Voelker *et al*. Isolating paleo relative humidity: dual isotopes $\delta^{18}O$ and δD as deuterium deviations from the global meteoric water line. *Ecological Applications*.

Appendix A. We used a variant of the steady state Craig-Gordon (C-G) model and the mechanistic framework for leaf water (Roden et al. 2000, Barbour et al. 2004) to predict $\delta^{18}O$ and δD and then calculated deuterium deviations (Δd) from the modeled $\delta^{18}O$ and δD. Model estimates of Δd were compared to measured Δd values from leaf water and cellulose to help understand the driving factors controlling the Δd variation. Fractionation factors differ between the two isotopes, but the structure of the leaf water model is the same for both. We predicted steady state isotopic variation, relative to source water, at the site of evaporation within a leaf [Δ_e for either of $\Delta^{18}O_e$ or ΔD_e,] according to the C-G model (Craig and Gordon 1965, Farquhar and Lloyd 1993):

$$\Delta_e = \varepsilon^+ + \varepsilon_k + (\Delta_v - \varepsilon_k)\frac{e_a}{e_i} \qquad \text{(A.1)}$$

where ε^+ is the temperature-dependent equilibrium fractionation factor between liquid and vapor water (Cappa et al. 2003), ε_k is the kinetic fractionation factor for water-vapor diffusion determined by the balance of stomatal and boundary layer conductance to water vapor, Δ_v is the isotope ratio of atmospheric water vapor (R_v) relative to source water (R_s) (i.e., $\Delta_v = ((R_v/R_s) - 1) \times 1000$), e_a is the ambient vapor pressure and e_i is the saturation vapor pressure at leaf temperature. Equation (A.1) predicts the enrichment above source water at the site of evaporation. However, water extracted from leaves includes a mixture of source water and water enriched by transpiration (Yakir et al. 1989). The isotopic signal recorded in photosynthates should reflect mixing of these pools in leaf lamina water, which can be described by the advection of [unenriched] xylem water into the leaf lamina being opposed by the diffusion of the

enriched Δ_e signal (Farquhar and Lloyd 1993). This advective/diffusive process can be described by a leaf's Péclet number ($\wp$):

$$\wp = \frac{LE}{CD} \qquad \text{(A.2)}$$

where E is the transpiration flux from the leaf (mmol m^{-2} s^{-1}), L is the effective pathlength for water through the leaf (m), C is the molar concentration of water (55,500 mol m^{-3}) and D is the diffusivity of heavy molecules in water (2.66 and 2.34 × 10^{-9} m^2 s^{-1} for molecules containing ^{18}O and D, respectively (Wang 1954)). In the present study, E was estimated as the product of stomatal conductance and the ratio of the leaf-to-atmosphere vapor pressure difference (i.e., $e_a - e_i$) over the barometric pressure. For the hardwoods and conifers investigated here, stomatal conductance was set to 0.2 and 0.1 mol m^{-2} s^{-1}, respectively. Although these values are arbitrary, they are representative of the maximum values measured in the field for mature *Quercus macrocarpa* (Voelker et al. 2013) and *Pseudotsuga menziesii* (Woodruff et al. 2010) and should roughly correspond to the difference between angiosperms and conifers (Lammertsma et al. 2011). For the results presented here, we assumed C and D to be constant. We set L to scale with transpiration rate following the empirical relationship in Song et al. (2013):

$$L = 0.094 E^{-1.20} \qquad \text{(A.3).}$$

This approximation of L appears to work relatively well for hardwood and conifer species (Song et al. 2013), but the underlying mechanisms are not fully understood. Following Farquhar and Lloyd (1993), the Péclet number can be applied to Δ_e to estimate the isotopic enrichment of leaf lamina water above source (i.e., xylem) water (Δ_l):

$$\Delta_l = \frac{\Delta_e\left(1 - e^{-\wp}\right)}{\wp} \qquad \text{(A.4).}$$

Once Δ_l and Δ_e were estimated for both isotopes, we calculated $\Delta d = \delta D - (8 \times \delta^{18}O + 10)$ of leaf lamina bulk water (Δd_l) and at the sites of evaporative enrichment in leaves (Δd_{le}).

With the leaf water signal defined as above, the isotopic signal of plant cellulose (δ_c) can be described after Roden et al. (2000) as:

$$\delta_c = f\,(\delta_s + \varepsilon_H) + (1 - f)(\delta_s + \Delta_l + \varepsilon_A) \qquad \text{(A.5)}$$

where f is the proportion of atoms that exchange with source water during post-photosynthetic metabolism leading to cellulose synthesis (subscripts s = source water and l = leaf water). The values of f for ^{18}O and D were set to 0.42 and 0.35, respectively. For ^{18}O the fractionation factor associated with carbonyl-water interactions during sucrose synthesis (i.e. autotrophic, ε_A) and cellulose synthesis (i.e. heterotrophic, ε_H) were set equal to 27‰. For δD, fractionation factors associated with carbohydrate metabolism, ε_A and ε_H, were set to -171‰ and +158‰, respectively (Sternberg et al. 1986, Yakir and DeNiro 1990, Luo and Sternberg 1992). For modeling leaf water from cellulose, the canopy integrated leaf temperature (T_l) was set to the mean daily May–August temperature.

