Oecologia (2002) 131:113–124
DOI 10.1007/s00442-001-0851-y

ECOSYSTEMS ECOLOGY

David R. Bowling · Nate G. McDowell
Barbara J. Bond · Beverly E. Law
James R. Ehleringer

^{13}C content of ecosystem respiration is linked to precipitation and vapor pressure deficit

Received: 19 April 2001 / Accepted: 23 October 2001 / Published online: 11 December 2001
© Springer-Verlag 2001

Abstract Variation in the carbon isotopic composition of ecosystem respiration ($\delta^{13}C_R$) was studied for 3 years along a precipitation gradient in western Oregon, USA, using the Keeling plot approach. Study sites included six coniferous forests, dominated by *Picea sitchensis*, *Tsuga heterophylla*, *Pseudotsuga menziesii*, *Pinus ponderosa*, and *Juniperus occidentalis*, and ranged in location from the Pacific coast to the eastern side of the Cascade Mountains (a 250-km transect). Mean annual precipitation across these sites ranged from 227 to 2,760 mm. Overall $\delta^{13}C_R$ varied from –23.1 to –33.1‰, and within a single forest, it varied in magnitude by 3.5–8.5‰. Mean annual $\delta^{13}C_R$ differed significantly in the forests and was strongly correlated with mean annual precipitation. The carbon isotope ratio of carbon stocks (leaves, fine roots, litter, and soil organic matter) varied similarly with mean precipitation (more positive at the drier sites). There was a strong link between $\delta^{13}C_R$ and the vapor saturation deficit of air (*vpd*) 5–10 days earlier, both across and within sites. This relationship is consistent with stomatal regulation of gas exchange and associated changes in photosynthetic carbon isotope discrimination. Recent freeze events caused significant deviation from the $\delta^{13}C_R$ versus *vpd* relationship, resulting in higher than expected $\delta^{13}C_R$ values.

Keywords Coniferous forest · Isotope · Oregon transect · OTTER · Precipitation transect

D.R. Bowling (✉) · J.R. Ehleringer
Stable Isotope Ratio Facility for Environmental Research, Department of Biology, University of Utah, 257 South 1400 East, Salt Lake City, UT 84112-0820, USA
e-mail: bowling@biology.utah.edu
Tel.: +1-801-5818917, Fax: +1-801-5814665

N.G. McDowell · B.J. Bond · B.E. Law
Department of Forest Science, Oregon State University, Corvallis, OR 97331, USA

Introduction

Two important parameters in the mass balance equations (e.g., Tans et al. 1993; Fung et al. 1997) that are used to assess the magnitude of the terrestrial carbon sink are photosynthetic discrimination (Δ) and the carbon isotopic composition of ecosystem respiration ($\delta^{13}C_R$). These are generally averaged or modeled over some temporal and spatial scale that corresponds to observations. (Δ and $\delta^{13}C_R$ correspond to ε_{lph} and δ_{lb}, respectively,in Tans et al. 1993, and to Δ and δ_b, respectively,in Fung et al. 1997). The extent to which Δ and $\delta^{13}C_R$ vary could potentially alter conclusions about the timing and nature of the terrestrial carbon sink (J.T. Randerson, personal communication). At present, we only marginally understand the magnitude of spatial and temporal variation in $\delta^{13}C_R$, and we know very little about how environmental factors might influence ecosystem-level Δ and $\delta^{13}C_R$ and the extent to which these parameters might be linked.

Many studies in the last decade have examined the carbon isotopic composition of CO_2 respired by terrestrial ecosystems using the two-component gas-mixing model introduced by Keeling (1958; see studies listed in Buchmann et al. 1998). Keeling's theory predicts that the integrated carbon isotope ratio of CO_2 produced by all respiring components of an ecosystem can be determined as the intercept of a regression of $\delta^{13}C$ versus $1/[CO_2]$ ($[CO_2]$ denotes mole fraction of CO_2), where both quantities are measured on air collected in the ecosystem at night. Nocturnal sampling avoids the possible complications of photosynthesis on the respiration signal.

Modeling studies indicate that large-scale isotope discrimination by photosynthesis in the terrestrial biosphere can vary dramatically (Lloyd and Farquhar 1994; Fung et al. 1997). Large variation in measured $\delta^{13}C_R$ has been reported across biomes, usually representing only a single point in time. While the difference between C_3- and C_4-dominated biomes provides the largest observed variation in $\delta^{13}C_R$ (Still 2000), there can be substantial (more than 10‰) variation in $\delta^{13}C_R$ within pure C_3 ecosystems (e.g., Buchmann et al. 1997b).

Relatively few studies have addressed the variability in $\delta^{13}C_R$ over time. Flanagan et al. (1996, 1999) examined changes in $\delta^{13}C_R$ in coniferous (*Picea*, *Pinus*) and deciduous (*Populus*) forests in Canada, and found little variation within a season except at their southernmost *Populus* site. Buchmann et al. (1997a) reported no difference in the isotopic signature of ecosystem respiration in a tropical forest in French Guiana in wet versus dry seasons. In contrast, Buchmann et al. (1997b) found marked variation in $\delta^{13}C_R$ in several Utah (USA) forests, including boxelder (*Acer*), aspen (*Populus*), and pine (*Pinus*). Because environmental stresses such as drought or cold alter CO_2 assimilation rate, stomatal conductance, and photosynthetic discrimination in predictable ways (Farquhar et al. 1989), there could be important changes in Δ in terrestrial ecosystems that are reflected in $\delta^{13}C_R$ when the assimilated carbon is respired. Recent studies have shown a direct and rather rapid link between photosynthetic assimilation, soil respiration rate, and ^{13}C in respired CO_2 (Ekblad and Högberg 2001; Högberg et al. 2001).

The aim of this study was to investigate the factors influencing variability in the isotopic composition of ecosystem respiration ($\delta^{13}C_R$). The degree to which $\delta^{13}C_R$ might vary at forested sites with widely differing water availability was examined at several sites across a precipitation gradient in western Oregon, USA, where precipitation varies quite strongly both spatially and seasonally (Peterson and Waring 1994).

We hypothesized that sites with higher precipitation would have more negative carbon isotope ratios in plant and soil organic matter, and that respired CO_2 signatures ($\delta^{13}C_R$) would therefore follow the same pattern. As water availability decreases, physiological adjustments by plants should change in response to soil and plant water status, affecting hydraulic and stomatal conductance, and thus photosynthetic discrimination (Ehleringer 1994; Ehleringer and Cerling 1995). Presumably, some portion of the CO_2 respired by an ecosystem must be composed of recently fixed carbon. Since a significant proportion of total ecosystem respiration may originate through heterotrophic decomposition of recalcitrant soil organic matter, the degree to which changes in Δ might be reflected in $\delta^{13}C_R$ is less clear. This study was designed to illuminate these issues.

Materials and methods

Study sites

This research was conducted along the Oregon transect (Peterson and Waring 1994), which is located in western Oregon, USA (Fig. 1). The transect is among the largest precipitation gradients in the world, with mean annual precipitation varying by nearly 3,000 mm within 250 km. This region is characterized by wet winters and dry summers. It is dominated by long-lived conifers (Waring and Franklin 1979), and vegetation patterns are linked to precipitation patterns (Fig. 1). Six coniferous forests were selected (Table 1); dominant species in the forests included *Picea sitchen-*

