Variability in the carbon isotopic composition of foliage carbon pools (soluble carbohydrates, waxes) and respiration fluxes in southeastern U.S. pine forests

Behzad Mortazavi,[1,2,3] Maureen H. Conte,[4,5] Jeffrey P. Chanton,[1] J. C. Weber,[5] Timothy A. Martin,[6] and Wendell P. Cropper Jr.[6]

Received 27 September 2011; revised 13 February 2012; accepted 18 February 2012; published 19 April 2012.

[1] We measured the $\delta^{13}C$ of assimilated carbon (foliage organic matter (δC_{OM}), soluble carbohydrates (δC_{SC}), and waxes (δC_W)) and respiratory carbon (foliage (δC_{FR}), soil (δC_{SR}) and ecosystem $^{13}CO_2$ (δC_{ER})) for two years at adjacent ecosystems in the southeastern U.S.: a regenerated 32 m tall mature *Pinus palustris* forest, and a mid-rotation 13 m tall *Pinus elliottii* stand. Carbon pools and foliage respiration in *P. palustris* were isotopically enriched by 2‰ relative to *P. elliottii.* Despite this enrichment, mean δC_{ER} values of the two sites were nearly identical. No temporal trends were apparent in δC_{SC}, δC_{FR}, δC_{SR} and δC_{ER}. In contrast, δC_{OM} and δC_W at both sites declined by approximately 2‰ over the study. This appears to reflect the adjustment in the $\delta^{13}C$ of carbon storage reserves used for biosynthesis as the trees recovered from a severe drought prior to our study. Unexpectedly, the rate of $\delta^{13}C$ decrease in the secondary C_{32-36} *n*-alkanoic acid wax molecular cluster was twice that observed for δC_{OM} and the predominant C_{22-26} compound cluster, and provides new evidence for parallel but separate wax chain elongation systems utilizing different carbon precursor pools in these species. δC_{FR} and δC_{ER} were consistently enriched relative to assimilated carbon but, in contrast to previous studies, showed limited variations in response to changes in vapor pressure deficit (*D*). This limited variability in respiratory fluxes and δC_{SC} may be due to the shallow water table as well as the deep taproots of pines, which limit fluctuations in photosynthetic discrimination arising from changes in *D*.

Citation: Mortazavi, B., M. H. Conte, J. P. Chanton, J. C. Weber, T. A. Martin, and W. P. Cropper Jr. (2012), Variability in the carbon isotopic composition of foliage carbon pools (soluble carbohydrates, waxes) and respiration fluxes in southeastern U.S. pine forests, *J. Geophys. Res.*, *117*, G02009, doi:10.1029/2011JG001867.

1. Introduction

[2] A key variable fundamental for using $\delta^{13}CO_2$ for investigating carbon transformations in the biosphere is photosynthetic carbon isotope discrimination (Δ) [*Farquhar et al.*, 1989]. At the leaf scale, Δ can be calculated from mass balance of $\delta^{13}C$ and CO_2 concentration of air entering and leaving a leaf chamber [*Evans et al.*, 1986] or from measurements of the ratio of intercellular to ambient air CO_2 concentrations (C_i/C_a) that can then be related to Δ [*Farquhar et al.*, 1989]. Measuring Δ at scales larger than the leaf or branches [*Wingate et al.*, 2007] remains challenging [*Wingate et al.*, 2010]. For scales larger than the leaf, proxies are used to infer Δ. At the ecosystem scale, for example, the $\delta^{13}C$ of products of photosynthesis such as leaf carbohydrates (δC_{SC}) [*Brugnoli et al.*, 1988], phloem sugars [*Scartazza et al.*, 2004; *Barbour et al.*, 2005], leaf water soluble organics [*Brandes et al.*, 2006], or bulk leaf organic matter (δC_{OM}) [*Farquhar et al.*, 1989] have been used to infer recent and seasonally integrated Δ. Additionally, the $\delta^{13}C$ of ablated leaf waxes (δC_W) have been used to infer Δ at the regional [*Conte et al.*, 2003] to the sub-continental scale [*Conte and Weber*, 2002]. Photosynthetic discrimination has also been estimated from eddy covariance measurements of water flux and a Penman-Monteith inversion [*Ogée et al.*, 2003] to calculate canopy conductance, or from biophysical models with varying degrees of complexity [*Chen and Chen*, 2007; *Aranibar et al.*, 2006; *Baldocchi and Bowling*, 2003].

[3] *Bowling et al.* [2002] demonstrated that the $\delta^{13}C$ of ecosystem respired CO_2 (δC_{ER}) responded to changes in vapor pressure deficit (*D*) in a nonlinear fashion and suggested that this relationship was consistent with changes in

[1]Department of Oceanography, Florida State University, Tallahassee, Florida, USA.
[2]Now at Department of Biological Sciences, University of Alabama, Tuscaloosa, Alabama, USA.
[3]Dauphin Island Sea Laboratory, Dauphin Island, Alabama, USA.
[4]Bermuda Institute of Ocean Sciences, St. Georges, Bermuda.
[5]Ecosystems Center, Marine Biological Laboratory, Woods Hole, Massachusetts, USA.
[6]School of Forest Resources and Conservation, University of Florida, Gainesville, Florida, USA.

0148-0227/12/2011JG001867

Δ resulting from adjustments in stomatal conductance to moisture deficit. Based on the analysis of 11 ecosystems, *Alstad et al.* [2007] concluded that the observed relationships between δC_{ER} and environmental variables was indeed consistent with changes in Δ through physiological control on C_i/C_a by adjustment of stomatal conductance and photosynthesis [*Ehleringer and Cerling*, 1995].

[4] When the $\delta^{13}C$ of carbon pools or respired CO_2 are used as proxies to infer Δ it is typically assumed that they reflect ^{13}C fractionation during photosynthesis [*Farquhar et al.*, 1982], as modified by a relatively fixed biosynthetic offset. However, recent findings on fractionation during post photosynthetic carbon transformations as well as respiration call into question the validity of this assumption [*Rascher et al.*, 2010; *Hobbie and Werner*, 2004; *Tcherkez et al.*, 2011; *Brüggemann et al.*, 2011]. For example, as a result of fractionation steps in the Calvin cycle, phloem sugars that result from transitory starch degradation at nighttime are $\delta^{13}C$ enriched, while the daytime phloem sugars that originate from triose phosphates following the aldolase/transkeolase reactions are $\delta^{13}C$ depleted [*Gessler et al.*, 2008; *Tcherkez et al.*, 2004; *Tcherkez et al.*, 2011]. The $\delta^{13}C$ of phloem sugars has therefore a diel variability that is superimposed on Δ.

[5] Isotopic labeling experiments furthermore have shown that soluble carbohydrates are partitioned into fast and slow turnover pools and that the relative size of these pools is species specific [*Mortazavi et al.*, 2009]. Thus, the larger and/or slower turnover pool will buffer the $\delta^{13}C$ signal of recent Δ imprinted on the plant's carbon pools. For example, leaf wax biosynthesis in the pine *Pinus elliottii* (slash pine) and in the deciduous tree *Liquidambar styraciflua* (sweetgum) draw significantly on carbon reserves as opposed to recently assimilated photosynthates, whereas in corn (*Zea mays*), which does not have significant reserves, wax $\delta^{13}C$ more closely reflects recently assimilated carbon [*Mortazavi et al.*, 2009]. Therefore, δC_{SC} and δC_W yield information on a temporally integrated Δ, but with an integration time that is species specific and will more than likely vary with the growth dynamics of the plant.

[6] Because substrates supporting autotrophic respiration are influenced by post photosynthetic fractionation [*Tcherkez et al.*, 2003] and supported by long turnover carbon pools [*Mortazavi et al.*, 2009; *Nogués et al.*, 2004)] the degree to which respired $\delta^{13}CO_2$ reflects Δ remains unclear. Foliage respiration, for example, can be enriched by several ‰ relative to δC_{SC} [*Ghashghaie et al.*, 2003]. In cases where aboveground respiration contributes significantly to nighttime ecosystem respiration [*Lavigne et al.*, 1997; *Mortazavi et al.*, 2006], estimate of Δ based upon δC_{ER} could be biased. In addition, δC_{ER} represents the flux-weighted contribution of heterotrophic and autotrophic respiration to ecosystem respiration. Because heterotrophic respiration is fueled by organic matter of different ages ranging from recent (e.g., litter) to decades old [*Trumbore*, 2000], the δC_{CR} contribution to δC_{ER} will reflect a temporally integrated $\delta^{13}C$ of carbon used for respiration that may differ significantly from recent Δ. *Bowling et al.* [2008] reported that with the exception of root respired CO_2, $\delta^{13}C$ fluxes in terrestrial ecosystems were enriched relative to δC_{OM}.

[7] Despite these limitations, numerous investigations have used the $\delta^{13}C$ of respiratory fluxes or carbon pools as proxies to estimate Δ at scales ranging from the leaf [*Brugnoli et al.*, 1988] to the ecosystem [*Wingate et al.*, 2010; *Buchmann et al.*, 1998; *Lai et al.*, 2005], and to the continental [*Conte and Weber*, 2002], and have related the temporal variability in the proxies of Δ to environmental variability [*Bowling et al.*, 2002; *Conte et al.*, 2003; *Knohl et al.*, 2005; *McDowell et al.*, 2004a, 2004b]. These investigations have yielded patterns that are consistent with the physiological understanding at the leaf level scale of assimilated carbon $\delta^{13}C$ [*Farquhar et al.*, 1989; *Dawson et al.*, 2002].

[8] Young pine forests and plantations of the southeastern U.S. appear to be among the largest terrestrial carbon sinks [*Lai et al.*, 2002; *Valentini et al.*, 2000; *Wofsy et al.*, 1993; *Clark et al.*, 1999; *Hollinger et al.*, 1994]. These ecosystems are the dominant vegetation type in a mosaic that also includes mature second growth deciduous and pine forests. In this study we examined the $\delta^{13}C$ of assimilated and respired carbon in a naturally regenerated *Pinus palustris* (Longleaf pine) forest and in a mid-rotation *Pinus elliottii* (Slash pine) plantation that are located 6 km apart in central Florida. Our goals were to examine the temporal variability in (i) foliage-, soil-, and ecosystem-respired $\delta^{13}CO_2$ reflected the $\delta^{13}C$ of foliage carbon pools (soluble carbohydrate and leaf waxes) and (ii) to determine to what extent the $\delta^{13}C$ of these carbon pools and of respired CO_2 reflected variability in environmental factors, for example, changes in *D.*

2. Methods

2.1. Study Sites

[9] Our study was conducted from June 2004 to June 2006 at two experimental forest stands, separated by ~6 km, that are managed by the School of Forest Resources and Conservation at the University of Florida. Soils at both sites are ultic alaquods with a shallow water table that fluctuates between the surface and 130 cm [*Clark et al.*, 2004]. Hurricane landfalls or severe droughts occur approximately every 15 years [*Gholz and Boring*, 1991], with droughts occasionally leading to wildfires.

[10] The first site, in Austin Cary Memorial Forest (AC) (29°44′N, 82°13′W), is an 85-year-old naturally regenerated forest with an open, 32 m tall overstory dominated by longleaf pine (*Pinus palustris*, 72% of basal area) and slash pine (*Pinus elliottii* var. *elliottii*, 28% of basal area) [*Gonzalez-Benecke et al.*, 2011; *Powell et al.*, 2008]. Prescribed fire is applied every 3–5 years to encourage natural regeneration and to control hardwood species. The stand was burned in January of 2003 prior to our study, and then again in January 2006.

[11] The second site, the Donaldson Tract (DT) (29°45′N, 82°10′W), is a mid-rotation *Pinus elliottii* var. *elliottii* tree plantation. The stand was planted in 1990 at a density of 2000 trees ha^{-1} following a clear-cut. Understory biomass at DT was about 5% of total ecosystem biomass [*Clark et al.*, 1999], and is estimated to contribute 7%–9% of total ecosystem net primary production [*Gholz and Cropper*, 1991].

[12] *Powell et al.* [2008] measured carbon exchange at these sites for four years between 2000 and 2002 and in 2004–2005, overlapping the beginning of our study. The 2000–2002 period corresponded to a period of record

drought for the region, with extended periods with a water table depth of >2 m at both sites. Extended spring droughts also occurred in both 2003 and 2004 and resulted in a large precipitation deficit that persisted until autumn 2004, just as our study began.

2.2. Meteorological Measurements

[13] Continuous measurements of photosynthetic photon flux density (PPFD) (LI-190, LI-COR, Inc.), incoming shortwave radiation (LI-200, LI-COR, Inc.), net radiation (Q7, Radiation and Energy Balance Systems, Inc.), air temperature and relative humidity (HMP35D-A, Vaisala, Inc.), wind speed and direction (R3, Gill Instruments, Ltd.), precipitation (TE525, Texas Instruments, Inc.) were collected at the top of walk-up towers at both sites. Additionally, water table depth was measured next to each tower with a Steven's water gauge (F-68, Leupold and Stevens, Inc.). D was calculated from measured temperature and humidity values.

2.3. Leaf to Bulk Air Vapor Pressure Gradient

[14] We calculated the leaf to bulk air vapor pressure gradient ($D_{\text{surf-air}}$), which reflects the evaporative demand as sensed at the leaf surface, incorporating departures of leaf temperature from air temperature. Average leaf temperature was approximated as surface temperature, which was estimated from weather station and eddy covariance data [*Bracho et al.*, 2012]:

$$T_s = T_{air} + \frac{H}{\rho_a C_p g_a}$$

where T_s is surface temperature (°C), T_{air} is measured air temperature (°C), H is sensible heat flux (W m^{-2}), ρa is air density (kg m^{-3}), Cp is specific heat capacity of air (J kg^{-1} $°C^{-1}$), and ga is aerodynamic conductance (m s^{-1}). At each site $D_{\text{surf-air}}$ was then calculated as the difference between saturation vapor pressure at T_s and air vapor pressure measured at the weather station.

2.4. Foliage and Soil Collections

[15] Foliage from *P. palustris* at AC and *P. elliottii* at DT was collected approximately every three weeks at sunset using a shotgun to obtain needles from mid-crown branches of several trees. Foliage collections were made on the same trees from which needles were used for foliage respiration (δC_{FR}) measurements (See Section 2.5.1). Needles collected in 2004 were analyzed for bulk organic matter (δC_{OM}) only. Needles collected in 2005–2006 were divided into three replicates for determination of δC_{OM}, δC_{SC} and δC_W and immediately placed in liquid nitrogen. In addition, foliage from the top of the canopy was also collected from a second group of *P. elliottii* trees at DT between February and August 2005. Needles from terminal branches of three trees were pooled and measured for total carbon and wax concentrations and isotopic compositions.

[16] Soil sample profiles were collected on 13 July 2004 at two locations at each site. Duplicate soils cores were collected and soil samples from 0 to 2, 4–5, 9–10, 14–15, 20–21 and 40–41 cm below the soil surface were collected. Roots were removed, samples were dried and ground to a fine power for the determination of carbon content and the $\delta^{13}C$ of soil organic matter.

