Age-related variations in $\delta^{13}C$ of ecosystem respiration across a coniferous forest chronosequence in the Pacific Northwest

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Summary  We tested the hypothesis that forest age influences the carbon isotope ratio ($\delta^{13}C$) of carbon reservoirs and CO$_2$ at local and regional levels. Carbon isotope ratios of ecosystem respiration ($\delta^{13}C_R$), soil respiration ($\delta^{13}C_{soil}$), bulk needle tissue ($\delta^{13}C_p$) and soil organic carbon ($\delta^{13}C_{SOC}$) were measured in > 450-, 40- and 20-year-old temperate, mixed coniferous forests in southern Washington, USA. Values of $\delta^{13}C_R$, $\delta^{13}C_{R-soil}$, $\delta^{13}C_p$ and $\delta^{13}C_{SOC}$ showed consistent enrichment with increasing stand age. Between the youngest and oldest forests there was a ~1‰ enrichment in $\delta^{13}C_p$ (at similar canopy depths), $\delta^{13}C_{SOC}$ (throughout the soil column), $\delta^{13}C_{R-soil}$ (during the wet season) and $\delta^{13}C_R$ (during the dry season). Mean values of $\delta^{13}C_R$ were $-25.9$, $-26.5$ and $-27.0$‰ for the 450-, 40- and 20-year-old forests, respectively. Both $\delta^{13}C_{R-soil}$ and the difference between $\delta^{13}C_R$ and $\delta^{13}C_{R-soil}$ were more $^{13}C$ enriched in older forests than in young forest: $\delta^{13}C_R - \delta^{13}C_{R-soil} = 2.3$, $1.1$ and $0.5$‰ for the 450-, 40- and 20-year-old forests, respectively. Values of $\delta^{13}C_p$ were proportionally more depleted relative to $\delta^{13}C_R$: $\delta^{13}C_P - \delta^{13}C_R = 0.5$, $2.2$ and $2.5$‰ for the 450-, 40- and 20-year-old forests, respectively. Values of $\delta^{13}C_p$ were most $^{13}C$-enriched at the top of the canopy and in the oldest forest regardless of season (overall values were $-26.9$, $-28.7$ and $-29.4$‰ for the 450-, 40- and 20-year-old forests, respectively). Values of $\delta^{13}C_{SOC}$ from shallow soil depths were similar to $\delta^{13}C_p$ values of upper- and mid-canopy needles. All $\delta^{13}C$ data are consistent with the hypothesis that a decrease in stomatal conductance associated with decreased hydraulic conductance leads to increased CO$_2$ diffusional limitations in older coniferous trees. The strong associations between $\delta^{13}C_p$ in needles with $\delta^{13}C_R$ and $\delta^{13}C_{R-soil}$ at the forest level suggest that $^{13}C$ observations scale between leaf and ecosystem levels.

Keywords: carbon isotopes, hydraulic conductance, stable isotope, water-use efficiency.

Introduction

Photosynthesis and respiration influence the carbon isotope ratio ($\delta^{13}C$) of atmospheric CO$_2$ within a forest canopy (Flanagan et al. 1996, 1999, Buchmann et al. 1997a, 1997b, Flanagan and Ehleringer 1997). During photosynthesis, plants preferentially incorporate $^{12}$CO$_2$, which has the net effect of increasing $^{13}$CO$_2$ remaining in the atmosphere. When plants respire, the CO$_2$ released back to the atmosphere has a $\delta^{13}C$ value that is depleted in $^{13}$CO$_2$, reflecting the carbon previously assimilated. The carbon assimilated in organic tissues can vary in $\delta^{13}C$ as a consequence of differences in the carbon isotope discrimination against $^{13}C$ ($\Delta_{leaf}$) during photosynthesis. Farquhar and Richards (1984) showed that the $\Delta_{leaf}$ of C$_3$ plants can be modeled as:

$$\Delta_{leaf} = a + (b - a)(c_i/c_a),$$

where $a$ is isotopic fractionation associated with diffusion of CO$_2$ in air (4.4‰), $b$ is net fractionation associated with carboxylation (27‰, Farquhar et al. 1989), $c_i$ is intercellular CO$_2$ concentration and $c_a$ is ambient CO$_2$ concentration. Farquhar et al. (1989) showed that typical $\Delta_{leaf}$ values were 16–22‰. Two processes control $c_i$: the rate of CO$_2$ consumption by photosynthesis and the rate of CO$_2$ supply via stomatal conductance. The value of $\Delta_{leaf}$ is related to the carbon isotope ratios of organic tissues ($\delta^{13}C_p$) and atmospheric CO$_2$ ($\delta^{13}C_a$):

$$\Delta_{leaf} = (\delta^{13}C_a - \delta^{13}C_p) / (1 - \delta^{13}C_p/1000).$$

