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Temporal variability in ^{13}C of respired CO_2 in a pine and a hardwood forest subject to similar climatic conditions

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Abstract Temporal variability in the ^{13}C of foliage ($\delta^{13}\text{C}_\text{F}$), soil ($\delta^{13}\text{C}_\text{S}$) and ecosystem ($\delta^{13}\text{C}_\text{R}$) respired CO_2 was contrasted between a 17.2-m tall evenly aged loblolly pine forest and a 35-m tall unevenly aged mature second growth mixed broadleaf deciduous forest in North Carolina, USA, over a 2-year period. The two forests are located at the Duke Forest within a kilometer of each other and are subject to identical climate and have similar soil types. The $\delta^{13}\text{C}_\text{F}$, collected just prior to dawn, was primarily controlled by the time-lagged vapor pressure deficit (VPD) in both stands; it was used for calculating the ratio of intercellular to ambient CO_2 (C_i/C_a). A remarkable similarity was observed in the relationship between C_i/C_a and time-lagged VPD in these two forests despite large differences in hydraulic characteristics. This similarity emerged as a result of physiological adjustments that compensated for differences in plant hydraulic characteristics, as predicted by a recently proposed equilibrium hypothesis, and has implications to ecophysiological models. We found that in the broadleaf forest, the $\delta^{13}\text{C}$ of forest floor CO_2 efflux dominated the $\delta^{13}\text{C}_\text{R}$, while in the younger pine forest, the $\delta^{13}\text{C}$ of foliage respired CO_2 dominated $\delta^{13}\text{C}_\text{R}$. This dependence resulted in a more variable $\delta^{13}\text{C}_\text{R}$ in the pine forest when compared to the broadleaf forest due to the larger photosynthetic contribution. Given the sensitivity of the atmospheric inversion models to $\delta^{13}\text{C}_\text{R}$, the results demonstrate that these models could be improved by accounting for stand characteristics, in addition to previously recognized effects of moisture availability, when estimating $\delta^{13}\text{C}_\text{R}$.

Keywords Carbon isotope · Coniferous forest · Hardwood forest · Vapor pressure deficit · Ecosystem respiration

Introduction

Partitioning the exchange of CO_2 between the biosphere and the atmosphere requires detailed understanding of the roles of the terrestrial and oceanic environments in the uptake and release of CO_2 . Stable isotopes of CO_2 (^{13}C , ^{18}O) and CO_2 concentrations have been used in the analysis of the global carbon cycle by inversion of atmospheric transport models (Ciais et al. 1995a; Rander-son et al. 2002) to partition the global CO_2 sink into the oceanic and terrestrial components. The atmospheric inversion models rely on estimates of photosynthetic discrimination (Δ) and the ^{13}C of ecosystem-respired CO_2 ($\delta^{13}\text{C}_\text{R}$) (Tans et al. 1993; Fung et al. 1997) to assess variations in the magnitude of the terrestrial carbon sink. Δ can be determined at the leaf level (Evans et al. 1986; Harwood et al. 1993; Gillon and Yakir 2000) and at the ecosystem level (Yakir and Sternberg 2000; Bowling et al. 2001; Buchmann and Kaplan 2001; Conte and Weber 2002). Whole ecosystem discrimination can be determined from the isotopic composition of CO_2 in the convective boundary layer, which includes the effects of respiration, photosynthesis and turbulent transport (Lloyd et al. 1996). A recent approach uses the isotopic composition of ablated leaf waxes to determine discrimination at the continental scale (Conte and Weber 2002). On the global scale, Δ and $\delta^{13}\text{C}_\text{R}$ are not measured directly and must be estimated from physiological-climate models (e.g., Lloyd and Farquhar 1994; Fung et al. 1997) or from atmospheric data (Bakwin et al. 1998) to which large corrections are applied to remove the isotopic imprint of anthropogenic sources.

Investigations at the ecosystem scale (e.g., Bowling et al. 2002) indicate that Δ and $\delta^{13}\text{C}_\text{R}$ do not remain constant, but reflect the consequences of changes in environmental factors on photosynthetic and respiratory

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processes. Interannual variability in Δ and $\delta^{13}\text{C}_R$ caused by nutrient stress, changes in C_3/C_4 productivity, or ENSO induced droughts (Hoerling and Kumar 2003), would be interpreted by inversion models as a shift in the terrestrial and oceanic sinks (Randerson et al. 2002). The variability in these two parameters will alter the conclusions about the timing and nature of the terrestrial carbon sink (Bowling et al. 2002). Fung et al. (1997) indicate that a 3% overestimate in the global value of Δ would result in a 20% underestimate in the magnitude of the biospheric sink.

Evidence for variability in $\delta^{13}\text{C}_R$ in response to changes in environmental factors (Pataki et al. 2003) such as vapor pressure deficit (VPD), precipitation, and soil moisture has been investigated for coniferous forest of the Northwest Pacific (Bowling et al. 2002; Fessenden and Ehleringer 2002, 2003), in a Florida pine plantation (Mortazavi and Chanton 2002a), and in the tropical forest of the Amazon basin (Ometto et al. 2002). The $\delta^{13}\text{C}_R$ has been found to be ^{13}C enriched during drier weather because of reduced stomatal conductance and lower photosynthetic discrimination (Bowling et al. 2002; Fessenden and Ehleringer 2002; Mortazavi and Chanton 2002a). Because of the sensitivity of global atmospheric inversion models to $\delta^{13}\text{C}_R$, these models should begin to incorporate the shifts in $\delta^{13}\text{C}_R$ based upon variations in moisture and stand characteristics (Ehleringer et al. 2002).

Northern mid-latitude forests are a large terrestrial carbon sink (Ciais et al. 1995b; Schimel 1995; Houghton et al. 1998; Tans and White 1998). In south-eastern USA, the young pine forests appear to be among the largest atmospheric terrestrial carbon sinks with published annual net ecosystem carbon exchange (NEE) values among the highest when compared to other published NEE values in Europe and North America (Wofsy et al. 1993; Hollinger et al. 1994; Clark et al. 1999; Valentini et al. 2000; Lai et al. 2002). These young pine forests are interlaced with second growth mature deciduous forests. The stands within the forest mosaic differ in characteristics such as age, tree height and their ability to assimilate CO_2 (Clark et al. 1999). To date, separating the effects of climatic conditions and soil type on carbon isotope discrimination from plant physiologic and hydraulic properties remains a challenge.

The objectives of this study were to investigate variations in the carbon isotope composition of ecosystem respiration ($\delta^{13}\text{C}_R$) and two of its main components, foliage respiration ($\delta^{13}\text{C}_F$) and soil respiration ($\delta^{13}\text{C}_S$) at two AmeriFlux sites in the Duke Forest of North Carolina, USA which were subject to identical climatic conditions: a maturing pine forest and an older, taller deciduous broadleaf forest. Tree height, through its control on stomatal conductance (Schäfer et al. 2000) influences variations in Δ , which is reflected in $\delta^{13}\text{C}_R$ (Bowling et al. 2002). To avoid potentially damaging effects of declining leaf water potential, stomatal conductance declines with tree height (Schäfer et al. 2000).

Because of hydraulic constraint and its impact on stomatal conductance we hypothesized that ^{13}C enriched

values of foliage respired CO_2 ($\delta^{13}\text{C}_F$) and foliage organic matter ($\delta^{13}\text{C}_P$) would be measured at the taller deciduous forest. We also hypothesized that because of lower assimilation rates (Ryan and Yoder 1997; P. Stoy et al., unpublished data) and higher soil CO_2 fluxes (Palmroth et al., unpublished data) at the older forest, the ^{13}C of ecosystem respired CO_2 would exhibit lower variability compared to the younger pine forest in response to changes in water availability. This would result from the relatively higher contribution of soil respired $^{13}\text{CO}_2$ to $\delta^{13}\text{C}_R$, which would buffer changes in $\delta^{13}\text{C}_R$ (Mortazavi and Chanton 2002a).

