

Hydrogen isotopic compositions of *n*-alkanes from terrestrial plants correlate with their ecological life forms

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Abstract Stable hydrogen isotopic compositions (δD) of compound-specific biomarkers, such as *n*-alkanes from plant leaf waxes, can be used as a proxy for paleoclimatic change. However, the relationship between hydrogen isotopes of plant leaf wax and plant ecological life forms is not well understood. Here, we report the δD of *n*-alkanes from 34 modern terrestrial plants, including twenty-one C_3 plants and thirteen C_4 plants from northwestern China, determined using gas chromatography/thermal conversion/isotope ratio mass spectrometry. Our data show that the stable hydrogen isotopes are poorly correlated with the plant photosynthetic pathway (C_3 vs. C_4) and that they do not give

clear regional precipitation signals. Together with a comparative analysis of published δD values from plant leaf waxes in other regions, we believe that the stable hydrogen isotope of plant leaf waxes is more closely related to ecological life forms of these terrestrial plants (i.e. tree, shrub, and grass). In general, the grasses have more negative δD values than the co-occurring trees and shrubs. Our findings suggest that the δD values of sedimentary leaf waxes from higher plants may record changes of a plant ecosystem under the influence of environmental alteration and imply that reconstruction of the paleoclimate using δD values from plant *n*-alkanes should be based upon specific plant taxa, and comparison should be made among plants with similar ecological life forms.

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Introduction

The hydrogen isotopic composition (δD) of terrestrial plants has been applied as a proxy to reconstruct past climatic changes and other paleoenvironmental conditions, such as relative humidity, soil water efficiency, and temperature (Epstein et al. 1977; Estep and Hoering 1980; Yapp and Epstein 1982; White et al. 1985; Sternberg 1988; Feng and Epstein 1994). However, hydrogen isotopic studies of geological record are largely limited to plant cellulose from tree rings and peat deposits (Smith and Ziegler 1990; Sternberg et al. 1984; Sternberg 1988; Feng and Epstein 1994; Terwilliger and DeNiro 1995; Roden et al. 2000). For terrestrial sediments, plant leaf wax is a reliable, widespread,

and stable molecular material that offers good potential for paleoenvironmental studies (Eglinton and Hamilton 1967; Goñi et al. 1997; Huang et al. 2002). Hydrogen isotope compositions of long-chain *n*-alkanes extracted from organic matter in terrestrial sediments, such as lake and soils, can be used to trace paleoclimatic evolution in deep geological times. With the development of gas chromatography (GC)/thermal conversion (TC)/isotope ratio mass spectrometry (IRMS) (Burgoyne and Hayes 1998; Hilkert et al. 1999), hydrogen isotopic signals of long-chain *n*-alkanes have recently emerged as a new paleoclimatic and paleohydrological proxy (Sessions et al. 1999; Sessions 2001; Xie et al. 2000; Andersen et al. 2001; Sauer et al. 2001; Huang et al. 2002, 2004; Yang and Huang 2003; Sachse et al. 2004a, b).

However, the δD values of long-chain *n*-alkanes in modern plants have not been systematically investigated, thus hydrogen isotopic signals of modern vegetation are poorly understood. In addition, the mechanism accounting for the δD of long-chain *n*-alkanes in modern plants has not been systematically examined until recently. Chikaraishi and Naraoka (2003) examined the δD of leaf wax *n*-alkanes of modern plants from Japan and Thailand, and found that C_4 plant-leaf waxes have slightly lower δD values than C_3 plant waxes. However, a preliminary analysis of modern plants from northwest China has shown that δD values seem to be primarily controlled by plant taxonomy (Liu and Huang 2005).

The aim of this work was to better understand possible causes and controls on δD values of the long-chain *n*-alkanes in terrestrial plants. We explore δD values of long-chain *n*-alkanes from plant leaf wax in modern woody and grass species, including both C_4 and C_3

plants, in arid and semi-arid areas of northwestern China. The hydrogen isotopic variations of long-chain *n*-alkanes in these plants were compared with those of plants with different photosynthetic pathways (C_3 and C_4) exposed to different climatic parameters (precipitation and temperature). Plants with different ecological life forms but growing at the same site were used for comparative analysis. We also compare our results with the published δD records of modern plants from Japan and Thailand.