2.5. Respiration Measurements

2.5.1. Foliage

[17] Gas samples of foliage respiration for determination of δC_{FR} were collected predawn on dates when foliage was collected for total carbon and wax analyses. Needles from mid-canopy branches of two trees at each site were measured. Needles from the same year cohort were excised and pooled to generate one sampling run. The respired CO_2 from the excised needles was measured using a sequenced-air-sampling system previously described in detail [*Prater et al.*, 2006].

2.5.2. Ecosystem

[18] δC_{ER} was calculated from measurements of CO_2 concentration and isotopic composition using the Keeling plot method, as previously described [*Mortazavi et al.*, 2005, 2006]. δC_{ER} was determined on or near the same dates as samples were collected for foliage respiration and carbon analyses. For each measurement, air samples were collected predawn at eight different heights (0.01, 1, 2, 10, 16, 20, 26 and 30 m) within the canopy at AC and at seven different heights (0.01, 0.5, 2, 6, 10, 12 and 18 m) at DT; total collection time was <1 h.

2.5.3. Soil

[19] At each site, soil CO_2 fluxes were measured using two replicate static chambers (65 × 65 cm base and a volume of 102 L, including the collar base) placed within 5 m of each other. The chamber collars were permanently inserted into the soil to a depth of 2 cm prior to the initiation of the study. A fan inside each chamber gently circulated the air. Replicate gas samples (125 ml) from the chamber headspace were drawn with a syringe from a sample port, while a port at the other end of the chamber was left open to the atmosphere to minimize variations in pressure. Gas samples were stored and processed according to *Mortazavi et al.* [2005, 2006]. Previous comparison of soil $\delta^{13}CO_2$ efflux determined with the chambers and open-topsoil-mini towers as well as soil probes have shown no bias in the chamber measurements in the sandy soils in Florida [*Mortazavi et al.*, 2004].

2.6. Laboratory Analyses

[20] The $\delta^{13}CO_2$ of ecosystem respiration, foliage respiration, and soil respiration samples was determined using a gas chromatograph isotope ratio mass spectrometer (GC-IRMS) (GC: Hewlett Packard 5890 Series II, IRMS: Finnigan Delta S operated in continuous flow mode (*Mortazavi and Chanton*, 2002). The CO_2 concentrations of the foliage and soil respiration samples were calculated from the CO_2 voltages obtained by the GC-IRMS [*Mortazavi and Chanton*, 2002; *Mortazavi et al.*, 2005]. CO_2 concentrations of the ecosystem respiration samples were determined with a LI-COR 6200 portable photosynthesis system (LI-COR, Inc.) calibrated with a NOAA reference gas (363.5 ppm CO_2). Isotopic ratios are presented in the δ notation and are reported relative to VPDB. Analytical precision for isotopic measurements was ±0.2‰ based on repeated measurements of a laboratory-working standard. The intercept of the Keeling plots for ecosystem-, foliage-, and soil-respired CO_2 was then determined with ordinary least square regressions

[*Zobitz et al.*, 2006] applied to the CO_2 isotopic ratios and associated $1/CO_2$ concentrations of the samples.

[21] δC_{SC} was determined on freeze-dried ground needles. The soluble carbohydrates were purified using an ion-exchange technique [*Göttlicher et al.*, 2006] as described in detail in *Mortazavi et al.* [2009]. Briefly, freeze-dried foliage samples (100 mg) were ground to a fine powder with a ball mill and then extracted with 1.5 ml of methanol\chloroform\ water (12:5:3, v\v\v) for 30 min at 70°C. The samples were cooled and then centrifuged (10,000 *g*, 3 min). The supernatant (0.8 ml) was removed and mixed with water (0.8 ml) and chloroform (0.25 ml). Samples were centrifuged (10,000 g, 2 min) to separate the chloroform and aqueous phases. The upper phase (1.2 ml) was removed and 0.5 ml of chloroform added. The sample was then mixed and centrifuged to separate the phases. The upper phase was transferred to a vial, evaporated to dryness, and then redissolved in DI water. The extracted samples were next run through a combination of ion exchange resins (cation-exchange: DOWEX 50 W × 8, 50–100 mesh prepared in the H^+-form, anion-exchange DOWEX 1 × 8, 50–100 mesh prepared in the formate-form). The eluate was collected, dried in vacuo and redissolved in 0.2 ml ethanol. An aliquot was removed to a tin capsule and dried for $\delta^{13}C$ determination at UC Davis Stable Isotope Facility. A working standard (freeze-dried foliage) was processed for every four experimental samples.

[22] Waxes were measured in both the 2004 and 2005 cohorts of needles obtained from mid-canopy branches of *P. elliottii* and of *P. palustris* and from the same branch as needles analyzed for δC_{OM} and δC_{SC}. Needles from two trees were pooled at each site. In addition, we also measured waxes in *P. elliottii* in needles from terminal branches at the top of the canopy (canopy top). Needles from three trees were pooled for the canopy data.

[23] Wax isotopic analytical procedures have been detailed in *Conte et al.* [2003] and *Mortazavi et al.* [2009]. Briefly, waxes were extracted in dichloromethane after addition of an internal standard mixture (21:0 *n*-alkanol, 23:0 *n*-alkanoic acid, 36:0 *n*-alkane, 5A cholestane). The total extract was evaporated to dryness, resuspended in 4:1 dichloromethane: methanol and passed through a minicolumn containing anhydrous Na_2SO_4 to remove residual water. The extract was evaporated just to dryness, resuspended in toluene and transesterified using 5% methanolic HCl (55°C, 12 h). The transesterified products were extracted into hexane, dried and then trimethylsylilated under N_2 using BSTFA + 1% TMCS in pyridine (55°C, 1 h). The individual compounds were quantified using a Fisons 8000 series GC fitted with a Chrompack CPSil5CB column (60 m × 0.25 mm, 0.25 μm film thickness, H_2 carrier gas). The GC programming was 50 to 150°C at 10°C min^{-1} and from 150 to 320°C at 4°C min^{-1} with a 30 min isothermal hold at 320°C.

[24] For isotopic analyses of the *n*-alkanoic acids, the samples were saponified, urea adducted to isolate the *n*-alkyl chain compounds and then purified by solid phase extraction (SPE) using the methods detailed in *Conte and Weber* [2002]. The $\delta^{13}C$ of the individual compounds was determined using a GV IsoPrime GC-mass spectrometer at the MBL Ecosystems Center Mass Spectrometry Facility. A standard mixture containing *n*-alkanoic acids of known isotopic composition was derivatized with the samples and was used to correct for the $\delta^{13}C$ of the derivatization group (CH_3). The median measurement error was 0.32‰ and did not differ significantly for the individual compounds.

[25] Bulk organic matter $\delta^{13}C$ was determined on freeze-dried ground needles at the MBL isotopic facility, using a Europa ANCA-SL elemental analyzer gas chromatograph preparation system attached to a continuous-flow Europa 20–20 gas source stable isotope ratio mass spectrometer.

3. Results

3.1. Environmental Conditions

[26] Mean daytime temperatures varied between 3 and 29°C (Figure 1a). Mean daytime *D* at DT and AC were 0.95 kPa and 0.97 kPa, respectively (Figure 2a and 2c). Maximum values of daytime *D* at DT and AC were 2.67 kPa and 2.59 kPa, respectively (Figure 2a and 2c). The mean values for water table depth were 93.0 and 69.7 cm at DT and AC, respectively (Figures 2b and 2d). Water table depth was at its deepest levels in June 2004 (Figure 2b and 2d) and precipitation deficit for 2004 was not relieved until the three tropical storms in later summer and early fall (Figure 1c). Annual precipitation in 2004 was 1496 mm, which exceeded the long-term (1975–2008) mean of 1226 mm (National Climatic Data Center, http://www.ncdc.noaa.gov/oa/ncdc.html, 2009). Annual precipitation in 2005 (1182 mm) was close to the long-term mean.

3.2. Carbon Isotope Ratios of Foliar Pools

[27] *Bulk organic matter:* Average δC_{OM} for the combined 2004 and 2005 flush data for *P. elliottii* at DT (−29.68 ± 0.23‰, mean ±1 SE, n = 50) was ^{13}C depleted on average by roughly 2‰ relative to *P. palustris* at AC (−27.33 ± 0.17‰, n = 49) (Figure 3a). The *P. elliottii* foliage collected at mid-canopy height at DT was ^{13}C depleted by roughly 1‰ in comparison to foliage collected at canopy height (Figure 3a). Additionally, the newly emergent 2005 flush of needles was $\delta^{13}C$ enriched by ∼1‰ relative to needles from the 2004 flush (Figure 3a).

[28] *Soluble carbohydrates:* δC_{SC} was $\delta^{13}C$ depleted on average by approximately 1‰ relative to δC_{OM} at both sites. Consistent with δC_{OM}, the δC_{SC} of *P. elliottii* at DT (−30.38 ± 0.10‰, n = 70) was also depleted by roughly 2‰ relative to *P. palustris* at AC (−28.38 ± 0.18‰, n = 28) (Figure 3b). No temporal trend in δC_{SC} was apparent over the study period.

[29] *Leaf waxes:* Temporal trends in needle wax *n*-alkanoic acid concentration are shown in Figure 4. Total needle wax concentration (sum of the $C_{20–36}$ *n*-alkanoic acids) at the beginning of the study period was 40% higher in *P. elliottii* at the DT site than in *P. palustris* at the AC site (700 versus 500 μg gdw^{-1}, respectively) (Figure 4a). Wax concentrations decreased during the study at both sites, but the decrease at the DT site (−0.37 μg/gdw d^{-1}), although more variable, was 80% faster than the AC site (−0.21 μg/gdw day^{-1}). Thus, by the end of the study wax concentrations in trees at both sites were nearly comparable.

[30] A striking difference was observed in the concentrations of the individual wax *n*-alkanoic acids between the sites, specifically the relative differences in the short-chain C_{20} and C_{22} compounds and in the long chain $C_{32–36}$

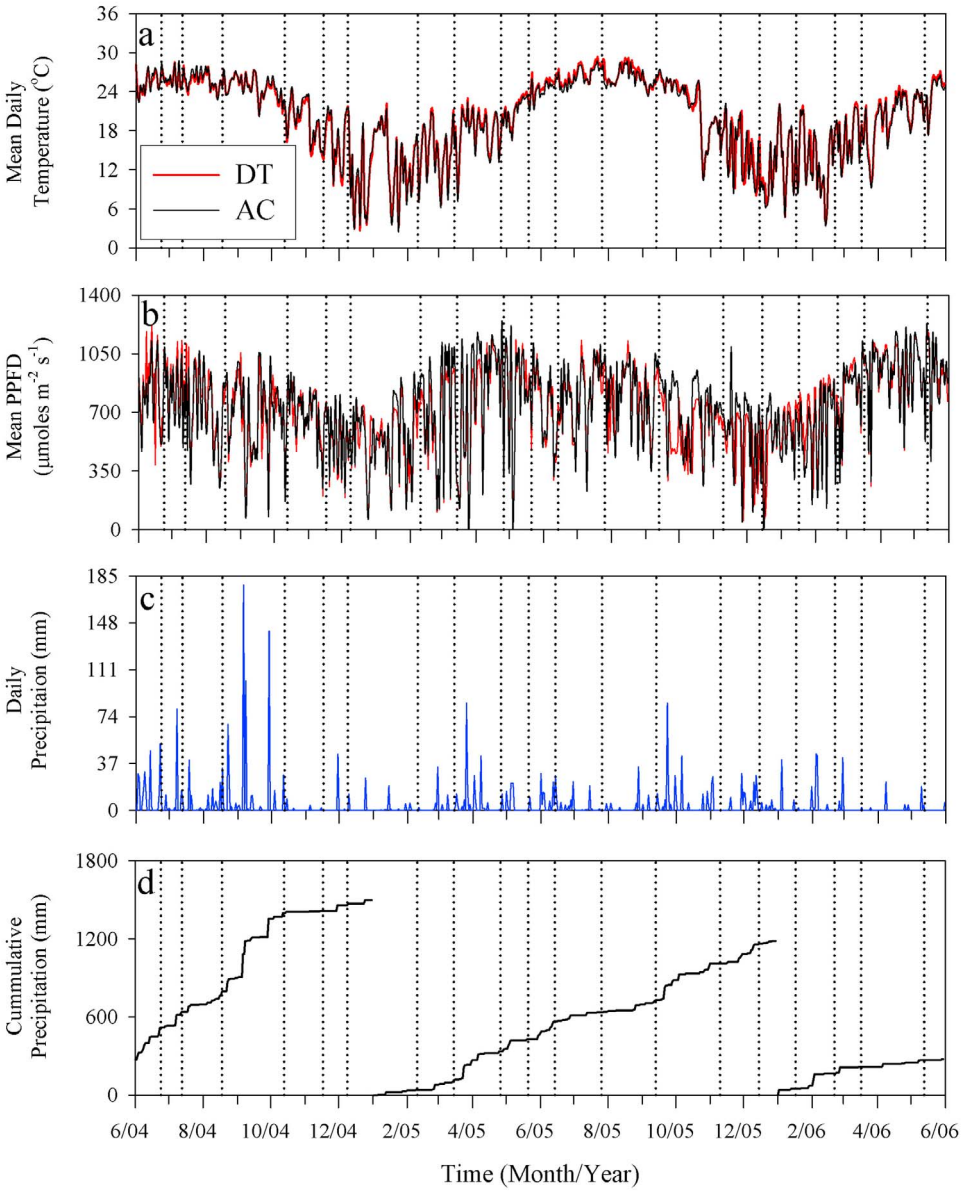

Figure 1. (a) Mean daily temperature and (b) photosynthetically active radiation (PPFD) at Donaldson Tract and Austin Cary, Fla.; (c) daily precipitation; and (d) cumulative precipitation. Vertical lines represent sampling events. Precipitation data is from *Bracho et al.* [2012].

compounds in the 2004 and 2005 needle flushes (Figures 4b and 4c). *P. elliottii* needles at the top of the canopy from the 2004 flush, but not the 2005 flush, were strongly enriched in $C_{20–22}$ *n*-alkanoic acids and depleted in $C_{32–36}$ *n*-alkanoic acids. Interestingly, the *P. elliottii* needles from the mid-canopy had similar trends but those from the 2004 flush were less enriched in $C_{20–22}$ *n*-alkanoic acids and similarly less depleted in $C_{32–36}$ *n*-alkanoic acids. In *P. palustris* at AC, mid-canopy needles from the 2004 flush were also notably depleted in $C_{32–36}$ *n*-alkanoic acids relative to the 2005 flush but only slightly enriched in $C_{20–22}$ *n*-alkanoic acids.

[31] At both sites, the concentrations of the $C_{32–36}$ *n*-alkanoic acids were maximal in newly emerged needles of the 2005 flush (May–June samples). In both *P. palustris* and *P. elliotti*, concentrations in mid-canopy needles then decreased by >50% before rising again in August (Figure 4c). This swing in concentration was not observed in needles from the top of the canopy. For the remainder of the study, concentrations of the $C_{32–36}$ *n*-alkanoic acids steadily decreased at a rate of ~0.2 μg/gdw d^{-1}. In contrast, no temporal trend in $C_{32–36}$ *n*-alkanoic acid concentration was apparent in needles from the 2004 flushes. Neither was there any temporal trend in the $C_{20–22}$ *n*-alkanoic acid concentrations in needles from the 2005 flush at either site.

