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Stable isotope variations from cultivated *Metasequoia* trees in the United States: A statistical approach to assess isotope signatures as climate signals

Abstract We measured bulk $\delta^{13}C$ and $\delta^{15}N$ values and carbon and nitrogen elemental concentrations of leaves collected from *Metasequoia glyptostroboides* Hu et Cheng trees cultivated at 39 sites across the United States under different latitudes and climatic regions. δD values from south-facing leaf *n*-alkanes of 27 trees were also determined. Climate data over the past 50 years (1950–2009) were compiled from stations near each site. Isotope data were cross plotted against each geographic and climatic parameter, including latitude, annual mean temperature (AMT), spring (February–May) mean temperature (SMT), annual mean precipitation (AMP), and spring mean precipitation (SMP). Statistical analyses revealed the following significant correlations: 1) a strong negative correlation between *n*-alkane δD and latitude; 2) statistically significant correlations between δD and both AMT and SMT; 3) a weaker but still significant correlation between δD and SMP; 4) statistically significant relationships between carbon concentration and both temperature and precipitation parameters, especially AMP; 5) an unexpected correlation between nitrogen concentration and SMP. These results bear strong implications for using $\delta^{13}C$ and δD values obtained from fossil *Metasequoia* as paleoclimatic and paleoenvironmental proxies.

Keywords: climate, cultivated *Metasequoia glyptostroboides* tree, stable isotope, statistics, United States

Introduction

Ever since the establishment of the fossil conifer genus *Metasequoia* Miki from a Cenozoic deposit in Japan (Miki, 1941) and the subsequent discovery of its living species, *M. glyptostroboides* Hu et Cheng, in south-central China (Hu & Cheng, 1948), the study of *Metasequoia* has attracted both neo- and paleobotanists for the past 70 years (for updated summaries see LePage et al., 2005a; Yang & Hickey, 2007). Elected as the "Tree of the 20th Century" by the Arnold Arboretum of Harvard University in 1999 (Yang, 1999), *M. glyptostroboides* trees have been widely cultivated around the globe, including a systemic seed dissimilation and germination across the United States as a result of a collaborative effort to protect this treasured species from extinction by Chinese and American scientists (Hu, 1948; Kuser et al., 1997; Merrill, 1948). Shortly after the discovery of the living species in China, seeds were collected from Moudao Town, where the type tree of the species is located, and the nearby Shui-shan Ba (the *Metasequoia* Valley, where the largest grove of *M. glyptostroboides* trees are located) in 1947 and then were redistributed to different botanical gardens and university campuses across the United States for plantation through the Arnold Arboretum of Harvard University and the University of California, Berkeley (Ma, 2003, 2007). Later, especially after 1979, more seeds collected from Hubei Province, China were brought over to the United States, resulting *M. glyptostroboides* being one of the most popular conifers in American botanical gardens and university campuses ranging from Alaska to Florida.

As a living fossil, the biology of *M. glyptostroboides* has been extensively studied. However, there is a lack of systematic research on its stable carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), and hydrogen (δD) isotope contents. As terrestrial plants fix their carbon and hydrogen by ultimately utilizing C from atmospheric CO_2 and H from environmental H_2O respectively, both $\delta^{13}C$ and δD values determined in plant tissues should provide an effective means of reconstructing various environmental conditions (Fig. 1). Meanwhile, the nitrogen isotope

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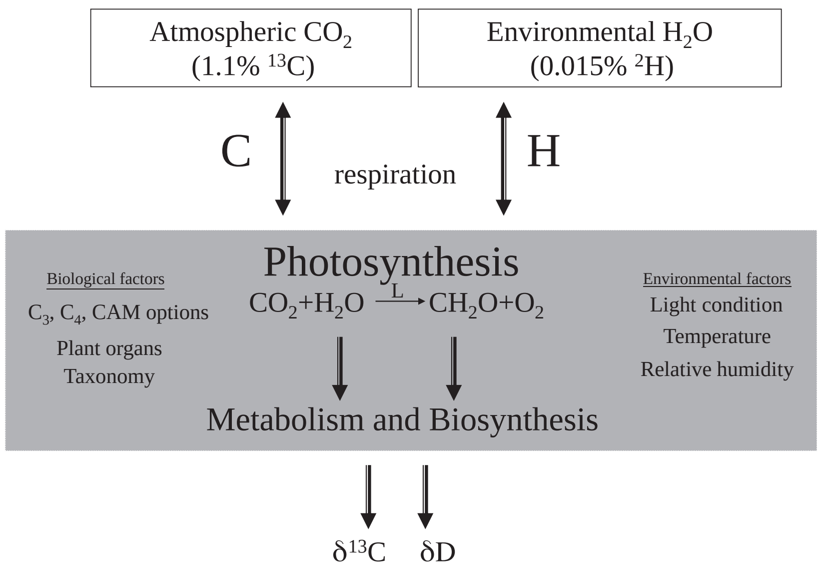

Fig. 1 Schematic diagram showing the incorporation of carbon and hydrogen into plant tissues during photosynthesis and major controlling factors for carbon and hydrogen isotope fractionations. L: light.

composition should reflect its nutrient level and provides insightful information about its growth. In addition, both $\delta^{13}C$ and δD values from *Metasequoia* fossil leaves have offered important clues for understanding paleoecology and paleophysiology of *Metasequoia* and have provided a powerful tool for paleoenvironmental and paleoclimatic interpretations (e.g., Jahren & Sternberg, 2003; Yang et al., 2009).

The $\delta^{13}C$ value of plant leaf tissues depends upon two main factors during carbon fixation (Farquhar et al., 1989): $\delta^{13}C$ value of the atmosphere and the ratio of concentrations of intercellular (C_i) to atmospheric CO_2 (C_i/C_a). As CO_2 concentration and $\delta^{13}C$ value of atmosphere are not significantly different at various open air sites for cultivated *M. glyptostroboides* trees in the United States, the change in $\delta^{13}C$ values from different trees may be mainly attributed to the change of C_i/C_a under various climatic (such as temperature and precipitation) and environmental (such as leaf aspects) conditions. Despite previous efforts examining the relationship between plant $\delta^{13}C$ and climatic parameters (i.e., Kloeppel et al., 1998), such an investigation on carbon isotope using a single species with known genetic source was rarely conducted.

Only recently, the development of online isotope ratio mass spectrometer (IRMS) made it possible to accurately measure molecular hydrogen isotope ratios (δD) from plant waxes (Yang & Leng, 2009). Our understanding of the distribution of molecular hydrogen isotope from plant leaves have just begun, and data concerning δD from leaf waxes from a single species across different latitudes were previously unavailable. Early studies of δD values of living plants focused on δD variation from various types of plants (Bi et al., 2005; Chikaraishi & Naraoka, 2003; Sessions et al., 1999; Yang & Huang, 2003), but subsequent studies have explored the relationship between *n*-alkane δD values and various environmental factors such as seasonality (Sachse et al., 2009; Sessions, 2006), plant ecological life forms (Liu et al., 2006), rooting patterns (Krull et al., 2006), and climate gradients (Duan & Wu, 2009; Sachse et al., 2006). Using the available global *n*-alkane δD record, Liu & Yang (2008) identified and ranked multiple controlling factors and their impacts on the variation of δD of higher plant leaf waxes. However, molecular hydrogen isotope signatures and variations of δD within a single species across different geographic and climatic gradient remain unknown. Thus, a systematic approach examining the relationship between *M. glyptostroboides* $\delta^{13}C$ and δD values and various climatic parameters would provide further insights into the understanding of climate-driven isotope changes in *Metasequoia* leaf tissues and their potential applications as proxies for the reconstruction of paleoclimate.

Metasequoia glyptostroboides trees cultivated in the United States provide several advantages for this kind of study. First, cultivated *M. glyptostroboides* trees in the United States were planted under various climate conditions over the past 60 years, stretching more than 30 degrees of latitudes with mean annual temperatures ranging from 5–20 °C. These trees offer unique plant material as a natural climatic archive that recorded long-term climate signals. Second, as the seed sources of these trees are from a narrow geographic range in China (Leng et al., 2007), and the species has a rather low genetic diversity (Li et al., 1999, 2003), stable isotopic variations from these cultivated trees are likely solely caused by environmental factors. Third, detailed climatic parameters in the United States for the past 50 years are readily available from stations near the growing sites of these *M. glyptostroboides* trees, providing critical data for investigating the relationships between stable isotope signals and long-term climatic parameters.

