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$\delta^{13}C$ and δD compositions of *n*-alkanes from modern angiosperms and conifers: An experimental set up in central Washington State, USA

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Abstract

This study investigates stable carbon and hydrogen isotope compositions of leaf wax *n*-alkanes from 3 angiosperm and 2 conifer species from a semi-desert natural environment. The $\delta^{13}C$ and δD *n*-C_{27} alkane data show a clear distinction between *Populus tremuloides*/*Syringa vulgaris* (−31.9 to −32.7‰ and −168 to −186‰, respectively) and *Pinus sylvestris*/*Picea pungens* (−28.8 to −30.6‰ and −190 to −212‰) throughout the 2005 growing season. Like the other angiosperm species, *Betula pendula* was D-enriched (−162 to −178‰) relative to the conifer species. However, its $\delta^{13}C$ values were more similar to those of *Pinus sylvestris* and *Picea pungens* in May, but had a ~4‰ positive shift later in the season. We suggest that the observed isotopic variations derive mainly from lower stomatal conductance for CO_2 and H_2O vapor in conifers relative to angiosperms. *Betula pendula* experienced severe environmental stress that affected its carbon metabolism for most of the season. Our results suggest that shifts in δD and $\delta^{13}C$ values of sedimentary leaf waxes may result not only from shifts in the paleoclimatic variables but also from temporal shifts in the distribution of angiosperms and conifers as well as from changes in the isotopic signatures of higher plants undergoing metabolic changes.

1. Introduction

Extending earlier applications of stable carbon isotopes from leaf waxes for investigating paleoclimate (e.g., climate related shifts in C_3/C_4 vegetation – Pancost and Boot, 2004; Tipple and Pagani, 2007, and references therein), hydrogen stable isotope signatures of higher plant leaf waxes are becoming increasingly popular in paleoclimate research. For example, Schefuß et al. (2005) used the ~20‰ D-enrichment for *n*-C_{29} alkane in a marine sediment core to infer a period of aridification in equatorial Africa during the Younger Dryas period. Schuman et al. (2006) used two positive shifts (up to ~40‰) in the δD values of leaf wax C_{28} *n*-acid from lacustrine sediments to suggest a shift towards a greater amount of precipitation during the plant growing season in comparison with the other seasons in the northeastern USA at ~8,200 cal yr BP. Pagani

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et al. (2006) interpreted the ~55‰ increase in the δD values of n-$C_{27/29}$ alkanes as reflecting decreased rainout during moisture transport from lower latitude and, as a result, increased moisture delivery to the Arctic at the onset of the Paleocene/Eocene thermal maximum. Additionally, the −4.5 to −6‰ shift in the $\delta^{13}C$ values of these compounds was used by the authors to argue for a substantially larger negative shift in the $\delta^{13}C$ values of atmospheric CO_2 than reported previously.

The spatial distribution of angiosperms and conifers is controlled by regional climate (e.g., Woodward, 1992) and is susceptible to climate change, even at the millennial and submillennial timescales (Tinner and Lotter, 2001; Williams et al., 2004). Thus, interpretation of leaf wax $\delta^{13}C$ and δD paleodata depends strongly on understanding the relative contribution and isotopic signatures of angiosperm and conifer plants in the sedimentary record. The importance of these variables was recently highlighted by Smith et al. (2007), who argued that a shift from a mixed angiosperm/conifer to a purely angiosperm plant community led to an amplified $\delta^{13}C$ signal (4–5‰, leaf wax n-alkanes in the Bighorn Basin, Wyoming, USA) in comparison with that typically recorded by marine carbonates for the carbon isotopic excursion during the Paleocene/Eocene thermal maximum.

Simultaneous investigation of $\delta^{13}C$ and δD values of leaf wax lipids could become a powerful tool for deciphering the dynamics of angiosperm and conifer communities in the sedimentary record, because of differences in stomatal structure between these plant types (Esau, 1977) and, as a result, potential differences in their isotopic signatures. Only a few studies have investigated leaf wax $\delta^{13}C$ and δD values of modern angiosperms and conifers simultaneously. These studies did not reveal clear differences in the $\delta^{13}C$/δD signal. Chikaraishi and Naraoka (2007) observed no trend among angiosperm and conifer species collected from a number of sites in Japan and Thailand. Hou et al. (2007) observed a weak trend in $\delta^{13}C$/δD values of plants with different water use efficiency (as was assumed by the authors based on $\delta^{13}C$ relationship among the plants) near Blood Pond, Massachusetts, USA, but the trend did not reveal clear differences between angiosperms and conifers.