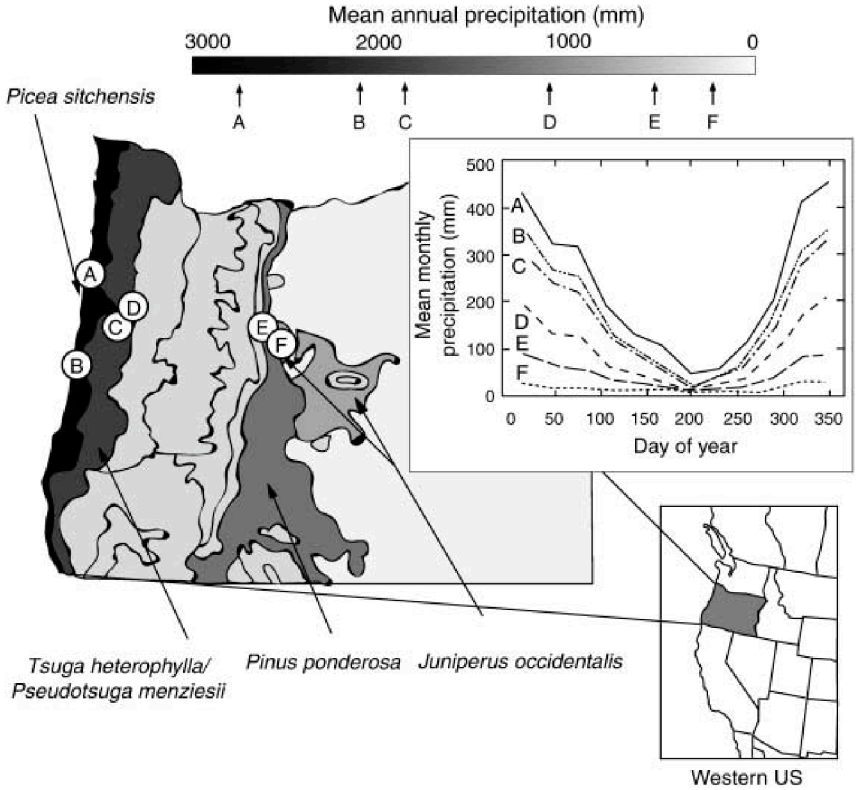

Fig. 1 Location of study sites shown on a map of major vegetation zones in western Oregon. *Arrows* at the top indicate the mean annual precipitation at each study site. The inset figure shows the strong seasonal pattern in mean monthly precipitation at each site. Adapted from Franklin and Dyrness (1988), and Taylor and Hannan (1999)

Table 1 Relevant site details, with sites arranged in order from wettest site to driest. Sites and site codes are unique to this project and do not correspond to sites on the original Oregon transect, except for A (Cascade Head, originally site 1O). Climate station names are consistent with Taylor and Hannan (1999) (*vpd* vapor saturation deficit of air)

Site code	Dominant species	Location and elevation	Approximate stand age (years)	Canopy height (m)	PRISM modeled 30-year mean precipitation (mm)	Nearest distance station with measured 30-year mean precipitation (mm) and from site	Nearest *vpd* station (km)	Years sampled	Site reference[a]
A	*Picea sitchensis*, *Tsuga hetorophylla*	45°03′ N, 123°57′ W, 240 m	150	50	2,760	2,470 (Otis 2 NE, 4 km)	None	1996, 1997	Gholz (1982), Harcombe et al. (1990), Peterson and Waring (1994)
B	*P. sitchensis*, *T. hetorophylla*	44°07′ N, 124°07′ W, 300 m	30	11	2,129	1,930 (Honeyman State Park, 13 km)	None	2000	None
C	*Pseudotsuga menziesii*	44°35′ N, 123°35′ W, 290 m	15	8	1,892	1,680 (Corvallis Water Bureau, 10 km)	Corvallis (18)	2000	None
D	*P. menziesii*	44°36′ N, 123°16′ W, 310 m	30	23	1,140	1,084 (Corvallis, 9 km)	Corvallis (9)	1996, 1997	Bond and Kavanaugh (1999)
E	*Pinus ponderosa*	44°30′ N, 121°37′ W, 941 m	45/250[b]	9/33[b]	523	602 (on site[c])	On site	1996, 1997, 2000	Anthoni et al. (1999), Law et al. (1999a, 1999b)
F	*Juniperus occidentalis*	44°18′ N, 121°20′ W, 930 m	75/230[b]	4/10[b]	227	217 (Redmond, 12 km)	Redmond (12)	1996, 1997, 2000	Gholz (1982), Miller et al. (1992)

[a] Site E is an AmeriFlux long-term CO_2 flux study site (Metolius Research Natural Area), and site F is near (but not identical to) the sites used by Gholz (1982) and Miller et al. (1992)

[b] Sites E and F have two age and height classes

[c] Measured precipitation at site E is the mean of 1996–2000

sis and *Tsuga heterophylla* (mixed Sitka spruce and western hemlock, two sites), *Pseudotsuga menziesii* (Douglas fir, two sites), *Pinus ponderosa* (ponderosa pine, one site), and *Juniperus occidentalis* (western juniper, one site). The sites differed in age and canopy structure, but only minimally in elevation. Two original sites (A and D) were inaccessible in 2000, and were replaced with nearby conspecific stands (B and C). We anticipated a strong environmental gradient effect on $\delta^{13}C_R$, and preliminary results at site A did not follow our expectations (Ehleringer and Cook 1998). Previous studies have shown that this particular stand is characterized by unusually low productivity (Runyon et al. 1994). We hypothesized that stand age might be important in determining $\delta^{13}C_R$, and therefore selected a younger stand to replace site A in 2000 (Table 1). The *P. ponderosa* site (E) is a long-term CO_2 flux-monitoring site (Metolius Research Natural Area) in the AmeriFlux network and has been studied extensively (Anthoni et al. 1999; Law et al. 1999a, 1999b, 2000). Further site details are available in Table 1 and references therein.

Measurements were conducted during 1996, 1997, and 2000. Annual precipitation during these years was 151, 95, and 86% of normal, averaged across all our sites.

Air sampling

Air samples were collected at night from a variety of heights within the canopy, depending on the forest. In total, 1,068 air samples were collected during 45 different time periods. Air samples were chemically dried with magnesium perchlorate during collection and saved in 100-ml glass flasks with Teflon stopcocks (34-5671; Kontes Glass Co., Vineland, N.J.). CO_2 mole fraction was measured in the field using a portable photosynthesis system (LI-6200; LI-COR, Inc., Lincoln, Neb.) during all sampling periods, and additionally in the laboratory using the method of Bowling et al. (2001a) during 2000. Based on the comparison of the two methods during 2000, we estimate the accuracy of the field measurements at 1.0 ppm, and of the laboratory measurements (year 2000 samples), 0.3 ppm.

Carbon isotope ratios of CO_2 in the flasks were measured 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 $\delta^{13}C$ was determined daily by comparison to known standards and was typically ±0.1‰. Corrections for the presence of ^{17}O were applied, and CO_2 was separated from N_2O by gas chromatography before analysis. We report all carbon isotope ratio values in this paper relative to the international PDB standard.

$\delta^{13}C_R$ was evaluated using the Keeling plot approach (Keeling 1958). We used geometric mean (GM) regressions on nocturnal data only, and report our uncertainties as the standard error of the intercept (Sokal and Rohlf 1995). Outliers were selected and removed (if necessary) on each Keeling plot based on a modification of the method of Lancaster (1990). This consisted of (1) performing a GM regression with all data points on the plot, (2) removing

any points where the absolute value of the residual was greater than 2 standard deviations (SDs) of all the absolute residuals, (3) recalculating the GM regression with the remaining points, and (4) repeating steps 2 and 3 until all residuals were within 2 SD. This resulted in the removal of 64 individual air samples from analysis (6.0% of the total).

Organic samples

The carbon isotopic composition of leaves, fine roots, litter, and bulk soil organic matter was monitored during 2000 at sites B, C, E, and F. Sun and shade needles were collected during each site visit in 2000 (five time periods, three replicates each). In January 2000, three soil pits were excavated at each site, and samples collected in 5-cm depth increments to a maximum depth of 25 cm. Litter was separated into fresh and old (largely decomposed but still recognizable as needles) categories. All organic samples were dried at 60°C to constant mass. Roots were removed from the soil samples and fine roots (<2 mm diameter) saved. Root-free soil samples were acid-washed (0.5 N HCl) to remove carbonates; roots were not treated with acid. Leaves, litter, roots, and bulk soil were ground to No. 20 mesh and 2- to 20-mg samples were combusted and analyzed for $\delta^{13}C$ on an IRMS (deltaS; Finnigan MAT). Measurement precision for the organic samples was 0.2‰, and data are presented as means and standard errors (SEs).

Weather and climate data

Weather and climate data at each site were provided by the Oregon Climate Service (OCS) at Oregon State University (http://www.ocs.orst.edu/). Site E is fully instrumented and on-site data were used whenever possible (details in Anthoni et al. 1999). We selected existing OCS climate stations as near to the field sites as possible (Table 1), but the climate stations and field sites in many cases differed in altitude. To better estimate precipitation at each site, we used the PRISM model (Daly et al. 1994, 1997), which interpolates between climate stations in mountainous terrain on a 0.040° grid. A comparison of PRISM estimates of 30-year mean annual precipitation with 30-year mean measurements at the nearest climate stations is shown in Table 1. We report our results relative to the 30-year mean PRISM estimates for each site. Air temperature and relative humidity data were also supplied by OCS and used to calculate the water vapor saturation deficit of air (which we refer to in this paper as vapor pressure deficit, or *vpd*). Not all OCS climate stations monitor these parameters, so in some cases, the distance to the *vpd* stations was larger than the distance to the precipitation stations (Table 1). No stations could be identified within the existing OCS network that provided representative air temperature and *vpd* data for our coastal sites (A and B), and thus we were forced to omit the coastal sites from some analyses. For some analyses, we used daytime mean values for *vpd*, and nocturnal mean or minimum values for air temperature. For these purposes, daytime was defined by the hours representing 90% of the half daylength on either side of solar noon (Campbell and Norman 1998), and night hours were defined as midnight to 4:00 a.m.

