Oecologia
DOI 10.1007/s00442-009-1321-1

PHYSIOLOGICAL ECOLOGY - ORIGINAL PAPER

Carbon and hydrogen isotope fractionation under continuous light: implications for paleoenvironmental interpretations of the High Arctic during Paleogene warming

Hong Yang · Mark Pagani · Derek E. G. Briggs · M. A. Equiza · Richard Jagels · Qin Leng · Ben A. LePage

Received: 8 July 2008 / Accepted: 26 February 2009

Abstract The effect of low intensity continuous light, e.g., in the High Arctic summer, on plant carbon and hydrogen isotope fractionations is unknown. We conducted greenhouse experiments to test the impact of light quantity and duration on both carbon and hydrogen isotope compositions of three deciduous conifers whose fossil counterparts were components of Paleogene Arctic floras: *Metasequoia glyptostroboides*, *Taxodium distichum*, and *Larix laricina*. We found that plant leaf bulk carbon isotopic values of the examined species were 1.75–4.63‰ more negative under continuous light (CL) than under diurnal light (DL). Hydrogen isotope values of leaf *n*-alkanes under continuous light conditions revealed a D-enriched hydrogen isotope composition of up to 40‰ higher than in diurnal light conditions. The isotope offsets between the two light regimes is explained by a higher ratio of intercellular to atmospheric CO_2 concentration (C_i/C_a) and more water loss for plants under continuous light conditions during a 24-h transpiration cycle. Apparent hydrogen isotope fractionations between source water and individual lipids ($\varepsilon_{lipid-water}$) range from −62‰ (*Metasequoia* C_{27} and C_{29}) to −87‰ (*Larix* C_{29}) in leaves under continuous light. We applied these hydrogen fractionation factors to hydrogen isotope compositions of in situ *n*-alkanes from well-preserved Paleogene deciduous conifer fossils from the Arctic region to estimate the δD value in ancient precipitation. Precipitation in the summer growing season yielded a δD of −186‰ for late Paleocene, −157‰ for early middle Eocene, and −182‰ for late middle Eocene. We propose that high-latitude summer precipitation in this region was supplemented by moisture derived from regionally recycled transpiration of the polar forests that grew during the Paleogene warming.

Keywords Arctic · Stable isotope · Paleogene · Paleoclimate · Deciduous conifers

Communicated by Todd Dawson.

H. Yang (✉)
Department of Science and Technology,
College of Arts and Sciences, Bryant University,
Smithfield, RI 02917, USA
e-mail: hyang@bryant.edu

M. Pagani · D. E. G. Briggs
Department of Geology and Geophysics,
Yale University, New Haven, CT 06520, USA

M. A. Equiza · R. Jagels
School of Forest Resources, University of Maine,
Orono, ME 04469, USA

Q. Leng
LPS, Nanjing Institute of Geology and Palaeontology,
CAS, Nanjing 210008, People's Republic of China

B. A. LePage
The Academy of Natural Sciences, 1900 Benjamin Franklin Parkway, Philadelphia, PA 19103, USA

B. A. LePage
PECO Energy Company, 2301 Market Avenue,
S9-1, Philadelphia, PA 19103, USA

Introduction

Carbon and hydrogen isotope fractionations under low intensity 24-h continuous light were unknown previously, even though they are critical to the interpretation of isotope signatures in plant fossils in the Paleogene High Arctic where the light cycle played a critical role. Photosynthesis by plants growing in the Arctic is impacted by continuous,

Published online: 08 April 2009

low angle, low intensity illumination during the summer months (Jagels and Day 2004; Osborne et al. 2004). Physiological studies and theoretical analysis predict that photosynthesis under variable light conditions should influence the opening of stomata and carbon assimilation rates, as well as the carbon and hydrogen isotopic compositions (Farquhar et al. 1989; Ögren and Sundin 1996; Osborne and Beerling 2003). But data on the influence of different light regimes on carbon isotope signals are limited (Ehleringer et al. 1986; Pearcy and Pfitsch 1991; Smith et al. 1976), and no study has been performed on the impact of these conditions on molecular hydrogen isotopes.

During the late Paleocene to middle Eocene (60–40 Ma), the Canadian High Arctic was characterized by a warm (Greenwood and Basinger 1994; Sluijs et al. 2006) and humid (Jahren and Sternberg 2003, 2008) growing season with elevated atmospheric CO_2 concentrations (Lowenstein and Demicco 2006; Pearson and Palmer 2000). This unique high-latitude paleoecosystem, illuminated by $\sim$4 months of continuous low intensity light during summer, nurtured extensive deciduous forests with productivities as high as those measured in modern temperate regions (Williams et al. 2003). Paleogene floras, recovered from the Canadian Arctic Archipelago as far north as 80°N, show forested ecosystems dominated by deciduous conifers such as *Metasequoia* Miki ex Hu et Cheng (Greenwood and Basinger 1994; McIver and Basinger 1999)—a paleo-endemic genus that its natural occurrence is presently restricted to low latitudes between 29°25′N and 30°10′N.

Paleocene and Eocene environmental reconstructions suggest that global warming was associated with an increase in polar temperatures (Sluijs et al. 2006) and a substantial increase in moisture delivery to the Arctic (Bowen et al. 2004; Pagani et al. 2006). This inference, based on the hydrogen isotopic compositions of sedimentary leaf wax lipids, requires knowledge of the hydrogen isotope fractionation that occurs during lipid biosynthesis. Commonly, an average value for the hydrogen isotope fractionation between source water and individual lipids ($\varepsilon_{lipid-water}$) of representative higher plants is applied to hydrological reconstructions. However, recent work indicates that magnitudes of $\varepsilon_{lipid-water}$ are genus-specific (Hou et al. 2007; Liu et al. 2006), and more precise evaluations of ancient precipitation δD are possible if the source of the terrestrial lipid is known and fractionation factors are constrained. If the unique Arctic light regime has an impact on stable isotope fractionations, then this influence should be accounted for in paleoenvironmental reconstructions.

For this study, we investigated the impact of light on carbon and hydrogen isotope fractionations by cultivating saplings of living representatives of the three deciduous conifers that dominated the Paleocene–Eocene Arctic landscape. Plants were grown in a greenhouse under controlled conditions and under different light regimes that simulated the conditions at 45°N and the High Arctic (80°N) to establish values of $\varepsilon_{lipid-water}$. These values were then used to determine the hydrological characteristics of the Paleogene Arctic based on isotopic analyses of three-dimensionally preserved fossil conifer leaves with well preserved labile biomolecules (Yang et al. 2005).

