

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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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 ($\delta D = -2$) leaf's growth and later using isotopically-labeled water ($\delta D = 1000$). 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.

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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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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.

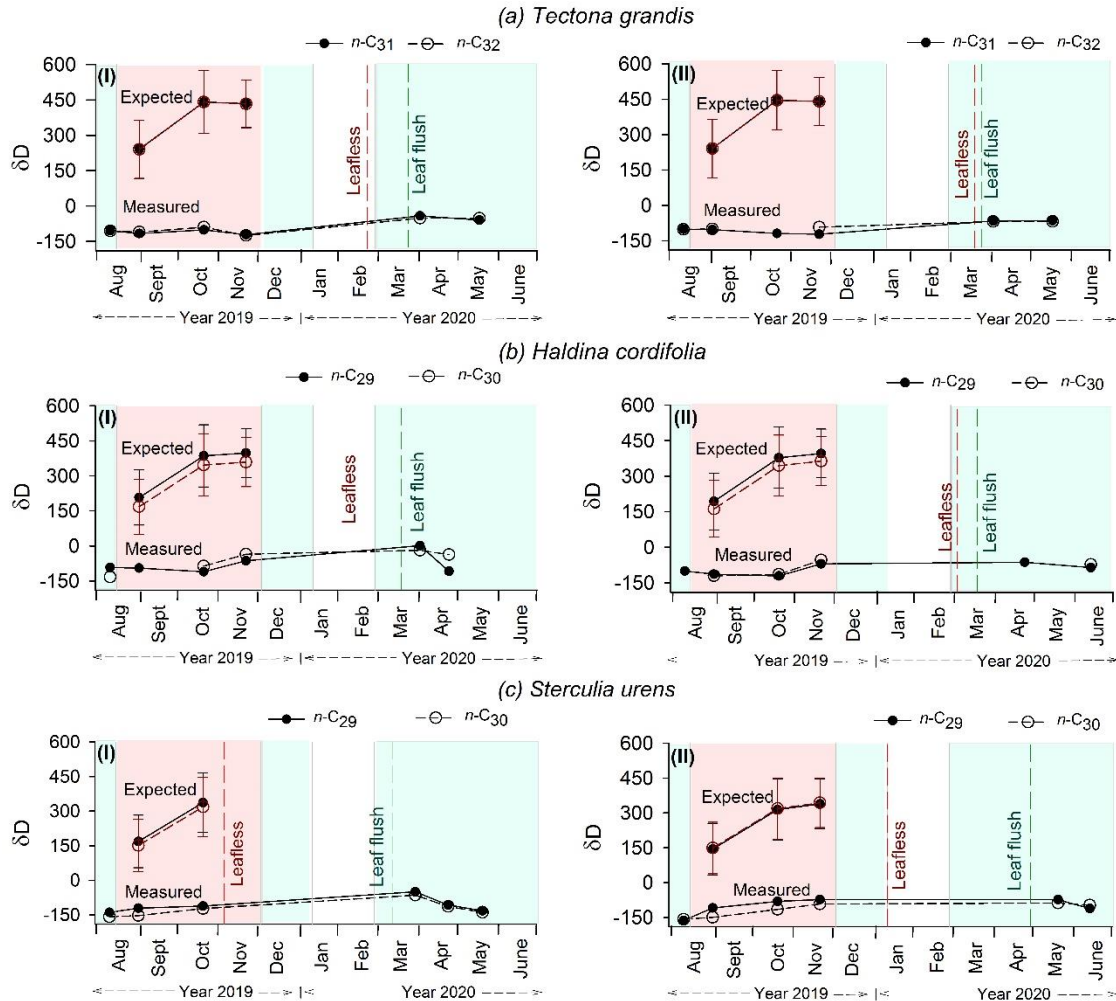
2.3.3 Uncertainty estimation

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

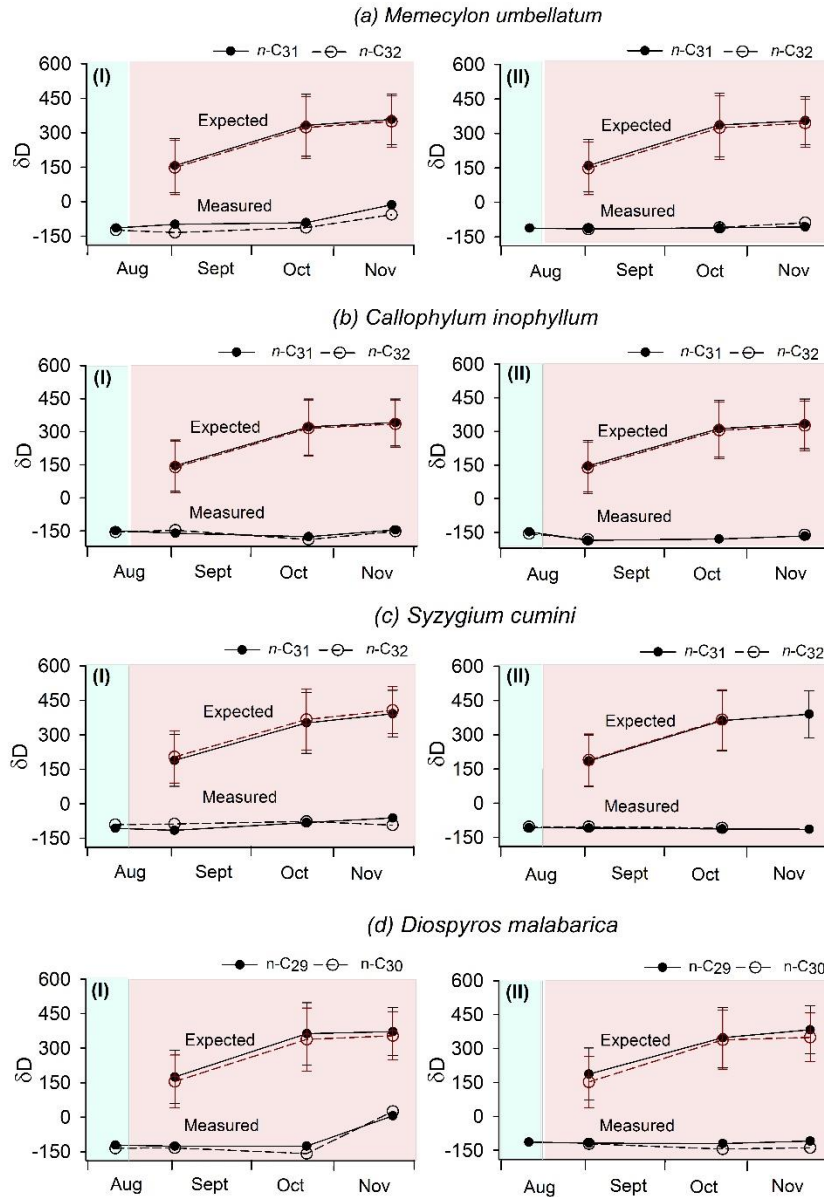
3.1 Temporal changes in the measured δD_{alk} and δD_{acid}

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 ($\delta D = -2\text{‰}$) water (green region) and
 222 isotopically-labeled ($\delta D = 1000\text{‰}$) 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

