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Environmental controls on the carbon isotope composition of ecosystem-respired CO_2 in contrasting forest ecosystems in Canada and the USA

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Summary We compared the carbon isotope composition of ecosystem-respired CO_2 ($\delta^{13}C_R$) from 11 forest ecosystems in Canada and the USA and examined differences among forest $\delta^{13}C_R$ responses to seasonal variations in environmental conditions from May to October 2004. Our experimental approach was based on the assumption that variation in $\delta^{13}C_R$ is a good proxy for short-term changes in photosynthetic discrimination and associated shifts in the integrated ecosystem-level intercellular to ambient CO_2 ratio (c_i/c_a). We compared $\delta^{13}C_R$ responses for three functional groups: deciduous, boreal and coastal forests. The $\delta^{13}C_R$ values were well predicted for each group and the highest R^2 values determined for the coastal, deciduous and boreal groups were 0.81, 0.80 and 0.56, respectively. Consistent with previous studies, the highest correlations between $\delta^{13}C_R$ and changes in environmental conditions were achieved when the environmental variables were averaged for 2, 3 or 4 days before $\delta^{13}C_R$ sample collection. The relationships between $\delta^{13}C_R$ and environmental conditions were consistent with leaf-level responses, and were most apparent within functional groups, providing support for our approach. However, there were differences among groups in the strength or significance, or both, of the relationships between $\delta^{13}C_R$ and some environmental factors. For example, vapor pressure deficit (VPD) and soil temperature were significant determinants of variation in $\delta^{13}C_R$ in the boreal group, whereas photosynthetic photon flux (PPF) was not; however, in the coastal group, variation in $\delta^{13}C_R$ was strongly correlated with changes in PPF, and there was no significant relationship with VPD. At a single site, comparisons between our $\delta^{13}C_R$ measurements in 2004 and published values suggested the potential application of $\delta^{13}C_R$ measurements to assess year-to-year variation in ecosystem physiological responses to changing environmental conditions, but showed that, in such an analysis, all environmental factors influencing carbon isotope discrimination during photosynthetic gas exchange must be considered.

Keywords: AmeriFlux, boreal forest, Fluxnet-Canada, Keeling plot, stable isotopes.

Introduction

Studying the ecosystem physiology of forests is challenging because forests are large, have diverse species composition and encompass broad physiological and environmental variation. Stable isotope techniques provide a tool for studying ecosystem physiology (Flanagan and Ehleringer 1998). The carbon isotope composition of leaves is useful for studying interactions among environmental and physiological factors that affect CO_2 assimilation in forest canopies because it provides information about photosynthetic characteristics integrated over the life of the leaf (Flanagan and Ehleringer 1998). In particular, the carbon isotope ratio of leaf tissue provides a photosynthesis-weighted mean of the ratio of intercellular (c_i) to ambient (c_a) CO_2 concentration (Farquhar et al. 1982). The c_i/c_a ratio is important because it is dependent on changes in photosynthetic capacity and stomatal conductance (Farquhar et al. 1989), and because c_i controls leaf physiological characteristics such as water- (Farquhar et al. 1989), light- (Farquhar and Sharkey 1982) and nitrogen-use efficiency (Field et al. 1983). How changes in environmental conditions cause changes in the ratio of photosynthetic capacity and stomatal conductance, with associated changes in leaf carbon isotope ratio ($\delta^{13}C$), is well documented (Ehleringer and Osmond 1989, Farquhar et al. 1989).

Studies have shown that carbon isotope ratios of ecosystem respiration ($\delta^{13}C_R$) are directly linked to factors that control leaf gas exchange activities, including vapor pressure deficit (Bowling et al. 2002, Knohl et al. 2005, Mortazavi et al. 2005, Werner et al. 2006), soil temperature during drought (McDowell et al. 2004), precipitation (Flanagan et al. 1996, Ometto et al. 2002, Pataki et al. 2003), stand structure (Buchmann et al. 1997) and soil water availability (Fessenden and Ehleringer 2003, Lai et al. 2005, Ponton et al. 2006, Werner et al. 2006). Based on these studies, we propose that measurements of $\delta^{13}C_R$ are a good proxy for canopy-level c_i/c_a, and that they provide insight into constraints on ecosystem productivity and the acclimation of ecosystem responses to climate change. The rationale for this proposition and the data that support it are briefly described.

Carbon isotope discrimination occurring during net ecosystem carbon uptake should reflect the photosynthesis-weighted mean of discrimination in all plants in the ecosystem. Assuming that no significant fractionation occurs during respiration, the autotrophic (plant and rhizosphere) component of ecosystem respiration should release CO_2 with an isotopic composition reflecting that of recently fixed carbohydrates (Amthor and Baldocchi 2001, Högberg et al. 2001). Total ecosystem respiration also includes carbon dioxide released from organic matter decomposition (heterotrophic respiration), but the carbon isotope ratio of CO_2 from soil litter and humus decomposition is unlikely to change on time scales of less than one year (Trumbore 2000), and so it should not substantially alter the carbon isotope signal associated with recent ecosystem photosynthesis. This proposition assumes that any partitioning and metabolism of molecules with different $^{13}C/^{12}C$ ratios in the shoots and roots are balanced on a whole-plant basis so that there is no significant difference in the carbon isotope composition of respired CO_2 and whole-plant biomass (Klumpp et al. 2005). Our approach is supported by recent studies demonstrating correlations between the carbon isotope ratio of ecosystem-respired CO_2 and changes in vapor pressure deficit, precipitation, soil water availability and canopy conductance (Ekblad and Högberg 2001, Bowling et al. 2002, Ometto et al. 2002, Fessenden and Ehleringer 2003, Pataki et al. 2003, McDowell et al. 2004, Lai et al. 2005, Ponton et al. 2006, Werner et al. 2006).

In this study, the carbon isotope composition ($^{13}C/^{12}C$) of ecosystem-respired CO_2 was considered a proxy for short-term changes in photosynthetic discrimination and associated shifts in integrated ecosystem-level c_i/c_a. Our primary objective was to compare 11 forest ecosystems in Canada and the USA and to test for differences among forest $\delta^{13}C_R$ responses to seasonal variation in environmental conditions from May to October 2004. We compared coniferous and deciduous forests, and forests in boreal and coastal environments. We assumed that the carbon isotope composition of ecosystem-respired CO_2 varies in response to significant changes in environmental conditions in a manner consistent with the responses documented in leaf-level studies (Farquhar et al. 1989). Specifically, the $\delta^{13}C$ of ecosystem-respired CO_2 should follow the theoretical response patterns observed at the leaf-level, where $\delta^{13}C$ values increase in response to declining soil water availability and precipitation, and increasing photosynthetic photon flux (PPF), temperature and vapor pressure deficit (VPD) (Figure 1). Relationships between $\delta^{13}C$ and changes in PPF, air temperature and VPD should be positive because an increase in these variables normally results in decreased c_i/c_a. In contrast, c_i/c_a normally increases in response to increasing soil water availability, and so a negative relationship between soil water availability and $\delta^{13}C$ was expected.