In this study, we report $\delta^{13}C$ and δD compositions of leaf wax n-alkanes from several angiosperm and conifer species sampled during May–October 2005 near Kittitas, central Washington State, USA. Our first data from this well-controlled experimental set up in a climatically stressful, semi-desert natural environment provide initial information for interpreting stable isotope signatures of higher plants that existed in such paleoenvironments.

2. Materials and methods

The climate in the study area is characterized as dry, with four definite seasons, and <10 cm of precipitation from May to October (Kittitas Country, 2004). Well water, drawn from 95 m depth, was the most important source of moisture for the plants under investigation during the sampling period. We sampled 3 angiosperm (*Betula pendula*, *Populus tremuloides* and *Syringa vulgaris*) and 2 gymnosperm (*Pinus sylvestris* and *Picea pungens*) plants. All five plants are approximately the same age (7–8 years old as of 2005), grow outdoors in close proximity to each other (<10 m apart) and receive an equal amount of sunlight. Trees were watered at the same time (over a period of 2–3 h) once or twice a week. Each tree received, on average, about 70 l. The water disappeared into the ground after 30 min. or less. No soil moisture measurements were carried out during 2005, so no quantitative data are available for estimating evaporative losses subsequent to watering of individual plants. Relative humidity and temperature were monitored using a VERITEQ SP-2000-20R logger installed in the shade, 2 m above ground. Fourteen well water and 7–10 leaf (or 3 shoots for conifers) samples per each tree were sampled from May to October 2005.

Leaf samples were extracted with hexane by sonication (30 min ×2). Lipid fractions were separated via column chromatography (70–230 mesh) using a hexane, hexane/dichloromethane (DCM; 9:1 v/v) and DCM/MeOH (2:1 v/v) sequence. The hexane fraction was analyzed for $\delta^{13}C$ and δD compositions using a Thermo Finnigan MAT 253 mass spectrometer interfaced to a Thermo Finnigan Trace GC Combustion III interface (for $\delta^{13}C$) and a high temperature conversion (for δD) systems. The δD values of well water samples were determined using the same instrument interfaced with a H-device. Individual n-alkanes were separated using a J&W Scientific DB-1 column (60 m × 0.25 mm × 0.25 μ film thickness). The GC oven was programmed from 60 °C (1 min) at 6 °C/min to 320 °C (25 min). The $\delta^{13}C$ values are expressed relative to Vienna Pee Dee belemnite (VPDB), based on an in-house reference gas calibrated against the

Table 1

Isotopic compositions of leaf-wax *n*-alkanes and well water in 2005, central Washington State, USA ($\delta^{13}C$ and δD values are in ‰ relative to VPDB and VSMOW, respectively; numbers in parenthesis represent an error (±) based on duplicate analyses)

