Temporal variations in the δD of leaf *n*-alkanes from four riparian plant species

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ABSTRACT

The hydrogen isotopic composition of terrestrial plant leaf waxes is widely used as a proxy for the isotopic composition of ambient water at the time of plant growth, yet there is considerable uncertainty about how environmental or plant-specific factors impact apparent hydrogen isotope fractionation. We sampled leaves from four riparian plant species (*Pinus strobus*, *Tsuga canadensis*, *Phalaris arundinacea* and *Corylus americana*) during the 2013 growing season (April to October) to evaluate the controls of hydrogen isotope fractionation in plant-derived *n*-alkanes. Our data show that plants from different taxonomic classes produce distinct seasonal patterns of isotopic change that could reflect differences in stomatal regulation, soil versus groundwater uptake, and timescales of wax synthesis. These patterns are distinct even for plants from the same functional group (i.e. angiosperms versus gymnosperms). *P. strobus* (gymnosperm tree) and *P. arundinacea* (angiosperm grass) are characterized by a significant increase in apparent fractionation during the growing season ($> 50\text{‰}$ change). In contrast, *n*-alkanes from *C. americana* (angiosperm) show a decrease in ϵ value from $-145 \pm 8\text{‰}$ during early season growth to $-111 \pm 6\text{‰}$ late in the season. *T. canadensis* (gymnosperm) exhibits a constant ϵ of $-123 \pm 10\text{‰}$ throughout the growing season with minimal temporal change. Sedimentary *n*-alkanes collected from the stream channel adjacent our plant sample locality show minor changes over the sampling period with a mean ϵ value of $-130 \pm 6\text{‰}$. Apparent fractionation differences between plant species greatly exceed variations in stream composition throughout the growing season, indicating that differences in the timing of wax production or groundwater δD cannot alone explain the variation in ϵ between different plant types. In contrast to individual plant leaves, fluvial sediments represent a time-integrated ecosystem average of *n*-

alkane δD that yields a more consistent isotopic record of changes in ambient ecosystem water than individual plants.

1. Introduction

Higher plants produce a range of organic molecules within, and on the surface of, leaves to provide protection from predation, minimize water loss, and add structural support for leaf architecture (Barthlott and Neinhuis, 1997; Barbour et al., 2000; Barbour et al., 2004; Jetter et al., 2006; Sachse et al., 2006; Nichols et al., 2010). One of the most abundant classes of plant-derived wax compounds is straight chain normal alkanes (*n*-alkanes). These compounds are produced on the surface of leaves and are preserved in sedimentary archives with minimal degradation or isotopic alteration at low temperatures ($\sim 150^\circ\text{C}$) over geologic time (Schimmelmann et al., 1999; Tipple and Pagani, 2010; Tipple et al., 2011; Sachse et al., 2012; Zhuang et al., 2014). As a result, *n*-alkanes constitute a valuable record of past environmental conditions.

Numerous studies have employed the stable hydrogen isotopes of plant-derived *n*-alkanes preserved in ancient sediments to interpret changes in paleoelevation (e.g., Polissar et al., 2009; Hren et al., 2010), isotope hydrology (Schefuß et al., 2005; Pagani et al., 2006; Jacob et al., 2007), ecosystem change (Douglas et al., 2012; Eley et al., 2014) and paleoenvironment (Sauer et al., 2001; Voelker et al., 2014). However, complications in reconstructing paleoenvironments result from uncertainty in the timing of wax production, sedimentary deposition and integration, and how isotopes of individual *n*-alkanes relate to ambient environmental conditions (i.e. temperature, precipitation, precipitation isotopes, moisture availability and ecosystem type).

