Carbon stable isotope composition of DNA isolated from an incipient paleosol

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ABSTRACT

We determined the carbon isotope ($\delta^{13}C$) value of double-stranded DNA (dsDNA) isolated from the organic horizons of a Delaware soil that is actively being covered by an encroaching sand dune. The soil belongs to a *Nymphaea odorata* Ait. (water lily) wetland, and we regard its active acquisition of a thick (~24 cm) surface mantle to embody the process of paleopedogenesis; therefore, we have termed it an "incipient paleosol." In this study, we compared the $\delta^{13}C$ value of paleosol dsDNA to the bulk $\delta^{13}C$ value of *N. odorata*, as well as to the $\delta^{13}C$ value of plants that had colonized the surface mantle. The isotopic offset between paleosol $\delta^{13}C_{dsDNA}$ and *N. odorata* $\delta^{13}C_{tissue}$ was identical to the relationship between $\delta^{13}C_{dsDNA}$ and $\delta^{13}C_{tissue}$ for tracheophytes, which we had previously determined. In contrast, the isotopic offset between paleosol $\delta^{13}C_{dsDNA}$ and the $\delta^{13}C_{tissue}$ of plants colonizing the surface mantle differed from this relationship by as much as 4‰. Similarly, the $\delta^{13}C$ value of bulk paleosol organic matter was extremely heterogeneous and varied across 6‰. All paleosol DNA polymerase chain reaction (PCR) products produced clear, sharp, 350 base-pair (bp) fragments of *rbcL*, a gene shared by all photosynthetic organisms. These results open the exciting possibility that stable isotope analysis of dsDNA isolated from paleosol organic matter can be used to infer the $\delta^{13}C$ value of the plant that dominated the nucleic acid contribution.

Keywords: paleosol, DNA, carbon, stable isotope.

INTRODUCTION

Paleosols preserve a myriad of characteristics that reflect the climate and environment of their time (e.g., Retallack, 2001). The carbon isotope ($\delta^{13}C$) value of paleosol bulk organic matter is central to many characterizations of paleoclimate; however, its isotopic signal is extensively modified by processes of decomposition (e.g., Bowen and Beerling, 2004), leading workers to increasingly focus on the isolation and analysis of specific compounds from bulk organics. Isolation of genetic material from paleosol organic matter has successfully resulted in reconstructions of plant taxonomic status (Mitchell et al., 2005; Willerslev et al., 2003). Because soil organic carbon is overwhelmingly dominated by contributions from vegetation (e.g., Jobbágy and Jackson, 2000), we set out to test the potential of paleosol nucleic acids to act as an isotopic proxy for the dominant vegetation that was growing in the soil. Toward this end, we compared the carbon stable isotope offset between plant nucleic acids isolated from paleosol organic horizons and the paleovegetation suggested by the geological context and palynological examination. We then compared the offset to our revised and taxonomically expanded data set of tracheophyte $\delta^{13}C_{DNA}$.

BOTANICAL GARDEN SAMPLING AND PALEOSOL SITE DESCRIPTION

Plant leaf tissues were collected from 12 species selected in order to highlight monocots and other groups not included in our previous work (Table 1; Jahren et al., 2004). The total data set now contains four gymnosperms (all conifers) and twenty angiosperms (five monocots and fifteen dicots). Within the angiosperms, the magnoliid group and many core eudicots were included, effectively sampling across the dicots. Plant species reported here strategically augment the previously reported sample set (Jahren et al., 2004), not only by the inclusion of monocots, but also by the inclusion of many herbs in addition to trees (Table 1). Because all sampled plants were under cultivation, they were largely shielded from stress, and many of these plants were growing within greenhouses.

The paleosol field site is located within Cape Henlopen State Park, Delaware (mean annual air temperature = 13.3 °C; mean annual precipitation = 112.5 cm), at the frontal margin of a large eolian sand dune that is actively migrating westward. As it migrates, the dune encroaches on a freshwater wetland, covering the organic-rich soils that formed in the moist environment. The wetland is dominated by *Nymphaea odorata* Ait. (water lily); in contrast, the encroaching sand dunes bring *Oxydendrum arboretum* (L.) DC. (swamp cranberry), *Pinus rigida* P. Mill (pitch pine), and *Acer rubrum* L. (red maple) plants and seedlings in large numbers.