Plant species	27 May		3 July		24 July		24 August		14 September		27 September		23 October	
	$\delta^{13}C$	δD	$\delta^{13}C$	δD	$\delta^{13}C$	δD	$\delta^{13}C$	δD	$\delta^{13}C$	δD	$\delta^{13}C$	δD	$\delta^{13}C$	δD
Betula pendula														
nC$_{25}$	−30.3(0.6)	−188(4)	−26.7(0.0)	−187(11)	−28.1(0.2)	−165(5)	−26.6(0.1)	−177(2)	−26.3(0.1)	−174(8)	−25.6(0.0)	−182(1)	−26.7(0.1)	−169(6)
nC$_{27}$	−30.1(0.1)	−163(4)	−26.7(0.0)	−166(2)	−27.9(0.0)	−162(3)	−26.9(0.0)	−172(3)	−26.6(0.1)	−175(3)	−26.3(0.1)	−178(2)	−26.1(0.1)	−171(1)
nC$_{31}$	−32.2(0.1)	n.d	n.d.	n.d.	−32.0(0.1)	−144(10)	−30.7(0.0)	−149(2)	−31.1(0.0)	−163(3)	−31.4(0.0)	−166(2)	−31.7(0.0)	−155(1)
Syringa vulgaris														
nC$_{27}$	−32.4(0.1)	−168(4)	−32.3(0.3)	−176(1)	−32.2(0.2)	−176(1)	−32.0(0.1)	−175(1)	−32.7(0.0)	−178(1)	−32.2(0.1)	−177(1)	−32.4(0.4)	−177(1)
nC$_{29}$	−32.3(0.3)	−186(2)	−32.5(0.1)	−196(1)	−32.6(0.3)	−192(1)	−32.6(0.0)	−192(0)	−33.4(0.3)	−195(0)	−32.5(0.3)	−190(2)	−32.6(0.1)	−188(1)
nC$_{31}$	−33.2(0.4)	−199(1)	−33.6(0.1)	−215(0)	−33.3(0.0)	−210(1)	−34.2(0.1)	−209(1)	−34.6(0.1)	−208(0)	−33.1(0.0)	−217(1)	−33.7(0.1)	−206(2)
nC$_{33}$	−33.3(0.2)	−203(0)	−33.5(0.1)	−212(1)	−33.4(0.1)	−209(1)	−34.2(0.1)	−209(1)	−34.6(0.0)	−209(1)	−33.2(0.1)	−206(0)	−33.5(0.1)	−206(1)
Populus tremuloides														
nC$_{23}$	n.d.	−171(2)	−33.1(0.1)	−178(3)	−33.5(0.1)	−172(3)	−33.2(0.1)	−166(4)	−32.8(0.1)	−180(1)	−33.0(0.0)	−181(4)	−33.7(0.0)	−175(1)
nC$_{25}$	−31.7(0.1)	−178(2)	−32.5(0.0)	−180(1)	−32.7(0.1)	−176(1)	−32.7(0.1)	−175(4)	−32.3(0.0)	−180(2)	−32.5(0.0)	−184(4)	−33.2(0.2)	−178(1)
nC$_{27}$	−32.0(0.0)	−169(4)	−32.5(0.1)	−179(1)	−32.7(0.1)	−177(2)	−32.3(0.0)	−177(4)	−31.9(0.1)	−183(2)	−32.3(0.0)	−186(3)	−32.7(0.0)	−181(1)
Pinus sylvestris														
nC$_{25}$	−28.2(0.0)	−184(0)	−30.3(0.0)	−186(2)	−29.8(0.1)	−184(4)	−29.1(0.1)	−179(1)	−29.4(0.0)	−185(3)	−30.0(0.1)	−191(1)	−29.2(0.1)	−187(1)
nC$_{27}$	−28.8(0.1)	−191(2)	−30.6(0.0)	−195(1)	−29.2(0.0)	−193(1)	−29.2(0.1)	−190(3)	−29.4(0.1)	−194(1)	−29.9(0.1)	−196(1)	−29.1(0.0)	−193(0)
nC$_{29}$	−28.5(0.0)	−208(3)	−28.8(0.0)	−210(1)	−27.6(0.1)	−209(1)	−28.4(0.0)	−205(4)	−28.8(0.1)	−208(1)	−29.5(0.0)	−210(1)	−28.4(0.0)	−202(1)
nC$_{31}$	−30.3(0.0)	−194(1)	−31.4(0.3)	−192(5)	−29.5(0.1)	−189(3)	n.d.	−185(2)	−29.8(0.0)	−190(4)	−31.3(0.2)	−196(0)	−29.9(0.1)	−182(4)
Picea pungens														
nC$_{23}$	−29.8(0.0)	−186(9)	−30.0(0.0)	−190(0)	−29.8(0.1)	−173(3)	−30.0(0.0)	−167(7)	−29.5(0.0)	−183(11)	−29.7(0.0)	−190(1)	−28.9(0.0)	−185(11)
nC$_{25}$	−30.3(0.0)	−206(1)	−30.8(0.1)	−212(5)	−30.1(0.0)	−207(3)	−30.7(0.0)	−204(1)	−30.3(0.1)	−207(6)	−30.4(0.0)	−210(0)	−30.0(0.0)	−201(8)
nC$_{27}$	−30.6(0.0)	−203(1)	−29.9(0.0)	−212(1)	−29.1(0.0)	−208(1)	−29.8(0.0)	−204(4)	−30.0(0.1)	−207(2)	−29.8(0.1)	−212(1)	−29.3(0.0)	−202(2)
nC$_{29}$	−29.9(0.1)	−210(5)	−28.6(0.1)	−212(2)	−27.7(0.0)	−213(1)	−28.5(0.1)	−211(2)	−28.8(0.0)	−214(4)	−28.4(0.1)	−216(2)	−27.9(0.0)	−202(7)
nC$_{31}$	−32.3(0.0)	n.d	−29.9(0.0)	−194(4)	−28.9(0.1)	−193(5)	−30.1(0.1)	−188(1)	−30.2(0.1)	n.d.	−30.0(0.1)	−198(3)	−29.5(0.1)	n.d.
Date	03/07	08/07	24/07	28/07	01/08	06/08	13/08	24/08	29/08	14/09	20/09	27/09	06/10	24/10
Well water, δD	−103(0)	−103(0)	−103(0)	−102(0)	−105(0)	−104(0)	−102(1)	−104(1)	−102(0)	−104(0)	−102(0)	−103(0)	−104(0)	−103(0)