[32] The within class distribution of the individual compounds is very similar in *P. palustris* and *P. elliottii* and is characterized by a primary peak at the C_{24} *n*-alkanoic acid and and secondary peak at the C_{34} *n*-alkanoic acid (Figures 5a–5c). The greater spread seen in the percent contribution of $C_{20–22}$ *n*-alkanoic acids and of $C_{32–36}$ *n*-alkanoic acids in these graphs reflects the switchover

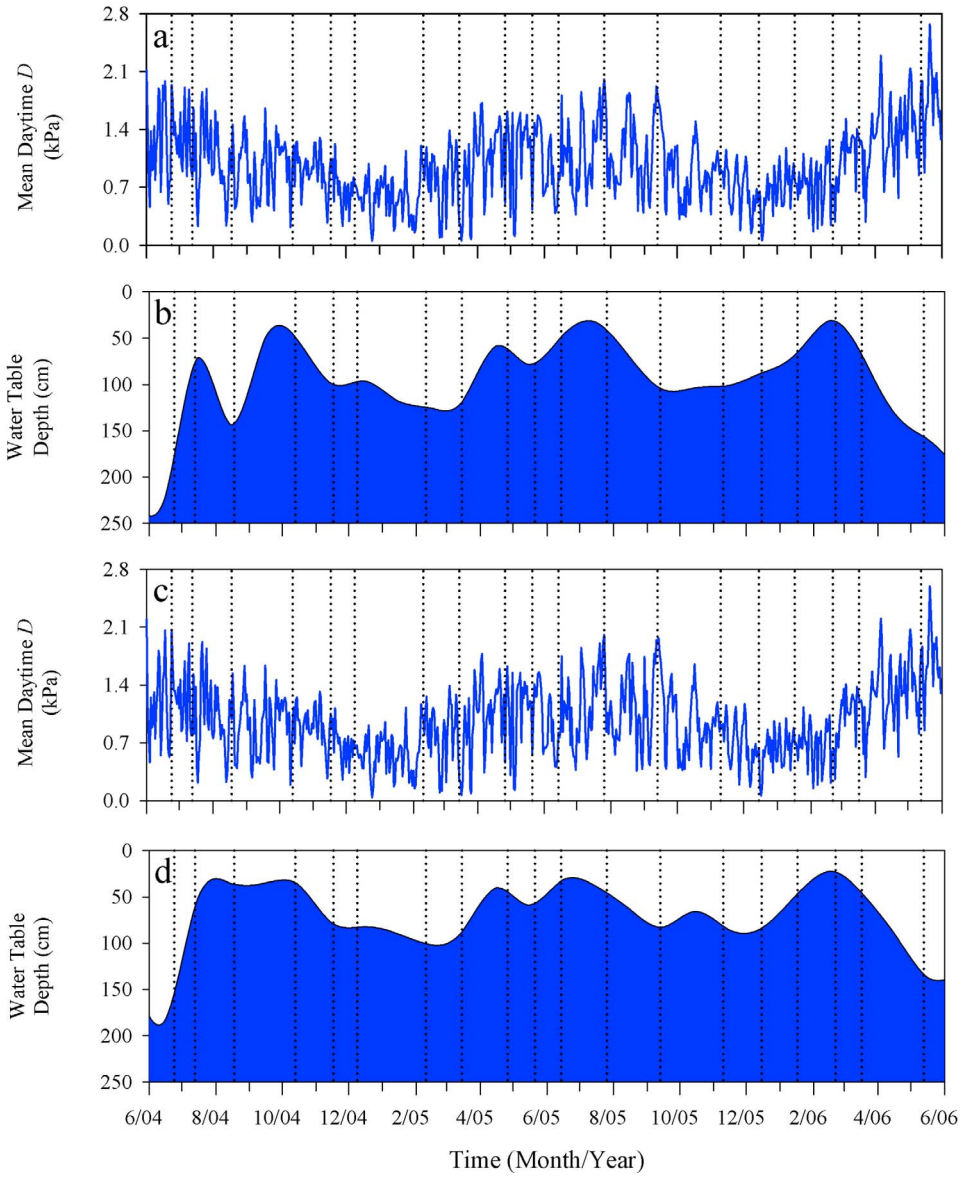

Figure 2. (a) Mean daily vapor pressure deficit and (b) mean monthly water table depth at Donaldson Tract; (c) mean daily vapor pressure deficit; and (d) mean monthly water table depth at Austin Cary.

previously described between concentrations of the $C_{20–22}$ *n*-alkanoic acids versus the $C_{32–36}$ *n*-alkanoic acids (Figure 4). In contrast to these compounds, the relative concentrations of the $C_{24–32}$ *n*-alkanoic acids are far less variable.

[33] Averaged over the entire study, the δC_W of the wax *n*-acids (the concentration-weighted average of the $C_{22–36}$ compounds) for *P. palustris* at AC and for *P. elliottii* at DT was ^{13}C depleted relative to δC_{OM} (Figure 3a) by 3.4 ± 0.3‰ and 3.2 ± 0.7‰, respectively. As observed for δC_{OM} and δC_{SC}, the needle wax of *P. elliottii* at DT was depleted by 2.3 ± 0.3‰ relative to the mature *P. palustris* at AC. This difference in δC_W between the two species mirrored that observed in δC_{OM} and δC_{SC} (Figure 3) and indicates that waxes are similarly recording the broad isotopic shifts that are associated with different Δ in these two species.

[34] As observed in wax concentration and composition, there are clear differences in temporal trends in the $\delta^{13}C$ of individual wax compounds in *P. palustris* and *P. elliottii* and, for *P. elliottii*, in the 2004 and 2005 flushes (Figure 6). In *P. palustris* at AC, there was a decrease in $\delta^{13}C$ over the study period for all compounds, with no distinct discontinuity in this trend apparent between the 2004 and 2005 flushes. The decline in $\delta^{13}C$ of the primary molecular cluster of C_{22}, C_{24} and C_{26} *n*-alkanoic acids during the study period (-0.002‰ to -0.004‰ d^{-1}), although variable, was comparable to the decline in $\delta^{13}C_{OM}$ (-0.004‰ d^{-1}) (Figure 6, Table 1). Quite unexpectedly, the rate of decline in $\delta^{13}C$ of the secondary molecular cluster of C_{32}, C_{34} and C_{36} compounds (-0.008‰ to -0.009‰ d^{-1}) was twice that of the $C_{22–26}$ compounds in the primary molecular cluster (Figure 6, Table 1). Additionally, the temporal trend in the $C_{32–36}$ *n*-alkanoic acids was far less variable than observed

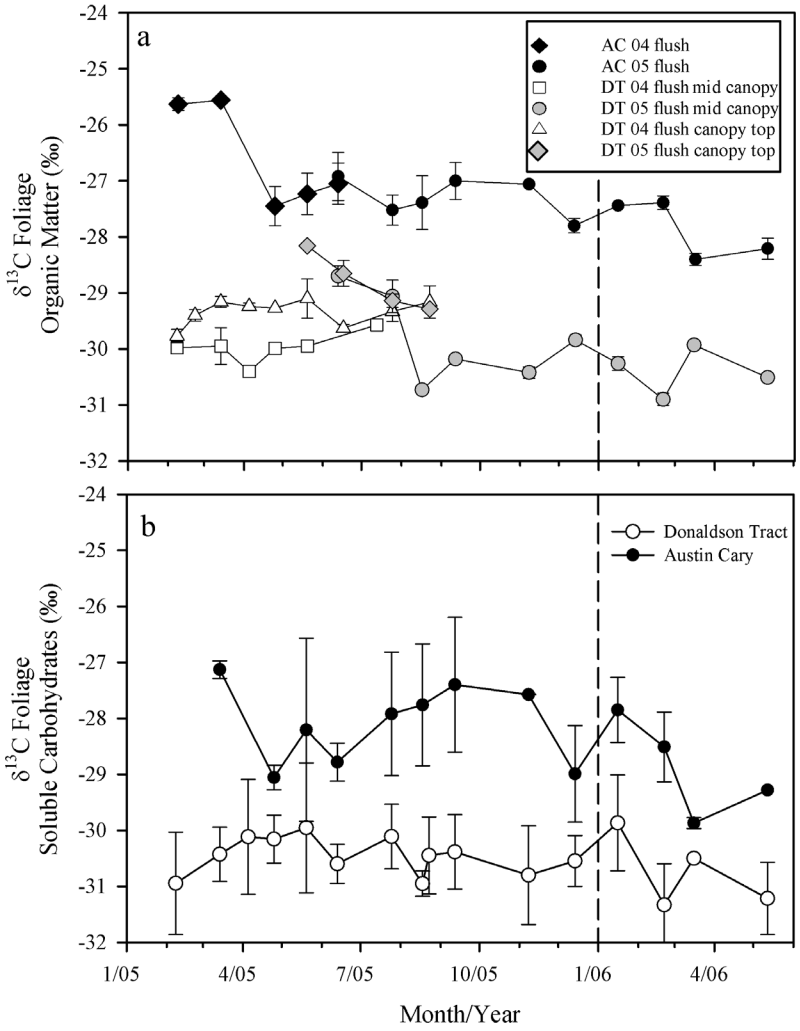

Figure 3. Seasonal trends in the $\delta^{13}C$ of (a) foliage organic matter (mean of 2–3 replicate ±1 SD), δC_{OM}, (b) foliage soluble carbohydrates (mean of 2–6 replicates ±1 SD, except in 03/06 at DT where $n = 1$), δC_{SC} at Donaldson Tract (*P. elliottii*) and Austin Cary (*P. palustris*).

for the shorter chain length compounds. For the C_{28} acid, a minor wax component (Figure 5), the rate of decline (-0.006‰ d^{-1}) fell between that of the primary and secondary molecular clusters (Table 1).

[35] In contrast to *P. palustris*, needles of the 2004 and 2005 flushes of *P. elliottii* at DT showed large isotopic differences in temporal trends of the individual compounds (Figure 6). For needles from the 2004 flush, there was no temporal trend in the $\delta^{13}C$ of the primary cluster of $C_{22–26}$ *n*-alkanoic acids. Additionally, the $C_{22–26}$ *n*-alkanoic acids were also isotopically depleted relative to the new flush of needles in 2005. However, needles from the 2005 flush clearly showed a decreasing trend, albeit noisy, in the $\delta^{13}C$ of $C_{22–26}$ *n*-alkanoic acids. These differences in the primary molecular cluster can be contrasted with those of the C_{28} to C_{36} compounds, in which both the 2004 and 2005 flushes showed a similar isotopic trend (Figure 6).

[36] A second striking observation was that the rates of decline in $\delta^{13}C$ of the secondary $C_{32–36}$ molecular cluster in *P. elliottii* at the DT site and in *P. palustris* at the AC site were indistinguishable (Table 1), although the data were much noisier at the DT site (Figure 6). The mass weighted average $\delta^{13}C$ of the $C_{32–36}$ molecular cluster in *P. palustris* at the AC and DT sites decreased at similar rates (Table 1).

3.3. Carbon Isotope Ratios of Foliar, Ecosystem, Soil Respired CO_2 and Soil Organic Matter

[37] Foliar respiration exhibited the greatest overall temporal variability in the $\delta^{13}C$ of respired CO_2 (Figure 7a). Consistent with differences observed in carbon pools, δC_{FR} of *P. elliottii* at DT (-28.49 ± 0.29‰, n = 35) was depleted by ~2‰ relative to *P. palustris* at AC (-26.34 ± 0.22‰, n = 34). No seasonal or long-term trend was apparent in δC_{FR} at either site. In contrast to δC_{FR}, δC_{ER} was not significantly different at the two sites (Figure 7b). Mean δC_{ER} at DT was -27.16 ± 0.20‰ (n = 18) and -26.94 ± 0.14‰ (n = 20) at AC. All δC_{ER} values at AC, and all but one (January 2006) at DT, were within 1‰ of the site average.

[38] On average, δC_{SR} at DT (-27.07 ± 0.13‰, n = 40) was only 0.7 ± 0.2‰ more depleted than at AC (-25.89 ± 0.55‰, n = 38). Minimum values of δC_{SR} were observed in the fall of 2004 at both sites (Figure 7c). Leaf

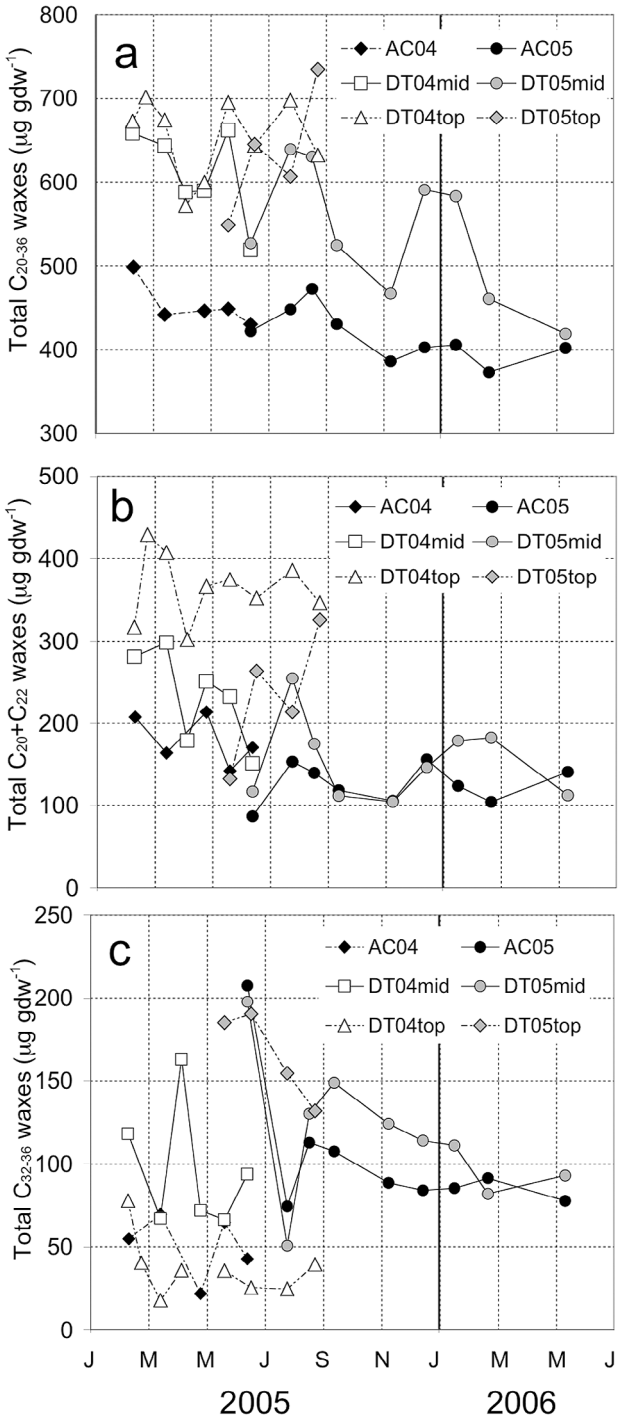

Figure 4. Seasonal trends in wax concentration of 2004 and 2005 needle flushes in *P. palustris* at Austin Carey (top of canopy needles) and in *P. elliottii* at Donaldson Tract (top of canopy and mid-canopy needles). (a) Total needle wax (sum of $C_{20–36}$ *n*-acids), (b) total $C_{20–22}$ *n*-acids, and (c) total $C_{32–36}$ *n*-acids.

temperature differed from air temperature by less than 0.55°C for 95% of daylight hours, resulting in little difference between $D_{surf\text{-}air}$ and D of bulk air (data not shown). The shortest time lag that yielded a significant correlation between δC_{SR} and D was 11 days at AC and 5 days at DT. However, no significant correlations of δC_{FR} or δC_{ER} were observed with temperature, PPFD or D for time lags of 0–21 days (data not shown).