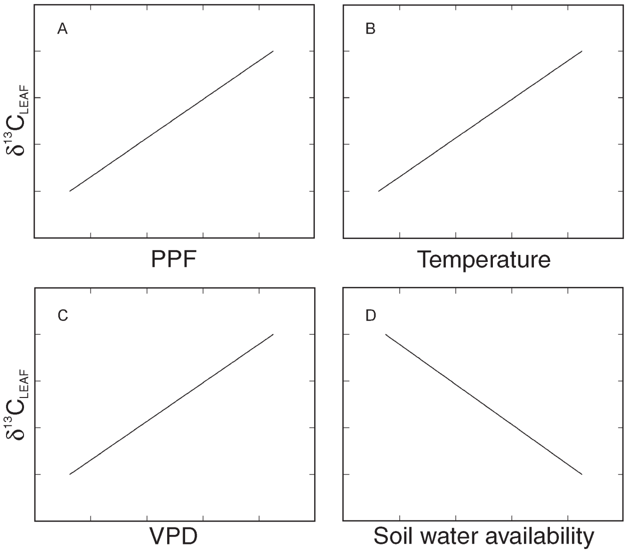

Figure 1. Illustration of anticipated general patterns of correlation between leaf-level carbon isotope composition ($\delta^{13}C$) and changes in photosynthetic photon flux (PPF), air temperature, vapor pressure deficit (VPD) and soil water availability. Relationships between $\delta^{13}C$ and changes in environmental variables were expected to be positive (PPF, air temperature and VPD) because an increase in these variables normally results in decreased leaf c_i/c_a. In contrast, c_i/c_a normally increases with soil water availability, and so a negative relationship between soil water availability and $\delta^{13}C$ was expected. These relationships are based on our understanding of leaf-level isotope effects during photosynthetic gas exchange (Farquhar et al. 1989).

We realize that simple, linear relationships between ecosystem-respired $\delta^{13}C$ and individual factors are unlikely because of the complex interactions between environmental conditions and physiological processes. The relationships depicted in Figure 1 represent anticipated general patterns of correlation between single environmental variables and $\delta^{13}C_R$. We applied linear regression models to simultaneously consider multiple environmental variables, thereby incorporating variable interactions, and to avoid misinterpretation of single variable responses. We employed variable reduction methods and collinear statistical indicators to identify and reduce collinearity among independent variables in the linear regression analyses.

Materials and methods

Study sites

Seven forest sites in Canada and four in the USA were studied. The Canadian sites are all part of the Fluxnet-Canada research network (FCRN; Coursolle et al. 2006) and the US sites are part of the AmeriFlux program. The location of the sites and some basic features are listed in Table 1. All sites are mature forests that have either regenerated naturally after fire or were planted after forest harvest.

Table 1. Characteristics of the 11 study sites.

Abbreviation	Site name AmeriFlux/FCRN	Province/state, country	Location (°N, °W)	Elevation (m)	Dominant species	Sub-dominant species	Age (years)	Canopy height (m)	Group	Reference
Wind River	Wind River	WA, USA	45.49, 121.58	355	*Pseudotsuga menziesii*	*Tsuga heterophylla*	450	62	Coastal	Paw U et al. 2004
Niwot	Niwot Ridge	CO, USA	40.033, 105.546	3050	*Abies lasiocarpa* *Picea engelmannii* *Pinus contorta*		97	11.4	None	Monson et al. 2002
Harvard	Harvard Forest	MA, USA	42.538, 72.171	340	*Quercus rubra* *Acer rubrum*	*Betula papyrifera* *Betula nigra* *Tsuga canadensis*	90	20–24	Deciduous	Goulden et al. 1996
Howland	Howland Forest	ME, USA	45.204, 68.740	60	*Picea rubens* *Tsuga canadensis*	*Abies balsamea* *Pinus strobus* *Thuja occidentalis*	90	20	None	Hollinger et al. 2004 Hollinger et al. 1999
BC	Campbell River 1949	BC, Canada	49.905, 125.336	300	*Pseudotsuga menziesii*	*Thuja plicata* *Tsuga heterophylla*	54	33	Coastal	Humphreys et al. 2003 Morgenstern et al. 2004
SK black spruce	Southern Old Black Spruce	SK, Canada	53.987, 105.117	629	*Picea mariana*		123	11	Boreal	Griffis et al. 2003
SK aspen	Old Aspen	SK, Canada	53.629, 106.200	601	*Populus tremuloides*		84	20.1	Deciduous Boreal	Barr et al. 2004 Chen et al. 1999
SK jack pine	Southern Old Jack Pine	SK, Canada	53.916, 104.690	579	*Pinus bankiana*		88	15.6	Boreal	Griffis et al. 2003
Ontario	Groundhog River	ON, Canada	48.217, 82.156	341	*Picea mariana* *Picea glauca* *Abies balsamea* *Populus tremuloides*		74	21.6	Boreal	Coursolle et al. 2006 McCaughey et al. 2006
Quebec	Chibougamau	QC, Canada	49.692, 74.342	393	*Picea mariana*		100	14	Boreal	Coursolle et al. 2006
New Brunswick	Nashwaak Lake	NB, Canada	46.472, 67.100	340	*Abies balsamea*		35	13.5	Coastal Boreal	Coursolle et al. 2006

Collection and analysis of atmospheric CO_2 samples

Atmospheric CO_2 samples were collected at night on several dates from May to October 2004. The isotopic composition of ecosystem-respired CO_2 was calculated by a modified Keeling plot approach (Miller and Tans 2003, Pataki et al. 2003). At the Canadian sites, samples were collected in 200-ml glass flasks equipped with high vacuum Teflon stopcocks. After sample collection, the flasks were shipped to the University of Lethbridge where CO_2 concentrations and carbon isotope ratios were analyzed as described by Ponton et al. (2006) with a GasBench II interface (ThermoFinnigan, Bremen, Germany) coupled to a gas isotope ratio mass spectrometer (Delta Plus, ThermoFinnigan). At the USA sites, samples were collected in 100-ml flasks equipped with high vacuum stopcocks. The flasks were shipped to the University of Utah where CO_2 concentrations and carbon isotope ratios were analyzed as described by Schauer et al. (2005) with a gas isotope ratio mass spectrometer (Delta Plus, ThermoFinnigan).

Carbon isotope ratios were expressed as $\delta^{13}C$ (‰):

$$\delta = \frac{R_{\text{sample}}}{R_{\text{std}}} - 1 \qquad (1)$$

where R is molar ratio of heavy to light isotope and the subscript std refers to the Vienna PeeDee Belemnite standard (VPDB). At the University of Lethbridge, measurement precision was 0.18 ppm for CO_2 concentration and 0.14‰ for $\delta^{13}C$ (Ponton et al. 2006). The corresponding values for measurements made at the University of Utah were 0.48 ppm and 0.06‰ (Schauer et al. 2005). At both laboratories, measurements of CO_2 concentration were referenced to the WMO scale and the $\delta^{13}C$ measurements were expressed on the VPDB scale (Coplen 1996).

At all but two study sites, an automatic flask sampling system collected atmospheric CO_2 samples (Schauer et al. 2003). The system included a data logger (CR23X, Campbell Scientific, Logan, UT) and accessories that controlled the operation of a multi-position valve (Valco Instruments Company, Houston, TX), solenoid valves on two manifolds, a pump and an infrared gas analyzer (LI-820, Li-Cor, Lincoln, NE). Air samples were manually collected at two of the Canadian sites (Ontario and New Brunswick) as described previously (Flanagan et al. 1996).

