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Note

Evidence for water use efficiency as an important factor in determining the δD values of tree leaf waxes

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Abstract

D/H ratios of sedimentary leaf waxes can provide useful information about past climate change. However, factors controlling δD values of higher plant leaf waxes (δD_{wax}) are poorly understood. Here we show that δD_{wax} values are negatively correlated with $\delta^{13}C$ values of leaf waxes ($\delta^{13}C_{wax}$), based on a study of 35 leaf samples of 11 tree species around Blood Pond, Massachusetts (USA). Our data suggest that plant water use efficiency exerts an important control on the δD_{wax} variation among tree species.
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1. Introduction

Hydrogen isotope ratios of plant leaf waxes (δD_{wax}) from lake and ocean sediments track δD values of environmental waters and have potential for paleoclimate reconstruction (e.g., Schefuß et al., 2005; Liu and Huang, 2005; Sachse et al., 2004; Shuman et al., 2006; Pagani et al., 2006). However, values of δD_{wax} show considerable variation among different plants from a single site receiving the same precipitation (Hou et al., 2007). While existing data suggest plant water use strategies may affect δD values of plant leaf waxes (Smith and Freeman, 2006; Sachse et al., 2006; Hou et al., 2007), there have been no data directly linking plant water use efficiency to hydrogen isotope ratios of leaf waxes. Carbon isotope ratios are well established indicators of plant water use efficiency (WUE), defined as the ratio of carbon assimilation, A, to the transpiration water loss from the plant, E (Farquhar et al., 1989; Bacon, 2004):

$$\mathrm{WUE} = \frac{A}{E} = \frac{(1-\phi)p_a}{1.6v(b-a)}(b - \Delta^{13}\mathrm{C}) = k'(b - \Delta^{13}\mathrm{C}) \quad (1)$$

where ϕ is the respired proportion of assimilated carbon; p_a is the atmospheric CO_2 pressure; v is the difference between intercellular and atmospheric water vapor pressure; the factor 1.6 is the ratio of diffusivity of water vapor and CO_2 in air; a is the fractionation occurring due to diffusion in air and b is the net fractionation caused by carboxylation; $\Delta^{13}C$ is the carbon isotopic discrimination of plant material relative to ambient CO_2; $\Delta^{13}C$ is defined as $(\delta^{13}C_a - \delta^{13}C_p)/\ (1 + \delta^{13}C_a)$, where $\delta^{13}C_a$ and

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$\delta^{13}C_p$ represent the $\delta^{13}C$ values of atmospheric CO_2 and plant material, respectively (Farquhar et al., 1989). Carbon isotope discrimination ($\Delta^{13}C$) is relevant to WUE via the extent of stomatal conductance and capacity of CO_2 fixation during photosynthesis. Smaller stomatal conductance causes smaller $\Delta^{13}C$ and larger WUE, while greater stomatal conductance causes larger $\Delta^{13}C$ and smaller WUE. WUE is a function of plant type and depends on environmental factors such as humidity, sunlight exposure and temperature. Here we compare carbon and hydrogen isotope ratios of tree leaf waxes to assess the relationship between tree water use efficiency and δD_{wax}.

2. Samples and methods

A total of 35 leaf samples from 11 tree species was collected near the shoreline of Blood Pond, Massachusetts in August 2005 (Table 1). All the samples were growing below the forest canopy and receiving dappled sunlight throughout the day. Hydrogen isotope ratio values of the leaf waxes from the trees have been reported by Hou et al. (2007). Carbon isotope analysis of individual leaf wax compounds was performed using gas chromatography–combustion–isotope ratio mass spectrometry (ThermoFinnigan). An HP 6890 GC was connected to a Finnigan MAT Delta^{+}-XL mass spectrometer via a combustion

Table 1
$\delta^{13}C$ and δD values of leaf wax compounds (*n*-acids) from trees around Blood Pond, Massachusetts

