

Multiple controls for the variability of hydrogen isotopic compositions in higher plant *n*-alkanes from modern ecosystems

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Abstract

We performed a global scale analysis of available leaf wax *n*-alkane δD data compiled from our new results, as well as from the literature and expressed as average values of D/H ratios from three common lipids of *n*-alkanes with odd carbon numbers (*n*-C₂₇, *n*-C₂₉, and *n*-C₃₁) from living higher plants. Our results clearly indicate multiple controls of hydrogen isotope composition and its variability in plants leaf wax. (1) At the global scale, precipitation δD values play a dominating factor that exercises the first order of control for hydrogen isotopic compositions in plant leaf wax. The hydrogen isotopic composition of plant leaf wax tracks the decreasing trend of precipitation δD with increasing latitude. (2) Because of different water acquisition systems, plant life form influences the hydrogen isotopic composition of leaf wax *n*-alkanes with woody plants and grasses having different responses to the change of global precipitation δD . (3) Physiological difference, due to different photosynthesis pathways or different water usage strategies, can leave an imprint on δD patterns of plant leaf waxes, causing δD variations among plants using the same source water. While these results better explain the variability of hydrogen isotope composition in leaf wax, they also have important implications for the interpretation of *n*-alkane δD data from fossils and ancient sediments.

Keywords: global variation, hydrogen isotopes, *n*-alkane, plant leave wax

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Introduction

With the advent of online gas chromatography/thermal conversion isotope ratio mass spectrometry (GC/TC/IRMS) (Burgoyne & Hayes, 1998; Hilkert *et al.*, 1999), hydrogen isotope geochemistry of specific compounds in natural organic matter has emerged as an effective means to trace paleoenvironmental change (Andersen *et al.*, 2001; Sauer *et al.*, 2001; Huang *et al.*, 2002, 2004; Radke *et al.*, 2005). Because terrestrial plants may regis-

ter the hydrogen isotopic information of source water and because of the stability, abundance, and known source of *n*-alkyl lipids, hydrogen isotopic composition of *n*-alkanes from plant leaf wax has been increasingly used as a favored biomarker for the reconstruction of paleovegetation and paleoclimate (Sessions *et al.*, 1999; Andersen *et al.*, 2001; Sauer *et al.*, 2001; Huang *et al.*, 2002, 2004; Yang & Huang, 2003; Dawson *et al.*, 2004; Sachse *et al.*, 2004a, b; Liu & Huang, 2005; Pagani *et al.*, 2006).

The large mass difference between D and H results in large isotopic fractionations and high variability in nature. While hydrogen isotope composition (δD) of plant leaf wax is expected to vary systematically at a global scale (Smith & Ziegler, 1990; Yakir, 1992), D/H ratios of homologous compounds from different plants

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growing on the same site exhibit significant variance (Bi *et al.*, 2005; Liu *et al.*, 2006; Hou *et al.*, 2007). In addition, hydrogen isotope composition from a single plant utilizing the source water with a constant δD value also varies throughout different seasons (Sessions, 2006). Efforts have been made to characterize and explain the significance of the δD variability in leaf *n*-alkanes and their possible controlling factors (Chikaraishi & Naraoka, 2001, 2003; Yang & Huang, 2003; Sessions & Hayes, 2005; Liu *et al.*, 2006; Sachse *et al.*, 2006; Smith & Freeman, 2006; Hou *et al.*, 2007). These studies, although using limited regional data, have suggested that δD in plant leaf wax is related to precipitation δD , humidity/aridity changes, as well as plant physiology and life forms. However, to date, there is a lack of global-scale analysis, and studies based upon local and regional datasets have yielded conflicting results on these controlling factors. For example, hydrogen isotopic composition of *n*-alkanes from modern plants in Japan and Thailand shows that the C_4 plant leaf waxes had slightly lower δD values than C_3 plant waxes (Chikaraishi & Naraoka, 2003). In contrast, δD data obtained from grasses grown in both greenhouses and in the field of US Great Plains indicated that *n*-alkanes from C_4 grasses are in fact enriched in D relative to those from C_3 grasses (Smith & Freeman, 2006). Our recent data from plants in northwestern China demonstrated that the hydrogen isotope of plant leaf waxes is strongly influenced by plant ecological life forms (i.e. tree, shrub, and grass) rather than plant photosynthetic pathway (C_3 vs. C_4) (Liu *et al.*, 2006).

With the rapid accumulation of global *n*-alkanes δD data from higher plants, it becomes possible to analyze the global pattern of hydrogen isotope composition of leaf waxes in relation to various biological and environmental factors. To further elucidate the distribution pattern of *n*-alkanes δD from higher plants and to better understand their controlling factors, an investigation of the distribution and variation of hydrogen isotope composition from higher plants with a global perspective is necessary. Here, based upon both published results and our new data, we compiled and analyzed all known *n*-alkane δD records from modern higher plants with the following three objectives. First, we perform a global analysis of leaf wax δD of higher plant *n*-alkanes in relation to latitudes and precipitation δD . Second, we attempt to explain the observed variability of global *n*-alkane δD data across geographic distribution and plant taxonomy. Third, we propose a multiple control model by estimating the range of δD variation under different factors. It is our hope that the analysis will shed further light on the mechanism that causes large δD variations in plant leaf wax, and the result would help better interpret *n*-alkane δD from modern as

well as ancient samples. To our knowledge, this study represents the first attempt to analyze plants *n*-alkane δD data from a global perspective.

Methods

The analyzed database is composed of hydrogen isotope compositions of *n*-alkanes from 233 higher plants around the globe, including both grass and woody species of different photosynthesis pathways (Fig. 1 and Table 1). The database was compiled from our previously published results (Liu *et al.*, 2006), as well as data from other parts of China (Bi *et al.*, 2005), Japan and Thailand (Chikaraishi & Naraoka, 2001, 2003), North America (Yang & Huang, 2003; Smith & Freeman, 2006; Hou *et al.*, 2007), and Europe (Sachse *et al.*, 2006).