[39] *Soil:* The average $\delta^{13}C$ of soil organic matter in the 0–40 cm soil interval (n = 7) was similar at both the AC and DT sites (−25.72 ± 0.37‰ and −25.32 ± 0.61‰, respectively) (Figure 8).

4. Discussion

4.1. Temporal Trends in Wax Isotopic Composition

[40] Significant temporal trends in wax concentrations and in molecular and isotopic composition were found in both *P. palustris* at AC and *P. elliottii* at DT over the course of this study. Additionally, there were clear differences between the 2004 and 2005 needle flushes, and in *P. elliottii*, between top of canopy and mid-canopy needles. These observations provide further evidence for rapid wax turnover [*Sachse et al.*, 2009; *Neinhuis and Barthlott*, 1998; *Hauke and Schreiber*, 1998] and for the dynamic adjustments in wax biosynthesis in response to environmental stressors such as drought [*Shepherd and Griffiths*, 2006; *Kosma et al.*, 2009; *Leide et al.*, 2011; *Percy et al.*, 2009; *Yang et al.*, 2011].

[41] The thickness and microstructure of the wax coating of leaves is critical to leaf photoprotection, to control cuticular respiration and to regulate other interactions with the environment [reviewed in *Shepherd and Griffiths*, 2006]. We found significantly higher wax loadings in needles of the 2004 flush which were formed during a very dry spring (Figure 2) [*Powell et al.*, 2008]. In particular, concentrations of shorter chain length C_{20} and C_{22} *n*-alkanoic acids were much higher than in needles of the 2005 flush that were formed under more typical spring conditions. This increase in wax loading in a drought year is consistent with other studies that have observed increased wax deposition in response to water stress and low humidity to reduce cuticular respiration [reviewed in *Shepherd and Griffiths*, 2006].

[42] Light and temperature have also been observed to influence wax chain length in several species. For example, higher levels of UV-B were associated with an increase in the proportion of shorter (C < 30) to longer (C > 30) chain *n*-alkanes in *Nicotiana tabacum* [*Barnes et al.*, 1996]. Thus, higher light levels and leaf temperature at the top of the canopy may have contributed to the wax compositional differences and higher levels of shorter chain length compounds in fully sunlit needles of *P. elliottii* as compared to the more shaded needles in the mid-canopy (Figure 5).

[43] Corresponding with the increase in $C_{20–22}$ *n*-alkanoic acid concentration was a decrease in $C_{32–36}$ *n*-alkanoic acid concentration. Previous studies have attributed changes in wax chain length composition in response to its growth conditions to an adjustment in channeling wax precursors into free fatty acids and the reductive and decarbonylation pathways [*Giese*, 1975; *Shepherd et al.*, 1995, 1997]. Our data suggests that a similar environmentally induced switchover in channeling carbon into biosynthetic pathways may be occurring here in both *P. palustris* at AC and *P. elliottii* at DT, as they show remarkably similar trends and changes in wax molecular distributions.

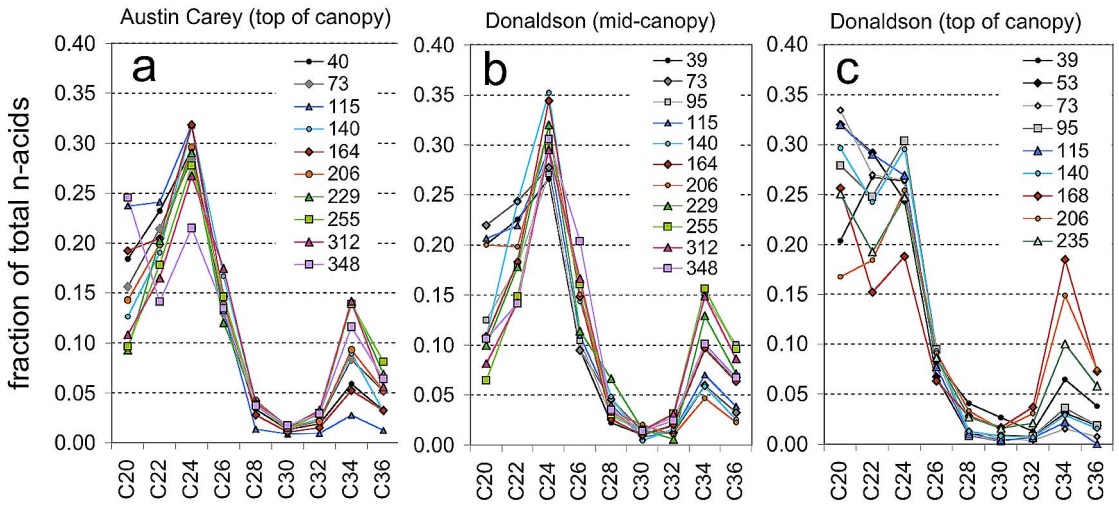

Figure 5. Within-class molecular distribution of wax *n*-alkanoic acids. The ID numbers are the sequence day of the individual sampling dates, starting 1 January 2005. (a) Top of canopy needles of *P. palustris* at Austin Carey, (b) top of canopy needles of *P. elliottii* at Donaldson Tract, and (c) mid-canopy needles of *P. elliottii* at Donaldson Tract.

[44] Waxes of differing chain lengths are biosynthesized via chain elongation within fatty acid elongase (FAE) complexes in the endoplasmic reticulum. In a simple model of wax biosynthesis [e.g., *Kolattukudy et al.*, 1976], the longer-chain length compounds are thought to be biosynthesized in the same FAE complexes from elongation of waxes of shorter-chain lengths, so both are sourced from the same wax precursors. This simple model predicts that the $<C_{30}$ *n*-alkanoic acids provide the substrate for elongation to produce the C_{32}, C_{34} and C_{36} *n*-alkanoic acids. Therefore, the average $\delta^{13}C$ of the C_1 to C_{28} carbons in the alkyl chain of the $>C_{28}$ *n*-alkanoic acids should be identical to that of the C_{28} *n*-alkanoic acids.

[45] Assuming this model, the average $\delta^{13}C$ of the terminal (i.e., $>C_{28}$) carbons of the C_{32}, C_{34} and C_{36} *n*-alkanoic acids can be calculated from a simple mass balance. The results (Figure 9) indicate that the $\delta^{13}C$ of the terminal carbons of the C_{32} to C_{36} *n*-acids declined by nearly 10‰ over the course of our study. The rate of decline is virtually indistinguishable between *P. palustris* at AC and *P. elliottii* at DT. For the combined data, the fitted regression for the calculated trend line is $\delta^{13}C = -28.63 - 0.017$ day^{-1} ($r^2 = 0.56$). However, the magnitude of the decline that is predicted using this mass balance approach seems unlikely given the minimal change in δC_{SC} and δC_{OM} over the study period (Figure 3) as it would require large internal isotopic fractionations during carbon remobilization and/or utilization.

[46] An alternative model of wax biosynthesis indicates that waxes of different chain lengths are synthesized independently [*Samuels et al.*, 2008; *Kunst and Samuels*, 2009]. For example, comparative studies of genetic mutants known to be defective in specific points in the elongation pathway have indicated that rather than a single pathway, there are sequential elongation systems with multiple isoforms of elongases or subunits, such as the condensing enzyme β-ketoacyl-CoA synthase (KCS). The KCS(s) in each of these systems appears to have a strict substrate specificity and catalyze only a few successive elongation steps.

[47] The isotopic differences observed among the waxes of different chain lengths (Figure 6), as well as the switch-over pattern observed in their production (Figure 4), are consistent with this alternative model, i.e. there are parallel but separate elongation systems that regulate the biosynthesis of the primary C_{20-28} molecular cluster and C_{32-36} molecular cluster. Our results furthermore suggest that these parallel elongation systems are supplied by distinct pools of wax precursors. This hypothesis is supported by evidence in some species for separate but parallel elongation systems linked with the different associated biosynthetic pathways that do not share a common pool of precursors [reviewed in *Shepherd and Griffiths*, 2006]. Furthermore, there may be changes in the relative activities of these parallel systems during plant growth and/or in response to environmental variables. For example, in corn the ED-I elongation system is more active in seedlings and produces very long-chain alcohols, aldehydes, acids and alkanes while a second parallel system, ED-II, is active over all stages of growth and produces shorter acyl chains primarily for ester formation [*Avato et al.*, 1984; *Avato et al.*, 1990; *Beattie and Marcell*, 2002]. In barley and wheat, ED-1 is also paralleled by a 3-ketoacyl elongation system. ED-I is more active in seedlings, and the 3-ketoacyl elongation system more active in mature plants [*Wettstein-Knowles*, 1995]. Physiological switching between parallel elongation systems that is induced by environmental conditions has been observed in several species. For example, a switch between parallel pathways of biosynthesis of alkanes and β-diketones appears to occur in some species in response to water or salinity stress [*Wettstein-Knowles*, 1972; *Bengtson et al.*, 1978; *Rao et al.*, 1981] and has been suggested to explain light-induced changes in wax chain length in barley [*Shepherd and Griffiths*, 2006].

[48] No matter what the biochemical explanation for the differences observed between the primary and secondary

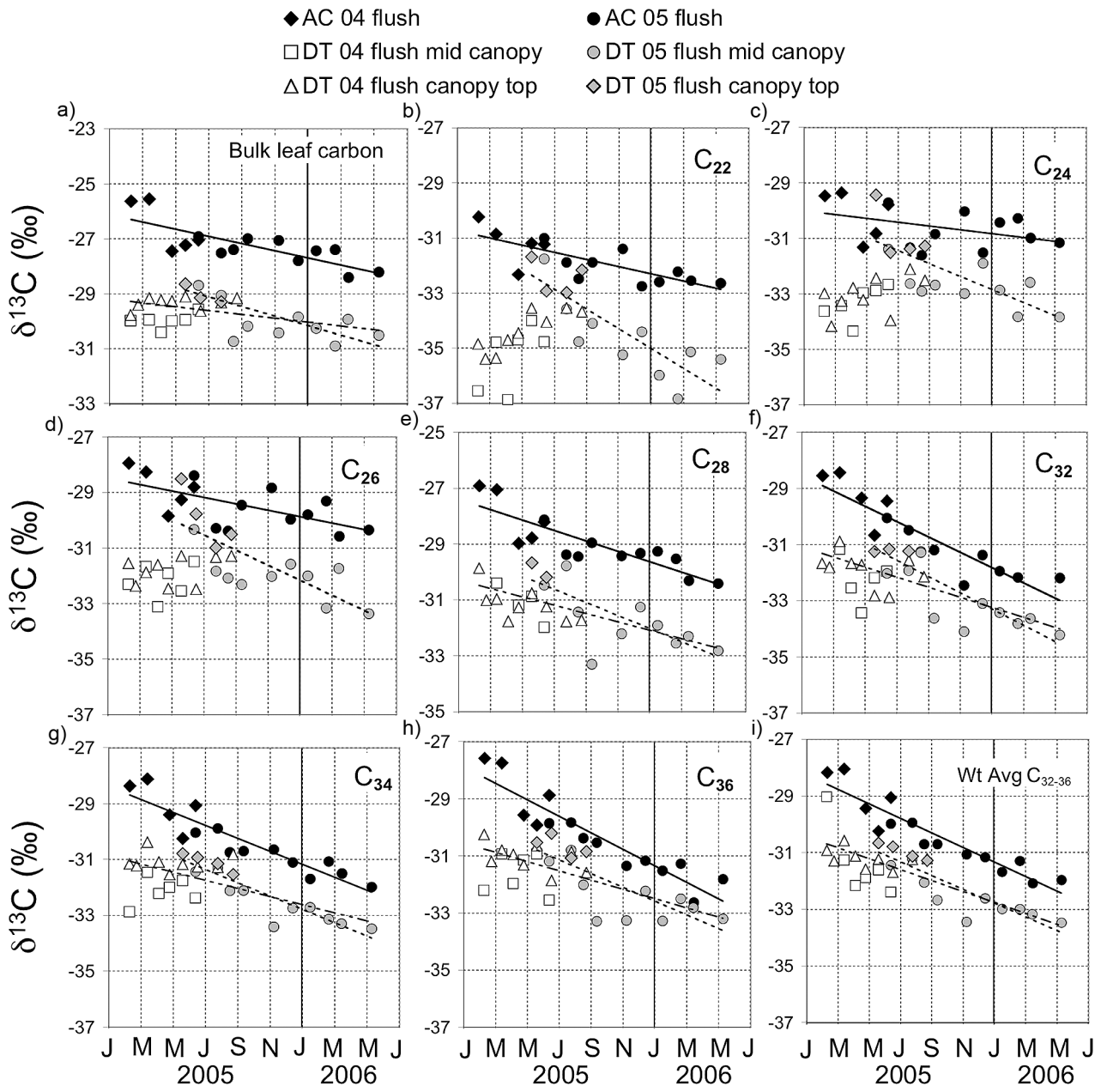

Figure 6. (a) The $\delta^{13}C$ of bulk carbon in needles shown for comparison with temporal trends in (b–h) the individual C_{22} through C_{36} *n*-alkanoic acids and in (i) the mass weighted average of the $C_{32–36}$ *n*-alkanoic acids.

molecular clusters, the decline in $\delta^{13}C_W$ during the study (Figure 6) indicates a slow decline in the $\delta^{13}C$ of carbon precursors(s) used for wax biosynthesis. As storage reserves provide the primary carbon source for wax biosynthesis in *P. elliottii* [*Mortazavi et al.*, 2009], the decline strongly suggests that the storage carbon reserves of both *P. palustris* at AC and *P. elliottii* at DT were becoming more ^{13}C depleted over the study period.

[49] The remarkably similar rate of decline in $\delta^{13}C_W$ at both sites (Figures 6i, Table 1) strongly suggests a uniform physiological response to a strong environmental driver rather than any site- or species-specific variable. An explanation consistent with environmental data is that the trees were recovering from the extreme drought of 2000–2002. This "drought of the century" depressed the water table to >2 m and resulted in severe water stress, as indicated by premature needle fall and reduced daytime carbon assimilation and net ecosystem exchange [*Powell et al.*, 2008] Extended spring droughts in both 2003 and 2004 also resulted in a large precipitation deficit that persisted until autumn 2004 (Figure 1).