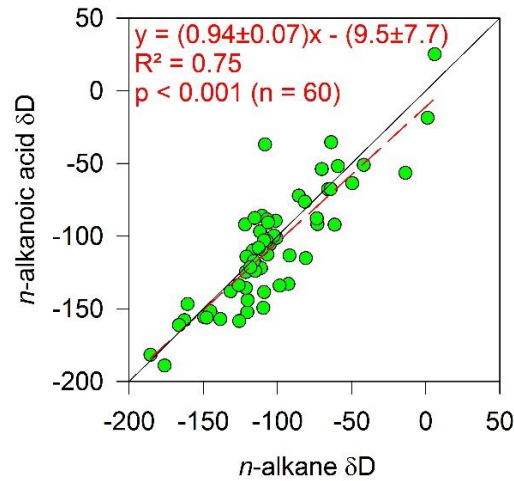


Fig. 3. Correlation between the observed δD_{alk} and δD_{acid} values. The black and red lines are 1:1 and linear best-fit lines, respectively.

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.

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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>

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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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Contents of this file

Text S1 to S6
Figures S1 to S6
Tables S1 to S7

Additional Supporting Information (Files uploaded separately)

NA

Introduction

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

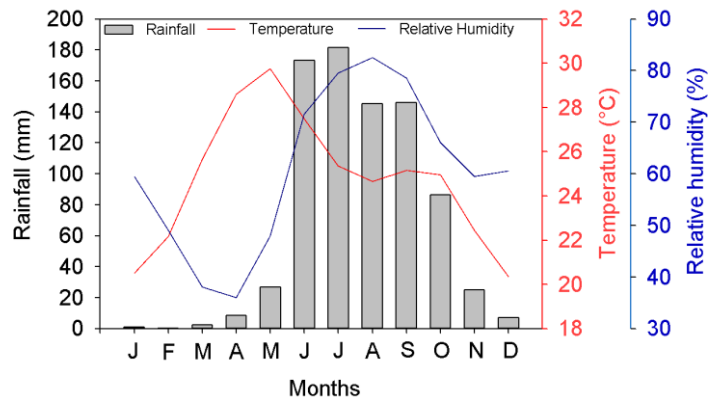


Fig S1. Monthly climatology of rainfall, temperature and relative humidity in the study area. [Data source: India Meteorological Department *Climatological Normals*: (1981 - 2010)]