For this study, we determine carbon, nitrogen, and hydrogen isotope compositions of leaves collected from cultivated *M. glyptostroboides* trees across the United States and investigate the relationships between these isotope values and various climatic parameters using a statistical approach. First, we present bulk carbon and nitrogen as well as compound-specific hydrogen isotope compositions from these cultivated *Metasequoia* trees. Elemental concentrations for both carbon and nitrogen are also measured. Second, we compile 50 years' climatic data at or near the growing sites and perform statistical analyses of the relationships between climatic parameters and stable isotope and elemental concentration data. Finally, we evaluate the registration of climatic signals as isotope signatures in these leaf tissues and discuss the source of variations of the stable isotope signals. This investigation represents the first comprehensive examination on various stable isotope values in trees of a single species with a narrow genetic source but growing in various climatic and environmental conditions. Climatic signals registered as stable isotopic values revealed by this study bear significance on paleoclimatic and paleoenvironmental interpretations of isotopic signals obtained from fossil *Metasequoia* tissues.

Material and methods

1. Leaf samples from *Metasequoia glyptostroboides* trees cultivated in the United States

Leaf samples were collected from *M. glyptostroboides* trees cultivated at 39 sites across the United States, ranging (from north to south) from Alaska (Lat 57.03°N) to Florida (Lat 29.64°N) in the summer of 2004 (Fig. 2). In addition, one sample (M-2) was collected from a cultivated tree in Uppsala University Botanical Garden in Sweden for comparison. Most of these trees were grown from the initial batch of seeds collected in 1947 from Moudao Town and the *Metasequoia* Valley (Table 1). Leaf samples were all taken from branches 1.5–2 m above the ground. While available, well defined sun leaves (south-facing leaves exposed directly to sunlight) and shade leaves (north-facing leaves under the shade) were collected from the same tree. Generally, multiple samples were collected from different individual trees grown at the same location while available. Two samples were analyzed for carbon and nitrogen isotopes, and standard deviations are reported among measurements of multiple samples. Upon collecting, leaf samples were kept in low temperature during transportation and stored in -20 °C refrigeration in the Laboratory of Terrestrial Environment at Bryant University until analyzed.

Fig. 2 Geographic map of the United States showing locations of sampled *Metasequoia glyptostroboides* cultivated trees selected for this study (except for M-80 sampled from the Japonski Island of Alaska and M-2 collected from the Uppsala University Botanical Garden in Sweden).

2. Stable isotope and elemental analyses

Leaf samples were cleaned with distilled water to remove dust particles, freeze-dried, crushed to fine powders, and extracted with dichloromethane. About 5 µg of lipid-free extracts were analyzed for carbon and nitrogen isotope measurements 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. Carbon and nitrogen elemental abundances were also measured simultaneously. Samples were analyzed in duplicate, and standard deviations of replicate samples were 0.1‰ for $\delta^{13}C$ and 0.2‰ for $\delta^{15}N$ respectively. Carbon isotopic composition ($\delta^{13}C$) is expressed relative to the Belemnites of the Peedee Formation (PDB standard) defined by the relationship in Equation 1, and nitrogen isotopic com-

Table 1 Data of *Metasequoia glyptostroboides* leaf samples used in this study, including sample number (while both shade/north-facing leaves and sun/south-facing leaves are used, the former is marked with “N” while the later as “S”), location, state, latitude (Lat., °N), longitude (Long., °E), leaf bulk carbon isotope composition ($\delta^{13}C$, ‰ PDB), carbon isotope offset between shade leaf and sun leaf ($\Delta^{13}C_{n\text{-}s}$, ‰ PDB), leaf carbon content (C%), leaf bulk nitrogen isotope composition ($\delta^{15}N$, ‰ N_{atm}), leaf nitrogen content (N%), and hydrogen isotope composition of leaf *n*-alkene (δD, ‰ VSMOW), an amount-weighted mean δD value of D/H ratio values from C_{27}, C_{29} and C_{31} *n*-alkanes. Measurements are followed by standard deviation (σ).