Hydrogen ($\delta D_{n\text{-alkane}}$) stable isotopes of terrestrial plant *n*-alkanes reflect the isotopic composition of ambient water modified by environmental parameters such as temperature, precipitation, stomatal regulation, water use efficiency and plant functional types (e.g., Lockheart et al., 1998; Chikaraishi and Naraoka 2003; Chikaraishi et al., 2004; Pagani et al., 2006; Hou et al., 2008; Tipple and Pagani 2010; Tipple et al., 2011; Yang et al., 2011; Sachse et al., 2012). Varied environmental factors affect ambient precipitation isotopes, the degree of soil or leaf-water evaporation, or the magnitude of biosynthetic fractionation, and alter the leaf water composition and thus, final *n*-alkane δD (Sessions et al., 1999; Feakins et al., 2010; Kahmen et al., 2012; Sachse et al., 2012; Tipple et al., 2013). A number of studies have examined apparent fractionation between plant *n*-alkane and leaf or xylem water (Sachse et al., 2006; Tipple et al., 2013; Gao et al., 2014). While this is informative of biological functions of plants, it does not fully address how plant *n*-alkanes isotopes relate to ambient precipitation or groundwater. In order to reliably utilize δD of individual leaf wax *n*-alkanes as a proxy for isotope hydrology, it is critical to relate plant wax *n*-alkane geochemistry to seasonal or annual environmental signals (Smith and Freeman 2006; Sachse et al., 2010; Douglas et al., 2012; Gao et al. 2013; etc.).

We examined the stable hydrogen isotopic compositions of *n*-alkanes from four C_3 plants (*Pinus strobus*, *Tsuga canadensis*, *Phalaris arundinacea* and *Corylus americana*) within the Fenton River watershed (Storrs, CT). This approach was tailored to examine how the hydrogen isotopic composition of *n*-alkanes from plants with different patterns of stomatal regulation (angiosperm, gymnosperm, grass and shrub), plant growth, and timescales of wax synthesis changes throughout the growing season. We focused on streamside vegetation with roots positioned visibly in the river water to minimize potential external differences in water stress and determine how resultant stomatal regulation is reflected in *n*-alkane isotopic composition. Our

data indicates the possibility that differences in stomatal regulation and/or function rather than the timescale of synthesis account for the large differences in *n*-alkane hydrogen isotopic composition throughout the growing season. Temporal variations in plant δD during the growing season greatly exceed seasonal variation in groundwater δD and most likely reflect biologic differences in stomatal regulation of transpiration and CO₂ fixation. In contrast to individual plant leaves, river sediments are temporal integrators that minimize heterogeneity in an ecosystem. Thus, we argue that soils or sediments provide the best ecosystem level record of the isotopic composition of ambient water during plant growth.

2. Sample collection and methods

2.1. Study catchment and sample collection

The Fenton River is a minimally altered ~89 km² drainage located in northeastern Connecticut and covered by a mixed deciduous forest. The climate is moist-temperate and receives ~120 cm/yr precipitation. Temperature ranges from a mean of –6 °C in January to 21 °C in July, with a mean annual temperature of 15.7 °C (Supplementary Table 1) and $\delta D_{\text{precipitation}}$ varies from ~ –30‰ to –100‰ throughout the year (Bowen and Revenaugh, 2003; Bowen, 2013).

We sampled modern leaves from four plants along the banks of the Fenton River (41°49.537 N, 72°14.222 W; Supplementary Fig. 1) on a weekly to biweekly basis initiated in April 2013, prior to the period of leaf-out of deciduous trees, through the period of leaf senescence in October 2013. Targeted species included (a) White Pine (*Pinus strobus*); (b) Eastern Hemlock (*Tsuga canadensis*); (c) Reed canary-grass (*Phalaris arundinacea*); and (d)

American Hazelnut (*Corylus americana*). In order to reduce heterogeneity in sampling, the same four trees were sampled from each sample period. Furthermore, during each sampling period, several leaves/needles/blades were sampled from various locations on each plant to get an average per plant, per week. All plants were situated immediately adjacent the river channel (< 1 m from bank full channel) and had roots visible in the stream channel below base-flow groundwater level. These species were chosen because they make up much of the young growth found along the river banks and, as such, are major contributors to sediment *n*-alkane concentrations. For conifers and grass, early season collection of leaves/needles contained mostly old growth, as that was all that was available. As the season progressed, we specifically targeted new growth for *P. strobus*, *T. canadensis*, and *P. arundinacea*. In addition to leaf samples, we collected sediment adjacent the plants in the stream channel and stream water for each period of leaf sample collection.