To determine the variability in $\delta^{13}\text{C}_F$, $\delta^{13}\text{C}_P$ and $\delta^{13}\text{C}_R$ for southeastern USA forests composed of different stands, we measured $\delta^{13}\text{C}_F$ from foliage collected before dawn, collected foliage for $\delta^{13}\text{C}_P$ and sampled canopy air from which nighttime $\delta^{13}\text{C}_R$ values were determined. Soil CO_2 fluxes and $\delta^{13}\text{C}_S$ were also determined with static chambers at both stands. The broader objective of this effort is to provide guidance for dealing with a forest mosaic for inverse atmospheric models that use stable isotopes to constrain regional sources and sinks.

Materials and methods

Study site

The study sites are located in the Blackwood Division of Duke Forest in Orange County, (35°58'41.430"N, 79°05'39.087"W), near Durham, N.C., USA. The 90-ha pine forest is a 17.2-m tall even-aged stand and was established from 3-year seedlings following clear-cutting and burning of the site in 1983. Loblolly pines (*Pinus taeda*, L.) from a Piedmont provenance were planted at 2×2.4 m spacing. Density of co-dominant pines in the study portion of the stand is approximately 1,600 trees ha⁻¹. The hardwood forest is a 35-m tall unevenly aged mature second growth deciduous hardwood mix. The oldest individuals exceed 180 years. The distance between the two towers is 986 m. The two stands have very distinct leaf area index (LAI) profiles in terms of leaf area density distribution near the top of the canopy. The LAI peak is at normalized height (z/h) of 0.8 and 0.6 for the hardwood and pine, respectively (Katul et al. 1997).

Weather and climate data

Mean air temperature and relative humidity were measured with Vaisala sensors (HMP35C, Campbell Scientific, Logan, Utah, USA) at two-third the canopy height. Soil moisture content was measured with four CS 615 sensors (Campbell Scientific) at the pine forest and 12 sensors at the hardwood forests. At the pine forest, the sensors measured the depth-integrated soil moisture across a 30-cm distance. At the hardwood forest, six sensors measured soil moisture at the 5–15-cm depth and six sensors measured soil moisture at the 20–25-cm depth.

The outputs from the 12 sensors were used to calculate mean soil moisture at the hardwood site. Oren et al. (1998) showed that 30 cm is sufficiently deep to encompass the root volume active in water uptake. Data were sampled every 1 min and averaged over a 30-min period.

Protocols for sample collection

¹³C of foliage respired CO₂

Foliage was collected just before dawn and put into vials and capped with Belco stoppers, which have been shown to be inert with respect to ¹³C and ¹⁸O of CO₂ (Mortazavi and Chanton 2002b). The measurements were timed to follow a long period of darkness to minimize the effects of isotopic fractionation, which may be associated with leaf respiration (Ghashghaie et al. 2001, 2003; Duranceau et al. 1999; Tcherkez et al. 2003).

Pine foliage was clipped from several branches. At the hardwood forest mid-canopy foliage was collected with the shotgun method. Three bottles were filled with 12 pine needles and three to five leaves from the hardwood species. Before adding the foliage to the vial, each vial was flushed with background air collected from above the canopy. This background air was analyzed for CO₂ concentration and δ¹³C as described below. After a 20-min dark incubation period at the ambient temperature, the bottles were put on ice. Our objective was to use the shortest incubation time that would yield sufficient respired CO₂ (5,000–10,000 ppmv) to make the background values inconsequential. Bottles were then brought to the laboratory where they were frozen and analyzed within 2 weeks of sample collection.

The δ¹³C of the vial headspace results from a combination of CO₂ respired by the foliage and a small amount of background CO₂ in the vial. A mass balance equation can be used to estimate δ¹³C_F:

$$C_V = C_B + C_F \quad (1)$$

$$\delta^{13}C_V \times C_V = \delta^{13}C_B \times C_B + \delta^{13}C_F \times C_F \quad (2)$$

where C_V , C_B and C_F correspond to the vial headspace, background CO₂ concentration and CO₂ produced by foliage respiration. $\delta^{13}C_V$, $\delta^{13}C_B$, and $\delta^{13}C_F$ correspond to the ¹³C ratio of vial headspace, background, and foliage respired CO₂. With Eqs. 1 and 2 and from measurement of the vial headspace and background CO₂ concentrations and isotopic ratios, δ¹³C_F can be solved for.

Precision of the method was estimated in November 2001. Foliage from a selected pine branch was collected and combined. Foliage subsample were then placed in five separate vials for determination of δ¹³C_F. Foliage collected from the same branch yielded a standard deviation (SD) of 0.34‰. On March 2002, we compared the bottle incubation procedure for determination of δ¹³C_F with a closed loop and vial system similar to that described by

Fessenden and Ehleringer (2003), and results between the two methods were not significantly different (B. Mortazavi et al, unpublished data). Additional comparisons between the foliage-vial technique and approaches for measuring the δ¹³C_F with Mylar balloons and leaf chambers to enclose whole branches have also shown agreement within 0.6±0.5 ‰ (J. Prater and J. Chanton, unpublished data).

Foliage ¹³C

The carbon isotopic composition of leaves was determined for samples collected before dawn. At the pine plantation, needles from mid canopy and top of the canopy from several branches were collected and pooled. At the hardwood site, samples from several trees were collected with the shotgun method and pooled. All organic matter was dried at 60°C, ground to a fine powder and analyzed for ¹³C on an isotope ratio mass spectrometer (IRMS) coupled to a CHN analyzer. Measurement precision for all organic matter was ~0.2‰. Data are presented as means and the 1/2 range of variability on duplicate measurements.

Tower profiles

Nighttime air samples were collected between 2 and 4 a.m. local time. At the pine forest, nighttime samples were collected within the canopy at 0.01, 0.5, 2, 5, and 10 m and above the canopy at 23 m above the ground. At the hardwood site nighttime air samples were collected within the canopy at 0.01, 0.5, 9, 20 m and above the canopy at 42 m above the ground. Duplicate daytime samples were also collected at local noontime from the tops of the towers. During the first two visits to the site, samples were only collected at one location (Pine forest: November 2000 and Hardwood forest: February 2001). During subsequent visits samples were collected during the same night at both locations, except in March 2002 at the hardwood site, when the CO₂ gradient was <20 ppm and canopy air samples were not collected. At both sites, the entire air sample collection time lasted <0.5 h. Samples were collected with a bellows pump, passed through a magnesium perchlorate trap to remove H₂O vapor and pressurized into pre-evacuated Summa canisters (Biospheric Research) (Mortazavi and Chanton 2002b). Ecosystem respired δ¹³CO₂ were determined by the geometric linear regression (Keeling plots) applied to the nighttime tower CO₂ isotope ratios and their respective 1/CO₂ concentrations (Pataki et al. 2003).

Soil flux

Soil CO₂ fluxes were measured within <12 h of the tower sampling with replicate static chambers placed within 5 m of each other at both forests. The chambers were

65×65 cm and enclosed a volume of 102 l, including the collar base. The collars were inserted into the soil to a depth of 2 cm and were placed permanently at the site during the initial visit. A fan inside the chamber gently circulated the air. Replicate gas samples (125 ml) from the chamber headspace were drawn with a syringe and stored in pre-evacuated glass vials with Belco stoppers for later analysis (Mortazavi and Chanton 2002b). Samples were collected at time 0 and every 15 min during a 45-min period for CO₂ concentration determination and isotopic analysis. During the sample withdrawal a sample port on the opposite side of the collection port was left open to the atmosphere to avoid pressure fluctuations. An in-line magnesium perchlorate trap was used to remove H₂O vapor when collecting headspace samples from the chambers. Samples were analyzed for CO₂ concentration and isotopic composition within a week of collection. Previous investigation has shown that the sample integrity is preserved within the time frame of the analysis (Mortazavi and Chanton 2002b). CO₂ concentrations increased linearly from background levels to approximately 1,000 ppm during the entire sample collection period. Soil respired $\delta^{13}\text{C}_{\text{CO}_2}$ was determined by the geometric linear regression (Keeling plots) applied to the chamber headspace CO₂ isotopic ratios and their respective 1/CO₂ concentrations.

