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Hydrogen isotopic variability in leaf waxes among terrestrial and aquatic plants around Blood Pond, Massachusetts (USA)

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Abstract

Paleoclimatic interpretation of the hydrogen isotope ratios of plant leaf waxes extracted from sediments requires a thorough understanding of the factors controlling the isotopic ratios. Existing studies have found relatively small variability in hydrogen isotope fractionation among plants of different photosynthetic pathways (C_3, C_4 and CAM) and between gymnosperms and angiosperms. However, there has been no systematic study at a single site to determine how leaf wax hydrogen isotope (D/H) ratios differ in different plant types under the same precipitation and environmental regime. Such data are nevertheless crucial for understanding the impact of past vegetation changes on the sedimentary hydrogen isotope records of leaf waxes. Here, we present a study of D/H ratios of leaf waxes from 48 species in seven types of terrestrial and aquatic C_3 plants around Blood Pond, Dudley, Massachusetts, USA. The δD values of leaf waxes differ by as much as 70‰ for different plant types, with those from trees and ferns having the highest values and those from grasses having the lowest values. The large isotopic variation indicates that the apparent hydrogen isotopic fractionation between leaf waxes and precipitation is not constant for different plant types. Our results indicate that inferring precipitation D/H ratios on the basis of sedimentary leaf waxes is only viable when significant vegetation change is absent or can be accounted for isotopically.

1. Introduction

Compound-specific hydrogen isotope (D/H) ratios of leaf waxes from lake surface sediments across large climatic gradients have been shown to track the D/H ratios of environmental water (Huang et al., 2004; Liu and Huang, 2005) or precipitation (e.g., Sachse et al., 2004, 2006; Dawson et al., 2004; Shuman et al., 2006). Long chain *n*-alkanes from different sites have been suggested to track D/H ratios of precipitation at nearly constant apparent isotopic fractionation (Sachse et al., 2004, 2006). Based on hydrogen isotopic analysis of both aquatic and terrestrial compounds, Hou et al. (2006) and Shuman et al. (2006) have constructed late glacial to Holocene climate reconstructions that are consistent with existing climate data (Huang et al., 2002; Shuman et al., 2004). However, despite these corroborated paleoclimate reconstructions, the reliability with which D/H ratios of sedimentary leaf waxes record ancient precipitation depends on an important premise, i.e., the natural hydrogen

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isotopic variability among different plants at a given site is relatively small. If, on the other hand, different plants at a given site produce leaf waxes with large hydrogen isotopic differences, vegetation changes through time could confound or even overpower the precipitation δD signal believed to be recorded by the sedimentary leaf waxes.

D/H ratios of leaf waxes in plants that use different photosynthetic pathways, and in several plant types (gymnosperms, angiosperms, ferns, aquatic plants) have been studied (e.g., Chikaraishi and Naraoka, 2003; Chikaraishi et al., 2004; Bi et al., 2005; Smith and Freeman, 2006). In contrast to the large carbon isotopic differences observed between C_3 and C_4 plants (Farquhar et al., 1989), differences in mean hydrogen isotopic ratios between C_3 and C_4 plants are relatively small (Chikaraishi and Naraoka, 2003; Bi et al., 2005). If only grasses are considered, C_4 *grasses* are enriched in deuterium by ~20‰ relative to C_3 *grasses* collected from the same site (Smith and Freeman, 2006). However, the plant samples studied by Chikaraishi and Naraoka (2003) were collected from a wide range of ecosystems, locations, and climate zones, making it difficult to ascertain the D/H ratios of plant source waters and to distinguish the effect of plant types from the effect of source water and environmental conditions (e.g., nutrients, soil types, evaporation, relative humidity, sun light, etc.) on the hydrogen isotopic fractionations. At different locations, plants may utilize soil water derived from precipitation falling during different seasons, and may incorporate different amounts of ground water for photosynthesis (White et al., 1985). Therefore, determining plant apparent hydrogen isotopic fractionation relative to either mean annual or summer precipitation may introduce significant uncertainty, especially when comparing sites with large climatic, hydrological and micro-environmental differences.