Sediments were collected from the streambed located on the northwest side of the small outcropping that the *P. arundinacea* was grown on. Samples were taken from here due to the accumulation of organic matter flowing through the catchment as a result of base flow deposition. A sample was collected concurrently with the plant samples throughout the growing season and heterogeneity was minimized by collecting from the same location and no deeper than 2–4 cm from the surface of the deposited materials. Samples were collected in ashed glassware and immediately frozen to minimize post-sampling alteration. The water was collected from the center of the stream in a PTFE bottle with a screw cap and sealed underwater in order to remove all headspace.

2.2. Extraction, separation and analytical methods

Leaves and sediments were frozen following sampling and then freeze-dried (Labconco Freezone 4.5) prior to *n*-alkane extraction. A minimum of 3–5 leaves were extracted via sonication using a 9:1 (v/v) dichloromethane-methanol (DCM:MeOH) solution. Sediments were freeze-dried and extracted using Soxhlet extraction. A minimum of 100 g of sediment was weighed and extracted using a 2:1 DCM:MeOH mixture for 24 h. TLE's were saponified with ~5 ml of 1M KOH in MeOH and heated for 2 h at 85 °C. The reaction was quenched using 5 ml of 5% NaCl. The neutral fraction was separated by liquid/liquid extraction for *n*-alkane separation.

n-Alkanes were separated by silica gel column chromatography and analyzed on a Thermo-Scientific Trace GC Ultra fitted with a split-splitless (SSL) injector and flame ionization detector (FID) using a BP-1 column (60 m × 0.25 mm i.d., 0.25 µm film thickness) with He as the carrier (1.5 ml/min). We calculated carbon preference indices (CPI) (Eq. 1) and Average Chain Length (ACL) (Eq. 2), and Average Higher Plant Chain Length (Eq. 3) to evaluate temporal changes in this distribution of alkanes. These equations are:

$$CPI = \frac{(C_{23}+C_{25}+C_{27}+C_{29}+C_{31}+C_{33})+(C_{25}+C_{27}+C_{29}+C_{31}+C_{33}+C_{35})}{2(C_{24}+C_{26}+C_{28}+C_{30}+C_{32}+C_{34})} \quad \text{Eq. 1}$$

$$ACL = \frac{(C_{25}(25)+C_{27}(27)+C_{29}(29)+C_{31}(31)+C_{33}(33))}{(C_{25}+C_{27}+C_{29}+C_{31}+C_{33})} \quad \text{Eq. 2}$$

$$AHPCL = \frac{(C_{27}(27)+C_{29}(29)+C_{31}(31))}{C_{27}+C_{29}+C_{31}} \quad \text{Eq. 3}$$

where C_x corresponds to the area of the individual *n*-alkane peak from the gas chromatogram.

2.3. Stable hydrogen isotope analyses

Hydrogen isotopes of individual *n*-alkanes were measured using a Thermo GC-Isolink attached to a Thermo Scientific Delta V Plus isotope ratio mass spectrometer with a 30 m × 0.25 μm ID, 0.25 μm film thickness BP-5 fused silica column with a helium carrier at a flow of 1.5 ml/min. The initial GC oven temperature was held at 50 °C for 1 min then heated to 180 °C at 12 °C/min, followed by a ramp to 320 °C at 6 °C/min and held at 320 °C for 4 min. We measured the H_3^+ factor daily prior to standard calibration and sample analysis. This averaged 6.4 over the duration of analysis.

The isotopic compositions of individual *n*-alkanes were determined using an in-house laboratory gas reference and a suite of *n*-alkanes of known isotopic composition that were measured multiple times at a range of concentrations (Mix A5 prepared by A. Schimmelmann). Typical standard deviation for repeated analysis of Mix A5 *n*-alkane isotope standard was less than 4‰.