Analysis

The ¹³C of CO₂ for samples collected along the tower and from the soil CO₂ flux chambers was determined with a gas chromatograph isotope ratio mass spectrometer (GC-IRMS) (Gas Chromatograph: Hewlett Packard 5890 Series II, IRMS: Finnigan Delta S) operating in continuous flow mode. We applied a simple modification to a commercially available GC-IRMS for rapid and precise determination of the stable isotopes (¹³C and ¹⁸O) of CO₂ at ambient CO₂ concentration. For a full description of the method see Mortazavi and Chanton (2002b). The GC was equipped with a Chromopack (Raritan, N.J., USA) Poroplot Q column (27.5-m long with a 2.5-m particle trap) to

separate N₂O from CO₂ and was operated at an oven temperature of 25°C. Transfer of the GC effluent to the MS was through a deactivated glass capillary.

The isotopic ratio of CO₂ in foliage bottles following the 20-min incubations was determined from 0.2 ml injections of headspace CO₂ into the GC-IRMS. The isotopic ratio of the background air CO₂ used to flush the bottles was determined as previously described for tower samples. The CO₂ concentrations of the headspace CO₂ after the incubation was determined with a LI-COR 6200 (LI-COR, Lincoln, Neb., USA) according to the procedures described by Davidson and Trumbore (1995). A volume of 100 μl of the headspace gas samples were injected into a CO₂ free air stream that carried the sample into a mixing chamber (150 ml) placed inline before the LI-COR 6200. The peak height was recorded and used to determine the CO₂ concentrations. A volume of 100 μl to 1 ml of certified 1% CO₂ were used to generate a calibration curve. The coefficient of variations for replicate injection of standard gases was less than 1%. CO₂ concentrations in the headspace following the incubation increased to between 1 and 2%. The initial CO₂ concentration of the background air used to flush the incubation bottles and the tower samples was determined with a LI-COR 6200. The LI-COR was calibrated with a NOAA reference gas (369 ppm). The CO₂ concentration from the soil CO₂ flux chambers samples were determined from the CO₂ voltage obtained from the GC-IRMS (Mortazavi and Chanton 2002b). A plot of CO₂ concentrations determined from the voltage versus CO₂ concentrations determined with the LI-COR 6200 resulted in a slope (0.99±0.02) that was not significantly different from unity (*t*-test, *p*<0.05, Mortazavi and Chanton 2002b).

Isotopic ratios data are presented in the δ notation, and are reported relative to V-PDB. External precision for isotopic measurements was ±0.2 based on repeated measurements of a laboratory-working standard.

Table 1 Environmental conditions at the pine and hardwood forests prior to sampling for nighttime CO₂ concentration and isotopic measurements. Soil moisture was measured with four and 12 sensors at the pine and hardwood forest, respectively. All data are half

hourly averages for the week prior to sampling except VPD values, which represent daytime averages (net radiation >0 W m⁻²) for days 3 and 4 prior to sampling. *ND* no data

Month	Air temperature (°C)		VPD (kPa)		Soil temperature (°C)		Soil moisture (m ³ m ⁻³)	
	Pine	Hardwood	Pine	Hardwood	Pine	Hardwood	Pine	Hardwood
15 Nov 2000	11.92	13.13	0.69	0.81	11.92	13.13	0.141	ND
21 Feb 2001	7.86	8.19	0.52	0.51	7.86	8.19	0.326	0.327
12 Jun 2001	22.35	22.82	0.70	0.89	22.35	22.82	0.227	0.242
29 Aug 2001	22.85	23.49	1.07	1.28	22.85	23.49	0.172	0.156
27 Nov 2001	9.94	11.74	0.23	0.37	9.94	11.74	0.141	0.143
15 Mar 2002	10.45	8.77	0.47	0.50	10.44	8.77	0.289	0.386
10 Jul 2002	25.85	26.16	2.01	1.98	25.85	26.16	0.131	0.146
5 Sep 2002	19.79	19.07	0.21	0.35	19.78	19.07	0.283	0.329

Results

Environmental conditions

Our sampling strategy covered the wide range in environmental conditions that occur at this site. Precipitation averages 1,151 mm annually and is well distributed throughout the year. July and August are normally the wettest months with an average of 118 mm of rainfall per month. October and November are normally the driest with an average of 80 mm of rainfall per month. Air temperatures at the site ranged from 7.86°C in February 2001 to a maximum of 26.16°C in July 2002 (Table 1). Air temperatures and VPD at the two sites were similar with the maximum difference measured at the two sites of 1.68°C in March 2002 and 0.21 kPa in August 2001 (Table 1). Minimum soil moisture content was measured in November 2001 at the hardwood forest, while maximum soil moisture was measured in February of 2001 at the pine forest (Table 1). VPD and soil moisture values varied during the study period by a factor of 9.77 and 2.39, respectively (Table 1).

Carbon isotope ratios of organic pools and respired CO₂

The range of variability in $\delta^{13}\text{C}_\text{P}$ was greater at the pine when compared to the hardwood forest (Table 2). $\delta^{13}\text{C}_\text{P}$ values compared for the same time periods were similar at both locations (Table 2). The most enriched $\delta^{13}\text{C}_\text{P}$ values at the pine stand were measured in July 2002, when soil moisture was minimum (Table 1). The temporal variability in $\delta^{13}\text{C}_\text{F}$ at both stands exceeded the variability in $\delta^{13}\text{C}_\text{P}$ (Table 2). The variability in $\delta^{13}\text{C}_\text{F}$ for the same time periods was greater at the pine compared to the hardwood stand. There was a greater range of variations in $\delta^{13}\text{C}_\text{F}$ than in $\delta^{13}\text{C}_\text{P}$ between any two-time points. For example, from July 2002 to September 2002, $\delta^{13}\text{C}_\text{F}$ varied by 2.1 and 2.8‰, while $\delta^{13}\text{C}_\text{P}$ varied by 0.4 and 1.3‰ at the hardwood and pine stands, respectively (Table 2).

We used the Keeling plot approach (Keeling 1958) to determine $\delta^{13}\text{C}_\text{R}$ from nighttime measurement of CO₂

concentrations and isotopic ratios. $\delta^{13}\text{C}_\text{R}$ at the pine forest varied from -31.0‰ in March 2002 to -26.4‰ in July 2002 (Table 2). $\delta^{13}\text{C}_\text{R}$ at the hardwood forest was ^{13}C enriched during the growing season, on average, by 1.4‰ relative to $\delta^{13}\text{C}_\text{R}$ at the pine forest for the same time periods (Table 2). The maximum change in $\delta^{13}\text{C}_\text{R}$ occurred from November 2001 to March 2002 at the pine forest (4.6‰, Table 2) and from July 2002 to September 2002 (1‰, Table 2) at the hardwood forest.

Mean $\delta^{13}\text{C}_\text{S}$ values were $-26.2\pm 0.8\%$ and $-26.9\pm 1.8\%$ at the hardwood and pine plantations (Table 2). The variability in $\delta^{13}\text{C}_\text{S}$ was less than the variability in $\delta^{13}\text{C}_\text{R}$ and $\delta^{13}\text{C}_\text{S}$ was generally enriched relative to $\delta^{13}\text{C}_\text{R}$ (Table 2). The most enriched $\delta^{13}\text{C}_\text{S}$ values were measured in August 2001 at the hardwood site and in July 2002 at the pine stand. From July 2002 to September 2002, $\delta^{13}\text{C}_\text{S}$ varied by 2.0 and 1.1‰ at the pine and hardwood forest, respectively (Table 2). During the same time period, $\delta^{13}\text{C}_\text{R}$ varied by 1.0 and 3.3‰ at the hardwood and pine stands, respectively (Table 2). In February 2001 and November 2001, $\delta^{13}\text{C}_\text{R}$ and $\delta^{13}\text{C}_\text{S}$ were measured at the hardwood forest, when foliage was absent (Table 2). In November 2001 $\delta^{13}\text{C}_\text{S}$ and $\delta^{13}\text{C}_\text{R}$ were nearly identical, while a larger difference was observed in February 2001 (Table 2).