During the compilation, we realized that there was a critical data gap concerning *n*-alkane δD from plants living in low latitudes. Thus, we generated new data from 16 plants with different life forms (trees, shrubs, and grasses) from a Chinese tropical area in Sanya (18°13'N, 109°30'E). In addition, to supplement our early results on middle latitudes, 20 plants from Yanan to Yuxian in northwestern China were also included for this study (Fig. 1 and Table 2). Sampling sites are constrained to in natural environments without irrigation and farming so that isotope composition of plants would reflect natural water and climatic conditions. The leaf was collected from trees and shrubs, and whole plant was collected for grass samples. To prepare samples for isotope analysis, plant leaves were first cleaned with distilled water to remove dust particles, dried at 40 °C, and then rinsed with DCM (dichloromethane) for three times. Lipid extraction and hydrogen isotope measurement were carried out as previously described (Liu *et al.*, 2006). Briefly, hydrogen isotope analyses were performed using a HP 6890 GC, interfaced via a high temperature conversion interface to a Finnigan MAT Delta + -XP mass spectrometer (Finnigan MAT GmbH, Bremen, Germany). The GC was held at 80 °C for 1 min and subsequently programmed from 80 to 180 °C at 3 °C min⁻¹. The final temperature was held at 300 °C for 10 min. Compounds separated by GC column were converted to H₂ by a pyrolysis reactor at 1445 °C. The determined δD values were against H₂ reference gas that is calibrated by a laboratory work standard. Typical standard deviation for the repeated analyses of standard heneicosane is smaller than $\pm 4\%$. For these new analyses, typical standard deviation for the repeats of standard heneicosane is smaller than $\pm 5\%$.

The resulting database includes samples from a wide geographic area spanning from 16.28°N to 69.5°N in latitude and from 6.52°E to 141.24°E and from 106.53°W to 71.56°W in longitude. The majority of the samples are

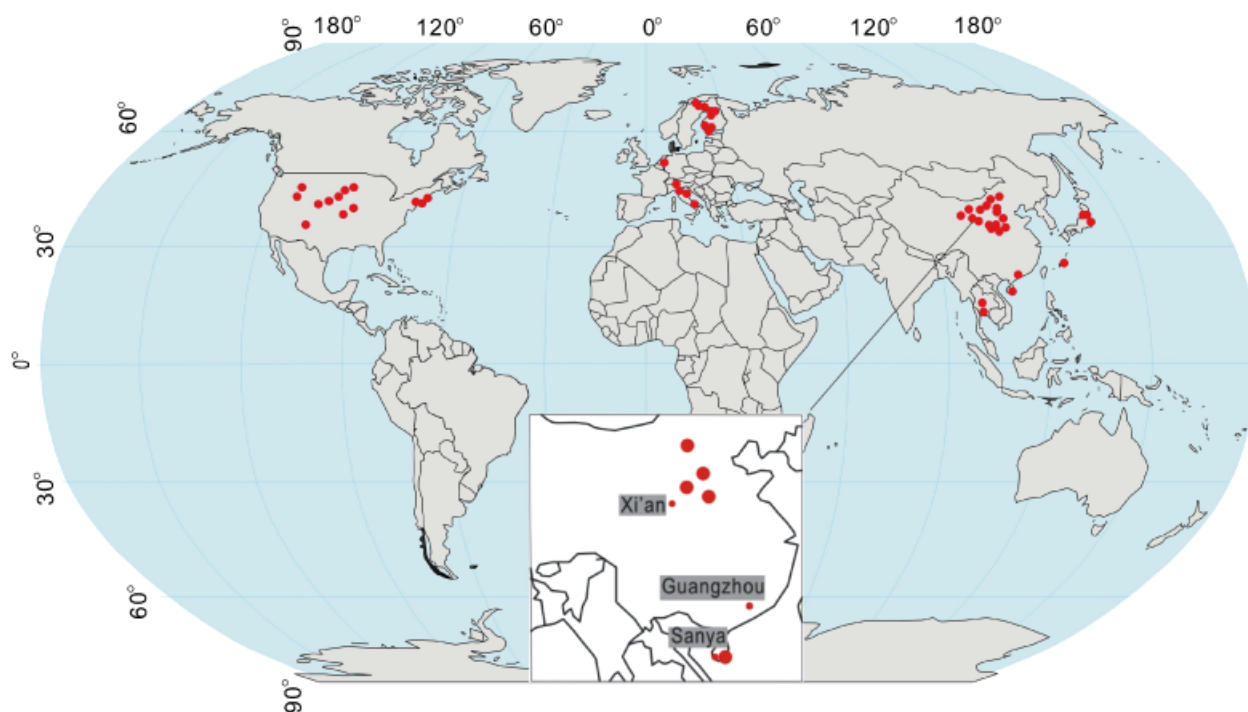


Fig. 1 Distribution of plant samples for hydrogen isotope of *n*-alkanes.

from plants living in the middle latitudes, but high latitude samples are represented by deciduous plants from Europe (Sachse *et al.*, 2006).

Statistical analyses were performed on the database. Pearson correlation was conducted to investigate various correlations between δD of *n*-alkanes and various geographic and environmental factors. One-way ANOVA tests were used to examine the statistical significance of different relationships. The apparent hydrogen fractionation factors between leaf *n*-alkane and source water ($\epsilon_{n\text{-alkane-water}}$) is calculated as in (1):

$$\epsilon_{n\text{-alkane-water}} = \left(\frac{\delta D_{n\text{-alkane}} + 1000}{\delta D_{\text{water}} + 1000} - 1 \right) \times 1000(\text{‰}).$$

