

## Response of the carbon isotopic content of ecosystem, leaf, and soil respiration to meteorological and physiological driving factors in a *Pinus ponderosa* ecosystem

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[1] Understanding the controls over ecosystem-respired  $\delta^{13}\text{C}$  ( $\delta^{13}\text{C}_R$ ) is important for applications of isotope-based models of the global carbon budget as well as for understanding ecosystem-level variation in isotopic discrimination ( $\Delta$ ). Discrimination may be strongly dependent on synoptic-scale variation in environmental drivers that control canopy-scale stomatal conductance ( $G_c$ ) and photosynthesis, such as atmospheric vapor pressure deficit (vpd) photosynthetically active radiation (PAR) and air temperature ( $T_{\text{air}}$ ). These potential relationships are complicated, however, due to time lags between the period of carbon assimilation and ecosystem respiration, which may extend up to several days, and may vary with tissue (i.e., leaves versus belowground tissues). Our objective was to determine if relationships exist over a short-term period (2 weeks) between meteorological and physiological driving factors and  $\delta^{13}\text{C}_R$  and its components, soil-respired  $\delta^{13}\text{C}$  ( $\delta^{13}\text{C}_{R\text{-soil}}$ ) and foliage-respired  $\delta^{13}\text{C}$  ( $\delta^{13}\text{C}_{R\text{-foliage}}$ ). We tested for these hypothesized relationships in a 250-year-old ponderosa pine forest in central Oregon, United States. A cold front passed through the region 3 days prior to our first sample night, resulting in precipitation (total rainfall 14.6 mm), low vpd (minimum daylight average of 0.36 kPa) and near-freeze temperature (minimum air temperature of  $0.18^\circ\text{C} \pm 0.3^\circ\text{C}$ ), followed by a warming trend with relatively high vpd (maximum daylight average of 3.19 kPa). Over this 2-week period  $G_c$  was negatively correlated with vpd ( $P < 0.01$ ) while net ecosystem  $\text{CO}_2$  exchange (NEE) was positively correlated with vpd ( $P < 0.01$ ), consistent with a vpd limitation to conductance and net  $\text{CO}_2$  uptake. Consistent with a stomatal influence over  $\Delta$ , a negative correlation was observed between  $\delta^{13}\text{C}_R$  and  $G_c$  measured 2 days prior (i.e., a 2-day time lag,  $P = 0.04$ ); however,  $\delta^{13}\text{C}_R$  was not correlated with other measured variables. Also consistent with a stomatal influence over discrimination,  $\delta^{13}\text{C}_{R\text{-soil}}$  was negatively correlated with  $G_c$  ( $P < 0.01$ ) and positively correlated with vpd and PAR measured one to 3 days prior ( $P = 0.01$  and  $0.04$ , respectively). In contrast,  $\delta^{13}\text{C}_{R\text{-foliage}}$  was not correlated with vpd or  $G_c$ , but was negatively correlated with minimum air temperature measured 5 days previously ( $P < 0.01$ ) supporting the idea that cold air temperatures cause isotopic enrichment of respired  $\text{CO}_2$ . The significant driving parameters differed for  $\delta^{13}\text{C}_{R\text{-foliage}}$  and  $\delta^{13}\text{C}_{R\text{-soil}}$  potentially due to different controls over the isotopic content of tissue-specific respiratory fluxes, such as differing carbon transport times from the site of assimilation to the respiring tissue or different reliance on recent versus old photosynthate. Consistent with  $G_c$  control over photosynthesis and  $\Delta$ , both  $\delta^{13}\text{C}_{R\text{-soil}}$  and  $\delta^{13}\text{C}_{R\text{-foliage}}$  became enriched as net  $\text{CO}_2$  uptake decreased (more positive NEE,  $P < 0.01$  for both). The  $\delta^{13}\text{C}$  value of *Pinus ponderosa* foliage ( $-27.1\text{‰}$ , whole-tissue) was 0.5 to 3.0‰ more negative than any observed respiratory signature, supporting the contention that foliage  $\delta^{13}\text{C}$  can be a poor proxy for the isotopic content of respiratory fluxes. The strong meteorological controls

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over  $G_c$  and NEE were associated with similar variation in  $\delta^{13}C_{R-soil}$  but only minor variation in  $\delta^{13}C_R$ , leading us to conclude that  $\delta^{13}C_R$  is not controlled solely by either canopy and belowground processes, but rather by their time-dependent interaction.

**INDEX TERMS:** 1615 Global Change: Biogeochemical processes (4805); 1040 Geochemistry: Isotopic composition/chemistry; **KEYWORDS:** carbon isotopes, conductance, ecosystems, eddy correlation, old growth, *Pinus ponderosa*

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## 1. Introduction

[2] Concerns over rising concentrations of atmospheric CO<sub>2</sub> ([CO<sub>2</sub>]) and subsequent effects on global warming have led to aggressive use of new techniques to resolve the global carbon budget [Canadell *et al.*, 2000]. Among these techniques, measurements of the stable isotope composition of atmospheric CO<sub>2</sub> coupled with mass balance calculations, inverse global models, and biogeochemical models [i.e., Ciais *et al.*, 1995; Battle *et al.*, 2000; Randerson *et al.*, 2002a] are being utilized to constrain the terrestrial carbon sink as well as determine its regional location. However, observed variation in carbon isotope discrimination ( $\Delta$ ) and subsequent variation in the carbon isotopic composition of ecosystem respiration ( $\delta^{13}C_R$ ) suggests that assuming constant values for these parameters may lead to inaccurate estimates of the land/ocean sink partitioning [Fung *et al.*, 1997; Randerson *et al.*, 2002b]. While a solid foundation exists for our understanding of  $\Delta$  at the leaf-scale [Farquhar *et al.*, 1989], our theoretical and empirical knowledge of controls and variability over  $\delta^{13}C_R$  is comparatively weak, thus causing uncertainty in our scaled estimates of  $\delta^{13}C_R$ .

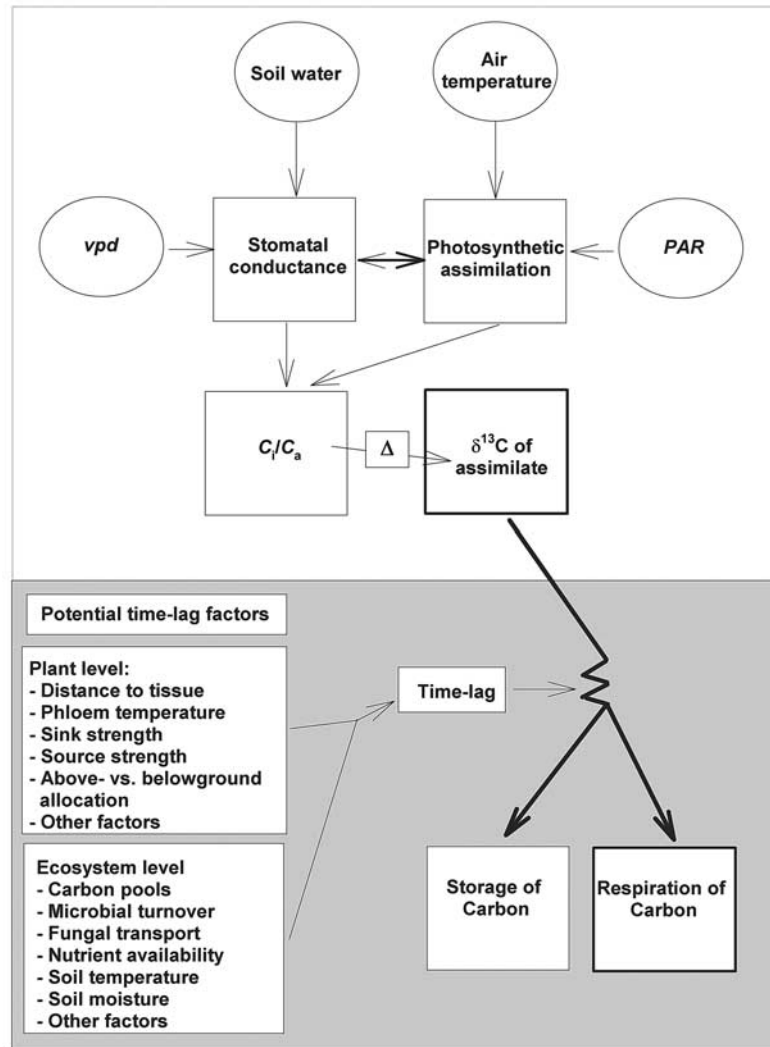
[3] Recent work has shown significant within-site variation in  $\delta^{13}C_R$  (up to 8.5‰) that appears to be driven by factors that influence leaf-level  $\Delta$  [Bowling *et al.*, 2002]. In that study, a nonlinear relationship between  $\delta^{13}C_R$  and vapor pressure deficit (vpd) was observed for a variety of coniferous forests along a precipitation gradient. Ekblad and Högberg [2001] found a similar link between  $\delta^{13}C$  of soil-respired CO<sub>2</sub> ( $\delta^{13}C_{R-soil}$ ) and atmospheric humidity. In both studies the isotopic response to humidity was in agreement with our traditional concept of a stomatal influence over  $\Delta$  at the leaf-level (Figure 1, top). Increasing vpd typically causes a reduction in stomatal conductance [Cowan, 1994; Hinckley and Braatne, 1994; Montieth, 1995; Oren *et al.*, 1999] and consequently the supply of atmospheric CO<sub>2</sub> to the stomatal pore is reduced, thereby causing the ratio of atmospheric to internal, or sub-stomatal CO<sub>2</sub> ( $c_i/c_a$ ) to decline (Figure 1). Reduced  $c_i/c_a$  subsequently forces a decrease in discrimination and hence an increase in the  $\delta^{13}C$  of photo-assimilate (Figure 1, and see Farquhar *et al.* [1989] or Ehleringer *et al.* [1993] for a review of  $c_i/c_a$  controls on  $\delta^{13}C$  of assimilated carbon).