Ancillary environmental data

Leaf predawn water potential was measured at sites B, C, E, and F during 2000 using a pressure chamber (PMS, Corvallis, Ore.). Soil water potential was estimated by correcting leaf water potentials for sampling height (0.01 MPa m^{-1}). Between four and ten replicates were measured at each site during each of five sampling periods throughout the year. Soil water content was monitored continuously at the *P. ponderosa* site (E) (details in Anthoni et al. 1999), and gravimetric soil water content in soil cores (5 cm diameter, 0–20 cm depth) was determined during two visits at each of the year 2000 sites.

Correlations between $\delta^{13}C_R$ and environmental variables

We investigated the possibility of a correlation between environmental variables and the carbon isotopic composition of respired CO_2. The environmental variables included factors expected to influence photosynthetic discrimination over a season or between years (precipitation, soil moisture, predawn leaf water potential), and other factors that might influence $\delta^{13}C_R$ more rapidly, such as air temperature or *vpd* (the latter possibly through changes in photosynthetic discrimination).

Since there is likely to be a delay between the time that carbon is fixed photosynthetically and the time it is respired by various ecosystem components, we examined correlations between $\delta^{13}C_R$ and *vpd* by examining *vpd* over varying time periods. We (1) calculated averages of daytime *vpd* for time periods varying between 1 and 5 days, and (2) shifted these averages back in time by 0–20 days (a subset of these data are reported here). In this fashion, we could examine how meteorological events lasting for 1, 2, or more days might affect $\delta^{13}C_R$ respired at some future time period (a lagged response). If on average *n* days were required for recently fixed carbon to serve as a substrate for respiration, then there should be a peak in the correlation coefficient between $\delta^{13}C_R$ and *vpd n* days earlier, provided *vpd* events affect photosynthetic discrimination. For example, a 2-day average and a 4-day shift associated with air sampling performed on Friday night would correspond to the average daytime *vpd* on the preceding Sunday and Monday. A 1-day average and a 0-day shift would correspond to the average daytime *vpd* on the day prior to the night of sampling. Based on initial analyses, periods that included recent freeze events (defined here as air temperatures <0.2°C within the last 7 days) were excluded from the *vpd* regression analysis.

Conductance calculations

To test whether the observed variation in $\delta^{13}C_R$ was consistent with stomatally induced changes in photosynthetic discrimination, we evaluated whether realistic canopy stomatal conductance values could be obtained from $\delta^{13}C_R$. First, we assumed $\delta^{13}C$ of the ambient CO_2 in each forest was –8‰. This assumption may fail in closed canopies (sites A–D in our case) as substantial vertical gradients in $\delta^{13}C$ exist at some times of the day (Buchmann et al. 1997b). However, the majority of photosynthesis occurs in the well-lit upper portions of the canopy, and flux-weighted averages of $\delta^{13}C$ near the canopy top can be quite close to –8‰ (Bowling et al. 2001b). Next, we used $c_i/c_a=(\delta^{13}C_R-(-8)+a)/(b-a)$ (Farquhar et al. 1982) to estimate an integrated c_i/c_a for the forest, where a=4.4‰ and b=27‰ represent the standard fractionations associated with C_3 photosynthesis, and c_i/c_a represents the ratio of intercellular to ambient CO_2. Assuming c_a=360 μmol mol^{-1}, we obtained c_i. Then, using A-c_i curves from the literature (*J. osteosperma*: Ehleringer et al. 1986) or from our own unpublished studies (*P. menziesii*, *P. ponderosa*), we estimated total conductance (stomatal and leaf boundary-layer) to CO_2 using the graphical approach of Farquhar and Sharkey (1982).

Results

Carbon isotopic composition of respired CO_2

Considerable variability was observed in $\delta^{13}C_R$ within and across sites (Fig. 2). Overall, $\delta^{13}C_R$ varied from –23.1 (*J. occidentalis*) to –33.1‰ (*P. menziesii*, site D), and within a single forest varied as little as 3.5‰ (*P. ponderosa*) to as much as 8.5‰ (*P. menziesii*, site D). There was a correlation (r^2=0.52, P=0.11, n=6) between the mean of all Keeling plot intercepts within a particular forest (mean $\delta^{13}C_R$) and the mean annual precipitation at that site

Table 2 Results of linear regressions of $\delta^{13}C_R$ versus (1) precipitation during the month preceding sampling, (2) mean nocturnal (midnight–4:00 a.m.) air temperature, (3) leaf predawn water potential, and (4) soil water content. Asterisks indicate levels of significance of the regression coefficient (*P<0.05, **P<0.01, ***P<0.001, Student's t-distribution). A *dash* indicates data not available

Site	Precipitation previous month		Nocturnal air temperature		Leaf predawn water potential		Soil water content	
	r^2	n	r^2	n	r^2	n	r^2	n
All sites	0.256***	45	0.014	31	0.364**	18	0.313*	17
Picea-Tsuga (A)	0.113	7	–	–	–	–	–	–
Picea-Tsuga (B)	0.295	5	–	–	0.839	4	–	–
P. menziesii (C)	0.954**	5	0.618	5	0.664	5	–	–
P. menziesii (D)	0.001	6	0.133	5	–	–	–	–
P. ponderosa (E)	0.001	11	0.001	11	0.015	5	0.067	11
J. occidentalis (F)	0.006	11	0.004	10	0.007	4	–	–

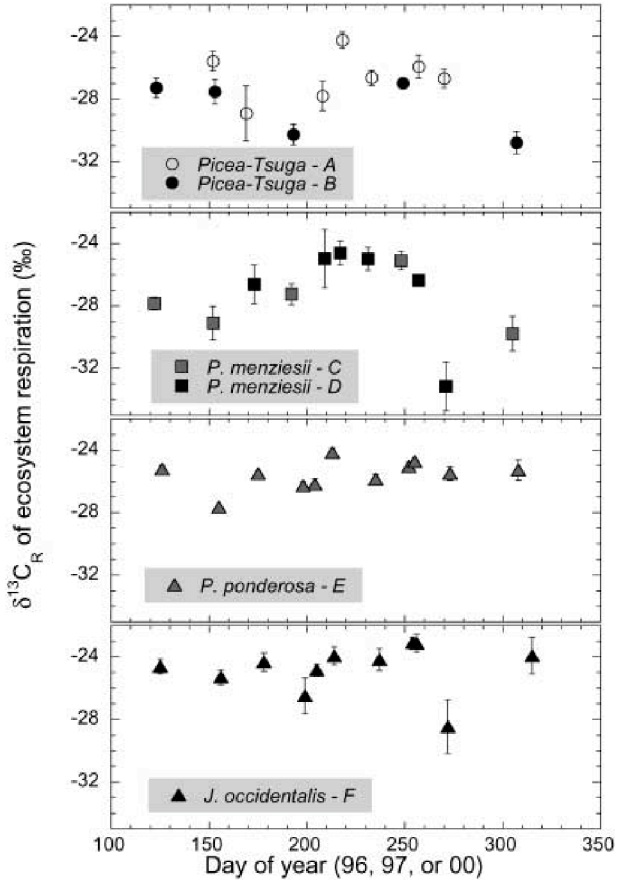

Fig. 2 Carbon isotopic composition of ecosystem respiration (Keeling plot intercept) versus day of year, from six forests in three different years (1996, 1997, and 2000). Sites are arranged top to bottom in order of decreasing annual precipitation