Results and discussion

Coherent changes of δD values from C_{27-31} *n*-alkanes

Our compiled global leaf wax δD database has recorded a wide range of hydrogen isotopic compositions from plant *n*-alkanes spanning from -87‰ to -235‰ for *n*- C_{27} , -114‰ to -273‰ for *n*- C_{29} , and -111‰ to -284‰ for *n*- C_{31} , respectively. The δD values of the three most abundant *n*-alkanes with odd C numbers, *n*- C_{27} , *n*- C_{29} , and *n*- C_{31} , are highly coherent, forming a tight linear correlation with average δD values of the

three compounds (Fig. 2). Two-tailed Pearson correlation tests indicate that with very few exceptions, the majority of samples in the three correlations fall within the 95% confident level. Hence, these correlations quantitatively confirm previous observations that hydrogen isotope ratios of individual *n*- C_{27} , *n*- C_{29} , and *n*- C_{31} respond similarly to climatic and environmental factors (Sessions *et al.*, 1999; Chikaraishi & Naraoka, 2003; Yang & Huang, 2003; Bi *et al.*, 2005; Liu *et al.*, 2006; Sessions, 2006; Hou *et al.*, 2007). Despite the previous observation that δD values from *n*- C_{29} lacks a significant correlation with the source water δD based upon the European dataset (Sachse *et al.*, 2006), at the global scale, hydrogen isotope compositions from individual *n*- C_{29} have a good correlation with the C_{27-31} average. Although the correlation between δD values of *n*- C_{27} and the average values has a relative lower R^2 value than that of both *n*- C_{29} and *n*- C_{31} , the average δD values of the three compounds (C_{27-31}) can be used to represent the general trend of δD values of *n*-alkanes from leaf waxes as they are applied separately as individual compounds. Thus, in this analysis, we treat average values of δD from *n*- C_{27-31} as representatives of hydrogen isotope compositions from leaf wax of higher plants. For all data presented here, the values of hydrogen isotopic composition of plant *n*-alkanes range from -278‰ to -99‰ . The most negative δD values (-277‰ and -278‰) were reported both from the grass *Bromus inermis* in the Great

Table 1 Hydrogen isotopic compositions of plants *n*-alkanes in China, 2007 [‰, relative to Standard Mean Ocean Water (SMOW)]

Location	Latitude	Longitude	Plant species	Photosynthetic pathway	Life form	δD (‰)		
						C ₂₇	C ₂₉	C ₃₁
Sanya	18°13'N	109°30'E	No identification	C4	Grass	-171	-171	-163
Sanya	18°13'N	109°30'E	No identification	C4	Grass	-148	-148	-151
Sanya	18°13'N	109°30'E	No identification	C3	Grass	-139	-155	-165
Sanya	18°13'N	109°30'E	No identification	C3	Grass	-148	-162	-155
Sanya	18°13'N	109°30'E	No identification	C4	Grass			-152
Sanya	18°13'N	109°30'E	No identification	C4	Grass	-165	-175	-178
Yuxian	37°05'N	113°02'E	<i>Heteropappus Less</i>	C3	Grass	-189	-197	-217
Yuxian	37°05'N	113°02'E	<i>Cleistogenes Keng</i>	C4	Grass	-194	-204	-226
Yuxian	37°05'N	113°02'E	<i>Stipa bungeana</i>	C3	Grass	-213	-218	-252
Yuxian	37°05'N	113°02'E	<i>Artemisia gmelinii</i>	C3	Grass	-161	-166	-163
Yuxian	37°05'N	113°02'E	<i>Artemisia giraldii</i>	C3	Grass	-168	-182	-186
Luochuan	35°49'N	109°30'E	<i>Bothriochloa ischaemum</i>	C4	Grass	-167	-160	-182
Luochuan	35°49'N	109°30'E	<i>Cleistogenes Keng</i>	C4	Grass	-176	-207	-204
Luochuan	35°49'N	109°30'E	<i>Themeda triandra</i>	C4	Grass	-200	-192	-181
Luochuan	35°49'N	109°30'E	<i>Stipa bungeana</i>	C3	Grass	-158	-168	-213
Luochuan	35°49'N	109°30'E	<i>Potentilla discolor</i>	C3	Grass	-177	-175	-181
Luochuan	35°49'N	109°30'E	<i>Heteropappus Less</i>	C3	Grass	-191	-189	-198
Luochuan	35°49'N	109°30'E	<i>Artemisia giraldii</i>	C3	Grass	-159	-173	-167
Ruicheng	34°44'N	110°25'E	<i>Wikstroemia chamaedaphne</i>	C3	Grass	-167	-184	-178
Ruicheng	34°44'N	110°25'E	<i>Stipa bungeana</i>	C3	Grass	-179	-179	-203
Ruicheng	34°44'N	110°25'E	<i>Cleistogenes Keng</i>	C4	Grass	-165	-179	-181
Sanya	18°13'N	109°30'E	No identification	C3	Tree		-131	-132
Sanya	18°13'N	109°30'E	No identification	C3	Tree		-144	-149
Sanya	18°13'N	109°30'E	No identification	C3	Tree	-140	-159	-143
Sanya	18°13'N	109°30'E	No identification	C3	Shrub		-129	-147
Sanya	18°13'N	109°30'E	No identification	C3	Tree	-134	-134	-141
Sanya	18°13'N	109°30'E	No identification	C3	Tree	-163	-160	-170
Sanya	18°13'N	109°30'E	<i>Spathodea campanulata</i>	C3	Tree	-139	-140	-134
Sanya	18°13'N	109°30'E	<i>Artocarpus heterophyllus</i> Lam.	C3	Shrub	-119	-119	-130
Sanya	18°13'N	109°30'E	<i>Michelia alba</i>	C3	Tree	-174	-186	-188
Sanya	18°13'N	109°30'E	<i>Tamarindus indica</i> Linn.	C3	Tree	-154	-154	-165
Yuxian	37°05'N	113°02'E	<i>Lespedeza davurica</i>	C3	Shrub	-148	-153	-153
Yuxian	37°05'N	113°02'E	Var. <i>spinosa</i> (Bunge)	C3	Shrub	-199	-156	-157
Luochuan	35°49'N	109°30'E	<i>Lespedeza davurica</i>	C3	Shrub	-148	-141	-148
Ruicheng	34°44'N	110°25'E	Var. <i>spinosa</i> (Bunge)	C3	Shrub	-171	-145	-139
Ruicheng	34°44'N	110°25'E	<i>Magonlia</i> Linn	C3	Grass	-162	-159	-164

Plains of the United States (Smith & Freeman, 2006); on the other hand, the most positive δD value (-99‰) was from *Amaranthus paniculatus* L. in Guangzhou of southern China (Bi *et al.*, 2005).