Most of the variation in $\delta^{13}C_p$ results from variations in $c_i/c_a$, although changes in $\delta^{13}C_a$ can be important in the understory and lower portions of the canopy (Buchmann et al. 1997b, 1998, Bowling et al. 2001c). Values of $\delta^{13}C_a$ vary over a limited range (~1‰) through the upper to middle portions of many canopies during the major photosynthetic periods of the day, particularly under turbulent conditions (Flanagan et al. 1996, Bowling et al. 2001c, Buchmann et al. 2002). Yet within forest canopies, vertical gradients in $\delta^{13}C_p$ are as large as 3–7‰ (Ehleringer et al. 1986, 1993, Farquhar et al. 1989, Flanagan et al. 1996, Buchman et al. 1997a, 1997b). These
variations in δ13Cp occur mainly as a result of vertical changes in photon flux density (PFD) and to a lesser amount from changes in δ13Cw (Farquhar et al. 1989, Ehleringer et al. 1993, Brooks et al. 1997, Buchmann et al. 1997b). Increased water stress and reduced hydraulic conductance can further increase δ13Cp values in both young and old trees (Ehleringer et al. 1986, 1993, Waring and Silvester 1994, Yoder et al. 1994, Panek and Waring 1995, 1997, Panek 1996, Ryan and Yoder 1997). During respiration, the δ13C of CO2 diffusing from leaves and roots reflects, in part, the carbon isotope ratio of the assimilated carbon (δ13Cr, Lin and Ehleringer 1997) and the isotope ratio of the most recently fixed carbohydrate (Bowling et al. 2001b, Ekblad and Högborg 2001). Yet, it should be noted that species-specific enrichment in δ13C has been observed between sucrose stores and respired CO2 (Duranseau et al. 1999, 2001, Ghashghaie et al. 2001).

A significant correlation between δ13C of aboveground components and soil organic carbon (SOC) is predicted. Balesdent and Mariotti (1996) have shown that δ13Cp and 13C SOC are tightly correlated across a wide range of input values. The residual value of δ13C in SOC (δ13C SOC) at greater soil depths is typically enriched relative to δ13Cr. The processes leading to this 13C-enrichment in soils are still not well delineated (Balesdent and Mariotti 1996, Buchmann et al. 1997b, Ehleringer et al. 2000).

The δ13C of ecosystem respiration (δ13Cr) can be viewed as a scaled measure of respiring components, integrating variability of the respiring pools at the ecosystem level (Flanagan and Ehleringer 1997, Yakir and Sternberg 2000). It defines how CO2 from the biosphere imparts an isotopic signal to the atmosphere. Values of δ13Cr can be constant if pool components show limited turnover or dynamic if short-term carbon pools respond rapidly to changes in environmental conditions. Although δ13Cr represents a nighttime measurement of how ecosystems are functioning (Flanagan and Ehleringer 1997), daytime atmospheric measurements of δ13C can be used to determine photosynthetic carbon isotope discrimination at the ecosystem scale (Lloyd et al. 1996, Yakir and Sternberg 2000, Bowling et al. 2001c).