OzTech standard ($\delta^{13}C = -40.61‰$). The margin of error for sample *n*-alkane $\delta^{13}C$ measurements was ±0.6‰ or better (Table 1). Pyrolytic conversion of organic hydrogen to H_2 was conducted at 1400 °C. Hydrogen isotopic compositions of *n*-alkanes are expressed relative to Vienna Standard Mean Ocean Water (VSMOW) based on an in-house reference gas adjusted daily using a 5α-androstane standard. The margin of error for sample *n*-alkane δD measurements was generally better than ±5‰, reaching ±11‰ in only a few cases (Table 1). Hydrogen isotopic compositions of well water samples are expressed relative to VSMOW and are normalized to Greenland Ice sheet Precipitation (GISP) and Standard Light Antarctic Precipitation (SLAP). The margin of error for well water δD measurement was ±1‰ or better.

3. Results and discussion

The 2005 data from central Washington State are shown in Table 1. Because *n*-C_{27} alkane was the only compound present in sufficient quantities in all five species for reliable $\delta^{13}C$/δD measurements, we restrict our discussion to this component. The *n*-C_{27} alkane data show clear separation between 2 conifers and 2 angiosperms (Fig. 1). From May to October, $\delta^{13}C$ and δD values of *Syringa vulgaris* and *Populus tremuloides* stayed within −31.9 to −32.7‰ and −168 to −186‰, respectively, showing a minor trend towards more D-depleted values at the end of the growing season (Fig. 2). However, $\delta^{13}C$ and δD values for *Pinus sylvestris* and *Picea pungens* ranged between −28.8 and −30.6‰, and between −190 and −212‰, with no major trend. Like the other angiosperm species, *B. pendula* was D-enriched (−162 to −178‰) relative to the conifer species. However, $\delta^{13}C$ values of *B. pendula* were more similar to those of *Pinus sylvestris* and *Picea pungens* in May, and had a positive ~4‰ shift from May to October, thus becoming even more ^{13}C-enriched in comparison with the other angiosperms by the end of the growing season.

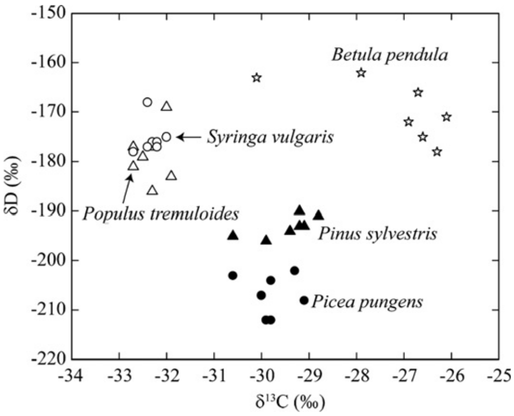

Fig. 1. The $\delta^{13}C$ and δD values of *n*-C_{27} alkane from five plant species, central Washington State, USA.