We excavated a profile set back from the leading edge of the dune where 24 cm of sand had buried the wetland soil; below this surface mantle we exposed three distinct soil horizons

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TABLE 1. $\delta^{13}C$ VALUES OF PLANT SPECIES AND EXTRACTED NUCLEIC ACIDS

Taxonomic group	Site of collection*	Latin name	Common name	A_{260}/A_{280}	$\delta^{13}C$ of bulk plant tissue $\pm\sigma$(‰)§	$\delta^{13}C$ of nucleic acids $\pm\sigma$(‰)§
Conifers	ATL	*Cryptomeria japonica* (L.f.) D. Don	Japanese cedar	1.462	−27.44 ± 0.06	−26.42 ± 0.15
	STR	*Cupressus sempervirens* L.†	Italian cypress	1.380	−29.68 ± 0.07	−28.54 ± 0.59
	UCD	*Pinus canariensis* C. Smith†	Canary Island pine	1.270	−27.40 ± 0.14	−26.11 ± 0.40
	UCR	*Pinus halepensis* P. Mill.†	Aleppo pine	1.600	−28.97 ± 0.06	−27.90 ± 0.29
Angiosperms						
Magnoliids	UKB	*Atriplex littoralis* L.	Grassleaf orache	1.422	−28.02 ± 0.04	−27.45 ± 0.20
	ATL	*Magnolia virginiana* L.†	Umbrella tree	1.610	−29.19 ± 0.11	−27.38 ± 0.06
Monocots	UKB	*Acorus calamus* L.	Calamus	1.416	−30.11 ± 0.04	−28.41 ± 0.20
	UKB	*Dietes grandiflora* N.E. Br.	Wild iris	1.418	−28.13 ± 0.06	−27.26 ± 0.01
	UKB	*Dypsis lutescens* (H. Wendl.)	Yellow butterfly palm	1.453	−30.40 ± 0.04	−28.85 ± 0.22
	UKB	*Lilium regale* E.E. Wilson	Regal lily	1.433	−27.67 ± 0.05	−26.27 ± 0.01
	UKB	*Phalaris arundinacea* L.	Reed canarygrass	1.414	−28.02 ± 0.06	−27.07 ± 0.04
Rosids	ATL	*Acer campestre* L.†	Hedge maple	1.710	−31.31 ± 0.05	−29.25 ± 0.08
	ATL	*Aesculus sylvatica* Bartr.†	Painted buckeye	1.590	−31.85 ± 0.14	−29.79 ± 0.07
	ATL	*Carya alba* L.†	Mockernut hickory	1.600	−28.15 ± 0.04	−26.29 ± 0.10
	UCR	*Cneoridium dumosum* Nutt.†	Bush rue	1.470	−30.74 ± 0.06	−29.41 ± 0.24
	UCD	*Eucalyptus robusta* Sm.	Swampmahogany	1.472	−28.66 ± 0.11	−27.30 ± 0.12
Asterids	ATL	*Camellia sasanqua* Thunb.	Sasanqua camellia	1.870	−30.74 ± 0.60	−30.03 ± 0.16
	STR	*Clethra mexicana* L.	Sweetpepperbush	1.444	−30.85 ± 0.01	−29.86 ± 0.33
	ATL	*Ilex decidua* Walt	Possumhaw	1.425	−30.24 ± 0.03	−28.62 ± 0.39
	ATL	*Kalmia latifolia* L.†	Mountain laurel	1.260	−29.83 ± 0.11	−28.91 ± 0.04
	UCR	*Laurus nobilis* L.†	Sweet bay	1.150	−28.25 ± 0.17	−27.67 ± 0.06
	FTG	*Manilkara zapota* (L.) van Royen†	Sapodilla	1.600	−30.25 ± 0.13	−29.25 ± 0.04
	UCR	*Umbellaria californica* L.	Oregon myrtle	1.550	−29.25 ± 0.17	−27.52 ± 0.06
Other Eudicots	ATL	*Hamamelis virginiana* L.†	American witch hazel	1.470	−30.26 ± 0.06	−28.75 ± 0.47

*Samples were collected at the following arboreta and botanical gardens during 2001 and 2003: Atlanta Botanical Gardens (ATL), University of Copenhagen Botanical Gardens (UKB), Fairchild Tropical Gardens (FTG), Strybing Arboretum and Botanical Gardens (STR), University of California at Davis Arboretum (UCD), University of California at Riverside Botanical Garden (UCR).
†Reported in Jahren et al. (2004).
§σ is the standard deviation seen in three replicates of the sample.