Materials and methods

Modern plant material and greenhouse light treatments

Greenhouse experiments were conducted at the University of Maine (45°N) during a 4-month-period from mid-May to mid-September in two consecutive years (2003–2004). Replicates of 3-year-old saplings of three deciduous conifers, *Metasequoia glyptostroboides* Hu et Cheng and *Taxodium distichum* (L.) L. C. Rich (Cupressaceae), and *Larix laricina* (Du Roi) K. Koch (Pinaceae), were grown in 22-dm^3 pots filled with peat:vermiculite:perlite (2:1:1) and fertilized with slow release fertilizer (Osmocote,19-5-8) containing minor and trace elements (Equiza et al. 2006a, b). These greenhouse plants were irrigated three times per day using water with a hydrogen isotope value of -65.7 ± 1.7‰. The greenhouse was divided into two light environmental treatments that were separated by buffer zones: a natural light regime ("diurnal light" treatment or DL, with a photoperiod characteristic of 45°N) and continuous light regime ("continuous light" treatment or CL, with a 24-h photoperiod). Illumination for the CL treatment during the night was provided by six metal-halide lamps; a shade-cloth with about 70% interception was placed on the outer surface of the greenhouse roof to reduce the photosynthetic photon flux density (PPFD) during the day to compensate for the extra light received from the lamps during the night. The mean maximum photosynthetic photon flux (PPF) in the CL treatment (447 ± 161 μmol m^{-2} s^{-1}) was about 35% lower than that in the DL treatment (676 ± 242 μmol m^{-2} s^{-1}), but the fluence remained similar between the two treatments at 15.7 ± 3.4 mol m^{-2} for CL and 15.4 ± 5.5 mol m^{-2} for DL, respectively (Equiza et al. 2006a). These values are within the range predicted for a high-latitude temperate environment (Jagels and Day 2004). The humidity and temperature of the greenhouse were maintained by an evaporative cooler at the south end and exhaust fans at the north end. Temperature (16.8–25.2°C for DL and 16.6–22.2°C for CL) and relative humidity (85% for DL and 92% for CL) did not differ significantly between the two light treatments ($P = 0.207$ for temperature, $P = 0.093$ for humidity, respectively) (Equiza et al. 2006b). The average

CO_2 concentrations in the greenhouse fluctuated between 357 and 361 μL L^{-1}. More detailed day-by-day measurements of these parameters in the greenhouse were provided by Equiza et al. (2006a, b, 2007).

Leaf transpiration was measured on fully expanded foliage from the upper third of the saplings with a portable open-flow photosynthesis system (LI-6400, LI-COR). Samples were equilibrated for 15 min to conditions within the cuvette (CO_2 concentration: 360–400 μL L^{-1}, leaf temperature 22–25°C, HR: 50–60%). Measurements were made every 4 h on randomly selected trees in each block-by-block treatment each day during the 24-h cycle for a 9-day period in early August. Leaf water loss per day was calculated by integrating the 4-h values.

Fossil plant material and geological setting

Three-dimensionally-preserved late Paleocene *Metasequoia* leaves were sampled from lignite beds at the top of Member 2 of the Iceberg Bay Formation, Stenkul Fiord, Ellesmere Island, Canadian Arctic Archipelago, with a paleolatitude of ~77–82°N (Christie 1988). Middle Eocene leaves of *Metasequoia* and *Larix* were collected from the Upper Coal member of the Buchanan Lake Formation, Axel Heiberg Island, Canadian Arctic Archipelago (Ricketts and Stephenson 1994), with a paleolatitude of ~74–80°N (Irving and Wynne 1991) (see Yang et al. 2005 for detailed location map of the two fossil sites). *Metasequoia* leaves were also sampled from leaf litters along a 50-cm-thick vertical section, at 6.25-cm intervals, in the upper section of middle Eocene beds on Axel Heiberg Island, representing a duration of between 313 and 625 years (Kojima et al. 1998). The Upper Coal Member of the Buchanan Lake Formation is a fluvial deposit with an age equivalent to the Uintan North American land mammal stage (41.3–47.5 Ma, i.e., middle Eocene) (Eberle and Storer 1999). The fossil-bearing sediments of the Iceberg Bay Formation are dated palynologically as late Paleocene–early Eocene, and Ricketts and McIntyre (1986) provided more detailed geological information on these two localities. Based upon recent carbon isotope analysis (Yang et al., unpublished data), we have located the tentative Paleocene–Eocene boundary and determined our fossil bed to be late Paleocene in age.

Stable isotope analysis

Fresh leaves of the three conifers under CL and DL greenhouse treatments were sampled (two samples per tree from different positions), freeze-dried, and crushed to a fine powder. Approximately 5 mg were analyzed for carbon isotope measurement on a Costech ECS 4010 elemental analyzer connected to a Thermo Finnigan DeltaPlus Advantage isotope ratio mass spectrometer at the Earth System Center for Stable Isotopic Studies (ESCSIS) of Yale University. Samples were analyzed in duplicate, and standard deviations of replicate samples were 0.05‰ for $\delta^{13}C$. The analytical accuracy and precision for all samples during measurement was ~0.17‰ or better. Carbon isotopic composition ($\delta^{13}C$) is expressed relative to the VPDB standard defined by the relationship in Eq. 1.

$$\delta^{13}C = 1{,}000 \times [(^{13}C/^{12}C_{sample})/(^{13}C/^{12}C_{VPDB}) - 1] \quad (1)$$

Individual fossil conifer leaves were isolated and identified under a dissecting microscope based upon gross morphology; the identity of selected samples was verified using cuticle characteristics observed under scanning electron microscopy (SEM). Total lipid extraction was carried out with a Dionex 300 Accelerated Solvent Extractor (ASE) five times at 125°C, 1,500 p.s.i. for 25 min using dichloromethane (DCM):methanol = 2:1 (v/v) buffer. Solid phase ion exchange columns (70–230 mesh) were used to separate free carboxylic acids from the neutral lipid fractions, which were further separated into hydrocarbons and alcohols by silica gel flash column chromatography using solvents of increasing polarity. Urea adduction was performed on the hydrocarbon fraction of fossil samples to separate branched and cyclic compounds (Pagani et al. 2006; Yang and Huang 2003). Concentrations of the individual compounds were determined using HP6890 gas chromatograph mass spectrometry (GC/MS). GC analysis (using a DB-1 capillary column, 60 m × 0.25 mm × 0.25 μm) of extracted *n*-alkanes demonstrated a strong odd over even peak distribution for all species, and major odd peaks were measured for D/H ratios. The D/H composition of the individual lipid compounds was determined by isotope ratio monitoring–gas chromatography/thermal conversion/mass spectrometry (irm–GC/TC/MS) (Hilkert et al. 1999) using a Thermo Finnigan MAT 253 mass spectrometer interfaced with a Thermo Finnigan high temperature conversion system (Pagani et al. 2006; Sessions et al. 1999). H_3 factors were calculated daily using H_2 reference gas. Three standards (*n*-C_{16} and *n*-C_{30} alkanes, and 5α-androstane; isotopic ratios were determined offline by A. Schimmelmann, Biogeochemical Laboratory at Indiana University) were co-injected to monitor the analytical accuracy; each sample was measured three times. The precision of isotopic measurements of H_2 reference gas after H_3 factor correction was 1‰ or better. The analytical errors for samples were less than 4‰. δD values are expressed relative to the VSMOW standard (Eq. 2), and the apparent hydrogen isotope fractionation factor ($\varepsilon_{lipid-water}$) between the source water and *n*-alkanes was calculated