Here, we present a systematic study of 48 species of terrestrial and aquatic plants (all C_3) around Blood Pond, MA (Hou et al., 2006) in order to examine the difference in the leaf wax δD values. By collecting plant samples within the close vicinity (within 50 m) of Blood Pond, we minimize the impact of various hydrological and environmental factors on leaf wax D/H ratios. We aimed to (1) systematically study the δD values of leaf waxes produced by different terrestrial and aquatic plants under the same hydrological and environmental system; (2) evaluate the influence of plant composition on the application of leaf waxes to climate reconstruction; and (3) determine the biosynthetic hydrogen isotopic fractionation between δD values of long chain *n*-acids and alkanes.

2. Samples and methods

2.1. Samples

Blood Pond (42.08°N, 71.96°W, 212.1 m a.s.l.) is a kettle pond located in the south-central Massachusetts town of Dudley (Hou et al., 2006). We collected 74 samples of plant leaves, representing 48 species of terrestrial and aquatic plants around the pond on August 11, 2005 (Table 1). There were 11 tree species (35 leaf samples), nine shrub species (11 leaf samples), three vine species (three leaf samples), nine herb species (nine leaf samples), seven grass species (seven samples), four fern species (four leaf samples) and five species of aquatic macrophytes (five leaf samples). All are C_3 plants. Multiple samples from different heights (6 m, 4.5 m, 3 m) of individual trees or shrubs (Table 1) were also collected to determine the hydrogen isotopic variability within the same plant.

2.2. Methods

All leaf samples were freeze-dried and ultrasonically extracted with CH_2Cl_2 (×3, 15 min). The extract was separated into neutral and acid fractions using solid phase extraction (Aminopropyl Bond Elute®). The acid fraction was methylated using anhydrous 2% HCl in MeOH. Hydroxy acids were removed using silica gel column chromatography (CH_2Cl_2 as solvent), in order to further purify the fatty acid methyl esters and avoid chromatographic coelution. The neutral fraction was further separated into hydrocarbon, ketone/aldehyde and alcohol fractions using silica gel chromatography, eluting with hexane, CH_2Cl_2, and ethyl acetate:hexane (v/v = 1/3), consecutively.

Quantification and identification were carried out using gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS). An HP 6890 chromatograph interfaced to a Finnigan Delta + XL stable isotope mass spectrometer through a high temperature pyrolysis reactor was used for hydrogen isotopic analysis (Huang et al., 2002, 2004). The H_3^+ factor was determined daily prior to sample analysis (average values 2.0 during this study). The precision (1σ) of triplicate analyses was $< \pm 2$‰. The accuracy was routinely checked