Materials and methods

Samples and isotopic analysis

Plant samples were collected from northwestern China, where the climate is arid and semi-arid (Fig. 1). The modern vegetation in the study area is dominated by grasses and shrubs of a mixed C_4 and C_3 ecosystem (Liu et al. 2003). A total of 26 plant samples, including seventeen C_3 plants and the nine C_4 plants were used in this study (Table 1). Several plant species were sampled from within 4-m² grids at the same site (in Yanan and Ertuoqeqi) in order to assess the variations of the δD of plant leaf wax from plants species with different ecological forms. The mean annual temperature ranges from 7.8 to 21°C and the annual precipitation ranges from 250 to 750 mm. The meteorological data were recorded from 1991 to 2001. The mean annual rainfall and mean annual temperature in the past 10 years (1991–2001) recorded by the Meteorological Centres of Shanxi and Guansu provinces are used for this study.

To prepare samples for isotope analysis, plant leaves were first cleaned with distilled water to remove dust

Fig. 1 Sampling sites of this study in northwestern China

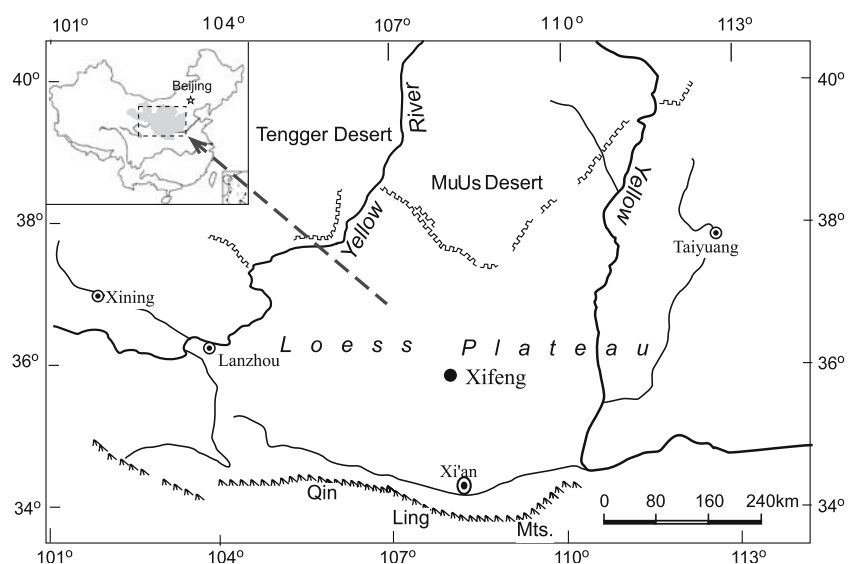


Table 1 Individual hydrogen isotopic compositions (δD) of *n*-alkanes from plants in northwestern China. MAP Mean annual precipitation, MAT mean annual temperature