Table 1 Carbon isotopic ratios of bulk leaf tissues and molecular D/H ratios of *n*-alkanes from living deciduous conifers (*Metasequoia*, *Taxodium*, and *Larix*) growing under diurnal light (DL) and continuous light (CL) and the calculated apparent hydrogen fractionation factors between lipid and water ($\varepsilon_{lipid-water}$)

	Metasequoia		*Larix*		*Taxodium*	
	$\delta^{13}C$ (‰)		$\delta^{13}C$ (‰)		$\delta^{13}C$ (‰)	
DL	−28.93 ± 0.15		−30.27 ± 0.17		−25.88 ± 0.05	
CL	−30.68 ± 0.08		−34.90 ± 0.10		−28.77 ± 0.05	
Difference	1.75 ± 0.11		4.63 ± 0.13		2.89 ± 0.05	
n-alkane	*Metasequoia*		*Larix*		*Taxodium*	
	δD (‰)	$\varepsilon_{lipid-water}$ (‰)	δD (‰)	$\varepsilon_{lipid-water}$ (‰)	δD (‰)	$\varepsilon_{lipid-water}$ (‰)
DL						
C_{25}	−154 ± 2.9	−95 ± 2.8	NA	NA	NA	NA
C_{27}	−164 ± 1.8	−105 ± 1.7	−164 ± 1.4	−105 ± 1.4	−155 ± 2.5	−96 ± 2.4
C_{29}	−166 ± 1.6	−105 ± 1.6	−165 ± 0.4	−106 ± 0.4	−159 ± 1.9	−100 ± 1.8
CL						
C_{25}	−126 ± 1.9	−64 ± 1.8	NA	NA	NA	NA
C_{27}	−124 ± 1.4	−62 ± 1.4	−138 ± 1.4	−77 ± 1.3	−135 ± 1.5	−74 ± 1.5
C_{29}	−124 ± 2.5	−63 ± 2.4	−148 ± 0.3	−87 ± 0.3	−127 ± 3.9	−66 ± 3.8

using Eq. 3. Source water used during the 2003–2004 growing season for greenhouse saplings was monitored using a Hydrogen Device (Finnigan, error less than 0.8‰).

$$\delta D = 1{,}000 \times [({}^2H/{}^1H_{sample})/({}^2H/{}^1H_{VSMOW} - 1)] \quad (2)$$

$$\varepsilon_{water\text{-}n\text{-}alkane} = 1{,}000 \times [(\delta D_{water} + 1{,}000)/(\delta D_{n-alkane} + 1{,}000) - 1] \quad (3)$$

Results

Leaf bulk carbon isotope fractionations and leaf intercellular CO_2 concentration under different light regimes

Leaf bulk $\delta^{13}C$ in these plants ranges from −25.88‰ (*Taxodium* DL) to −34.9‰ (*Larix* CL). Leaf bulk $\delta^{13}C$ from CL leaves consistently displayed more negative values than those from their DL counterparts for all three genera tested. The carbon isotope offset between the two light conditions ($\delta^{13}C_{CL}$–$\delta^{13}C_{DL}$) varied among species. The difference was up to 4.63‰ in *Larix*, but only 1.75‰ in *Metasequoia* (Table 1). We obtained average values of intercellular CO_2 concentration (C_i) of 335 ± 7 μL L^{-1} (CL) and 310 ± 8 μL L^{-1} (DL) for *Taxodium* and 336.8 ± 4 μL L^{-1}(CL) and 321.5 ± 7 μL L^{-1} (DL) for *Larix*. These values lead to a small but statistically significant ($P < 0.01$) higher ratio of the concentration of intercellular to atmospheric CO_2 (C_i/C_a) of 0.930 ± 0.02 and 0.933 ± 0.01 under CL treatment in comparison with 0.861 ± 0.02 and 0.891 ± 0.02 under DL conditions for *Taxodium* and *Larix*, respectively.

Lipid D/H, $\varepsilon_{water\text{-}n\text{-}alkane}$ values and leaf transpiration under different light regimes

For all three genera tested, *n*-alkanes from CL leaves displayed more positive D/H values than those from their DL counterparts. Leaf *n*-alkane δD in these plants ranges from −166‰ (*Metasequoia* C_{29} DL) to −124‰ (*Metasequoia* C_{29} CL). For both CL and DL leaves, *n*-alkanes with even carbon-chain length lipids were usually about 15–20‰ more D-enriched than those of odd-carbon chain lengths, a result similar to those obtained in other studies (Chikaraishi and Naraoka 2003; Liu et al. 2006; Yang and Huang 2003). The magnitude of the *n*-alkane D/H offset between CL and DL leaves varied slightly among different conifers. CL leaves were more D-enriched than their DL counterparts: in *Metasequoia* by +15 to 40‰, in *Larix* by +15 to 30‰, and in *Taxodium* by +20 to 30‰. D/H values of homologous *n*-alkanes between different genera varied up to 25‰ under the same conditions (either CL or DL). For a particular genus, D/H ratios in both C_{27} and C_{29} were similar, a result consistent with previous findings (Liu and Yang 2008; Liu et al. 2006; Smith and Freeman 2006). A one-way analysis of variance (ANOVA) test indicated that the differences in *n*-alkane δD in leaves grown under the two different light treatments were statistically significant ($P < 0.001$).

Depending upon the genus and individual compounds, calculated $\varepsilon_{lipid-water}$ values ranged from −62‰

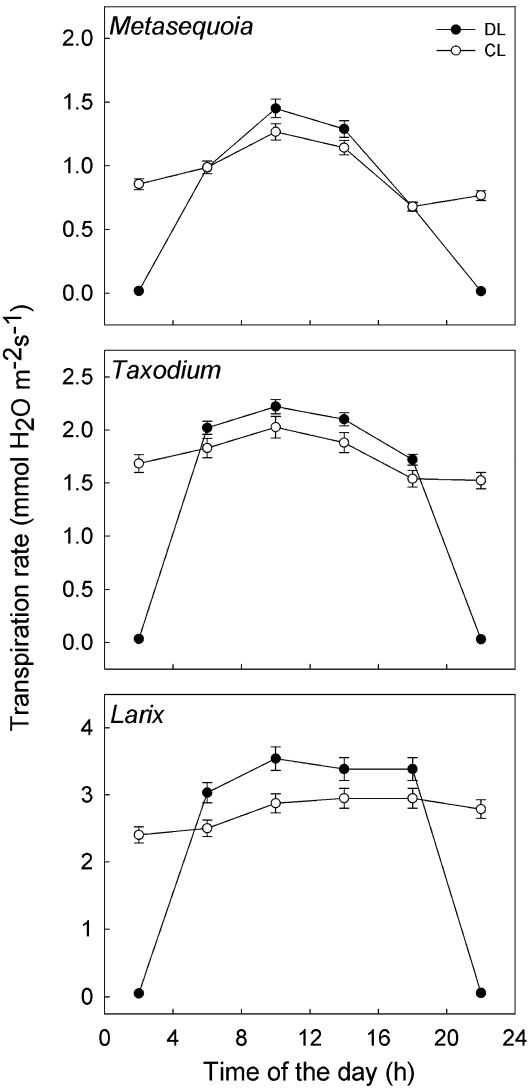

Fig. 1 Daily patterns of leaf transpiration rates in *Metasequoia*, *Taxodium*, and *Larix* saplings grown in continuous light (CL) or diurnal light (DL) treatments. *Bars* indicate standard errors