Sample	Location	State	Lat.	Long.	$\delta^{13}C$	σ	$\Delta^{13}C_{n\text{-}s}$	C%	σ	$\delta^{15}N$	σ	N%	σ	δD	σ
M-23N	Arnold Arboretum, Jamacia Plain	MA	42.60	71.20	-29.19	0.08		43.70	0.20	3.78	0.03	2.45	0.04		
M-23S	Arnold Arboretum, Jamacia Plain	MA	42.60	71.20	-27.85	0.05	1.34	45.52	0.11	6.04	0.01	2.10	0.00	-152	1.1
M-29N	Thuja Garden, Northwest Harbor	ME	44.17	68.16	-27.79	0.15		47.64	0.33	1.44	0.15	2.08	0.05		
M-29S	Thuja Garden, Northwest Harbor	ME	44.17	68.16	-27.29	0.04	0.50	48.60	0.07	3.86	0.04	2.06	0.02	-133	2.1
M-31S	South Portland	ME	43.63	70.28	-27.87	0.06		41.22	3.74	11.61	0.27	2.33	0.26		
M-60N	Bailey Arboretum, Locust Valley	NY	40.53	73.35	-28.96	0.14		47.67	0.76	2.81	0.14	2.30	0.02		
M-60S	Bailey Arboretum, Locust Valley	NY	40.53	73.35	-28.67	0.04	0.29	47.84	0.05	3.19	0.04	2.38	0.03	-145	3.2
M-61	Rutgers Garden	NJ	40.28	74.25	-28.19	0.10		45.04	0.96	-0.51	0.08	2.24	0.01	-138	4.1
M-62N	Winterthur Gardens, Winterthur	DE	39.48	75.35	-28.43	0.05		47.18	0.13	1.73	0.05	2.05	0.02		
M-62S	Winterthur Gardens, Winterthur	DE	39.48	75.35	-28.81	0.08	-0.38	47.22	0.15	2.65	0.03	2.28	0.01	-143	1.3
M-63S	National Arboretum, Washington DC	DC	38.54	76.57	-29.84	0.00		47.13	0.21	1.87	0.00	2.41	0.09	-140	1.6
M-64N	Longwood Gardens, Kennett Square	PA	39.52	75.40	-27.83	0.12		47.21	0.10	-0.78	0.04	2.61	0.00		
M-64S	Longwood Gardens, Kennett Square	PA	39.52	75.40	-27.80	0.05	0.03	47.94	0.05	-0.49	0.04	2.97	0.11	-150	3.2
M-65	Brooklyn Botanical Garden	NY	40.39	73.57	-29.11	0.00		47.68	0.01	1.88	0.01	2.18	0.06	-154	2.2
M-66N	Broadmead, Princeton Boro	NJ	40.20	74.38	-27.80	0.09		43.39	3.81	3.82	0.02	2.10	0.15		
M-66S	Broadmead, Princeton Boro	NJ	40.20	74.38	-27.46	0.12	0.34	46.62	0.38	4.57	0.03	2.23	0.03		
M-67S	Princeton University	NJ	40.20	74.38	-28.59	0.06		46.71	0.01	3.24	0.00	2.60	0.08	-150	2.2
M-68N	Morris Arboretum, Philadelphia	PA	39.97	75.16	-28.84	0.02		44.93	0.06	4.38	0.01	2.78	0.02		
M-68S	Morris Arboretum, Philadelphia	PA	39.97	75.16	-28.86	0.02	-0.02	46.98	0.07	5.24	0.09	2.46	0.03	-142	1.7
M-69N	University of Florida, Gainesville	FL	29.64	82.35	-28.91	0.04		48.15	0.11	1.63	0.02	2.47	0.09		
M-69S	University of Florida, Gainesville	FL	29.64	82.35	-28.48	0.10	0.43	47.15	0.24	2.50	0.04	2.32	0.01	-130	4.1
M-70N	Cave Hill Cemetarym, Louisville	KY	38.20	85.80	-27.41	0.04		49.75	0.04	1.95	0.02	1.80	0.05		
M-70S	Cave Hill Cemetarym, Louisville	KY	38.20	85.80	-27.12	0.05	0.29	48.46	0.08	3.99	0.03	2.32	0.03	-164	2.2
M-71N	249 Narragansett Bay, Warwick	RI	41.40	71.22	-27.48	0.07		47.09	0.36	3.71	0.04	2.67	0.07		
M-71S	249 Narragansett Bay, Warwick	RI	41.40	71.22	-27.57	0.17	-0.09	46.54	0.49	3.38	0.01	1.80	0.11	-148	3.3
M-72	Connecticut College	CT	41.35	72.10	-29.01	0.21		47.84	0.34	1.82	0.03	2.21	0.07	-143	1.1
M-73	Duke University Durham	NC	36.00	78.56	-27.42	0.00		45.06	0.27	3.41	0.03	1.94	0.04	-142	4.1
M-74	Tulane University, New Orleans	LA	29.95	90.12	-28.80	0.11		45.64	0.40	1.04	0.11	2.04	0.06		
M-75	Dawes Arboretum, Newark	OH	40.20	82.30	-26.67	0.16		46.49	0.16	5.86	0.13	1.87	0.05		
M-76	Secrest Arboretum, Wooster	OH	40.80	81.97	-28.37	0.00		46.16	0.07	0.51	0.01	2.55	0.02	-164	2.7
M-77N	Bernheim Forest Arboretum	KY	38.00	85.80	-28.03	0.04		48.04	0.04	0.22	0.01	1.94	0.10		
M-77S	Bernheim Forest Arboretum	KY	38.00	85.80	-27.21	0.05	0.82	46.91	0.18	0.77	0.01	2.23	0.04	-156	2.2
M-78N	Hoyt Arboretum, Portland	OR	45.50	122.60	-29.39	0.02		46.62	0.24	-3.44	0.03	2.05	0.00		
M-78S	Hoyt Arboretum, Portland	OR	45.50	122.60	-28.88	0.02	0.51	44.16	0.29	-3.02	0.03	2.32	0.01		
M-79N	Biltmore House, Asheville	NC	35.50	82.70	-28.35	0.03		46.08	0.05	1.00	0.01	2.74	0.01		
M-79S	Biltmore House, Asheville	NC	35.50	82.70	-28.50	0.02	-0.15	46.61	0.22	1.07	0.05	2.73	0.02	-137	3.3
M-80N	Japonski Island, Sitka	AK	57.03	135.21	-28.24	0.01		45.83	2.08	0.91	0.01	2.15	0.10		
M-80S	Japonski Island, Sitka	AK	57.03	135.21	-26.40	0.09	1.84	48.30	0.65	1.30	0.09	2.04	0.00	-173	2.9
M-81N	Smith College, Northampton	MA	42.50	73.80	-28.42	0.13		45.49	0.17	3.18	0.02	2.26	0.09		
M-81S	Smith College, Northampton	MA	42.50	73.80	-27.12	0.03	1.30	44.76	3.39	2.02	0.03	2.45	0.04		
M-82N	Coker College	SC	33.43	80.08	-28.90	0.25		46.31	1.55	0.63	0.25	1.91	0.10		
M-82S	Coker College	SC	33.43	80.08	-27.37	0.06	1.53	49.97	0.08	0.39	0.06	2.02	0.02		
M-83	Mount Auburn Cemetary, Cambridge	MA	42.60	71.20	-27.31	0.17		48.04	0.11	2.04	0.00	2.01	0.04		
M-84N	Marsh Garden, New Haven	CT	41.19	72.55	-28.79	0.03		46.35	0.02	0.88	0.02	1.71	0.06		
M-84S	Marsh Garden, New Haven	CT	41.19	72.55	-27.80	0.16	0.99	48.88	0.10	1.31	0.01	1.57	0.01	-143	1.5
M-85	University of Georgia, Athens	GA	34.20	84.10	-27.43	0.01		47.28	0.24	-0.48	0.02	1.40	0.01	-133	2.4
M-86N	NCSU, Raleigh	NC	35.70	78.70	-29.23	0.04		44.34	0.02	2.77	0.01	2.37	0.00		
M-86S	NCSU, Raleigh	NC	35.70	78.70	-28.19	0.03	1.04	43.55	0.19	3.34	0.00	2.42	0.01	-133	2.1
M-87N	College of William and Mary	VA	37.16	76.42	-27.66	0.11		45.53	0.12	2.59	0.04	1.89	0.02		
M-87S	College of William and Mary	VA	37.16	76.42	-26.83	0.02	0.83	46.69	1.81	2.07	0.02	2.05	0.02	-169	3.1
M-88N	Callaway Gardens, Pine Mountain	GA	32.70	85.20	-29.64	0.31		46.80	0.37	1.80	0.00	1.90	0.28	-134	2.2
M-88S	Callaway Gardens, Pine Mountain	GA	32.70	85.20	-29.47	0.02	0.17	46.30	0.55	2.50	0.01	1.85	0.23		
M-89	Oregon State University, Corvallis	OR	44.50	122.80	-29.33	0.02		45.45	0.26	0.26	0.04	1.42	0.02	-158	3.7
M-90	LA State and County Arboretum, LA	CA	33.80	118.20	-28.68	0.01		43.82	0.31	2.02	0.05	2.18	0.03	-142	2.2
M-91N	UCLA Botanical Garden, Los Angeles	CA	33.80	118.20	-29.74	0.04		43.63	0.52	1.93	0.04	2.13	0.05		
M-91S	UCLA Botanical Garden, Los Angeles	CA	33.80	118.20	-28.93	0.03	0.81	42.83	0.28	2.36	0.02	2.90	0.05		
M-92	Peavy Arboretum, Corvallis	OR	44.50	122.80	-28.37	0.00		44.90	1.66	4.00	0.05	1.52	0.06		
M-93	Auburn University	AL	32.59	85.48	-28.38	0.06		45.69	0.01	4.86	0.01	1.73	0.03	-131	1.1
M-94	University of California, Berkeley	CA	36.80	121.90	-25.58	0.02		46.41	0.07	4.06	0.02	1.10	0.01		

position ($\delta^{15}N$) is expressed relative to the N_2 in the atmosphere (Natm standard) defined by the relationship in Equation 2.

$$\delta^{13}C = 1000 \times [(^{13}C/^{12}C_{sample})/(^{13}C/^{12}C_{PDB}) - 1] \quad (1)$$
$$\delta^{15}N = 1000 \times [(^{15}N/^{14}N_{sample})/(^{15}N/^{14}N_{atm}) - 1] \quad (2)$$

For molecular hydrogen isotope analysis, extracted *n*-alkanes of leaves of south-facing trees from 27 sites (Table 1) were purified by column chromatograph and then evaluated by Gas Chromatography (GC) analysis using a DB-1 capillary (60 m × 0.25 mm × 0.25 μm) column. Hydrogen isotope analyses were performed using a HP 6890 GC, interfaced via a high-temperature conversion interface to a Thermo Finnigan MAT 253 mass spectrometer (Hilkert et al., 1999). The GC was held at 80 °C for 1 min and subsequently programmed from 80 °C to 180 °C at 3 °C/min and then to 300 °C at 8 °C/min. The final temperature was held at 300 °C for 10 min. Compounds separated by GC column were converted to H_2 by a pyrolysis reactor at 1445 °C. The determined δD values were against H_2 reference gas that is calibrated by a co-injected laboratory working standard (*n*-C_{16} and *n*-C_{30} alkanes, and 5α-androstane; isotopic ratios were determined offline by A. Schimmelmann, Biogeochemical Laboratory at Indiana University). Each sample was analyzed three times. H_3 factors were calculated daily using the same H_2 reference gas. The precision of isotopic measurements of H_2 reference gas after H_3 factor correction was 1‰ or better. Analytical error was less than 4‰ for our samples. δD values are expressed relative to the Vienna Standard Mean Ocean Water (VSMOW standard) using Equation 3.

$$\delta D = 1000 \times [(^{2}H/^{1}H)_{sample}/(^{2}H/^{1}H)_{VSMOW} - 1] \quad (3)$$

3. Climate data

Temperature and precipitation data (Appendix I) consisting of monthly mean temperature and average monthly precipitation for a period of 50 years (1950–2009) was obtained from the NCDC Station Historical Listing for NWS Cooperative Network (http://www.wrcc.dri.edu/Climsum.html) and the NOAA Global Historical Climatology Network (ftp://ftp.ncdc.noaa.gov/pub/data/ghcn/v2). Climate data from the nearest stations where these trees are growing were compiled and analyzed. Further averages were used, such as annual mean temperature (AMT), annual mean precipitation (AMP), spring mean temperature (SMT), and spring mean precipitation (SMP). As in its native land in China, *Metasequoia glyptostroboides* started its pollination process in February (Fu et al., 1999), here spring is defined to be the months of February–May rather than March–May. Isotopic concentrations were compared to climatic variables (temperature and precipitation) to determine the magnitude and significance of any relationship between them.

4. Statistical methods

Least-squares regression analysis was performed for several variable comparisons, and the corresponding *p*-value significance tests were conducted on the coefficients to determine whether there is sufficient evidence to infer a relationship between isotopic/elemental values and the climatic variables. In addition, the relationship between $\delta^{13}C$ and δD values was also explored, and a comparison between isotopic composition and latitude was performed. Because our primary concern is detecting whether there is any evidence for a non-zero trend in the prediction of climatic variables from the isotopic data, we primarily present *p*-value significance values, which gives us a measure of how confident we are that the slope is non-zero, and that there is a relationship between the two variables. We take the standard convention that $p < 0.01$ is highly significant, $p < 0.05$ is significant, and $p < 0.1$ is weakly significant. In addition, we present the values of the R^2 values to assess the completeness of the linear regression model.