No.	Location	MAP (mm)	MAT (°C)	Species	Family	Life form	Photosynthetic pathway	δD (C ₂₇) (‰)	δD (C ₂₉) (‰)	δD (C ₃₁) (‰)	δD (C ₂₇ , C ₂₉ , C ₃₁) (‰)
1	Yanan	462	10.5	<i>Artemisia scoparia</i>	Chenopodiaceae	Shrub	C ₃	-140	-142	-133	-138
2	Yanan	462	10.5	<i>Bothriochloa ischaemum</i>	Gramineae	Grass	C ₄	-157	-152	-186	-165
3	Yanan	462	10.5	<i>Vitex negundo</i>	Verbenaceae	Shrub	C ₃	-107	-145	-150	-148
4	Yanan	462	10.5	<i>Stipa grandis</i>	Gramineae	Grass	C ₃	-112	-146	-149	-134
5	Yanan	462	10.5	<i>Svictifolia hance</i>	Leguminosae	Shrub	C ₃	-113	-115	-113	-113
6	Yanan	462	10.5	<i>Stipa bungeana</i>	Gramineae	Grass	C ₃	-140	-154	-197	-176
7	Yanan	462	10.5	<i>Dracocephalum moldavica</i>	Labiatae	Shrub	C ₃	-140	-167	-183	-163
8	Yanan	462	10.5	<i>Pennisetum flaccidum</i>	Gramineae	Grass	C ₄	-117	-117	-138	-127
9	Yanan	462	10.5	<i>Prinsepia</i>	Rosaceae	Shrub	C ₄	-156	-156	-178	-167
10	Ertuokeqi	243	7.8	<i>Agropyron desertorum</i>	Gramineae	Grass	C ₃	-168	-185	-184	-179
11	Ertuokeqi	243	7.8	<i>Citibetica kom</i>	Leguminosae	Shrub	C ₃	-145	-165	-164	-158
12	Ertuokeqi	243	7.8	<i>Asparagus officinalis</i>	Liliaceae	Shrub	C ₃	-131	-164	-154	-149
13	Ertuokeqi	243	7.8	<i>Corispermum hyssopifolium</i>	Chenopodiaceae	Shrub	C ₄	-123	-116	-124	-121
14	Ertuokeqi	243	7.8	<i>Oxytropis aciphylla</i>	Labiatae	Shrub	C ₃	-142	-162	-158	-154
15	Ertuokeqi	243	7.8	<i>Achnatherum splendens</i>	Gramineae	Grass	C ₃	-173	-182	-180	-178
16	Ertuokeqi	243	7.8	<i>Stipa bungeana</i>	Gramineae	Grass	C ₃	-156	-167	-197	-173
17	Ertuokeqi	243	7.8	<i>Cleistogenes squarrosa</i>	Gramineae	Grass	C ₄	-149	-161	-151	-154
18	Ertuokeqi	243	7.8	<i>Stipa glareosa</i>	Gramineae	Grass	C ₃	-162	-159	-179	-167
19	Ertuokeqi	243	7.8	<i>Caragana stenophylla</i>	Leguminosae	Shrub	C ₃	-133	-133	-153	-143
20	Baishui	510	11.9	<i>Bothriochloa ischaemum</i>	Gramineae	Grass	C ₄	-173	-170	-188	-177
21	Huanglong	542	9.0	<i>Pinus tabulaeformis</i>	Pinaceae	Tree	C ₃	-136	-136	-136	-136
22	Huanglong	542	9.0	<i>Pinus tabulaeformis</i>	Pinaceae	Tree	C ₃	-150	-137	-155	-147
23	Huining	347	7.5	<i>Stipa bungeana</i>	Gramineae	Grass	C ₃	-171	-173	-188	-177
24	Jitantai	243	7.8	<i>Haloxylon ammodendron</i>	Chenopodiaceae	Shrub	C ₄	-152	-140	-145	-145
25	Jitantai	243	7.8	<i>Haloxylon ammodendron</i>	Chenopodiaceae	Shrub	C ₄	-113	-114	-129	-119
26	Lanzhou	375	6.9	<i>Salsola collina</i>	Chenopodiaceae	Shrub	C ₄	-128	-130	-130	-129
27	Lanzhou	375	6.9	<i>Pennisetum flaccidum</i>	Gramineae	Grass	C ₄	-194	-195	-200	-196
28	Luochuan	556	10.1	<i>Bothriochloa ischaemum</i>	Gramineae	Grass	C ₄	-163	-157	-173	-164
29	Lantian	673	13.2	<i>Heteropappus less</i>	Compositae	Grass	C ₃	-185	-186	-206	-192
30	Neimeng			<i>Stipagrandis</i>	Gramineae	Grass	C ₃	-200	-205	-226	-210
31	Pengyang	413	7.7	<i>Cleistogenes squarrosa</i>	Gramineae	Grass	C ₄	-176	-178	-174	-176
32	Yanchi	301	8.9	<i>Peganum harmala</i>	Oxalidaceae	Shrub	C ₃	-158	-160	-134	-150
33	Yijun	645	9.8	<i>Lespedeza davurica</i>	Leguminosae	Shrub	C ₃	-133	-136	-131	-133
34	Yingchuan	349	8.7	<i>Oxytropis aciphylla</i>	Labiatae	Shrub	C ₃	-148	-158	-153	-153

particles, dried at 40°C, and then rinsed 3 times with dichloromethane. The *n*-alkane fraction was fractionated from total lipids by column chromatography (100–200 mesh silica gel) using a hexane solution. The concentrations of individual compounds were determined using a Hewlett-Packard 6890 GC with a 30-m capillary column (320 mm diameter, 0.25 mm film thickness).

Hydrogen isotope analyses were performed using a GC/TC/IRMS (Burgoyne and Hayes 1998; Hilkert et al. 1999). An HP 6890 GC, interfaced via high temperature conversion to a Finnigan MAT Delta+XP mass spectrometer at the Lanzhou Institute of Geology, Chinese Academy of Sciences was employed for compound-specific isotope analysis. Injection was performed in the splitless mode to deliver typical sample amounts of compound to the column. The GC was held at 80°C for 1 min and programmed to increase subsequently from 80 to 180°C at 3°C/min. 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 1,445°C. Each sample was measured twice and a set of *n*-alkanes with known δ D values was determined daily to ensure the accuracy of the instrumentation. A typical SD for the repeated analyses of standard heneicosane is smaller than $\pm 4\%$.

Results and discussion

δ D values of *n*-alkanes

The hydrogen isotope values of C₂₇, C₂₉, and C₃₁ *n*-alkanes from the 34 modern C₃ or C₄ plants from northwestern China are given in Table 1. These results also include the δ D values of eight plant samples that were previously determined (Liu and Huang 2005).