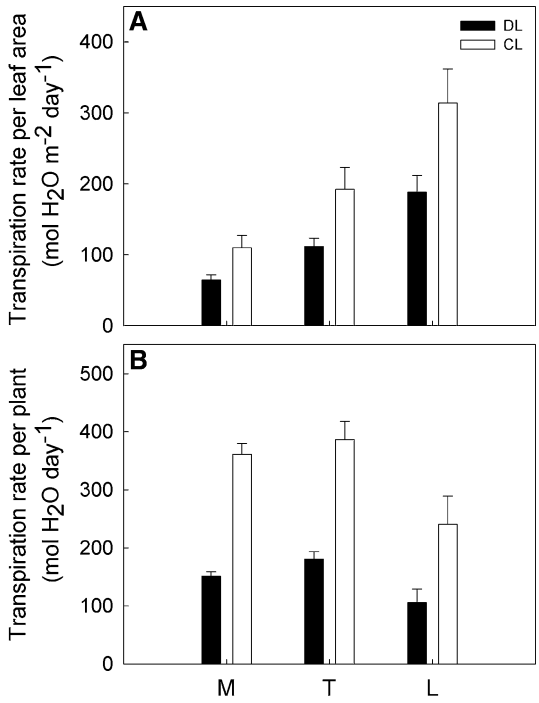

Fig. 2 Water loss under different light treatments (continuous light, CL, or diurnal light, DL) during a 24-h transpiration cycle for the three conifers calculated as per leaf area per day (**a**) and per plant per day (**b**). M *Metasequoia*, T *Taxodium*, and L *Larix*

(*Metasequoia* C_{27}) to −87‰ (*Larix* C_{29}) for CL leaves and −96‰ (*Taxodium* C_{27}) to −106‰ (*Larix* C_{29}) for DL leaves (Table 1). In all cases, the magnitude of $\varepsilon_{\text{lipid–water}}$ was smaller for leaves under CL conditions than for those under DL conditions, with *Metasequoia* under CL conditions yielding the smallest $\varepsilon_{\text{lipid–water}}$ (−62‰) among the three tested conifer genera.

The leaf instantaneous transpiration rates, expressed as mmol water loss per square meter leaf per second (mmol H_2O m^{-2} s^{-1}), were similar in the three tested genera. Transpiration rates were slightly higher in DL plants throughout the diurnal cycle (Fig. 1). However, CL leaves transpired at almost the same rate during the night as during the day when transpiration in DL leaves was shut down. Calculated water loss on per leaf unit area per day basis yields values 1.7 times higher in CL leaves than in DL leaves (Fig. 2a). Furthermore, because CL plants developed 1.2–1.4 times higher total leaf areas than DL plants (Equiza et al. 2006a), the difference in water loss per plant during 24 h was even greater. Figure 2b shows that, on a whole-plant basis, CL plants lost 2.1–2.4 times more water per day than DL plants (Fig. 2b). This calculation is obtained by multiplying daily transpiration rates (Fig. 1) measured as water loss per unit leaf area per day (Fig. 2a) by the total leaf area.

Hydrogen isotope compositions in fossil conifers and the reconstruction of D/H in Paleogene Arctic summer precipitation

Hydrogen isotope analysis of in situ *n*-alkanes extracted from the late Paleocene fossil *Metasequoia* leaves yielded *δD* values ranging from −246 to −257‰ (Table 2). D/H ratios of C_{25}, C_{27}, and C_{29} from the lower middle Eocene *Metasequoia* displayed similar values (−218 to −229‰) that were slightly more D-enriched than those from co-occurring *Larix* (−237 and −226‰). The seven *Metasequoia* samples from the 50 cm upper middle Eocene sequence yielded *n*-alkane *δD* values ranging from −237 to −251‰ (Table 3).

The hydrogen isotopic composition of late Paleocene precipitation averages −186‰, and that of early middle Eocene summer precipitation averages −156‰ (Fig. 3).

Table 2 The D/H ratios from in situ fossil *n*-alkanes (δD_{lipid}) and the calculated average δD of the summer precipitation (δD_{water}) during late Paleocene and middle Eocene

n-alkane	Middle Eocene				Late Paleocene	
	Metasequoia		*Larix*		*Metasequoia*	
	δD_{lipid} (‰)	δD_{water} (‰)	δD_{lipid} (‰)	δD_{water} (‰)	δD_{lipid} (‰)	δD_{water} (‰)
C_{25}	−229 ± 3.9	−154 ± 3.9	NA	NA	−257 ± 0.2	−182 ± 0.2
C_{27}	−218 ± 1.0	−155 ± 1.0	−237 ± 1.5	−159 ± 1.5	−252 ± 1.2	−190 ± 1.2
C_{29}	−222 ± 2.4	−159 ± 2.4	−226 ± 0.6	−154 ± 0.6	−246 ± 0.3	−184 ± 0.3
Average		−157		−156		−186
SD		2.5		2.6		3.3

SD Standard deviation

Table 3 The D/H ratios from in situ fossil *Metasequoia* *n*-alkanes and the calculated average δD of the summer precipitation during a 700-year sequence during the middle Eocene

n-alkane	BA-1097 δD_{lipid} (‰)	BA-897 δD_{lipid} (‰)	BA-797 δD_{lipid} (‰)	BA-697 δD_{lipid} (‰)	BA-597 δD_{lipid} (‰)	BA-497 δD_{lipid} (‰)	BA-397 δD_{lipid} (‰)
Stratigraphic sequence							
C_{27}	−248 ± 1.2	−242 ± 1.5	−251 ± 0.1	−249 ± 0.7	−240 ± 2.8	−250 ± 3.2	−242 ± 1.5
C_{29}	−247 ± 0.3	−240 ± 0.2	−249 ± 0.2	−249 ± 0.1	−237 ± 3.5	−243 ± 0.6	−238 ± 0.7
n-alkane	δD_{water} (‰)	δD_{water} (‰)	δD_{water} (‰)	δD_{water} (‰)	δD_{water} (‰)	δD_{water} (‰)	δD_{water} (‰)
Reconstructed D/H in ancient precipitation							
C_{27}	−185 ± 1.2	−179 ± 1.5	−189 ± 0.1	−187 ± 0.7	−178 ± 2.8	−188 ± 3.2	−180 ± 1.5
C_{29}	−184 ± 0.3	−178 ± 0.2	−187 ± 0.2	−187 ± 0.1	−174 ± 3.5	−181 ± 0.6	−176 ± 0.7
Average	−185	−179	−188	−187	−176	−184	−178
SD	0.67	1.36	1.48	0.18	2.8	5.16	2.9