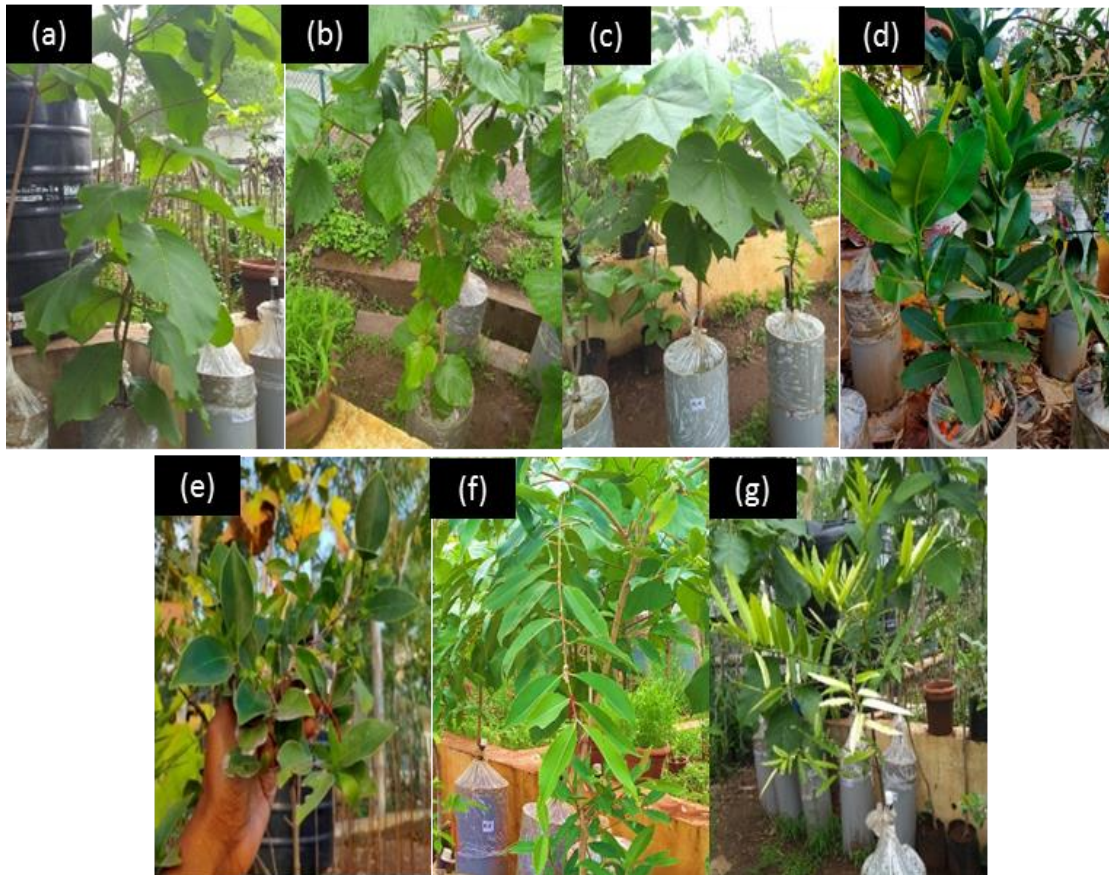


Fig S2. Deciduous [*Tectona grandis* (a), *Haldina cordifolia* (b), *Sterculia urens*(c)] and evergreen [*Callophylum inophyllum* (d), *Memecylon umbellatum* (e), *Syzygium cumini* (f), and *Diospyros malabarica* (g)] species considered in the present study.