The δ D values of long-chain *n*-alkanes range from –200 to –103‰ (C₂₇), –205 to –115‰ (C₂₉), and –226 to –113‰ (C₃₁), respectively. The average δ D values of long-chain *n*-alkanes from C₂₇, C₂₉, and C₃₁ range from –210 to –113‰. These ranges are generally consistent with previously published values of compound-specific hydrogen isotopes from modern plants from other parts of the world (Chikaraishi and Naraoka 2003; Yang and Huang 2003).

At both Yanan and Ertuoqeqi, the δ D values of *n*-alkanes from different species sampled in the 4-m² grids showed large variations. For example the range for δ D values of C₂₉ *n*-alkanes is 50‰ for the nine species in Yanan, and 71‰ for the ten species in Ertuoqeqi. Furthermore, the range of variation is similar in all samples in the study area. This led us to believe that such variations may be taxonomically related, and

ecological life forms may play an important role in controlling hydrogen isotopic variations in the leaf waxes of terrestrial plants.

δ D of plant *n*-alkanes and mean annual precipitation

The δ D of leaf water can be enriched in deuterium by direct transpiration of leaves and/or indirectly by evaporation of soil water (Flanagan et al. 1991). Similar to hydrogen isotopic fractionation of wood cellulose (Yapp and Epstein 1982; White et al. 1985; Roden and Ehleringer 1999), the δ D values of plants *n*-alkanes are directly linked to leaf water and therefore should, in theory, preserve signatures of δ D values of precipitation and the environmental humidity under which the plant species has grown.

In northwestern China, environmental moisture is largely controlled by the amount of precipitation from the Pacific and Indian Ocean (Ding 1994). From the southeast to the northwest of the study region, humidity progressively decreases with a reduction of mean annual rainfall, which coincides with increasing distance from the coast of the South China Sea and the Indian Ocean.

Figure 2 summarises average δ D values of long-chain *n*-alkanes (C₂₇, C₂₉, and C₃₁) from plant leaf waxes in the study area along a southeast–northwest climatic gradient, with the mean annual precipitation (MAP) ranging from 700 to 250 mm and average δ D values of the MAP ranging from –65‰ in the northwest to –51‰ in the southeast (IAEA/WMO 2000). No significant correlation between δ D values of plant *n*-alkanes and MAP was found in the study area. These

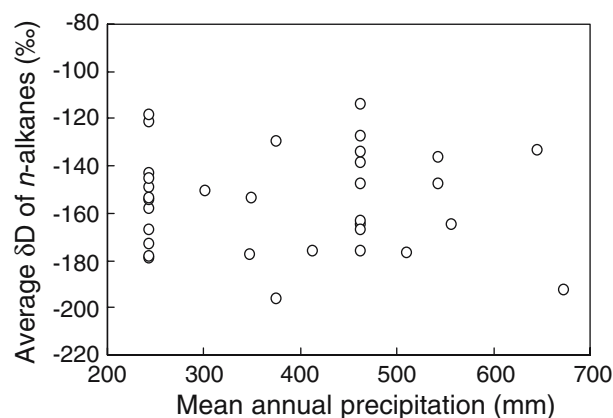
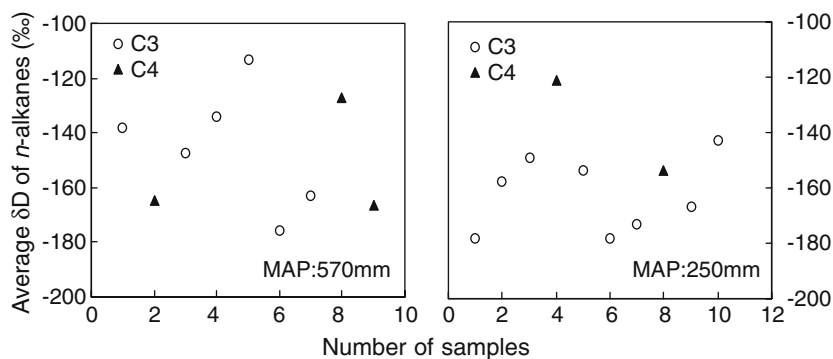


Fig. 2 Hydrogen isotopic variations of plant *n*-alkanes versus mean annual precipitation (MAP) in northwestern China. No significant correlation between δ D values of plant *n*-alkanes and MAP was found in the study area. Changes in δ D values of plant *n*-alkanes with plant species cover those changing with MAP in the study sites

Fig. 3 A comparison of hydrogen isotopic variations of modern plants from a less humid site (MAP: 250 mm) and a relatively humid site (MAP: 570 mm) in northwestern China. Larger changes in the δD values of plant *n*-alkanes from either site cannot be clearly explained in terms of different plant photosynthetic pathways (C_3 and C_4) or environmental humidity



data suggest that although variations of annual precipitation and air humidity at the growth site determine δD values of source water, other factors, such as plant taxonomy and ecological forms may have played significant roles in controlling δD values of long-chain *n*-alkanes of terrestrial plants.