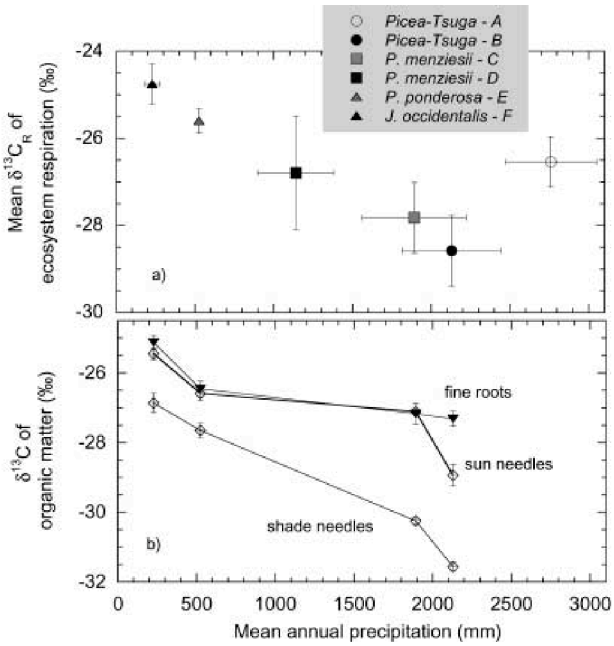

Fig. 3 **a** $\delta^{13}C_R$ of ecosystem respiration (means±1 SE) versus mean annual precipitation (means±1 SD) for the six forests. **b** $\delta^{13}C$ (means±1 SE, n=15) of sun leaves, shade leaves, and fine roots, collected at the year 2000 study sites. *Error bars* are smaller than the symbols in some cases

(Fig. 3a), regardless of whether the PRISM estimates or nearby OCS station precipitation measurements were used (Table 1). This correlation was considerably stronger and highly significant when the wettest site (*Picea-Tsuga*, site A) was excluded (r^2=0.99, P<0.01, n=5). In general, $\delta^{13}C_R$ was more negative at the wettest sites. Although the sites do differ somewhat in altitude (Tab0le 1), the range in altitude is likely not great enough to cause major changes in discrimination (Marshall and Zhang 1994).

Correlations with other environmental and biological variables likely to influence plant water relations were generally weak (Table 2). Significant correlations were observed between $\delta^{13}C_R$ and (1) the precipitation in the month preceding air sampling, (2) leaf predawn water potential on the night of sampling, and (3) concurrent soil water content, but only when all sites were considered together. Considering data within a single site only, one site (*P. menziesii*, site C) showed a significant correlation with precipitation of the preceding month. All other correlations were non-significant (Table 2).

Leaf, litter, and soil organic matter

The pattern of wetter sites having more negative isotope ratios was also observed in leaf tissue and in fine roots

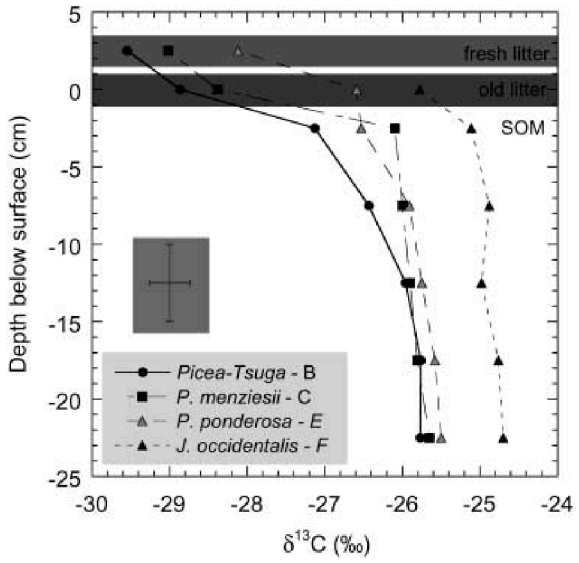

Fig. 4 Mean $\delta^{13}C$ of forest litter and bulk soil organic matter (*SOM*) profiles, in 5-cm depth increments (0–5, 5–10 cm, etc.), for the year 2000 study sites. A typical *error bar* (5-cm depth range, and $\delta^{13}C$ values±1 SE) is shown in the *box*. There was no fresh litter at site F during sampling

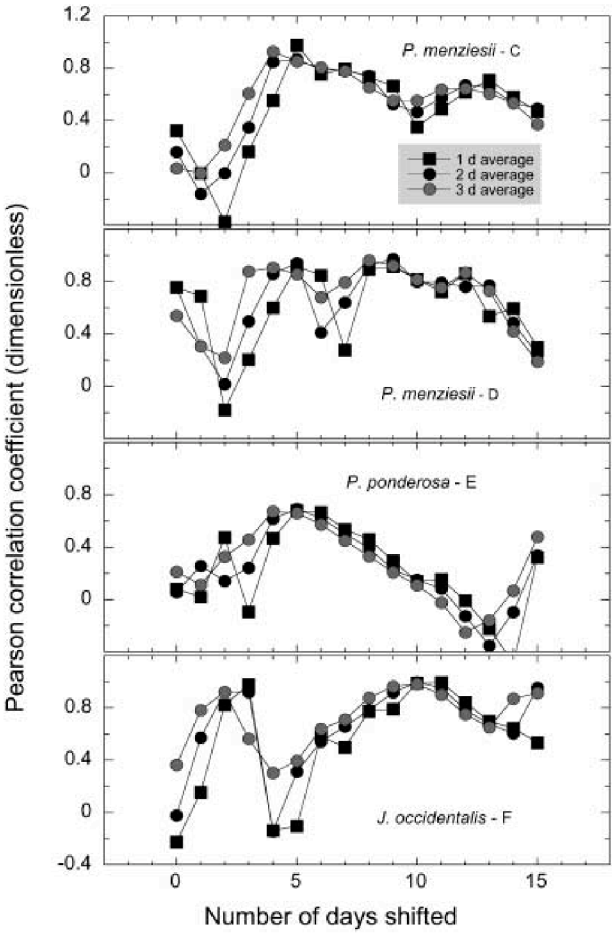

Fig. 5 Pearson product-moment correlation coefficients for linear regressions of $\delta^{13}C_R$ versus (averaged and time-lagged) air vapor saturation deficit (*vpd*). *Vpd* data used in this and subsequent analyses are daytime hours only, and recent freeze events are excluded. The different symbols in the legend correspond to number of days averaged, and the abscissa corresponds to number of days shifted back in time, relative to the date of air sampling. A 1-day average with a 0-day shift corresponds to the *vpd* on the day immediately preceding sampling (all sampling occurred at night)

(Fig. 3b). Bulk soil organic matter (SOM) showed substantial enrichment in ^{13}C with depth (Fig. 4) in the first 10 cm of soil at the wetter sties (B and C), which have a more developed soil profile. The $\delta^{13}C$ of SOM was fairly constant at all sites below 10 cm depth. In general, the wettest of the year 2000 sites (*Picea-Tsuga*, site B) had the most negative SOM values and the driest site (*J. occidentalis*, site F) the most positive. Below 10 cm, the SOM $\delta^{13}C$ values were indistinguishable at the *Picea-Tsuga* (B), *P. menziesii* (C), and *P. ponderosa* (E) sites. Within each site, there was a large enrichment in ^{13}C between fresh and old litter at three of the sites, and between litter and SOM at the wetter sites (Fig. 4).

Relationship between $\delta^{13}C_R$ and *vpd*

Strong correlations were observed between *vpd* and $\delta^{13}C_R$ at all sites where *vpd* data were available (C, D, E, and F), with the correlation coefficient in some cases approaching 1 after a time shift (Fig. 5). There were broad general peaks in the correlation coefficient that appeared at consistent time shifts regardless of the averaging time. In some cases, multiple peaks were observed (sites D and F) that corresponded with different time lags, while in others, a single peak was apparent (site E).

Two-week time series of half-hourly *vpd* at the *P. ponderosa* (E) site are shown in Fig. 6. These include the sampling periods with the maximum (–24.2‰) and minimum (–27.7‰) observed Keeling plot intercepts at this site. Arrows denote the nights when air sampling occurred (lined up on the *x*-axis for comparison purposes), and the shaded boxes indicate the 5-day shift and 2-day average that provided the maximum correlation in Fig. 5. These boxes indicate the relevant *vpd* events associated with observed $\delta^{13}C_R$ values. Note that the most positive $\delta^{13}C_R$ was associated with strong atmospheric demand (high *vpd*), and the most negative with the lowest *vpd*.