Table 1
δD values of leaf wax compounds extracted from leaf samples

Type [a]	Symbol [b]	Scientific name	*n*-Alkanes			*n*-Acids		
			C_{27}	C_{29}	C_{31}	C_{26}	C_{28}	C_{30}
TR	BEPO-1(*a*)	*Betula populifolia* Marsh.	−191	−182	−177	−153	−159	−131
TR	BEPO-1(*b*)	*Betula populifolia* Marsh.	−186	−176	−174	−155	−163	−142
TR	BEPO-1(*c*)	*Betula populifolia* Marsh.	−198	−190	−182	−163	−167	−151
TR	BEPO-2(*a*)	*Betula populifolia* Marsh.	−192	−178	−176	−147	−161	−135
TR	BEPO-2(*b*)	*Betula populifolia* Marsh.	−192	−177	−180	−125	−144	−122
TR	QUVE	*Quercus velutina* Lam.	−152	−167		−130	−152	−147
TR	ACRU-1	*Acer rubrum* L.	−172	−194	−180	−136	−159	−159
TR	ACRU-2	*Acer rubrum* L.	−157	−195	−198	−158	−168	−162
TR	ACRU-3(*a*)	*Acer rubrum* L.		−206	−213	−158	−173	−172
TR	ACRU-3(*b*)	*Acer rubrum* L.		−219	−210	−142	−171	−165
TR	BELE-1(*a*)	*Betula lenta* L.	−182	−182		−149	−167	−158
TR	BELE-1(*b*)	*Betula lenta* L.		−197	−178			
TR	BELE-1(*c*)	*Betula lenta* L.	−181	−181		−165	−167	−153
TR	BELE-2(*a*)	*Betula lenta* L.	−188	−168		−144	−163	−155
TR	BELE-2(*b*)	*Betula lenta* L.	−193	−174		−137	−160	−156
TR	BELE-2(*c*)	*Betula lenta* L.	−204	−174		−138	−164	−153
TR	CARYA-1(*a*)	*Carya* sp. Nutt.		−194	−172	−158	−162	−160
TR	CARYA-1(*b*)	*Carya* sp. Nutt.		−188	−175	−144	−152	−147
TR	CARYA-1(*c*)	*Carya* sp. Nutt.		−176	−185	−138	−144	−145
TR	CARYA-2	*Carya* sp. Nutt.	−190	−208	−203	−175	−179	−182
TR	PIST (*a*)	*Pinus strobus* L.	−183	−192	−195	−184	−188	−181
TR	PIST(*b*)	*Pinus strobus* L.	−173	−201	−200	−175	−154	−184
TR	PIST(*c*)	*Pinus strobus* L.	−178	−200	−199	−182	−179	−190
TR	PRSE(*a*)	*Prunus serotina* Ehrh.		−184	−166	−155	−153	−156
TR	PRSE(*b*)	*Prunus serotina* Ehrh.	−201	−173				
TR	QURU(*a*)	*Quercus rubra* L.	−167	−165	−150	−157	−164	−161
TR	QURU(*c*)	*Quercus rubra* L.	−168	−173	−157	−153	−165	−166
TR	FRAM2(*a*)	*Fraxinus americana* L.		−185	−162	−109	−124	−121
TR	FRAM2(*b*)	*Fraxinus americana* L.		−182	−177	−101	−122	−129
TR	FRAM2(*c*)	*Fraxinus americana* L.		−187	−172	−115	−125	−139
TR	TSCA(*b*)	*Tsuga canadensis* L.	−159	−160	−155	−159	−156	−158
TR	TSCA(*c*)	*Tsuga canadensis* L.	−163	−165	−156	−126	−131	−142
TR	NYSY(*a*)	*Nyssa sylvatica* Marsh.		−180		−124	−126	−133
TR	NYSY(*b*)	*Nyssa sylvatica* Marsh.		−187		−126	−130	−134
TR	NYSY(*c*)	*Nyssa sylvatica* Marsh.		−188		−114	−125	−131
SH	HAVI4-1	*Hamamelis virginiana* L.	−166	−188		−140	−155	−158
SH	HAVI4-2	*Hamamelis virginiana* L.	−179	−170		−145	−168	−152
SH	HAVI4-3	*Hamamelis virginiana* L.	−171	−168	−161	−147	−157	−160
SH	LOTA	*Lonicera tatarica* L.		−185	−179	−141	−151	−164
SH	RUAL	*Rubus allegheniensis*	−178	−187	−180	−177	−177	−158
SH	VIAC	*Viburnum acerifolium* L.		−187		−134	−145	−163
SH	CLAL3	*Clethra alnifolia* L.		−197		−172	−176	−175
SH	LIBE3	*Lindera benzoin* (L.)	−189	−200	−182	−178	−198	−187
SH	DEVE	*Decodon verticillatus* (L.) Elliot				−149	−179	−197
SH	ILVE	*Ilex verticillata* (L.) Gray				−160	−163	−157
SH	RHODO	*Rhododendron* sp. L.	−190	−190	−168	−136	−156	−172
VI	SMHE	*Smilax herbacea* L.				−141	−175	−170
VI	SMRO	*Smilax rotundifolia* L.		−202	−175	−185	−172	−154
VI	VIAE	*Vitis aestivalis* Michx.	−179	−193	−167	−148	−155	−153
HB	ASDI	*Aster Divaricatus* L.		−176	−181	−176	−177	−175
HB	DACA6	*Daucus carota* L.	−188	−170	−156	−167	−186	−184
HB	PLMA2	*Plantago major* L.		−204	−208	−210	−213	−211
HB	TRPR2	*Trifolium pratense* L.	−196	−207	−190	−176	−185	−198
HB	UNKN1	Unknown				−165	−181	−174
HB	UNKN2	Unknown		−188	−190	−186	−162	−193
HB	IMCA	*Impatiens capensis*	−193	−187		−194	−194	−164