Finally, the post-photosynthetic portion of the Roden et al. (2000) model can be applied such that cellulose $\delta^{18}O$ and δD can be used to predict deuterium deviations in leaf lamina water (Δd_c). For modeling Δd_c from cellulose, Equation (A.5) was solved for Δ_l and then δ_s was added to Δ_l to obtain each leaf water isotope value. For $\delta^{18}O$ and δD this simplifies as:

$$\Delta_l = \frac{\delta_c - f(\delta_s + \varepsilon_H)}{1 - f} + \varepsilon_A \qquad \text{(A.6).}$$

For modeling deuterium deviations at the sites of evaporative enrichment (Δd_{ce}), both components of E, e_a - e_i and canopy-integrated stomatal conductance, may be unknowns. Nonetheless, for comparing Δd_c versus Δd_{ce}, we calculated the difference between Δ_e and Δ_l using equations A.1-A.4 as described above and added this value to Δ_l (as estimated from cellulose) to yield estimates of Δ_e (as estimated from cellulose).

Evidence suggests that ε_H varies with temperature (Sternberg and Ellsworth 2012), which would predict Δd_c or Δd_{ce} to be somewhat less negative than that predicted by the model described above at temperatures of cellulose synthesis lower than about 17.3° C and slightly more negative above 17.3° C. For studies using paleo cellulose, temperatures are likely to be poorly constrained so it would be difficult to incorporate this effect. Preliminary analyses using known growing season temperatures also suggested that slopes of Δd_c or Δd_{ce} vs. RH as well as the amount of variation predicted between these variables within or across sites did not change appreciably after incorporating this effect. As such the Δd_c or Δd_{ce} data we report here do not incorporate temperature effects on ε_H.

LITERATURE CITED

Barbour M. M. J. S. Roden, G. D. Farquhar, and J. R. Ehleringer. 2004. Expressing leaf water and cellulose oxygen isotope ratios as enrichment above source water reveals evidence of a Péclet effect. Oecologia 138:426–435.

Cappa, C. D., M. B. Hendricks, D. J. DePaolo, and R. C. Cohen. 2003. Isotope fractionation of water during evaporation. Journal of Geophysical Research 108:(D16), 4525, doi 4510.1029/2003JD003597

Craig, H., and L. I. Gordon 1965. Deuterium and oxygen-18 variations in the ocean and the marine atmosphere. *In* Proceedings of a Conference on Stable Isotopes in Oceanographic Studies and Palaeotemperatures (ed. E. Tongiorgi), pp. 9-130.Lischi and Figli, Pisa.

Farquhar, G. D., and J. Lloyd. 1993. Carbon and oxygen isotope effects in the exchange of carbon dioxide between terrestrial plants and the atmosphere. *in* Stable Isotopes and Plant Carbon-Water Relations. J. R. Ehleringer, A. E. Hall, and G. D. Farquhar), pp. 47–70. Academic Press, San Diego, California, USA.

Lammertsma, E. I. H. Jan de Boer, S. C. Dekker, D. L. Dilcher, A. F. Lotter, and F. Wagner-Cremer. 2011. Global CO_2 rise leads to reduced maximum stomatal conductance in Florida vegetation. Proceedings of the National Academy of Sciences 108:4041–4046.

Luo, Y. H., and L. Sternberg. 1992. Hydrogen and oxygen isotope fractionation during hetertrophic cellulose synthesis. Journal of Experimental Botany 43:7–50.

Roden, J. S., G. Lin, and J. R. Ehleringer. 2000. A mechanistic model for interpretation of hydrogen and oxygen isotope ratios in tree-ring cellulose. Geochimica et Cosmochimica Acta 64:21–35.

Sternberg, L., M. J. DeNiro, and R. A. Savidge. 1986. Oxygen isotope exchange between metabolites and water during biochemical reactions leading to cellulose synthesis. Plant Physiology 82:423–427.

Song, X., M. M. Barbour, G. D. Farquhar, D. R. Vann, and B. R. Helliker. 2013. Transpiration rate relates to within- and across species variations in effective path length in a leaf water model of oxygen isotope enrichment. Plant, Cell and Environment. doi: 10.1111/pce.12063

Sternberg, L., and P. F. V. Ellsworth. 2012. Divergent biochemical fractionation, not convergent temperature, explains cellulose oxygen isotope enrichment across latitudes. PLoS ONE 6: doi:10.1371/journal.pone.0028040.

Voelker, S. L., F. C. Meinzer, B. Lachenbruch, J. R. Brooks, and R. P. Guyette. 2013. Drivers of radial growth and carbon isotope discrimination of bur oak (*Quercus macrocarpa* Michx.) across continental gradients in vapor pressure deficit and irradiance. Plant, Cell & Environment. *In Press*.

Wang, J. H. 1954. Theory of self diffusion of water in protein solutions: a new method for studying the hydration and shape of protein molecules. Journal of the American Chemical Society 76:4755–4763.

Woodruff, D. R., F. C. Meinzer, and K. A. McCulloh. 2010. Height-related trends in stomatal sensitivity to leaf-to-air vapour pressure deficit in a tall conifer. Journal of Experimental Botany 61:203–210.

Yakir, D., M. J. DeNiro, and P. W. Rundel. 1989. Isotopic inhomogeneity of leaf water: Evidence and implications for the use of isotopic signals transduced by plants. Geochimica et Cosmochimica Acta 53:2769–2773.

Yakir, D., and M. J. DeNiro. 1990. Oxygen and hydrogen isotope fractionation during cellulose metabolism in *Lemna gibba* L. Plant Physiology 93:325–332.