We also compared δD values of plant *n*-alkanes in two study sites with distinctive humidity: the arid Etuoqeqi site with 250 mm MAP and the semi-arid Yanan site with 570 mm MAP (Fig. 3). The average δD values of long-chain *n*-alkanes (C_{27} , C_{29} , and C_{31}) in the Etuoqeqi site range from -179 to -121‰ , and in Yanan from -176 to -113‰ . Although increasing aridity has resulted in stronger evaporation that leads to the enrichment of deuterium in soil water (Dansgaard 1964; Waterhouse et al. 2002), the δD values of *n*-alkanes from the arid site cannot be clearly distinguished from those at the relatively humid site. We believe that the large δD variations of *n*-alkanes (i.e. C_{31} with -64 and -84‰ in Etuoqeqi and in Yanan, respectively) can be explained by the physiological controls on hydrogen isotopic fractionations of individual plant species or ecological life forms.

δD values of *n*-alkanes and photosynthetic pathways (C_3 vs. C_4)

Hydrogen isotope values of C_4 and C_3 plants are expected to co-vary with oxygen isotope compositions since both are controlled by the same source water. It has been reported that the $\delta^{18}O$ 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 and DeNiro 1983; Sternberg et al. 1984; Leaney et al. 1985; Helliker and Ehleringer 2000, 2002). If similar mechanisms are applied to the hydrogen isotope fractionation of a plant species, the δD of C_4 plants is expected to be more deuterium-enriched than that of C_3 plants.

Sternberg et al. (1983, 1984) determined δD values of lipids from CAM, C_3 , and C_4 plants, and pointed out

that there was no significant difference in δD values among plants with different biosynthesis pathways. Recently, Chikaraishi and Naraoka (2003) investigated δD values of leaf wax *n*-alkanes of modern plants in Japan and Thailand, and found that leaf wax δD values of C_4 plants (-171‰) were slightly more negative than those of C_3 plants (-152‰ for angiosperms and -149‰ for gymnosperms). The hydrogen isotopic difference in C_3 and C_4 plant was interpreted to reflect isotope effects associated with evapotranspiration and *n*-alkane biosynthesis. As shown in Figs. 3 and 4, no significant differences in the δD values of C_3 and C_4 plants were found. In Fig. 4, the one-way ANOVA shows that there is no significant difference between average δD values of *n*-alkanes in C_3 and C_4 plants among the three kinds of plants, i.e. trees, shrubs, and grasses ($P = 0.315$). Although C_4 species are not as sensitive to relative humidity as their C_3 counterparts, different photosynthetic pathways may not be the dominating factor controlling δD values of plant *n*-alkanes in leaf waxes.

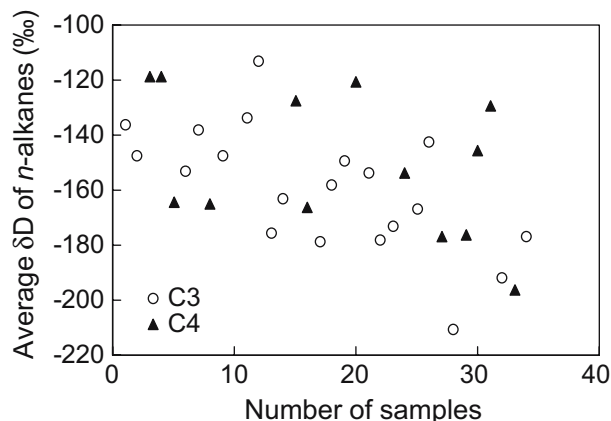


Fig. 4 Hydrogen isotopic variations of plants *n*-alkanes versus photosynthetic pathways (C_3 and C_4) in northwestern China. There is no significant difference between average δD values of *n*-alkanes in C_3 and C_4 plants among the three kinds of plants (trees, shrubs, and grasses) ($P = 0.315$)

However, we also noted that δD values of C_4 and C_3 grasses from Japan and Thailand (Chikaraishi and Naraoka 2003) and from China (Liu and Huang 2005 and this study) differ, with those of C_4 grasses being slightly positive (Fig. 5). The average δD values of long-chain n -alkanes (C_{27} , C_{29} , and C_{31}) varied from -210 to -148‰ for C_3 plants and from -196 to -127‰ for C_4 plants. The δD values of n -alkanes of C_4 grasses from these areas have slightly more positive values than C_3 grasses from the same areas. One-way ANOVA also showed that the average δD values of plant n -alkanes differed to a small extent between C_4 and C_3 grasses ($P = 0.080$), although photosynthetic pathway (C_3 and C_4) may not be the dominant factor determining the δD values of plants.