(*continued on next page*)

Table 1 (*continued*)

Type [a]	Symbol [b]	Scientific name	*n*-Alkanes			*n*-Acids		
			C_{27}	C_{29}	C_{31}	C_{26}	C_{28}	C_{30}
HB	MIRI	*Mimulus ringens* L.		−173	−168	−144	−155	−156
HB	TYLA	*Typha latifolia* L.	−195	−195	−189	−170	−164	−191
GR	CAPE6	*Carex pensylvanica* Lam.				−193	−210	−217
GR	AGRO	*Agropyron* sp.	−175	−231	−228	−217	−220	−220
GR	DAGL	*Dactylis glomerata* L.	−202	−218	−221	−194	−207	−182
GR	JUTE	*Juncus tenuis* Willd.	−216	−217	−198	−192	−185	−182
GR	PHPR3	*Phleum pratense* L.	−232	−226	−217	−218	−226	−227
GR	POAC	*Poaceae*	−206	−232	−238	−226	−220	−221
GR	ALOPE	*Alopecurus sp.* L.	−171	−186	−188	−168	−179	−184
FE	DEPU2	*Dennstaedtia punctilobula*				−129	−123	−126
FE	POAC4	*Polystichum acrostichoides*	−162	−170	−165	−136	−143	−140
FE	OSCI	*Osmunda cinnamomea* L.	−152	−159		−129	−147	−147
FE	ONSE	*Onoclea sensibilis* L.	−156	−156		−114	−143	−135
AQ	BRSC	*Brasenia schreberi* J. F. Gmel				−154	−162	−171
AQ	LEMNA	*Lemna* sp. L.	−172	−200		−169	−169	−178
AQ	NYMPH	*Nymphaea* L.				−161	−165	−173
AQ	NYMPH	*Nymphaea* L.				−169	−173	−183
AQ	POCO14	*Pontederia cordata* L.	−188	−179		−151	−159	−165

[a] TR, tree; SH, shrub; VI, vine; HB, herb; GR, grass; FE, fern; AQ, aquatic macrophyte.

[b] Symbols from USDA PLANTS database (http://plants.usda.gov/). Number following the symbol indicates individual plants; the letters (*a*), (*b*), (*c*) indicate the height of the leaf samples above the ground ($a = 6$ m, $b = 4.5$ m, $c = 3$ m). For example, BEPO-1(*a*) indicates the leaf sample was taken from the first *Betula populifolia* at 6 m above the ground.

by injection of laboratory isotopic standards between every six measurements. The δD values obtained for individual acids (as methyl esters) were corrected by mathematically removing the isotopic contributions from the added group. The δD value of the added Me group was determined by acidifying and then methylating (along with the samples) the disodium salt of succinic acid with a predetermined δD value (Huang et al., 2002). Previous studies (Yang and Huang, 2003) have shown that kinetic isotopic fractionation and hydrogen exchange during the methylation are negligible.