Our interest in understanding how stand age might influence δ13Cr was prompted by recent data sets suggesting that δ13Cp increases with stand age. To assess how physiological and stand-age components might influence these results, we examined effects of stand age on δ13C patterns on both interseasonal and interannual bases. We measured δ13C of different carbon pools and of respired CO2 in 20-, 40- and 450-year-old coniferous forests located within a 3-km radius near Carson, Washington (45°49′ N, 121°57′ W, elevation 360 m). Douglas-fir and western hemlock are the dominant species in these forests, although red alder (Alnus rubra Bong.) is prevalent at the 40-year-old forest and western red cedar (Thuja plicata Donn ex D. Don) is significant in the 450-year-old forest (Wind River Canopy Crane research site). A detailed description of the climate, soils and vegetation is given by D. Shaw (University of Washington, Wind River Canopy Crane Research Facility, personal communication), and a further description of the soil and nutrient variation among sites is presented by Klopatek (2002). Briefly, the climate is transitional between maritime and continental with mean annual temperatures of 8.7 °C and precipitation of ~250 cm (D. Shaw, unpublished data). Winters are cold and wet (181 cm) with much of the precipitation coming as snowfall. Fall and spring are warmer, with the bulk of the precipitation falling as rain. Summers are dry with less than 10% of the annual precipitation falling between June and September. Soils are defined as sandy loams with volcanic tephra and have a top layer of decomposing organic material (ranging from 2 to 4 cm depending on location). Soil nutrients (carbon and nitrogen) are highest and soil respired CO2 fluxes are lowest at the 40-year-old site (Klopatek 2002).

Understory vegetation is most extensive in the 450-year-old forest with common species such as western hemlock, vine maple (Acer circinatum Pursh), salal (Gaultheria shallon Pursh), Oregon grape (Berberis nervosa Pursh), vanilla leaf (Achlys triphylla Smith) and various bryophytes. The 20- and 40-year-old Douglas-fir forests originated from clear-cutting followed by replanting, whereas the 450-year-old forest is thought to have regenerated following a fire (Franklin and DeBell 1988). The height of the canopy (tallest trees) averaged 60, 32 and 15 m at the 450-, 40- and 20-year-old forests, respectively. Access to the upper portions of the canopies was by means of towers at the 20- and 40-year-old forests and a construction crane at the 450-year-old forest. The tower at the 40-year-old forest was removed on October 24, 1999, preventing collection of needle tissues from the top of the canopy at this forest thereafter. Stand leaf area index (LAI) was estimated as 8.6 ± 1.1 at the 450-year-old forest (Thomas and Winner 2000), 5.6 at the 40-year-old forest and 6.1 at the 20-year-old forest (N. McDowell, Oregon State University, Corvallis, OR, unpublished data).

Sample collection and preparation

Organic carbon measurements Current-year foliage was collected at top- (full sun, upper third of canopy) and mid-canopy (middle third) positions from three to four Douglas-fir trees and from three to four 2-m-tall western hemlock seedlings growing at each site. The same branches were resampled.

Materials and methods

Study site

Canopy CO2, soil CO2, and bulk needle and soil organic carbon were collected for δ13C analyses from 20-, 40- and 450-year-old coniferous forests located within a 3-km radius near Carson, Washington (45°49′ N, 121°57′ W, elevation 360 m). Douglas-fir and western hemlock are the dominant species in these forests, although red alder (Alnus rubra Bong.) is prevalent at the 40-year-old forest and western red cedar (Thuja plicata Donn ex D. Don) is significant in the 450-year-old forest (Wind River Canopy Crane research site). A detailed description of the climate, soils and vegetation is given by D. Shaw (University of Washington, Wind River Canopy Crane Research Facility, personal communication), and a further description of the soil and nutrient variation among sites is presented by Klopatek (2002). Briefly, the climate is transitional between maritime and continental with mean annual temperatures of 8.7 °C and precipitation of ~250 cm (D. Shaw, unpublished data). Winters are cold and wet (181 cm) with much of the precipitation coming as snowfall. Fall and spring are warmer, with the bulk of the precipitation falling as rain. Summers are dry with less than 10% of the annual precipitation falling between June and September. Soils are defined as sandy loams with volcanic tephra and have a top layer of decomposing organic material (ranging from 2 to 4 cm depending on location). Soil nutrients (carbon and nitrogen) are highest and soil respired CO2 fluxes are lowest at the 40-year-old site (Klopatek 2002).

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throughout the experiment, with care made to collect branch tips growing in sun-pockets on the south side of the sampled tree. One to three large branches were sampled at each canopy position with 5–10 clusters of needles (about 20 needles per cluster) collected and pooled to provide a single measurement for a given height. Needles were dried at 80 °C for 48 h immediately after collection. Foliage was then ground to a fine powder with mortar and pestle, and δ13C measured by isotope ratio mass spectrometry (details below). Because the tower at the 40-year-old forest was removed in October 1999, branches growing at the top of the canopy could not be resampled in May and September 2000. Therefore, needles from three to four Douglas-fir trees growing at the forest edge were substituted, with the assumption that sun-exposed foliage at the forest edge was in similar light conditions as foliage growing at the top of the canopy. One to three branches growing at a height of 2 m were sampled (5–10 clusters of current year needles).