Results

1. Leaf carbon and nitrogen concentrations and bulk $\delta^{13}C$ and $\delta^{15}N$ values

Carbon and nitrogen concentrations as well as bulk $\delta^{13}C$ and $\delta^{15}N$ values of *M. glyptostroboides* trees from each site can be found in Table 1. Our results indicate that leaf carbon content ranges from 49.97% (M-82S from Coker College in South Carolina) to 41.11% (M-31S from South Portland in Maine), with a tendency of increasing carbon content with the decrease of latitude. Leaf N concentrations range from 1.10% (M-94 from University of California, Berkeley campus) to 2.97% (M-64S from Longwood Gardens in Pennsylvania). Leaf bulk $\delta^{13}C$ values in these trees range from -25.58‰ (M-94 from University of California, Berkeley campus) to -30.04‰ (M-88N from the Galloway Gardens in Georgia), falling into the range of a typical C_3 plant. When both shade leaves (north-facing leaves) and sun leaves (south-facing leaves) were collected from the same tree, the carbon isotope offset between shade and sun leaves ($\Delta^{13}C_{N-S}$) varies within the same species, with most of the sun leaves containing more positive $\delta^{13}C$ values. The offset was up to 1.84‰ in M-80 from the Japonski Island in Alaska but only -0.38‰ (slightly more positive in shade leaves) in

M-62 from the Winterthur Gardens in Delaware. Leaf bulk $\delta^{15}N$ values in these samples range from 11.61‰ (M-31S from South Portland in Maine) to -3.44‰ (M-78 from the Hoyt Arboretum in Portland, Oregon).

2. Molecular hydrogen isotope (δD) from leaf lipids

As leaf wax D/H ratio values from C_{27}, C_{29} and C_{31} *n*-alkanes are highly correlated (Liu & Yang, 2008; Liu et al., 2006; Smith & Freeman, 2006), here we report an amount-weighted mean δD value of the three compounds. Similarly, as the difference in hydrogen isotope compositions between sun leaves and shade leaves from any site was relatively small (Yang & Leng, 2009), we measured δD from samples of south-facing leaves. Leaf *n*-alkane δD from measured trees (n = 27) ranges from -173‰ (M-80 from the Japonski Island in Alaska) to -131‰ (M-93 from Auburn University in Alabama), with *n*-alkanes from high latitude leaves displaying more negative δD values than those from their lower latitude counterparts (Table 1), a result similar to that obtained in other studies (Chikaraishi & Naraoka, 2003; Liu & Yang, 2008; Liu et al., 2006; Yang & Huang, 2003; Yang et al., 2011). A cross-plotting between leaf bulk $\delta^{13}C$ and *n*-alkane δD values from the same trees show no significant relationship (p = 0.25).

3. Statistical tests

The annual mean temperature (AMT) gradient for these samples spans from 5 °C (Uppsala, Sweden) to 20 °C (Gainesville in Florida and New Orleans in Louisiana), and the annual mean precipitation (AMP) ranges from 2180 mm (the Japonski Island in Alaska) to 500 mm (Pasadena in California). For several sites with low AMP, such as in California and Oregon, little precipitation occurs in the summer. To assess the quality of extracted climate data, climatic parameters for the year of 2004 were singled out and compared against the past 50 years of the NOAA data. The results revealed that the 2004 and the NOAA data displayed almost identical trends in both temperature and precipitation (data not shown), indicating that the sampling year of 2004 was a climatically normal year among the past 50 years.

The comparisons between δD values and the four climatic variables (AMT, AMP, SMT, and SMP) show a significant relationship with temperature: both AMT (0.1500 ± 0.0412, p = 0.00117, R^2 = 0.338) and SMT (0.1595 ± 0.0495, p = 0.0034, R^2 = 0.285) (Table 2, Fig. 3), although only a weakly significant relation with SMP (2.0760 ± 1.1482, p = 0.0822, R^2 = 0.112) was detected. In contrast, the leaf carbon content shows a significant relationship to precipitation, AMP (59.406 ± 20.58, p = 0.0057, R^2 = 0.184) and SMP (11.1052 ± 5.4268, p = 0.046, R^2 = 0.225), and a less significant relationship with temperature, SMT (-0.6874 ± 0.3395, p = 0.0481, R^2 = 0.075) and AMT (-0.5266 ± 0.2917, p = 0.0768, R^2 = 0.059). No relationship with latitude was found. Leaf δD values show a strong correlation with latitude (-0.3442 ± 0.0706, p = 0.000047, R^2 = 0.478), while $\delta^{13}C$ values do not show a pronounced relationship. Although $\delta^{15}N$ values show very little effect on predictions of either temperature or precipitation, there is a significant relationship between the nitrogen concentration and SMP (-68.16 ± 22.061, p = 0.0032, R^2 = 0.225).

Table 2 Results of slopes for linear regressions for comparisons between isotopic and bulk composition and climatic variables

Climatic variable	δD (‰ vs VSMOW)	$\delta^{13}C$ (‰ vs PDB)	$\delta^{15}N$ (‰ vs N_{atm})	C concentration (%)	N concentration (%)
Latitude (°N)	-0.3442 ± 0.0706 p = 0.00005 ** R^2 = 0.478	0.9799 ± 0.7144 p = 0.17595 R^2 = 0.034	-0.1445 ± 0.3426 p = 0.67493 R^2 = 0.003	0.4213 ± 0.4220 p = 0.32269 R^2 = 0.021	-0.4653 ± 1.7154 p = 0.78729 R^2 = 0.021
Annual mean temperature (°C)	0.1500 ± 0.0412 p = 0.00117 ** R^2 = 0.338	-0.8228 ± 0.4998 p = 0.10563 R^2 = 0.049	-0.1067 ± 0.2415 p = 0.66053 R^2 = 0.004	-0.5266 ± 0.2917 p = 0.07677 ˜ R^2 = 0.059	-0.1378 ± 1.1857 p = 0.90795 R^2 = 0.059
Spring mean temperature (°C)	0.1595 ± 0.0495 p = 0.00343 ** R^2 = 0.285	-0.8435 ± 0.5903 p = 0.15891 R^2 = 0.037	-0.2103 ± 0.2826 p = 0.45998 R^2 = 0.010	-0.6874 ± 0.3395 p = 0.04805 * R^2 = 0.075	-0.7107 ± 1.3803 p = 0.60879 R^2 = 0.075
Annual mean precipitation (mm)	1.8206 ± 4.3454 p = 0.67868 R^2 = 0.007	54.8217 ± 38.0980 p = 0.15604 R^2 = 0.038	-7.5092 ± 18.3058 p = 0.68331 R^2 = 0.003	59.4061 ± 20.5802 p = 0.00566 ** R^2 = 0.184	-130.6723 ± 83.6660 p = 0.12439 R^2 = 0.184
Spring mean precipitation (mm)	2.0760 ± 1.1482 p = 0.08217 ˜ R^2 = 0.112	8.7749 ± 10.4357 p = 0.40421 R^2 = 0.013	-1.3594 ± 4.9562 p = 0.78494 R^2 = 0.001	11.1052 ± 5.4268 p = 0.04579 * R^2 = 0.225	-68.1566 ± 22.0617 p = 0.00322 ** R^2 = 0.225

Multiple regression results are given for the data with both carbon and nitrogen measurements. Significance is denoted by **, *, and ˜ for significance at the levels of 0.01, 0.05, and 0.10 respectively.