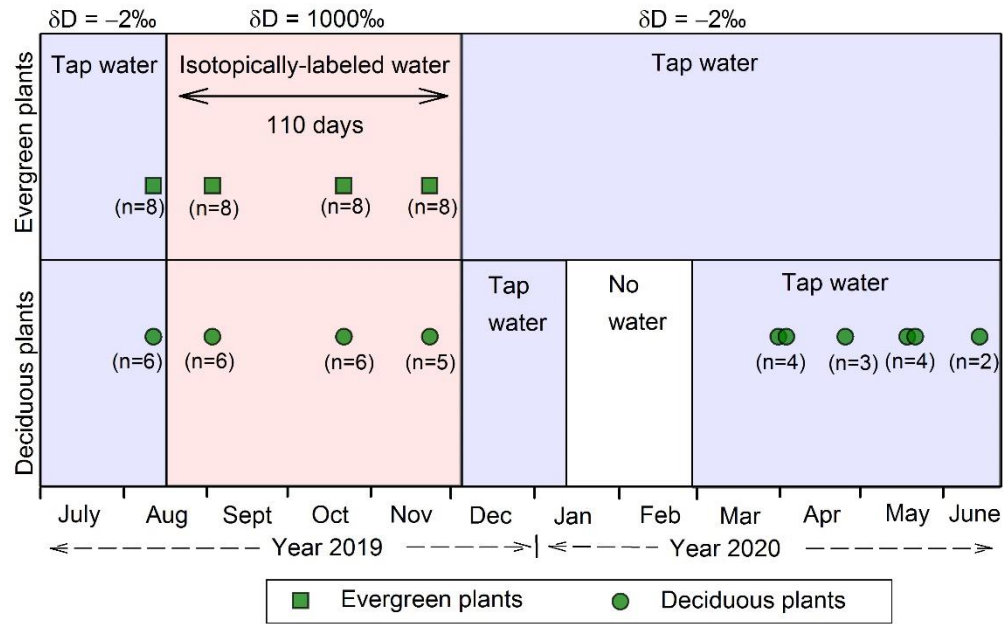


Fig S3. A schematic illustration of the irrigation regime employed and sampling carried out in this study. The purple and beige colors indicate the period of application of normal and tracer water, respectively. After the leafshed, the deciduous species were not irrigated for 47 days (from the 13th of 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.

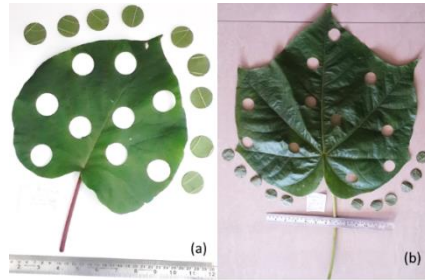


Fig S5. Different parts of leaves were sampled for LMA measurements. For example, (a) *Haldina cordifolia*, and (b) *Sterculia urens*.

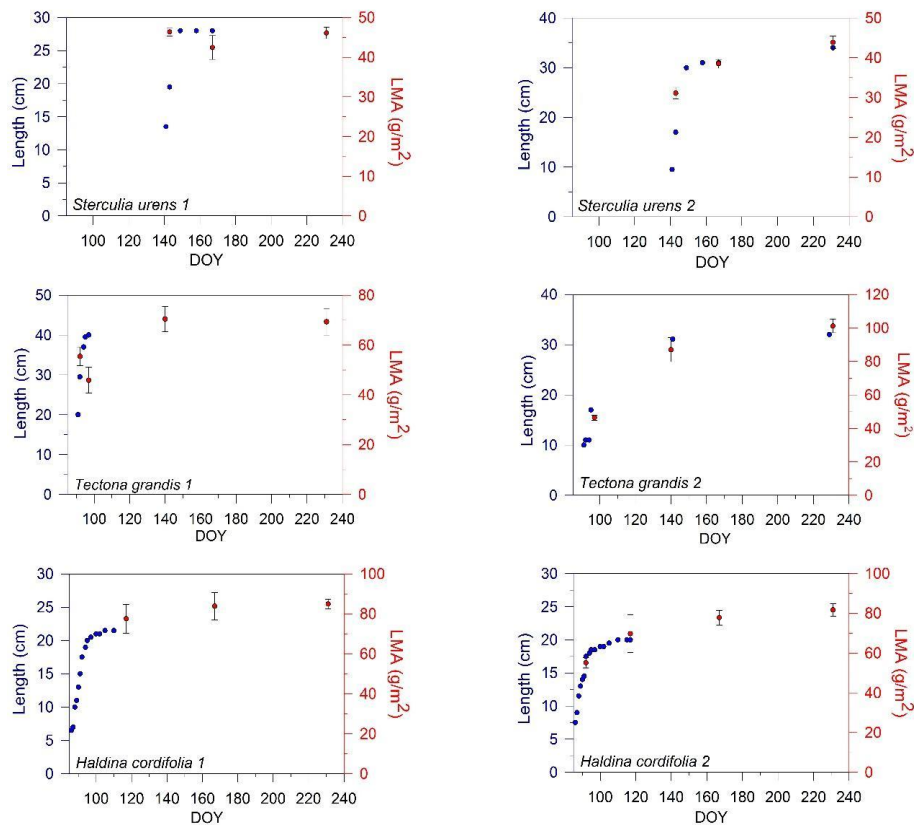


Fig S6. Leaf lengths (blue circles) and LMA (red circles) variation in deciduous leaves.

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 μl^{-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 ($\sim 1 \text{ km}$) 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 ($^{\circ}\text{C}$) [#]	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>Su 2</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.

	Plants	$\delta D^*_{alk} - \delta D_{alk}$			$\delta D^*_{acid} - \delta D_{acid}$		
		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.