(Table 2). The uppermost horizon ($1O_e$) was 18 cm thick and was composed of primarily hemic organic materials. Mixed with the organic material was medium-grained quartz sand, accounting for 20% of the bulk material by mass. The second horizon (1A) was 11 cm thick and classified as a mineral horizon, with 80% of the bulk material as medium-grained quartz sand and the remainder made up of hemic organic material. We interpret the first ($1O_e$) and second (1A) horizons to comprise a buried Typic Endoaquent. Radiocarbon dating of fine-grained organic (<0.1 μm particles) yielded an age of 1900–present (Table 2), which is in keeping with maps of the migration of the shoreline (Division of Parks and Recreation, Dover, Delaware, 2005, personal commun.). Based on these features, the buried horizons comprise an incipient paleosol—a model for the inception of paleopedogenesis. An examination of pollen isolated from horizons $1O_e$ and 1A revealed the presence of *N. odorata* to the exclusion of other species. The third horizon ($2O_e$) was 13 cm thick and had the highest organic content of the three horizons; however, there was evidence of extensive bioturbation within this horizon, including evidence of fluctuating water table. For this study, we performed nucleic acid extractions from the $1O_e$ and 1A horizons because

TABLE 2. FIELD, ISOTOPIC, AND DNA DESCRIPTIONS OF THE INCIPIENT PALEOSOL

Typic Endoaquent

Horizon	Depth (cm)	Color (moist)	Structure	pH	Boundary	NOSAMS accession #	$F_m \pm \alpha$*	$\Delta^{14}C$	Age	$\%C_{bulk} \pm \sigma$†	Range in $\delta^{13}C_{bulk}$ (‰)
Surface mantle	0–24	2.5Y 6/2	single grain	6.5	gradual smooth	N.D.§					
$1O_e$	24–42	2.5Y 3/1	hemic	5.5	clear smooth	OS-45719	1.0151 ± 0.0036	8.5	1950–present	32.7 ± 4.0 (n=59)	−32.54 to −25.85 (n=59)
1A	42–53	2.5Y 5/2	single grain	4.5	clear smooth	OS-45720	1.0174 ± 0.0037	10.8	1950–present	3.0 ± 0.3 (n=46)	−31.47 to −27.10 (n=46)
$2O_e$	53–66	10YR 2/1	hemic	4.5		N.D.					

Horizon	Sample	$\delta^{13}C$ of extracted DNA $\pm \sigma$† (‰)
$1O_e$	1	−25.70 ± 0.30 (n=6)
	2	−25.58 ± 0.20 (n=5)
	3	−25.63 ± 0.20 (n=5)
1A	1	−25.96 ± 0.70 (n=3)

Carbon Isotope Composition of Plants Growing at the Site

Latin name	Common name	$\delta^{13}C$ of bulk plant tissue $\pm \sigma$† (‰)
Nymphaea odorata Ait. stem	Water lily stem	−27.05 ± 0.47 (n=3)
Nymphaea odorata Ait. leaf	Water lily leaf	−26.11 ± 0.17 (n=3)
Acer rubrum L.	Red maple	−29.35 ± 0.30 (n=3)
Oxydendrum arboretum (L.) DC.	Swamp cranberry	−30.68 ± 0.80 (n=3)
Pinus rigida P. Mill	Pitch pine	−30.55 ± 0.16 (n=3)

*Fraction modern $^{14}C \pm$ error (reported according to conventions described by Siuiver and Polach [1977] and Stuvier [1980]).
†σ is the standard deviation seen in three replicates of the sample.
§N.D. = not determined.