3. Results and discussion

3.1. Hydrogen isotopic variation among plant types

Hydrogen isotopic values of C_{27}, C_{29}, C_{31} *n*-alkanes and C_{26}, C_{28}, C_{30} *n*-acids of the plant samples are listed in Table 1. Because δD values are strongly correlated for C_{27}, C_{29} and C_{31} *n*-alkanes and for C_{26}, C_{28}, C_{30} *n*-acids (Fig. 1; Table 2), discussion focusses on the C_{29} *n*-alkane and C_{30} *n*-acid. To facilitate comparison, we also group plants into seven types: tree, shrub, vine, herb, grass, fern and aquatic macrophyte based on growth habit (Raven et al., 2003; Fig. 2). The definitions of growth habit and the acronyms (e.g., BEPO for *Betula populifolia* Marsh.) used in Table 1 are obtained from the PLANTS database on the United States Department of Agriculture website (USDA, 2006).

The δD values of leaf waxes from the different plant types sampled at Blood Pond vary significantly (Table 1; Fig. 2). Long chain *n*-acids and *n*-alkanes from trees and ferns have the highest average values, whereas grasses have the lowest. The hydrogen isotopic difference between ferns/trees and grasses is as large as 60–70‰. Shrubs, vines, herbs, and macrophytes have intermediate values. There is also significant variation in δD value within each plant type (e.g., trees), but the range of variation is clearly smaller than those observed among different plant types. Trees, grasses and herbs show greater isotopic variability than the other types. For example, among the trees, δD values of C_{30} *n*-acid range from −190‰ (CARYA-2, *Carya* sp. Nutt) to −121‰ (FRAM2(*a*), *Fraxinus americana* L., Table 1). The values of C_{30} *n*-acid in grasses range from −227‰ (PHPR3, *Phleum pratense* L.) to −182‰ (DAGL, *Dactylis glomerata* L. and JUTE, *Juncus tenuis* Willd.). The large variability observed for trees and grasses may partially reflect the relatively large number of samples taken from trees and grasses in this study.