Hydrogen isotopes of stream waters were analyzed using a Thermo Scientific Temperature Conversion Elemental Analyzer (TC/EA) coupled to a Thermo Delta V Plus. Water samples were measured in triplicate the mean values are presented. Samples are expressed relative to VSMOW by comparison with a suite of International and laboratory standards (e.g., SMOW, SLAP, GISP). Repeat analyses of water standards yield a precision of ±3‰.

The calculated enrichment factor (ϵ) is used to characterize the hydrogen isotopic fractionation between source and product. This may be defined by Eq. 4:

$$\epsilon = \left[\left(\frac{\delta D_{n-alkanes} + 1}{\delta D_{water} + 1} \right) - 1 \right] \quad \text{Eq. 4}$$

and includes a variety of fractionations associated with physical and biochemical processes. In the above formulation (after Coplen, 2011), the δ equation excludes the commonly utilized

multiplier of 1000 for delta calculations and is defined by Eq. 5 for the calculation of epsilon values:

$$\delta D_{n-alkane} = \left[\left(\frac{R_{sample}}{R_{standard}} \right) - 1 \right] \quad \text{Eq. 5}$$

3. Results and discussion

3.1. Abundance, Carbon Preference Index (CPI), and chain length distribution of *n*-alkyl lipids

3.1.1. Leaf *n*-alkane abundances

We normalized the abundances and distribution of *n*-alkanes in plant extracts relative to the total mass of the extracted leaves (Fig. 1a-d, Supplementary Table 2). The *n*-alkane concentrations in *P. strobus* and *T. canadensis* are characterized most prominently by large spikes in *n*-alkane concentration mid-season (days 200–250) (Fig. 1a, b). The angiosperms *P. arundinacea* and *C. americana* peak early in the season (days 100–150) and remain fairly constant throughout the growing season (Fig. 1c, d).

Increases in *n*-alkane concentration during the period of growth (*P. strobus*, *T. canadensis*) suggest continued wax production throughout the growing season. The spikes seen in both species occur immediately after a period of heavy rain, which could possibly indicate increases production of *n*-alkanes after they have been physically removed by natural processes. Thus, changes in *n*-alkane $\delta D_{nC27-31}$ of *P. strobus* and *T. canadensis* likely reflect changes in internal leaf water throughout the growing season. The relative stability of *n*-alkane concentrations after leaf flush (*P. arundinacea*, *C. americana*) indicates that for these angiosperm plants, the bulk of leaf waxes are formed early in the growing season. For *P.*

arundinacea, peak *n*-alkane production occurs during late April as the weather warms and daylight hours increase, while for *C. americana*, waxes are produced with the bud and decrease with expansion of leaf mass during leaf flush. For these two angiosperms, a temporally biased period of formation indicates that *n*-alkanes from these two plants should reflect early season water δD .

3.1.2. Sediment *n*-alkane abundances

The distribution of sedimentary *n*-alkanes mirrors those of the plants, with maximum abundances of *n*-C₂₉ to *n*-C₃₃, and the most abundant *n*-alkane being *n*-C₂₉ (Fig. 2a, b). Angiosperms exhibit far higher concentrations of long-carbon chain *n*-alkanes than the gymnosperm samples (317 $\mu g/g$ and 3.6 $\mu g/g$ for C₂₉ for *C. americana* and *P. strobus* respectively) and may bias the sedimentary pool (Fig. 3a-d). Indeed, sedimentary *n*-alkanes mirror the distribution in *C. americana* and other similar angiosperm vegetation. Soil *n*-alkane abundances show an almost identical pattern to *C. americana* (Figs. 2b and 3d). This could be due to the fact that *C. americana*, and other deciduous trees shed alkane-rich leaf material each Fall, adding more sedimentary *n*-alkanes than conifers with smaller leaf areas and lower normal alkane abundances. However, the overall concentration and distribution of *n*-alkanes preserved in fluvial sediments will vary as a function of both leaf-wax concentration and overall representation within an ecosystem.