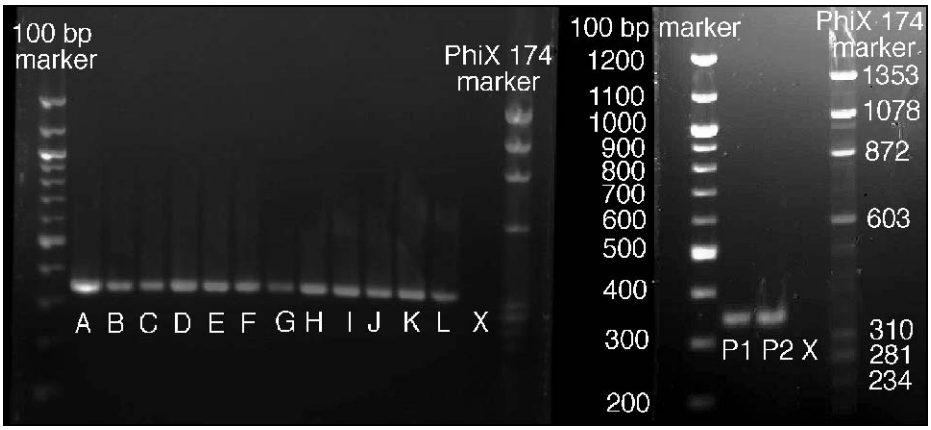

Figure 1. Amplification of ~350 base-pair (bp) fragment from *rbcL* gene within isolated nucleic acids: electrophoresis images for both modern plants and incipient paleosol horizons. A—*Acorus calamus*; B—*Atriplex littoralis*; C—*Camellia sasanqua*; D—*Clethra mexicana*; E—*Cryptomeria japonica*; F—*Dietes grandiflora*; G—*Dypsis lutescens*; H—*Eucalyptus robusta*; I—*Ilex decidua*; J—*Lilium regale*; K—*Phalaris arundinacea*; L—*Hamamelis virginiana*; P1—horizon $1O_e$; P2—horizon 1A; X—negative control (blank). Size markers are PhiX 174 and 100 bp increments. Fragments were stained with ethidium bromide and resolved by gel electrophoresis in 3.0% agarose gels. Resulting polymerase chain reaction products were visualized under 302 nm of light at intensity of 800 μW/cm².

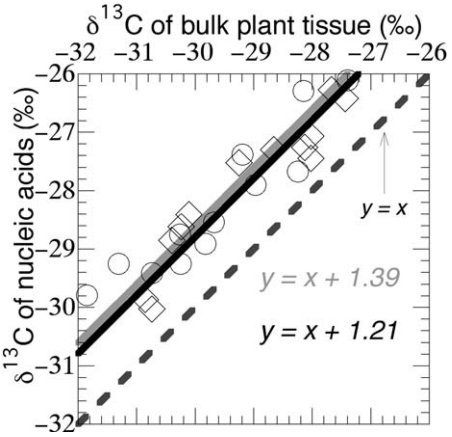

Figure 2. $\delta^{13}C$ value of bulk plant tissue versus $\delta^{13}C$ value of nucleic acids extracted from same plant species: Circles—species (largely dicots; n = 12) reported in Jahren et al. (2004); diamonds—species (largely monocots; n = 12) included in this study. Isotopic offsets are displayed for each data set: +1.39‰ (gray line—Jahren et al., 2004); +1.21‰ (black line—additional data); offset for entire data set is +1.30‰.

they best approximated the buried organic material of a classical paleosol and provided a wide comparison of soil organic matter content (Table 2).

MATERIALS AND METHODS

Descriptions of stable carbon isotope analyses, as well as of DNA extraction and purification, can be found in the GSA Data Repository material.[1] Within this file, we include an explicit description of the several measures that were taken to ensure against DNA contamination during the procedures. Note that all of the DNA templates, from both the plant and soil samples, produced clear, sharp, and reproducible bands of ~350 base pair (bp) (Fig. 1), confirming the presence of *rbcL* in the vast majority of dsDNA from both plant and soil samples.

RESULTS AND DISCUSSION

Table 1 presents the $\delta^{13}C$ values of all plant tissues sampled and nucleic acids extracted from these tissues in triplicate, in addition to the data for plants reported in Jahren et al. (2004). Carbon isotope compositions of bulk paleosol material, isolated paleosol dsDNA, and bulk tissue of plants growing at the Cape Henlopen site are presented in Table 2.