Variability in $\delta^{13}\text{C}_\text{F}$, $\delta^{13}\text{C}_\text{B}$, $\delta^{13}\text{C}_\text{S}$ and $\delta^{13}\text{C}_\text{R}$ in response to VPD and soil moisture

There was an increase in $\delta^{13}\text{C}_\text{F}$ with increasing VPD at both locations (Fig. 1a). Here, VPD is the average daytime (net radiation $>0.0 \text{ W m}^{-2}$) VPD corresponding to days 3 and 4 prior to nighttime sampling. This time lag was selected as that which provides highest correlation between the isotopic composition of respired CO₂ and VPD, after Bowling et al. (2002). For example, the correlation coefficient between $\delta^{13}\text{C}_\text{F}$ and VPD increased from 0.55 (for VPD for the day prior to sampling) to 0.77 (for VPD two days prior to sampling), and to 0.88 for average VPD for days 3 and 4 prior to sampling. Beyond this time period the correlation decayed.

In contrast to Fig. 1a, there was no relation between $\delta^{13}\text{C}_\text{F}$ and soil moisture (Fig. 1b). There was a mild

Table 2 ^{13}C of ecosystem- ($\delta^{13}\text{C}_\text{R}$), soil ($\delta^{13}\text{C}_\text{S}$) and foliage-respired ($\delta^{13}\text{C}_\text{F}$) CO₂ and foliage organic matter ($\delta^{13}\text{C}_\text{P}$) at the hardwood and pine forest in North Carolina. $\delta^{13}\text{C}_\text{R}$ (‰) and $\delta^{13}\text{C}_\text{F}$

(‰) and $\delta^{13}\text{C}_\text{P}$ (‰) were determined from samples collected at nighttime. $\delta^{13}\text{C}_\text{S}$ (‰) was determined within 12 h of the tower sampling

Date	$\delta^{13}\text{C}_\text{R}$		$\delta^{13}\text{C}_\text{S}$		$\delta^{13}\text{C}_\text{F}$		$\delta^{13}\text{C}_\text{P}$	
	Hardwood	Pine	Hardwood	Pine	Hardwood	Pine	Hardwood	Pine
15 Nov 2000	–	-28.9±1.3	–	-27.0±1.6	–	-28.7±0.7	–	-28.9±1.2
21 Feb 2001	-24.3±1.5	–	-26.0±0.5	–	–	–	–	–
12 Jun 2001	-26.8±0.3	-28.8±1.2	-25.8±0.2	-26.4±0.2	-27.6±0.1	-28.9±0.5	-27.8±0.7	-29.2±0.8
29 Aug 2001	-26.5±0.3	-27.0±0.4	-25.4±0.2	-27.0±0.3	–	–	-28.1±0.6	-29.6±0.7
27 Nov 2001	-26.8±0.1	-26.9±0.4	-26.5±0.3	-26.7±0.1	–	-31.0±1.1	–	-29.2±0.6
15 Mar 2002	–	-31.0±1.4	-25.9±0.3	-27.7±0.4	–	-29.9±0.6	–	-29.7±0.5
10 Jul 2002	-26.0±0.3	-26.4±0.4	-26.2±0.1	-25.7±0.5	-27.2±1.4	-27.3±0.4	-28.7±0.5	-27.7±1.2
5 Sep 2002	-27.0±0.5	-29.7±0.7	-27.3±0.3	-27.7±0.1	-29.3±1.1	-30.1±0.8	-29.1±0.8	-29.0±0.9

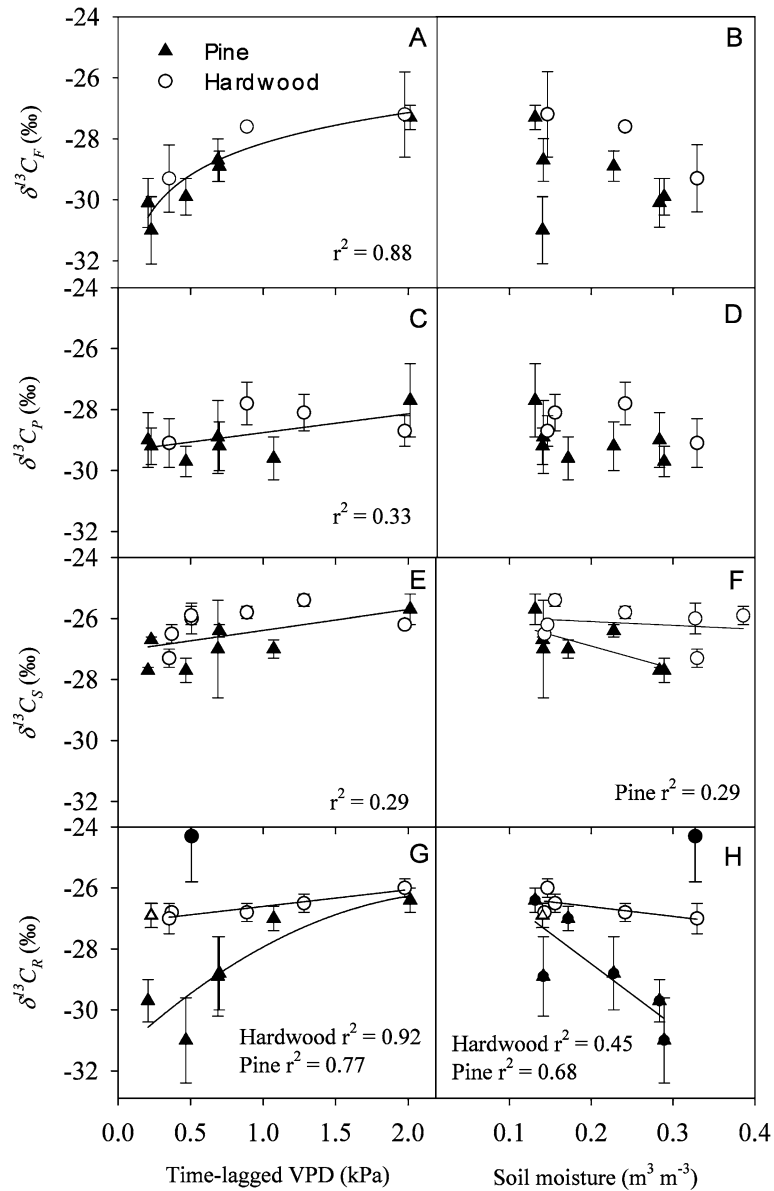


Fig. 1 a $\delta^{13}C_F$ (mean \pm SD) versus time lagged daytime (net radiation $> 0 W m^{-2}$) VPD (average of daytime VPD for days 3 and 4 prior to sampling) for the pine and hardwood forest ($\delta^{13}C_F = -28.1466 \times VPD^{-0.0523}$). b $\delta^{13}C_F$ (mean \pm SD) versus soil moisture for 1 week prior to sampling at the two stands. c $\delta^{13}C_P$ (mean \pm SD) versus time lagged daytime VPD (average of daytime VPD for days 3 and 4 prior to sampling) for the pine and hardwood forest. d $\delta^{13}C_P$ (mean \pm SD) versus soil moisture for 1 week prior to sampling. e $\delta^{13}C_S$ (mean $\pm 1/2$ range in variability) versus time lagged daytime VPD (average of daytime VPD for days 3 and 4 prior to sampling) for the pine and hardwood forest. f $\delta^{13}C_S$ (mean $\pm 1/2$ range in variability) versus soil moisture (θ) for 1 week prior to sampling at the two stands. g $\delta^{13}C_R$ versus time lagged daytime VPD (average