Global distributions of δD in precipitation and plant *n*-alkanes

Meteoric water serves as the ultimate source of hydrogen and deuterium for terrestrial plants (Gat, 1996). Thus, its hydrogen isotope composition, which is largely controlled by a simple Rayleigh distillation model of atmospheric vapor, plays a major role in affecting D/H ratios in tissues of terrestrial plants (Estep &

Dabrowski, 1980; Estep & Hoering, 1980; Yapp & Epstein, 1982; Hobson, 1999). The global distribution of δD values in precipitation exhibits spatial variations that are believed to be driven primarily by initial hydrogen isotope compositions of source water, global circulation of atmospheric moisture, and global temperature gradient (Dansgaard, 1964; Bowen & Revenaugh, 2003). Similar to oxygen stable isotope composition in precipitation (Bowen & Wilkinson, 2002), a general trend of decreasing δD values in precipitation is observed with the increase of latitude (Rozanski *et al.*, 1993; Bowen & Revenaugh, 2003). We fit a curved negative regression line between annual average δD values of global precipitation and the absolute value of latitudes by using

Table 2 Data sources and distribution of plant *n*-alkane D/H values used in this study

Site	Location		No. of samples		Data source*
	Latitude	Longitude	Woody	Grass	
Japan	36°28'N	138°52'E		9	1
Japan	35°42'N	139°43'E	4		1
Japan	26°16'N	127°08'E	1		1
Japan	36°28'N	138°54'E	12		1
Thailand	16°06'N	101°00'E	1	2	1
China	34°10'N	109°19'E	1	1	2
China	35°16'N	108°38'E		1	2
China	43°57'N	116°03'E		1	2
China	35°40'N	106°36'E		1	2
China	36°03'N	103°47'E	1	1	2
China	35°41'N	105°02'E		1	2
China	35°49'N	109°30'E		1	2
China	36°36'N	109°03'E	1	4	2
China	38°23'N	107°30'E	5	5	2
China	18°13'N	109°30'E	10	6	3
China	37°05'N	113°02'E	2	5	3
China	35°49'N	109°30'E	1	7	3
China	34°44'N	110°25'E	2	3	3
China	23.1°N	113.3°E	12	10	4
Great plain	47°09'N	99°18'W		6	5
Great plain	46°50'N	100°55'W		6	5
Great plain	43°58'N	101°52'W		7	5
Great plain	40°42'N	104°47'W		3	5
Great plain	40°00'N	105°16'W		16	5
Blond Pond, USA	42.08°N	71.96°W	44	16	6
USA	42°N	71°W	1		7
USA	43°N	70°W	1		7
USA	47°N	116°W	3		7
USA	43°N	116°W	3		7
Europe	69°00'16"N	20°53'52"E	1		8
Europe	68°50'55"N	21°10'50"E	1		8
Europe	68°40'07"N	21°30'58"E	1		8
Europe	67°22'44"N	26°51'03"E	1		8
Europe	67°21'23"N	27°10'06"E	1		8
Europe	67°10'20"N	26°51'35"E	1		8
Europe	61°50'55"N	24°16'46"E	1		8
Europe	61°11'37"N	25°08'29"E	2		8
Europe	61°03'36"N	25°05'55"E	1		8
Europe	50°07'10"N	6°52'44"E	1		8
Europe	43°50'00"N	10°18'49"E	2		8
Europe	42°45'14"N	10°55'22"E	1		8
Europe	42°36'46"N	11°46'09"E	1		8

*Data source: 1, Chikaraishi & Naraoka (2001), *Phytochemistry* 63; 2, Liu *et al.* (2006) *Oecologia*, 150; 3, this work; 4, Bi *et al.* (2005) *Organic Geochemistry*, 36; 5, Smith & Freeman (2006), *GCA*, 70; 6, Hou *et al.* (2007), *Organic Geochemistry*; 7, unpublished data by Y. Hong; Sachse *et al.* (2006) *Organic Geochemistry*, 37.

water hydrogen isotope data from the Global Network for Isotopes in Precipitation (GNIP), sponsored by the International Atomic Energy Agency and the World Meteorological Organization (IAEA/WMO, 2000) (Fig. 3a). Superimposed on the global precipitation δD distribution, we plotted our entire database of plant

n-alkane δD values against the latitudinal gradient. In general, the plant *n*-alkane δD values decrease with increasing latitude, responding to the latitudinal effect of δD in precipitation at the global scale (Fig. 3a). Although the correlation is poor ($R^2 = 0.1432$) and high variation is observed at a given latitudinal range, the

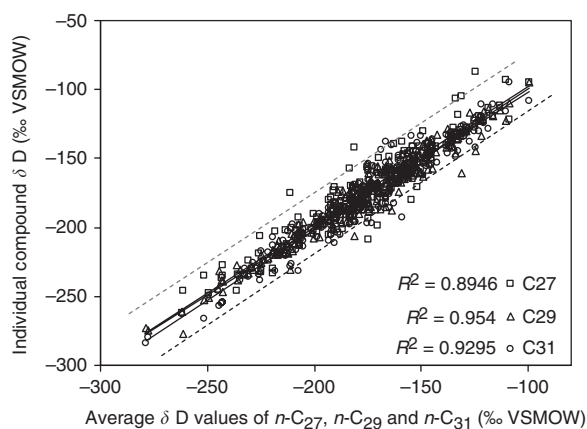


Fig. 2 Global correlation between δD values of individual n -alkanes (C_{27} , C_{29} , and C_{31}) and average δD values (arithmetic mean value) among n - C_{27} , n - C_{29} , and n - C_{31} in modern plants, showing the consistency for change of leaf wax n -alkanes δD values among n - C_{27} , n - C_{29} , and n - C_{31} . The dotted line shows the 95% confidence interval (only shown for n - C_{27} with the lowest R^2 value).

general trend is obvious. However, if we break down the entire database into woody plant and grass categories (Fig. 3b), then it becomes more apparent that (1) while hydrogen isotope composition of both woody and grass species tend to be more negative along with the increase of latitudes, the rates of their changes follow different slopes; (2) at a given latitudinal range, δD values from grasses tend to be more D-depleted than from woody species. In addition, although δD data from grass living at high latitudes are currently lacking, the steep slope of the grass δD trend ($y = -2.5x - 101$) predicts highly negative n -alkane δD values from high latitude grass.