At all sites, air was sampled about 1 m above ground and midway between the ground and the top of the tree canopy. Samples were collected at night (starting one hour after PPF decreased below 100 μmol m^{-2} s^{-1}). We attempted to maximize the range of CO_2 concentrations in air samples to minimize the uncertainty in the modified Keeling plot slope calculation (Miller and Tans 2003, Pataki et al. 2003). All samples for a given Keeling plot calculation were collected during a maximum of three successive nights, with the majority of samples collected during one night.

Our sampling protocol likely constrained the footprint of the Keeling plot analysis for two reasons. First, samples were collected both near the ground (about 1 m or less) and at mid-canopy height. The height of the near-ground inlet assured that the footprint of these samples was small. Air samples were collected on calm nights (a requirement to reduce statistical uncertainties for Keeling plot analysis, see Pataki et al. 2003 and Miller and Tans 2003), which reduced the contribution of respiration from adjacent ecosystems. Second, all the study sites are eddy covariance flux sites that have large areas of homogeneous vegetation (in contrast to agricultural areas and low stature crops). This minimized variation in land use, plant species composition and disturbance.

Our measurements were potentially affected more heavily by belowground respiration. Carbon dioxide concentrations were higher in the samples collected close to the ground than in the samples collected at mid-canopy height, which may have biased $\delta^{13}C_R$ to more negative values. However, this should have no effect on our cross-site comparison because the collection procedure was the same at all sites.

The carbon isotope composition of ecosystem-respired CO_2 ($\delta^{13}C_R$) was calculated from CO_2 concentration and carbon isotope composition by the modified Keeling plot approach described by Miller and Tans (2003):

$$\delta_{\text{obs}} c_{\text{obs}} = \delta_{\text{R}} c_{\text{obs}} - c_{\text{bg}}(\delta_{\text{bg}} - \delta_{\text{R}}) \qquad (2)$$

where c is CO_2 concentration, δ is carbon isotope composition of CO_2 and the subscripts obs, bg and R refer to observed, background and ecosystem-respired CO_2, respectively. Equation 2 describes a simple linear function with slope δ_R and intercept $-c_{bg}(\delta_{bg} - \delta_R)$. A geometric mean linear regression was performed to estimate $\delta^{13}C_R$ (Pataki et al. 2003). Uncertainty in the slope was calculated as described by Miller and Tans (2003) and Ponton et al. (2006). For the Canadian site data, the linear regressions for the $\delta^{13}C_R$ calculations had a mean (± SD) $r^2 = 0.993 \pm 0.006$ (minimum $r^2 = 0.971$, maximum $r^2 = 0.999$) (42 $\delta^{13}C_R$ values). For the USA site data, mean $r^2 = 0.966$ (90 $\delta^{13}C_R$ values; minimum $r^2 = 0.947$, maximum $r^2 = 1.000$).

The slope method (Miller and Tans 2003) differs from the intercept method originally proposed by C.D. Keeling (Pataki et al. 2003). In addition, a geometric mean regression can result in a systematic negative bias in $\delta^{13}C_R$ estimates (Zobitz et al. 2006) . We compared $\delta^{13}C_R$ values calculated by the slope method with $\delta^{13}C_R$ values calculated by a least-squares regression. For the seven Canadian sites, there was close agreement between the techniques. A scatter plot of the 42 Canadian $\delta^{13}C_R$ values had the following linear regression statistics: intercept method (y) = 1.0154 × slope method (x) + 0.5238, $r^2 = 0.995$. For the USA $\delta^{13}C_R$ values, the linear regression statistics were: intercept method (y) = 1.0120 × slope method (x) + 0.3745, $r^2 = 0.987$. We concluded that the choice of the $\delta^{13}C_R$ calculation method had no effect on our interpretation of the results.

Statistical analyses

All study sites were included in an initial ANOVA to determine if there were significant differences ($P \leq 0.05$) in $\delta^{13}C_R$ values among sites, and to examine the relationships between single environmental variables and $\delta^{13}C_R$ (Table 2). The environmental variables included in these analyses were: daily maximum PPF; daily maximum air temperature (T_{air}); daily maximum soil temperature (T_{soil}); mean soil water availability (SWA, volumetric soil water content expressed on a relative scale); and cumulative total precipitation (PPT). Before conducting the statistical analyses, the environmental measurements for each site were averaged for different time periods (lag times) that varied from one to six days before, and including, the $\delta^{13}C_R$ sample collection date. Because the initial ANOVA revealed site-specific differences in the effects of environmental variables on $\delta^{13}C_R$ (Table 2), forests were grouped as boreal, coastal or deciduous (Table 1). A second ANOVA was run separately for each group to test for site differences within each group (Tables 3–5). A series of regression analyses were conducted for each ecosystem group separately to determine the statistical significance of relationships between single environmental variables and $\delta^{13}C_R$ (for all lag times). Such an analysis was complicated because of correlations among the environmental factors studied. For example, the highest correlations observed within the coastal group (for the 4-day lag time) occurred between VPD and T_{air} ($r = 0.90$), between VPD and PPF ($r = 0.84$) and between T_{air} and PPF ($r = 0.82$). Similar high correlations were observed within the deciduous group between VPD and PPF ($r > 0.81$), with the second highest correlation between T_{soil} and SWA ($r < -0.77$). The correlations between environmental variables were lowest for the boreal group, with the highest correlation observed between T_{soil} and T_{air} ($r = 0.78$) for the 4-day lag time.

This correlation or collinearity among supposedly independent variables can lead to distortions in regression analyses, especially when sample size is low or when models are poorly designated (Mason and Perreault, Jr. 1991). To identify collinearity distortions in our analyses, we used two diagnostic indicators based on the eigenstructure of the data matrix: the variance inflation factor (VIF) and the condition index (CI). Although there is no consensus, several authors have suggested that collinearity effects are theoretically inconsequential when VIF values are below 10 (Hair et al. 1995, Neter et al. 1996, Chatterjee et al. 2000). Belsey et al. (1980) and Johnston (1984) suggest that CI values (the square root of the ratio of the largest eigenvalue to each individual eigenvalue) of 5–10 indicate weak collinearity and CI values above 30 indicate strong linear dependencies. However, confounding effects of collinear predictors on regression coefficients were demonstrated even when VIF and CI values were below these "rule-of-thumb" values (Mason and Perreault, Jr. 1991, Mela and Kopalle 2002). Because our group sample sizes were small (28–30 per group), the regression results were carefully considered. In addition, we examined the proportion of variance of the parameter estimate associated with each eigenvalue in the corresponding variance-decomposition tables. A high proportion of two or more coefficients associated with the same eigenvalue indicates that linear dependencies between the corresponding variables were distorting the regression analysis.