The first portion of this study was designed to augment the $\delta^{13}C$ analyses on plant tissue reported in Jahren et al. (2004) with analyses of monocots and additional dicots in order to produce an expanded plant dsDNA $\delta^{13}C$ data set, as well as a $\delta^{13}C_{plant}$ versus $\delta^{13}C_{dsDNA}$ relationship that can be considered germane to the tracheophytes. Jahren et al. (2004; note erratum in Table 1) reported that the $\delta^{13}C$ value of the bulk leaf tissue ranged from −31.85‰ to −27.40‰, with a mean value of −29.66‰ (n = 12); our additional data set yielded bulk leaf tissue ranging from −30.85‰ to −27.44‰, with a mean value of −29.13‰ (n = 12). All measured $\delta^{13}C_{plant}$ values coincided with $\delta^{13}C$ values classically reported for C3 plants (Smith and Epstein, 1971). In addition, Jahren et al. (2004) reported that the $\delta^{13}C$ value of nucleic acids extracted using the methods described in the supplementary material (see footnote one) ranged from −29.79‰ to −26.11‰, with a mean value of −28.27‰ (n = 12); our additional data set yielded nucleic acids ranging from −30.03‰ to −26.27‰, with a mean value of −27.92‰ (n = 12). Figure 2 presents the $\delta^{13}C$ value of bulk leaf tissue plotted against the $\delta^{13}C$ value of nucleic acids extracted from the same plant species for both Jahren et al. (2004) data and the newly included additional species. The carbon isotope difference between bulk leaf tissue and nucleic acids ($\Delta = \delta^{13}C_{plant} - \delta^{13}C_{nucleic\ acids}$) reported in Jahren et al. (2004) ranged from −2.06‰ to −0.58‰, with a mean value of −1.39‰ (n = 12; σ = 0.48‰), and showed a linear trend such that $\delta^{13}C_{nucleic\ acids} = 0.85(\delta^{13}C_{plant}) - 3.10$ (‰; $R^2 = 0.87$). The carbon isotope offset between the bulk tissue and the isolated nucleic acids within plants new to this study ranges from −1.73‰ to −0.57‰, with a mean value of −1.21‰ (n = 12; σ = 0.40‰), and has a linear trend of $\delta^{13}C_{nucleic\ acids} = 0.91(\delta^{13}C_{plant}) - 1.31$ (‰; $R^2 = 0.90$). As we noted previously, the nucleic acids resulted from identical biochemical pathways in each plant species, so a standard isotopic offset between bulk leaf tissue and nucleic acids is more likely than $\delta^{13}C_{nucleic\ acids}$ being dependent on $\delta^{13}C_{plant}$. Jahren et al. (2004) characterized this isotopic offset as $\Delta = \delta^{13}C_{plant} - \delta^{13}C_{nucleic\ acids} = -1.39‰$. Given the additional data displayed in Table 1 and Figure 2, we now believe that this offset is best characterized as $\Delta = \delta^{13}C_{plant} - \delta^{13}C_{nucleic\ acids} = -1.30‰$, which is a minor modification given the breadth of the plant species added to the data set. Jahren et al. (2004) discussed this offset as different from that reported for unicellular organisms; to date, our work is still the only comparison between $\delta^{13}C_{bulk\ tissue}$ and $\delta^{13}C_{nucleic\ acids}$ for multicellular organisms.

When we apply this isotopic offset to the $\delta^{13}C$ of nucleic acids isolated from horizons $1O_e$ and 1A, a clear pedogenic relationship emerges. Examination of the geological context, as well as the palynology of horizons $1O_e$ and 1A, suggests that the incipient paleosol supported a monoculture of *N. odorata*. Because all plants studied at Cape Henlopen shared a common C3 photosynthetic pathway and were subject to approximately the same value of $\delta^{13}CO_2$, species differences in bulk leaf tissue $\delta^{13}C$ value (Table 2) are likely a reflection of species differences in the ratio of intercellular to atmospheric pCO_2 (c_i/c_a; Ehleringer, 1993). It is notable that *N. odorata* produced a higher tissue $\delta^{13}C$ value than other plants: the aquatic habitat likely altered the rate of diffusion of ^{13}C versus ^{12}C to carbon-fixing tissues (Hobbie and Werner, 2004). The $\delta^{13}C$ value of the paleo-plant community is indicated by the $\delta^{13}C$ value of nucleic acids isolated from paleosol horizons $1O_e$ and 1A:

[1]GSA Data Repository item 2006076, DNA extraction and isotope analyses, is available online at www.geosociety.org/pubs/ft2006.htm, or on request from editing@geosociety.org or Documents Secretary, GSA, P.O. Box 9140, Boulder, CO 80301-9140, USA.