[50] Although no isotopic data were collected during the drought of 2000–2002, it can be speculated that the water stress depleted tree reserves and resulted in a build-up of $\delta^{13}C$ enriched carbon that was assimilated during the drought. As water stress was subsequently alleviated in

Table 1. Regression Parameters (a + bx) of Temporal Trend Lines for $\delta^{13}C$ of Bulk Leaf Carbon, Individual *n*-Alkanoic Acids and Mean of C_{32-36} Compounds (Mass Weighted Average) Shown in Figure 6[a]

		n-Alkanoic Acid							
Parameter	Bulk Leaf Carbon	C_{22}	C_{24}	C_{26}	C_{28}	C_{32}	C_{34}	C_{36}	Weight Average C_{32-36}
				Austin Carey					
a	−26.12	−30.74	−30.01	−28.47	−27.40	−28.55	−28.39	−27.88	−28.25
b	−0.004	−0.004	−0.002	−0.004	−0.006	−0.009	−0.008	−0.009	−0.008
r^2	0.61	0.59	0.17	0.41	0.74	0.81	0.85	0.86	0.87
				Donaldson Tract (All)					
a	No Trend	No Trend	No Trend	No Trend	−30.30	−31.09	−30.88	−30.55	−30.44
b	NT	NT	NT	NT	−0.005	−0.006	−0.005	−0.005	−0.006
r^2	NT	NT	NT	NT	0.43	0.46	0.46	0.46	0.61
				Donaldson Tract (2005 Flush Only)					
a	−28.07	−30.70	−30.03	−28.90	−29.22	−29.83	−29.92	−29.70	−29.87
b	−0.006	−0.012	−0.008	−0.009	−0.008	−0.009	−0.008	−0.008	−0.008
r^2	0.56	0.70	0.55	0.60	0.56	0.71	0.84	0.62	0.79

[a]The Austin Carey regressions are for combined data for both 2004 and 2005 flushes. The Donaldson Tract regressions are given for combined data and for the 2005 flush only.

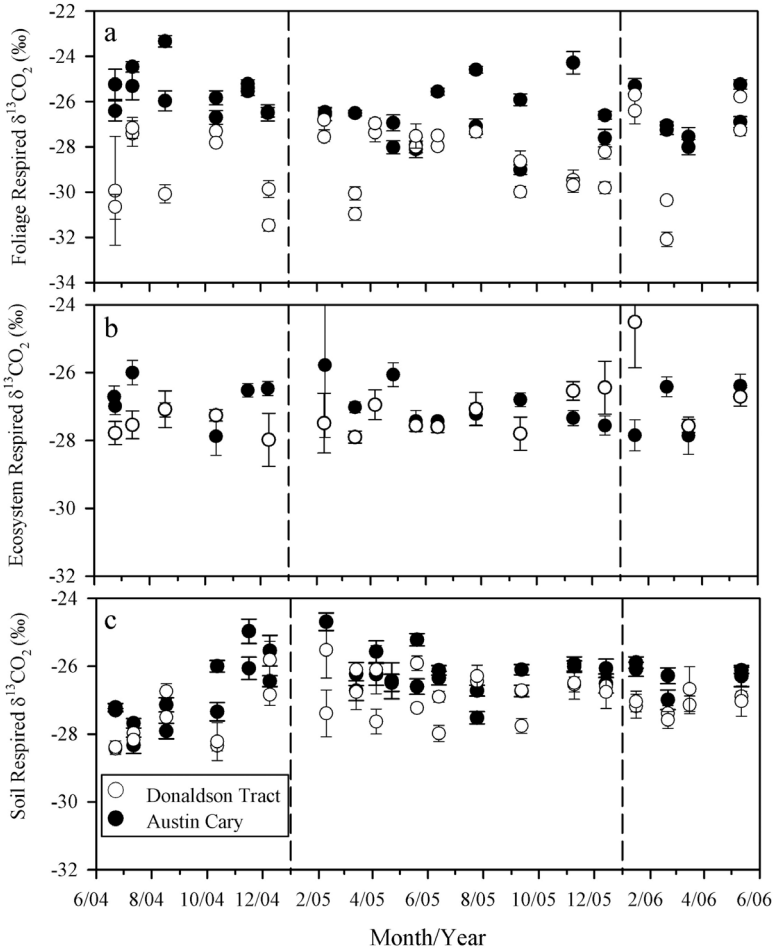

Figure 7. Temporal trends in the $\delta^{13}C$ of (a) foliage (δC_{FR}), (b) ecosystem δC_{ER}, and (c) soil-respired (δC_{SR}) CO_2 at the Donaldson Tract (*P. elliotii*) and Austin Cary (*P. palustris*) sites. The error bars indicate the standard error of Keeling plots intercepts.

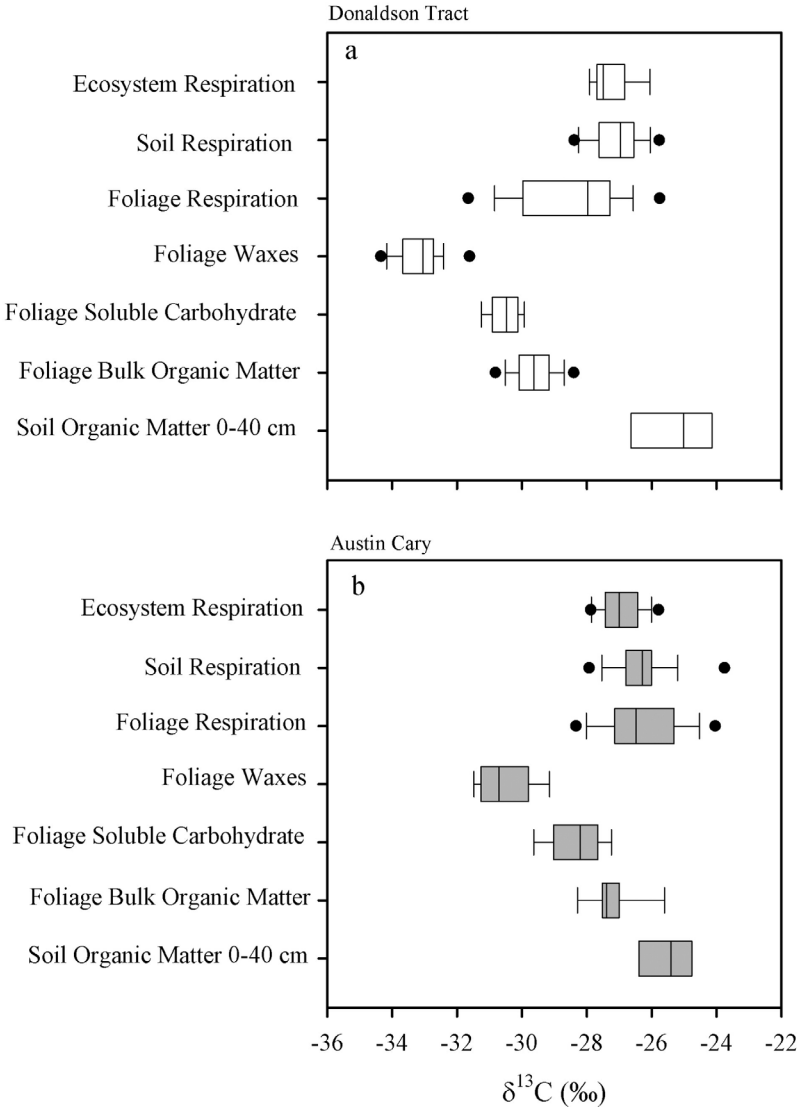

Figure 8. Synthesis showing the $\delta^{13}C$ of the various pools and CO_2 fluxes measured for (a) *P. elliottii* at the Donaldson Tract and (b) *P. palustris* at Austin Cary. The median, 25% upper/lower quartiles as well as the 5% and 95% limits of the data are shown.

late 2004, Δ would have reverted back to typical values as the trees would assimilate more ^{13}C depleted photosynthates compared to those assimilated during the drought. This addition of more $\delta^{13}C$ depleted carbon would slowly decrease the $\delta^{13}C$ of the carbon storage reserve(s) that would then supply the wax biosynthetic precursors.

[51] Our interpretation that the isotopic trend in $\delta^{13}C_W$ reflects a slow change in the $\delta^{13}C$ of the tree's carbon reserves is supported in other studies of conifers and deciduous trees that have found a large influence of storage reserves on the $\delta^{13}C$ of carbon pools. The labeling experiment of *Kagawa et al.* [2006] found that larch (*Larix gmelinii*) needles drew nearly half of their carbon from material assimilated during the prior year and stored within the branch. Label was still detectable within the needle starch pool three years post-labeling, suggesting the presence of significant long-term metabolic reserves in this species. The isotopic labeling experiments of *Mortazavi et al.* [2009] found that in addition to *P. elliottii*, the deciduous sweetgum tree *L. styraciflua* also heavily utilized stored carbon for biosynthesis. Labeled carbon from storage reserves was still detectable in waxes of new leaves that were produced the year after the trees were removed from the labeled CO_2. Similarly, in a whole-tree labeling experiment in a natural temperate hardwood forest, *Körner et al.* [2005] observed that carbon reserves from the prior year accounted for ~60% of the foliage organic matter in *Quercus petraea*, 30%–35% in *Fagus sylvatica* and *Acer campestre* and 20% in *Carpinus betulus.* A small (~1%), but still measurable, fraction of carbon from long-term storage reserves was detectable 4 years post-labeling.

[52] The dichotomy between the temporal trends in the isotopic composition of the $C_{22–26}$ and $C_{32–36}$ molecular clusters of wax *n*-alkanoic acids (Figure 6) highlights the gaps in our knowledge of how assimilated carbon is subsequently redistributed within the plant tissues to support

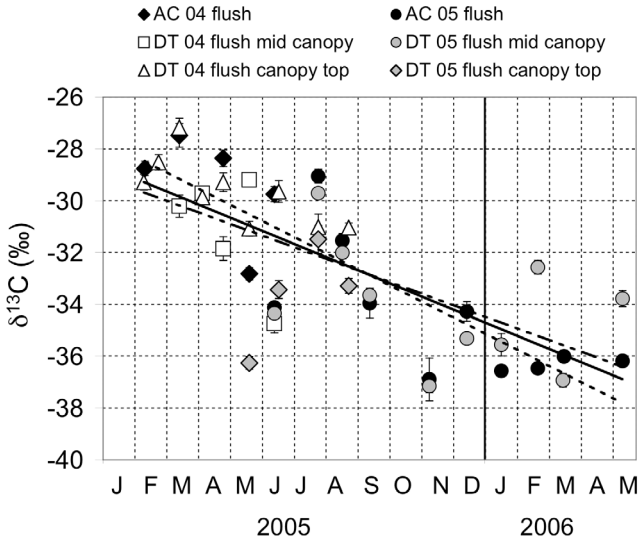

Figure 9. Estimated mean $\delta^{13}C$ of the $C_{32–36}$ terminal carbons in the long-chain waxes for a hypothetical model that assumes these are synthesized via chain elongation of C_{28} precursor *n*-acids having the same $\delta^{13}C$ as that measured for the C_{28} *n*-acid. The regression for the combined data (solid line): $\delta^{13}C = -28.63–0.017$ month^{-1}, $r^2 = 0.56$. The short and long dashed lines show the separate regressions for *P. palustris* and *P. elliottii*, respectively.

biosynthesis. Although current knowledge sheds no light on reasons why the $\delta^{13}C$ of the carbon precursor pools for the primary $C_{20–28}$ and secondary $C_{32–36}$ molecular cluster may differ, we speculate that newly assimilated carbon may be channeled more strongly into the precursor pool supplying the wax biosynthetic pathway for the secondary $C_{32–36}$ molecular cluster. In contrast, the precursor pools that supply the pathway for the primary $C_{20–28}$ molecular cluster appear to be sourced mainly from the tree's carbon storage reserves, a speculation that is supported by the similarity between the isotopic trend in the C_{20} to C_{26} *n*-alkanoic acids and bulk leaf carbon (Figure 6, Table 1). The physiological basis for different isotopic fluxes and turnover rates in wax precursor pools is currently an enigma and a ripe topic for future studies.

4.2. Controls on the $\delta^{13}C$ Respired CO_2

[53] For both species δC_{FR} measured prior to dawn remained on average ~2‰ enriched relative to δC_{SC} and by a ~1‰ relative to δC_{OM} (Figure 3). Enrichment in δC_{FR} relative to δC_{SC} has been observed in sweetgum [*Mortazavi et al.*, 2009], oak [*Hymus et al.*, 2005], in beans, tobacco, sunflower [*Tcherkez et al.*, 2003; *Xu et al.*, 2004; *Ghashghaie et al.*, 2003] and as well as herbaceous species [*Werner et al.*, 2009]. The enrichment in δC_{FR} and δC_{ER} relative to δC_{SC}, the carbon substrate presumably used for autotrophic respiration is consistent with other studies conducted at the leaf [*Ghashghaie et al.*, 2003] and ecosystem [*Bowling et al.*, 2008] scale. Isotopic enrichment in δC_{FR} has been explained by post photosynthetic fractionation that results in enriched $\delta^{13}CO_2$ during foliage respiration [*Ghashghaie et al.*, 2003]. In *P. elliottii* [*Mortazavi et al.*, 2006; *Prater et al.*, 2006] as well as other species [*Barbour et al.*, 2007]. *Tcherkez et al.* [2003] suggested that the enrichment in δC_{FR} results from the non-random distribution of ^{13}C in glucose and molecular fragmentation that occurs during respiration. δC_{FR} is highly enriched immediately upon darkening and this enrichment declines exponentially to stable values within a few hours [*Mortazavi et al.*, 2006]. *Gessler et al.* [2008] demonstrated that the $\delta^{13}C$ enrichment observed in dark respired CO_2 for light acclimated foliage arises from excess malate previously synthesized by the leaf in the light.

[54] Variability in δC_{FR} (Figure 7a) was significantly greater than that in δC_{SC} (Figure 3b), the presumed carbon source used during foliage respiration. Predawn δC_{SC} in *P. elliotii* consists of fast and slow turnover substrate pools, with the fast turnover pool (~3–4 d half-life) accounting for only 1%–2% of the total [*Mortazavi et al.*, 2009]. Therefore, δC_{sc} reflects temporally integrated values of Δ, with the isotopic values of recently assimilated carbon overshadowed by the contribution of a large carbon pool with a long turnover rate [*Mortazavi et al.*, 2009].

[55] The greater variability in δC_{FR} relative to that for δC_{SC} is consistent with other observations [*Hymus et al.*, 2005; *Mortazavi et al.*, 2009]. *Tcherkez et al.* [2003] related the changes in δC_{FR} to switching of carbon substrates and metabolic pathways as foliage respiration proceeds. The pool of carbohydrates declines with respiratory losses, and the fraction of ^{13}C-enriched CO_2 that is produced declines. With the reduction in carbohydrates there is a greater rate of fatty acid oxidation coupled to the Krebs cycle, and subsequently the fraction of $\delta^{13}C$-depleted CO_2 produced increases. This mechanism can account for 8‰–9‰ short-term variability (hours) observed in δC_{FR} [*Sun et al.*, 2009; *Hymus et al.*, 2005; *Mortazavi et al.*, 2006; *Prater et al.*, 2006]. Therefore, the greater variability in δC_{FR} compared to δC_{SC} reflects the contribution of different metabolic pools to predawn respiration rather than changes in Δ.

[56] In contrast to the isotopic enrichment of foliar carbon pools and foliar respiration in *P. palustris* at AC in comparison to *P. elliottii* at DT, δC_{SR} was only slightly more enriched at AC site (-25.89 ± 0.55‰ at versus -27.07 ± 0.13‰, respectively) (Figure 7c). Interestingly, there was an increase in δC_{SR} from June to December 2004. This increase coincided with a steep rise in the water table (Figures 2b and 2d) and declining respiration rates (data not shown). The increase in δC_{SR} could reflect changes in autotrophic/heterotrophic respiration or the contribution of anoxic respiration as soil pore spaces became saturated following the recovery from the drought.