SD Standard deviation

For the reconstruction of middle Eocene precipitation δD, *Metasequoia*- and *Larix*-specific hydrogen isotopic compositions yielded the same δD value (within technical errors) for summer precipitation at any particular time (−156 and −157‰, respectively). The time-series analysis of summer precipitation during the late middle Eocene, over a period of 313–625 years (Kojima et al. 1998), indicates that the δD of the precipitation ranged from −178 to −187‰ with a variation of less than 10‰ over 50–90 years (Table 3).

Discussion

The impact of low intensity continuous light on leaf carbon isotope compositions

In contrast to previous studies (e.g., Pearcy and Pfitsch 1991; Smith et al. 1976; Zimmerman and Ehleringer 1990), our experimental design examined both light intensity and the duration of illumination over 24-h periods. The 1.75‰ (*Metasequoia*) to 4.6 ‰ (*Larix*) depletion of $\delta^{13}C$ in leaf bulk tissues observed between DL and CL conditions with low light intensity are in agreement in both fractionation direction and magnitude with previous studies using low light illumination. More critically, they also mirror depletion levels in ^{13}C values in wood α-cellulose determined for *Metasequoia* and *Larix* between 45°N extant trees and 80°N Paleocene fossil logs. Jagels and Day (2004) found a 1.89‰ depletion for *Metasequoia* and a 3.1‰ depletion for *Larix*. Smith et al. (1976) documented that low light intensity resulted in ^{13}C-depletion of 1.8–4.6‰ in *Acacia farnesiana* (L.) Willd., and Zimmerman and Ehleringer (1990) reported a 4‰ negative shift in bulk carbon isotope ratios in leaves of *Catasetum viridiflavum* Hook under low light intensity compared with its high-light irradiation counterparts. As our experimental design maintained similar temperature, humidity, salinity, and both concentration and carbon isotope composition of source CO_2, the observed difference in leaf bulk $\delta^{13}C$ for a particular species under different treatments can be attributed to the impact of the quantity and quality of light.

Ehleringer et al. (1986) and Pearcy and Pfitsch (1991) proposed that the depletion of ^{13}C in plant tissues grown

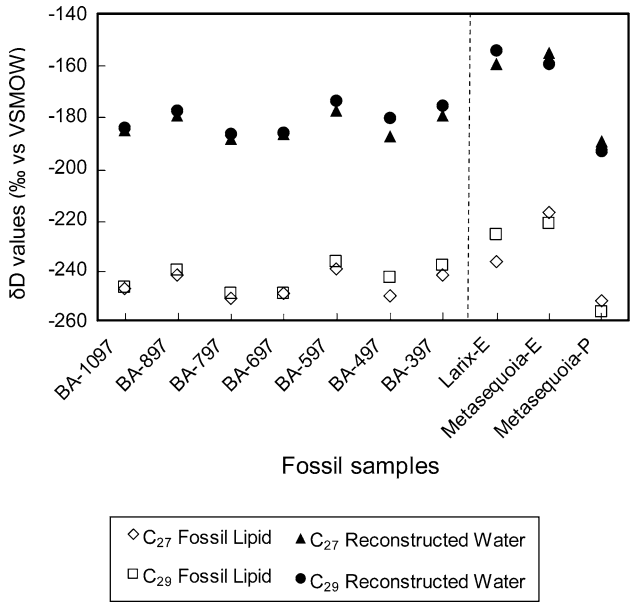

Fig. 3 D/H ratios of late Paleocene and middle Eocene summer precipitation from the High Arctic based on *n*-alkanes *δD* values obtained from fossil leaves of *Metasequoia* and *Larix*. Late Paleocene: *Metasequoia*-P; middle Eocene: *Larix*-E and *Metasequoia*-E; middle Eocene: BA-397-1097 (*Metasequoia* deposit sequence). *Dashed line* separates sequence samples from outcrop samples. *Open diamond and square symbols* measured molecular lipid *δD*, *solid circle and triangle symbols* reconstructed *δD* in ancient source water (per mil vs VSMOW)

under low light intensity was a result of leaf response to the increase in intercellular CO_2 pressure. The $\delta^{13}C$ value of a leaf depends upon three main factors (Farquhar et al. 1982, 1989): atmospheric CO_2 concentration (C_a), $\delta^{13}C$ value of the atmosphere, and the ratio of concentrations of intercellular (C_i) to atmospheric CO_2 (C_i/C_a). As both CL and DL treatments utilized the same source of CO_2, atmospheric CO_2 concentration ($C_a = 360 \pm 3$ μL L^{-1}) and $\delta^{13}C$ value of atmosphere are the same for CL and DL leaves. Thus, the observed depletion in ^{13}C in CL leaves can be attributed to a higher C_i/C_a ratio in these leaves compared to their DL counterparts. Indeed, previous leaf gas exchange measurements in *Metasequoia* indicated an increase in internal CO_2 concentrations (317 ± 2 μL L^{-1} under CL compared with 304 ± 8 μL L^{-1} under DL) (Equiza et al. 2006b), resulting in values of $C_i/C_a = 0.880 \pm 0.01$ under CL and $C_i/C_a = 0.844 \pm 0.02$ under DL treatments. In the present study we found that *Taxodium* and *Larix* follow the same trend, yielding C_i/C_a ratios with values that are significantly higher in CL leaves than in DL leaves. However, bulk $\delta^{13}C$ offsets between CL and DL leaves are larger than $\delta^{13}C$ differences calculated on the basis of changes in C_i/C_a ratios in models proposed by Farquhar et al. (1982, 1989). Limited measurements of intercellular CO_2 concentration (C_i) under different light conditions may not have captured the full range of C_i variation in the population. Alternatively, the larger bulk $\delta^{13}C$ offset between CL and DL conditions may be a result of intrinsic discrimination of $^{13}CO_2$ between intercellular spaces and the site of carboxylation due to photorespiration under CL conditions. Farquhar et al. (1982) discussed the impact of factors other than C_i/C_a ratios on plant bulk $\delta^{13}C$ but more experimental data are needed to understand fully how these factors influence plant $\delta^{13}C$ under CL conditions.