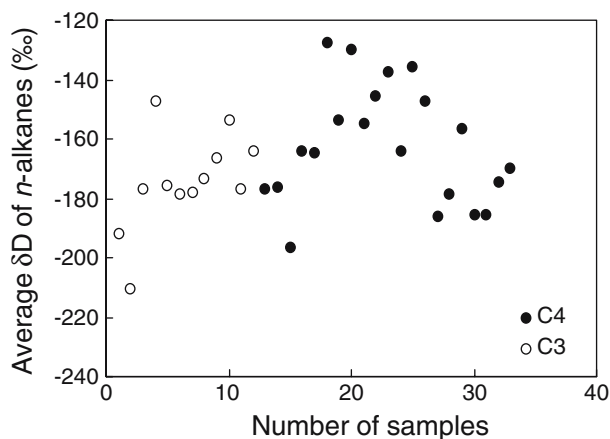


Fig. 5 Hydrogen isotopic variations of n -alkanes of C_4 and C_3 grasses from Japan, Thailand (Chikaraishi and Naraoka 2003) and China (this study). Although the photosynthetic pathway (C_3 and C_4) may not be the dominant factor determining the δD values of plants, the average δD values of n -alkanes differed to a small extent between C_3 and C_4 grasses ($P = 0.080$)

δD values of n -alkanes and ecological life forms (woody plants vs. grasses)

Although δD values of precipitation, soil moisture, and the plant photosynthetic pathway may contribute to the δD of n -alkanes from plant leaf waxes, we believe that the utilization of different source waters due to different ecological life forms plays a critical role in this. Hydrogen in leaf wax n -alkanes is derived from leaf water during photosynthesis and leaf water is further controlled by soil water, which originates from precipitation. In arid and semi-arid areas, strong evaporation results in soil water which is more deuterium-enriched than precipitation (Sternberg et al. 1988). Thus, the δD values of leaf water can be directly enriched in deuterium by transpiration and indirectly by evaporation of soil waters. In contrast, the δD of precipitation varies with moisture source, latitude, and degree of continentality. Thus, the δD of n -alkanes should register integrated signals that have resulted from collective effects of evapotranspiration, precipitation δD values, and other environmental changes.

The δD values of 34 modern plants from northwestern China also show that δD values of grasses are more depleted in deuterium than those of woody plants (tree and shrubs) (Fig. 6a). These results are consistent with our early observations (Liu and Huang 2005) which showed that δD values of plant leaf n -alkanes from eight modern plants are more closely related to plant taxonomy (woody plants or grasses) and ecological life forms than to plant photosynthetic pathway (C_3 and C_4).

In contrast to our results, the δD values of plant n -alkanes from modern plants in Japan and Thailand show that the C_4 plants have slightly more negative values than the C_3 plants (Chikaraishi and Naraoka 2003).

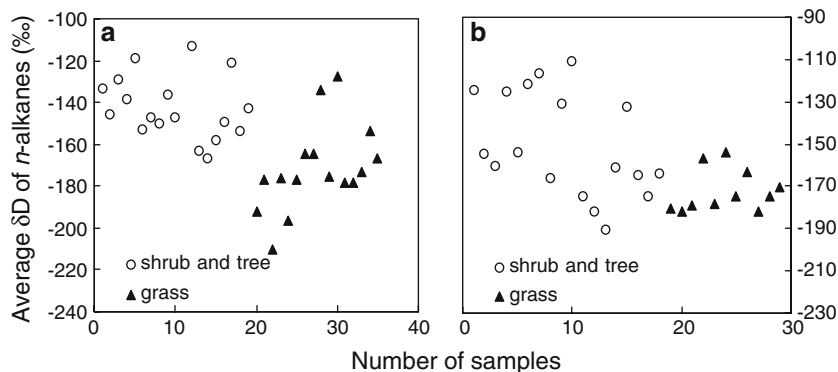


Fig. 6a, b A comparison of the hydrogen isotopic variation of modern woody plants and grasses. **a** The δD values of grass n -alkanes from western China are more depleted in deuterium than those of woody plants (trees and shrubs). **b** δD values of

plant n -alkanes from Japan and Thailand (Chikaraishi and Naraoka 2003) also show that δD values of grasses are more depleted in deuterium than those of woody plants (tree and shrubs)

We examined δD values and the taxonomy of the corresponding plants recorded by Chikaraishi and Naraoka (2003); most of the C_3 plants recorded from Japan and Thailand were trees and shrubs (see Table 2), whereas the C_4 plants were from the Gramineae. Figure 6b plots δD values of plant n -alkanes from trees against those of shrubs and grasses. In general, the woody species have more positive δD values than the grasses.