3.1.3. Carbon Preference Index

The CPI of plant *n*-alkanes is a measure of the distribution of odd and even carbon chain compounds and reflects variations in production and loss of different *n*-alkanes. *T. canadensis*

shows modest change in CPI over the growing season (CPI = 5–10), while CPI for *P. strobus* increases steadily (5 to 20) and *P. arundinacea* decreases (25 to 10) (Fig. 4a, b, c). *C. americana* is unique because it shows a sharp increase from below 10 on day 113 to above 50 on day 116, followed by a steep drop to approximately 10 on day 144, corresponding to leaf flush (Fig. 4d). These data point to variations in the timing of wax synthesis and/or degradation for each of the sampled species.

3.1.4. Chain-length distribution of *n*-alkanes in plant samples

Previous studies of *n*-alkanes indicate a relationship between ACL or AHPCL and extreme climate conditions (Dodd and Poveda, 2003; Rommerskirchen et al., 2003). For *P. strobus*, *T. canadensis*, and *P. arundinacea*, there are only minute changes throughout the growing season. The AHPCL for each of these range from 29.1 to 30.3, 28.9 to 30.0, and 28.8 to 30.1 respectively (Fig. 4a–c). *C. americana* experiences an increase in AHPCL over the first 6 weeks from 28.1 to 29.9 where it remained stable for the duration of the season (Fig. 4d).

The chain length range observed for our *P. strobus* is slightly larger than that reported by Tipple and Pagani (2013) in their study of plants along a North American transect. These differences may stem from differences in climate, summer precipitation and ambient relative humidity. Since our plants have roots directly in the water system, we suspect that our specimens have reduced water stress compared to upland plant growth or vegetation in more arid environments. Reduced water stress and/or higher relative humidity may minimize the need to produce longer chain lengths, which have lower vapor pressure and higher melting point, during peak summer conditions. AHPCL in *C. americana* increases from 28.1 to 29.7 from day 113 to

144 (during leaf flush) where it stabilizes for the remainder of the growing season. This trend is similar to other broadleaf angiosperms reported by Tipple et al. (2013) of ~27.5 to 28.9. This indicates that the composition of *n*-alkanes changes most substantially during leaf bud and leaf flush. Coupled with concentration and CPI data, this suggests there is little to no leaf wax production after leaf flush and all the long chain *n*-alkanes are formed at that time (Tipple et al., 2013).

3.2. Seasonal trends in δD values

3.2.1. Stream water isotopes

Plants utilize water from a range of depths within the critical zone (e.g., Dawson and Ehleringer, 1991). We measured the hydrogen isotopic composition of stream water during the sampling period to constrain changes in composition of ambient water available to plant roots below the water table. All sampled plants had visible roots below base-flow groundwater level (less than 1 m below the surface), providing direct access to stream and a means of minimizing potential water stress. Stream δD varied little over the period of sampling, ranging from $-48 \pm 1.7\text{‰}$ in the early spring (days 100–150) to $-42 \pm 2.5\text{‰}$ in peak summer (days 200–300). Water δD varied most during days 150–200 to approximately -57‰ , a 10‰ difference from previous weeks (Fig. 5). This drop followed three weeks of intense rainfall and flooding.

3.2.2. $\delta D_{nC27-nC33}$ for individual plants

Environmental water is the primary hydrogen source for *n*-alkanes synthesized within the leaves (Sachse et al., 2012; Yao and Liu, 2014). Greenhouse and field-based studies show no isotopic change during water uptake in roots (Ellsworth and Williams, 2007 and references therein). As a result, changes in the δD of plant source water should be evident in changes in the composition of *n*-alkanes (e.g., Sachse et al., 2006; Nichols et al., 2010; Eley et al., 2014). Our data show dramatically larger isotopic changes in *n*-alkanes than in source water. This can only result from fractionation after uptake of groundwater due to evaporative loss during transpiration, which would yield isotopically enriched leaf water relative to groundwater, or storage of cold season soil water that is used in biosynthesis early in the growing season. We believe that the latter is unlikely because early season *n*-alkanes show the most enriched δD values, when winter water should be the most depleted.