Hydrogen isotope fractionations factors under different light regimes

This investigation represents the first attempt to examine molecular hydrogen isotope fractionations under low-light intensity with 24-h continuous illumination. Our results clearly demonstrate that leaf wax *n*-alkanes from all three species under CL conditions were D-enriched by 15–40‰, depending upon molecular chain-length. Although the isotope response is by no means large, it falls within the range of *n*-alkane *δD* response to ecological changes (Liu and Yang 2008). We attribute the positive shift of D/H values in CL leaves to enhanced water loss during prolonged leaf transpiration under 24-h continuous light, resulting in D-enrichment of leaf tissue water that was used to synthesize lipid molecules. While detailed models of hydrogen isotope fractionation in leaves are still lacking, considerations of plant oxygen isotope fractionation during transpiration (recently reviewed in Farquhar et al. 2007; Barbour 2007) indicate that decreasing stomatal conductance and increasing the duration of transpiration will result in enrichment of heavy water at the sites of evaporation within leaves. This observation corresponds well with our measured leaf transpiration rate under CL treatment as well as calculated total water loss during a 24-h cycle. Although leaves under CL have a slightly lower transpiration rate during the day, they continue to transpire during the night (Fig. 1). This results in more net water loss per leaf area per day under CL conditions during the 24-h transpiration cycle than under the DL regime (Fig. 2a). Calculated water loss on a per plant basis shows that CL leaves lose 2.1–2.4 times more water than DL leaves during a 24-h cycle (Fig. 2b). Although this calculation is based upon an extrapolation of transpiration data obtained from the upper third of the canopy (Equiza et al. 2006a) to the entire foliage, we believe that such an estimate is reasonable, given the size of these young plants, as a basis for illustrating the effects of 24-h transpiration on CL leaves.

Our results indicate that the magnitude of $\varepsilon_{lipid-water}$ values in deciduous conifers grown under diurnal light (−96‰ for *Taxodium* C_{27}; −106‰ for *Larix* C_{29}) is similar to those reported for *n*-alkanes from modern C_3

deciduous trees in natural systems (Chikaraishi and Naraoka 2003; Sachse et al. 2006; Sessions et al. 1999; Smith and Freeman 2006). In contrast, continuous light conditions resulted in substantially smaller $\varepsilon_{\text{lipid–water}}$ values (from −62‰ in *Metasequoia* C_{27} to −87‰ in *Larix* C_{29}), reflecting greater evaporative D-enrichment of leaf water through transpiration. Chikaraishi and Naraoka (2003) showed values of −116 ± 13‰ for $\varepsilon_{\text{lipid–water}}$ in C_3 gymnosperms from Japan, and Sachse et al. (2006) reported an average $\varepsilon_{\text{lipid–water}}$ of −122‰ (ranging from −86 to −166‰) between August precipitation and mean *n*-alkane values for deciduous trees (both angiosperms and gymnosperms) in northern Europe. However, in previous studies the D/H composition of source water, required to estimate $\varepsilon_{\text{lipid–water}}$ values, lacked rigorous constraints or was extrapolated from on-line precipitation models (Bowen and Revenaugh 2003; Sachse et al. 2006). Chikaraishi and Naraoka (2003), for example, used an average δD of −42‰ in meteoric water to calculate $\varepsilon_{\text{lipid–water}}$ for *n*-alkanes from various higher plants living in Japan even though the D/H values of the meteoric waters at this site vary by more than 60‰ (−9.7 to −74‰) during the growing season.

Despite the reduced photosynthetic rate under CL conditions, Equiza et al. (2006a, 2007) observed higher foliar biomass production, as measured in leaf dry weight, in all three genera under CL compared with their DL counterparts. On a daily basis, under the CL treatment, the maintenance of a lower photosynthetic rate throughout the 24-h photoperiod resulted in a net higher carbon fixation per leaf area: the ratio of carbon fixed per leaf area per day in the CL regime to DL regime was estimated as 1.71, 1.54, and 1.43 mmol H_2O m^{-2} s^{-1} in *Metasequoia*, *Taxodium*, and *Larix*, respectively. Equiza et al. (2006a, b) posited that this apparent paradox in *Metasequoia* reflects an ability to minimize photosynthetic down-regulation by creating a carbon sink under CL conditions. However, more water is required to meet the demand of higher growth rates under CL conditions. While water supply is not limited and an efficient hydraulic system exists in *Metasequoia* (Jagels et al. 2003), a prolonged transpiration with more water loss during the 24-h cycle is a viable strategy to sustain carbon uptake and rapid redistribution during periods of high productivity. This may explain the large offset of hydrogen fractionation factors in *Metasequoia* between CL and DL conditions.

Although we acknowledge that these interpretations are based on a limited experimental dataset obtained from young plants growing in greenhouse conditions, our results provide the best available experimental information for interpreting hydrogen isotope signals obtained from fossil equivalents of these genera that grew under similar light conditions.

Implications for the interpretation of precipitation in the Paleogene High Arctic

Celebrated as "living fossils," *Metasequoia* and *Larix* are well known for their slow evolutionary rates and morphological stasis since the Paleogene (LePage and Basinger 1991; LePage et al. 2005; Yang and Jin 2000), and it is likely that their physiological characteristics were conserved through time (Jagels and Day 2004; Jagels et al. 2003). Therefore, genus-specific $\varepsilon_{\text{lipid–water}}$ determined under controlled conditions offers the best hydrogen fractionation factors for reconstructing the δD values of ancient source waters.

Modern day δD values of summer growing season precipitation for Ellesmere and Axel Heiberg Islands are estimated at approximately −172‰ (Ellesmere Island) and −165‰ (Axel Heiberg Island), and precipitation during the winter and early spring months is substantially more D-depleted (approximately −265‰ during December–March) (Bowen and Revenaugh 2003). Our results (late Paleocene: −186‰; early part of middle Eocene: −157‰; late part of middle Eocene: −178 to −187‰) (Fig. 3), based on the leaves of fossil *Metasequoia* and *Larix* recovered from the same region, indicate that the Paleocene–Eocene Arctic precipitation in this region was similar to or slightly D-depleted relative to modern values. *Metasequoia*- and *Larix*-specific hydrogen isotopic compositions yielded the same δD value for summer precipitation at any particular time and this, in conjunction with their genus-specific CL $\varepsilon_{\text{lipid–water}}$ values, strongly supports the reliability of our approach. These results, which are in agreement with a recent wood-cellulose based $\delta^{18}O$ study (Richter et al. 2008), are surprising in the light of the expectation that High Arctic precipitation should be more D-enriched during periods of global warming and reduced meridional temperature gradients (Pagani et al. 2006).

The isotope composition of modern high-latitude precipitation is largely a function of (1) isotopic distillation during progressive rainout, and (2) changes in air temperature and its effect on the magnitude of isotopic fractionation between vapor and liquid phases as moisture is transported north- and southward from tropical and subtropical regions (Bowen and Revenaugh 2003; Bowen and Wilkinson 2002). If the atmospheric circulation patterns in the Paleogene were similar to those today, a reduction in rainout events and generally warmer Arctic temperatures would have resulted in substantially D-enriched precipitation. The prevalence of D-depleted Arctic precipitation during a period in Earth's history when the polar regions were warm and humid suggests that different mechanisms of vapor transport and/or moisture sources

affected high-latitude summer precipitation during the Paleogene.