[4] However, the relationship between  $\delta^{13}C$  of photo-assimilate and  $\delta^{13}C$  of respiratory fluxes may not be direct or immediate. An important result of the studies by Ekblad and Högberg [2001] and Bowling *et al.* [2002] is that respired  $\delta^{13}C$  was correlated with humidity measured multiple days prior to the collection of the isotope data,

rather than with humidity on the same day as the isotopic collections. This time lag indicates that the transport time of assimilate from foliage to the bulk of respiring tissue is relatively rapid, but not immediate. A similar, multiple-day time lag between assimilation and soil respiration was observed in the girdling study by Högberg *et al.* [2001]. As hypothesized in the bottom of Figure 1, the time lag may be associated with the transport time of carbon between the site of assimilation (foliage) and the respiring tissue. This time lag can be influenced by plant physiological factors including, but not limited to, transport distance, phloem temperature, sink or source strength, allocation of carbon between tissues or between respiration and dry matter production (Figure 1). Likewise, the time lag may be influenced by ecosystem level factors including, but not limited to, controls over soil respiration such as carbon transport from roots to fungi, hyphal transport time, microbial turnover, nitrogen availability, and soil temperature and moisture (Figure 1).

[5] In some ecosystems, however, little variation in  $\delta^{13}C_R$  has been observed [Flanagan *et al.*, 1996; Buchmann *et al.*, 1998]. Such constancy of observed  $\delta^{13}C_R$  may be due to limited sampling, a lack of variation in or insensitivity to environmental driving variables, a balancing effect of driving variables on  $\Delta$  (i.e., if both stomatal conductance and assimilation rise in proportion causing constancy of  $c_i/c_a$ ) or to a decoupling of  $\Delta$  and  $\delta^{13}C_R$ . Decoupling may occur if the substrate used for respiration was not assimilated in recent days. For example, microbial respiration may switch from current (i.e., assimilated in the last few days) to relatively older (weeks to years old) photosynthate if cold temperatures reduce phloem transport rates, if freezing temperatures or (high vpd) cause stomatal closure and reduced photosynthetic rates [Smith *et al.*, 1984], or if soil moisture or oxygen availability becomes limiting to microbial metabolism [Paul and Clark, 1989]. In such a case, variation in  $\Delta$  may not be observed in  $\delta^{13}C_R$ .

[6] Ecosystem-respired CO<sub>2</sub> results from the combined flux of CO<sub>2</sub> from the soil surface, foliage, stems and woody debris within the ecosystem. At the *Pinus ponderosa* site used in this study, ecosystem respiration is dominated by soil CO<sub>2</sub> flux (~76%) with the remainder dominated by foliar respiration [Law and Ryan, 1999]. Foliar and soil surface respiration should have different time lags between the moment of assimilation of a given carbon atom and the moment of respiration of the organic compounds containing that atom due purely to the transport distance from the site of photosynthetic assimilation to mitochondrial respiration. Simultaneous measurements of  $\delta^{13}C_R$ ,  $\delta^{13}C_{R-soil}$ , and



**Figure 1.** Theoretical representation of factors that may influence  $\delta^{13}\text{C}_R$ . (top) A simplified view of factors that may influence  $c_i/c_a$ ,  $\Delta$  and subsequent  $\delta^{13}\text{C}$  of photo-assimilate. (bottom) Some factors that may influence the signature of  $\delta^{13}\text{C}_R$  as well as the temporal lag between the time of carbon assimilation and respiration. The term "other factors" refers to factors that may yet be discovered.

$\delta^{13}\text{C}_{R\text{-foliage}}$  may provide insight into the controls over the time lag of  $\delta^{13}\text{C}_R$  behind  $\Delta$ , thus providing insight regarding the mechanisms that affect carbon allocation and transport.

[7] Combining measurements of the isotopic content of respiratory fluxes with estimates of net ecosystem exchange (NEE) can also provide insight into the potential relationships between  $\delta^{13}\text{C}_R$  and ecosystem carbon flux. NEE is the ecosystem-scale balance of carbon assimilation and respiration,

$$\text{NEE} = R - A, \tag{1}$$

where  $R$  is respiration and  $A$  is photosynthetic assimilation, both at the ecosystem-scale. Equation (1) is written such that more negative values indicate greater terrestrial  $\text{CO}_2$  uptake. Canopy-averaged stomatal conductance ( $G_c$ ) reg-

ulates both  $A$  and  $\Delta$  and therefore should link NEE to  $\delta^{13}\text{C}_R$ . This prediction is described using the following equations. Leaf-level studies have shown that  $A$  is directly coupled to  $G_c$  [e.g., Meinzer *et al.*, 1993] because  $G_c$  controls  $\text{CO}_2$  diffusion from the atmosphere to the stomatal pore, thereby controlling substrate availability to photosynthetic enzymes. In simple terms,

$$A \approx f(G_c). \tag{2}$$

In addition to its potentially dominant effect over NEE,  $G_c$  regulates  $\Delta$  because  $G_c$  affects  $c_i$ ,

$$c_i = c_a - \frac{A}{G_c}, \tag{3}$$

and  $c_i$  regulates  $\Delta$  [Farquhar *et al.*, 1989],

$$\Delta = a + (b - a) \cdot \frac{c_i}{c_a}. \quad (4)$$

Last, the  $\delta^{13}\text{C}$  composition of photosynthate results directly from  $\Delta$  [Brugnoli *et al.*, 1988] such that

$$\delta^{13}\text{C}_R \approx \delta^{13}\text{C}_a - \Delta, \quad (5)$$

where  $\delta^{13}\text{C}_a$  is the carbon isotope signature of atmospheric  $\text{CO}_2$ . Equation (5) is a simplified version of the ecosystem-scale discrimination equation suggested by Buchmann *et al.* [1998] and should hold for  $\delta^{13}\text{C}_R$  if  $\delta^{13}\text{C}_R$  results directly from  $\delta^{13}\text{C}$  of photosynthate. In other words, equation (5) holds if canopy-scale  $\Delta$  is the direct control over  $\delta^{13}\text{C}_R$ , for example, no fractionation occurs during phloem loading or respiration, and ecosystem-respired  $\text{CO}_2$  is derived entirely from current photosynthate. Even though these assumptions are unlikely to be fully satisfied, we hypothesized that NEE and  $\delta^{13}\text{C}_R$  are to some extent linked to  $G_c$ .

[8] The simple relationship between  $G_c$ , NEE, and  $\delta^{13}\text{C}_R$  may be more complicated if  $\delta^{13}\text{C}$  of photosynthate does not directly transfer to the isotopic fluxes of the various above and belowground components of the ecosystem, for example, due to time lags between canopy  $\Delta$  and respired- $\delta^{13}\text{C}$ . For example, the time lag between  $G_c$  and  $\delta^{13}\text{C}_R$  should be longer than for  $G_c$  and NEE (if any lag exists) because  $\delta^{13}\text{C}_R$  is associated with respiration and hence the lag is controlled by within-ecosystem carbon transport, whereas NEE is associated with both respiration and photosynthesis, the latter of which should be tightly coupled with  $G_c$ . This could be further confounded, however, if ecosystem respiration rate is correlated with time-lagged  $G_c$ .

[9] In this study, we measured  $\delta^{13}\text{C}_R$ ,  $\delta^{13}\text{C}_{R\text{-soil}}$ , and  $\delta^{13}\text{C}_{R\text{-foliage}}$  nightly over a 2-week period with the objective of examining relationships between these fluxes and meteorological and physiological driving factors. Our two primary hypotheses were that (1) short-term fluctuations in vpd would be correlated with  $\delta^{13}\text{C}_R$ ,  $\delta^{13}\text{C}_{R\text{-soil}}$ , and  $\delta^{13}\text{C}_{R\text{-foliage}}$ , and (2) the mechanism underlying these correlations would be vpd regulation of  $G_c$  and subsequent  $G_c$  affects on  $\delta^{13}\text{C}_R$ ,  $\delta^{13}\text{C}_{R\text{-soil}}$ , and  $\delta^{13}\text{C}_{R\text{-foliage}}$ .

## 2. Methods

### 2.1. Site

[10] The study was conducted between days 179 and 191, 2001. The study site is a ponderosa pine (*Pinus ponderosa*) dominated forest located in the Metolius Research Natural Area near Sisters, Oregon (44°30'N, 121°37'W). The site is located at an elevation of 940 m on a nearly flat slope (2 to 6%). Ponderosa pine dominated forest extends for at least 12 km in all directions. The stand has two dominant age-classes of trees consisting of ~250-year-old trees and ~50-year-old trees, and a minor contribution (in regards to biomass) of saplings and seedlings. Understory vegetation is sparse. The canopy is open (leaf area index ~2.0 m<sup>2</sup> half surface area needles per m<sup>2</sup> ground), and vpd in the sub-canopy is similar to that measured above the canopy [Law and Baldocchi, 1999]. The soil is a sandy loam and is low

in nutrients. Climate at this site is characterized by warm, dry summers and wet, cool winters, with mean annual precipitation of 523 mm. This site is a member of the AmeriFlux network, and more extensive site details are given by Law and Ryan [1999], Law *et al.* [2001], and Anthoni *et al.* [2002].

### 2.2. Keeling Plots

[11] We used the Keeling plot approach [Keeling, 1958] to assess the isotopic composition of  $\text{CO}_2$  in respiratory fluxes. This approach uses a two-component mixing model that consists of the carbon isotope ratio of  $\text{CO}_2$  respired from all organisms within the forest and  $\delta^{13}\text{C}$  of  $\text{CO}_2$  in the background atmosphere. The intercept of a linear regression of  $\delta^{13}\text{C}$  of atmospheric  $\text{CO}_2$  versus  $1/[\text{CO}_2]$  (where  $[\text{CO}_2]$  is the mole fraction of  $\text{CO}_2$ ) provides an estimate of  $\delta^{13}\text{C}_R$ . We used geometric mean (model II) regressions [Sokal and Rohlf, 1995]. Outliers were determined on each individual Keeling plot as described by Bowling *et al.* [2002]. We assumed no changes in  $\delta^{13}\text{C}$  of the end-members during the sampling period for each individual Keeling plot. See Pataki *et al.* [2003] for more details on the application of Keeling plots in ecosystem science.

[12] Keeling plots were used to estimate  $\delta^{13}\text{C}_R$ ,  $\delta^{13}\text{C}_{R\text{-soil}}$  and  $\delta^{13}\text{C}_{R\text{-foliage}}$ .  $\delta^{13}\text{C}_R$  and  $\delta^{13}\text{C}_{R\text{-soil}}$  were sampled each night from day 179 to 191, and  $\delta^{13}\text{C}_{R\text{-foliage}}$  was sampled each night from day 187 to 191. Year 2001 foliage from shoots neighboring those used for  $\delta^{13}\text{C}_{R\text{-foliage}}$  was collected on day 179 for measurement of  $\delta^{13}\text{C}$  of whole-tissue. Approximately three fascicles per branch were collected.