In July 1999, soil-depth profiles were collected at three locations in each forest for SOC analyses. Three soil pits were dug and 50 g of soil was removed from each depth and a total of 4–6 depths collected per pit (0 cm (surface litter), 2-cm depth (decomposing bottom litter) and 10, 20, 30, and between 45 and 50-cm depth). Soil was dried at 80 °C for 48 h immediately after collection. Both coarse and fine roots were removed using a 30-µm mesh and hand picking with tweezers and a magnifying glass. Carbonates were removed by incubating the soils in 0.1 M HCl for 48 h at room temperature. The resulting SOC was then rinsed with deionized H2O and dried at 70 °C overnight, followed by measurement of δ13C SOC.

**Air measurements** To measure δ13C a for use in estimating δ13C R, canopy air was sampled after dark when CO2 gradients between the top and bottom of the canopy exceeded 50 ppm. Four canopy positions were sampled in the 40- and 450-year-old forest and three positions in the 20-year-old forest: top of the canopy, one to two mid-canopy heights, and at the ground surface. Five samples were taken per canopy position per sampling with the aid of Dekoron® tubes that were secured to the access towers at the sites supporting the 20- and 40-year-old forests and directly onto a Douglas-fir at the 450-year site. Specifically, air was collected at 0, 6.7 and 18 m at the 20-year site; 0, 15, 26 and 38.3 m at the 40-year site; and 0, 2, 24 and 53 m at the 450-year site. The air was dried by passing it through a magnesium perchlorate column, its CO2 concentration ([CO2]) measured with an LI-6200 (Li-Cor, Lincoln, NE) infrared gas analyzer, and then isolated in 100-ml glass flasks for laboratory analyses of δ13C (Ehleringer and Cook 1998) and additional [CO2] measurements (Bowling et al. 2001a). For c/ICa calculations, δ13C a was measured at each canopy height during the midday period when PFD was highest (Table 1).

Soil CO2 efflux (respired CO2 from the soil surface) was sampled from a series of soil collars (PVC rings) inserted 5 cm in the ground (1–3 cm within the mineral layer). The soil collars were installed 24 h before collection (three per forest). Measurements were made with a closed gas-exchange system coupled with a portable respiration system (Li-Cor LI-6200) that was equipped with a Li-Cor LI-6009 soil chamber. Soil-derived CO2 was circulated from the chamber, through a magnesium perchlorate trap, then diverted equally through six 100-ml flasks to the Li-Cor LI-6200, and back to the chamber above the soil. Air samples were collected at intervals of about 50 ppm in chamber [CO2] by sequentially closing one of the six glass flasks that were arranged in parallel. A total of six samples was collected per collar per measurement period (total collection time ranged between 3 and 6 min). Concentrations of CO2 were measured in the field with the Li-Cor LI-6200, and again in the laboratory with a Li-Cor LI-6262 attached to a compressible volume system (for a description of the laboratory [CO2] measurements see Bowling et al. 2001a).

**Isotope analyses**

We measured δ13C with a Finnigan MAT isotope ratio mass spectrometer (Model 252, Thermo Finnigan MAT, Bremen, Germany), equipped with a trace gas concentrator (PreCon) for analyses of atmospheric CO2 and with an elemental analyzer operating in continuous-flow mode for analyses of organic materials. Carbon isotope ratios are expressed as:

$$\delta^{13}C = \left( \frac{R_{sample}}{R_{standard}} - 1 \right) 1000\%e,$$

where δ13C is carbon isotope ratio expressed in ‰, R sample is 13C/12C ratio in the sample and R standard is 13C/12C ratio of the Pee Dee Belemnite (PDB) standard. Overall, long-term precision of the δ13C measurements was ± 0.11‰ for δ13CP and ± 0.03‰ for δ13C a.