Weighted $\delta D_{nC27-nC31}$ of *P. strobus* and *P. arundinacea* both decrease throughout the growing season. *P. strobus* shows fairly constant $\delta D_{nC27-nC31}$ ($\sim -194 \pm 5\text{‰}$) until early July, when values decrease to $-215 \pm 11\text{‰}$, while *P. arundinacea* decreases early in the season from $-196 \pm 16\text{‰}$ to a steady value of $\sim -218 \pm 5\text{‰}$ (Fig. 6a, c). In contrast, *T. canadensis* $\delta D_{nC27-nC31}$ remains constant at $\sim -160\text{‰}$ (Fig. 6b). For *C. americana*, $\delta D_{nC27-nC31}$ is initially depleted ($\sim -186 \pm 7\text{‰}$), followed by an increase in δD value throughout leaf flush (days 113–144) to a stable $\sim -152 \pm 8\text{‰}$ (Fig. 6d).

For *P. strobus*, peak 2013 summer temperatures coincided with the transition to more isotopically depleted *n*-C₂₅–*n*-C₃₃ values (days 178 to 248). Previous work shows that conifers have difficulty maintaining open stomata in the daytime during periods of high temperature and low precipitation, but open their stomata at night to allow CO₂ diffusion to the sites of CO₂ fixation (Zweifel et al., 2007). This could lead to a decrease in leaf-water evaporation due to a

change in transpiration. However, since the roots of the pine are directly in the water source, the plant may experience reduced water stress, allowing the plant to maximize CO₂ diffusion into the stomata rather than minimizing water loss, which can be confirmed through a growth chamber study. For *C. americana* and *P. arundinacea*, the most significant changes in *n*-alkane δ D occur during days 113 to 144 of plant growth during leaf flush and peak wax synthesis.

Noise in isotope data from individual plants may result from a mixture of old growth and new growth, however recent high-resolution temporal studies also show strong seasonal variations and heterogeneity within an individual plant (Kahmen et al., 2011; Tipple et al., 2013). This heterogeneity may derive from variability in the source of water used in biosynthesis, the leaf life cycle and timescale of wax synthesis, and plant-specific factors that influence stomatal regulation and transpiration of leaf water (e.g., Chikaraishi and Naraoka, 2003; Smith and Freeman 2006; Hou et al., 2007; Zweifel et al., 2007; Liu and Yang 2008; Pedentchouk et al., 2008; Diefendorf et al., 2011; Gao et al., 2011; McInerney et al., 2011; Garcin et al., 2012; Guenther et al., 2013; Rosado et al., 2013; Wang et al., 2013).

3.2.3. $\delta D_{nC27-nC33}$ for sediment samples

We measured the δ D of individual *n*-alkanes preserved in fluvial sediments collected adjacent to the plant sample locality. The mean seasonal abundance-weighted *n*-C₂₇–*n*-C₃₃ δ D was $-170 \pm 4\%$ (Fig. 7, Supplementary Table 3). The integration of multiple plants over time leads to a more stable isotopic signature than individual plants, which vary greatly over a single growing season. Several studies have observed a spatial correlation between sediment *n*-alkane δ D values and climate variables (Sachse et al., 2004; Polissar and Freeman, 2010; Douglas et al.,

2012; Garcin et al., 2012). Our sediments are less affected by short-term changes in isotopic values than plants, yielding a better record of general paleoenvironmental conditions than data from individual leaves. Fig. 7 shows a comparison of the δD values of the sediments with the weighted δD values of the four sampled plants. Sediments appear to show a muted temporal change over the period of sampling, and smaller than changes observed in all the plants. We calculated the abundance weighted δD of *n*-alkanes for the four sampled plants to assess how well this compares with sediment *n*-alkane δD . Sedimentary organic matter is derived from both terrestrially sourced plant matter as well as microbially cycled organic matter. However, we focused on long-carbon chain *n*-alkanes that are dominantly produced by plants and integrated into the sedimentary flux within the small Fenton catchment. The mean *n*-alkane δD calculated from weekly individual *n*-alkane δD (weighted for δD values and *n*-alkane abundances for each plant) varies throughout the sampling period. This pattern is similar to the δD of *n*-alkanes extracted from the sediment samples, however the magnitude of change is reduced in sediments compared to the abundance weighted calculated value derived from our four plants (Fig. 7). It should be noted that the sediment *n*-alkane δD values are an accumulation of a broader area with more plants. This may explain the slight differences in the patterns and the muted changes expressed in the sediments. These data suggest that sediments are biased by short timescale variability, but it is a second order effect relative to the long-term (multi-year) integration of *n*-alkanes from a variety of plants across a catchment.