When all six environmental factors were included in the preliminary linear regression models, the coastal and deciduous groups had the highest VIF and CI values (coastal: VIF > 18 and CI ≅ 11 for T_{air}; deciduous: VIF ≅ 15 and CI ≅ 10 for T_{air} and T_{soil}). The VIF values for the boreal group were lower (VIF ≅ 10, and CI ≅ 7). Because collinearity effects likely confounded some of our statistical analyses, we conducted additional analyses to remove these effects. The simplest approach to reducing the marginal statistic derived from regression analyses of collinear data is to drop the collinear variables from the model (Legendre and Legendre 1998, Philippi 1993). We used a variable-reduction method to derive statistical models that describe the relationship between environmental factors and $\delta^{13}C_R$ variation for each group, and in many cases this variable-reduction process reduced the collinearity among independent variables (Graham 2003). An "all possible subsets" approach to variable-selection was chosen, rather than a stepwise method, because collinearity can bias the selection of variables in stepwise analyses (Mason and Perreault, Jr. 1991, Graham 2003). The variable subset with the greatest fit was identified based on Akaike's Information Criteria (AIC), de-

Table 2. Parameters and variable *P* values of ANOVA models for all sites combined. Six separate ANOVAs were conducted; each model compares the lag-time-averaged climate variables (1–6-day lags) with the carbon isotope composition of ecosystem-respired CO_2.

	Number of lag days before the sampling date					
	1	2	3	4	5	6
Model parameters						
P value	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
R^2	0.53	0.53	0.53	0.53	0.52	0.52
Variable P values						
Photosynthetic photon flux	0.17	0.27	0.25	0.15	0.17	0.24
Vapor pressure deficit	0.39	0.46	0.75	0.76	0.56	0.57
Daily maximum air temperature	0.74	0.89	0.89	0.86	0.71	0.83
Daily maximum soil temperature	0.07	0.08	0.05	0.06	0.09	0.17
Mean soil water availability	0.09	0.26	0.33	0.33	0.31	0.30
Cumulative total precipitation	0.80	0.61	0.27	0.43	0.87	0.58
Site	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

Table 3. Boreal, coastal and deciduous group model parameters and variable *P* values for two separate ANOVAs for each group; each model compares the lag-time-averaged climate variables (3- and 4-day lag times shown) with the carbon isotope composition of ecosystem-respired CO_2. Site factor was not significant in the ANOVA models (not shown) for 1-, 2-, 5- and 6-day lag times for all three groups.

	Number of lag days before the sampling date					
	Boreal		Coastal		Deciduous	
	3	4	3	4	3	4
Model parameters						
P value	0.06	0.04	< 0.01	< 0.01	< 0.01	< 0.01
R^2	0.58	0.61	0.85	0.82	0.79	0.79
Variable P values						
Photosynthetic photon flux	0.77	0.35	0.01	0.01	0.07	0.07
Vapor pressure deficit	0.23	0.21	0.72	0.62	0.02	0.02
Daily maximum air temperature	0.62	0.81	0.37	0.22	< 0.01	< 0.01
Daily maximum soil temperature	0.14	0.28	0.69	0.79	0.03	0.03
Mean soil water availability	0.77	0.91	0.18	0.30	0.60	0.60
Cumulative total precipitation	0.80	0.85	0.21	0.73	0.53	0.53
Site	0.89	0.76	0.11	0.19	0.89	0.89

Table 4. Boreal group model parameters and variable *P* values for six separate linear regression analyses; each model compares the lag-time-averaged environmental variables (1–6-day lag times) with the carbon isotope composition of ecosystem-respired CO_2. Model selection was conducted based on Akaike's Information Criteria.

	Number of lag days before the sampling date					
	1	2	3	4	5	6
Model parameters						
Akaike's Information Criteria	–46.9	–49.9	–48.8	–45.1	–50.2	–50.3
Condition Index	4.1	4.4	1.3	1.1	1.3	1.4
P value	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
R^2	0.51	0.56	0.45	0.54	0.53	0.53
Variable P values						
Vapor pressure deficit	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Daily maximum air temperature	0.02	0.05	–	–	–	–
Daily maximum soil temperature	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Mean soil water availability	0.07	0.03	0.03	0.04	0.01	0.01

Table 5. Coastal group model parameters and variable *P* values for six separate linear regression analyses; each model compares the lag-time-averaged environmental variables (1–6-day lag times) with the carbon isotope composition of ecosystem-respired CO_2. Model selection was conducted based on Akaike's Information Criteria.

	Number of lag days before the sampling date					
	1	2	3	4	5	6
Model parameters						
Akaike's Information Criteria	–45.8	–47.8	–53.9	–50.4	–49.1	–50.1
Condition Index	3.0	3.1	3.6	4.3	3.5	3.7
P value	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
R^2	0.69	0.71	0.81	0.76	0.65	0.69
Variable P values						
Photosynthetic photon flux	0.02	0.02	< 0.01	< 0.01	< 0.01	< 0.01
Vapor pressure deficit	0.06	0.05	–	–	0.85	0.66
Daily maximum air temperature	–	–	–	0.07	–	–
Daily maximum soil temperature	–	–	0.02	–	–	–
Mean soil water availability	–	–	0.01	0.03	–	–
Cumulative total precipitation	–	–	0.02	–	–	–

fined as 2ln$L$ + 2k, where L represents the maximum likelihood estimate and k the number of free parameters. Thus, higher likelihood-estimation values are associated with better fitting models, and correspond to smaller AIC numbers (Akaike 1973, Neter et al. 1996). After performing AIC model selection analysis, CI values were reviewed for each selected model to confirm that no significant collinearity effect existed. Where CI values were still above the threshold values, or where the proportion of two or more coefficients were associated with the same eigenvalue, an additional ridge regression variable-reduction method was conducted to adjust for the associated collinearity. This procedure was conducted only for the deciduous group. The ridge regression method adds an additional constant K, with values between 0 and 1, to the diagonal elements of the correlation matrix, effectively lowering the standard errors of the regression coefficients (Neter et al. 1996). Thus, the ridge regression method adds some bias, but reduces the inflated variance resulting from collinearity and should derive a better estimate of the model (Bare and Hann 1981). Use of ridge regression as a screening tool for variable reduction involves examining the change in the standardized regression coefficients over a range of K values (called a ridge trace) to identify which combination of model variables is most stable (Bare and Hann 1981).

Before statistical model selection, Studentized-residuals were derived for $\delta^{13}C_R$ values from each group by ANOVA including all associated environmental variables, and the Shapiro-Wilk statistic tested for normal distribution. Extreme outliers having a Studentized-residual value > 2.5 were removed from each population; five $\delta^{13}C_R$ values were removed from the boreal group, three from the coastal group and two from the deciduous group (i.e., a total of eight $\delta^{13}C_R$ values were removed from the whole data set, because some sites were included in more than one group). Regression coefficients are all based on Type III Sum of Squares for unbalanced ANOVA analyses (Neter et al. 1996). All $\delta^{13}C_R$ values and corresponding environmental factors that were included for year-to-year comparisons were included in the summary statistics, including the outliers removed before regression analyses. All observations were included in the correlation analyses of $\delta^{13}C_R$ and sample collection date.

Results

A total of 123 (after eight outliers were removed) $\delta^{13}C_R$ values were calculated from the 11 forest sites, with an overall mean $\delta^{13}C_R$ of −27.1 ± 0.10‰ (± SE), and individual site values ranged from −24.0 to −30.4‰. Temporal variation in $\delta^{13}C_R$ at a given site during the growing season was moderate for most sites (Figure 2). No significant correlations were found between $\delta^{13}C_R$ and day of collection for any of the sites or groups, although the Saskatchewan aspen, Harvard and Niwot sites showed trends toward negative relationships (Saskatchewan aspen, $r = -0.72$, $P = 0.10$ $n = 7$; Harvard, $r = -0.40$, $P = 0.06$, $n = 24$; Niwot, $r = -0.38$, $P = 0.09$, $n = 21$). The highest seasonal variations in $\delta^{13}C_R$ occurred at the British Columbia (3.6‰) and Howland (3.9‰) sites, and the lowest seasonal variations occurred at the Ontario (1.0‰) and the three Saskatchewan (0.9‰) sites.