Plant life forms and leaf wax δD

Based upon hydrogen isotope composition of n -alkanes from plants in northern China, we have proposed that plant life forms have a profound influence on leaf wax δD values of higher plants (Liu *et al.*, 2006). In a given geographic region that receives the same precipitation as source water, difference in water absorption systems in various plant forms plays an important role that cause more negative δD values in n -alkanes from grasses than those from woody plants. Similar results were recently obtained in the New England area of northern America (Hou *et al.*, 2007). However, it is uncertain whether this correlation exists at the global scale. Applying our global database, we observe distinguishable distribution patterns between leaf wax δD values from woody plants and grasses with respect

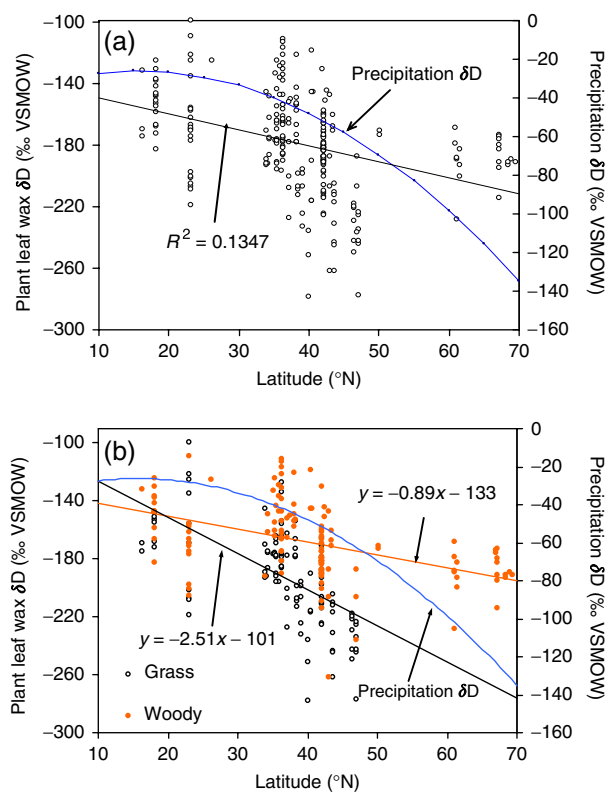


Fig. 3 Global scale variation of hydrogen isotopic compositions in leaf wax n -alkanes of modern plants and in precipitation along a latitudinal gradient in the northern hemisphere. The precipitation average δD is fit with a curved line, presented as $\delta D = -0.037 \times (\text{latitude})^2 + 1.1674 \times (\text{latitude}) - 35.423$, based on the data from the International Atomic Energy Agency and the World Meteorological Organization (IAEA/WMO, 2000) (a). Comparison between woody plants and grasses, noting different slopes between the two life forms ($P < 0.001$) (b).

to global precipitation δD . The results indicate more D-depleted values from grasses (Fig. 4). The δD values of plant leaf wax in woody plants range from -109‰ to -262‰ , with an average of -169‰ ; whereas, the average δD values from grasses vary from -99‰ to -278‰ , with an average of -192‰ . This relationship becomes more apparent when woody plant samples from high latitudes are removed from the analysis due to the lack of high latitudinal grass samples for comparison, as well as because of their unique physiological responses at high latitudes. Hydrogen isotope compositions from both woody and grasses decrease along with the decrease of precipitation δD ; but the woody plants and grasses follow different rates of reduction, with a slope of 1.13 for woody plants and 2.21 for grasses, respectively (Fig. 4b). This observed relationship at the global scale further supports the idea that plant life form plays

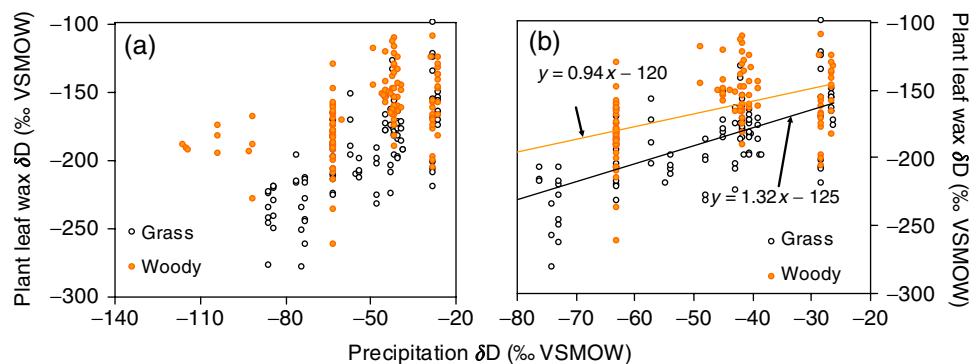


Fig. 4 Relationship between leaf wax *n*-alkane δD values and precipitation δD values: plants from the entire database (a), and after the removal of high latitudinal data ($>60^{\circ}\text{N}$) (b).

a significant role in controlling hydrogen isotopic variations of plant leaf wax (Liu *et al.*, 2006).