### 2.3. The Carbon Isotope Ratio of Ecosystem Respiration ( $\delta^{13}\text{C}_R$ )

[13] We sampled air from 0.2 m, 0.8 m and 11.4 m above the ground surface using Dekoron tubing (Dekoron/Uni-therm Cable USA, Cape Coral, Fla.) placed on a scaffolding tower. We also had an inlet tube located above the top of the canopy (~33 m); however, previous sampling at this site showed no isotopic difference in air from the 11.4- and 33-m inlets. Air was pulled through magnesium perchlorate to remove water vapor prior to collection. Samples were then contained within 100-mL glass flasks with Teflon stopcocks (34-5671; Kontes Glass Co., Vineland, N. J.). Samples were collected nocturnally to avoid confounding influences of photosynthesis on  $\delta^{13}\text{C}_R$ . We typically collected air samples from 2100 to 2400 local time (LT) each night and obtained  $[\text{CO}_2]$  ranges of 75 to 110  $\mu\text{mol mol}^{-1}$ . Large  $[\text{CO}_2]$  ranges improve estimates of  $\delta^{13}\text{C}_R$  because the error around the regression intercept is negatively related to the  $[\text{CO}_2]$  range [Pataki *et al.*, 2003].

### 2.4. The Carbon Isotope Ratio of Soil Respiration ( $\delta^{13}\text{C}_{R\text{-soil}}$ )

[14] We assessed  $\delta^{13}\text{C}_{R\text{-soil}}$  using samples collected from a soil respiration chamber. A custom closed, dynamic soil chamber (70 cm × 70 cm × 10 cm tall, 49 L volume) with small internal fans (D249L, Micronel, Vista, Calif.) was placed in series with an infrared gas analyzer (LI-6262, Licor, Inc., Lincoln, Nebr.), a pump (UNMP50KNDC, KNF Neuberger, Inc., Trenton, N. J.), a magnesium perchlorate

water trap, and an assembly of six 100-mL sample flasks (connected to each other in parallel). First, all flask stopcocks were opened, and the pump was run for several minutes to flush the flasks and tubing with ambient forest air near the ground. Efforts were made to avoid contaminating the system with human breath. The chamber was then placed into a groove that had been previously cut through the litter layer to allow contact between the mineral soil and the chamber edge. The chamber was gently placed on the ground, then five of the flasks were closed sequentially in roughly  $30 \mu\text{mol mol}^{-1}$  increments as  $[\text{CO}_2]$  rose from near ambient to  $\sim 150 \mu\text{mol mol}^{-1}$  above ambient. Collection times were approximately 2 min. Three separate chamber locations were used each evening, shortly after dusk. The chamber locations were chosen to represent the three major stand-structure classes for this site: (1) open canopy with few, large trees, (2) closed canopy with dense stocking of small trees and few large trees, and (3) the boundary between the first and second classes. The soil chamber locations were approximately 75 m from the site of flask collection for  $\delta^{13}\text{C}_R$ . Nightly comparison of arithmetic averages of Keeling plot intercepts from the three chamber locations versus intercepts derived by pooling all soil chamber data for a single Keeling plot showed no significant differences, so data were pooled from all three soil chambers to generate a single Keeling plot for each night.

## 2.5. The Carbon Isotope Ratio of Foliage Respiration ( $\delta^{13}\text{C}_{R\text{-foliage}}$ )

[15] We refer to this measurement as  $\delta^{13}\text{C}_{R\text{-foliage}}$ ; however, both woody stem tissue subtending the foliage and the foliage itself were included in the samples. Foliage and stems of two, 250-year-old ponderosa pine trees was accessed using a scaffolding tower. The samples were located approximately 25 m above the ground surface, and 125 m horizontally from the site of flask collection for  $\delta^{13}\text{C}_R$ . Ponderosa pine foliage is arranged in a spherical cluster around the end of the shoot. We wrapped entire foliage clusters in flexible bags (party balloons, Anagram International, Inc., Minneapolis, Minn.) with an internal layer of polyethylene. These bags have been tested for isotopic integrity and show no effect on  $\delta^{13}\text{C}$  of gas samples after 60 min of gas residence within the bags (an order of magnitude longer than our samples resided within the bags). Details on the bags and isotopic tests are given by *Bowling et al.* [2003]. We cut holes in the bottom of the bags large enough for the foliage and attached the cut end of the bag to the shoot using putty (between the bag and shoot) and bungee cords wrapped on the outside of the bag/putty/shoot structure. Bags were attached to the branch just moments before sampling began and were removed after sampling completion. A small fan and inlet and outlet tubes (Dekoron) were placed within the bag. The tubes were run down the tower to the pump located on the ground surface. Samples were collected using a similar six-flask system as described for  $\delta^{13}\text{C}_{R\text{-soil}}$ . A single foliage cluster was measured every night, and on three nights (near the beginning, middle and end of the experiment) we measured five foliage clusters. All branches were located on the south-side of the trees and the foliage was therefore “sun” foliage.

[16] Although this forest has a roughly equal amount of canopy leaf area contributed by the younger (50-year-old) age class, we were forced to constrain our  $\delta^{13}\text{C}_{R\text{-foliage}}$  measurements to the old trees due to time, sample size, and access constraints. Therefore, our  $\delta^{13}\text{C}_{R\text{-foliage}}$  measurements cannot be considered representative of the entire forest canopy.

## 2.6. Laboratory Analyses

[17] We measured carbon isotope ratios of flask samples on a continuous-flow isotope ratio mass spectrometer (IRMS; Finnigan MAT 252 or DELTAplus, San Jose, Calif.) as described by *Ehleringer and Cook* [1998]. Precision for  $\delta^{13}\text{C}$  was determined daily by comparison to known standards and averaged  $0.13\text{‰}$  (standard deviation). Corrections for the presence of  $^{17}\text{O}$  were applied.  $\text{CO}_2$  was separated from  $\text{N}_2\text{O}$  by gas chromatography before analysis. Foliage tissue was ground to number 20 mesh and 2- to 20-mg samples were combusted and analyzed for  $\delta^{13}\text{C}$  on an IRMS (deltaS, Finnigan MAT). Measurement precision for organic samples was  $0.2\text{‰}$ . All  $\delta^{13}\text{C}$  values are reported relative to the international PDB standard. Flask  $[\text{CO}_2]$  was measured using the method of *Bowling et al.* [2001], and World Meteorological Organization  $\text{CO}_2$  standards were used. Measurement precision was  $\pm 0.2 \mu\text{mol mol}^{-1}$ .

## 2.7. Meteorological, Micrometeorological, and Eddy Correlation Measurements

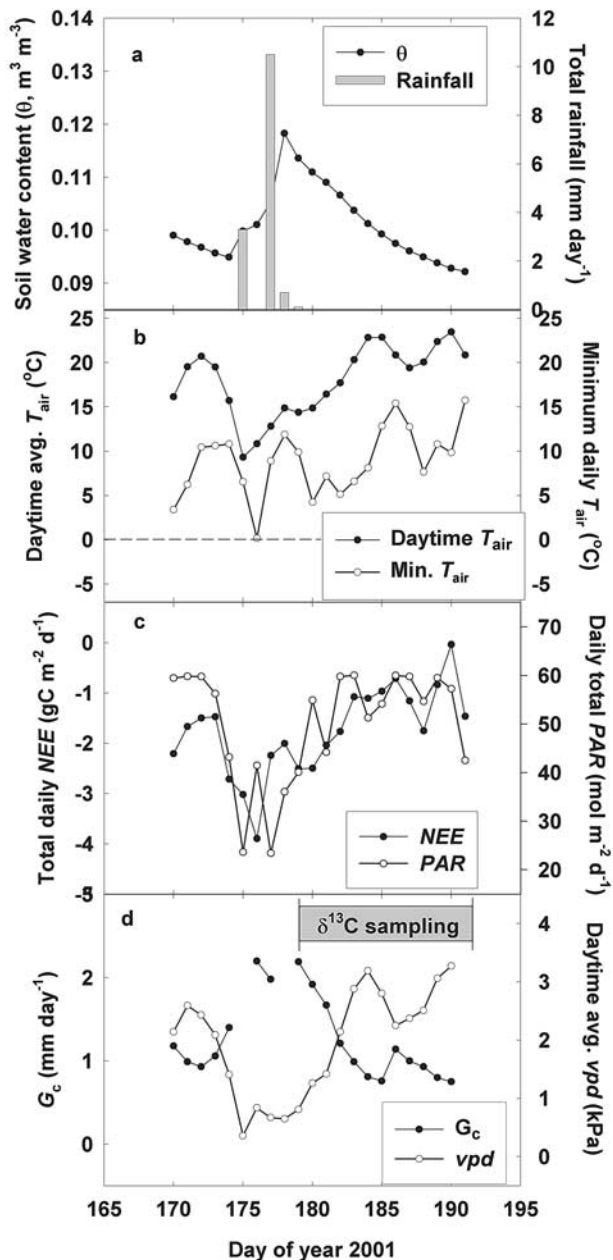
[18] We collected meteorological and micrometeorological measurements at half-hourly intervals during the experiment. Measured parameters included air temperature, relative humidity, soil temperature, soil water content, photosynthetically active radiation, and rainfall. The eddy covariance method was used to determine half-hourly fluxes of  $\text{CO}_2$  and water vapor above the forest canopy. Details of the meteorological and eddy covariance measurements are described by *Law et al.* [2001] and *Anthoni et al.* [2002].

## 2.8. Canopy Stomatal Conductance

[19] Mean midday canopy stomatal conductance ( $G_c$ ) was estimated with a simplified form of Penman-Monteith equation [*Jarvis and McNaughton*, 1986] where whole tree sap flux measurements averaged between 1100 and 1300 LT were used to determine canopy transpiration. See work by *Irvine et al.* [2002] for more details.