D-depleted Arctic precipitation during a period of Paleogene global warming can be explained by invoking much longer tropical vapor trajectories (Jahren and Sternberg 2002). However, such a scenario would greatly reduce moisture delivery to the north due to progressive rainout and could not support a wet Paleogene Arctic (Boyle 1997; Caballero and Langen 2005). Both paleobotanical (Greenwood and Basinger 1994) and isotope geochemical (Jahren and Sternberg 2003, 2008) evidence indicates a wet and humid Arctic during the Paleogene. Alternatively, it is possible that the source waters that supplied vapor to the Arctic were influenced by evapotranspiration and frequent fog events. The large vegetated landmass (Jahren 2007), high productivity (Williams et al. 2003), and high marine water to land area ratio (Brinkhuis et al. 2006), combined with the warm air temperature conditions (Greenwood and Basinger 1994; Sluijs et al. 2006), would have promoted frequent advective fogs and high rates of transpiration in the Paleogene High Arctic, generated by the extensive deciduous conifer forests growing under CL conditions. Prolonged 24-h transpiration would have enhanced convective precipitation (Brubaker et al. 1993), creating high relative humidity (Jahren and Sternberg 2003, 2008), while evaporation from large water bodies (Brinkhuis et al. 2006) would have promoted additional contributions of D-depleted water in the form of fog and rain. Conifers are particularly well adapted to scavenging water from fog (Dawson 1998). Water input to the forests resulting from these processes would have been substantially D-depleted relative to precipitation transported from the tropics and sub-tropics, making the δD value of the regional precipitation more negative than if sourced from low-latitude moisture alone. This alternative explanation integrates the role of 24-h transpiration during the summer months in the extensive polar deciduous forests and reconciles the documented paleoenvironmental conditions with the D-depleted hydrogen isotope signatures obtained from in situ biomolecules.

Conclusion

Our study demonstrates that low intensity, 24-h continuous light conditions impact both the bulk $\delta^{13}C$ of leaf tissues and the molecular δD of leaf *n*-alkanes in these deciduous conifers. The opposing shifts in bulk carbon and molecular hydrogen isotope fractionations under continuous light conditions compared to their diurnal light counterparts can be explained physiologically by the change of carbon isotope discrimination and water loss during 24-h photosynthesis and transpiration. The empirically determined apparent hydrogen isotope fractionations between source water and individual lipids have critical implications for reconstructing the hydrogen isotope compositions of ancient source water in the polar area using in situ *n*-alkanes from well-preserved Paleogene deciduous conifer fossils from the Arctic region.

Acknowledgments This project was funded in part by the CAS/SAFEA International Partnership Program for Creative Research Teams, the Pilot Project of Knowledge Innovation, CAS (KZCX2-YW-105), the Major Basic Research Projects (2006CB806400), the National Science Foundation of China (40402002 and 40872011), the American Chemical Society Petroleum Research Funds, and Bryant University Summer Research Fund. Most of the work presented in this paper was carried out during H.Y.'s sabbatical year at Yale University with M.P. and D.E.G.B. We thank Jingfeng Wang (MIT) for discussion of the recycling model, Liang Xiao (Bryant University) for preparation of diagrams, and Gerard Olack (Yale University) for technical supports. This paper is the contribution 200901 for the Laboratory of Terrestrial Environment of Bryant University. We declare that the experiments comply with the current laws of the United States in which the study was performed.

References

Barbour MM (2007) Stable oxygen isotope composition of plant tissue: a review. Funct Plant Biol 34:83–94

Bowen GJ, Revenaugh J (2003) Interpolating the isotopic composition of modern meteoric precipitation. Water Resour Res 39:1299

Bowen GJ, Wilkinson B (2002) Spatial distribution of $\delta^{18}O$ in meteoric precipitation. Geology 30:315–318

Bowen GJ, Beerling DJ, Koch PL, Zachos JC, Quattlebaum T (2004) A humid climate state during the Palaeocene/Eocene thermal maximum. Nature 432:495–499

Boyle EA (1997) Cool tropical temperatures shift the global $\delta^{18}O$–T relationship: an explanation for the Ice Core $\delta^{18}O$/borehole thermometry conflict? Geophys Res Lett 24:273–276

Brinkhuis H et al (2006) Episodic fresh surface waters in the Eocene Arctic Ocean. Nature 441:606–609

Brubaker KL, Entekhabi D, Eagleson PS (1993) Estimation of continental precipitation recycling. J Clim 6:1077–1089

Caballero R, Langen PL (2005) The dynamic range of poleward energy transport in an atmospheric general circulation model. Geophys Res Lett 32:L02705. doi:02710.01029/02004GL021581

Chikaraishi Y, Naraoka H (2003) Compound-specific δD-$\delta^{13}C$ analyses of *n*-alkanes extracted from terrestrial and aquatic plants. Phytochemistry 63:361–371

Christie RL (1988) Field studies of "fossil forest" sites in the Arctic Islands. Geol Surv Can Pap 88-1D:57–60

Dawson TE (1998) Fog in the California redwood forest: ecosystem inputs and use by plants. Oecologia 117:476–485

Eberle J, Storer JE (1999) Northernmost record of brontotheres, Axel Heiberg Island, Canada-implications for age of the Buchanan Lake Formation and brontothere paleobiology. J Paleontol 73:979–983

Ehleringer JR, Field CB, Lin Z-F, Kuo C-Y (1986) Leaf carbon isotope and mineral composition in subtropical plants along an irradiance cline. Oecologia 70:520–526

Equiza MA, Day ME, Jagels R, Li XC (2006a) Photosynthetic downregulation in the conifer *Metasequoia glyptostroboides* growing under continuous light: the significance of carbohydrate sinks and paleoecophysiological implications. Can J Bot 84:1453–1461

Equiza MA, Day ME, Jagels R (2006b) Physiological responses of three deciduous conifers (*Metasequoia glyptostroboides*, *Taxodium distichum* and *Larix laricina*) to continuous light: adaptive implications for the early Tertiary polar summer. Tree Physiol 26:353–364

Equiza MA, Jagels R, Cirelli D (2007) Differential carbon allocation in *Metasequoia glyptostroboides*, *Taxodium distichum* and *Sequoia sempervirens* growing under continuous light. Bull Peabody Mus Nat Hist 48:269–280

Farquhar GD, O'Leary MH, Berry JA (1982) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. Aust J Plant Physiol 9:121–137

Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. Ann Rev Plant Physiol Plant Mol Biol 40:503–537

Farquhar GD, Cernusak LA, Barnes B (2007) Heavy water fractionation during transpiration. Plant Physiol 143:11–18