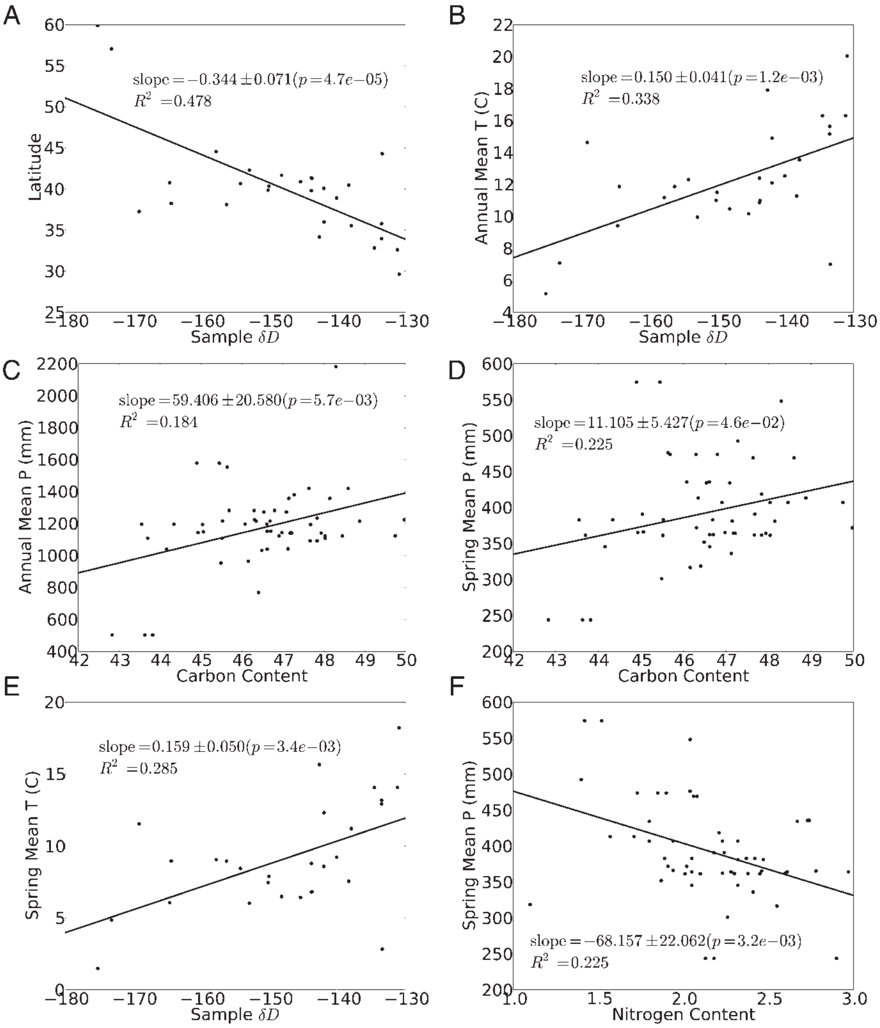

Fig. 3 Correlations between isotope/elemental concentrations and climatic variables. — A: δD values against latitude, B: δD values against annual temperatures, C: bulk carbon against annual precipitation, D: bulk carbon against spring time temperatures, E: δD values against spring time temperatures, F: bulk nitrogen against spring time precipitation.

Discussion

Our statistical analyses provide further insights into how long-term climate signals may be registered in leaf tissues in the forms of stable isotopic compositions as well as elemental concentrations. The results include both predicted relationships as well as some surprises. An unexpected finding is that there is no obvious relationship found between *M. glyptostroboides* leaf $\delta^{13}C$ values and any climate parameters. This is rather surprising given that among other factors, water availability in particular is rather important in controlling ^{13}C discrimination during photosynthesis (Warren et al., 2001), as indicated in previous studies using plant foliar (Hartman & Danin, 2010; Kloeppel et al., 1998) and tree ring (Loader et al., 2007) material. A recent global survey revealed strong relationship between plant $\delta^{13}C$ values and annual mean precipitation (AMP) (Diefendorfa et al., 2010). We believe that it may be due to the unique water availability in these botanical gardens and university campuses where these cultivated trees have been cared by supplementary artificial water (also see discussion below). On the other hand, we noticed that the carbon concentration is correlated with all four climate parameters with the strongest support for a positive relationship with the annual mean precipitation (AMP) (Fig. 3C). To our knowledge, this is the first time when such an observation is made, and it may reflect the particular growing habit of *M. glyptostroboides* that is largely dependent on large water supplies. It is known that the rapid growth of drought-intolerant *M. glyptostroboides* trees is heavily dependent on water availability (Vann, 2005). Both field observations (Kuser, 1982) and greenhouse experiments (Jagels & Day, 2004; Jagels et al., 2003) demonstrated

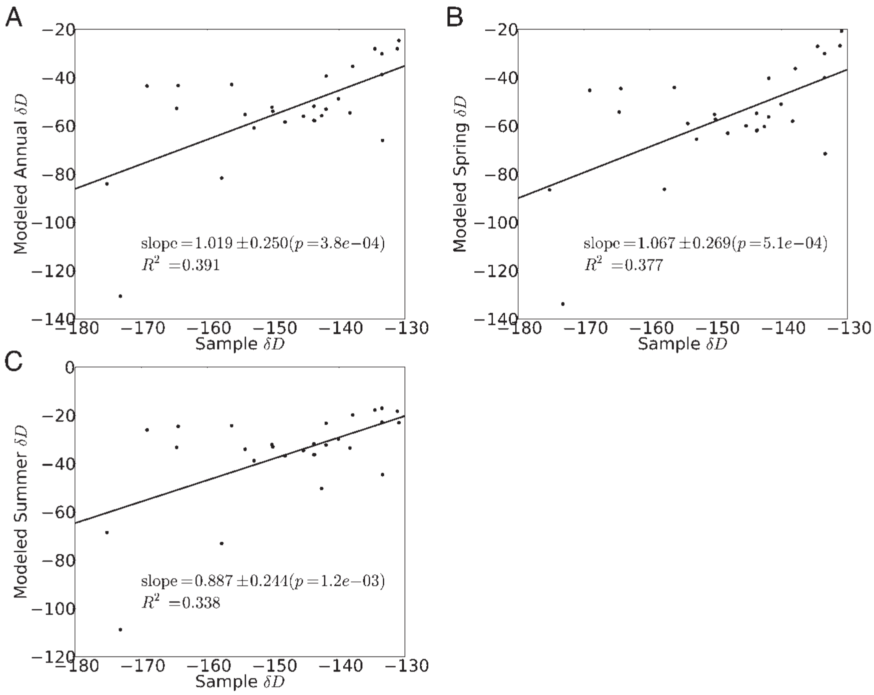

Fig. 4 Correlations between *Metasequoia glyptostroboides* lipid δD and annual (A), spring (B), and summer (C) precipitation δD values. Modeled precipitation δD data for these sites were derived from the Online Isotopes in Precipitation Calculator (Bowen, 2010).

the importance of water supplies to the buildup of the biomass for this species. The positive relationship between carbon concentration and AMP/SMP further sustain the critical role that water plays in the buildup of primary production for *M. glyptostroboides* during the growing season. It should be noted that the R^2 value is not particularly high (around 20% of the variance) which indicates that there are other factors that influence the ability for us to predict precipitation from carbon concentration. These factors could be explored in another study, but the connection with carbon content and precipitation is still significant.

We are puzzled by the strong negative correlation between leaf nitrogen concentration and SMT. While this relationship deserves further investigations, we speculate that rather than reflecting a relationship between plant nutrient and precipitation, the correlation may reflect either foliar uptake of anthropogenic atmospheric nitrogen pollution (Vallano & Sparks, 2007) or the change of metabolic activities during development (Evans, 1989); both of which are influenced by temperature during spring growing season.

Our statistical tests identify temperature as a dominant control on hydrogen isotopes in *M. glyptostroboides* leaf wax. Both annual mean temperature (AMT) and spring mean temperature (SMT) are significantly correlated with *n*-alkane δD values, accounting for approximately 30% of the variance, and it is also well reflected in the strong relationship between *n*-alkane δD values and latitude, accounting for nearly half of the variance in the data (Fig. 3A). This result is consistent with previous analysis on plant tree rings indicating a close relationship between temperature and δD from tree cellulose (Burk & Stuiver, 1981; Epstein et al., 1977; Yapp & Epstein, 1982). Our study further confirms that such a relationship also exists at the molecular isotope level. Meanwhile, we found a significant negative correlation between *n*-alkane δD values and latitude where these trees are growing. This evidence along with the strong relationships between *n*-alkane δD values and modeled precipitation δD values (Fig. 4) further supports the notion that global precipitation δD patterns under current temperature gradient exercise a strong control for the *n*-alkane δD distribution in leaf tissues of higher plants. This provides additional supports as well as a case example for our recent global analysis of precipitation δD as the first order of control for hydrogen isotope composition in plant leaf wax (Liu & Yang, 2008; Yang et al., 2011). Furthermore, while lipid δD values from *M. glyptostroboides* are compared with modeled annual, spring (February–May), and summer (June–September) precipitation δD derived from the Online Isotopes in Precipitation Calculator (Bowen, 2010), it becomes apparent that the strongest correlation was found between plant lipid δD with spring precipitation δD values (1.067 ± 0.269, p = 0.00051, R^2 = 0.377). The relationship is weaker but still significant for the summer precipitation δD values (0.887 ± 0.244, p = 0.0012, R^2 = 0.338) and for the annual precipitation δD values (1.019 ± 0.25, p = 0.0038, R^2 = 0.391) (Fig. 4). Further analysis would be needed to test if there is a significant difference between the influence of spring and summer precipitation. However, these data further suggest that hydrogen isotope values of seasonal precipitation may exercise influence on plant lipid δD, and precipitation during spring times, when *M. glyptostroboides* experiences its fastest growth, may play more important role in affecting the plant lipid δD signatures. It remains to be tested whether this new observation reflects *Metasequoia*'s unique ecological strategy or it is common in other terrestrial plants.