[57] The shortest time lags that yielded any correlation between δC_{SR} and *D* were 11 and 5 days at AC and DT sites, respectively (data not shown). Our results are in line with other recent studies that indicate multiple controls on δC_{SR}, in addition to change in the $\delta^{13}C$ of substrates supplied by autotrophic respiration [e.g., *Ekblad and Högberg*, 2001]. *Wingate et al.* [2010] observed in a maritime pine forest that δC_{SR} was at times decoupled from short-term variability in canopy dynamics. For example, immediately following rainy days soil respired CO_2 flux increased and became $\delta^{13}C$ enriched while all other ecosystem components became $\delta^{13}C$ depleted. *Moyes et al.* [2010] showed that much of the variability in δC_{SR} was due to change in heterotrophic processes

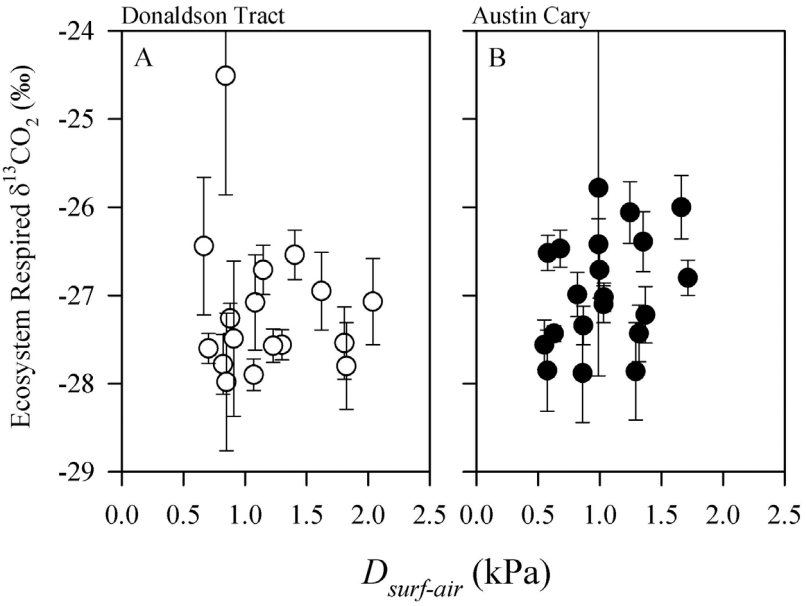

Figure 10. The ^{13}C of ecosystem respired CO_2 (δC_{ER}) at Donaldson Tract (*P. elliotii*) and Austin Cary (*P. palustris*) as a function of daytime average site $D_{surf\text{-}air}$ for days 1–4 prior to sampling (correlations were not significant, $P > 0.05$). The error bars indicate the standard error of Keeling plots intercepts.

and mechanisms that alter the ratio of soil autotrophic to heterotrophic respiration. Similarly, *Powers et al.* [2010] measured a 5‰ depletion in δC_{SR} in a semi-arid woodland after water addition to the soil and suggested that changes in the relative contribution of heterotrophic and autotrophic respiration were responsible for the large changes in soil CO_2 flux and δC_{SR}. In addition to short-term changes in soil respiratory components, diel variability in δC_{SR} can also arise due to transient diffusive isotopic fractionations [*Moyes et al.*, 2010; *Maseyk et al.*, 2009; *Kayler et al.*, 2010]. In the absence of high frequency measurements such as those by *Wingate et al.* [2010], the mechanisms leading to variability in δC_{SR} at these sites remains unclear.

[58] The relative contribution of soil and folaige to nighttime ecosystem respiration influnces $\delta C_{ER.}$ Despite the higher mean δC_{SC} and δC_{FR} values for *P. palustris* at AC compared to *P. elliottii* at DT, mean δC_{ER} values were nearly identical (Figure 8). δC_{ER} at AC is nearly identical to δC_{SR} (-25.98 ± 0.55‰) and δC_{FR} (-26.34 ± 0.22‰). However, δC_{ER} at DT is more similar to δC_{SR} (-27.07 ± 0.13‰) than it is to δC_{FR} (-28.49 ± 0.29‰), potentially indicating a greater influence of soil respiration to nighttime ecosystem respiration. This interpretation is consistent with results in a Loblolly pine plantation, where soil respiration was 4.6 times higher than plant respiration [*Lai et al.*, 2002].

[59] The relation between δC_{ER} and $D_{surf\text{-}air}$ at the sites are shown in Figure 10. The close coupling between conditions at the leaf surface and bulk air results from high boundary layer conductance in our systems at both the leaf and canopy scales resulted in $D_{surf\text{-}air}$ and D of bulk air being similar. At the leaf scale, small needle dimensions result in large boundary layer conductances to heat transfer, causing conifer needle temperatures to seldom, if ever, differ from air temperature by more than one degree [e.g., *Jarvis et al.*, 1976; *Angell and Miller*, 1994]. At the canopy scale, pine forests have high aerodynamic conductance due to their height and low leaf area index, resulting in minimal gradients in temperature and humidity from bulk air through the canopy space, and dominance of convective versus latent heat transfer [*Baldocchi and Vogel*, 1996; *Ewers and Oren*, 2000; *Powell et al.*, 2005].

[60] The lack of a clear relation between δC_{ER} and $D_{surf\text{-}air}$ (Figure 10) is in contrast to those observed for a plantation of the pine *P. taeda* at Duke Forest, North Carolina [*Mortazavi et al.*, 2005], and in a coniferous forest in the NW Pacific [*Bowling et al.*, 2002]. *Alstad et al.* [2007] compared the relationship between δC_{ER} and D in several forest ecosystems. The authors noted that while the overall patterns were consistent with a physiological response of lowered stomatal conductance (and C_i/C_a) to increasing D, there were important differences in the strength of the relationship among plant functional groups. For example, coastal sites exhibited little variability in comparison with continental boreal sites, which they attributed to the more moderate maritime climate of coastal regions that minimizes the seasonal variations in temperature and D.

[61] With increasing D, C_i/C_a is expected to decline due to reduction in stomatal conductance, resulting in assimilates becoming $\delta^{13}C$ enriched. The magnitude of the trend between stomatal conductance and D, however, exhibits a high degree of variability among species and for individuals within the same species located at different sites [*Whitehead et al.*, 1981; *Pataki et al.*, 1998; *McNaughton and Jarvis*, 1991]. The empirical analysis of *Oren et al.* [1999] shows a consistent and robust relationship between stomatal conductance at low D and the sensitivity of the stomatal closure response to increasing D. This analysis indicates that individuals, species, and stands with low stomatal conductance at low D exhibit a lower sensitivity to D [*Oren et al.*, 1999]. Thus, the lack of a clear response of δC_{ER} to D in this study is consistent with physiological studies in seedlings

[*Johnson et al.*, 1995] and mature trees [*Teskey et al.*, 1994] that find a limited influence of *D* on net photosynthesis and stomatal conductance in *P. elliottii* and *P. palustris* [*Gonzalez-Benecke et al.*, 2011]. This limited influence of *D* is further supported by a limited variability in δC_{SC} (Figure 3b) despite large variations in *D* during the study period (Figures 2a and 2c). δC_{ER} is reflecting the $\delta^{13}C$ of assimilated photosynthates but with an offset (Figure 8).

[62] Much of the insensitivity of δC_{SC} and δC_{ER} to *D* at the Florida sites compared to that observed in a Loblolly pine at Duke Forest [*Mortazavi et al.*, 2005] is likely related to the differences in the soil water availability patterns. In the flat spodosols common in the lower coastal plain of Florida, the surficial water table is generally within 1 m of the surface except during episodic drought [*Abrahamson and Hartnett*, 1990; *Powell et al.*, 2008]. During the study period, depth to the water table varied from 22 to 224 cm (Figures 2b and 2d) and averaged 101 and 74 cm at DT and AC, respectively. The fine roots of *P. palustris* reach 2 m [*Addington et al.*, 2006] and in the Florida sandy soil have taproots that can extend at least 4 m [*Heyward*, 1933]. The pine tree taproots are usually therefore in contact with saturated soils, mediating any effects of *D* or soil drying in upper layers. Integrative modeling studies [*McMurtrie et al.*, 1994; *Cropper and Gholz*, 1993] and production ecology investigations [*Martin and Jokela*, 2004; *Neary et al.*, 1990] find that *P. elliottii* productivity, for example, at this site is seldom limited by atmospheric or soil water stress except during periods of extreme drought. The consequences of extreme droughts are severe, however, in these ecosystems and appear to leave a long lasting metabolic impact as evidenced by δC_W (Figures 4 and 6). For example, the drought of 2000–2002 which resulted in the water table being depressed to >2 m for extended time periods caused premature needle fall in May and June of 2001 that was equivalent to 50% of the annual needlefall [*Powell et al.*, 2005]. Access to groundwater at a Douglas-fir forest was also suggested by *McDowell et al.* [2004a] to have buffered variability in canopy gas exchange from drought.

[63] The low ranges of variability in $\delta^{13}C$ of the foliage metabolic pools and in the respiratory fluxes observed here (Figures 3b, 8 and 10) are consistent with studies that have shown a lower sensitivity in stomatal conductance to *D* for deep rooting species [*Bréda et al.*, 1993; *Bréda et al.*, 1995], but a high sensitivity in stomatal conductance to *D* for species in areas where rooting depth is limited [*Pataki et al.*, 1998; *Bréda et al.*, 1993]. The contrasting results between this study in the coastal plain Florida and the Duke forest in North Carolina supports this view, as the Duke forest has a clay pan at 35 cm depth that isolates a surficial soil layer from which 90% of water for vegetation transpiration is derived from [*Oren et al.*, 1999]. Thus, canopy leaf conductance at Duke Forest is strongly related to soil moisture availability in the upper soil layer [*Oren et al.*, 1999] and C_i/C_a and δC_{ER} vary predictably in response to *D* [*Mortazavi et al.*, 2005].

[64] The range of δC_{ER} values for the identical *D* values (Figure 10) highlights the influence of additional factors besides stomatal conductance on δC_{ER}. Changes in the relative contribution of soil and aboveground biomass-to-ecosystem respiration, as well as variability in the ratio of autotrophic to heterotrophic respiration ultimately influence δC_{ER}. Seasonal changes in source-to-sink [*Kozlowski*, 1992], for example, influence carbohydrate flow in the phloem [*Peuke et al.*, 2006] will therefore influence the temporal coupling between assimilated and respired $\delta^{13}CO_2$. As suggested by *Wingate et al.* [2010] understanding the temporal variability δC_{ER} and how it varies with environmental variables that influence Δ will require explicit accounting for how recently assimilated carbon is mixed into a larger carbon pool that serves as the substrate for respiration.

5. Conclusions

[65] The persistent availability of moisture due to a shallow water table and deep taproots dampen fluctuations in the $\delta^{13}C$ of either the assimilated (and subsequently respired) CO_2 at the study sites that might have been observed in areas with more limited moisture availability. We found a similar 2‰ enrichment in δC_{OM}, δC_{SC} and δC_W in *P. palustris* relative to *P. elliotii*, indicating that these isotopic proxies, on average, similarly record species-level differences in Δ. However, the $\delta^{13}C$ of total needle carbon and waxes $\delta^{13}C$ in *P. elliottii* and *P. palustris* appear to more strongly reflect long-term trends in the $\delta^{13}C$ of storage reserves used for biosynthesis rather than any short-term variability in Δ, and thus provide a temporally integrated signal of photosynthetic discrimination. Differences in molecular and isotopic trends observed in the primary $C_{20–26}$ and secondary $C_{32–36}$ wax *n*-alkanoic acids provides evidence for the existence of parallel but separate chain elongation systems for compounds of differing chain lengths in these species, each utilizing different carbon precursor pools. Our results point to a need for targeted isotopic labeling experiments that focus on the internal carbon cycling of long-lived species with substantial storage reserves, as currently we have little understanding of the processes by which carbon is shunted internally to supply the cell's biosynthetic pathways and how this affects the isotopic signals that are recorded in different carbon pools and in respiration.

[66] **Acknowledgments.** We thank M. Smith and J. Malheur for field and laboratory assistance, and R. Mitchell for comments on an earlier version of this manuscript. We also thank L. Samuels for stimulating discussions on wax biosynthesis. This work was supported by a NSF grants DEB-0343604, DEB-0344562 and DEB-0552202, and DOE grant DE-FC02-06ER64156/06-SC-NICCR-1063. We would like to thank 3 anonymous referees, the associate editor and D. Baldocchi for their comments and suggestions on a previous version of this manuscript.

References

Abrahamson, W. G., and D. C. Hartnett (1990), Pine flatwoods and dry prairies, in *Ecosystems of Florida*, edited by R. L. Myers and J. J. Ewel, pp. 103–149, Univ. of Central Florida Press, Orlando.

Addington, R. N., L. A. Donovan, R. J. Mitchell, J. M. Vose, S. D. Pecot, S. B. Jack, U. G. Hacke, J. S. Sperry, and R. Oren (2006), Adjustments in hydraulic architecture of *Pinus palustris* maintain similar stomatal conductance in xeric and mesic habitats, *Plant Cell Environ.*, *29*, 535–545, doi:10.1111/j.1365-3040.2005.01430.x.

Alstad, K. P., C. T. Lai, L. B. Flanagan, and J. R. Ehleringer (2007), Environmental controls on the carbon isotope composition of ecosystem-respired CO_2 in contrasting forest ecosystems in Canada and the USA, *Tree Physiol.*, *27*, 1361–1374, doi:10.1093/treephys/27.10.1361.

Angell, R. F., and R. F. Miller (1994), Simulation of leaf conductance and transpiration in *Juniperus occidentalis*, *For. Sci. [Washington D. C.]*, *40*, 5–17.

Aranibar, J. N., J. A. Berry, W. J. Riley, D. E. Pataki, B. E. Law, and J. R. Ehleringer (2006), Combining meteorology, eddy fluxes, isotope measurements, and modeling to understand environmental controls of

carbon isotope discrimination at the canopy scale, *Global Change Biol.*, *12*, 710–730, doi:10.1111/j.1365-2486.2006.01121.x.

Avato, P., G. Bianchi, E. Gentinetta, and F. Salamini (1984), Effect of trichloroacetic acid on wax composition of normal and mutant maize (*Zea mays* L.), *J. Exp. Bot.*, *35*, 245–251, doi:10.1093/jxb/35.2.245.

Avato, P., G. Bianchi, and N. Pogna (1990), Chemosystematics of surface lipids from maize and some related species, *Phytochemistry*, *29*, 1571–1576, doi:10.1016/0031-9422(90)80124-Y.

Baldocchi, D. D., and D. R. Bowling (2003), Modelling the discrimination of $^{13}CO_2$ above and within a temperate broad-leaved forest canopy on hourly to seasonal time scales, *Plant Cell Environ.*, *26*, 231–244, doi:10.1046/j.1365-3040.2003.00953.x.

Baldocchi, D. D., and C. A. Vogel (1996), Energy and CO_2 flux densities above and below a temperate broad-leaved forest and a boreal pine forest, *Tree Physiol.*, *16*, 5–16, doi:10.1093/treephys/16.1-2.5.