The collection site factor had a significant effect on $\delta^{13}C_R$ variation in the initial ANOVA including all study sites, but environmental influences did not (Table 2). These findings were consistent for all lag times (1–6 days). Differences in site characteristics apparently masked environmental influences on $\delta^{13}C_R$. Therefore, we grouped sites with similar characteristics before analyzing the effects of environmental variation on $\delta^{13}C_R$. Subsequent ANOVAs were conducted after nine of the 11 sites were classified into one of three groups (boreal, deciduous or coastal). The Niwot and Howland sites were not included in these groups.

Collection site was not a significant factor for any of the groups for any lag times considered (results for 3- and 4-day lag times shown in Table 3), supporting our choice of criteria to group sites with similar characteristics. There was no significant correlation between environmental effects and $\delta^{13}C_R$ for the Niwot or Howland sites, likely because the sample size was small ($n = 23$ for both sites).

For the boreal group, after application of the statistical selection procedure, all models had CI values below five, indicating minimal effects of collinearity (Table 4). All 6 lag time regression models were significant, with the 2- and 4-day lag time models having the highest R^2 values (0.56 and 0.54, respectively; Table 4). The VPD and T_{soil} factors were significant in all lag-time models, and SWA was significant for all but the 1-day lag-time model (Table 4). As predicted, $\delta^{13}C_R$ was positively correlated with VPD and T_{soil} (Figure 3). Contrary to expectations, SWA was positively correlated with $\delta^{13}C_R$ when data from all boreal sites was considered together (Figure 4). However, this trend was weak when data from each site within the boreal group were examined separately (Figure 4).

After application of the statistical selection procedure, all models for the coastal group had CI values below five, indicating minimal effects of collinearity (Table 5). The regression models derived from the coastal group data were significant for all six lag times (Table 5). The 3-day lag-time models yielded the highest R^2 values ($R^2 = 0.81$) and all models had $R^2 > 0.65$. Photosynthetic photon flux was significant in all regression models for the coastal group, whereas SWA was significant only for the 3- and 4-day lag-time models. Values of $\delta^{13}C_R$ were positively correlated with PPF and T_{soil}, as shown for the 3-day lag-time data set in Figure 5 (panels A and B, respectively). There was a negative correlation between $\delta^{13}C_R$ and 3-day lag-time PPT (Figure 5D). There was a weak negative correlation between $\delta^{13}C_R$ and SWA when all sites within the group were considered (Figure 5C), but a strong correlation for the Wind River data only ($r = -0.73$, $P < 0.01$; Figure 5C).

Condition Index values were higher for the deciduous group than for the boreal and coastal groups, ranging between 6 and 11 for the 4- to 6-day lag-time models (Table 6), indicating that collinearity was likely to have a significant effect on the statistical analysis in this group. All lag-time models for the deciduous group were significant, with the 3-day lag-time model yielding the highest R^2 ($R^2 = 0.78$). Air temperature,

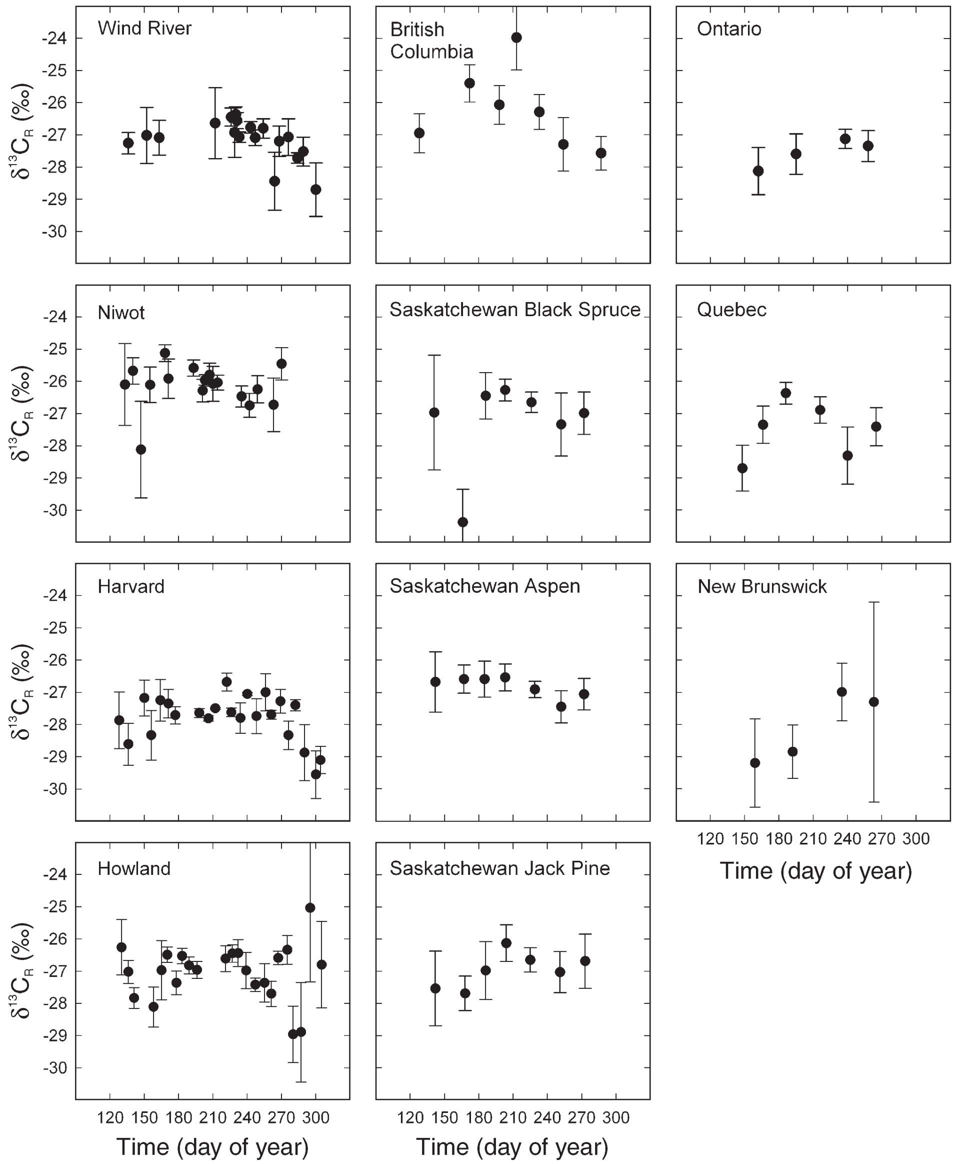

Figure 2. Seasonal variation of the carbon isotope composition of ecosystem-respired CO_2 ($\delta^{13}C_R$) measured at the study sites from May–October 2004.

PPF and VPD were significant factors in the regression models selected for the deciduous group and were positively correlated with $\delta^{13}C_R$ (Figure 6).