We have observed that the $\delta^{13}C$ values in sun leaves tend to be more positive than those in shade leaves. This result is consistent with previous findings (Lockheart et al., 1997; Lockheart et al., 1998; Nguyen Tu et al., 2004) and can be explained by the difference between C_i/C_a ratios in sun and shade leaves due to different levels of irradiation (Lockheart et al., 1998). On the other hand, $\delta^{15}N$ values seem randomly distributed in sun and shade leaves, indicating leaf aspects exercise little control on nitrogen isotope of leaf tissues.

While cultivated *M. glyptostroboides* trees in the United States provide a unique opportunity for the assessment of stable isotope compositions of a conifer grown under different climates, the fact that most of these trees were nurtured in botanical gardens or university campuses may have created a source of variation for their stable isotope compositions. In contrast to natural settings which rely only on precipitation as the primary water source, artificial water may be used for irrigation in botanical gardens or university campuses, interfering both water supply quantity and stable isotope signals and causing large variations for the measured *n*-alkane δD values of *M. glyptostroboides* leaf tissues in this study. While it may have less impact on water δD as recent studies indicate that the large scale pattern of δD in tap water is similar to annual mean precipitation δD (Bowen et al., 2007; Bowen & Revenaugh, 2003), it may have a larger impact on $\delta^{13}C$ values obtained from these leaf tissues as they may not truly reflect the level of water stress at these sites. Meanwhile, if applied, artificial fertilization may also affect both nitrogen concentration and $\delta^{15}N$ from these trees. In addition, the source of moisture, whether it came from the Pacific Ocean (for plants living in the West Coast) or from the Atlantic Ocean (for trees along the East Coast) may be a factor in affecting hydrogen isotopes from *M. glyptostroboides* leaves.

Nonetheless, despite these uncertainties for the source of variations for the obtained stable isotope contents and the elemental concentrations from *M. glyptostroboides* trees cultivated in the United States, certain patterns emerged from this study provide valuable information for a better understanding of ecology and physiology of these trees outside of their native habitats in China and yield qualitative data toward paleoclimatic and paleoenvironmental interpretations using *Metasequoia* as a model plant (LePage et al., 2005b). In contrast to the current narrow temperature (~12.7 °C MAT) and precipitation (1260 mm MAP) ranges for the native *M. glyptostroboides* in south-central China (Wang, 1984), it is clear that cultivated *M. glyptostroboides* can tolerate much larger temperature and precipitation variations, making them popular trees cultivated around the globe (Ma, 2007). This is also consistent with what *Metasequoia*'s fossil record has told us: the genus used to live under a much larger range of temperature and precipitation in the geological past, covering every continent of the Northern Hemisphere (Yang & Jin, 2000; LePage et al., 2005b). Thus, it is rather naive to believe that a fossil flora containing *Metasequoia* as a major component would indicate a narrow temperature and/or precipitation as its native range in current south-central China, as the nearest living species (NLS) method is applied to the interpretation of fossil record. Although the large isotope variations and uncertainties prevent us from deriving a quantitative formula leading to a mathematical model to characterize the relationship between any stable isotope composition or elemental content with a particular climate parameter, the qualitative relationships between *n*-alkane δD values and carbon concentrations with various climate parameters remain strong. Such relationships would provide useful indication for paleoclimatic and paleoenvironmental indications using *Metasequoia* fossils.

Conclusion

This study presents the largest stable isotope (bulk $\delta^{13}C$, $\delta^{15}N$, and *n*-alkane δD) dataset as well as carbon and nitrogen elemental concentration measurements from *M. glyptostroboides* trees (n = 39) cultivated across the United States. Statistical analyses performed using this dataset against 50 years of climatic variables indicate strong relationships between δD values and carbon concentration with various climate parameters, while $\delta^{13}C$ values were found not correlated with these variables. Our results indicate that temperature gradient exercises a strong control for the distribution of *M. glyptostroboides* leaf δD values, which is further modified by hydrogen isotope in seasonal precipitation. The strong relationship between carbon content and precipitation as well as the consistent correlations between three isotopic/elemental values (δD value, carbon content, and nitrogen content) and spring mean precipitation indicate that precipitation, particularly spring precipitation, may have played an important role in the growth of cultivated *Metasequoia* trees. Despite the large uncertainties, possibly caused by artificial irrigations at botanical gardens and university campuses, our data reveal general patterns between *Metasequoia* isotope and elementary signals and various climate parameters. Such relationships can serve as a qualitative guide for paleoclimatic and paleoenvironmental interpretations using *Metasequoia* fossil record.

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Appendix I. Fifty year average climatic data compiled from the NCDC Station Historical Listing for NWS Cooperative Network (http://www.wrcc.dri.edu/Climsum.html) and the NOAA Global Historical Climatology Network (ftp://ftp.ncdc.noaa.gov/pub/data/ghcn/v2) for the *Metasequoia glyptostroboides* growing sites. The data list sample number (while both shade/north-facing leaves and sun/south-facing leaves are used, the former is marked with "N" while the later as "S"), location, state, annual mean temperature (AMT, °C), annual mean precipitation (AMP, mm), spring mean temperature (SMT, °C), spring mean precipitation (SMP, mm), and monthly breakdowns.