Deuterium enrichment in environmental water due to preferential evaporation of the light isotope can be thought at two levels: (1) evaporation of soil water on the ground, and (2) evapotranspiration of leaf water on the plant. Although information on δD in vertical water profile is limited, studies of soil water profile near surface have shown that δD values in soil water usually increase with depth (Griew *et al.*, 2001). Differences in water absorption systems between grass and woody plants may be accounted for the more positive δD values in woody species as they usually have longer and deeper roots, allowing them to utilize water from deeper soil horizons that contain D-enriched water (Dawson & Ehleringer, 1991). Recent studies have indicated that hydrogen isotope fractionation may occur during water uptake in root for woody xerophytes (Ellsworth & Williams, 2007), but such hydrogen isotope effect is rather small ($<10\%$). On the other hand, the disparity between woody and grass hydrogen isotope composition may also be due to the difference in evapotranspiration controlled by microhydraulic systems of tree leaves, creating different relative humidity around plants. The isotope effect due to leaf morphology is plausible as up to 20‰ of lipid δD variation has been observed within a single leaf (Sessions, 2006).

The evapotranspiration of leaf water may also influence the disparity of hydrogen isotope compositions between woody and grass. It is possible that leaf water evaporation in trees is stronger than grasses due to the thickset leaf of woody plants or high WUE. The leaf wax δD values analyzed in our study also shows that hydrogen isotope composition of *n*-alkanes from grass and woody plants are different in most of the mid- to high latitudes, but the disparity is rather weak (statistically insignificant) in the low latitudes with relative warm and humid climate.

Photosynthetic pathways and *n*-alkane δD

Both geologists and biologists are interested in how plants with different photosynthetic pathways (C_4 vs. C_3 plants) would respond isotopically to environmental changes. It has been noted that the ranges of oxygen and hydrogen isotope composition in C_4 and C_3 plants may not be a diagnostic indicator of plant photosynthetic pathways, although carbon isotope composition has been used to define such photosynthetic pathways (Bi *et al.*, 2005). The $\delta^{18}\text{O}$ of C_4 plants is more positive than that in C_3 plants because C_4 species are able to photosynthesize under dry conditions and are not as sensitive to relative humidity as C_3 species (Sternberg & Deniro, 1983; Sternberg *et al.*, 1984; Leaney *et al.*, 1985; Sternberg, 1988; Helliker & Ehleringer, 2000, 2002). Thus, hydrogen isotope composition of C_4 and C_3 plants are hypothesized to behave similarly. Previous data indicated that the δD values in C_4 grasses are indeed more D-enriched than those in C_3 grasses, but the differences are rather small (Bi *et al.*, 2005; Liu *et al.*, 2006; Smith & Freeman, 2006). Based upon our compiled global database, one-way ANOVA tests show no apparent difference in plant leaf wax δD values between C_4 and C_3 grasses at the global scale ($P = 0.644$) (Fig. 5a). However, if we only compare C_4 and C_3 Gramineae plants from the same site in both northern China and the Great Plains in the United States, our data indicate that *n*-alkanes from C_4 Gramineae are δD enriched relative to C_3 Gramineae. This difference, ranging from 2‰ to 35‰ with an average of 18‰, is statistically slight significant ($P = 0.085$) (Fig. 5b). A plot of the relationship between average δD values in between C_3 and C_4 Gramineae from some sampling sites is also given in Fig. 5c. Based on this figure, we find that the δD values all fall below the 1:1 line, and this relationship clearly shows that the δD value of C_4 Gramineae is less negative than C_3 Gramineae if they are growing in the

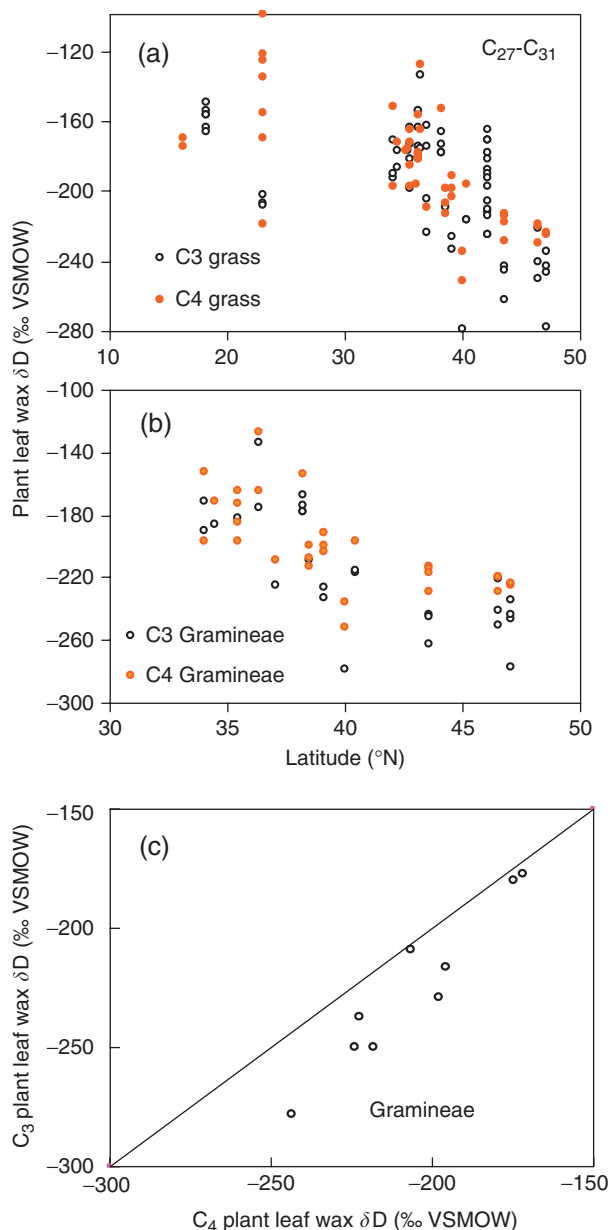


Fig. 5 Hydrogen isotopic variations of leaf wax *n*-alkanes from C₄ and C₃ grasses: showing no significant δ D difference between C₄ and C₃ grasses ($P = 0.644$) at the global scale (a), but they can be distinguished in the same sites in middle latitudes (30–50°N) ($P = 0.085$) (b), and a plot of δ D values of C₃ grass vs. C₄ grass from the same sites in middle latitudes (30–50°N) (c).

same condition. Such a variation has been attributed to the fact that C₄ Gramineae tend to exclusively use warm season precipitation for photosynthesis, resulting in more positive δ D values due to stronger evaporative enrichment of leaf water (Smith & Freeman, 2006). These data are also consistent with previous observations on δ D from plant tissue water in C₃ and C₄ plants (Ziegler *et al.*, 1976; Leaney *et al.*, 1985; Flanagan *et al.*, 1991).