## 2.9. Statistical Analyses

[20] We conducted correlation analyses to test our two primary hypotheses, that  $\delta^{13}\text{C}_R$ ,  $\delta^{13}\text{C}_{R\text{-soil}}$ , and  $\delta^{13}\text{C}_{R\text{-foliage}}$  were coupled to vpd and that this relationship was due to  $G_c$  effects on discrimination (see section 1). We also used correlation analyses to determine the relationships between  $\delta^{13}\text{C}_R$ ,  $\delta^{13}\text{C}_{R\text{-soil}}$ , and  $\delta^{13}\text{C}_{R\text{-foliage}}$ , as well as relationships between these isotopic signatures and other variables expected to influence  $\Delta$  or respiratory processes, including vpd,  $T_{\text{air}}$ ,  $T_{\text{min}}$ ,  $T_{\text{soil}}$ , PAR,  $\theta$ , and NEE. Because correlations between these variables and  $\delta^{13}\text{C}$  of respiratory fluxes may be lagged in time due to a delay between the time a given carbon atom is assimilated and respired, we conducted the correlations over a range of time lags. To do this, we



**Figure 2.** Meteorological and physiological data for days 170–191, 2001. (a) Average daily rainfall (solid bars) and  $\theta$  (0 to 30 cm depth, solid circles). (b) Daytime  $T_{\text{air}}$  (solid symbols) and minimum  $T_{\text{air}}$  (open symbols).  $T_{\text{air}}$  reached a nocturnal minimum of  $0.18^{\circ}\text{C} \pm 0.3^{\circ}\text{C}$  on day 176. Zero Celsius is indicated by the dashed line. (c) The 24-hour total NEE (solid symbols) and daylight total PAR (open symbols). (d) Average daily  $G_c$  (solid symbols) during daylight periods. Daylight period vpd is shown as the open symbols.  $G_c$  data are omitted on days 175 and 178 because the canopy was wet on those days making those estimates suspect. The period of flask sampling for isotopic analyses is indicated by the shaded bar in Figure 2d.

calculated averages of a given independent factor from 1 to 5 days, and then shifted these averages back in time by zero to 15 days (a subset of these results are reported). See work by *Bowling et al.* [2002] for a more detailed description of lag analysis. SYSTAT 10.0 was used for statistical analyses.

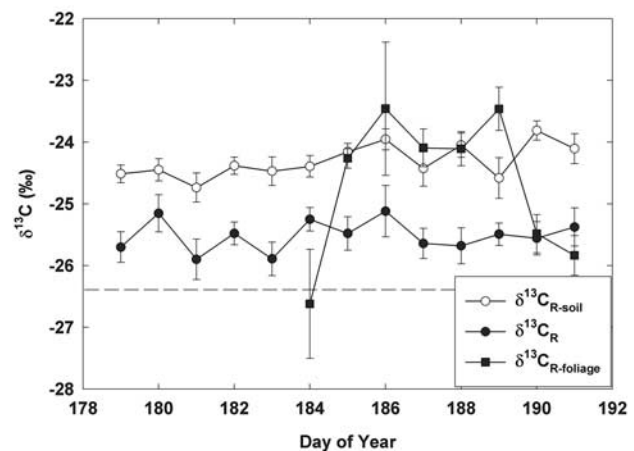
### 3. Results

#### 3.1. Meteorological and Physiological Patterns

[21] A cold front passed through central Oregon on days 175 through 178 (June 24–27), producing precipitation totaling 14.6 mm and increasing  $\theta$  by  $0.03 \text{ m}^3 \text{ m}^{-3}$  (Figure 2a). The minimum  $T_{\text{air}}$  (half-hourly average) during the cold-front was  $0.18^{\circ}\text{C} (\pm 0.3^{\circ}\text{C})$  (Figure 2b). Total daily PAR was reduced substantially by the cloud-cover during the storm, and this reduction in PAR was associated with high rates of net  $\text{CO}_2$  exchange (more negative NEE, Figure 2c). The large net uptake on days 175 to 179 was associated with high  $G_c$  in conjunction with relatively low vpd (Figure 2d). As vpd increased from day 180 to 190, both  $G_c$  and net  $\text{CO}_2$  uptake declined.  $G_c$  and NEE were strongly correlated, with high values of  $G_c$  nonlinearly associated with more negative NEE (i.e., more net  $\text{CO}_2$  uptake,  $P < 0.01$ , data not shown). The  $G_c$  data are omitted on days 175 and 178 because the canopy was wet on those particular days and sapflow-based estimates of  $G_c$  are erroneous when the canopy is wet. Therefore, analyses with  $G_c$  data were conducted without data from days 175 and 178.

#### 3.2. The $\delta^{13}\text{C}_R$ , $\delta^{13}\text{C}_{R\text{-soil}}$ , and $\delta^{13}\text{C}_{R\text{-foliage}}$

[22] The isotopic contents of respiratory fluxes each day are shown in Figure 3. Observed  $\delta^{13}\text{C}_R$  varied from  $-25.1$  to  $-25.8\text{‰}$ ,  $\delta^{13}\text{C}_{R\text{-soil}}$  varied from  $-23.8$  to  $-24.7\text{‰}$ , and  $\delta^{13}\text{C}_{R\text{-foliage}}$  ranged from  $-23.4$  to  $-26.6\text{‰}$ . Neither  $\delta^{13}\text{C}_R$  nor  $\delta^{13}\text{C}_{R\text{-foliage}}$  showed a time-trend over the duration of our measurements (regression  $P = 0.66$  and  $0.63$ , respectively). The  $\delta^{13}\text{C}_{R\text{-soil}}$  was positively correlated with day of



**Figure 3.** Observed  $\delta^{13}\text{C}_R$  (solid circles),  $\delta^{13}\text{C}_{R\text{-soil}}$  (open circles) and  $\delta^{13}\text{C}_{R\text{-foliage}}$  (solid squares) versus day of year, 2001. The dashed line is  $\delta^{13}\text{C}$  of whole-tissue foliage collected from old pine trees on day 179. Error bars represent standard errors.

**Table 1.** Coefficients of Determination ( $r^2$ ) From Linear Regression Analysis of  $\delta^{13}\text{C}_R$ ,  $\delta^{13}\text{C}_{R\text{-soil}}$ , and  $\delta^{13}\text{C}_{R\text{-foliage}}$  Versus Environmental and Physiological Driving Factors<sup>a</sup>

Component	vpd	$T_{\text{air}}$	$T_{\text{min}}$	$T_{\text{soil}}$	PAR	$\theta$	$G_c$	NEE
$\delta^{13}\text{C}_R$	0.01	0.06	0.06	0.05	0.03	0.02	0.00	0.01
$\delta^{13}\text{C}_{R\text{-soil}}$	0.19	0.20	0.20	0.45 <sup>b</sup>	0.04	0.39 <sup>b</sup>	0.25 <sup>c</sup>	0.3 <sup>b</sup>
$\delta^{13}\text{C}_{R\text{-foliage}}$	0.06	0.05	0.06	0.01	0.49 <sup>b</sup>	0.01	0.28	0.04

<sup>a</sup>All regressions presented in this table were done using a zero-day lag and a 1-day average. For example,  $\delta^{13}\text{C}_R$  was correlated with single-day average vpd from the day of flask collection. All presented correlations were positive.

<sup>b</sup>Regression significance  $P = 0.05$ .

<sup>c</sup>Regression significance  $P = 0.10$ .

year ( $r^2 = 0.38$ ,  $P = 0.03$ ). Observed  $\delta^{13}\text{C}_R$  was 1.2 permil; more negative than  $\delta^{13}\text{C}_{R\text{-soil}}$  on average, and 0.85‰ more negative than  $\delta^{13}\text{C}_{R\text{-foliage}}$  on average. The  $\delta^{13}\text{C}$  of foliage tissue collected on day 179 was  $-27.1\text{‰}$  (Figure 3).

[23] Observed  $\delta^{13}\text{C}_R$  was not correlated with  $\delta^{13}\text{C}_{R\text{-soil}}$  or  $\delta^{13}\text{C}_{R\text{-foliage}}$ . The best fit was between  $\delta^{13}\text{C}_R$  and  $\delta^{13}\text{C}_{R\text{-soil}}$  (1-day lag, 1-day average,  $r^2 = 0.14$ ,  $P = 0.20$ ,  $n = 13$ ). A correlation was observed between  $\delta^{13}\text{C}_{R\text{-soil}}$  and  $\delta^{13}\text{C}_{R\text{-foliage}}$ , however, the correlation was strong due to clustering of four points at one end of the regression line and a single point at the other end (3-day lag, 1-day average,  $r^2 = 0.61$ ,  $P = 0.03$ ,  $n = 5$ ).

[24] Coefficients of determination between  $\delta^{13}\text{C}_R$ ,  $\delta^{13}\text{C}_{R\text{-soil}}$ , or  $\delta^{13}\text{C}_{R\text{-foliage}}$  and meteorological and physiological variables expected to influence them are shown in Tables 1 and 2. In Table 1, the correlation analyses included regressions of  $\delta^{13}\text{C}$  of respiratory fluxes versus the parameter of interest measured that same day (a zero-day lag) and with single-day averaging. Table 2 shows the results of regressions with the same independent and dependent factors but with lags ranging from zero to 15 days, and with averaging periods of 1 to 5 days. The direction of the relationships (positive versus negative) and significance of the regressions are also noted. Comparison of Tables 1 and 2 shows that, in general, relationships between isotopic signatures of respiration and driving parameters are quite poor when no lag period is accounted for. Only two regression combinations,  $\delta^{13}\text{C}_{R\text{-soil}}$  versus  $\theta$  and  $\delta^{13}\text{C}_{R\text{-foliage}}$  versus PAR, exhibited the highest coefficients of determination for zero-day lags with 1-day averages (Table 1). All other regression combinations exhibited improved statistical fits if the driving parameter from a few days prior to the flask collections was used rather than from the day of the flask collection.