Barbour, M. M., J. E. Hunt, R. J. Dungan, M. H. Turnbull, G. W. Brailsford, G. D. Farquhar, and D. Whitehead (2005), Variation in the degree of coupling between $\delta^{13}C$ of phloem sap and ecosystem respiration in two mature *Nothofagus* forests, *New Phytol.*, *166*, 497–512, doi:10.1111/j.1469-8137.2005.01329.x.

Barbour, M. M., N. G. McDowell, G. Tcherkez, C. P. Bickford, and D. T. Hanson (2007), A new measurement technique reveals rapid post-illumination changes in the carbon isotope composition of leaf-respired CO_2, *Plant Cell Environ.*, *30*, 469–482, doi:10.1111/j.1365-3040.2007.01634.x.

Barnes, J. D., K. E. Percy, N. D. Paul, P. Jones, C. K. McLaughlin, P. M. Mullineaux, G. Creissen, and A. R. Wellburn (1996), The influence of UV-B radiation on the physicochemical nature of tobacco (*Nicotiana tabacum* L.) leaf surfaces, *J. Exp. Bot.*, *47*, 99–109, doi:10.1093/jxb/47.1.99.

Beattie, G. A., and L. M. Marcell (2002), Effect of alterations in cuticular wax biosynthesis on the physiochemical properties and topography of maize leaf surfaces, *Plant Cell Environ.*, *25*, 1–16, doi:10.1046/j.0016-8025.2001.00804.x.

Bengtson, C., S. Larsson, and C. Liljenberg (1978), Effects of water stress on cuticular transpiration rate and amount and composition of epicuticular wax in seedlings of six oat varieties, *Physiol. Plant.*, *44*, 319–324, doi:10.1111/j.1399-3054.1978.tb01630.x.

Bowling, D. R., N. G. McDowell, B. J. Bond, B. E. Law, and J. R. Ehleringer (2002), ^{13}C content of ecosystem respiration is linked to precipitation and vapor pressure deficit, *Oecologia*, *131*, 113–124, doi:10.1007/s00442-001-0851-y.

Bowling, D. R., D. E. Pataki, and J. T. Randerson (2008), Carbon isotopes in terrestrial ecosystem pools and CO_2 fluxes, *New Phytol.*, *178*, 24–40, doi:10.1111/j.1469-8137.2007.02342.x.

Bracho, R. G., G. Starr, H. Gholz, T. A. Martin, W. P. Cropper Jr., and H. W. Loescher (2012), Controls on carbon dynamics by ecosystem structure and climate for southeastern U.S. Slash pine plantations, *Ecol. Monogr.*, doi:10.1890/11-0587.1, in press.

Brandes, E., N. Kodama, K. Whittaker, C. Weston, H. Rennenberg, C. Keitel, M. A. Adams, and A. Gessler (2006), Short-Term variation in the isotopic composition of organic matter allocated from the leaves to the stem of *Pinus sylvestris*: Effects of photosynthetic and postphotosynthetic carbon isotope fractionation, *Global Change Biol.*, *12*, 1922–1939, doi:10.1111/j.1365-2486.2006.01205.x.

Bréda, N., H. Cochard, E. Dreyer, and A. Granier (1993), Field comparison of transpiration, stomatal conductance and vulnerability to cavitation of *Quercus petraea* and *Quercus robur* under water stress, *Ann. Sci. For.*, *50*, 571–582, doi:10.1051/forest:19930606.

Bréda, N., A. Granier, F. Barataud, and C. Moyne (1995), Soil water dynamics in an oak stand, *Plant Soil*, *172*, 17–27, doi:10.1007/BF00020856.

Brüggemann, N., et al. (2011), Carbon allocation and carbon isotope fluxes in the plant-soil-atmosphere continuum: A review, *Biogeosciences*, *8*, 3457–3489, doi:10.5194/bg-8-3457-2011.

Brugnoli, E., K. T. Hubick, S. von Caemmerer, S. C. Wong, and G. D. Farquhar (1988), Correlation between the carbon isotope discrimination in leaf starch and sugars of C3 plants and the ratio of intercellular and atmospheric partial pressures of carbon dioxide, *Plant Physiol.*, *88*, 1418–1424, doi:10.1104/pp.88.4.1418.

Buchmann, N., J. R. Brooks, L. B. Flanagan, and J. R. Ehleringer (1998), Carbon isotope discrimination of terrestrial ecosystem, in *Stable Isotopes-Integration of biological ecological and geochemical processes*, edited by H. Griffiths, pp. 203–221, Bios Scientific, Oxford.

Chen, B., and J. M. Chen (2007), Diurnal, seasonal and interannual variability of carbon isotope discrimination at the canopy level in response to environmental factors in a boreal forest ecosystem, *Plant Cell Environ.*, *30*, 1223–1239, doi:10.1111/j.1365-3040.2007.01703.x.

Clark, K. L., H. L. Gholz, J. B. Moncrieff, F. Cropley, and H. W. Loescher (1999), Environmental controls over net exchanges of carbon dioxide from contrasting Florida ecosystems, *Ecol. Appl.*, *9*, 936–948, doi:10.1890/1051-0761(1999)009[0936:ECONEO]2.0.CO;2.

Clark, K. L., H. L. Gholz, and M. S. Castro (2004), Carbon dynamics along a chronosequence of slash pine plantations in north Florida, *Ecol. Appl.*, *14*, 1154–1171, doi:10.1890/02-5391.

Conte, M. H., and J. C. Weber (2002), Plant biomarkers in aerosols record isotopic discrimination of terrestrial photosynthesis, *Nature*, *417*, 639–641, doi:10.1038/nature00777.

Conte, M. H., J. C. Weber, P. J. Carlson, and L. B. Flanagan (2003), Molecular and carbon isotopic composition of leaf wax in vegetation and aerosols in a northern prairie ecosystem, *Oecologia*, *135*, 67–77, doi:10.1007/s00442-002-1157-4.

Cropper, W. P., and H. L. Gholz (1993), Simulation of the carbon dynamics of a Florida slash pine plantation, *Ecol. Modell.*, *66*, 231–249, doi:10.1016/0304-3800(93)90115-9.

Dawson, T. E., S. Mambelli, A. H. Plamboeck, P. H. Templer, and K. P. Tu (2002), Stable isotopes in plant ecology, *Annu. Rev. Ecol. Syst.*, *33*, 507–559, doi:10.1146/annurev.ecolsys.33.020602.095451.

Ehleringer, J. R., and T. E. Cerling (1995), Atmospheric CO_2 and the ratio of intercellular to ambient cohymus concentrations in plants, *Tree Physiol.*, *15*, 105–111, doi:10.1093/treephys/15.2.105.

Ekblad, A., and P. Högberg (2001), Natural abundance of ^{13}C in CO_2 respired from forest soils reveals speed of link between tree photosynthesis and root respiration, *Oecologia*, *127*, 305–308, doi:10.1007/s004420100667.

Evans, J. R., T. D. Sharkey, J. A. Berry, and G. D. Farquhar (1986), Carbon isotope discrimination measured concurrently with gas exchange to investigate CO_2 diffusion in leaves of higher plants, *Aust. J. Plant Physiol.*, *13*, 281–292, doi:10.1071/PP9860281.

Ewers, B. E., and R. Oren (2000), Analyses of assumptions and errors in the calculation of stomatal conductance from sap flux measurements, *Tree Physiol.*, *20*, 579–589, doi:10.1093/treephys/20.9.579.

Farquhar, G. D., M. H. O'Leary, and J. A. Berry (1982), On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves, *Aust. J. Plant Physiol.*, *9*, 121–137, doi:10.1071/PP9820121.

Farquhar, G. D., J. R. Ehleringer, and K. T. Hubick (1989), Carbon isotope discrimination and photosynthesis, *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, *40*, 503–537, doi:10.1146/annurev.pp.40.060189.002443.

Gessler, A., G. Tcherkez, A. D. Peuke, J. Ghashghaie, and G. D. Farquhar (2008), Experimental evidence for diel variations of the carbon isotope composition in leaf, stem and phloem sap organic matter in *ricinus communis*, *Plant Cell Environ.*, *31*, 941–953, doi:10.1111/j.1365-3040.2008.01806.x.

Ghashghaie, J., F. W. Badeck, G. Lanigan, S. Nogues, G. Tcherkez, E. Deleens, G. Cornic, and H. Griffiths (2003), Carbon isotope fractionation during dark respiration and photorespiration in C3 plants, *Phytochem. Rev.*, *2*, 145–161, doi:10.1023/B:PHYT.0000004326.00711.ca.

Gholz, H. L., and L. Boring (1991), Characterizing the site: Environment, associated vegetation and site potential, in *Forest Regeneration Manual*, edited by M. L. Duryea and P. M. Dougherty, pp. 163–182, Kluwer Acad., Dordrecht, Netherlands.

Gholz, H. L., and W. P. Cropper (1991), Carbohydrate dynamics in mature *pinus elliottii var. Elliottii* trees, *Can. J. For. Res.*, *21*, 1742–1747, doi:10.1139/x91-240.

Giese, B. N. (1975), Effects of light and temperature on the composition of epicuticular wax of barley leaves, *Phytochemistry*, *14*, 921–929, doi:10.1016/0031-9422(75)85160-0.

Gonzalez-Benecke, C. A., T. A. Martin, and W. P. Cropper Jr. (2011), Whole-Tree water relations of co-occurring mature *pinus palustris* and *pinus elliottii* var. *Elliottii*, *Can. J. For. Res.*, *41*, 509–523, doi:10.1139/X10-230.

Göttlicher, S., A. Knohl, W. Wanek, N. Buchmann, and A. Richter (2006), Short-Term changes in carbon isotope composition of soluble carbohydrates and starch: From canopy leaves to the root system, *Rapid Commun. Mass Spectrom.*, *20*, 653–660, doi:10.1002/rcm.2352.

Hauke, V., and L. Schreiber (1998), Ontogenetic and seasonal development of wax composition and cuticular transpiration of ivy (*Hedera helix* L.) Sun and shade leaves, *Planta*, *207*, 67–75, doi:10.1007/s004250050456.

Heyward, L. (1933), The root system of longleaf pine on the deep sands of western Florida, *Ecology*, *14*, 136–148, doi:10.2307/1932880.

Hobbie, E. A., and R. A. Werner (2004), Intramolecular, compound-specific, and bulk carbon isotope patterns in C_3 and C_4 plants: A review and synthesis, *New Phytol.*, *161*, 371–385, doi:10.1111/j.1469-8137.2004.00970.x.

Hollinger, D. Y., F. M. Kelliher, J. N. Byers, J. E. Hunt, T. M. McSeveny, and P. L. Weir (1994), Carbon dioxide exchange between an undisturbed

old-growth temperate forest and the atmosphere, *Ecology*, *75*, 134–150, doi:10.2307/1939390.

Hymus, G. J., K. Maseyk, R. Valentini, and D. Yakir (2005), Large daily variation in ^{13}C-enrichment of leaf-respired CO_2 in two *Quercus* forest canopies, *New Phytol.*, *167*, 377–384, doi:10.1111/j.1469-8137.2005.01475.x.

Jarvis, P. G., G. B. James, and J. J. Landsberg (1976), Coniferous forest, in *Vegetation and the Atmosphere 2: Case Studies*, edited by J. L. Monteith, pp. 171–240, Academic, London.

Johnson, J. D., D. P. Byers, and T. J. Dean (1995), Diurnal water relations and gas exchange of two slash pine (*Pinus elliottii*) families exposed to chronic ozone levels and acidic rain, *New Phytol.*, *131*, 381–392, doi:10.1111/j.1469-8137.1995.tb03075.x.

Kagawa, A., A. Sugimoto, and T. C. Maximov (2006), Seasonal course of translocation, storage and remobilization of ^{13}C pulse-labeled photoassimilate in naturally growing *Larix gmelinii* saplings, *New Phytol.*, *171*, 793–804, doi:10.1111/j.1469-8137.2006.01780.x.

Kayler, Z. E., L. Ganio, M. Hauck, T. G. Pypker, E. W. Sulzman, A. C. Mix, and B. J. Bond (2010), Bias and uncertainty of $\delta^{13}CO_2$ isotopic mixing models, *Oecologia*, *163*, 227–234, doi:10.1007/s00442-009-1531-6.

Knohl, A., R. A. Werner, W. A. Brand, and N. Buchmann (2005), Short-Term variations in $\delta^{13}C$ of ecosystem respiration reveals link between assimilation and respiration in a deciduous forest, *Oecologia*, *142*, 70–82, doi:10.1007/s00442-004-1702-4.

Kolattukudy, P. E., R. Crotea, and J. S. Buckner (1976), Biochemistry of plant waxes, in *Chemistry and Biochemistry of Natural Waxes*, edited by P. E. Kolattukudy, pp. 289–347, Elsevier Sci., Amsterdam.

Körner, C., R. Asshoff, O. Bignucolo, S. Hättenschwiler, S. G. Keel, S. Peláez-Riedl, S. Pepin, R. T. Siegwolf, and G. Zotz (2005), Carbon flux and growth in mature deciduous forest trees exposed to elevated CO_2, *Science*, *309*, 1360–1362, doi:10.1126/science.1113977.

Kosma, D. K., B. Bourdenx, A. Bernard, E. P. Parsons, S. Lü, J. Joubès, and M. A. Jenks (2009), The impact of water deficiency on leaf cuticle lipids of arabidopsis, *Plant Physiol.*, *151*, 1918–1929, doi:10.1104/pp.109.141911.

Kozlowski, T. T. (1992), Carbohydrate sources and sinks in woody plants, *Bot. Rev.*, *58*, 107–222, doi:10.1007/BF02858600.

Kunst, L., and L. Samuels (2009), Plant cuticles shine: Advances in wax biosynthesis and export, *Curr. Opin. Plant Biol.*, *12*, 721–727, doi:10.1016/j.pbi.2009.09.009.

Lai, C. T., G. Katul, J. Butnor, D. Ellsworth, and R. Oren (2002), Modelling night-time ecosystem respiration by a constrained source optimization method, *Global Change Biol.*, *8*, 124–141, doi:10.1046/j.1354-1013.2001.00447.x.

Lai, C. T., Jr., A. J. Ehleringer, P. P. Schauer, D. Y. Tans, K. T. Hollinger, U. Paw, J. W. Munger, and S. C. Wofsy (2005), Canopy-Scale $\delta^{13}C$ of photosynthetic and respiratory CO_2 fluxes: Observations in forest biomes across the United States, *Global Change Biol.*, *11*, 633–643, doi:10.1111/j.1365-2486.2005.00931.x.

Lavigne, M. B., et al. (1997), Comparing nocturnal eddy covariance measurements to estimates of ecosystem respiration made by scaling chamber measurements at six coniferous boreal sites, *J. Geophys. Res.*, *102*, 28,977–28,985, doi:10.1029/97JD01173.

Leide, J., U. Hildebrandt, G. Vogg, and M. Riederer (2011), The positional sterile (ps) mutation affects cuticular transpiration and wax biosynthesis of tomato fruits, *J. Plant Physiol.*, *168*, 871–877, doi:10.1016/j.jplph.2010.11.014.