Selecting the shift (5, 9, 5, and 10 days for sites C, D, E, and F, respectively) that provided the maximum correlation in Fig. 5 for a 2-day average, $\delta^{13}C_R$ is plotted versus *vpd* for all four sites in Fig. 7. A consistent relationship is apparent for all sites, and within each site, there was a wide range of $\delta^{13}C_R$ and associated *vpd* values. There were ten sampling periods at the *P. ponderosa* and *J. occidentalis* sites that deviated significantly from the pattern shown in Fig. 7; these points are not shown explicitly in the figure, but their location is indicated by the ellipse in the upper left corner. All of these sampling pe-

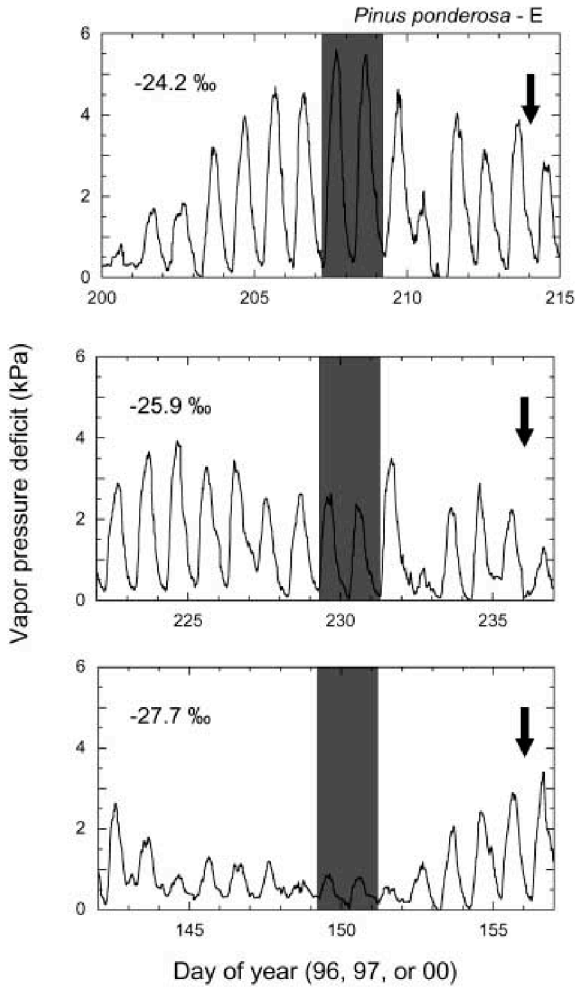

Fig. 6 Time series of half-hourly air vapor saturation deficit for the 2-week period preceding air sampling on selected nights at the *P. ponderosa* site, showing the events of importance in determining $\delta^{13}C_R$. The most positive and most negative $\delta^{13}C_R$ data at this site are shown, along with an intermediate point. The night of air sampling in each case is denoted with an *arrow*, and the *shaded box* corresponds to the 2-day average and 5-day shift that provided the maximum correlation in Fig. 5. $\delta^{13}C_R$ values for each sampling night are indicated on each plot

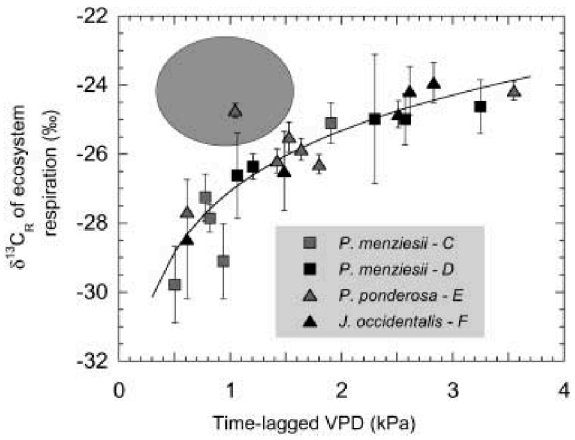

Fig. 7 Relationship between the carbon isotopic composition of ecosystem respiration and (averaged and time-lagged) air vapor saturation deficit. Two-day averages were used in each case, and time lags of 5, 9, 5, and 10 days were used for the *P. menziesii* (C), *P. menziesii* (D), *P. ponderosa* (E), and *J. occidentalis* (F) sites, respectively. The effect of recent freeze events (minimum air temperature <0.2°C in the last 7 days) on Keeling plot intercept is indicated by the *ellipse*; all data points which were excluded from the *vpd* analysis due to recent cold temperatures fall within the ellipse. Of the 33 Keeling plot intercepts at these four sites shown in Fig. 2, 10 were omitted here due to recent freeze events, and 2 were omitted due to missing weather data. The line drawn is a simple logarithmic fit (y=2.54ln$x$–27.08, r^2=0.79, n=22)

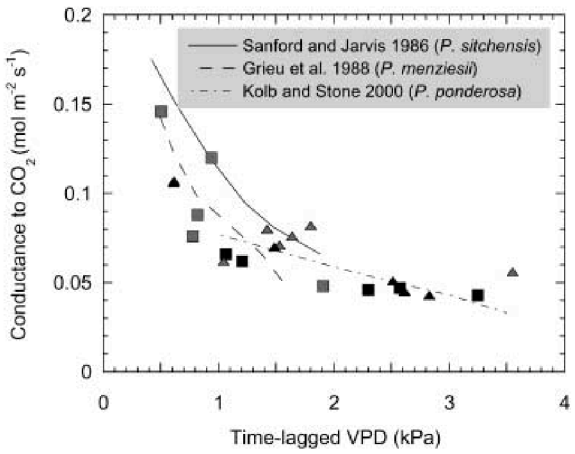

Fig. 8 Total (stomatal+boundary layer) conductance to CO_2 versus air vapor saturation deficit. Conductance was estimated from A-c_i curves for each species using the approach of Farquhar and Sharkey (1982), with c_i/c_a derived from the Keeling plot intercepts, assuming c_a=360 ppm. Also shown are leaf-level stomatal conductance-*vpd* relationships adapted from Sanford and Jarvis (1986), Grieu et al. (1988), and Kolb and Stone (2000) for three of our study species

riods occurred during or just after a period of freezing temperatures. There is a clear distinction between sampling periods with versus without freezing air temperatures. (The apparent outlier at the ponderosa pine site within the ellipse in Fig. 7 was not associated with a recent freeze, but falls within the region associated with other freeze events.)

Conductance

Leaf diffusive conductance, estimated from the $\delta^{13}C_R$ values, decreased as *vpd* increased (Fig. 8). This is consistent with observations of stomatal responses to *vpd* from the literature made on individual leaves for three of our study species.

Discussion

Consistent patterns were found between precipitation and the carbon isotope ratios of plant tissues, soil organic

components, and ecosystem respiration. In general, the drier sites exhibited more positive $\delta^{13}C$ than the wetter sites (Figs. 3, 4). This is consistent with our hypothesis that site water availability affects stomatal conductance, which is reflected in photosynthetic discrimination differences between the sites, and ultimately affects the isotopic content of carbon released during respiration.

Several other studies have examined the variation in isotopic composition of plant tissues across moisture or humidity gradients (Guy et al. 1980; Farquhar et al. 1989; Stewart et al. 1995; Schulze et al. 1996a, 1996b, 1998). Stewart et al. (1995) examined leaf carbon isotope ratios for hundreds of herbaceous and arboreal species in 12 communities across a rainfall gradient in eastern Australia. Leaf $\delta^{13}C$ for individual species, as well as community averages at each site, were strongly negatively correlated to precipitation. In contrast, Schulze et al. (1998) found that community-averaged leaf-level discrimination of a wide variety of tree species along a northern Australian moisture gradient was constant at rainfalls above 475 mm. The lack of significant change in $\delta^{13}C$ at the community scale with changing rainfall was also observed in Patagonian ecosystems consisting of several plant functional types (Schulze et al. 1996b) and in C_4 grasses in relatively drier sites in Namibia (Schulze et al. 1996a). Schulze et al. (1998) suggested that (for their northern Australian transect), changes in species composition may have acted to maintain a relatively constant carbon isotope discrimination across a rainfall gradient.

The results of the present study (Figs. 3, 4) suggest that there is substantial variation in discrimination based on water availability in coniferous forests in Oregon. While several of our sites have been logged within the last 30 years (sites B, C, D), the dominant species at each site are natives and consistent with those in natural ecosystems of Oregon with similar rainfall amounts. We did not find major differences in ^{13}C content of SOM at depth at all sites (Fig. 4), which would be a strong indicator of community-level differences in photosynthetic discrimination between sites over a long time period. However, differences in $\delta^{13}C$ of leaf and root tissue support this hypothesis for the existing vegetation.