To further explore the effects of collinearity on the statistical analysis of the deciduous group data, the variance-decomposition tables associated with each deciduous group model were

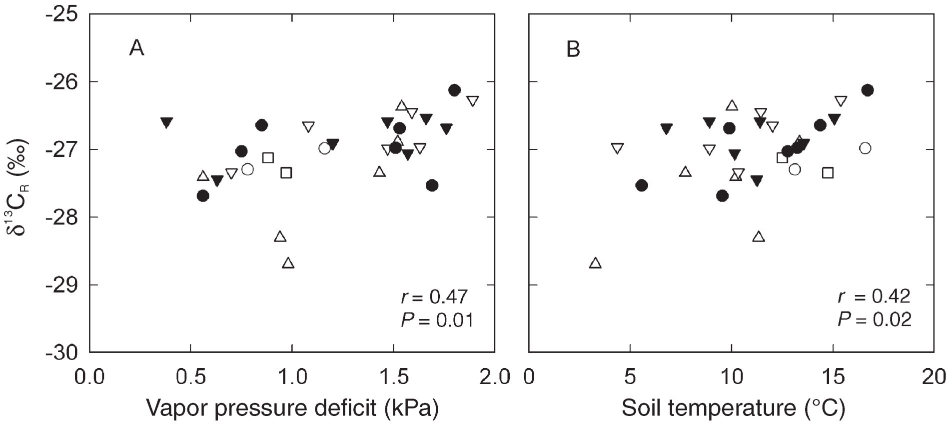

Figure 3. Relationships between the carbon isotope composition of ecosystem-respired CO_2 ($\delta^{13}C_R$) for the boreal group and (A) 4-day lag-time vapor pressure deficit and (B) 4-day lag-time soil temperature. Different symbols represent different sites, and the correlation coefficient (*r*) and *P* values represent calculations for all sites combined. Symbols: ○, New Brunswick; □, Ontario; △, Quebec; ▼, Saskatchewan aspen; ▽, Saskatchewan black spruce; and ●, Saskatchewan jack pine.

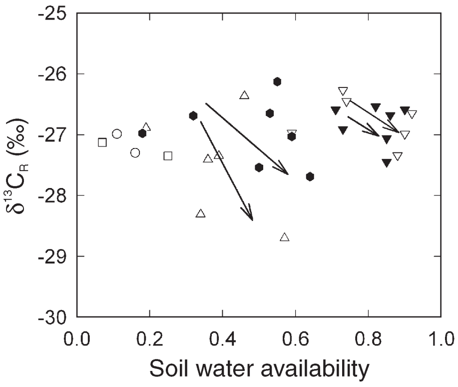

Figure 4. Relationships between the carbon isotope composition of ecosystem-respired CO_2 ($\delta^{13}C_R$) and 4-day lag-time soil water availability for the boreal group. Symbols: ○, New Brunswick; □, Ontario; △, Quebec; ▼, Saskatchewan aspen; ▽, Saskatchewan black spruce; and ●, Saskatchewan jack pine.

examined (data not shown). For all models, the highest proportion of variation was split between T_{air} and T_{soil} (e.g., values of 0.88 and 0.92 for the 4-day lag-time model). A ridge trace method determined that, for all six models, the PPT, SWA and VPD variables were unstable, and these variables were eliminated from the model stepwise in this order. The resulting regression models for the deciduous group were significant for all lag times tested, and R^2 values were above 0.5 for all but the 1-day lag-time model (Table 7). Both T_{air} and T_{soil} were significant factors in the regression models for the deciduous group (Table 7, Figure 6), and PPF was significant in the 3-day regression model (Table 7).

Discussion

In general, $\delta^{13}C_R$ was correlated with environmental changes as predicted by known leaf-level responses (i.e., c_i/c_a response to environmental changes, see Figure 1). We observed positive relationships between $\delta^{13}C_R$ and PPF in the coastal and deciduous groups (Figures 5 and 6), VPD in the boreal group (Figures 3), T_{air} and T_{soil} in the deciduous group and T_{soil} in the boreal and coastal groups (Figures 3, 5 and 6). There was a negative correlation between $\delta^{13}C_R$ and PPT in the coastal group (Figure 5D). These results agree with those of previous studies (Ekblad and Högberg 2001, Bowling et al. 2002, Ometto et al. 2002, Fessenden and Ehleringer 2003, McDowell et al. 2004, Scartazza et al. 2004, Lai et al. 2005, Knohl et al. 2005, Ponton et al. 2006), and are consistent with the assumption that the majority of ecosystem-respired CO_2 comes from the breakdown of recently-fixed carbon compounds whose carbon isotope composition reflects that of the conditions during photosynthetic gas exchange.

There are time lags between photosynthetic carbon fixation and subsequent carbon release to the atmosphere by respiration. The magnitude of the time lags should vary among ecosystem components, being shortest for foliage and root and rhizosphere respiration and longest for litter and soil organic matter respiration and decomposition. When correlating seasonal changes in $\delta^{13}C_R$ with changes in environmental condi-

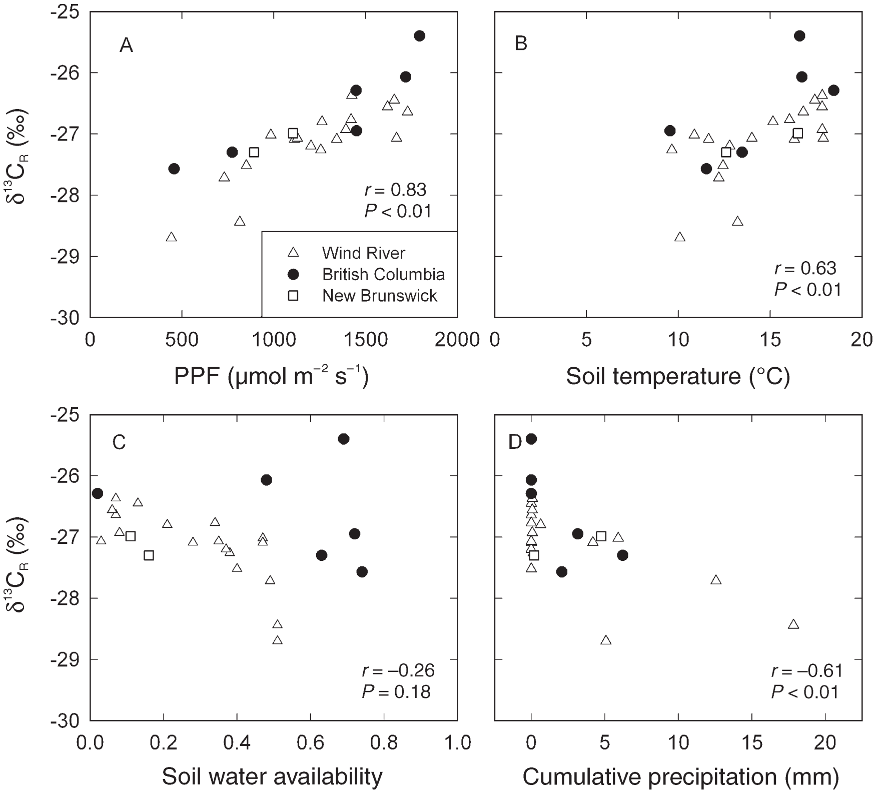

Figure 5. Relationships between the carbon isotope composition of ecosystem-respired CO_2 ($\delta^{13}C_R$) for the coastal group and (A) 3-day lag time photosynthetic photon flux (PPF), (B) 3-day lag time soil temperature, (C) 3-day lag time soil water availability and (D) 3-day lag time cumulative precipitation. Different symbols represent different sites, and the correlation coefficient (r) and P values represent calculations for all sites combined.