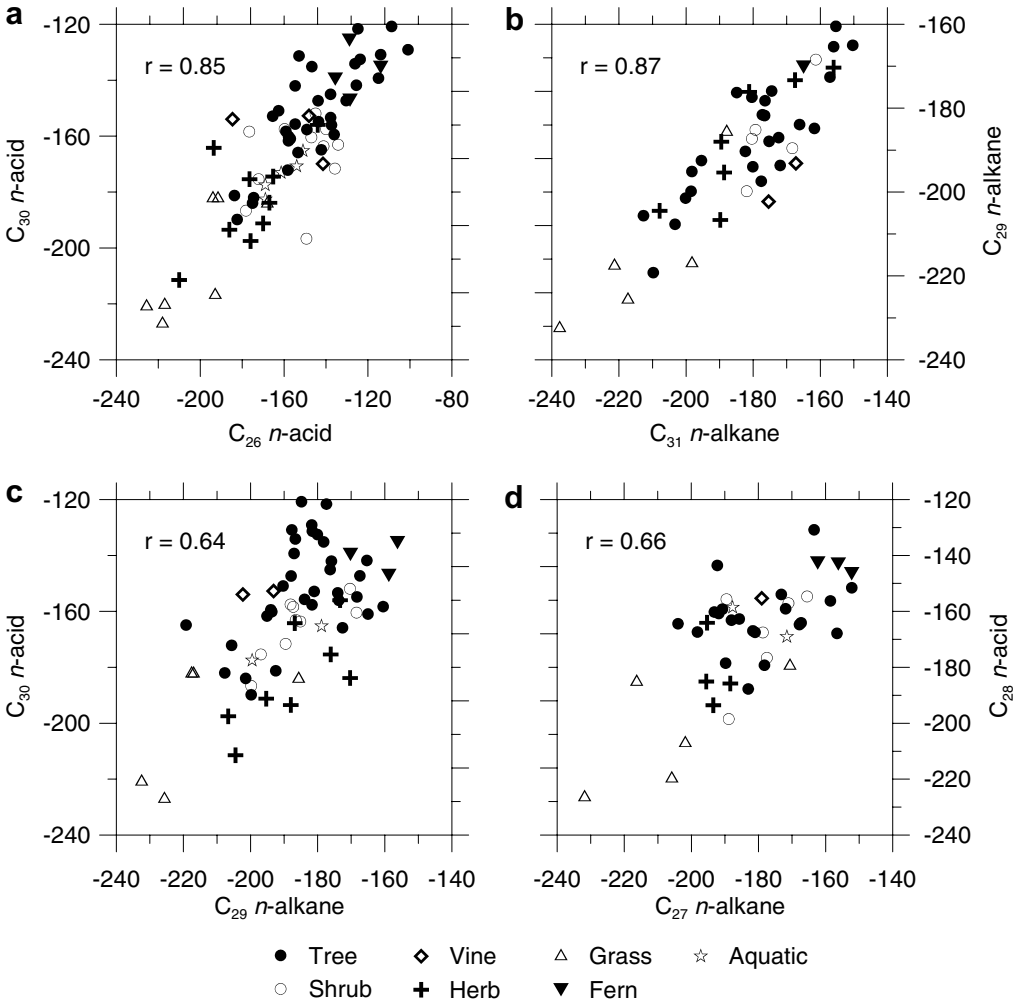

Fig. 1. Correlation between δD values of leaf wax compounds.

Table 2
Correlation coefficients (*r* values) between δD values of individual leaf waxes

Compound	C_{26} *n*-acid	C_{28} *n*-acid	C_{30} *n*-acid	C_{27} *n*-alkane	C_{29} *n*-alkane	C_{31} *n*-alkane
C_{26} *n*-acid	1					
C_{28} *n*-acid	0.91	1				
C_{30} *n*-acid	0.85	0.86	1			
C_{27} *n*-alkane	0.54	0.66	0.43	1		
C_{29} *n*-alkane	0.63	0.60	0.64	0.61	1	
C_{31} *n*-alkane	0.64	0.67	0.64	0.60	0.87	1

3.2. Factors leading to D/H variability in different plants

A number of factors may affect the D/H ratios of leaf waxes. The source water used by plants may differ, even though the regional precipitation is the same. Different trees are rooted to different depths and may have access to water with different D/H ratio values. In general, shallow soil water should have higher D/H ratio values due to evaporation (Barnes and Turner, 1998). Trees, having more extensive root systems, may tap into deeper groundwater than grasses and herbs. Our data do not, however, suggest that this is an important mechanism