Sample	City	State	AMT	SMT	Temperature (°C)											
					Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
M-23N	Jamacia Plain	MA	9.96	6.02	-3.09	-1.80	2.64	8.76	14.49	19.69	22.68	21.81	17.30	11.35	6.04	-0.36
M-23S	Jamacia Plain	MA	9.96	6.02	-3.09	-1.80	2.64	8.76	14.49	19.69	22.68	21.81	17.30	11.35	6.04	-0.36
M-29N	Northwest Harbor	ME	7.01	2.84	-5.98	-5.17	-0.42	5.58	11.37	16.34	19.46	18.77	14.54	8.90	3.56	-2.86
M-29S	Northwest Harbor	ME	7.01	2.84	-5.98	-5.17	-0.42	5.58	11.37	16.34	19.46	18.77	14.54	8.90	3.56	-2.86
M-60N	Locust Valley	NY	10.17	6.43	-2.14	-1.07	3.22	9.24	14.34	19.46	22.39	21.50	17.41	11.32	6.13	0.30
M-60S	Locust Valley	NY	10.17	6.43	-2.14	-1.07	3.22	9.24	14.34	19.46	22.39	21.50	17.41	11.32	6.13	0.30
M-61	New Brunswick	NJ	11.28	7.56	-1.00	0.18	4.40	10.14	15.51	20.68	23.40	22.58	18.58	12.37	7.13	1.39
M-62N	Winterthur	DE	12.40	8.80	0.04	1.22	5.57	11.48	16.92	21.95	24.46	23.66	19.71	13.54	7.90	2.32
M-62S	Winterthur	DE	12.40	8.80	0.04	1.22	5.57	11.48	16.92	21.95	24.46	23.66	19.71	13.54	7.90	2.32
M-63S	Washington	DC	12.53	9.23	0.45	1.80	6.14	11.85	17.11	21.99	24.53	23.68	19.68	13.18	7.66	2.35
M-64N	Kennett Square	PA	11.01	7.46	-1.43	-0.09	4.23	10.17	15.54	20.70	23.24	22.27	18.17	11.88	6.66	0.74
M-64S	Kennett Square	PA	11.01	7.46	-1.43	-0.09	4.23	10.17	15.54	20.70	23.24	22.27	18.17	11.88	6.66	0.74
M-65	Brooklyn	NY	12.31	8.45	-0.10	0.99	5.12	11.14	16.55	21.57	24.44	23.75	19.73	13.80	8.30	2.43
M-66N	Princeton	NJ	11.51	7.89	-0.62	0.55	4.82	10.43	15.77	20.82	23.47	22.58	18.67	12.57	7.36	1.70
M-66S	Princeton	NJ	11.51	7.89	-0.62	0.55	4.82	10.43	15.77	20.82	23.47	22.58	18.67	12.57	7.36	1.70
M-67S	Princeton	NJ	11.51	7.89	-0.62	0.55	4.82	10.43	15.77	20.82	23.47	22.58	18.67	12.57	7.36	1.70
M-68N	Philadelphia	PA	12.11	8.59	0.01	1.15	5.58	11.20	16.43	21.55	24.18	23.24	19.24	13.15	7.50	2.09
M-68S	Philadelphia	PA	12.11	8.59	0.01	1.15	5.58	11.20	16.43	21.55	24.18	23.24	19.24	13.15	7.50	2.09
M-69N	Gainesville	FL	20.04	18.22	11.91	13.28	16.50	19.66	23.42	26.18	27.20	27.04	25.33	20.88	16.36	12.71
M-69S	Gainesville	FL	20.04	18.22	11.91	13.28	16.50	19.66	23.42	26.18	27.20	27.04	25.33	20.88	16.36	12.71
M-70N	Louisville	KY	11.87	8.96	-0.86	1.04	6.03	11.87	16.88	21.50	23.46	22.80	19.15	12.81	6.54	1.23
M-70S	Louisville	KY	11.87	8.96	-0.86	1.04	6.03	11.87	16.88	21.50	23.46	22.80	19.15	12.81	6.54	1.23
M-71N	Warwick	RI	10.48	6.50	-1.76	-0.79	3.29	9.09	14.41	19.64	22.76	22.01	17.75	11.88	6.66	0.79
M-71S	Warwick	RI	10.48	6.50	-1.76	-0.79	3.29	9.09	14.41	19.64	22.76	22.01	17.75	11.88	6.66	0.79
M-72	New London	CT	10.87	6.80	-0.99	-0.10	3.82	9.18	14.31	19.34	22.67	22.22	18.25	12.57	7.44	1.67
M-73	Durham	NC	14.89	12.32	4.22	5.74	9.56	14.87	19.11	23.29	25.42	24.79	21.16	14.96	10.15	5.45
M-74	New Orleans	LA	21.34	19.52	12.62	14.19	17.52	21.28	25.08	27.94	28.83	28.77	26.85	22.16	17.17	13.68
M-75	Newark	OH	10.65	7.48	-2.23	-0.66	4.28	10.41	15.86	20.58	22.55	21.81	18.03	11.59	5.62	-0.01
M-76	Wooster	OH	9.42	6.07	-3.53	-2.12	2.86	9.07	14.45	19.36	21.37	20.64	16.77	10.64	4.75	-1.19
M-77N	Clermont	KY	11.87	8.96	-0.86	1.04	6.03	11.87	16.88	21.50	23.46	22.80	19.15	12.81	6.54	1.23
M-77S	Clermont	KY	11.87	8.96	-0.86	1.04	6.03	11.87	16.88	21.50	23.46	22.80	19.15	12.81	6.54	1.23
M-78N	Portland	OR	10.78	8.83	3.61	5.44	7.34	9.62	12.92	15.76	18.44	18.33	15.87	11.18	6.85	4.06
M-78S	Portland	OR	10.78	8.83	3.61	5.44	7.34	9.62	12.92	15.76	18.44	18.33	15.87	11.18	6.85	4.06
M-79N	Asheville	NC	13.54	11.22	3.43	4.95	8.68	13.39	17.85	21.59	23.58	22.95	19.43	13.73	8.56	4.39
M-79S	Asheville	NC	13.54	11.22	3.43	4.95	8.68	13.39	17.85	21.59	23.58	22.95	19.43	13.73	8.56	4.39
M-80N	Sitka	AK	7.08	4.86	1.16	2.25	3.09	5.56	8.53	11.33	13.34	13.92	11.66	7.79	4.14	2.24
M-80S	Sitka	AK	7.08	4.86	1.16	2.25	3.09	5.56	8.53	11.33	13.34	13.92	11.66	7.79	4.14	2.24
M-81N	Northhampton	MA	8.64	4.85	-5.60	-4.17	1.08	8.24	14.25	19.29	21.84	20.79	16.27	10.01	4.19	-2.56
M-81S	Northhampton	MA	8.64	4.85	-5.60	-4.17	1.08	8.24	14.25	19.29	21.84	20.79	16.27	10.01	4.19	-2.56
M-82N	Hartsville	SC	17.59	15.44	7.79	9.34	13.33	17.49	21.60	25.24	27.00	26.55	23.51	17.63	12.84	8.72
M-82S	Hartsville	SC	17.59	15.44	7.79	9.34	13.33	17.49	21.60	25.24	27.00	26.55	23.51	17.63	12.84	8.72
M-83	Cambridge	MA	9.96	6.02	-3.09	-1.80	2.64	8.76	14.49	19.69	22.68	21.81	17.30	11.35	6.04	-0.36
M-84N	New Haven	CT	10.99	6.83	-0.73	-0.20	3.71	9.33	14.48	19.76	22.68	22.11	18.45	12.90	7.48	1.93
M-84S	New Haven	CT	10.99	6.83	-0.73	-0.20	3.71	9.33	14.48	19.76	22.68	22.11	18.45	12.90	7.48	1.93
M-85	Athens	GA	15.16	12.92	4.81	6.61	10.55	15.25	19.29	23.00	24.96	24.54	21.12	15.49	10.39	5.94
M-86N	Raleigh	NC	15.64	13.19	5.23	6.63	10.65	15.57	19.89	23.96	26.01	25.25	21.80	15.75	10.61	6.33
M-86S	Raleigh	NC	15.64	13.19	5.23	6.63	10.65	15.57	19.89	23.96	26.01	25.25	21.80	15.75	10.61	6.33
M-87N	Williamsburg	VA	14.63	11.55	3.57	4.64	8.85	14.01	18.69	23.07	25.47	24.83	21.39	15.47	10.24	5.33
M-87S	Williamsburg	VA	14.63	11.55	3.57	4.64	8.85	14.01	18.69	23.07	25.47	24.83	21.39	15.47	10.24	5.33
M-88N	Pine Mountain	GA	16.31	14.07	6.37	8.05	11.91	16.01	20.32	24.18	25.85	25.43	22.31	16.51	11.36	7.43
M-88S	Pine Mountain	GA	16.31	14.07	6.37	8.05	11.91	16.01	20.32	24.18	25.85	25.43	22.31	16.51	11.36	7.43
M-89	Corvallis	OR	11.19	9.06	4.39	6.06	7.68	9.69	12.82	15.84	18.91	18.91	16.53	11.55	7.19	4.63
M-90	Los Angeles	CA	17.91	15.67	12.68	13.73	14.53	16.27	18.14	20.64	23.68	24.05	22.97	19.71	15.75	12.83
M-91N	Los Angeles	CA	17.91	15.67	12.68	13.73	14.53	16.27	18.14	20.64	23.68	24.05	22.97	19.71	15.75	12.83
M-91S	Los Angeles	CA	17.91	15.67	12.68	13.73	14.53	16.27	18.14	20.64	23.68	24.05	22.97	19.71	15.75	12.83
M-92	Corvallis	OR	10.02	8.08	3.55	5.30	6.60	8.71	11.70	14.63	17.45	17.23	14.83	10.29	6.21	3.78
M-93	Auburn	AL	16.31	14.07	6.37	8.05	11.91	16.01	20.32	24.18	25.85	25.43	22.31	16.51	11.36	7.43
M-94	Berkeley	CA	13.85	12.55	9.77	11.03	11.69	12.82	14.68	16.30	17.24	17.51	17.30	15.53	12.36	10.00
M-2	Uppsala	Sweden	5.17	1.50	-4.20	-4.50	-1.90	3.30	9.10	14.30	16.60	15.10	10.80	5.50	0.50	-2.60

Appendix I. (continued)