The c/ICa ratio was calculated based on δ13CP and δ13Ca (Equations 1 and 2). The δ13C of ecosystem respiration was calculated with a simple mixing model (Keeling 1958, 1961):

$$\delta^{13}C_a = M \frac{1}{[CO_2]_a} + \delta^{13}C_{R},$$

where M is ([CO2] trop(δ13C trop − δ13C a)), [CO2] trop is [CO2] above the forest boundary layer, δ13C trop is isotope ratio of CO2 above the forest boundary layer and δ13CR is the carbon isotope ratio of ecosystem respiration. A linear relationship exists between δ13CP and 1/[CO2] a with a slope, M, and intercept value of δ13CR. We used this linear extrapolation of [CO2] and δ13CP to obtain δ13CR. We also used this approach to determine the carbon isotope ratio of respired CO2 (δ13C R-soil).

**Results**

**Carbon isotope ratios of needles and SOC**

Forest age and δ13CP of needle tissues of Douglas-fir were positively related (mid- and upper-canopy positions in Table 1). This difference in δ13CP among needles from different-aged forests was greater during dry periods than during the wetter period of the year. For example, differences in δ13CP of foliage between the old-growth and young forest stands (δ13C P450-year-old − δ13C P20-year-old) were 3.7‰ in May 2000, 2.6‰ in September 1999 and 3.0‰ in September 2000. The 40-year-old...
Table 1. Variations in measured midday atmospheric carbon dioxide ($c_a$), the carbon isotope ratio of that carbon dioxide ($\delta^{13}C_p$), and the carbon isotope ratio of needles ($\delta^{13}C_{i}$) at different positions in dominant trees across stands of different ages. Also shown are the calculated $\Delta_i/\Delta_c$ values for carbon isotope discrimination ($\Delta_{\text{leaf}}$), the ratio of intercellular to ambient carbon dioxide ($c_i/c_c$) and the intercellular carbon dioxide concentration ($c_i$). NR = not recorded.

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<th>Date</th>
<th>Height</th>
<th>Age (year)</th>
<th>Sample</th>
<th>$c_a$ (ppm)</th>
<th>$\delta^{13}C_a$ (‰)</th>
<th>$\delta^{13}C_p$ (‰)</th>
<th>$\Delta_{\text{leaf}}$ (‰)</th>
<th>$c_i/c_a$</th>
<th>$c_i$ (ppm)</th>
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<td>Bottom</td>
<td>450</td>
<td>Tsuga heterophylla</td>
<td>419</td>
<td>-10.70</td>
<td>-32.5 ± 0.7</td>
<td>22.5</td>
<td>0.80</td>
<td>355</td>
</tr>
<tr>
<td></td>
<td>(seedlings)</td>
<td>40</td>
<td>Tsuga heterophylla</td>
<td>378</td>
<td>-8.67</td>
<td>-31.9 ± 0.1</td>
<td>23.9</td>
<td>0.86</td>
<td>327</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>Tsuga heterophylla</td>
<td>374</td>
<td>-8.73</td>
<td>-31.5 ± 0.4</td>
<td>23.5</td>
<td>0.85</td>
<td>316</td>
</tr>
</tbody>
</table>

1 Values of current-year foliage $\delta^{13}C_p$ (whole needles) and $\delta^{13}C_a$ were used to calculate $\Delta_{\text{leaf}}$ and $c_i/c_a$ for the dominant coniferous species at each forest during September 1999, May 2000 and September 2000. Errors (SD) for $\Delta_{\text{leaf}}$, $c_i/c_a$, and $c_a$ once propagated, will reflect those for $\delta^{13}C_p$. Values for $c_a$ were determined based on atmospheric [CO$_2$] taken at midday from each height where vegetation was sampled. Values for $c_i$ and $\delta^{13}C_a$ are point measurements. Vegetation was not collected at the mid-canopy height in the 40-year-old forest because vegetation did not grow at this height. The access tower was removed from the 40-year site in October 1999, so values for sun-exposed foliage (2 m at canopy-edge) were substituted for the canopy-top values in May and September 2000.

Forest had $\delta^{13}C_p$ values intermediate between those of the 20- and 450-year-old forests with differences in $\delta^{13}C_p$ ($\delta^{13}C_{(P20-year-old)} - \delta^{13}C_{(P450-year-old)}$) of 1.3‰ in September 1999, 0.6‰ in May 2000 and 0.4‰ in September 2000. The observed differences in $\delta^{13}C_p$ must have been associated with differences in $c_i/c_p$, because $\delta^{13}C_a$ values at the canopy tops were similar at the three sites. Calculated $c_i/c_a$ and $\Delta_{\text{leaf}}$ were lower in needles from older forests relative to young forests.