3.2.4. *n*-Alkanes and apparent fractionation

Plants with different photosynthetic pathways exhibit distinct apparent fractionation ($\epsilon_{n-alkane}$) due to differences in timing of water synthesis or water use strategies, with C_3 grasses

averaging $\sim -170\text{‰}$, C_4 grasses $\sim -145\text{‰}$, and C_3 trees and shrubs averaging $\sim -120\text{‰}$ (Chikaraishi and Naraoka, 2003; Bi et al., 2005; Liu et al., 2006; Sachse et al., 2006; Smith and Freeman 2006; Hou et al., 2007; Pedentchouk et al., 2008; Tipple et al., 2013). The variability and magnitude of leaf water enrichment can be attributed to various environmental parameters and plant physiological processes (Sachse et al., 2009). One of the primary goals of this experiment was to create a modern day proxy that compared $\delta D_{n\text{-alkane}}$ to δD_{water} in order to better understand how they are related, for use in reconstructing paleohydrology.

The ϵ value for *P. strobus* is relatively constant at $-152 \pm 11\text{‰}$ throughout the early growing season, then decreased steadily to $-174 \pm 9\text{‰}$ in early fall (Fig. 6a). *P. arundinacea* shows a decrease in ϵ early in the growing season with an average of $-156 \pm 16\text{‰}$ over weeks 1 through 9 and achieves a constant value of $-182 \pm 6\text{‰}$ (Fig. 6c). These fractionations are larger than nearly all previously published data for similar plant classes ($\epsilon_{\text{Pinus strobus}} = -128 \pm 12$, $\epsilon_{\text{grass}} \sim -145\text{‰}$; Tipple and Pagani, 2013 and references therein). In contrast, ϵ values for *T. canadensis* remain constant at $-123 \pm 10\text{‰}$ with respect to stream water, while for *C. Americana* they increase from $\sim -145\text{‰}$ during leaf flush to $-111 \pm 6\text{‰}$, after which it remains stable (Fig. 6b,d). Both *T. canadensis* and *C. americana* have ϵ values similar to those published previously.

Eley et al. (2014) suggest that for a constant water source, apparent fractionation should remain constant throughout a growing season since source water is the only hydrogen source. However, the only specimen in our study with effectively constant ϵ is *T. canadensis*. *P. strobus*, *C. americana* and *P. arundinacea* all show a large change in ϵ throughout the growing season despite all accessing the same potential water pool. *C. americana* is the first to attain steady state values ($\sim$ day 130), followed by *P. arundinacea* (day 158) and by *P. strobus* (day 256). We cannot exclude uptake of shallow soil water by individual plants, however the temporal patterns

observed in our *n*-alkane data are not easily explained by temporal shifts in precipitation δD during the growing season. Our data suggest that the major difference in the hydrogen isotopic composition of higher plant *n*-alkanes is not strictly a difference in the timing of wax synthesis, but rather regulation of stomata in response to environmental stressors.

Tipple et al. (2013) observed that for deciduous C_3 species such as *C. americana*, the δD of *n*-alkanes will only reflect the environment at the time of wax synthesis. This interpretation was supported by *n*-alkane concentration, ACL, and δD data. Our data for angiosperms shows that during leaf flush, *n*-alkane concentrations peak, indicating maximum production. After several weeks, concentrations decrease to a stable value. ACL and δD of the *n*-alkanes follow a similar pattern. However, distinct trends in *n*-alkane δD for different plant species and approximately constant groundwater δD show that plants from the same functional group may be located adjacent each other yet show distinct temporal patterns. These data are most plausibly explained by differences in individual plant stomatal regulation in response to environmental variability throughout the growing season. Such variability requires records that integrate heterogeneity between plants in order to properly constrain the ecosystem-level geochemical signals, which can be provided by fluvial sediments.