Scientific name	Height (m)	δD (‰, VSMOW)[a]			$\delta^{13}C$ (‰, VPDB)		
		C_{26}	C_{28}	C_{30}	C_{26}	C_{28}	C_{30}
Betula populifolia Marsh. (1)	6	−153	−159	−131	−33.6	−34.9	−36.2
Betula populifolia Marsh. (1)	4.5	−155	−163	−142	−34.3	−35.1	−37.4
Betula populifolia Marsh. (1)	3	−163	−167	−151	−35.7	−36.6	
Betula populifolia Marsh. (2)	6	−147	−161	−135	−34.0	−35.1	−38.7
Betula populifolia Marsh. (2)	4.5	−125	−144	−122	−34.8	−35.7	−39.3
Betula lenta L. (1)	6	−144	−163	−155	−36.1	−34.6	−36.4
Betula lenta L. (1)	4.5	−137	−160	−156	−35.2	−33.7	−35.6
Betula lenta L. (1)	3	−138	−164	−153	−36.2	−35.4	−37.3
Betula lenta L. (2)	6	−149	−167	−158	−33.8	−33.1	−35.1
Betula lenta L. (2)	4.5				−34.2	−33.7	−34.4
Betula lenta L. (2)	3	−165	−167	−153	−33.4	−33.3	−35.3
Quercus velutina Lam.	3	−130	−152	−147			
Quercus rubra L. (1)	6	−157	−164	−161	−33.9	−34.1	−34.0
Quercus rubra L. (1)	4.5	−153	−165	−166	−34.1	−34.0	−34.5
Acer rubrum L. (1)	3	−136	−159	−159	−34.0	−34.8	−36.9
Acer rubrum L. (2)	3	−148	−168	−162		−32.9	−35.5
Acer rubrum L. (3)	6	−158	−173	−172	−33.4	−34.3	−35.8
Acer rubrum L. (3)	4.5	−142	−171	−165	−34.8	−34.3	−36.2
Carya sp. Nutt. (1)	6	−158	−162	−160		−33.2	−34.9
Carya sp. Nutt. (1)	4.5	−144	−152	−147		−33.4	−34.4
Carya sp. Nutt. (1)	3	−138	−144	−145	−33.1	−33.3	−34.6
Carya sp. Nutt. (2)	3	−175	−179	−182			
Pinus strobus L. (1)	6	−184	−188	−181		−33.0	−33.4
Pinus strobus L. (1)	4.5	−175	−154	−184	−35.9	−32.9	−33.4
Pinus strobus L. (1)	3	−182	−179	−190	−32.8	−33.7	−34.5
Prunus serotina Ehrh. (1)	6				−34.4	−35.2	−36.8
Prunus serotina Ehrh. (1)	4.5	−155	−153	−156	−34.8	−35.6	−37.0
Fraxinus americana L. (1)	6	−109	−124	−121	−35.3	−35.3	
Fraxinus americana L. (1)	4.5	−101	−122	−129		−37.3	−36.8
Fraxinus americana L. (1)	3	−115	−125	−139	−38.0	−35.9	−35.8
Tsuga canadensis L. (1)	4.5	−159	−156	−158	−32.8	−33.1	
Tsuga canadensis L. (1)	3	−126	−131	−142	−33.7	−33.5	
Nyssa sylvatica Marsh. (1)	6	−124	−126	−133	−37.3	−38.0	−39.3
Nyssa sylvatica Marsh. (1)	4.5	−126	−130	−134	−37.8	−38.7	−39.7
Nyssa sylvatica Marsh. (1)	3	−114	−125	−131	−38.2	−38.1	−39.1

[a] δD data first published by Hou et al. (2007).

interface. He was the carrier gas, operating in constant flow mode at 1.1 mL min^{-1}. The oven program was: 40–200 °C @ 20 °C/min – 315 @ 5 °C/min, hold 20 min. Compounds separated on the GC column were converted to CO_2 and H_2O through a combustion furnace operated at 940 °C and loaded with CuO and Pt wires as oxidant and catalyst, respectively. A small stream of 1% O_2 in He was added right in front of the reactor to maintain the oxidation capacity of the CuO. Six pulses of CO_2 reference gas of known $\delta^{13}C$ value were injected via the interface to the isotope ratio mass spectrometer for computation of $\delta^{13}C$ values of sample compounds. Samples were analyzed in duplicate, with a standard deviation less than ±0.2‰. The $\delta^{13}C$ values obtained for individual acids (as methyl esters) were corrected by mathematically removing the isotopic contribution from the added group. The $\delta^{13}C$ values are reported relative to the Vienna Pee Dee Belemnite (VPDB) standard.