**Table 2.** Results of Lag Analysis of  $\delta^{13}\text{C}_R$ ,  $\delta^{13}\text{C}_{R\text{-soil}}$ , and  $\delta^{13}\text{C}_{R\text{-foliage}}$  Versus Environmental and Physiological Driving Factors<sup>a</sup>

Component	vpd	$T_{\text{air}}$	$T_{\text{min}}$	$T_{\text{soil}}$	PAR	$\theta$	$G_c$	NEE
$\delta^{13}\text{C}_R$	0.10 (+7, 1)	0.11 (+1, 1)	0.17 (+, 7, 1)	0.05 (+, 0, 1)	0.25 (+, 4, 1) <sup>b</sup>	0.06 (+, 6, 2)	0.35 (-, 2, 1) <sup>c</sup>	0.15 (+, 7, 1)
$\delta^{13}\text{C}_{R\text{-soil}}$	0.46 (+, 1, 3) <sup>c</sup>	0.52 (+, 1, 1) <sup>b</sup>	0.24 (+, 1, 1) <sup>b</sup>	0.45 (+, 0, 1) <sup>c</sup>	0.46 (+, 3, 2) <sup>c</sup>	0.39 (-, 0, 1) <sup>c</sup>	0.48 (-, 1, 2) <sup>c</sup>	0.48 (+, 1, 1) <sup>c</sup>
$\delta^{13}\text{C}_{R\text{-foliage}}$	0.30 (+, 3, 1)	0.64 (-, 5, 2) <sup>c</sup>	0.71 (-, 5, 2) <sup>c</sup>	0.22 (+, 1, 1)	0.49 (+, 0, 1) <sup>c</sup>	0.38 (+, 7, 1) <sup>b</sup>	0.30 (-, 4, 1)	0.69 (+, 2, 2) <sup>c</sup>

<sup>a</sup>The coefficient of determination ( $r^2$ ) is presented for each lag/average combination that provided the best fit. The sign of the relationship (plus or minus) is presented in parentheses along with the number of days lagged and number of days averaged. For example, 0.46 (+, 3, 2) is a positive correlation with an  $r^2$  of 0.46 and is a 3-day lag with a 2-day average. A zero-day lag with a 1-day average (0, 1) is identical to saying that a climatic factor yesterday was regressed against last night's isotopic signature.

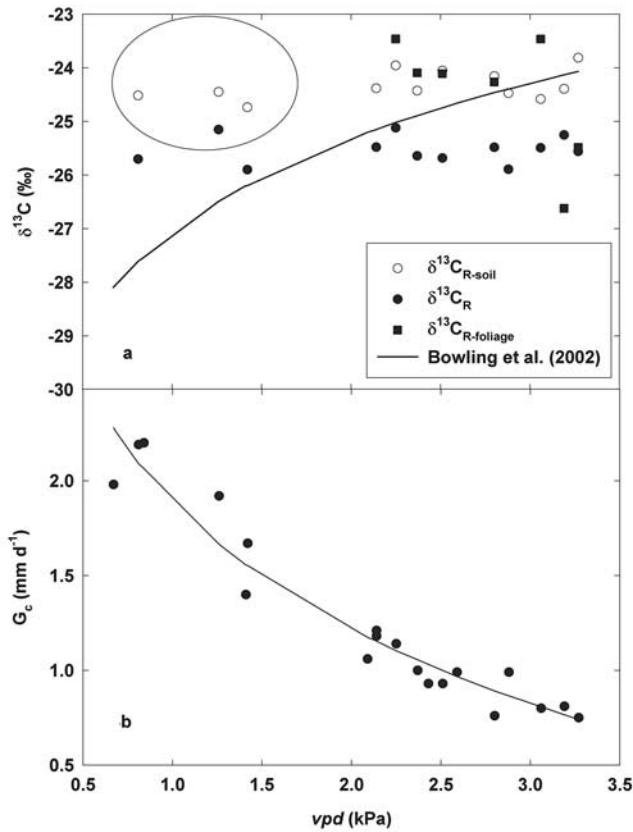
<sup>b</sup>Regression significance  $P = 0.10$ .

<sup>c</sup>Regression significance  $P = 0.05$ .

[25] Even after determining the appropriate lag and averaging periods, relatively poor correlations between  $\delta^{13}\text{C}_R$  and measured driving parameters were observed, with only a significant relationship ( $P = 0.04$ ) observed with  $G_c$ , and a marginally significant relationship ( $P = 0.08$ ) with PAR (Table 2). Observed  $\delta^{13}\text{C}_{R\text{-soil}}$  and  $\delta^{13}\text{C}_{R\text{-foliage}}$  were more strongly correlated with measured driving parameters than  $\delta^{13}\text{C}_R$ . The  $\delta^{13}\text{C}_{R\text{-soil}}$  correlations were generally noisy ( $r^2 \sim 0.24$  to 0.48), but were also relatively significant. The  $\delta^{13}\text{C}_{R\text{-soil}}$  correlations with the tested parameters were all consistent with a stomatal control over  $\Delta$ , including positive relationships with vpd, temperature, and PAR and negative relationships with  $\theta$  and  $G_c$ . Observed  $\delta^{13}\text{C}_{R\text{-foliage}}$  exhibited mixed results; being strongly, negatively correlated with minimum air temperature measured 5 days previously, but positively correlated with PAR measured the same day (Table 2). Shorter time lags were observed for  $\delta^{13}\text{C}_{R\text{-soil}}$  than  $\delta^{13}\text{C}_{R\text{-foliage}}$ . Both  $\delta^{13}\text{C}_{R\text{-soil}}$  and  $\delta^{13}\text{C}_{R\text{-foliage}}$  were positively correlated with NEE, indicating that reduced net  $\text{CO}_2$  uptake was correlated with isotopically enriched respiratory fluxes.

[26] We also assessed if there was a common lag/averaging combination that provided consistently high statistical fits for both  $\delta^{13}\text{C}_R$  and  $\delta^{13}\text{C}_{R\text{-soil}}$ . We expected that these two isotopic signatures should share common lag/averaging periods because belowground respiration is the dominant respiratory flux in this ecosystem [Law and Ryan, 1999]. Both signatures shared relatively high statistical fits with PAR; a 4-day lag, single-day average, and 2-day lag, 2-day average both gave relatively strong fits for both components (data not shown). However, for no other driving parameter did we observe lag/averaging combinations that were shared by  $\delta^{13}\text{C}_{R\text{-soil}}$  and  $\delta^{13}\text{C}_R$ .

[27] The results of our explicit hypothesis test that  $\delta^{13}\text{C}$  of respiratory fluxes is related to short-term variation in vpd is shown in Figure 4. Figure 4a shows the previously observed relationship between vpd and  $\delta^{13}\text{C}_R$  during periods when air temperatures were above  $0.2^\circ\text{C}$  (shown as a solid line) and when air temperatures were below  $0.2^\circ\text{C}$  (shown as the circled area) along with measurements from the present study (the solid line and circled area are from the work of Bowling *et al.* [2002]). Because no significant time lag was observed between  $\delta^{13}\text{C}_R$  and vpd in the present study, the data is plotted with no lag (i.e., vpd from day 180 is plotted versus  $\delta^{13}\text{C}_R$  from day 180). Alternatively, plotting the data with a 5-day lag, as observed by Bowling *et al.* [2002], causes little change in the figure (data not shown). The measured  $\delta^{13}\text{C}_R$  values do fall within the range observed by Bowling *et al.* [2002];



**Figure 4.** (a) Observed  $\delta^{13}\text{C}_R$  (solid circles),  $\delta^{13}\text{C}_{R\text{-soil}}$  (open circles), and  $\delta^{13}\text{C}_{R\text{-foilage}}$  (solid squares) versus vpd. Because there was not a significant relationship between time-lagged vpd and  $\delta^{13}\text{C}_R$ , vpd on the x axis is not lagged. The solid line is the predicted relationship between  $\delta^{13}\text{C}_R$  and vpd in the absence of freezing air temperatures, and the circled area is the prediction for  $\delta^{13}\text{C}_R$  if freezing air temperatures occur [from *Bowling et al.*, 2002]. (b)  $G_c$  versus vpd (no lag). The line is  $G_c = -0.97\ln(\text{vpd}) + 1.89$ ,  $r^2 = 0.94$ ,  $P < 0.01$ .

however, they do not track the previously published vpd response. In contrast,  $G_c$  did exhibit the expected negative relationship with vpd (Figure 4b).

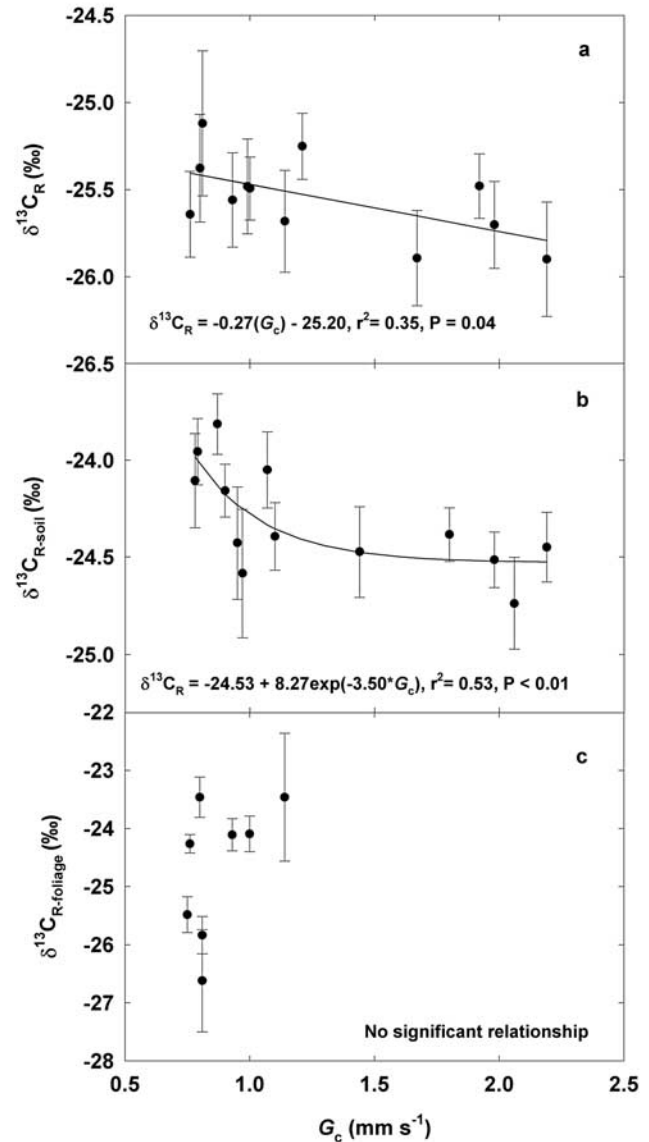
[28] Figure 5 shows the results of our explicit hypothesis test that  $G_c$  influences variation in isotopic content of respiratory fluxes.  $G_c$  is time lagged according to the best fit from Table 2 for  $\delta^{13}\text{C}_R$  (2-day lag, Figure 5a) and  $\delta^{13}\text{C}_{R\text{-soil}}$  (1-day lag, Figure 5b), and with no lag for  $\delta^{13}\text{C}_{R\text{-foilage}}$  since that relationship was not significant ( $P = 0.18$ , Figure 5c).