Hydrogen isotope fractionation and the high latitude effect

Using the δ D data of known local precipitation for the Great Plain and Europe, and global precipitation for other sites where local precipitation δ D data is lacking, we have documented a large variation of apparent hydrogen isotope fractionation between D/H ratios of plant *n*-alkanes and source water ($\epsilon_{n\text{-alkane-water}}$, simplified as ϵ), ranging from –73‰ to –242‰ (average of –156‰) for grasses and from –57‰ to –220‰ (average of –120‰) for woody species (Fig. 6a). These ϵ values, in woody species and grasses, have greater variations in absolute values than previously reported values of 91–152‰ for *n*-alkyl lipids relative to ambient water (Chikaraishi *et al.*, 2004). Previous investigations have also suggested that there maybe a constant hydrogen isotopic biological fractionation about 160‰ for higher plants (Sessions *et al.*, 1999; Chikaraishi *et al.*, 2004; Sachse *et al.*, 2004b, 2006). Our compiled *n*-alkane δ D data indicated that while the average ϵ in the grasses (–157‰) is close to what has been reported for the biological fractionation (ca. –160‰ in Sessions *et al.*, 1999; Sachse *et al.*, 2004b, 2006), it is unlikely that a constant hydrogen isotopic fractionation factor between leaf wax *n*-alkanes and corresponding environmental water would hold across plant taxa. In general, while $\epsilon_{n\text{-alkane-water}}$ in both woody plants and grasses tend to be more negative with the decrease of precipitation δ D, the average of ϵ value are less positive in grasses (–156‰) than those in woody plants (–120‰), reflecting an enhanced biological fractionation for hydrogen isotope in grasses (Sessions *et al.*, 1999; Chikaraishi *et al.*, 2004; Sessions, 2006).

We have noticed that hydrogen isotopic composition of *n*-alkanes from woody plants living in relatively high latitudes (>60°N) tend not to follow the projected trajectory for leaf wax δ D and precipitation δ D (Figs 3b and 4a). Woody plants, especially deciduous trees, in higher latitudes exhibit similar or small hydrogen isotope fractionation factors than their middle latitude counterparts (Fig. 6a). When we plot global *n*-alkane δ D data against calculated apparent hydrogen isotope fractionation factors ($\epsilon_{n\text{-alkane-water}}$), a linear relationship emerges between $\epsilon_{n\text{-alkane-water}}$ and *n*-alkane δ D in plants from middle and low latitudes, with more depleted δ D in leaf wax corresponding to more negative $\epsilon_{n\text{-alkane-water}}$. Apparently, plants from high latitudes fall off of this global relationship (Fig. 6b). The detrended samples from plants at higher latitudes usually have 40–50‰ more positive $\epsilon_{n\text{-alkane-water}}$ than their lower latitude counterparts. In addition, analysis based on our dataset shows a positive correlation between latitude and apparent hydrogen isotope fractionation factor, with more positive ϵ obtained from deciduous

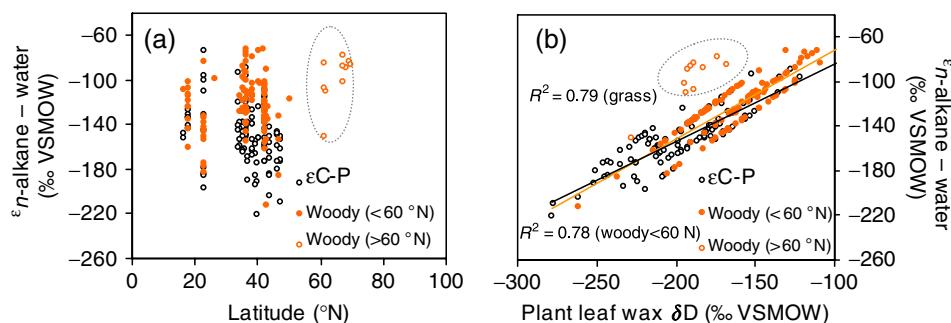


Fig. 6 Variations of hydrogen isotopic fractionation factor (ϵ) for leaf wax n -alkane relative to precipitation with latitude (a) and plant leaf wax δD (b). Note the offset in ϵ between grass and woody species ($P < 0.001$). In general, the ϵ values of leaf wax in woody plants (circled) were more positive than those in grasses.

plants at higher latitudes. This is in agreement with previous observations by Smith & Freeman (2006) and Sachse *et al.* (2006) based upon regional data in North America and Europe, respectively.

In addition to the source water control and the influence of plant life forms (Liu *et al.*, 2006), we propose that environmental factors associated with the temperate climatic zone of higher latitudes, such as the quantity and quality of light irradiation and the contrast of day/evening temperatures, may have a strong influence on the physiology of deciduous plants, resulting in the observed abnormality of $\epsilon_{n\text{-alkane-water}}$ in these plants. Experimental studies have predicted that the intensity and duration of sun light irradiation would affect stable isotope behavior of deciduous plants by controlling the rate of photosynthesis and evapotranspiration. Recent greenhouse-based studies have revealed that leaves from deciduous conifers under simulated arctic light of 3 months of continuous irradiation would yield an average of 25–33‰ D enrichment, resulting in more positive $\epsilon_{n\text{-alkane-water}}$ by up to 40‰ (H. Yang, unpublished data). This level of influence by light irradiation, while not necessarily the sole cause of δD variation, provides at least one plausible explanation for the abnormally positive $\epsilon_{n\text{-alkane-water}}$ calculated for high-latitude deciduous plants (Fig. 6b).