3. Results and discussion

3.1. δD and $\delta^{13}C$ variation in tree leaf waxes

The δD values of three long chain *n*-acids (C_{26}, C_{28}, C_{30}) from different trees show an overall range of 60–70‰ variation (Table 1). C_{30} *n*-acid values range from −190 to −120‰, C_{28} *n*-acid from −179 to −116‰ and C_{26} *n*-acid from −184 to −101‰. The leaf waxes from evergreen trees appear to have lower δD values than those from deciduous trees [e.g., the δD_{wax} values for the evergreen *Pinus strobus* L. (∼ −180 to −190‰) are 20–50‰ lower than those of deciduous trees (Table 1)].

The $\delta^{13}C$ values of the long chain *n*-acids ($\delta^{13}C_{wax}$) range from −39.7 to −32.9‰ and show strong inter-correlation ($r^2 = 0.85$ for C_{30} and C_{28} *n*-acids, 0.79 for C_{28} and C_{26} *n*-acids and 0.55 for C_{30} and C_{26} *n*-acids, respectively). The variation in $\delta^{13}C$ value is not a result of different biosynthetic pathways as all trees are C_3 plants. The $\delta^{13}C_{wax}$ values of different leaf samples from individual trees also show some variability. However, there is no clear correlation between $\delta^{13}C_{wax}$ and the height of leaf samples in this study.

Hydrogen and carbon isotope ratios show significant negative correlation for the three individual *n*-acids (Fig. 1). The correlation coefficients between δD_{wax} and $\delta^{13}C_{wax}$ are $R^2 = 0.55$ for C_{30} *n*-acid ($n = 27$, $p < 0.001$), 0.40 for C_{28} *n*-acid ($n = 31$, $p < 0.001$) and 0.34 for C_{26} *n*-acid ($n = 26$, $p < 0.005$), respectively.

3.2. WUE and $\delta^{13}C$ values of leaf waxes

Published data have shown that there is a strong negative correlation between $\Delta^{13}C$ and WUE for field samples. For example, Hubick et al. (1986) demonstrated such a correlation for peanuts grown under wet and dry conditions. Ismail and Hall (1992) showed a strong linear relationship between WUE and $\Delta^{13}C$ for different cowpea genotypes. The relationship is also observed among different tree species. For example, higher WUE for evergreen tree species is accompanied by higher $\delta^{13}C$ values (or smaller $\Delta^{13}C$) relative to deciduous trees (Garten and Taylor, 1992). The difference in WUE and $\delta^{13}C$ values between evergreen and deciduous trees was modeled by Zhang and Marshall, 1994. The results suggest that $\Delta^{13}C$ can be used as an estimate of WUE for plants from a given region where environmental variables (e.g., soil type, temperature, humidity) are relatively constant.

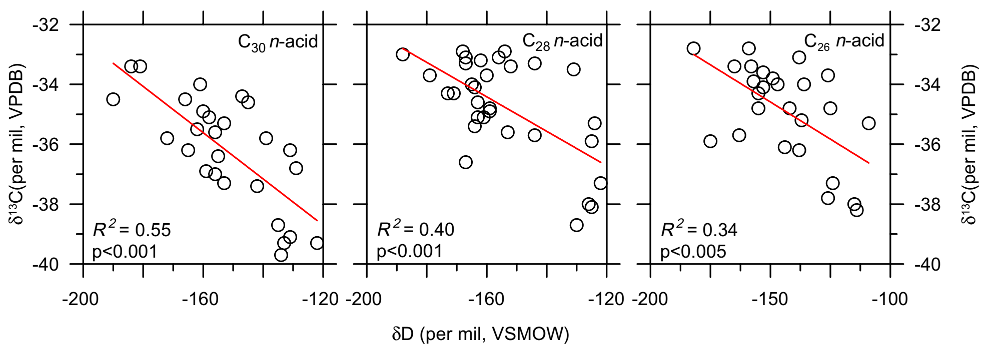

Fig. 1. Correlations between $\delta^{13}C$ and δD values of leaf wax compounds (C_{30}, C_{28}, and C_{26} *n*-acids) from natural trees around Blood Pond, Massachusetts.