of daytime VPD for days 3 and 4 prior to sampling) for the hardwood ($\delta^{13}C_R = -27.153 + 0.548 \times VPD$), the value corresponding to the time the foliage was absent (*closed circle*) was excluded from the regression and pine forest ($\delta^{13}C_R = -31.443 + 4.425 \times VPD - 0.919 \times VPD^2$), the value corresponding to the freeze event (*open triangle*) was excluded from the regression. h $\delta^{13}C_R$ versus soil moisture (θ) for 1 week prior to sampling at the hardwood ($\delta^{13}C_R = -25.969 - 3.202 \times \theta$), the value corresponding to the time the foliage was absent (*closed circle*) was excluded from the regression and at the pine forest ($\delta^{13}C_R = -24.458 - 20.134 \times \theta$), the value corresponding to the freeze event (*open triangle*) was excluded from the regression

increase in $\delta^{13}C_P$ with time-lagged VPD as well (Fig. 1c), but no clear pattern was present with soil moisture (Fig. 1d). There was minor variability in $\delta^{13}C_S$ with time-lagged VPD (Fig. 1e) and soil moisture (Fig. 1f). Mikan et al. (2000) radiolabelled *Populus* trees and found peak activity in soil ^{14}C after 3–4 day. This corresponds well to our time lag.

The relation between $\delta^{13}C_R$ and time-lagged VPD for the two forest types exhibited distinct patterns with a greater range of variability in $\delta^{13}C_R$ for the pine forest compared to the hardwood forest (Fig. 1g). At the hardwood forest, $\delta^{13}C_R$ did not demonstrate large variability in the range of observed soil moisture values

(Fig. 1h). At the pine forest, in contrast, there was a decline in $\delta^{13}\text{C}_R$ with increasing soil moisture (Fig. 1h).

Soil CO₂ flux

Soil CO₂ fluxes showed a seasonal pattern at both locations (Table 3). Minima in soil CO₂ flux were measured during the winter months, while maxima were measured during the growing season (Table 3). With the exception of September 2002 soil CO₂ fluxes were higher at the hardwood forest and averaged 4.86 ± 1.33 and 3.52 ± 0.39 $\mu\text{moles CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ at the hardwood and pine forests, respectively (Table 3).

Discussion

This study is the first to compare the temporal variability in the carbon isotopic composition of respired CO₂ for two forest types located in close proximity and exposed to the same climatic conditions and with similar soils. To address the study objectives, we first evaluate the causes for differences in long-term carbon isotopic discrimination between pine and hardwood forests, focusing on mean $\delta^{13}\text{C}_F$ and $\delta^{13}\text{C}_P$ because of their linkage to conductance and photosynthesis. We follow with an analysis of the causes of temporal variability in isotopic discrimination at each forest and their linkage to environmental drivers such as VPD and soil moisture. The co-variation amongst the three isotopic discrimination variables is also analyzed.

Leaf-level isotopic differences between pine and hardwood stands

As stated earlier, $\delta^{13}\text{C}_F$ collected just prior to dawn, increased with increasing VPD for both stands (Fig. 1a). At the leaf level, photosynthetic discrimination is related to $[C_i/C_a]$, where C_i is the canopy-scale intercellular CO₂ concentration and C_a is the ambient CO₂ concentration, which is affected by environmental factors (Farquhar et al. 1989), such as VPD, through their effect on photosynthe-

Table 3 Soil Respiration rates ($\mu\text{moles CO}_2 \text{ m}^{-2} \text{ s}^{-1}$; mean \pm 1/2 range of variability of duplicate chambers). Errors were propagated for calculation of the grand mean

Date	Hardwood	Pine
15 Nov 2000	–	1.42 \pm 0.16
21 Feb 2001	1.44 \pm 0.03	–
12 Jun 2001	6.25 \pm 0.22	4.76 \pm 0.04
29 Aug 2001	7.82 \pm 0.46	4.02 \pm 0.11
27 Nov 2001	4.26 \pm 0.41	3.72 \pm 0.23
15 Mar 2002	2.98 \pm 0.33	2.08 \pm 0.21
10 Jul 2002	5.95 \pm 0.95	2.39 \pm 0.12
5 Sep 2002	5.33 \pm 0.58	6.25 \pm 0.00
Mean \pm error	4.86 \pm 1.33	3.52 \pm 0.39

sis or canopy conductance. The highest correlation between $\delta^{13}\text{C}_F$ and VPD occurred for a few days (average of day 3 and day 4) prior to sampling, indicating that pre-dawn $\delta^{13}\text{C}_F$ represents the isotopic composition of assimilated carbon during few days prior to sampling, while $\delta^{13}\text{C}_P$ is the temporally integrated value of the ^{13}C of structural material at the time they were assimilated. Once the structural material of the foliage is assimilated there are minor changes with time in its isotopic composition (Fessenden and Ehleringer 2002; Fig. 1c).

Long-term differences

The $\delta^{13}\text{C}_F$ and $\delta^{13}\text{C}_P$ values in both stands are likely to be affected by differences in plant hydraulic and physiological characteristics because of the similarity in soil and climate. Given the canopy height differences between the two forests and, in turn, expected differences in stomatal conductance (Ryan and Yoder 1997; Schäfer et al. 2000), the similarity in long-term mean values of $\delta^{13}\text{C}_F$ and $\delta^{13}\text{C}_P$ was surprising. Such similarity can materialize only if hydraulic and physiological compensation exists, and is explored next.

The effect of plant hydraulics on canopy conductance is given by

$$G_s = K(T) \times \left(k_s \times \frac{A_s}{A_1} \times \frac{1}{h} \right) \times (\Delta\Psi_{1-s} - 0.01h) \times \left(\frac{1}{\text{VPD}} \right) \quad (3)$$

where G_s is stomatal conductance, $K(T)$ are temperature dependent constants (see Phillips and Oren 1998), T is air temperature, k_s is a tissue-specific hydraulic conductivity averaged from the soil to the stomata cells, A_s/A_1 ($\text{cm}^2 \text{ m}^{-2}$) is sapwood to leaf area ratio, h is tree height as a surrogate for the path length for water flow, $\Delta\Psi_{1-s}$ is the potential gradient for water in megaPascal between the soil and substomata cells, and $0.01 h$ (h is in m) is a correction for the effect of gravity. Using this equation to calculate G_s assumes a good coupling between the leaves and the air in the canopy volume where VPD is measured.

Based on Table 1, T and VPD are similar in both stands as are the soil type and soil moisture; hence differences in G_s should reflect the combined effects of sapwood to leaf area ratio, canopy height, minimum leaf water potential, and tissue-specific hydraulic conductivity. The A_s/A_1 in the pine ranges from ~ 5.9 before bud-break to ~ 4.0 in September. It ranges from 4.4 (Pataki and Oren 2003) to 5.3 (Oren and Pataki 2001) in similar hardwood stands. Thus, we assume that A_s/A_1 is similar at these two stands. The canopy heights in the pine and hardwood forests are 17.2 and 35 m, respectively. We assume that the minimum leaf water potential is -1.5 MPa for the hardwood species and -2.0 MPa for the pine (Cruziat et al. 2002), and that

the soil is near saturation. We further assume that the ratio of pine to hardwood k_s is 0.53 (Becker et al. 1999). Combining the effect of all these factors produces an expected G_s ratio of pine to hardwood of about 1.7. This G_s ratio falls approximately in the middle of the wide range in canopy transpiration ratios (1.1–2.1) estimated in these pine and hardwood stands (Pataki and Oren 2003).