#### 4. Discussion

[29] The large ranges of vpd and  $G_c$  that occurred over the 2-week experiment (Figure 2) provided an ideal test of the hypothesis that short-term variation in vpd affects  $\delta^{13}\text{C}_R$  via changes in canopy-level stomatal conductance. This test was conducted in order to determine (1) if the vpd- $\delta^{13}\text{C}_R$  relationship observed over 3 years and across a 250-km transect in Oregon [*Bowling et al.*, 2002] would also occur at a single site over a 2-week period, and (2) if canopy-level

stomatal conductance was the mechanism controlling  $\delta^{13}\text{C}_R$ . While the overall results are inconclusive, the data shown in Figure 4a fail to support our first hypothesis. Over a 2-week period during the summer of 2001,  $\delta^{13}\text{C}_R$  showed very little variation (Figures 3 and 4a) despite large changes in meteorological conditions and  $G_c$  (Figures 2 and 4b). The second hypothesis test, that  $G_c$  is related to  $\delta^{13}\text{C}_R$ , was marginally significant (Figure 5).

[30] Our failure to accept our first hypothesis, that  $\delta^{13}\text{C}_R$  is affected by short-term variation in vpd, was due to relatively constant  $\delta^{13}\text{C}_R$  over the 2-week period (Figures 3 and 4a).



**Figure 5.** (a) Observed  $\delta^{13}\text{C}_R$  versus  $G_c$ , where  $G_c$  is lagged by 2 days with a single-day average (Table 2). (b) Observed  $\delta^{13}\text{C}_{R\text{-soil}}$  versus  $G_c$ , where  $G_c$  is lagged by 1 day with a 2-day average (Table 2). A nonlinear fit is shown for reference. (c) The  $\delta^{13}\text{C}_{R\text{-foilage}}$  versus  $G_c$ , in which  $G_c$  is not lagged because no significant relationship was observed (Table 2). Error bars represent standard errors.



Of the theories proposed in section 1 to explain the lack of  $\delta^{13}\text{C}_R$  variation observed in some other studies [i.e., Flanagan *et al.*, 1996; Buchmann *et al.*, 1998], we can exclude (1) limited sampling or (2) a lack of variation in or insensitivity to environmental driving variables. Our sampling was relatively intensive (nightly) over the 2-week period, and variation in environmental driving variables and resulting variation in  $G_c$  and NEE was large (Figures 2 and 4b). At least two alternative mechanisms exist that may contribute to the lack of variation in  $\delta^{13}\text{C}_R$ . From the plant physiological scale, a balancing effect of driving variables on  $c_i/c_a$  (i.e., if both  $G_c$  and assimilation rise in proportion causing constancy of  $c_i/c_a$ ) or from the plant and ecosystem scales, to a decoupling of  $c_i/c_a$  and  $\delta^{13}\text{C}_R$  (Figure 1).

[31] A balancing effect of driving variables that results in no variation in  $c_i/c_a$  could theoretically occur if freezing air temperatures are followed by a period of high vpd. The near freezing air temperature that occurred on day 176 (0.18°C, Figure 2b) could cause stomatal closure resulting in reduced conductance of  $\text{CO}_2$  and hence reduced  $c_i/c_a$  [Kaufmann, 1976; Fahey, 1979; Smith *et al.*, 1984; Kozlowski *et al.*, 1991; Strand *et al.*, 2002] and subsequent enrichment of  $\delta^{13}\text{C}_R$  at low vpd (see circled area in Figure 5a and see work of Bowling *et al.* [2002]). However,  $G_c$  showed no sensitivity to the cold air temperature on day 176 (Figures 2d and 4b), invalidating air temperature effects on  $G_c$  as the mechanism for enrichment of  $\delta^{13}\text{C}_R$ .

[32] Constant  $c_i/c_a$  may also occur if changes in  $G_c$  are mirrored by proportional changes in photosynthesis. We examined this theory by calculating  $c_i/c_a$  using  $G_c$  coupled with canopy photosynthesis data from the same 2-week period. Canopy photosynthesis was calculated using the measured daytime NEE data with respiration subtracted using a nocturnally based relationship between air temperature and respiration (P. Anthoni, unpublished data, 2003). Over the 2-week experiment, calculated  $c_i/c_a$  exhibited a wide range, from  $>0.70$  to  $<0.50 \mu\text{mol mol}^{-1}$ , which is equivalent to  $\delta^{13}\text{C}$  variation of  $<-28.0$  to  $>-24.0\text{‰}$ . This suggests that  $\delta^{13}\text{C}$  of photo-assimilates varied by 4.0‰ or more over the 2-week experiment, and subsequently, equivalent variation in  $\delta^{13}\text{C}_R$  should have occurred if  $\delta^{13}\text{C}_R$  is directly linked to  $\delta^{13}\text{C}$  of photo-assimilate.

[33] A decoupling of  $c_i/c_a$  and  $\delta^{13}\text{C}_R$  could occur via mechanisms operating at both the plant physiological and ecosystem scales. For example,  $\delta^{13}\text{C}$  of photo-assimilates may be modified between assimilation and the time (and location) of respiration, or alternatively, tissue- or location-specific variation in respiratory substrate may have a canceling effect on the net isotopic signature respired at the ecosystem scale. It is well documented that  $c_i/c_a$  is directly related to  $\delta^{13}\text{C}$  of photo-assimilates [Brugnoli *et al.*, 1988; Lauteri *et al.*, 1993; Brugnoli *et al.*, 1998], and furthermore, no fractionation occurs during mitochondrial respiration [Lin and Ehleringer, 1997]. However, shifts in the isotopic composition of assimilates prior to respiration could occur [Duranceau *et al.*, 1999] leading to constant  $\delta^{13}\text{C}_R$ . At the ecosystem-scale, tissue-specific variation in respiratory substrate could occur for multiple reasons, including differential time lags for carbon transport to and respiration from foliage versus roots versus microbial bio-

mass, changes in carbon allocation or sink strength, or a shift of respiration from labile- to recalcitrant carbon pools in the soil [Schönwitz *et al.*, 1986] such as may occur with changes in water content of the litter layer (B. Law, personal communication, 2003). We examined the theory that differences in  $\delta^{13}\text{C}$  respired from aboveground versus belowground sources balanced at the ecosystem level by scaling the component fluxes of  $\delta^{13}\text{C}_R$  using the following equation:

$$\delta^{13}\text{C}_R \cdot R_{\text{ecosystem}} = \delta^{13}\text{C}_{R\text{-foliage}} \cdot R_{\text{-foliage}} + \delta^{13}\text{C}_{R\text{-soil}} \cdot R_{\text{-soil}}, \quad (6)$$

where R stands for the fraction of total ecosystem respiration from the ecosystem (100%), foliage, or soil (the latter two summing to 100%). On the basis of conservation of mass, the sum of the right-hand side of equation (1) equals  $\delta^{13}\text{C}_R \cdot R_{\text{ecosystem}}$ . We used a range of values for  $R_{\text{-foliage}}$  and  $R_{\text{-soil}}$ , ranging from 24 and 76% of  $R_{\text{ecosystem}}$ , respectively [Law and Ryan, 1999] to 50% and 50% of  $R_{\text{ecosystem}}$ , respectively. However, all scaling combinations of the component fluxes predicted  $\delta^{13}\text{C}_R$  substantially more enriched than measured  $\delta^{13}\text{C}_R$  (1.0 to 2.5‰). Therefore, we have little evidence of a decoupling of  $c_i/c_a$  and  $\delta^{13}\text{C}_R$ .

[34] The objective of our study was to examine temporal patterns of the isotopic signatures of  $\text{CO}_2$  fluxes rather than to close an isotopic mass balance. Because of this objective and because of logistical constraints, we limited our foliage sampling to old trees. This exclusion of young trees may be responsible for the lack of mass balance because foliage tissue of young trees typically has more depleted  $\delta^{13}\text{C}$  than old trees [Yoder *et al.*, 1994; McDowell *et al.*, 2002] and presumably more depleted  $\delta^{13}\text{C}_{R\text{-foliage}}$ . Measurement error in  $\delta^{13}\text{C}_{R\text{-soil}}$  may also be responsible.  $\text{CO}_2$  beneath the soil surface is enriched by up to 4.4‰ above soil surface  $\text{CO}_2$  efflux due to fractionation during diffusion through the soil [Cerling *et al.*, 1991; Davidson, 1995]. Perturbation of pressure within the chamber headspace can cause advection of  $\text{CO}_2$  out of the soil [Fang and Moncrieff, 1996; Lund *et al.*, 1999], potentially allowing enriched  $\text{CO}_2$  to advect from the soil without the complete 4.4‰ fractionation. Such enrichment of  $\text{CO}_2$  flux would result in a more positive Keeling plot intercept and hence a more positive estimate of  $\delta^{13}\text{C}_{R\text{-soil}}$ . Future work should address pump artifacts on  $\delta^{13}\text{C}_{R\text{-soil}}$  as well as spatial variability in  $\delta^{13}\text{C}_{R\text{-soil}}$  and  $\delta^{13}\text{C}_{R\text{-foliage}}$ .