Figure 7 shows the δD values of plant n -alkanes from both woody (including four C_3 and one C_4 plants) and grass (including four C_3 and one C_4 plants) species from the Etuokeqi site and the Yanan site. Under the same climatic conditions, such as humidity and temperature etc., the average δD values of long-chain n -alkanes (C_{27} , C_{29} , and C_{31}) of shrubs range from -158 to -121‰ for the Etuokeqi site and from -167 to -113‰ for the Yanan site. In contrast, δD values for the grass species vary from -179 to -154‰ , and from -176 to -127‰ , respectively. A similar dataset was also compiled for the Gunma site (data from Chikaraishi and

Naraoka 2003). In all these sites, the leaf waxes of grass species tend to have more negative δD values.

Observations of water in the soil profile near the soil surface have shown that soil water δD values usually increase with depth (Griew et al. 2001). Grasses with a short root use surface soil water from recent precipitation in a strongly seasonal fashion, and thus tend to have more negative hydrogen isotopic values. In contrast, trees and shrubs usually have longer and deeper roots, allowing them to absorb water from deeper soil horizons, resulting in more positive δD values. This difference in plant water absorption system may be one of the major reasons for grasses to have more negative δD values than woody plants at the same site.

As previously discussed, we suggest that the δD values of C_4 and C_3 grasses from Japan and Thailand (Chikaraishi and Naraoka 2003), and China (Liu and Huang 2005 and this study) can be better explained if we consider their ecological life forms (Fig. 8). One-way ANOVA showed that the average δD values of plant n -alkanes differed significantly ($P < 0.001$) among

Table 2 Individual δD values of n -alkanes (Chikaraishi and Naraoka 2003)

No.	Individual δD values of n -alkanes (Chikaraishi and Naraoka 2003)						
	Species	Life form	Photosynthesis pathway	δD (C_{27}) C_{27}	δD (C_{29}) C_{29}	δD (C_{31}) C_{31}	δD (C_{33}) C_{33}
C_4 plants							
1	<i>Zea mays</i>	Grass	C_4	-160	-153	-156	-158
2	<i>Zoysia japonica</i>	Grass	C_4	-177	-177	-180	-180
3	<i>Miscanthus sinensis</i>	Grass	C_4	-181	-196	-179	-156
4	<i>Saccharum officinarum</i>	Grass	C_4	-186	-188	-185	-169
5	<i>Miscanthus sinensis</i>	Grass	C_4	-191	-186	-179	-173
6	<i>Saccharum officinarum</i>	Grass	C_4	-173	-173	-176	-176
7	<i>Sorghum bicolor</i>	Grass	C_4	-162	-171	-176	-172
C_3 plants							
8	<i>Quercus acutissima</i>	Tree	C_3	-87	-145	-142	-133
9	<i>Camellia sasanqua</i>	Shrub	C_3	-166	-167	-159	-146
10	<i>Chamaecyparis obtuse</i>	Tree	C_3	-180	-150	-134	-123
11	<i>Pinus thunbergii</i>	Tree	C_3	-167	-164	-151	-146
12	<i>Albizia julibrissin</i>	Tree	C_3	-123	-124	-128	-126
13	<i>Benthamidia japonica</i>	Shrub	C_3	-157	-158	-168	
14	<i>Cryptomeria japonica</i>	Tree	C_3	-165	-156	-141	-140
15	<i>Acer carpinifolium</i>	Tree	C_3	-120	-134	-111	-140
16	<i>Acer argutum</i>	Tree	C_3	-119	-119	-111	-113
17	<i>Phragmites communis</i>	Grass	C_3	-142	-206	-196	-178
18	<i>Benthamidia japonica</i>	Shrub	C_3	-149	-170	-176	
19	<i>Prunus jamasakura</i>	Shrub	C_3	-159	-183	-183	-183
20	<i>Cryptomeria japonica</i>	Tree	C_3	-178	-168	-153	-146
21	<i>Acer carpinifolium</i>	Tree	C_3	-105	-161	-127	-128
22	<i>Acer argutum</i>	Tree	C_3	-93	-123	-116	-133
23	<i>Taraxacum officinale</i>	Grass	C_3	-151	-155	-155	
24	<i>Plantago asiatica</i>	Grass	C_3	-177	-178	-176	-169
25	<i>Artemisia princeps</i>	Grass	C_3	-172	-157	-163	-161
26	<i>Acer palmatum</i>	Tree	C_3	-174	-171	-179	-177
27	<i>Quercus mongolica</i>	Tree	C_3	-186	-188	-172	
28	<i>Quercus dentata</i>	Tree	C_3	-194	-193	-184	
29	<i>Manihot utilissima</i>	Shrub	C_3	-129	-134	-133	-138