Having estimated the effect of plant hydraulics, we proceed to assess the physiological effects on gas exchange. Eddy covariance measurements in 2001 suggest that the ecosystem level photosynthesis (A_c) ratio of pines to hardwoods is about 1.45 (P. Stoy et al., in preparation). Combining this information with the G_s ratio, allows us to estimate the expected ratio of $[Ci/Ca]_{\text{pine}}$ to $[Ci/Ca]_{\text{hardwood}}$, given by

$$\frac{1 - [Ci/Ca]_{\text{hardwood}}}{1 - [Ci/Ca]_{\text{pine}}} = \frac{[G_s]_{\text{pine}}}{[G_s]_{\text{hardwood}}} \times \frac{[A_c]_{\text{hardwood}}}{[A_c]_{\text{pine}}} = 1.7 \times \frac{1}{1.45} \approx 1.17 \quad (4)$$

To compare this estimate with measured $\delta^{13}C_F$, we calculated $[Ci/Ca]_{\text{pine}}$ and $[Ci/Ca]_{\text{hardwood}}$ from the $\delta^{13}C_F$ (Table 2) and averaged the values to obtain long-term means of 0.74 and 0.70, respectively. The ratio $[Ci/Ca]$ was calculated from

$$\delta^{13}C_F = \delta^{13}C_A - a - (b - a) \times [Ci/Ca] \quad (5)$$

where $\delta^{13}C_F$ is pre-dawn foliage respired $\delta^{13}CO_2$, $\delta^{13}C_A$ = background air $\delta^{13}CO_2$ measured at the tower top in daytime, $a=4.4\%$ diffusional fractionation, and $b=27\%$ fractionation during carboxylation (Farquhar et al. 1982). Prater et al. (submitted) tested the hypothesis that pre-dawn determination of Ci/Ca from $\delta^{13}C_F$ are representative of daytime integrated Ci/Ca in pine species. Prater et al. (submitted) suggested that at pre-dawn as the substrate pool used for respiration was depleted in sucrose and glucose the apparent fractionation (Ghashghaie et al. 2003) effects caused by the non-statistical distribution of ^{13}C in those molecules (Rossmann et al. 1991) would be minimized. They used a series of $\delta^{13}C_F$ and photosynthetic uptake measurements on foliage over a diurnal cycle to calculate the ^{13}C of net assimilated carbon during a diurnal cycle. Their results showed excellent agreement between daytime integrated Ci/Ca measured with a gas exchange system and Ci/Ca determined from pre-dawn $\delta^{13}C_F$ using Eq. 5.

With these Ci/Ca estimates replaced in the left-hand-side of Eq. 4, a ratio of 1.15 is obtained, close to the 1.17 value predicted from plant hydraulics and eddy-covariance-based photosynthesis. If the calculations are repeated with Ci/Ca estimated from $\delta^{13}C_P$ (0.71 and 0.73 for the hardwood and pine, respectively), the ratio in Eq. 4 drops from 1.17 to 1.07, perhaps reflecting the differences in photosynthesis and conductance integrated over different

seasons for the evergreen pine versus the deciduous hardwood. Although the similarity in $[Ci/Ca]_{\text{pine}}$ and $[Ci/Ca]_{\text{hardwood}}$ appears surprising, it does not reflect similarities in gas exchange parameters; rather, it reflects compensating changes in hydraulics and photosynthetic properties as predicted by the water-carbon equilibrium hypothesis in Katul et al. (2003).

Intra-annual differences

The analysis above focused on the long-term average of $\delta^{13}C_F$ and $\delta^{13}C_P$. However, the long-term means may mask short-term differences in responses to environmental variables such as VPD and soil moisture. In Fig. 1, the δC of all ecosystem components is presented for both forest types in relation to time-lagged VPD and soil moisture variations. Both $\delta^{13}C_F$ and $\delta^{13}C_P$ decreased with decreasing VPD with the relationship being more significant for $\delta^{13}C_F$, as expected, given its shorter integration time. The relationship of these isotopic discrimination variables with soil moisture was weak to none. The implication of the dependence of $\delta^{13}C_F$ on VPD, and the similarity in the response in both forest types, is discussed below in terms of the dependence of Ci/Ca on VPD.

Implications to leaf-level physiological models

Starting with the definition of water use efficiency (WUE) to be $WUE \approx A/E$, where A is leaf-level photosynthesis and E is water vapor flux, replacing a Fickian diffusion parameterization for these fluxes, and after some simplifications, the WUE reduces to

$$WUE = \frac{C_a \left(1 - \frac{C_i}{C_a}\right)}{1.6 \times VPD} \quad (6)$$

Upon rearranging,

$$\frac{C_i}{C_a} = 1 - \frac{WUE}{C_a} 1.6 \times VPD \quad (7)$$

which suggests the following: (1) when the WUE is constant (e.g., as in Cowan and Farquhar 1977), Ci/Ca decays linearly with increasing VPD, (2) when the WUE varies with VPD^{-1} , which occurs when A is independent of VPD but E is linearly varying with VPD (e.g., see Katul et al. 2000), $Ci/Ca \rightarrow$ constant.

The above theoretical predictions are supported by the Ci/Ca determined from $\delta^{13}C_F$ for both stands. Notice in Fig. 2 that:

For $VPD < 1$ kPa, Ci/Ca decays approximately linearly (and similarly) with VPD for both stands.

For $VPD > 1$ kPa, Ci/Ca is approximately constant, and equal to an earlier stable-isotope based estimate (=0.66) in the pine forest (see Katul et al. 2000, 2003).

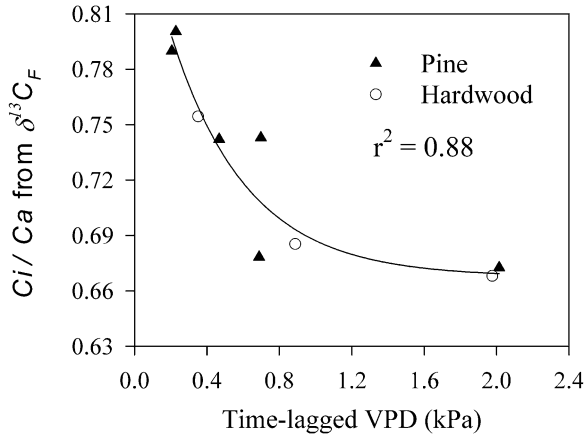


Fig. 2 C_i/C_a versus time lagged daytime VPD (average of daytime VPD for days 3 and 4 prior to sampling) at the pine and hardwood forest ($[C_i/C_a]=0.6676+0.2122 \times e^{(-2.38\Gamma^3 \times VPD)}$). C_i/C_a was derived from $\delta^{13}C_F$ values and the equation in Farquhar et al. (1982) (see text). The long-term mean C_i/C_a determined from foliage organic matter ^{13}C is 0.66 (Katul et al. 2000)

To place the observed C_i/C_a dependence on VPD in the context of leaf-level photosynthesis models, we consider a simplified Leuning (1995) formulation, now widely used in ecological models, given by

$$g = m \frac{A}{C_a} f(\text{VPD}) + b' \quad (8)$$

$$A = g(C_a - C_i) \quad (9)$$

$$f(\text{VPD}) = \frac{1}{1 + \frac{\text{VPD}}{D_o}} \quad (10)$$

where g is the leaf-level conductance to CO_2 , m and b' are species- and condition-specific constants, and D_o is a VPD sensitivity parameter. Neglecting b' (when compared to g) results in

$$\frac{C_i}{C_a} = \left(1 - \frac{1}{m}\right) - \frac{1}{mD_o} \text{VPD} \quad (11)$$

It is not our intent here to use Eqs. 9, 10, 11 in a prognostic manner; rather we seek a diagnostic description of the C_i/C_a dependence on VPD. Clearly, the model described by Eq. 11 is not correct over the entire range of VPD because it predicts a continuous linear decline in C_i/C_a with increasing VPD. The data in Fig. 2 shows an asymptotic behavior with increasing VPD. Similar deficiencies emerge with the Cowan and Farquhar (1977) model, which assumes a constant WUE and predicts a continuous linear decline in C_i/C_a with increasing VPD. Another model proposes a constant WUE defined as $\lambda_{cf} = \partial E / \partial A$ (rather than A/E as we did above), and uses a linearized $A-C_i$ curve (Lloyd and Farquhar 1994). With these simplifications, it can be shown that (Katul et al. 2000):

$$\frac{C_i}{C_a} = 1 - \sqrt{\frac{1.6 \text{VPD}(C_a - \Gamma)}{\lambda_{cf} C_a^2}} \quad (12)$$

where Γ is the CO_2 compensation point. In short, this model predicts that

$$\frac{C_i}{C_a} = 1 - m' \sqrt{\text{VPD}} \quad (13)$$

where m' is a constant, that depends on Γ , λ_{cf} , and C_a . While this model exhibits some nonlinear dependence on VPD, the curvature is too mild to represent the observed pattern in C_i/C_a versus VPD shown in Fig. 2.