Foliage $\delta^{13}C_p$ became more $^{13}$C depleted with depth into the canopy (from canopy top to bottom) (Table 1). Needles from trees sampled from the 450-year-old forest showed the greatest change in $\delta^{13}C_p$ with canopy position ($\delta^{13}C_{(Pcanopy top)} - \delta^{13}C_{(Pcanopy bottom)}$ = 6.9 ‰), followed by the 40-year-old forest ($\delta^{13}C_{(Pcanopy top)} - \delta^{13}C_{(Pcanopy bottom)}$ = 5.2‰), and the 20-year-old forest ($\delta^{13}C_{(Pcanopy top)} - \delta^{13}C_{(Pcanopy bottom)}$ = 3.7 ‰). Calculated $c_i/c_a$ and $\Delta_{\text{leaf}}$ were lowest at the top of the canopy and highest in the understory canopy with the greatest difference in $\Delta_{\text{leaf}}$ with canopy position ($\Delta_{\text{leaf}(canopy top)} - \Delta_{\text{leaf}(canopy bottom)}$) observed in the 450-year-old forest (6.1‰). We note that needle samples collected from the bottom of the canopy (2 m height) were from western hemlock seedlings, because western hemlock was the only coniferous species growing in the understory.

Values of $\delta^{13}C_p$ of overwintering current-year needles did not change between September 1999 and May 2000 (Figure 1a), even though these trees have been shown to photosynthesize over the winter (Winner and Thomas 1997). However, bud break did not occur until 2 weeks after the May 2000 sampling (D. Shaw, personal communication). When current-year
needles were reevaluated in September 2000, their \( \delta^{13}C_p \) values were enriched relative to the values of the spring samples. Although both the May and September 2000 measurements were made on current-year needles, the new needles formed in 2000 and sampled in September 2000 likely reflected, in part, a carbon source from the year before, as well as input from current-year photosynthesis. These data suggest that (1) there were differences between the isotopic composition of soluble carbohydrates that were stored overwinter in needles and transported out in spring for new needle growth, or (2) the growing conditions differed between 1999 and 2000 (varying growing conditions in this area have been documented by M. Unsworth, Oregon State University, Corvallis, OR, personal communication). This apparent seasonal shift in \( \delta^{13}C_p \) of \( \sim 2\%e \) between May and September 2000 was observed in needles from all forest ages, suggesting a similar environmental factor influencing \( \delta^{13}C_p \) regardless of tree age.

Enrichment of \( \delta^{13}C \) occurred with increasing soil depth and stand age in all forests (Figure 2). Depth profiles of \( \delta^{13}C_{SOC} \) from each forest showed an average difference of 1.6\%e between \( \delta^{13}C_{SOC} \) of the litter layer and the deepest sample in the soil profile (30- to 50-cm depth depending on the forest sampled). The greatest \( \delta^{13}C_{SOC} \) change consistently occurred between the 0- and 10-cm depths. The \( \delta^{13}C_{SOC} \) values were enriched in \( ^{13}C \) throughout the entire soil column as forest age increased. On average, a 0.75\%e enrichment in \( \delta^{13}C_{SOC} \) was observed between the 20- and 40-year-old forests and a 0.98\%e enrichment between the 40- and 450-year-old forests. The upper soil (top 5 cm) was composed of a decomposing leaf and needle litter layer that had \( ^{13}C \) values similar to the top-most and mid-canopy needles of the canopy above. Between 5 and 50 cm in the soil column, the fine root content decreased significantly and the mineral composition (sandy loams) increased.