Because all five plants experienced the same environmental conditions and were irrigated with the same water, we suggest that the observed isotopic variations derive mainly from physiological differences. Relative ^{13}C-enrichment and D-depletion for *P. sylvestris* and *P. pungens* could have resulted from their lower stomatal conductance for CO_2 and H_2O vapor and thus greater water use efficiency (WUE) in comparison with *Syringa vulgaris* and *P. tremuloides*. We currently have no field data for stomatal conductance for the plants, but published studies – either for the same species (*P. sylvestris* and *P. tremuloides*) or for the species from the same genus (*P. sitchensis* and *B. alleghaniensis*) – show lower stomatal conductance in conifers relative to angiosperms in outdoors environments (Körner et al., 1979; Fig. 3).

Apparent hydrogen fractionation factor between *n*-C_{27} alkane and well water used for irrigation (Fig. 4) provides further evidence for physiological differences between angiosperm and conifer species in our study. Assuming that biosynthetic fractionation between *n*-C_{27} alkane and water is constant, species with greater stomatal conductance would experience greater evaporative D-enrichment of leaf water through transpiration, consistent with our observations for *Syringa vulgaris* and *P. tremuloides* in comparison with *Pinus sylvestris* and *Picea pungens*.

Our results indicate a much wider range of apparent hydrogen fractionation factors for both angiosperm and conifer species (ranging between ca. −120 and −65‰, Fig. 4) than previously reported (Chikaraishi and Naraoka, 2003; Bi et al., 2005; Sachse et al., 2006). These other studies estimate rather invariable and more negative apparent hydrogen fractionation factors (ca. −120‰) for both types of plants. The stark differences between our study and previous works could be attributed to the fact that our experimental design in eastern WA state represents an unusually dry environment in comparison with that typically experienced by the species used in this study. Thus, our data may

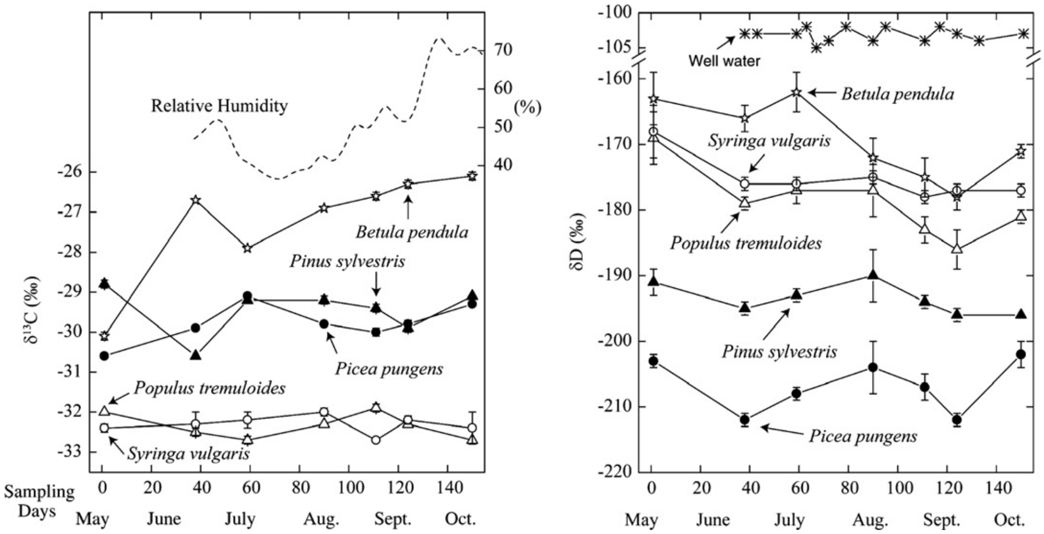

Fig. 2. Seasonal variability in $\delta^{13}C$ and δD values of n-C_{27} alkane from five plant species, and δD of well water during 2005, central Washington State, USA.

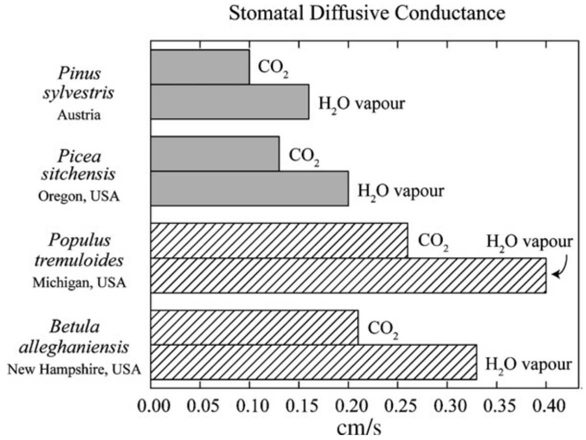

Fig. 3. Maximum stomatal diffusive conductance. Data from Körner et al. (1979). Gray shaded pattern – conifer species, diagonal lines – angiosperm species.