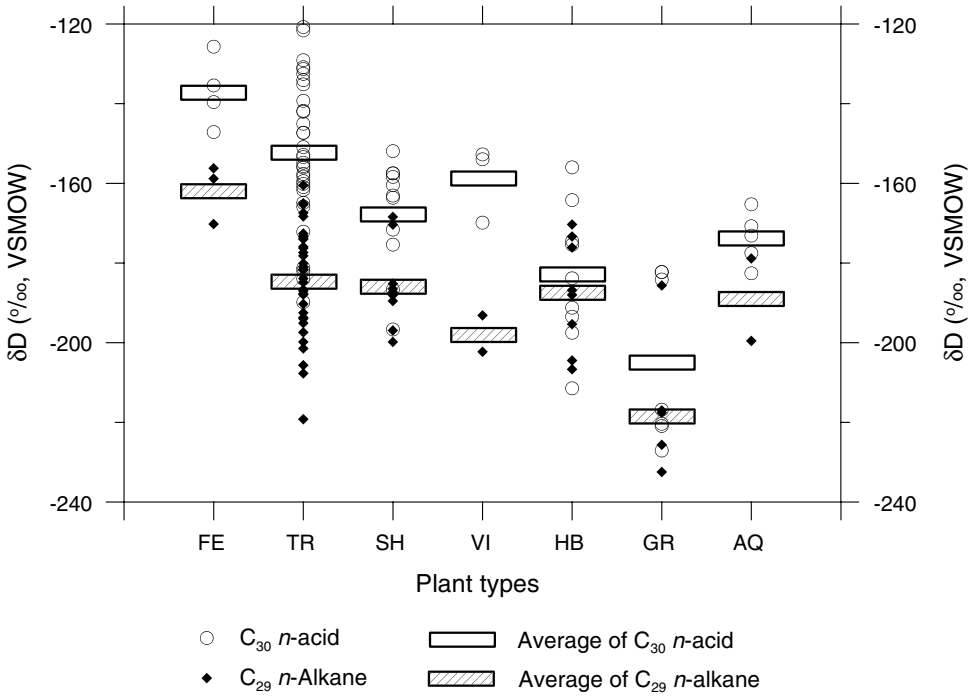

Fig. 2. Hydrogen isotope ratios of leaf waxes collected around Blood Pond. δD values of C_{29} *n*-alkanes (diamonds) and C_{30} *n*-acids (circles) for each plant are shown, and the respective average values are depicted as horizontal bars. FE, ferns; TR, trees; SH, shrubs; VI, vines; HB, herbs; GR, grasses; AQ, aquatic macrophytes.

controlling the D/H ratios of leaf waxes from trees, grasses, and herbs, since grasses and herbs have on average 60–70‰ lower δD values than trees, opposite to what would be expected if source water depth exerted a primary control.

The higher δD values for trees relative to grasses and herbs may result from differences in the microclimate around the tree crown and grasses, leading to different degrees of evapotranspiration. The strong impact of plant transpiration on the hydrogen isotopic ratios of plant leaf waters has been demonstrated by field and modeling studies (e.g., Flanagan et al., 1991). Grasses and herbs are more likely to be shaded by trees and shrubs in the forest. In contrast, most trees receive direct sunlight and experience more windy conditions (which also facilitate more evapotranspiration). The temperature at the surface of tree leaves should be higher than that at the grasses due to the direct sunlight on tree leaves and the shading effect on grasses. The deep roots of trees also enhance transpiration (Raven et al., 2003). The relative humidity around tree leaves may also be lower than that around grasses because of the moisture from soil water evaporation. Lower humidity would act to increase the kinetic isotopic effect for trees relative to grasses. The evapotranspiration effect, which enriches deuterium in the leaf water, could thus be smaller for the grasses than for trees. Differences in evapotranspiration may therefore be an important factor leading to higher hydrogen isotopic ratios for the leaf waxes in trees than grasses. It is also possible that differences in plant physiology and biochemistry (e.g., biosynthetic fractionation) could contribute to the hydrogen isotopic differences. For example, difference in leaf morphology has been suggested to affect the isotopic composition of leaf water and may contribute the observed isotopic variations (Grice et al., 2005). However, we currently do not have sufficient data to determine the specific factors causing the observed isotopic differences between various plant types.

Leaf samples collected from different heights of individual plants do not show significant variation (Table 1). The leaves of BELE-2, PIST, NYSY show little change in *n*-acid δD values with height. There is a slightly increasing trend (~9‰) in δD value with height for BEPO-1 and FRAM2, but the trend is reversed for BEPO-2 (same plant species).

3.3. Implication for reconstructing past precipitation D/H ratios

The mean annual δD value of precipitation at Blood Pond (Dudley, MA) is calculated to be $-63‰$, while summer δD is $-40‰$, using the online precipitation isotope calculator (Bowen and Revenaugh, 2003). Since all our samples were collected within 50 m of Blood Pond, all plants from this study have received the same precipitation. Thus, the differences in δD value for the leaf samples (Table 1 and Fig. 2) are equivalent to the differences in the *apparent* hydrogen isotopic fractionation relative to precipitation. The apparent hydrogen isotopic fractionation between leaf waxes and precipitation must be known in order to reconstruct past precipitation D/H ratios from leaf waxes preserved in lake sediments. Based on our data, the apparent H isotopic enrichment relative to summer precipitation for trees ranges from $-156‰$ to $-85‰$ for *n*-acids (mean $= -117‰$), and $-180‰$ to $-115‰$ for *n*-alkanes (mean $= -150‰$). Grasses show much larger apparent isotopic fractionation, with *n*-acids ranging from $-195‰$ to $-148‰$ (mean $= -171‰$) and *n*-alkanes ranging from $-206‰$ to $-154‰$ (mean $= -183‰$). The lower average δD values for grasses and herbs relative to trees may also help explain the lower δD values of *n*-alkanes from lake sediments than those from the surrounding trees (Sachse et al., 2006).