Table 6. Deciduous group model parameters and variable *P* values for six separate linear regression analyses; each model compares the lag-time averaged environmental variables (1–6-day lag times) with the carbon isotope composition of ecosystem-respired CO_2. Model selection was conducted based on Akaike's Information Criteria.

	Number of lag days before the sampling date					
	1	2	3	4	5	6
Model parameters						
Akaike's Information Criteria	–32.5	–38.0	–40.0	–42.6	–45.1	–45.5
Condition Index	2.7	2.7	9.1	6.3	6.3	6.4
P value	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
R^2	0.71	0.75	0.78	0.67	0.71	0.72
Variable P values						
Photosynthetic photon flux	0.11	0.11	0.02	< 0.01	< 0.01	0.01
Vapor pressure deficit	–	–	0.04	< 0.01	< 0.01	< 0.02
Daily maximum air temperature	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Daily maximum soil temperature	–	–	0.10	< 0.01	< 0.01	< 0.01
Mean soil water availability	< 0.01	< 0.01	0.06	–	–	–

tions during photosynthesis, it is necessary to consider the environmental conditions before $\delta^{13}C_R$ sample collection. We conducted statistical tests for a variety of lag times. In general, all lag times had similar patterns of statistical significance, though the highest R^2 values occurred for the 2-, 3- and 4-day lag times. This 2–4-day peak in lag times was consistent with previous studies that examined carbon residence times in terrestrial ecosystems (Horwarth et al. 1994, Trumbore 2000). The peak lag times we found were similar to those found for a temperate deciduous forest (4–5 days; Knohl et al. 2005), a boreal conifer forest (1–4 days; Ekblad and Högberg 2001) and a ponderosa pine forest in Oregon (3 days; McDowell et al. 2004). In contrast, Bowling et al. (2002) found a strong correlation between $\delta^{13}C_R$ and VPD in Oregon conifer forests with 5–10-day lag times.

To analyze the effects of environmental variation on seasonal changes in $\delta^{13}C_R$, we classified the study sites into three broad-scale ecological groups (boreal, coastal, deciduous). Examining the $\delta^{13}C_R$ responses of the combined sites within each forest type provided a way to pool samples to derive sta-

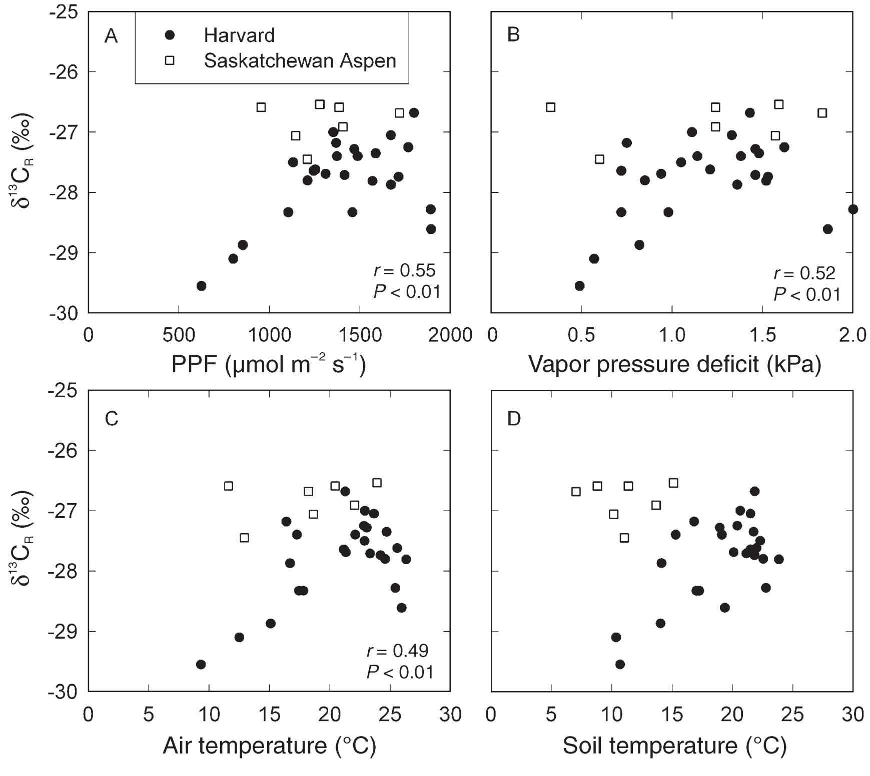

Figure 6. Relationships between the carbon isotope composition of ecosystem-respired CO_2 ($\delta^{13}C_R$) for the deciduous group and (A) 3-day lag time photosynthetic photon flux (PPF), (B) 3-day lag time vapor pressure deficit, (C) 3-day lag time air temperature and (D) 3-day lag time soil temperature. Different symbols represent different sites, and the correlation coefficient (*r*) and *P* values represent calculations for all sites combined.

Table 7. Parameters and variable *P* values from the ridge regression analyses of the deciduous group, where separate trace analyses were compared for each of the six lag-time-averaged environmental variables (1–6-day lag times) with the carbon isotope composition of ecosystem-respired CO_2.

	Number of lag days before the sampling date					
	1	2	3	4	5	6
Model parameters						
Condition Index	4.3	3.7	3.3	3.5	3.5	3.6
P value	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
R^2	0.36	0.51	0.55	0.56	0.62	0.64
Variable P values						
Photosynthetic photon flux	0.08	0.06	0.04	0.09	0.12	0.20
Daily maximum air temperature	0.02	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Daily maximum soil temperature	0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

tistically viable population sizes, and facilitated analysis of $\delta^{13}C_R$ responses with a single statistical model. This approach also minimized the possible misinterpretation of significant correlations between $\delta^{13}C_R$ and non-independent environmental factors (Aranibar et al. 2006). In addition, our functional-type approach provided a large-scale view, demonstrating variation in $\delta^{13}C_R$ responses among the different forest groups.

There were differences among the forest groups in the strength or significance, or both, of the relationships between $\delta^{13}C_R$ and particular environmental factors. For example, VPD and soil temperature were significant determinants of variation in $\delta^{13}C_R$ in the boreal group, but PPF was not a significant factor (Table 4). By contrast, in the coastal group, variation in $\delta^{13}C_R$ was strongly correlated with changes in PPF (Figure 5), but there was no significant relationship with VPD. Coastal regions have more cloudy days than continental boreal sites; thus, daily variation in PPF may influence leaf photosynthetic gas exchange processes and carbon gain at coastal sites. The moderate maritime climate of coastal regions may also minimize seasonal variations in temperature and VPD relative to continental boreal sites. The larger day-to-day and seasonal variations in temperature in the boreal sites, which have more consistently high PPF compared with coastal sites, suggests that temperature, and indirectly VPD, have stronger effects on photosynthetic gas exchange and carbon isotope discrimination in boreal ecosystems than in coastal ecosystems.

