356±105	148±115	325±138	345±105
	<i>Sc1</i>	189±113	352±132	392±102	204±113	367±132	407±102
	<i>Sc2</i>	185±114	362±132	390±103	189±114	366±132	394±103
	<i>Ci1</i>	146±117	322±127	342±107	139±117	316±127	335±107
	<i>Ci2</i>	146±114	313±127	334±111	138±114	305±127	326±111
	<i>Dm1</i>	175±117	363±135	372±104	155±115	338±137	354±104
	<i>Dm2</i>	187±115	348±134	383±107	152±114	338±132	349±108

Table S5. Modeled δD values of *n*-alkanes and *n*-alkanoic acids in mature leaves of each plant, if the new leaf wax was synthesised using tracer water alone during September, October and November. Species abbreviations as in Table S3.

	$\delta D^*_{\text{alk}} - \delta D_{\text{alk}}$				$\delta D^*_{\text{acid}} - \delta D_{\text{acid}}$		
	Plants	Sept	Oct	Nov	Sept	Oct	Nov
Deciduous	<i>Tg1</i>	358±124	542±133	555±101	350±124	530±133	558±101
	<i>Tg2</i>	343±124	565±127	563±102	341±124	-	533±102
	<i>Hc1</i>	302±118	496±133	462±105	-	433±133	394±105
	<i>Hc2</i>	308±120	499±129	466±103	282±120	459±129	418±103
	<i>Su1</i>	289±114	448±129	-	304±114	441±129	-
	<i>Su2</i>	253±111	395±131	412±107	298±111	434±131	436±107
Evergreen	<i>Mu1</i>	256±118	424±135	372±111	282±118	436±135	405±111
	<i>Mu2</i>	276±115	448±138	463±105	265±115	434±138	434±105
	<i>Sc1</i>	304±113	433±132	453±102	292±113	443±132	499±102
	<i>Sc2</i>	294±114	475±132	505±103	292±114	474±132	-
	<i>Ci1</i>	307±117	498±127	487±107	286±117	505±127	487±107
	<i>Ci2</i>	332±114	493±127	500±111	320±114	305±127	487±111
	<i>Dm1</i>	301±117	489±135	366±104	289±115	497±137	329±104
	<i>Dm2</i>	305±115	468±134	492±107	274±114	482±132	488±108

Table S6. Differences between the expected and measured δD values of *n*-alkanes and *n*-alkanoic acids for each plant for September, October and November. Species abbreviations as in Table S3.

Plants		f_{new_alk} (%)			f_{new_acid} (%)		
		Sept	Oct	Nov	Sept	Oct	Nov
Deciduous	<i>Tg1</i>	-3±1	1±1	-3±1	-1±1	3±1	-4±1
	<i>Tg2</i>	0±1	-3±1	-4±1	0±1	2±1	2±1
	<i>Hc1</i>	-1±1	-4±1	6±1	4±2	10±3	20±4
	<i>Hc2</i>	-5±2	-4±1	6±1	4±2	4±1	16±3
	<i>Su1</i>	6±2	6±2	13±3	2±1	7±2	13±3
	<i>Su2</i>	18±6	17±5	18±4	3±1	9±3	13±3
Evergreen	<i>Mu1</i>	6±3	5±2	21±5	-4±2	2±1	14±3
	<i>Mu2</i>	-1±1	0±1	1±1	3±2	3±1	7±2
	<i>Sc1</i>	-3±2	5±2	9±2	1±1	3±1	0±1
	<i>Sc2</i>	-1±1	-1±1	-2±1	0	-1±1	2±1
	<i>Ci1</i>	-4±2	-6±2	1±1	3±2	-7±2	1±1
	<i>Ci2</i>	-13±5	-7±2	-4±1	-9±4	-7±2	-1±1
	<i>Dm1</i>	-2±1	-1±1	26±5	1±1	-5±2	33±7
	<i>Dm2</i>	-2±1	-2±1	1±1	5±2	-2±1	-1±1

Table S7. The estimated fraction of newly synthesized n -alkanes (f_{new_alk}) and n -alkanoic acids (f_{new_acid}) for September, October and November. Species abbreviations as in Table S3.