Fig. 7 Comparison of hydrogen isotopic variations of *n*-alkanes from woody and grass species growing under the same climatic conditions in the Ertuoqeqi (a) and Yanan sites, China (b) and in the Gunma site, Japan (c)

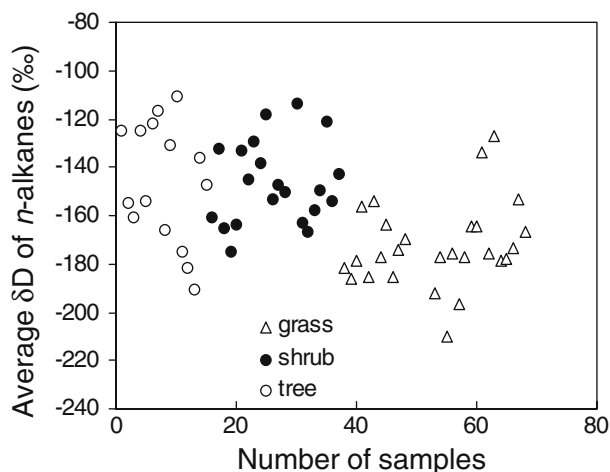
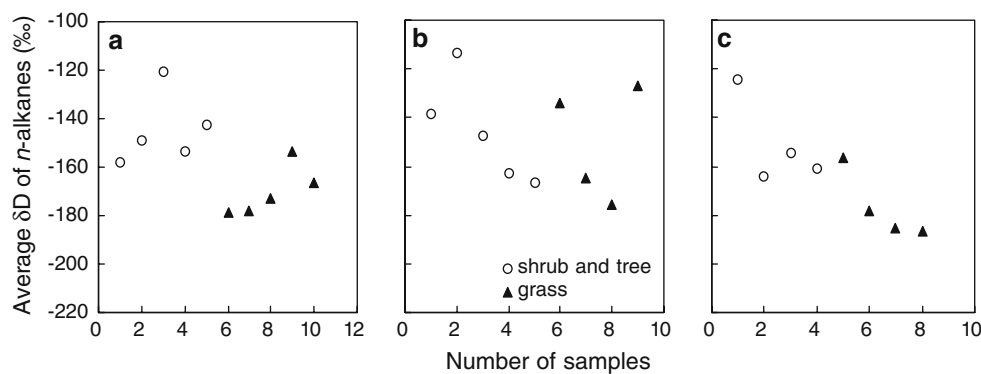


Fig. 8 Hydrogen isotopic variations of *n*-alkanes from woody (trees and shrubs) and grass species from Japan and Thailand (Chikaraishi and Naraoka 2003), and China. Average δD values of plant *n*-alkanes of grasses differed significantly from those of shrubs and trees ($P < 0.001$), but no significant difference was found for the δD values between the shrubs and trees ($P < 0.897$)

the three kinds of plants, and that the δD values of grasses differed significantly ($P < 0.001$) from those of shrubs and trees. However, one-way ANOVA revealed no significant difference in the δD values between the shrubs and trees ($P < 0.897$). We believe that the difference in δD values of plant leaf wax *n*-alkanes between C_4 and C_3 plants is largely due to their fundamental ecophysiological difference in terms of water use. The effect of the photosynthetic pathway (C_3 vs. C_4) on the δD of plant *n*-alkanes is relatively weak in comparison with the effect of their life form (i.e. woody plants vs. grasses).

Conclusion

δD values of plant leaf wax *n*-alkanes can be affected by multiple factors, such as taxonomy, plant photosynthetic pathways (C_3 and C_4), precipitation δD values,

and various plant ecological life forms. Our analysis of δD values of plant *n*-alkanes from China, together with re-analysis of values from Japan and Thailand, show that the physiological life form (woody plants and grass) may play a dominant role in determining the δD of *n*-alkanes in leaf waxes of terrestrial plants. If δD values of plant *n*-alkanes are used to trace aridity vs. plant species change, it is ideal to compare hydrogen isotopic signals of *n*-alkanes from a single species or genus across temporal and spatial ranges. If it is not feasible to design such an experiment, comparison should be made among plants that have similar ecological life forms.

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