We found major differences in the isotopic signature of ecosystem respiration across the transect (Fig. 2), and the drier sites exhibited more positive $\delta^{13}C_R$ (Fig. 3a). This result is consistent with the notion that photosynthetic discrimination varies in response to availability of water to plants (Ehleringer and Cerling 1995), and that differences in discrimination are conferred to $\delta^{13}C_R$. The wettest site (A) was a distinct exception to this pattern. Site A is a 150-year-old mixed stand of *P. sitchensis* and *T. heterophylla*. Aboveground net primary production of forests is now well-established to decline with stand age (Ryan and Waring 1992; Yoder et al. 1994; Gower et al. 1996), and decreasing aboveground production in this stand has been directly confirmed (Harcombe et al. 1990). While the reasons for decline in productivity with age are an area of active research (Ryan and Yoder 1997; Becker et al. 2000), hydraulic limitations to water uptake in old trees have been directly implicated (Hubbard et al. 1999). Changes in hydraulic conductance have been shown to affect stomatal conductance (Hubbard et al. 2001) and leaf $\delta^{13}C$ (Panek 1996), and the ecosystem-wide carbon isotope discrimination at site A has likely decreased with age. Similar enrichment in $\delta^{13}C_R$ with increasing stand age has been observed in *P. menziesii* forests ranging in age from 20 to >450 years in Washington, USA (J.E. Fessenden and J.R. Ehleringer, unpublished data). While the overstory trees in our *P. ponderosa* and *J. occidentalis* sites are also old (Table 1), each has a much younger age component in the stand, and our air samples contain CO_2 from all respiring components. Because water is more limiting at these sites, the influence of water availability is potentially more important than stand age in determining $\delta^{13}C_R$.

Examining individual periods across all sites, variations in $\delta^{13}C_R$ values were correlated most significantly with precipitation during the month preceding sampling, but also with leaf predawn water potential and soil water content (Table 2). Even so, a component of the overall variation in $\delta^{13}C_R$ was not explained by these factors. Within a site, each of these variables had limited predictability in accounting for the variability in $\delta^{13}C_R$ (site C was an exception for precipitation; Table 2). Across any extreme climatic gradient, substantial covariation in environmental and biological factors is expected; such similarities do not necessarily imply a causal relationship. However, the combination of the leaf, root, litter, and SOM isotope data, and the observation that mean $\delta^{13}C_R$ is strongly correlated with mean annual precipitation, strongly implicates differences in photosynthetic isotope discrimination across the Oregon transect as the cause for observed variation in $\delta^{13}C$ of carbon stocks and fluxes.

There are at least three possible mechanisms that could cause variation in $\delta^{13}C_R$ within a given ecosystem over time. First, since respiration rates in general are strongly dependent on temperature, seasonal or diurnal changes in air and soil temperatures may change the relative contributions of each ecosystem component (root respiration and decomposition belowground, litter decomposition, foliar and sapwood respiration) to the total ecosystem respiration flux. These changes might result solely from changes in the balance of the component flux *rates* and do not necessarily involve changes in the isotopic composition of the components of the CO_2 flux. Second, the isotopic signatures of the components of the respiration flux may change over time. For example, soil moisture at our sites changes dramatically throughout the year (Gholz 1982; Miller et al. 1992; Anthoni et al. 1999; Law et al. 2000), affecting C and N mineralization rates and altering the organic substrates available for decomposition. Activity of soil microbes, macrofauna, and mycorrhizae are likely to differ seasonally in response to soil moisture and nutrient availability (e.g., Law et al. 2001), and their isotopic contribution to the total ecosys-

tem CO_2 flux will change as different substrates are utilized. Third, photosynthetic discrimination may change in response to changes in environmental factors such as irradiance, air temperature, and humidity. Bulk leaf $\delta^{13}C$ has long been used as an indicator of photosynthetic discrimination, and variation of leaf $\delta^{13}C$ within year 2000 at our sites was minimal (data not shown, but see standard errors in Fig. 3b). However, leaf $\delta^{13}C$ provides a long-term estimate of leaf Δ during the time that the carbon in leaves was fixed, and that is a poor indicator of day-to-day variability in c_i/c_a (Brooks et al. 1997). The isotopic content of phloem sap has been shown to be related to seasonal rainfall patterns in *Eucalyptus globulus*, in a manner consistent with expected seasonal changes in Δ (phloem sap $\delta^{13}C$ was more positive during water stress; Pate and Arthur 1998).

The ^{13}C content of ecosystem respiration in the present study was not correlated with nocturnal air temperature either across or within sites (Table 2), but was strongly correlated with *vpd* at all sites where this comparison was possible (Figs. 5, 6). The correlation with daytime *vpd* on the day that sampling occurred (1-day average, 0-day shift, Fig. 5) was generally weak, but strong correlations were apparent with the *vpd* some days before sampling (5- to 10-day shift, Fig. 5). Stomatal conductance and photosynthetic discrimination are directly affected by changes in humidity (Madhavan et al. 1991; Comstock and Ehleringer 1992; Ehleringer and Cerling 1995), and recently produced leaf starch and sugars are closely linked to c_i/c_a (Brugnoli et al. 1988). Thus photosynthate produced during periods of water stress is likely to be enriched in ^{13}C. To the extent that recently fixed photosynthate is used as a substrate for respiration, either above or below ground, such signals might be apparent in the ^{13}C signature of the ecosystem respiration flux some time later.

However, when such a signal might be expected in the integrated respiration flux of an ecosystem is unclear. Clearly, some lag would be expected, since such factors as phloem loading, phloem transport time, phloem unloading, plant carbon allocation, leaf and root phenology, root exudation, mycorrhizal and microbial activity, and fine-root turnover must all play a role. There is some indication of reasonable timing expectations in the literature. Horwath et al. (1994) radiolabeled small (3-m) *Populus* trees, and found a peak in ^{14}C activity of the soil respiration flux 2 days later, along with a smaller, second peak after 10 days. A similar experiment with *Populus* found ^{14}C in the soil CO_2 flux several hours after labeling, with a peak after 3–4 days (Mikan et al. 2000). Root growth and respiration rate in grasslands are dependent on the cumulative solar radiation flux 2–10 days earlier (Fitter et al. 1998, 1999), and a ^{13}C label applied to the air of a 15-year old *Pinus taeda* forest in North Carolina, USA, was detected in the soil respiration flux within 1 week of fumigation (Andrews et al. 1999).

We found peaks in the correlation coefficients between $\delta^{13}C_R$ and *vpd* at 5, 9, 5, and 10 days for sites C–F, respectively (Fig. 5). These are compatible with the time periods mentioned in the above studies; thus, the respiration flux from an ecosystem likely consists in large part of recently fixed carbon (assimilated within the last 10 or so days). This is in direct agreement with Ekblad and Högberg (2001), who found a distinct correlation between atmospheric humidity and the isotopic signature of soil-respired CO_2 2–4 days later, in a boreal mixed coniferous forest in Sweden.

We do not expect a single lag time to be associated with a given forest. Since the rate of translocation of photosynthate is likely dependent on temperature, the time for fixed carbon to be exuded by the roots or consumed by rhizospheric bacteria or distant mycorrhizae is not likely to be fixed. We would expect the isotopic signature of foliar respiration to respond much more quickly to a given atmospheric stress event than heterotrophic respiration in the soil that might be dependent on root exudates. Furthermore, the amount of carbon accumulated will differ under differing weather events, since assimilation is so strongly linked to light availability. The fact that there is not a single, obvious lag time at each site (Fig. 5) is probably a consequence of these factors. However, no matter which lag time is chosen, there is almost always a high degree of correlation between $\delta^{13}C_R$ and *vpd* (Fig. 5).

Examination of the most extreme $\delta^{13}C_R$ periods at the *P. ponderosa* site (Fig. 6) suggests that the isotopic signature of the respiration flux may be quite dynamically linked to recent meteorological events. A period with high *vpd* was followed 5 days later by a fairly positive respired CO_2 signature (–24.2‰). In contrast, a relatively cool moist period was associated with more negative $\delta^{13}C_R$ (–27.7‰; Fig. 6). Anthoni et al. (1999) have shown that this forest exhibits interesting shifts in the balance between photosynthesis and respiration during the summer. During a year of extreme drought (half the normal annual rainfall), hot dry days resulted in a net daily release of CO_2 via respiration, and cool, humid periods were associated with net uptake. Air temperature and *vpd* can change markedly over the span of a few days, and the isotope ratio of respired CO_2 appears to change concomitantly. This *P. ponderosa* forest canopy is very open, with a one-sided leaf area index of 2.1 m^2 m^{-2} (Law et al. 2001). About 75% of annual ecosystem respiration (R_e) comes from root and microbial respiration in the soil, and 20% from foliage. Autotrophic respiration accounts for ~55% of annual R_e (Law et al., in press), suggesting that only half of the respiration response to *vpd* was from autotrophs.