[35] Our second hypothesis, that  $G_c$  influences  $\delta^{13}\text{C}$  of respiratory fluxes of carbon, was supported by the measurements of  $\delta^{13}\text{C}_R$  and  $\delta^{13}\text{C}_{R\text{-soil}}$  (Figures 5a and 5b, Table 2). It is surprising that the relationship was even weakly significant between  $\delta^{13}\text{C}_R$  and  $G_c$  (Figure 5a,  $r^2 = 0.35$ ,  $P = 0.04$ ) considering the limited range of  $\delta^{13}\text{C}_R$ . The  $\delta^{13}\text{C}_{R\text{-soil}}$  results support a canopy-level control more strongly, both directly through the relationship with  $G_c$  and indirectly through the relationships with environmental factors known to affect  $G_c$  such as  $\theta$ , PAR, and vpd (Table 2). However,  $T_{\text{soil}}$  was also well-correlated with  $\delta^{13}\text{C}_{R\text{-soil}}$ . This may be causal due to temperature regulation of rates and sources of belowground respiration, or it may be spurious due to the inherent relationship between soil drying and soil temperature. The  $\delta^{13}\text{C}_{R\text{-foliage}}$  correlations provided a less intuitive set of results, with some supporting a temperature influence

(negative relationships with temperature) or supporting a positive effect of PAR (Table 2). To our knowledge, ours are the first isotopic measurements of foliar respiratory fluxes that have been conducted in a field setting; therefore we cannot compare our methodology or results to other studies at this time.

[36] The fact that  $\delta^{13}\text{C}_{\text{R-soil}}$  exhibited relatively strong relationships with driving factors leads us to suggest that belowground carbon fluxes may be critical in regulating  $\delta^{13}\text{C}_{\text{R}}$  variation. *Mortazavi and Chanton* [2002] made a similar conclusion in a study of a slash pine forest in southeastern United States, in which  $\delta^{13}\text{C}_{\text{R-soil}}$  actually acted to buffer  $\delta^{13}\text{C}_{\text{R}}$  from isotopic variation of aboveground respiration. However, the lack of consistency between  $\delta^{13}\text{C}_{\text{R-soil}}$ ,  $\delta^{13}\text{C}_{\text{R-foilage}}$ , and  $\delta^{13}\text{C}_{\text{R}}$ , either real or due to methodological issues, makes strong conclusions about the controls over  $\delta^{13}\text{C}_{\text{R}}$  in this study impossible.

[37] An interesting result is that correlations of  $\delta^{13}\text{C}_{\text{R-soil}}$  with driving parameters have shorter lags than for  $\delta^{13}\text{C}_{\text{R-foilage}}$  or  $\delta^{13}\text{C}_{\text{R}}$  (Table 2). The average lag period for all parameters shown in Table 2 is 1.1 days for  $\delta^{13}\text{C}_{\text{R-soil}}$ , compared to 3.7 and 4.9 for  $\delta^{13}\text{C}_{\text{R-foilage}}$  and  $\delta^{13}\text{C}_{\text{R}}$ . This is somewhat surprising given that foliage is located much closer to the source of assimilation than soil and hence should have a shorter delay between the time of assimilation and respiration if transport distance controls time lags. One possible interpretation is that the short time lags for  $\delta^{13}\text{C}_{\text{R-soil}}$  may be due to a direct response of  $\delta^{13}\text{C}_{\text{R-soil}}$  to changes in conditions at the soil level rather than via changes in canopy gas exchange. This would allow  $\delta^{13}\text{C}_{\text{R-soil}}$  to respond immediately to meteorological changes as long as those forcing factors are transferred into changes in soil conditions. Indeed,  $\delta^{13}\text{C}_{\text{R-soil}}$  was well correlated not only with factors that constrain canopy gas exchange, but it was also correlated with  $T_{\text{soil}}$  (Table 2). This may be spurious in that  $T_{\text{soil}}$  is likely to rise as  $\theta$  and  $G_c$  decline, or it may be causal in that changes in  $T_{\text{soil}}$  could drive changes in the sources and certainly rates of respiration belowground. Unfortunately, comparison of the component fluxes is difficult because  $\delta^{13}\text{C}_{\text{R-foilage}}$  appears to be coupled to different driving factors than  $\delta^{13}\text{C}_{\text{R-soil}}$  and  $\delta^{13}\text{C}_{\text{R}}$  (as shown in Table 2). Averaging periods did not differ substantially for the different components.

[38] The positive relationship between NEE and  $\delta^{13}\text{C}_{\text{R-soil}}$  and  $\delta^{13}\text{C}_{\text{R-foilage}}$  (Table 2) is consistent with the idea that periods of low assimilation (associated with low  $G_c$ ) can cause isotopically enriched carbon isotope ratios of photo-assimilate and subsequent respiratory fluxes [*Randerson et al.*, 2002b]. There are at least two possible causes of these relationships: (1) NEE and  $\delta^{13}\text{C}$  of photo-assimilate are indirectly correlated because both are directly linked to  $G_c$ , or (2) increased rates of ecosystem respiration are associated with isotopic changes in the carbon substrate used for respiration (i.e., a switch to more enriched substrates). There are a myriad of potential mechanisms for the second option including both autotrophic and heterotrophic tissues. However, the first option, that  $G_c$  causes simultaneous shifts in  $\delta^{13}\text{C}$  of photo-assimilate and NEE is supported by the relationships between NEE, the isotopic content of respiratory  $\text{CO}_2$ , and  $G_c$ . Long-term data sets comparing  $\delta^{13}\text{C}_{\text{R}}$ ,

NEE and  $G_c$  will be necessary to further test the relationships and mechanisms controlling rates and signatures of carbon fluxes.

[39] The shorter time lag for the response of NEE to  $G_c$  (zero-day lag) than for either  $\delta^{13}\text{C}_{\text{R}}$  to  $G_c$  (2-day lag, 1-day average, Table 2) or  $\delta^{13}\text{C}_{\text{R-soil}}$  to  $G_c$  (1-day lag, 2-day average, Table 2) suggests that  $\text{CO}_2$  flux rates and isotopic signatures are temporally decoupled. We suspect decoupling of rates and signatures is due to variation in within-ecosystem carbon transport after carbon assimilation. Future work on the relationships between fluxes and isotopic signatures should examine the controls and temporal variation of within-ecosystem carbon transport.

[40] A noteworthy result of this study is that none of the respiratory fluxes, including the foliar fluxes, isotopically matched the  $\delta^{13}\text{C}$  of whole-leaf tissue (Figure 3). Leaf tissue  $\delta^{13}\text{C}$  was 0.5 to 3.0‰ more negative than any of the observed fluxes, indicating an isotopic disequilibrium between stored and respired carbon. Similar results have been observed by *Pate and Arthur* [1998], *Ometto et al.* [2002], and *Pataki et al.* [2003], among others. A potential cause of this discrepancy is that the carbon in leaf tissue is predominately derived from photosynthesis during spring months when the climate is wet and mild and  $c_i/c_a$  is high (resulting in depleted  $\delta^{13}\text{C}$ ). Our  $\delta^{13}\text{C}$ -flux measurements were conducted at least 1 month after the foliar carbon was assimilated, after conditions had become hotter and drier. Future work comparing  $\delta^{13}\text{C}$  of stocks and fluxes would benefit from a time series analysis starting before bud break with repeated measurements throughout the period of leaf elongation.

[41] An important point must be made about the limited variation in  $\delta^{13}\text{C}_{\text{R}}$  observed in this study. While it may be tempting to consider this result as evidence of constancy of  $\delta^{13}\text{C}_{\text{R}}$ , large variation in  $\delta^{13}\text{C}_{\text{R}}$  at this site does occur, as observed between 1997 and 2000 [*Bowling et al.*, 2002] and as observed through weekly Keeling plots in 2001–2002 (N. G. McDowell et al., unpublished data, 2003:  $\sim 8.0\%$  variation annually). At this site, variability in  $\delta^{13}\text{C}_{\text{R}}$  is minimal during the rain-free summer period but is much larger during periods when precipitation is present, in the autumn, winter, and spring. We suspect the summer-period constancy in  $\delta^{13}\text{C}_{\text{R}}$  observed in the current study and in the N. G. McDowell et al. unpublished data is due to groundwater access via deep rooting of the forest trees, which buffers the ecosystem from drought effects. Although we cannot yet conclude exactly what factors regulate  $\delta^{13}\text{C}_{\text{R}}$ , it is clear that periods of constancy as well as variability in  $\delta^{13}\text{C}_{\text{R}}$  occur at this forest.

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## References

- Anthoni, P. M., M. H. Unsworth, B. E. Law, J. Irvine, D. Baldocchi, and D. Moore (2002), Seasonal differences in carbon and water vapor exchange in young and old-growth ponderosa pine ecosystems, *Agric. For. Meteorol.*, *111*, 203–222.