4. Conclusions

We sampled leaves from four co-occurring plant species in the Fenton River catchment in Storrs, CT throughout the 2013 growing season to evaluate controls on the δD of individual plant *n*-alkanes for different plant functional types (*P. strobus*, *T. canadensis*, *P. arundinacea* and *C. americana*). We specifically selected plants that were closely associated in space and had roots

directly in the river water to evaluate how well these organic proxies reflect ambient water in the environment. River water and sediment samples were collected concurrently with the plant specimens. Our data show distinct timescales of wax synthesis and growth, and temporal shifts in $\epsilon_{n\text{-alkane}}$. The deciduous angiosperm *C. americana* appears to show dominant wax production during leaf flush, followed by minimal change throughout the remainder of the growing season. *P. strobus*, *T. canadensis*, and *P. arundinacea* all show signs of continual leaf wax synthesis but distinct patterns of hydrogen isotopes.

The ϵ values for our plants change significantly throughout the season, with the exception of *T. canadensis*. Observed changes are far larger than the change in river/groundwater δD and indicate strong control of factors such as stomatal regulation, biosynthesis, or timescales of wax synthesis. Proximity to groundwater source may also impact apparent fractionation, as measured ϵ values in this study are larger than studies from upland sites (with the exception of *T. canadensis*) (Chikaraishi and Naraoka, 2003; Bi et al., 2005; Liu et al., 2006; Sachse et al., 2006; Smith and Freeman 2006; Hou et al., 2007; Pedentchouk et al., 2008; Tipple et al., 2013). Sediments integrate biomass from different plant types and possibly represent a weighted ecosystem average of *n*-alkanes. Given the heterogeneity between plants in our study that share a close proximity to the stream edge, our data indicates that the temporal and spatial integration provided by fluvial sediments produces the best organic molecular archive of ecosystem level paleoenvironment and paleohydrology, though this is likely biased by differences in wax production and timescales of integration.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi.org/10.XXX

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Figure Captions

Fig. 1. Leaf *n*-alkane concentrations for (a) *P. strobus*, (b) *T. canadensis*, (c) *P. arundinacea*, and (d) *C. americana* throughout the growing season. Concentrations represent the total *n*-alkane concentration relative to leaf dry weight (ng/g). For *C. americana* (d) leaf flush occurred during days 113 to 137, as indicated by the gray box.

Fig. 2. (a) Total *n*-alkane abundances from collected sediments throughout the growing season in ng/g of dry material. Since sediments are assumed to incorporate the organic matter from the surrounding environment, (b) shows the weighted abundances of each *n*-alkane of all sampled plant species combined throughout the growing season in ng/g of dry material. Similar abundance patterns are seen in both graphs (C₂₉ has the largest abundance for both).

Fig. 3. The carbon preference index (CPI) and average higher plant chain length (AHPCL) for (a) *P. strobus*, (b) *T. canadensis*, (c) *P. arundinacea*, and (d) *C. americana* throughout the growing season. Leaf flush occurred during days 113 to 137, as indicated by the gray box.

Fig. 4. δD of stream water throughout collection period. Water was collected from the center of the stream and measured in triplicate with standard precision $\pm 3\text{‰}$.

Fig. 5. Abundance weighted $\delta D_{C27-C33}$ for sediments throughout the growing season compared with abundance weighted $\delta D_{C27-C33}$ for all plant species measured.

Fig. 6. Isotopic composition ($\delta D_{C27-C33}$) and apparent fractionation (ϵ) of: (a) *P. strobus*, (b) *T. canadensis*, (c) *P. arundinacea*, and (d) *C. americana* throughout the growing season. Leaf flush occurred during days 113 to 137, as indicated by the gray box.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7