To summarize, from stable isotopes, we show that the current empirical physiological models do not represent well *the nonlinear dependence of C_i/C_a on VPD across the entire VPD range*. Furthermore, the similarity in the C_i/C_a measurements in Fig. 2 suggests that for a given VPD,

$$\begin{aligned} \frac{A_{\text{pine}}}{A_{\text{hardwood}}} &\approx \frac{E_{\text{pine}}}{E_{\text{hardwood}}} = \frac{g_{\text{pine}}}{g_{\text{hardwood}}} \\ &= \frac{g_{\text{ref,pine}} \times f_{\text{pine}}(\theta) \times [1 - 0.6 \log(\text{VPD})]}{g_{\text{ref,hardwood}} \times f_{\text{hardwood}}(\theta) \times [1 - 0.6 \log(\text{VPD})]} \\ &\approx 1.7 \frac{f_{\text{pine}}(\theta)}{f_{\text{hardwood}}(\theta)} \end{aligned} \quad (14)$$

where g_{ref} is the reference conductance at $\text{VPD}=1$ kPa (Oren et al. 1999), and $f(\theta) \in [0,1]$ is the stomatal conductance sensitivity to soil moisture. In the above formulation, the conductance was modeled after Jarvis (1976) with g sensitivity to VPD set proportional to g_{ref} (Oren et al. 1999). Based on this formulation, the ratio of assimilation rates at these two forests will be constant for a given soil moisture condition regardless of the variation in VPD, but will change depending on the sensitivity of those two stands to soil moisture. Previous studies have shown that the conductance in the pine forest is more sensitive to soil moisture content depletion than the hardwood forest (Oren et al. 1998; Oren and Pataki 2001; Pataki and Oren 2003).

Influence of aboveground processes on $\delta^{13}C_S$

$\delta^{13}C_S$ increased with increasing $\delta^{13}C_F$ ($r^2=0.87$, freeze event omitted, nighttime temperatures dropped to below 0.0°C 3 days prior to sampling), but soil respired CO_2 was at all times ^{13}C enriched relative to foliage respired CO_2 , with the largest difference between $\delta^{13}C_F$ and $\delta^{13}C_S$ measured after the freeze event (Fig. 3). The relative contributions of heterotrophic respiration of soil organic matter and autotrophic respiration will both contribute to the composition and the variability in $\delta^{13}C_S$. $\delta^{13}C_F$ results from the respiration of recent photosynthate, which will have a variable ^{13}C composition ($-31.0\text{‰} < \delta^{13}C_F <$

–27.2‰, Table 1) depending on the environmental conditions (Fig. 1a). $\delta^{13}\text{C}_\text{S}$ results from respiration of recent photosynthate transported to the root system as well as respiration of a relatively older carbon pool with a relatively constant isotopic composition (Trumbore 2000). Soil organic matter at 0–15 cm depth has an estimated mean residence time of 20 years and a mean ^{13}C value of –26.24‰ (range –25.83 to –27.14‰ for the <0.53 μm and coarse fractions, respectively) (Schlesinger and Lichter 2001). While $\delta^{13}\text{C}_\text{F}$ will tend to introduce temporal variability in δC_s , respiration of the soil organic matter with a relatively long residence time and a constant isotopic composition will tend to maintain $\delta^{13}\text{C}_\text{S}$ relatively constant (Fig. 1e, f). This buffering effect was more apparent at the hardwood forest (Fig. 1f). However, the high degree of correlation between $\delta^{13}\text{C}_\text{S}$ and $\delta^{13}\text{C}_\text{F}$ highlights the influence of aboveground processes on the isotopic composition of soil respired CO_2 .

Temporal variations in $\delta^{13}\text{C}_\text{S}$ have also been reported in a number of studies. For example, Ekbald and Högberg (2001) measured a 5‰ difference in $\delta^{13}\text{C}_\text{S}$ during the June–October period in a boreal mixed coniferous forest. Fessenden and Ehleringer (2003) also measured variability in $\delta^{13}\text{C}_\text{S}$ of approximately 6‰ in an old growth coniferous forest in the northwest Pacific during an annual period. Maxima in $\delta^{13}\text{C}_\text{S}$ values were measured during drier periods (Ekbald and Högberg 2001; Fessenden and Ehleringer 2003) suggesting some soil moisture control on the variability of $\delta^{13}\text{C}_\text{S}$. The fact the $\delta^{13}\text{C}_\text{S}$ at the Duke Forests shows lower temporal variations compared to $\delta^{13}\text{C}_\text{F}$ (Table 2, Fig. 1a,e,f) suggests that at the two stands a large fraction of soil-respired CO_2 results from respiration of organic matter with a relatively long residence time.

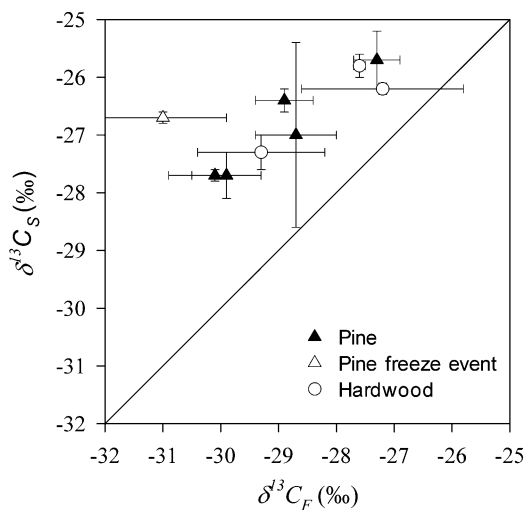


Fig. 3 Scatter plot of $\delta^{13}\text{C}_\text{S}$ versus $\delta^{13}\text{C}_\text{F}$ ($r^2=0.87$, freeze event omitted). The *solid line* represents the 1:1 relation. Notice that $\delta^{13}\text{C}_\text{S}$ is at all times enriched relative to $\delta^{13}\text{C}_\text{F}$, with the largest difference observed after the freeze event at the pine forest. There was a greater range of variability in $\delta^{13}\text{C}_\text{F}$ at both forests compared to $\delta^{13}\text{C}_\text{S}$. The contribution of heterotrophic respiration of soil organic matter with a relatively constant isotopic composition buffers changes induced in $\delta^{13}\text{C}_\text{S}$ by $\delta^{13}\text{C}_\text{F}$

Temporal and spatial variability in $\delta^{13}\text{C}_\text{R}$ at the Duke Forest

$\delta^{13}\text{C}_\text{R}$ was correlated to time-lagged VPD at the pine and hardwood stands (Fig. 1g). This is in agreement with earlier findings (Bowling et al. 2002; Fessenden and Ehleringer 2003), and suggests that $\delta^{13}\text{C}_\text{R}$ is enriched during high VPD and low soil moisture conditions (Fig. 1g,h). The $\delta^{13}\text{C}_\text{R}$ is thought to reflect ecosystem scale photosynthetic discrimination (Buchmann et al. 1998; Flanagan and Ehleringer 1998), and should be influenced by environmental variables such as VPD and soil moisture that affect stomatal conductance (Farquhar et al. 1989). Stomatal responses at diurnal and daily scales are mainly influenced by atmospheric conditions, whereas longer-term responses are likely to be controlled by variations in soil water content and rooting characteristics (Schulze 1986; Pataki 1998). Bowling et al. (2002) have observed a significant relation between $\delta^{13}\text{C}_\text{R}$ and time-lagged VPD (between 5 and 9 days) for four Pacific Northwest coniferous forests. The relation between $\delta^{13}\text{C}_\text{R}$ and VPD for the four forests followed the same general pattern. Fessenden and Ehleringer (2002) also measured a difference in $\delta^{13}\text{C}_\text{R}$ of 0.7‰ along a chronosequence of coniferous forest (20–450 years) in the Northwest Pacific and attributed the ^{13}C enriched $\delta^{13}\text{C}_\text{R}$ values at the older forest to decreased stomatal conductance in the older trees. Ometto et al. (2002) and Pataki et al. (2003) reported a significant relation between $\delta^{13}\text{C}_\text{R}$ and precipitation on seasonal and annual scales, respectively, and attributed this to the influence of soil moisture on stomatal conductance (Pataki et al. 2003).