Implications for the interpretation of n -alkane δD data

Hydrogen isotope composition of long-chain n -alkanes from sediments, such as lakes and soils, have been increasingly used as paleoclimatic and paleohydrological proxies (Andersen *et al.*, 2001; Sauer *et al.*, 2001; Sessions, 2001; Huang *et al.*, 2002, 2004, 2007; Sachse *et al.*, 2004a, b; Liu & Huang, 2005). The complicity of hydrogen isotope compositions of precipitation and hydrogen isotope fractionation from source water to lipid synthesis in terrestrial plants resulted in hydrogen isotope variations that made paleoenvironmental inter-

pretations less straightforward. Our analysis revealed multiple levels of controls for the variability of hydrogen isotope compositions of n -alkanes in modern higher plants. While their controlling mechanisms are currently not fully understood, it is essential to consider the intricacy of these factors and their influence on the range of δD variations before reliable interpretations of n -alkane δD signals from sediments can be made.

Latitudinal influence of global precipitation δD along a latitudinal gradient, as results from moisture transport from low to high altitudes (Caballero & Langen, 2005), exercises the first order of controls that may result in the variation of n -alkane δD over 100‰ at the global scale. Except for high latitude deciduous plants, the hydrogen isotopic compositions of leaf wax in higher plants, in general, carry isochronous changes with precipitation δD values (Fig. 3). This global trend is important as n -alkane δD data are increasingly used in the study of paleohydrological systems (Schefuß *et al.*, 2005; Pagani *et al.*, 2006; Huang *et al.*, 2007). A better understanding of global paleoclimatic patterns can be achieved by analyzing leaf wax δD from same-aged samples at different latitudes. Equally important is the influence of plant life forms on hydrogen isotopic fractionation between plant and environmental water as different plant life forms are capable of producing n -alkane δD variation up to 60–70‰ (Liu *et al.*, 2006; Hou *et al.*, 2007). Photosynthetic pathways can be a source for generating δD variations but the contribution is relatively small, in the range of <35‰ (Liu *et al.*, 2006; Smith & Freeman, 2006). Unless n -alkanes are isolated from known *in situ* fossil species (e.g. Yang & Huang, 2003), sedimentary δD data represent a mixed signal from local vegetation; thus, hydrogen isotope variations caused by changes of vegetation have to be considered. Hence, as it is critical to separate the vegetation effect from climate signals, and complimentary paleobotanic information, in terms of the diversity of the flora, relative abundance of dominant species, and the

constitution of different plant life forms and photosynthetic pathways, would aid the interpretation of δD from sediment *n*-alkanes. On the other hand, when independent evidence suggests that precipitation δD is relatively stable (e.g. long periods of climate stability or contemporary samples from small geographic regions), the δD value of leaf wax *n*-alkane extracted from ancient sediments may be used to indicate ecosystem evolution with respect to its ecological functions and diversity.

In addition to precipitation, other environmental factors, such as temperature contrast, may influence δD in leaf wax, especially for deciduous plants in temperate zones, and such impacts can cause variation in δD in the range of 10–40‰. Because of the physiological difference, D/H from homologous molecules of different plants with the same life form can vary up to 50‰ [e.g. between *Myrtus* and *Betula* in Sachse *et al.* (2006)]. Sessions (2006) has documented 40‰ decrease in *n*-alkanes in C_4 grass *Spartina alterniflora* during summer months, and this seasonal change has been attributed to biosynthetic fractionations due to the use of different sources for the same organic substrate for biosynthesis.

Our analysis further supports the idea that a major forcing for $\epsilon_{n\text{-alkane-water}}$ is plant anatomy and site condition (Sachse *et al.*, 2006; Smith & Freeman, 2006). Thus, the interpretation of ancient *n*-alkane δD data must take plant physiology into account. Our global analysis reconfirmed a recent observation that is contrary to what has been previously suggested (Chikaraishi & Naraoka, 2003), a consistent biological hydrogen isotope fractionation factor unlikely exists across different taxonomic groups (Sessions & Hayes, 2005; Liu *et al.*, 2006; Hou *et al.*, 2007). Thus, rather than using a universal $\epsilon_{n\text{-alkane-water}}$, taxon-specific apparent fractionation factors need to be calibrated before applying to paleoclimate reconstruction. In addition, as no data have suggested that major plant physiology changed through time, plant taxa with known conserved physiology (such as 'living fossils') should be examined for climatic signals. When this is not available, at least comparison should be made among plants having similar life form/photosynthesis pathways.

Conclusions

Our global analysis of available leaf wax δD data, represented by average values of D/H ratios from three common lipids of *n*-alkanes with long carbon chains of odd carbon numbers (*n*- C_{27} , *n*- C_{29} , and *n*- C_{31}) from living higher plants, clearly indicates multiple control of hydrogen isotope composition and its variability in plants leaf wax. These different levels of controls imposed different magnitudes of variation on plant δD

and can be divided into the following categories. (1) Precipitation δD values play a dominating factor that exercises the first order of control for hydrogen isotopic composition of plant leaf wax at the global scale. (2) Plant life form is an important factor in controlling hydrogen isotopic composition of leaf wax *n*-alkanes by tapping into different water pools and by different mechanisms of biological fractionation. On a global scale, woody plants and grasses have different responses to global precipitation δD changes. (3) Physiological differences, including different photosynthesis pathways or different water-use efficiency, can also leave an imprint on δD patterns of plant leaf waxes, causing δD variations among plants using the same source water. Thus, the biological significance of δD values from plant *n*-alkanes is dependent upon a better understanding of these factors, and better interpretation of δD data from compound-specific analysis of hydrogen isotope composition from sediments geological samples requires accounting for these influences.

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