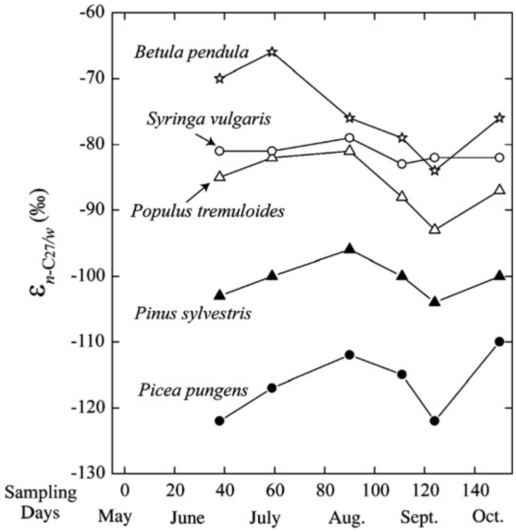

Fig. 4. Apparent fractionation factor (ε) between average n-C_{27} alkane δD values of five plant species and well water; $\varepsilon_{n\text{-C27/w}} = ((R_{n\text{-C27}}/R_{\text{w}} - 1) \times 1000‰$, where $R = D/H$. The same δD_w value was used for each plant species at a particular point in time.

represent an extreme case, so generalization of these results should be done with caution.

It is not clear why *B. pendula* displays such a different trend in $\delta^{13}C$ values in comparison with the other angiosperm species. One possible explanation is that *B. pendula*, typically accustomed to moist soil in a more humid northern climate, experienced severe environmental stress, with the worst effects in July–August when the relative humidity was lowest. This stress could have affected carbon metabolism in *B. pendula* for the rest of the season. Indirect evidence for this comes from the fact that it is the only species that showed a significant increase in the proportion of n-C_{31} relative to all the other prominent n-alkanes during the growing season (Fig. 5) – a possible mechanism for building a thicker more rigid wax layer to avoid drought (Kozlowski and Pallardy, 1997).

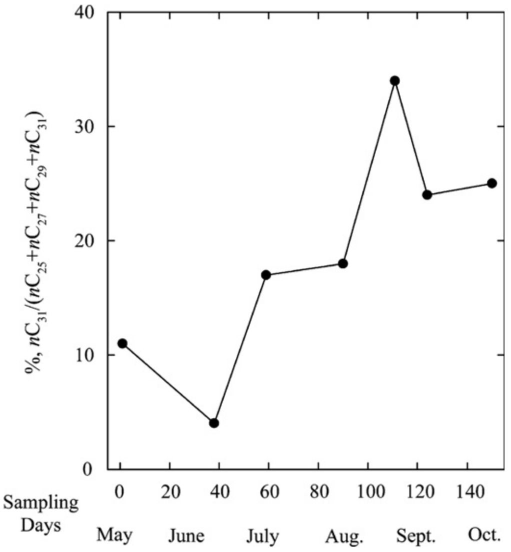

Fig. 5. Proportion of n-C_{31} alkane relative to the sum of all prominent n-alkanes (n-C_{25}, n-C_{27}, n-C_{29}, and n-C_{31}) from *B. pendula* leaves collected in 2005, central Washington State, USA. The ratio was calculated based on individual peak areas using GC FID (flame ionization detector) data.

4. Conclusions

The initial 2005 results from a semi-desert environment in central Washington State indicate that well-adapted higher plant species maintain relatively constant δD and $\delta^{13}C$ values of n-alkanes during a single growing season. However, certain poorly adapted species, *B. pendula* in this case, may undergo large shifts in $\delta^{13}C$ value due a possible alteration in carbon metabolism under environmental stress. Additionally, the results suggest that shifts in δD and $\delta^{13}C$ values of sedimentary n-alkanes may result not only from shifts in paleoclimatic variables but also from temporal shifts in the distribution of angiosperms and conifers as well as from changes in the isotopic signatures of higher plants undergoing metabolic changes.

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