As discussed in the introduction, WUE and $\Delta^{13}C$ are negatively correlated, with $\Delta^{13}C$ negatively correlated with $\delta^{13}C_{wax}$ values. Therefore, $\delta^{13}C_{wax}$ values show a positive correlation with WUE, i.e., higher $\delta^{13}C_{wax}$ values should correspond to higher water use efficiency in trees. For example, the $\delta^{13}C$ values of leaf waxes from *P. strobus* L. are higher than those from *Betula lenta* L. and *Nyssa sylvatica* Marsh., suggesting that *P. strobus* L. has a higher water use efficiency than *B. lenta* L. and *N. sylvatica* Marsh. Leaf waxes of evergreen trees have been shown to have higher $\delta^{13}C$ values than deciduous trees (Chikaraishi and Naraoka, 2003; Huang et al., 2006). These data are consistent with the previous observation that evergreen species tend to have higher water use efficiency than co-occurring deciduous species (e.g., DeLucia et al., 1988; Gower and Richards, 1990; DeLucia and Schlesinger, 1991; Garten and Taylor, 1992).

3.3. WUE and δD_{wax}

The significant negative correlation between $\delta^{13}C_{wax}$ and δD_{wax} (Fig. 1) in the leaf samples in this study indicates that tree WUE is likely to be an important control on hydrogen isotope ratios of leaf waxes. D/H ratios of plant leaf waxes are influenced by WUE in addition to δD values of precipitation. Other factors, such as temperature, relative humidity and photosynthetic pathways may also affect δD_{wax} (Smith and Freeman, 2006; Sachse et al., 2006). However, since all our samples were collected within 50 m of Blood Pond's shoreline, precipitation, temperature, humidity, soil and source water should be very similar for all the trees. The observed hydrogen isotopic variation among different trees is therefore likely related to the WUE of the different trees.

Trees with higher WUE (as inferred from higher $\delta^{13}C_{wax}$ values) were found to have lower δD_{wax} values. This is readily understandable. Plants with higher WUE require transpiration of less water to produce the same amount of leaf wax as plants with lower WUE. As water evaporates from the sub-stomatal cavity, the remaining leaf water becomes enriched in deuterium (Farquhar et al., 1989). Smaller transpiration rates result in less hydrogen isotopic enrichment of leaf water, resulting in the subsequent synthesis of leaf wax with lower δD values. For example, δD values of C_{30} *n*-acid from *B. lenta* L. and *N. sylvatica* Marsh. are $-155‰$ and $-133‰$, respectively. The δD of C_{30} *n*-acid from *P. strobus* L. is around $-185‰$. The higher δD values indicate that *Betula* and *Nyssa* have lower WUE than *Pinus*, consistent with higher $\delta^{13}C$ values observed for *Pinus*.

Chikaraishi and Naraoka (2006) measured both δD and $\delta^{13}C$ values of plant leaf waxes, but did not observe the relationship we report here. The samples used by Chikaraishi and Naraoka (2006) were, however, collected from multiple sites in Japan and Thailand. Environmental factors such as temperature, humidity and soil type at the different sites may affect the carbon and hydrogen isotope ratios of leaf waxes in complex ways, thereby complicating comparisons between δD_{wax} and $\delta^{13}C_{wax}$. Contrary to our findings, Bi et al. (2005) show a positive correlation between weighted mean δD_{wax} and $\delta^{13}C_{wax}$ values for nine tree samples from a well maintained botanic garden in China. However, the relationship is defined by one data point relative to a cluster of 8 points. Plants in the botanic garden are grown under varying degrees of artificial irrigation, fertilization and other soil treatment (e.g., adjustment of pH), which could also complicate the relationship between δD_{wax} and $\delta^{13}C_{wax}$.

4. Conclusions

The $\delta^{13}C$ and δD values of leaf waxes from 35 leaf samples from 11 tree species from Blood Pond, MA (USA) show a statistically significant negative correlation, suggesting that water use efficiency exerts an important control on δD values of leaf waxes in trees. Plants with higher WUE display lower δD_{wax} values. The correlation is not perfect, reflecting the fact that other independent factors also affect δD_{wax}. Nevertheless, our study represents the first step in deciphering the complex mechanisms controlling the variation in leaf wax δD values in terrestrial plants.

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