The individual $\delta^{13}C_R$ values for all time periods in the analysis are shown in Fig. 7, plotted versus the time-lagged *vpd* that provided the highest correlation for each forest (individually) in Fig. 5. A consistent relationship was found across all four sites. These sites range in mean annual precipitation by nearly an order of magnitude (Table 1), their mean $\delta^{13}C_R$ differs quite strongly with precipitation (Fig. 3a), and yet a similar *vpd* relationship is apparent for all. Circled in Fig. 7 is the loca-

tion of the data points excluded from the *vpd* analysis (a total of ten time periods, all from the *P. ponderosa* or *J. occidentalis* sites). These periods were associated with recent freeze events, and in every case, the isotope ratio of respired CO_2 was more enriched than the *vpd* relationship would predict. This is likely a result of prolonged stomatal closure following cold air temperatures, which has been observed in several conifers in the Rocky Mountains of the United States (Smith et al. 1984, and references therein).

Notable in Fig. 7 is the lack of data below and to the right of the line. Assuming that stomatal closure in response to *vpd* is influencing Δ, and that this result is transferred to $\delta^{13}C_R$, data points in this region would indicate exceedingly high stomatal conductance for a given *vpd*. The absence of data in this region is consistent with a hydraulic operational setpoint, whereby plants regulate stomatal aperture to prevent embolism of xylem conduits in either the shoots or the roots (Tyree and Sperry 1988; Hacke et al. 2000).

If photosynthetic discrimination indeed changes in response to atmospheric humidity, then $\delta^{13}C_R$ should reflect to some degree the stomatal or canopy conductance of the entire ecosystem. When conductance to CO_2 is estimated using $\delta^{13}C_R$, a realistic relationship with time-lagged *vpd* emerges (Fig. 8). Conductance is highest at low *vpd* and declines substantially as *vpd* increases. These $\delta^{13}C_R$-derived estimates of conductance are directly comparable in magnitude to leaf-level observations from the literature for three of our study species (Fig. 8). We were unable to find leaf area-based *g-vpd* curves for *T. heterophylla* or *J. occidentalis* in the literature, but Law and Waring (1994) reported full stomatal closure at 1.4 kPa for *J. occidentalis*. We stress that our purpose was not to use isotopic measurements to evaluate ecosystem conductance. Rather, we use the conductance/*vpd* relationship to demonstrate the likelihood of Δ changing in response to atmospheric humidity deficits. This is a necessary condition to support our assertion that Δ is changing and that this change is reflected in respired CO_2 some time later. If this is correct, then we stand to gain considerable insight about ecosystem physiology from analyses of Keeling plots.

Acknowledgements Thanks to P. Anthoni and J. Irvine for sharing data and several thoughtful discussions, C. Hannan of the Oregon Climate Service at Oregon State University for supplying meteorological data, S. Kincaid for extensive laboratory and field help, R. Brooks, Z. Brooks, C. Hatt, M. Zwieniecki, M. Martin, and A. Schauer for assistance with field work, and to C. Cook, M. Lott, S. Bill, and E. Perro-Grande for sharing their analytical expertise. We are grateful to A. Ekblad and R. Hubbard for sharing unpublished manuscripts. D. Pataki and B. Helliker reviewed and improved earlier versions of this manuscript. Special thanks to Starker Forests, Inc., for kindly allowing us to work at the Tum Tum tree farm (site C). This work was supported by a grant from the United States Department of Agriculture (99-35101-7772). Any opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect the views of USDA.

References

Andrews JA, Harrison KG, Matamala R, Schlesinger WH (1999) Separation of root respiration from total soil respiration using carbon-13 labeling during free-air carbon dioxide enrichment (FACE). Soil Sci Soc Am J 63:1429–1435

Anthoni PM, Law BE, Unsworth MH (1999) Carbon and water vapor exchange of an open-canopied ponderosa pine ecosystem, Agric For Meteorol 95:151–168

Becker P, Meinzer FC, Wullschleger SD (2000) Hydraulic limitation of tree height: a critique. Funct Ecol 14:4–11

Bond BJ, Kavanagh KL (1999) Stomatal behavior of four woody species in relation to leaf-specific hydraulic conductance and threshold water potential. Tree Physiol 19:503–510

Bowling DR, Cook CS, Ehleringer JR (2001a) Technique to measure CO_2 mixing ratio in small flasks with a bellows/IRGA system. Agric For Meteorol 109:61–65

Bowling DR, Tans PP, Monson RK (2001b) Partitioning net ecosystem carbon exchange with isotopic fluxes of CO_2. Global Change Biol 7:127–145

Brooks JR, Flanagan LB, Varney GT, Ehleringer JR (1997) Vertical gradients in photosynthetic gas exchange characteristics and refixation of respired CO_2 within boreal forest canopies. Tree Physiol 17:1–12

Brugnoli E, Hubick KT, Caemmerer S von, Wong SC, Farquhar GD (1988) Correlation between the carbon isotope discrimination in leaf starch and sugars of C_3 plants and the ratio of intercellular and atmospheric partial pressures of carbon dioxide. Plant Physiol 88:1418–1424

Buchmann N, Guehl J-M, Barigah TS, Ehleringer JR (1997a) Interseasonal comparison of CO_2 concentrations, isotopic composition, and carbon dynamics in an Amazonian rainforest (French Guiana) Oecologia 110:120–131

Buchmann N, Kao W-Y, Ehleringer JR (1997b) Influence of stand structure on carbon-13 of vegetation, soils, and canopy air within deciduous and evergreen forests in Utah, United States. Oecologia 110:109–119

Buchmann N, Brooks JR, Flanagan LB, Ehleringer JR (1998) Carbon isotope discrimination of terrestrial ecosystems. In: Griffiths H (ed) Stable isotopes, integration of biological, ecological, and geochemical processes. BIOS, Oxford, pp 203–222

Campbell GS, Norman JM (1998) An introduction to environmental biophysics. Springer, Berlin Heidelberg New York

Comstock JP, Ehleringer JR (1992) Correlating genetic variation in carbon isotopic composition with complex climatic gradients. Proc Natl Acad Sci USA 89:7747–7751

Daly C, Neilson RP, Phillips DL (1994) A statistical-topographic model mapping climatological precipitation over mountainous terrain. J Appl Meteorol 33:140–158.

Daly C, Taylor GH, Gibson WP (1997) The PRISM approach to mapping precipitation and temperature. In: 10th Conference on Applied Climatology, Reno, Nev. American Meteorological Society, Boston, Mass, pp 10–12

Ehleringer JR (1994) Variation in gas exchange characteristics among desert plants. In: Schulze E-D, Caldwell MM (eds) Ecophysiology of photosynthesis. Springer, Berlin Heidelberg New York, pp 361–392

Ehleringer JR, Cerling TE (1995) Atmospheric CO_2 and the ratio of intercellular to ambient CO_2 concentrations in plants. Tree Physiol 15:105–111

Ehleringer JR, Cook CS (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 JR, Cook CS, Tieszen LL (1986) Comparative water use and nitrogen relationships in a mistletoe and its host, Oecologia 68:279–284