Unlike the boreal and coastal groups, the initial statistical analysis for the deciduous group showed that variation in $\delta^{13}C_R$ had similar positive correlations with temperature, PPF and VPD (Figure 6, Table 6), although strong collinearity effects were noted for this analysis. After ridge regression variable reduction, air and soil temperature were the most important environmental factors explaining variation in $\delta^{13}C_R$ in all lag-time models for the deciduous group (Table 7). However, the $\delta^{13}C_R$ dataset for the Harvard site was much larger than that of the Saskatchewan aspen site, and so the Harvard data dominated the patterns observed in the combined deciduous dataset (Figure 6). In addition, the environmental conditions at the two deciduous sites differed greatly, and so the observed correlations are likely biased by effects at the Harvard site and may not represent deciduous forests in general.

In the coastal group, seasonal variations in precipitation and soil water availability significantly affected $\delta^{13}C_R$ (3- and 4-day lag time models; Table 5, Figure 5). These findings are consistent with previous studies in coastal forests showing significant relationships between $\delta^{13}C_R$ and precipitation (Bowling et al. 2002, McDowell et al. 2004) and between $\delta^{13}C_R$ and soil water availability (Fessenden and Ehleringer 2003, Lai et al. 2005). Water availability in coastal forests in western North America typically fluctuates seasonally, with heavy spring and fall precipitation bracketing summer droughts. In contrast, there was no strong correlation between $\delta^{13}C_R$ and precipitation or soil water availability at the other study sites or for other groups. For the Saskatchewan sites, total growing season precipitation in 2004 was much higher than in 2003 (451 versus 193 mm) and may have minimized the effects of seasonal drought on $\delta^{13}C_R$ (Ponton et al. 2006). At the Harvard site, there was only a minor difference between the seasonal mean $\delta^{13}C_R$ value observed in 2003 (–27.0 ± 0.3‰, Lai et al. 2005) and 2004 (–27.8 ± 0.2‰), and growing season precipitation in 2003 was only slightly higher than in 2004 (709 versus 678 mm). The Niwot site also had similar seasonal mean $\delta^{13}C_R$ values in 2003 (–26.5 ± 0.1‰, Bowling et al. 2005) and 2004 (–26.2 ± 0.1‰), despite less growing season precipitation in 2003 compared to 2004 (215 versus 468 mm). Environmental factors other than precipitation and soil water availability also influenced leaf-level photosynthetic gas exchange, with effects on seasonal and annual variations in $\delta^{13}C_R$. It is possible that variation in temperature at the Harvard site confounded comparisons of mean $\delta^{13}C_R$ values between 2003 and 2004, because air temperature was a primary control on $\delta^{13}C_R$ values in the deciduous group. This comparison within and between years suggests the potential application of $\delta^{13}C_R$ values to assess year-to-year variation in ecosystem physiological responses to changing environmental conditions, but also shows that all environmental factors influencing carbon isotope discrimination during photosynthetic gas exchange need to be considered in such an analysis.

An alternative to the statistical approach we adopted would be to use a mechanistic model that incorporates known isotope effects during photosynthetic gas exchange to analyze the effects of the environmental variables on $\delta^{13}C_R$ (Aranibar et al. 2006). Some of our group (T. Cai and L.B. Flanagan, unpublished data) have recently developed an ecosystem-scale model for one study site that calculates the $\delta^{13}C$ values of ecosystem-respired CO_2, and excellent agreement between modeled and measured $\delta^{13}C_R$ values have been observed during three separate study years. To accurately predict measured $\delta^{13}C_R$ values with the model, however, it was found that accurate calculations of leaf c_i/c_a were required for the forest canopy and this involved modeling leaf CO_2 assimilation, stomatal conductance and chloroplast CO_2 concentration separately for sunlit and shaded leaves within multiple canopy layers. The stomatal conductance model calculations were linked to differences in water potential and resistances in the hydraulic pathway between the soil and the tree foliage. In addition, the model made use of prior knowledge of mesophyll or internal conductance for CO_2 from the leaf intercellular air spaces to the chloroplast. Thus, the model requires a great deal of prior information in order to apply it at one site and so it was well beyond the scope of this analysis to apply it at eleven study sites. However, the ecosystem model produced data that closely resembled the conceptual relationships shown in Figure 1, consistent with the statistical approach taken in this study.

The mean $\delta^{13}C_R$ value based on samples from all sites was calculated to be –27.1 ± 0.1‰ (equivalent to a discrimination value of about 19.1‰, assuming that the $\delta^{13}C$ of source atmospheric CO_2 is –8.0‰). Lai et al. (2004) showed that, to infer ecosystem discrimination from measurements of $\delta^{13}C_R$, using a global mean of atmospheric $\delta^{13}C$ creates no bias (also see Flanagan et al. 1996). In addition, the variation in $\delta^{13}C$ of atmospheric CO_2 collected at three NOAA Global Monitoring Division sites demonstrated little variation during May–October, which corresponds to our field collection period.

Our mean discrimination value of about 19.1‰ for 11 North American forests was consistent with calculations from a global model by Lloyd and Farquhar (1994), who determined discrimination values of 19.6‰ for cool/cold deciduous forests, 19.0‰ for evergreen warm mixed forests, 18.3‰ for cool/cold mixed forests and 15.4‰ for cool/cold conifer forests. In a synthesis of $\delta^{13}C_R$ values from North American temperate conifer and boreal forests, Pataki et al. (2003) calculated a mean value of –26.5‰ (equivalent to a discrimination value of about 18.5‰), which was similar to our result. In contrast, Kaplan et al. (2002), in a global modeling analysis, calculated discrimination values of 16.6‰ for temperate deciduous forests, 19.2‰ for cold deciduous forests, 17.8‰ for temperate evergreen needle-leaf forests and 18.4‰ for cold evergreen needle-leaf forests. All forest types in our study had similar mean $\delta^{13}C_R$ values despite being in a range of different geographic regions with contrasting climates.

In conclusion, we have provided additional evidence that the carbon isotope composition of ecosystem-respired CO_2 is indicative of physiological responses at the ecosystem-level. The relationships we observed between $\delta^{13}C_R$ and environmental factors were consistent with known leaf-level responses, and these relationships were apparent within each functional group. However, there were differences among the functional groups in the strength or significance, or both, of the relationships between $\delta^{13}C_R$ and particular environmental factors. For example, although VPD and soil temperature were significant determinants of variation in $\delta^{13}C_R$ in the boreal group, PPF was not a significant factor. By contrast, in the coastal group, variation in $\delta^{13}C_R$ was strongly correlated with changes in PPF, and there was no significant relationship with VPD. This suggests that seasonal variations in VPD and soil temperature were the most prominent environmental limitations on photosynthetic gas exchange in the boreal group, whereas seasonal changes in PPF most strongly limited photosynthetic gas exchange in the coastal group, which included sites where seasonal changes in temperature and VPD were moderate. At a single site, comparisons between our $\delta^{13}C_R$ measurements in 2004 and published values indicate the potential application of $\delta^{13}C_R$ values to assess year-to-year variation in ecosystem physiological responses to changing environmental conditions. They also showed that all environmental factors influencing carbon isotope discrimination during photosynthetic gas exchange need to be considered in such an analysis.

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