Greenwood DR, Basinger JF (1994) The paleoecology of high-latitude Eocene swamp forests from Axel-Heiberg Island, Canadian High Arctic. Rev Palaeobot Palynol 81:83–97

Hilkert AW, Douthitt CB, Schlüter HJ, Brand WA (1999) Isotope ratio monitoring gas chromatography/mass spectrometry of D/H by high temperature conversion isotope ratio mass spectrometry. Rapid Commun Mass Spectrom 13:1226–1230

Hou J-Z, D'Andrea WJ, MacDonald D, Huang Y-S (2007) Hydrogen isotopic variability in leaf waxes among terrestrial and aquatic plants around Blood Pond, Massachusetts (USA). Org Geochem 38:977–984

Irving E, Wynne PJ (1991) The paleolatitude of the Eocene fossil forests of Arctic Canada. Geol Surv Can Bull 403:209–211

Jagels R, Day ME (2004) The adaptive physiology of *Metasequoia* to Eocene high-latitude environment. In: Hemsley AR, Poole I (eds) The evolution of plant physiology. Elsevier, Boston, MA, pp 401–425

Jagels R, Visscher GE, Lucas J, Goodell B (2003) Palaeo-adaptive properties of the xylem of *Metasequoia*: mechanical/hydraulic compromises. Ann Bot 92:79–88

Jahren AH (2007) The Arctic forest of the middle Eocene. Annu Rev Earth Planet Sci 35:509–540

Jahren AH, Sternberg LSL (2002) Eocene meridional weather patterns reflected in the oxygen isotopes of Arctic fossil wood. GSA Today 12:4–9

Jahren AH, Sternberg LSL (2003) Humidity estimate for the middle Eocene Arctic rain forest. Geology 31:463–466

Jahren AH, Sternberg LSL (2008) Annual patterns within tree rings of the Arctic middle Eocene (ca. 45 Ma): isotopic signatures of precipitation, relative humidity, and deciduousness. Geology 36:99–102

Kojima S, Sweda T, LePage BA, Basinger JF (1998) A new method to estimate accumulation rates of lignites in the Eocene Buchanan Lake Formation, Canadian Arctic. Palaeogeogr Palaeoclimatol Palaeoecol 141:115–122

LePage BA, Basinger JF (1991) Early Tertiary *Larix* from the Buchanan Lake Formation, Canadian Arctic Archipelago, and a consideration of the phytogeography of the genus. Geol Surv Can Bull 403:67–82

LePage BA, Yang H, Matsumoto M (2005) The evolution and biogeographic history of *Metasequoia*. In: LePage BA, Williams CJ, Yang H (eds) The geobiology and ecology of *Metasequoia*. Springer, Dordrecht, pp 3–114

Liu W-G, Yang H (2008) Multiple controls for the variability of hydrogen isotopic compositions in higher plant *n*-alkanes from modern ecosystems. Glob Chang Biol 14:2166–2177

Liu W-G, Yang H, Li L-W (2006) Hydrogen isotopic compositions of *n*-alkanes from terrestrial plants correlate with their ecological life forms. Oecologia 150:330–338

Lowenstein TK, Demicco RV (2006) Elevated Eocene atmospheric CO_2 and its subsequent decline. Science 313:1928

McIver EE, Basinger JF (1999) Early Tertiary floral evolution in the Canadian High Arctic. Ann Mo Bot Gard 86:523–545

Ögren E, Sundin U (1996) Photosynthetic responses to variable light: a comparison of species from contrasting habitats. Oecologia 106:18–27

Osborne CP, Beerling DJ (2003) The penalty of a long, hot summer. Photosynthetic acclimation to high CO_2 and continuous light in "living fossil" conifers. Plant Physiol 133:803–812

Osborne CP, Royer DL, Beerling DJ (2004) Adaptive role of leaf habit in extinct polar forests. Int For Rev 6:181–186

Pagani M et al (2006) Arctic hydrology during global warming at the Palaeocene/Eocene thermal maximum. Nature 442:671–675

Pearcy RW, Pfitsch WA (1991) Influence of sunflecks on the $\delta^{13}C$ of *Adenocaulon bicolor* plants occurring in contrasting forest understory microsites. Oecologia 86:457–462

Pearson PN, Palmer MR (2000) Atmospheric carbon dioxide concentrations over the past 60 million years. Nature 406:695–699

Richter SL, Johnson AH, Dranoff MM, LePage BA, Williams CJ (2008) Oxygen isotope ratios in fossil wood cellulose: isotopic composition of Eocene- to Holocene-aged cellulose. Geochim Cosmochim Acta 72:2744–2753

Ricketts BD, McIntyre DJ (1986) The Eureka Sound Group of eastern Axel Heiberg Island: new data on the Eurekan Orogeny. Geol Surv Can Pap 86-1B:405–410

Ricketts BD, Stephenson RA (1994) The demise of Sverdrup Basin: late Cretaceous–Paleogene sequence stratigraphy and forward modeling. J Sediment Res B64:516–530

Sachse D, Radke J, Gleixner G (2006) δD values of individual *n*-alkanes from terrestrial plants along a climatic gradient—implications for the sedimentary biomarker record. Org Geochem 37:469–483

Sessions AL, Burgoyne TW, Schimmelman A (1999) Fractionation of hydrogen isotope in lipid biosynthesis. Org Geochem 30:1193–1200

Sluijs A et al (2006) Subtropical Arctic Ocean temperatures during the Palaeocene/Eocene thermal maximum. Nature 441:610–613

Smith FA, Freeman KH (2006) Influence of physiology and climate on δD of leaf wax *n*-alkanes from C_3 and C_4 grasses. Geochim Cosmochim Acta 70:1172–1187

Smith BN, Oliver J, McMillan C (1976) Influence of carbon source, oxygen concentration, light intensity, and temperature on $^{13}C/^{12}C$ ratios in plant tissues. Bot Gaz 137:99–104

Williams CJ, Johnson AH, LePage BA, Vann DR, Sweda T (2003) Reconstruction of Tertiary *Metasequoia* forests. II. Structure, biomass, and productivity of Eocene floodplain forests in the Canadian Arctic. Paleobiology 29:271–292

Yang H, Huang Y-S (2003) Preservation of lipid hydrogen isotope ratios in Miocene lacustrine sediments and plant fossils at Clarkia, northern Idaho, USA. Org Geochem 34:413–423

Yang H, Jin J-H (2000) Phytogeographic history and evolutionary stasis of *Metasequoia*: geological and genetic information contrasted. Acta Palaeontol Sin 39(suppl):288–307

Yang H, Huang Y-S, Leng Q, LePage BA, Williams CJ (2005) Biomolecular preservation of Tertiary *Metasequoia* fossil *lagerstätten* revealed by comparative pyrolysis analysis. Rev Palaeobot Palynol 134:237–256

Zimmerman JK, Ehleringer JR (1990) Carbon isotope ratios are correlated with irradiance levels in the Panamanian orchid *Catasetum viridiflavum*. Oecologia 83:247–249