Interestingly the relation between $\delta^{13}\text{C}_\text{R}$ and VPD in our stands that are within a kilometer of each other exhibited distinct patterns (Fig. 1g). While $\delta^{13}\text{C}_\text{R}$ at the hardwood was linearly related to time-lagged VPD, $\delta^{13}\text{C}_\text{R}$ at the pine forest exhibited a non linear dependence on VPD (Fig. 1g). Furthermore, at the hardwood forest, in contrast to the pine forest, there was only minor variability in $\delta^{13}\text{C}_\text{R}$ in response to changes in VPD and soil moisture conditions (Fig. 1g,h). The distinct patterns in $\delta^{13}\text{C}_\text{R}$ in response to water availability at the two stands results from the relative influence of $\delta^{13}\text{C}_\text{S}$ and $\delta^{13}\text{C}_\text{F}$ on $\delta^{13}\text{C}_\text{R}$. At the pine forest, $\delta^{13}\text{C}_\text{S}$ and soil moisture were correlated, ($r^2=0.52$) but no relation existed between the two parameters at the hardwood forest. This lower variability in $\delta^{13}\text{C}_\text{S}$ compared to $\delta^{13}\text{C}_\text{F}$ will buffer changes in $\delta^{13}\text{C}_\text{R}$ (Mortazavi and Chanton 2002a). This was particularly apparent in changes in $\delta^{13}\text{C}_\text{R}$ at the hardwood forest from July to September 2002: while $\delta^{13}\text{C}_\text{F}$ changed by 2.1‰, $\delta^{13}\text{C}_\text{R}$ only varied by 1.0‰, a change that is similar to that of $\delta^{13}\text{C}_\text{S}$ (Table 2).

From comparisons of $\delta^{13}\text{C}_\text{R}$ with its components (Fig. 4), it is clear that in the pine forest $\delta^{13}\text{C}_\text{R}$ is controlled primarily by $\delta^{13}\text{C}_\text{F}$, while at the hardwood forest the primary contributor to $\delta^{13}\text{C}_\text{R}$ is forest floor respiration. Higher soil CO_2 fluxes at the hardwood forest (Table 3) and 45% lower assimilation rates (discussed earlier, P. Stoy et al., in preparation) compared to the pine

forest, combined with relatively constant $\delta^{13}\text{C}_\text{S}$ despite variations in VPD (Fig. 1a) and soil moisture (Fig. 1b), contribute to maintaining a relatively constant $\delta^{13}\text{C}_\text{R}$ at the hardwood forest. These isotopic discrimination measurements are consistent with the observations of higher photosynthesis in the pine forest (discussed earlier) and greater forest floor respiration in the hardwood forest (Table 3).

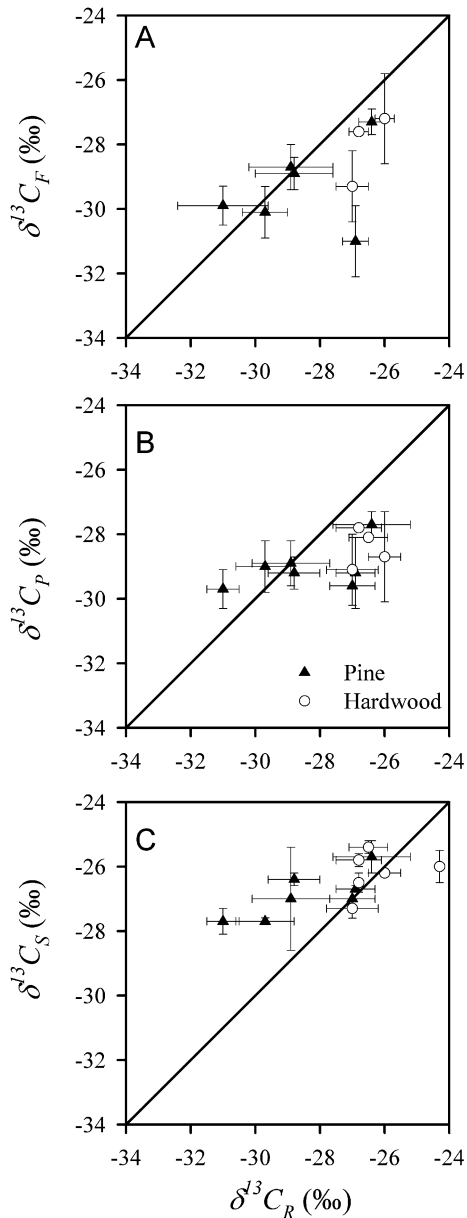


Fig. 4a–c Scatter diagram **a** $\delta^{13}\text{C}_\text{F}$ versus $\delta^{13}\text{C}_\text{R}$, **b** $\delta^{13}\text{C}_\text{P}$ versus $\delta^{13}\text{C}_\text{R}$ and **c** $\delta^{13}\text{C}_\text{S}$ versus $\delta^{13}\text{C}_\text{R}$. The *solid lines* represent the 1:1 relation. Notice that while at the pine forest $\delta^{13}\text{C}_\text{R}$ is mainly influenced by $\delta^{13}\text{C}_\text{F}$, at the hardwood forest $\delta^{13}\text{C}_\text{R}$ is mainly influenced by $\delta^{13}\text{C}_\text{S}$. This is consistent with higher assimilation rates at the pine and lower assimilation rates and higher soil CO_2 fluxes at the hardwood forest

Conclusions

This study quantified variations in $\delta^{13}\text{C}_\text{R}$ and its components in adjacent pine and the hardwood forests in southeastern USA. We demonstrated a remarkable similarity in the relationship between C_i/C_a and VPD in the two forests despite large differences in hydraulic characteristics. This similarity is the outcome of physiological adjustments that compensated for the differences in hydraulic characteristics between the forests as expected based on a recent equilibrium hypothesis (Katul et al. 2003). Results indicate that in a mosaic of forests composed of different stands, different factors contribute to the variations in $\delta^{13}\text{C}_\text{R}$: (1) in deciduous broadleaf stands, the isotopic composition of the forest floor CO_2 efflux dominates $\delta^{13}\text{C}_\text{R}$ while in a younger pine forest, the isotopic composition of foliage respired CO_2 dominates $\delta^{13}\text{C}_\text{R}$; (2) given the relatively low variability of $\delta^{13}\text{C}_\text{S}$, dominated by long residence time soil organic matter, $\delta^{13}\text{C}_\text{R}$ in the hardwood forest was nearly constant; (3) given the relatively variable nature of $\delta^{13}\text{C}_\text{F}$, dominated by the effect of VPD on stomatal conductance, $\delta^{13}\text{C}_\text{R}$ in the pine forest was variable. Because of the sensitivity of the atmospheric inversion models to $\delta^{13}\text{C}_\text{R}$, these models merit incorporation of the effect of stand characteristics on $\delta^{13}\text{C}_\text{R}$, in addition to previously recognized effects of moisture availability (Bowling et al. 2002; Ometto et al. 2002) and age (Fessenden and Ehleringer 2002).

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