- Battle, M., M. L. Bender, P. P. Tans, J. W. C. White, J. T. Ellis, T. Conway, and R. J. Francey (2000), Global carbon sinks and their variability inferred from atmospheric O<sub>2</sub> and δ<sup>13</sup>C, *Science*, 287(5462), 2467–2470.
- Bowling, D. R., C. S. Cook, and J. R. Ehleringer (2001), Technique to measure CO<sub>2</sub> mixing ratio in small flasks with a bellow/IRGA system, *Agric. For. Meteorol.*, 109, 61–65.
- Bowling, D. R., N. G. McDowell, B. J. Bond, B. E. Law, and J. R. Ehleringer (2002), <sup>13</sup>C content of ecosystem respiration is linked to precipitation and vapor pressure deficit, *Oecologia*, 131, 113–124.
- Bowling, D. R., D. E. Pataki, and J. R. Ehleringer (2003), Critical evaluation of micrometeorological methods for measuring ecosystem-atmosphere isotopic exchange of CO<sub>2</sub>, *Agric. For. Meteorol.*, 116, 159–179.
- Brugnoli, E., K. T. Hubick, S. von Caemmerer, S. Chin Wong, and G. D. Farquhar (1988), Correlation between the carbon isotope discrimination in leaf starch and sugars of C<sub>3</sub> plants and the ration of intercellular and atmospheric partial pressures of carbon dioxide, *Plant Physiol.*, 88, 1418–1424.
- Brugnoli, E., A. Scartazza, M. Lauteri, and M. C. Monteverdi (1998), Carbon isotope discrimination in structural and non-structural carbohydrates in relation to productivity and adaptation to unfavourable conditions, in *Stable Isotopes*, edited by H. Griffiths, pp. 133–146, BIOS Sci., Oxford, UK.
- Buchmann, N., J. R. Brooks, L. B. Flanagan, and J. R. Ehleringer (1998), Carbon isotope discrimination by terrestrial ecosystems, in *Stable Isotopes*, edited by H. Griffiths, pp. 203–222, BIOS Sci., Oxford, UK.
- Canadell, J. G., et al. (2000), Carbon metabolism of the terrestrial biosphere: A multitechnique approach for improved understanding, *Ecosystems*, 3, 115–130.
- Cerling, T. E., D. K. Solomon, J. Quade, and J. R. Bowman (1991), On the isotopic composition of carbon in soil carbon dioxide, *Geochim. Cosmochim. Acta*, 55, 3403–3405.
- Ciais, P., P. P. Tans, M. Trolier, J. W. C. White, and R. J. Francey (1995), A large Northern Hemisphere terrestrial CO<sub>2</sub> sink indicated by the <sup>13</sup>C/<sup>12</sup>C ratio of atmospheric CO<sub>2</sub>, *Science*, 269, 1089–1102.
- Cowan, I. R. (1994), As to the mode of action of guard cells in dry air, in *Ecophysiology of Photosynthesis*, edited by E. D. Schulze and M. M. Caldwell, pp. 205–229, Springer-Verlag, New York.
- Davidson, G. R. (1995), The stable isotopic composition and measurement of carbon in soil CO<sub>2</sub>, *Geochim. Cosmochim. Acta*, 59, 2485–2489.
- Duranceau, M., J. Ghashghaie, F. Badeck, E. Deleens, and G. Cornic (1999), δ<sup>13</sup>C of CO<sub>2</sub> respired in the dark in relation to δ<sup>13</sup>C of leaf carbohydrates in *Phaseolus vulgaris* L. under progressive drought, *Plant Cell Environ.*, 22, 515–523.
- Ehleringer, J. R., and C. S. Cook (1998), Carbon and oxygen isotope ratios of ecosystem respiration along an Oregon conifer transect: Preliminary observations based on small-flask sampling, *Tree Physiol.*, 18, 513–519.
- Ehleringer, J. R., A. E. Hall, and G. D. Farquhar (1993), *Stable Isotopes and Plant Carbon-Water Relations*, Academic, San Diego, Calif.
- Ekblad, A., and P. Höglberg (2001), Natural abundance of <sup>13</sup>C in CO<sub>2</sub> respired from forest soils reveals speed of link between tree photosynthesis and root respiration, *Oecologia*, 127, 305–308.
- Fahey, T. J. (1979), The effect of night fronts on the transpiration of *Pinus contorta* ssp. *latifolia*, *Oecol. Plant.*, 14, 483–490.
- Fang, C., and J. B. Moncrieff (1996), An improved dynamic chamber technique for measuring CO<sub>2</sub> efflux from the surface of soil, *Funct. Ecol.*, 10, 297–305.
- Farquhar, G. D., J. R. Ehleringer, and K. T. Hubick (1989), Carbon isotope discrimination and photosynthesis, *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 40, 503–537.
- Flanagan, L. B., R. J. Brooks, G. T. Varney, S. C. Berry, and J. R. Ehleringer (1996), Carbon isotope discrimination during photosynthesis and the isotope ratio of respired CO<sub>2</sub> in boreal forest ecosystems, *Global Biogeochem. Cycles*, 10(4), 629–640.
- Fung, I., et al. (1997), Carbon 13 exchanges between the atmosphere and biosphere, *Global Biogeochem. Cycles*, 11(4), 507–533.
- Hinckley, T. M., and J. H. Braatne (1994), Stomata, in *Plant-Environment Interactions*, edited by R. E. Wilkinson, pp. 323–355, Marcel Dekker, New York.
- Höglberg, P., A. Nordgren, N. Buchmann, A. F. S. Taylor, A. Ekblad, M. N. Höglberg, G. Nyberg, M. Ottosson-Löfvenius, and D. J. Read (2001), Large-scale forest girdling shows that current photosynthesis drives soil respiration, *Nature*, 411, 789–792.
- Irvine, J., B. E. Law, and P. M. Anthoni (2002), Water limitations to carbon exchange in old-growth and young ponderosa pine stands, *Tree Physiol.*, 22, 189–196.
- Jarvis, P. G., and K. G. McNaughton (1986), Stomatal control of transpiration: Scaling up from leaf to region, *Adv. Ecol. Res.*, 15, 1–49.
- Kaufmann, M. R. (1976), Stomatal responses of Engelmann spruce to humidity, light, and water stress, *Plant Physiol.*, 57, 898–901.
- Keeling, C. D. (1958), The concentration and isotopic abundances of atmospheric carbon dioxide in rural areas, *Geochim. Cosmochim. Acta*, 13, 322–334.
- Kozlowski, T. T., P. J. Kramer, and S. G. Pallardy (1991), *The Physiological Ecology of Woody Plants*, 657 pp., Academic, San Diego, Calif.
- Lauteri, M., E. Brugnoli, and L. Spaccino (1993), Carbon isotope discrimination in leaf soluble sugars and in whole-plant dry matter in *Helianthus annuus* L. grown under different water conditions, in *Stable Isotopes and Plant Carbon-Water Relations*, edited by J. R. Ehleringer, A. E. Hall, and G. D. Farquhar, pp. 93–108, Academic, San Diego, Calif.
- Law, B. E., and D. D. Baldocchi (1999), Below-canopy and soil CO<sub>2</sub> fluxes in a ponderosa pine forest, *Agric. For. Meteorol.*, 94, 171–188.
- Law, B. E., and M. G. Ryan (1999), Seasonal and annual respiration of a ponderosa pine ecosystem, *Global Change Biol.*, 5, 169–182.
- Law, B. E., P. Thornton, J. Irvine, and S. Van Tuyl, P. Anthoni (2001), Carbon storage and fluxes in ponderosa pine forests at different developmental stages, *Global Change Biol.*, 7, 755–777.
- Lin, G., and J. R. Ehleringer (1997), Carbon isotopic fractionation does not occur during dark respiration in C<sub>3</sub> and C<sub>4</sub> plants, *Plant Physiol.*, 114, 391–394.
- Lund, C. P., W. J. Riley, L. L. Pierce, and C. B. Field (1999), The effects of chamber pressurization on soil-surface CO<sub>2</sub> flux and the implications for NEE measurements under elevated CO<sub>2</sub>, *Global Change Biol.*, 5, 269–281.
- McDowell, N. G., N. Phillips, C. K. Lunch, B. J. Bond, and M. G. Ryan (2002), Hydraulic limitation and compensation in large, old Douglas-fir trees, *Tree Physiol.*, 22, 763–774.
- Meinzer, F. C., G. Goldstein, and D. A. Grantz (1993), Carbon isotope discrimination and gas exchange in coffee during adjustment to different soil moisture regimes, in *Stable Isotopes and Plant Carbon-Water Relations*, edited by J. R. Ehleringer, A. E. Hall, and G. D. Farquhar, pp. 327–348, Academic, San Diego, Calif.
- Montieth, J. L. (1995), A reinterpretation of stomatal responses to humidity, *Plant Cell Environ.*, 18, 357–364.
- Mortazavi, B., and J. P. Chanton (2002), Carbon isotopic discrimination and control of nighttime canopy δ<sup>18</sup>O-CO<sub>2</sub> in a pine forest in the southeastern United States, *Global Biogeochem. Cycles*, 16(1), 1008, doi:10.1029/2000GB001390.
- Ometto, J. P. H. B., L. B. Flanagan, L. A. Martinelli, M. Z. Moreira, N. Higuchi, and J. R. Ehleringer (2002), Carbon isotope discrimination in forest and pasture ecosystems of the Amazon Basin, Brazil, *Global Biogeochem. Cycles*, 16(4), 1109, doi:10.1029/2001GB001462.
- Oren, R., J. S. Sperry, G. G. Katul, D. E. Pataki, B. E. Ewers, N. Phillips, and K. V. R. Schafer (1999), Survey and synthesis of intra- and inter-specific variation in stomatal sensitivity to vapour pressure deficit, *Plant Cell Environ.*, 22, 1515–1526.
- Pataki, D. E., J. R. Ehleringer, L. B. Flanagan, D. Yakir, D. R. Bowling, C. J. Still, N. Buchmann, J. O. Kaplan, and J. A. Berry (2003), The application and interpretation of Keeling plots in terrestrial carbon cycle research, *Global Biogeochem. Cycles*, 17(1), 1022, doi:10.1029/2001GB001850.
- Pate, J., and D. Arthur (1998), δ<sup>13</sup>C analysis of phloem sap carbon: Novel means of evaluating seasonal water stress and interpreting carbon isotope signatures of foliage and trunk wood of *Eucalyptus globules*, *Oecologia*, 117, 301–311.
- Paul, E. A., and F. E. Clark (1989), *Soil Microbiology and Biochemistry*, 275 pp., Academic, San Diego, Calif.
- Randerson, J. T., et al. (2002a), Carbon isotope discrimination of arctic and boreal biomes inferred from remote atmospheric measurements and a biosphere-atmosphere model, *Global Biogeochem. Cycles*, 16(3), 1028, doi:10.1029/2001GB001435.
- Randerson, J. T., G. J. Collatz, J. E. Fessenden, A. D. Munoz, C. J. Still, J. A. Berry, I. Y. Fung, N. Suits, and A. S. Denning (2002b), A possible global covariance between terrestrial gross primary production and <sup>13</sup>C discrimination: Consequences for the atmospheric <sup>13</sup>C budget and its response to ENSO, *Global Biogeochem. Cycles*, 16(4), 1136, doi:10.1029/2001GB001845.
- Schönwitz, R., W. Stichler, and H. Ziegler (1986), δ<sup>13</sup>C values of CO<sub>2</sub> from soil respiration on sites with crops of C<sub>3</sub> and C<sub>4</sub> type of photosynthesis, *Oecologia*, 69, 305–308.
- Smith, W. K., D. R. Young, G. A. Carter, J. L. Hadley, and G. M. McNaughton (1984), Autumn stomatal closure in six conifer species of the Central Rocky Mountains, *Oecologia*, 63, 237–242.
- Sokal, R. R., and F. J. Rohlf (1995), *Biostatistics*, W. H. Freeman, New York.
- Strand, M., T. Lundmark, I. Söderbergh, and P. Mellander (2002), Impacts of seasonal air and soil temperatures on photosynthesis in Scots pine trees, *Tree Physiol.*, 22, 839–847.

Yoder, B. J., M. G. Ryan, R. H. Waring, A. W. Schoettle, and M. R. Kaufmann (1994), Evidence of reduced photosynthetic rates in old trees, *For. Sci.*, 40(3), 513–527.

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