Martin, T. A., and E. J. Jokela (2004), Developmental patterns and nutrition impact radiation use efficiency components in southern pine stands, *Ecol. Appl.*, *14*, 1839–1854, doi:10.1890/03-5262.

Maseyk, K., L. Wingate, U. Seibt, J. Ghashghaie, C. Bathellier, P. Almeida, R. Lobo de Vale, J. S. Pereira, D. Yakir, and M. Mencuccini (2009), Biotic and abiotic factors affecting the $\delta^{13}C$ of soil respired CO_2 in a Mediterranean oak woodland, *Isotopes Environ. Health Stud.*, *45*, 343–359, doi:10.1080/10256010903388212.

McDowell, N. G., D. R. Bowling, A. Schauer, J. Irvine, B. J. Bond, B. E. Law, and J. R. Ehleringer (2004a), Associations between carbon isotope ratios of ecosystem respiration, water availability and canopy conductance, *Global Change Biol.*, *10*, 1767–1784, doi:10.1111/j.1365-2486.2004.00837.x.

McDowell, N. G., D. R. Bowling, B. J. Bond, J. Irvine, B. E. Law, P. Anthoni, and J. R. Ehleringer (2004b), Response of the carbon isotopic content of ecosystem, leaf, and soil respiration to meteorological and physiological driving factors in a *Pinus ponderosa* ecosystem, *Global Biogeochem. Cycles*, *18*, GB1013, doi:10.1029/2003GB002049.

McMurtrie, R. E., H. L. Gholz, S. Linder, and S. T. Gower (1994), Climatic factors controlling the productivity of pine stands: A model-based analysis, *Ecol. Bull.*, *43*, 173–188.

McNaughton, K. G., and P. G. Jarvis (1991), Effects of spatial scale on stomatal control of transpiration, *Agric. For. Meteorol.*, *54*, 279–302, doi:10.1016/0168-1923(91)90010-N.

Mortazavi, B., and J. P. Chanton (2002), Carbon isotopic discrimination and control of nighttime canopy $\delta^{18}O$-CO_2 in a pine forest in the southeastern United States, *Global Biogeochem. Cycles*, *16*(1), 1008, doi:10.1029/2000GB001390.

Mortazavi, B., J. L. Prater, and J. P. Chanton (2004), A field-based method for simultaneous measurements of the $\delta^{18}O$ and $\delta^{13}C$ of soil CO_2 efflux, *Biogeosciences*, *1*, 1–9, doi:10.5194/bg-1-1-2004.

Mortazavi, B., J. P. Chanton, J. L. Prater, A. C. Oishi, R. Oren, and G. Katul (2005), Temporal variability in ^{13}C of respired CO_2 in a pine and a hardwood forest subject to similar climatic conditions, *Oecologia*, *142*, 57–69, doi:10.1007/s00442-004-1692-2.

Mortazavi, B., J. P. Chanton, and M. C. Smith (2006), Influence of ^{13}C enriched foliage respired CO_2 on $\delta^{13}C$ of ecosystem-respired CO_2, *Global Biogeochem. Cycles*, *20*, GB3029, doi:10.1029/2005GB002650.

Mortazavi, B., M. H. Conte, J. P. Chanton, M. C. Smith, J. C. Weber, J. Crumsey, and J. Ghashghaie (2009), Does the ^{13}C of foliage-respired CO_2 and biochemical pools reflect the ^{13}C of recently assimilated carbon?, *Plant Cell Environ.*, *32*, 1310–1323, doi:10.1111/j.1365-3040.2009.01999.x.

Moyes, A. B., S. J. Gaines, R. T. Siegwolf, and D. R. Bowling (2010), Diffusive fractionation complicates isotopic partitioning of autotrophic and heterotrophic sources of soil respiration, *Plant Cell Environ.*, *33*, 1804–1819, doi:10.1111/j.1365-3040.2010.02185.x.

Neary, D. G., D. L. Rockwood, N. B. Comerford, B. F. Swindel, and T. E. Cooksey (1990), Importance of weed control, fertilization, irrigation, and genetics in slash and loblolly pine early growth on poorly drained spodosols, *For. Ecol. Manage.*, *30*, 271–281, doi:10.1016/0378-1127(90)90142-X.

Neinhuis, C., and W. Barthlott (1998), Seasonal changes of leaf surface contamination in beech, oak, and ginkgo in relation to leaf micromorphology and wettability, *New Phytol.*, *138*, 91–98, doi:10.1046/j.1469-8137.1998.00882.x.

Nogués, S., G. Tcherkez, G. Cornic, and J. Ghashghaie (2004), Respiratory carbon metabolism following illumination in intact french bean leaves using $^{13}C/^{12}C$ isotope labeling, *Plant Physiol.*, *136*, 3245–3254, doi:10.1104/pp.104.048470.

Ogée, J., P. Peylin, P. Ciais, T. Bariac, Y. Brunet, P. Berbigier, C. Roche, P. Richard, G. Bardoux, and J.-M. Bonnefond (2003), Partitioning net ecosystem carbon exchange into net assimilation and respiration using $^{13}CO_2$ measurements: A cost-effective sampling strategy, *Global Biogeochem. Cycles*, *17*(2), 1070, doi:10.1029/2002GB001995.

Oren, R., J. S. Sperry, G. G. Katul, D. E. Pataki, B. E. Ewers, N. Phillips, and K. V. R. Schafer (1999), Survey and synthesis of intra-and interspecific variation in stomatal sensitivity to vapour pressure deficit, *Plant Cell Environ.*, *22*, 1515–1526, doi:10.1046/j.1365-3040.1999.00513.x.

Pataki, D. E., R. Oren, G. Katul, and J. Sigmon (1998), Canopy conductance of *Pinus taeda*, *Liquidambar styraciflua* and *Quercus phellos* under varying atmospheric and soil water conditions, *Tree Physiol.*, *18*, 307–315, doi:10.1093/treephys/18.5.307.

Percy, K. E., S. Manninen, K. H. Häberle, C. Heerdt, H. Werner, G. W. Henderson, and R. Matyssek (2009), Effect of 3 years' free-air exposure to elevated ozone on mature norway spruce (*Picea abies* (L.) Karst.) Needle epicuticular wax physicochemical characteristics, *Environ. Pollut.*, *157*, 1657–1665, doi:10.1016/j.envpol.2008.12.016.

Peuke, A. D., C. Windt, and H. Van As (2006), Effects of cold-girdling on flows in the transport phloem in *Ricinus communis*: Is mass flow inhibited?, *Plant Cell Environ.*, *29*, 15–25, doi:10.1111/j.1365-3040.2005.01396.x.

Powell, T. L., G. Starr, K. L. Clark, T. A. Martin, and H. L. Gholz (2005), Ecosystem and understory water and energy exchange for a mature, naturally regenerated pine flatwoods forest in north Florida, *Can. J. For. Res.*, *35*, 1568–1580, doi:10.1139/x05-075.

Powell, T. L., H. L. Gholz, K. L. Clark, G. Starr, W. P. Cropper, and T. A. Martin (2008), Carbon exchange of a mature, naturally regenerated pine forest in north Florida, *Global Change Biol.*, *14*, 2523–2538, doi:10.1111/j.1365-2486.2008.01675.x.

Powers, H. H., J. E. Hunt, D. T. Hanson, and N. G. McDowell (2010), A dynamic soil chamber system coupled with a tunable diode laser for online measurements of $\delta^{13}C$, $\delta^{18}O$, and efflux rate of soil-respired CO_2, *Rapid Commun. Mass Spectrom.*, *24*, 243–253, doi:10.1002/rcm.4380.

Prater, J. L., B. Mortazavi, and J. P. Chanton (2006), Diurnal variation of the $\delta^{13}C$ of pine needle respired CO_2 evolved in darkness, *Plant Cell Environ.*, *29*, 202–211, doi:10.1111/j.1365-3040.2005.01413.x.

Rao, G. G., S. K. M. Basha, and G. R. Rao (1981), Effect of NaCl salinity on amount and composition of epicuticular wax and cuticular transpiration rate in peanut *arachis hypogaea* L, *Indian J. Exp. Biol.*, *19*, 880–881.

Rascher, K. G., C. Máguas, and C. Werner (2010), On the use of phloem sap $\delta^{13}C$ as an indicator of canopy carbon discrimination, *Tree Physiol.*, *30*, 1499–1514, doi:10.1093/treephys/tpq092.

Sachse, D., A. Kahmen, and G. Gleixner (2009), Significant seasonal variation in the hydrogen isotopic composition of leaf-wax lipids for two deciduous tree ecosystems (*Fagus sylvativa* and *Acer pseudoplatanus*), *Org. Geochem.*, *40*, 732–742, doi:10.1016/j.orggeochem.2009.02.008.

Samuels, L., L. Kunst, and R. Jetter (2008), Sealing plant surfaces: Cuticular wax formation by epidermal cells, *Annu. Rev. Plant Biol.*, *59*, 683–707, doi:10.1146/annurev.arplant.59.103006.093219.

Scartazza, A., C. Mata, G. Matteucci, D. Yakir, S. Moscatello, and E. Brugnoli (2004), Comparisons of $\delta^{13}C$ of photosynthetic products and ecosystem respiratory CO_2 and their responses to seasonal climate variability, *Oecologia*, *140*, 340–351, doi:10.1007/s00442-004-1588-1.

Shepherd, T., and D. W. Griffiths (2006), The effects of stress on plant cuticular waxes, *New Phytol.*, *171*, 469–499, doi:10.1111/j.1469-8137.2006.01826.x.

Shepherd, T., G. W. Robertson, D. W. Griffiths, A. N. E. Birch, and G. Duncan (1995), Effects of environment on the composition of epicuticular wax from kale and swede, *Phytochemistry*, *40*, 407–417, doi:10.1016/0031-9422(95)00281-B.

Shepherd, T., G. W. Robertson, D. W. Griffiths, and A. N. E. Bircht (1997), Effects of environment on the composition of epicuticular wax esters from kale and swede, *Phytochemistry*, *46*, 83–96, doi:10.1016/S0031-9422(97)00272-0.

Sun, W., V. Resco, and D. G. Williams (2009), Diurnal and seasonal variation in the carbon isotope composition of leaf dark-respired CO_2 in velvet mesquite (*Prosopis velutina*), *Plant Cell Environ.*, *32*, 1390–1400, doi:10.1111/j.1365-3040.2009.02006.x.

Tcherkez, G., S. Nogués, J. Bleton, G. Cornic, F. Badeck, and J. Ghashghaie (2003), Metabolic origin of carbon isotope composition of leaf dark-respired CO_2 in french bean, *Plant Physiol.*, *131*, 237–244, doi:10.1104/pp.013078.

Tcherkez, G., G. Farquhar, F. Badeck, and J. Ghashghaie (2004), Theoretical considerations about carbon isotope distribution in glucose of C-3 plants, *Funct. Plant Biol.*, *31*, 857–877, doi:10.1071/FP04053.

Tcherkez, G., A. Mahé, and M. Hodges (2011), $^{12}C/^{13}C$ fractionations in plant primary metabolism, *Trends Plant Sci.*, *16*, 499–506, doi:10.1016/j.tplants.2011.05.010.

Teskey, R. O., H. L. Gholz, and W. P. Cropper Jr. (1994), Influence of climate and fertilization on net photosynthesis of mature slash pine, *Tree Physiol.*, *14*, 1215–1227, doi:10.1093/treephys/14.11.1215.

Trumbore, S. (2000), Age of soil organic matter and soil respiration: Radiocarbon constraints on belowground C dynamics, *Ecol. Appl.*, *10*, 399–411, doi:10.1890/1051-0761(2000)010[0399:AOSOMA]2.0.CO;2.

Valentini, R., et al. (2000), Respiration as the main determinant of carbon balance in European forests, *Nature*, *404*, 861–865, doi:10.1038/35009084.

Werner, C., F. Wegener, S. Unger, S. Nogués, and P. Priault (2009), Short-term dynamics of isotopic composition of leaf-respired CO_2 upon darkening: Measurements and implications, *Rapid Commun. Mass Spectrom.*, *23*, 2428–2438, doi:10.1002/rcm.4036.

Wettstein-Knowles, P. (1972), Genetic control of β-diketone and hydroxy-β-diketone synthesis in epicuticular waxes of barley, *Planta*, *106*, 113–130, doi:10.1007/BF00383991.

Wettstein-Knowles, P. (1995), Biosynthesis and genetics of waxes, in *Waxes: Chemistry, Molecular Biology and Functions*, edited by R. J. Hamilton, pp. 91–129, Oily Press, Dundee, U. K.

Whitehead, D., D. U. U. Okali, and F. E. Fasehun (1981), Stomatal response to environmental variables in two tropical forest species during the dry season in Nigeria, *J. Appl. Ecol.*, *18*, 571–587, doi:10.2307/2402418.

Wingate, L., U. Seibt, J. B. Moncrieff, P. G. Jarvis, and J. Lloyd (2007), Variations in ^{13}C discrimination during CO_2 exchange by *picea sitchensis* branches in the field, *Plant Cell Environ.*, *30*, 600–616, doi:10.1111/j.1365-3040.2007.01647.x.

Wingate, L., J. Ogée, R. Burlett, A. Bosc, M. Devaux, J. Grace, D. Loustau, and A. Gessler (2010), Photosynthetic carbon isotope discrimination and its relationship to the carbon isotope signals of stem, soil and ecosystem respiration, *New Phytol.*, *188*, 576–589, doi:10.1111/j.1469-8137.2010.03384.x.

Wofsy, S. C., M. L. Goulden, J. W. Munger, S. M. Fan, P. S. Bakwin, B. C. Daube, S. L. Bassow, and F. A. Bazzaz (1993), Net exchange of CO_2 in a mid-latitude forest, *Science*, *260*, 1314–1317, doi:10.1126/science.260.5112.1314.

Xu, C. Y., G. H. Lin, K. L. Griffin, and R. N. Sambrotto (2004), Leaf respiratory CO_2 is ^{13}C-enriched in five species of C_3 plants, *New Phytol.*, *163*, 499–505, doi:10.1111/j.1469-8137.2004.01153.x.

Yang, H., W. Liu, Q. Leng, M. T. Hren, and M. Pagani (2011), Variation in *n*-alkane δD values from terrestrial plants at high latitude: Implications for paleoclimate reconstruction, *Org. Geochem.*, *42*, 283–288, doi:10.1016/j.orggeochem.2011.01.006.

Zobitz, J. M., J. P. Keener, H. Schnyder, and D. R. Bowling (2006), Sensitivity analysis and quantification of uncertainty for isotopic mixing relationships in carbon cycle research, *Agric. For. Meteorol.*, *136*, 56–75, doi:10.1016/j.agrformet.2006.01.003.

J. P. Chanton, Department of Oceanography, Florida State University, Tallahassee, FL 32306, USA.

M. H. Conte, Bermuda Institute of Ocean Sciences, St. Georges GE01, Bermuda.

W. P. Cropper Jr. and T. A. Martin, School of Forest Resources and Conservation, University of Florida, Gainesville, FL 32611, USA.

B. Mortazavi, University of Alabama, Department of Biological Sciences, Tuscaloosa, AL 34587, USA. (bmortazavi@ua.edu)

J. C. Weber, Ecosystems Center, Marine Biological Laboratory, 7 MBL St., Woods Hole, MA 02543, USA.