Sachse et al. (2004) suggest that D/H ratios of leaf wax *n*-alkanes in surface sediments are fractionated relative to precipitation by ca. $130‰$. However, the results from this study suggest large differences in hydrogen isotopic fractionation for different types of plants at a single site. Leaf waxes are transported to lake sediments primarily by wind. Sedimentary leaf waxes are thus an integral of those produced by all terrestrial and aquatic plants. Our data indicate the apparent H isotopic enrichment can differ by as much as 60–$70‰$ between trees and grasses. Therefore, it is inappropriate to assume constant fractionation between sedimentary leaf waxes and precipitation over intervals where there have been significant changes in vegetation. Naturally, vegetation is often sensitive to climate change. Hence, paleoclimate interpretations based on leaf wax δD values in sediments must also consider the isotopic variation induced by changing vegetation type. In regions where vegetation is less variable, such as arid grasslands, it is possible that a relatively invariable H isotopic fractionation could be applied to sedimentary leaf wax records to obtain precipitation D/H ratios. In cases where plant transpiration is not a factor in affecting δD values, such as short chain *n*-alkanes from sphagnum species (which have no stomata) in peat bogs (Xie et al., 2004), hydrogen isotope ratios of sphagnum biomarkers may more faithfully track isotopic changes in peat bog waters.

3.4. H isotopic difference between n-acids and n-alkanes

The C_{30} *n*-acid is the biosynthetic precursor of the C_{29} *n*-alkane in plants. There is a biosynthetic isotopic fractionation during the decarboxylation process. The fractionation for various plants has not been reported. Our data (Table 1; Fig. 2) show that the C_{29} *n*-alkane is depleted in D by up to $30‰$ relative to the C_{30} *n*-acid. This indicates that decarboxylation discriminates against D, as expected. Grasses and herbs appear to show smaller D depletion of C_{29} alkane relative to C_{30} acid ($< 13‰$) than trees, although we cannot exclude the possibility that such a difference is a result of sampling bias at the time. The C_{28} *n*-acid and C_{27} *n*-alkane from the same plant show a similar difference in δD value (Table 1, Fig. 1d). In comparison, Chikaraishi et al. (2004) reported a much larger hydrogen isotopic effect (up to $100‰$) during enzymatic desaturation of fatty acids.

4. Conclusions

We systematically studied the hydrogen isotopes of leaf waxes from 48 species of seven types of terrestrial and aquatic C_3 plants around Blood Pond, Massachusetts. The δD values of leaf waxes show large differences among plant types. The difference between average δD value of ferns, trees and grasses is as large as $70‰$. We attribute the hydrogen isotopic difference to different degrees of evapotranspiration as a result of micro-environmental differences for different plants, such as sunlight exposure, temperature, humidity and turbulence (wind). Differences in plant physiology and biochemical fractionation may also be important, but we currently do not have data to demonstrate their impact on hydrogen isotopic fraction of leaf waxes. The data indicate that caution must be taken in attempting to use sediment leaf wax D/H ratios to reconstruct past precipitation isotopic ratios. Variable

fractionation among different plants must be considered to minimize the effect of vegetation change under different climate conditions. It is possible that combining isotopic and pollen stratigraphy will allow more accurate paleoclimate interpretations based on leaf wax δD values in sediments. In regions with more monotonic plant types (e.g., arid regions, Liu and Huang, 2005), hydrogen isotopic ratios of sedimentary leaf waxes may more faithfully track precipitation D/H ratios.

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