Sample	City	State	AMP	SMP	Precipitation (mm)											
					Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
M-23N	Jamacia Plain	MA	1108.40	361.38	97.49	75.23	95.81	96.47	93.87	87.31	87.60	83.95	94.28	96.08	106.58	93.73
M-23S	Jamacia Plain	MA	1108.40	361.38	97.49	75.23	95.81	96.47	93.87	87.31	87.60	83.95	94.28	96.08	106.58	93.73
M-29N	Northwest Harbor	ME	1420.25	469.27	129.89	115.42	124.97	122.55	106.33	99.98	88.60	74.86	104.57	128.58	180.98	143.53
M-29S	Northwest Harbor	ME	1420.25	469.27	129.89	115.42	124.97	122.55	106.33	99.98	88.60	74.86	104.57	128.58	180.98	143.53
M-60N	Locust Valley	NY	1264.48	419.98	94.52	84.69	114.80	111.71	108.78	102.49	97.56	108.36	109.77	109.31	113.80	108.68
M-60S	Locust Valley	NY	1264.48	419.98	94.52	84.69	114.80	111.71	108.78	102.49	97.56	108.36	109.77	109.31	113.80	108.68
M-61	New Brunswick	NJ	1194.16	382.12	89.83	74.46	105.35	102.40	99.92	97.24	123.58	108.32	102.15	89.62	98.71	102.57
M-62N	Winterthur	DE	1190.95	386.06	88.54	74.32	108.60	101.39	101.75	104.55	118.18	103.96	110.39	89.55	91.47	98.25
M-62S	Winterthur	DE	1190.95	386.06	88.54	74.32	108.60	101.39	101.75	104.55	118.18	103.96	110.39	89.55	91.47	98.25
M-63S	Washington	DC	1047.72	335.86	70.83	61.52	86.32	84.10	103.92	96.61	106.00	99.47	96.34	85.63	76.91	80.07
M-64N	Kennett Square	PA	1177.76	371.00	87.41	74.89	101.04	94.30	100.77	100.74	111.83	104.47	115.08	89.62	98.49	99.11
M-64S	Kennett Square	PA	1177.76	371.00	87.41	74.89	101.04	94.30	100.77	100.74	111.83	104.47	115.08	89.62	98.49	99.11
M-65	Brooklyn	NY	1188.10	388.21	86.93	78.35	105.39	104.09	100.39	96.92	107.59	107.91	99.95	96.91	104.58	99.09
M-66N	Princeton	NJ	1152.64	362.37	82.76	69.08	98.73	96.96	97.60	97.18	119.90	115.69	102.83	89.09	89.65	93.16
M-66S	Princeton	NJ	1152.64	362.37	82.76	69.08	98.73	96.96	97.60	97.18	119.90	115.69	102.83	89.09	89.65	93.16
M-67S	Princeton	NJ	1152.64	362.37	82.76	69.08	98.73	96.96	97.60	97.18	119.90	115.69	102.83	89.09	89.65	93.16
M-68N	Philadelphia	PA	1142.88	365.33	80.93	67.62	101.20	97.40	99.12	94.92	117.26	120.11	97.58	87.54	88.56	90.65
M-68S	Philadelphia	PA	1142.88	365.33	80.93	67.62	101.20	97.40	99.12	94.92	117.26	120.11	97.58	87.54	88.56	90.65
M-69N	Gainesville	FL	1357.67	381.16	99.05	93.50	114.52	81.31	91.83	172.91	175.93	178.34	143.35	69.71	56.91	80.31
M-69S	Gainesville	FL	1357.67	381.16	99.05	93.50	114.52	81.31	91.83	172.91	175.93	178.34	143.35	69.71	56.91	80.31
M-70N	Louisville	KY	1122.82	406.98	79.94	69.07	107.53	106.69	123.70	91.61	113.31	94.92	77.87	74.71	85.81	97.67
M-70S	Louisville	KY	1122.82	406.98	79.94	69.07	107.53	106.69	123.70	91.61	113.31	94.92	77.87	74.71	85.81	97.67
M-71N	Warwick	RI	1165.40	396.61	99.06	89.10	112.59	105.52	89.41	80.97	78.31	97.10	95.06	96.24	111.78	110.27
M-71S	Warwick	RI	1165.40	396.61	99.06	89.10	112.59	105.52	89.41	80.97	78.31	97.10	95.06	96.24	111.78	110.27
M-72	New London	CT	1234.09	418.66	103.59	92.63	119.39	108.82	97.81	82.66	86.40	106.51	102.03	103.46	113.93	116.87
M-73	Durham	NC	1174.30	388.95	95.66	92.19	111.23	87.75	97.78	101.80	111.03	116.60	100.50	86.86	86.95	85.94
M-74	New Orleans	LA	1553.71	476.33	125.78	119.55	122.58	115.50	118.71	157.77	181.61	157.44	145.50	78.58	102.40	128.29
M-75	Newark	OH	1032.12	352.05	75.14	61.63	87.19	99.57	103.65	98.97	115.59	90.51	77.28	67.64	80.66	74.29
M-76	Wooster	OH	964.57	316.62	62.86	50.57	74.10	87.93	104.02	97.46	106.85	96.58	83.10	64.76	71.09	65.25
M-77N	Clermont	KY	1122.82	406.98	79.94	69.07	107.53	106.69	123.70	91.61	113.31	94.92	77.87	74.71	85.81	97.67
M-77S	Clermont	KY	1122.82	406.98	79.94	69.07	107.53	106.69	123.70	91.61	113.31	94.92	77.87	74.71	85.81	97.67
M-78N	Portland	OR	1039.16	345.49	157.86	110.50	101.59	73.49	59.91	43.10	16.04	25.28	42.67	87.15	153.92	167.65
M-78S	Portland	OR	1039.16	345.49	157.86	110.50	101.59	73.49	59.91	43.10	16.04	25.28	42.67	87.15	153.92	167.65
M-79N	Asheville	NC	1404.18	467.63	108.56	109.94	141.61	106.53	109.55	126.33	125.68	140.27	117.29	96.99	106.86	114.56
M-79S	Asheville	NC	1404.18	467.63	108.56	109.94	141.61	106.53	109.55	126.33	125.68	140.27	117.29	96.99	106.86	114.56
M-80N	Sitka	AK	2181.86	548.13	183.64	159.77	150.62	120.90	116.84	84.07	108.20	171.96	279.91	334.26	249.68	222.00
M-80S	Sitka	AK	2181.86	548.13	183.64	159.77	150.62	120.90	116.84	84.07	108.20	171.96	279.91	334.26	249.68	222.00
M-81N	Northhampton	MA	953.32	301.15	63.87	56.57	78.68	77.65	88.26	88.82	90.60	88.73	82.81	81.55	79.97	75.81
M-81S	Northhampton	MA	953.32	301.15	63.87	56.57	78.68	77.65	88.26	88.82	90.60	88.73	82.81	81.55	79.97	75.81
M-82N	Hartsville	SC	1223.60	371.91	96.66	89.06	111.24	78.30	93.31	124.80	127.39	155.47	117.09	78.50	63.09	88.68
M-82S	Hartsville	SC	1223.60	371.91	96.66	89.06	111.24	78.30	93.31	124.80	127.39	155.47	117.09	78.50	63.09	88.68
M-83	Cambridge	MA	1108.40	361.38	97.49	75.23	95.81	96.47	93.87	87.31	87.60	83.95	94.28	96.08	106.58	93.73
M-84N	New Haven	CT	1091.91	361.87	84.00	72.11	95.42	104.37	89.98	88.46	89.32	96.93	92.84	91.79	92.01	94.69
M-84S	New Haven	CT	1091.91	361.87	84.00	72.11	95.42	104.37	89.98	88.46	89.32	96.93	92.84	91.79	92.01	94.69
M-85	Athens	GA	1379.92	492.54	138.05	123.91	154.17	110.09	104.37	104.32	115.14	100.12	110.70	92.89	104.39	121.77
M-86N	Raleigh	NC	1196.24	382.95	94.02	91.70	105.81	84.99	100.45	105.09	148.45	118.09	112.64	79.07	77.56	78.37
M-86S	Raleigh	NC	1196.24	382.95	94.02	91.70	105.81	84.99	100.45	105.09	148.45	118.09	112.64	79.07	77.56	78.37
M-87N	Williamsburg	VA	1215.63	383.00	93.26	84.70	107.23	84.68	106.39	95.67	138.14	127.59	113.51	89.12	87.16	88.18
M-87S	Williamsburg	VA	1215.63	383.00	93.26	84.70	107.23	84.68	106.39	95.67	138.14	127.59	113.51	89.12	87.16	88.18
M-88N	Pine Mountain	GA	1281.72	473.90	117.08	124.35	142.69	115.28	91.58	92.35	140.57	85.57	90.62	71.64	90.88	119.11
M-88S	Pine Mountain	GA	1281.72	473.90	117.08	124.35	142.69	115.28	91.58	92.35	140.57	85.57	90.62	71.64	90.88	119.11
M-89	Corvallis	OR	1079.50	359.49	179.46	126.93	113.53	66.47	52.57	33.72	10.18	17.10	33.92	83.99	167.30	194.34
M-90	Los Angeles	CA	503.21	243.87	115.04	115.30	81.43	37.20	9.94	3.89	0.77	3.00	9.11	16.40	48.24	62.89
M-91N	Los Angeles	CA	503.21	243.87	115.04	115.30	81.43	37.20	9.94	3.89	0.77	3.00	9.11	16.40	48.24	62.89
M-91S	Los Angeles	CA	503.21	243.87	115.04	115.30	81.43	37.20	9.94	3.89	0.77	3.00	9.11	16.40	48.24	62.89
M-92	Corvallis	OR	1578.02	574.37	221.32	160.27	174.93	133.18	105.98	73.43	18.41	31.72	56.29	131.74	229.15	241.60
M-93	Auburn	AL	1281.72	473.90	117.08	124.35	142.69	115.28	91.58	92.35	140.57	85.57	90.62	71.64	90.88	119.11
M-94	Berkeley	CA	768.57	318.64	165.40	140.90	106.02	54.61	17.11	4.86	2.01	2.26	8.52	33.58	95.19	138.11
M-2	Uppsala	Sweden	541.10	122.40	33.30	25.80	25.70	29.60	41.30	49.90	67.50	71.70	55.30	53.80	47.30	39.90