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

Farquhar GD, Sharkey TD (1982) Stomatal conductance and photosynthesis, Annu Rev Plant Physiol 33:317–345
Farquhar GD, O'Leary MH, Berry JA (1982) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. Aust J Plant Physiol 9:121–137
Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. Annu Rev Plant Physiol Plant Mol Biol 40:503–537
Fitter AH, Graves JD, Self GK, Brown TK, Bogie DS, Taylor K (1998) Root production, turnover, and respiration under two grassland types along an altitudinal gradient: influence of temperature and solar radiation. Oecologia 114:20–30
Fitter AH, Self GK, Brown TK, Bogie DS, Graves JD, Benham D, Ineson P (1999) Root production and turnover in an upland grassland subjected to artifical soil warming respond to radiation flux and nutrients, not temperature. Oecologia 120:575–581
Flanagan LB, Brooks JR, Varney GT, Berry SC, Ehleringer JR (1996) Carbon isotope discrimination during photosynthesis and the isotope ratio of respired CO_2 in boreal forest ecosystems. Global Biogeochem Cycles 10:629–640
Flanagan LB, Kubien DS, Ehleringer JR (1999) Spatial and temporal variation in the carbon and oxygen stable isotope ratio of respired CO_2 in a boreal forest ecosystem. Tellus 51B: 367–384
Franklin JF, Dyrness CT (1988) Natural vegetation of Oregon and Washington. Oregon State University Press, Corvallis
Fung I, Field CB, Berry JA, Thompson MV, Randerson JT, Malmstrom CM, Vitousek PM, Collatz GJ, Sellers PJ, Randall DA, Denning AS, Badeck F, John J (1997) Carbon 13 exchanges between the atmosphere and biosphere. Global Biogeochem Cycles 11:507–533
Gholz HL (1982) Environmental limits on aboveground net primary production, leaf area, and biomass in vegetation zones of the Pacific Northwest. Ecology 63:469–481
Gower ST, McMurtrie RE, Murty D (1996) Aboveground net primary production decline with stand age: potential causes. Trends Ecol Evol 11:378–382
Grieu P, Guehl JM, Aussenac G (1988) The effects of soil and atmospheric drought on photosynthesis and stomatal control of gas exchange in three coniferous species. Physiol Plant 73:97–104
Guy RD, Reid DM, Krouse HR (1980) Shifts in carbon isotope ratios of two C3 halophytes under natural and artificial conditions. Oecologia 44:241–247
Hacke UG, Sperry JS, Ewers BE, Ellsworth DS, Schäfer KVR, Oren R (2000) Influence of soil porosity on water use in *Pinus taeda*. Oecologia 124:495–505
Harcombe PA, Harmon ME, Greene SE (1990) Changes in biomass and production over 53 years in a coastal *Picea sitchensis-Tsuga heterophylla* forest approaching maturity. Can J For Res 20:1602–1610
Högberg P, Nordgren A, Buchmann N, Taylor AFS, Ekblad A, Högberg MN, Nyberg G, Ottosson-Löfvenius M, Read DJ (2001) Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature 411:789–792
Horwath WR, Pretziger KS, Paul EA (1994) ^{14}C allocation in tree-soil systems. Tree Physiol 14:1163–1176
Hubbard RM, Bond BJ, Ryan MG (1999) Evidence that hydraulic conductance limits photosynthesis in old *Pinus ponderosa* trees. Tree Physiol 19:165–172
Hubbard RM, Stiller V, Ryan MG, Sperry JS (2001) Stomatal conductance and photosynthesis vary linearly with plant hydraulic conductance in ponderosa pine. Plant Cell Environ 24:113–121
Keeling CD (1958) The concentration and isotopic abundances of atmospheric carbon dioxide in rural areas. Geochim Cosmochim Acta 13:322–334
Kolb TE, Stone JE (2000) Differences in leaf gas exchange and water relations among species and tree sizes in an Arizona pine-oak forest. Tree Physiol 20:1–12
Lancaster J (1990) Carbon-13 fractionation in carbon dioxide emitting diurnally from soils and vegetation at ten sites on the North American continent. PhD thesis, University of California, San Diego
Law BE, Waring RH (1994) Combining remote sensing and climatic data to estimate net primary production across Oregon. Ecol Appl 4:717–728
Law BE, Baldocchi DD, Anthoni PM (1999a) Below-canopy and soil CO_2 fluxes in a ponderosa pine forest. Agric For Meteorol 94:171–188
Law BE, Ryan MG, Anthoni PM (1999b) Seasonal and annual respiration of a ponderosa pine ecosystem. Global Change Biol 5:169–182
Law BE, Williams M, Anthoni PM, Baldocchi DD, Unsworth MH (2000) Measuring and modelling seasonal variation of carbon dioxide and water vapour exchange of a *Pinus ponderosa* forest subject to soil water deficit. Global Change Biol 6:613–630
Law BE, Van Tuyl S, Cescatti A, Baldocchi DD (2001) Estimation of leaf area index in open-canopy ponderosa pine forests at different successional stages and management treatments in Oregon. Agric For Meteorol 108:1–14
Law BE, Thornton P, Irvine J, Van Tuyl S, Anthoni PM (2001) Carbon storage and fluxes in ponderosa pine forests at different developmental stages. Global Change Biol 7: 755–777
Lloyd J, Farquhar GD (1994) ^{13}C discrimination during CO_2 assimilation by the terrestrial biosphere. Oecologia 99:201–215
Madhavan S, Treichel I, O'Leary MH (1991) Effects of relative humidity on carbon isotope fractionation in plants. Bot Acta 104:292–294
Marshall JD, Zhang J (1994) Carbon isotope discrimination and water-use efficiency in native plants of the north-central Rockies. Ecology 75:1887–1895
Mikan CJ, Zak DR, Kubiske ME, Pregitzer KS (2000) Combined effects of atmospheric CO_2 and N availability on the belowground carbon and nitrogen dynamics of aspen mesocosms. Oecologia 124:432–445
Miller PM, Eddleman LE, Miller JM (1992) The seasonal course of physiological processes in *Juniperus occidentalis*. For Ecol Manage 48:185–215
Panek JA (1996) Correlations between stable carbon-isotope abundance and hydraulic conductivity in Douglas-fir across a climate gradient in Oregon, USA. Tree Physiol 16:747–755
Pate J, Arthur D (1998) $\delta^{13}C$ analysis of phloem sap carbon: novel means of evaluating seasonal water stress and interpreting carbon isotope signatures of foliage and trunk wood of *Eucalyptus globulus*. Oecologia 117:301–311
Peterson DL, Waring RH (1994) Overview of the Oregon Transect Ecosystem Research Project. Ecol Appl 4:211–225
Runyon J, Waring RH, Goward SN, Welles JM (1994) Environmental limits on net primary production and light-use efficiency across the Oregon transect. Ecol Appl 4:226–237
Ryan MG, Waring RH (1992) Maintenance respiration and stand development in a subalpine lodgepole pine forest. Ecology 73:2100–2108
Ryan MG, Yoder BJ (1997) Hydraulic limits to tree height and tree growth. BioScience 47:235–242
Sanford AP, Jarvis PG (1986) Stomatal responses to humidity in selected conifers. Tree Physiol 2:89–103
Schulze E-D, Ellis R, Schulze W, Trimborn P, Ziegler H (1996a) Diversity, metabolic types and $\delta^{13}C$ carbon isotope ratios in the grass flora of Namibia in relation to growth form, precipitation and habitat conditions. Oecologia 106:352–369
Schulze E-D, Mooney HA, Sala OE, Jobbagy E, Buchmann N, Bauer G, Canadell J, Jackson RB, Loreti J, Oesterheld M, Ehleringer JR (1996b) Rooting depth, water availability, and vegetation cover along an aridity gradient in Patagonia. Oecologia 108:503–511
Schulze E-D, Williams RJ, Farquhar GD, Schulze W, Langridge J, Miller JM, Walker BH (1998) Carbon and nitrogen isotope discrimination and nitrogen nutrition of trees along a rainfall gradient in northern Australia, Aust J Plant Physiol 25:413–425

Smith WK, Young DR, Carter GA, Hadley JL, McNaughton GM (1984) Autumn stomatal closure in six conifer species of the Central Rocky Mountains. Oecologia 63:237–242

Sokal RR, Rohlf FJ (1995) Biometry. Freeman, New York

Stewart GR, Turnbull MH, Schmidt S, Erskine PD (1995) ^{13}C natural abundance in plant communities along a rainfall gradient: a biological integrator of water availability. Aust J Plant Physiol 22:51–55

Still CJ (2000) C-4 plants and the carbon cycle: studies at regional and global scales. PhD thesis, Stanford University

Tans PP, Berry JA, Keeling RF (1993) Oceanic $^{13}C/^{12}C$ observations: a new window on ocean CO_2 uptake. Global Biogeochem Cycles 7:353–368

Taylor GH, Hannan C (1999) The climate of Oregon, from rainforest to desert. Oregon State University Press, Corvallis

Tyree MT, Sperry JS (1988) Do woody plants operate near the point of catastrophic xylem dysfunction caused by dynamic water stress? Plant Physiol 88:574–580

Yoder BJ, Ryan MG, Waring RH, Schoettle AW, Kaufmann MR (1994) Evidence of reduced photosynthetic rates in old trees. For Sci 40:513–527

Waring RH, Franklin JF (1979) Evergreen coniferous forests of the Pacific Northwest. Science 204:1380–1386