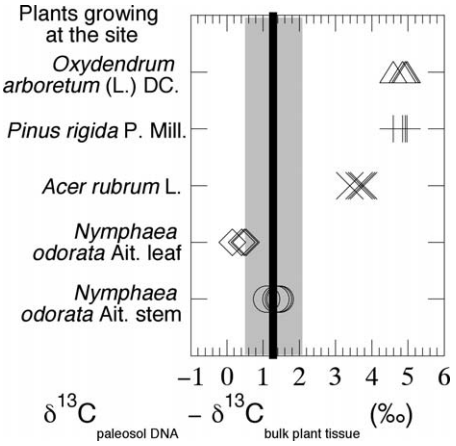

Figure 3. $\delta^{13}C$ offset measured between nucleic acids extracted from paleosol horizons $1O_e$ and 1A and tissues of plants growing at field site now; black line indicates mean offset (+1.30‰; *n* = 24) observed between plant tissues and plant-extracted double-stranded (ds)DNA (Fig. 2); gray shading represents standard deviation associated with mean. Both $\delta^{13}C_{dsDNA}$ values of paleosol horizons, as well as geological context indicate that paleosol dsDNA is dominated by *Nymphaea odorata* plants.

Figure 3 and Table 2 illustrate the comparison between the carbon isotope composition of plants growing at the field site at present and the $\delta^{13}C$ value of dsDNA extracted from the incipient paleosol. In particular, analysis of $\delta^{13}C_{paleosol\ dsDNA} - \delta^{13}C_{plant\ dsDNA}$ values reveals that the offset seen for *N. odorata* (stem and leaf) coincides with the offset we have characterized for tracheophytes (Fig. 3). In contrast, values of $\delta^{13}C_{paleosol\ dsDNA} - \delta^{13}C_{plant\ dsDNA}$ for plants growing on the encroaching sand dune (i.e., the surface mantle; *O. arboretum*, *P. rigida*, *A. rubrum*) fall well outside (i.e., 2‰–4‰) the range of offsets determined by our expanded data set (Fig. 2). We note also that the bulk organic matter $\delta^{13}C$ value of $1O_e$ and 1A is very heterogeneous (Table 2), ranging across values seen in plants on the encroaching sand dune as well as the values obtained for *N. odorata*. Although the results presented here reflect a very simple system with one dominant plant paleo-input, this study opens an exciting possibility: that stable isotope analysis of dsDNA isolated from paleosol organic matter can be used to infer the $\delta^{13}C$ value of the organism that dominated the nucleic acid contribution. The organism in question might then be discernible through standard polymerase chain reaction (PCR) and sequencing techniques.

CONCLUSIONS

Sequences of *rbcL* have been extensively used to shed light on evolutionary patterns within the major groups of land plants (e.g., Sanderson and Doyle, 2001). Plant DNA can be recovered and sequenced from Quaternary sediments: a 130 bp fragment of *rbcL* was recovered from 400,000-yr-old sediments and used to infer the taxonomic status of ancient plants (Willerslev et al., 2003). Fossil soils are emerging as a legitimate source of nucleic acids worthy of sequencing (Hebsgaard et al., 2005; Perkins, 2003; Willerslev and Cooper, 2005); it has long been known that nearly all soil carbon is ultimately derived from plant tissue (e.g., Jobbágy and Jackson, 2000). Provided that sufficient amounts of nucleic acids can be isolated from a soil substrate (≥2900 ng; discussed in Jahren et al., 2004), this study suggests that the $\delta^{13}C$ value of the nucleic acids can be used to infer the $\delta^{13}C$ value of the dominant plant through DNA sequencing. Changes in the $\delta^{13}C$ value of plants through time can be used to argue for changes in water stress (e.g., Toft et al., 1989), salinity (e.g., Guy et al., 1980), light levels (e.g., Madhavan et al., 1991), and a wealth of other potential ecological and environmental effects (i.e., Dawson et al., 2002), depending on geological and taxonomic context. Because of the myriad of environmental processes that influence the $\delta^{13}C$ value of plant tissues, the methods we present here have the potential to yield genetically specific information on changes in plant ecology during the Quaternary, which has been recorded in the $\delta^{13}C_{dsDNA}$ of paleosol organic matter.

ACKNOWLEDGMENTS

We thank W.M. Hagopian, C. Hansen, L. Knudsen, and J.W. Mundy for help and advice in the laboratory; we thank S.P. Werts and C. Bennett for help in the field. We are grateful to the State of Delaware Department of Natural Resources for providing access to the field site, and to the arboreta and botanical gardens listed in Table 1 for allowing us to sample their plants. This project was supported by the Danish-American Fulbright Association and the Howard Hughes Medical Institute (awards to Jahren). The National Ocean Sciences Accelerator Mass Spectrometry Facility (NOSAMS) is supported by National Science Foundation grant OCE-9807266.

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Manuscript received 21 November 2005
Revised manuscript received 21 December 2005
Manuscript accepted 22 December 2005

Printed in USA