Carbon isotope ratios of canopy CO\(_2\)
We used the Keeling plot approach (Equation 4), to measure \( \delta^{13}C \) of respired CO\(_2\) from the whole ecosystem based on nighttime observations. In May 2000, \( \delta^{13}C_{R} \) at the 450-year-old forest was \(-26.2 \pm 0.2\%e\) (Figure 3). Though not shown, regression \( r^2 = 0.97–0.99 \) in the Keeling plots for all forests and sampling times. The \( \delta^{13}C_{R} \) value was enriched by about 0.7\%e more on average in the old-growth forest relative to the younger forests (Figure 4). The difference in \( \delta^{13}C_{R} \) between the old-growth and young forests increased during dry periods ((\( \delta^{13}C_{R}(450\text{-year-old}) - \delta^{13}C_{R}(20\text{-year-old}) \)) = 1.3\%e for September 1999, 0.8\%e for May 2000 and 0.9\%e for September 2000).
Based on the same mixing model approach, we calculated δ¹³C of CO₂ effluxed from the soils (δ¹³Cᵣ-soil). Within each forest, variability was low and replicate measurements of δ¹³Cᵣ-soil were statistically indistinguishable (e.g., May 2000 from the 450-year-old forest, Figure 5). In contrast, δ¹³Cᵣ-soil of CO₂ effluxing from soil differed from that of the entire ecosystem. For example, in May 2000, δ¹³Cᵣ-soil in the 450-year-old stand was -23.0‰, whereas δ¹³Cᵣ was -26.2‰. This pattern of δ¹³Cᵣ-soil increasing with stand age held true for all forest stands (Figure 6). The extent of the enrichment in δ¹³Cᵣ-soil relative to δ¹³Cᵣ appeared to depend on season, especially in the 450-year-old forest (5.2‰ in May 2000 and 2.5‰ in September).

Discussion

Ryan and Yoder (1997). Our δ13C values of Douglas-fir throughout the canopy profile, across different-aged stands, and between seasons are consistent with these previously published observations of Pacific Northwest conifers at the needle scale. Furthermore, differences in species found with canopy position (from Douglas-fir to western hemlock at the mid- to bottom-canopy transition) may cause some of the differences in δ13CO2 observed with height (δ13CO2 can be 1‰ lower in western hemlock (Marshall and Zhang 1994)).

Yoder et al. (1994) noted that same-aged foliage of ponderosa pine (Pinus ponderosa Dougl. ex Laws) trees became enriched in 13C as trees grew older and larger. This change could not be explained by differences in light environment or photosynthetic capacity between old and young trees, and it corresponded with decreased mean stomatal conductance in the older trees. Yoder et al. (1994) and Ryan and Yoder (1997) hypothesized that, as trees age and grow taller, resistance to water transport increases, because of increases in overall path length, tortuosity and gravity effects. Consistent with this hypothesis, Phillips et al. (2002) observed that leaf-specific hydraulic conductance decreased as tree size and age increased for the same three sites used in our study, especially in the late summer. On the other hand, sap flow per unit leaf area (Phillips et al. 2002) and stomatal conductance of current-year foliage (N. McDowell, unpublished data) was greater in 450-year-old trees than in 40-year-old trees. Leaf nitrogen concentration was higher in the 40-year-old trees compared with both older and younger trees (Klopatek 2002), but McDowell (N. McDowell, unpublished data) did not observe any differences in photosynthetic capacity among trees in the three different-aged stands. Therefore, we interpret the observed tree-age-dependent variations in δ13C as indications of partial stomatal closure and decreased c/ci, although it is not clear why this trend was not observed in measurements of sap flow or stomatal conductance in the oldest trees.

The apparent impacts of increased δ13C in older trees on ecosystem-scale processes are also evident in the soil organic carbon profiles. Litter and upper soil layers reflect the elevated δ13C inputs from older trees, so shifts in upper soil δ13C values paralleling the observed differences in δ13C values were predicted. Perhaps what is not expected is that the younger 20- and 40-year-old stands should also have exhibited such a strong relationship between δ13C and δ13C of soil carbon (i.e., δ13C of soil carbon). If these relationships hold, then it would suggest that δ13C values of soil carbon are influenced by the age and growth of the canopy, which is consistent with our findings. However, it is possible that other factors, such as soil management practices, may also contribute to the observed differences in δ13C values.

Both δ13C and δ13C of soil carbon influence the δ13C through addition of respired CO2. In turn, it is now clear that these bio-sphere signals influence the δ13C values of regional and tropospheric CO2 (δ13C of tropospheric CO2) (Francey et al. 1995, Schimel 1995, Bakwin et al. 1998). By using Keeling plot analyses, we can determine the δ13C of CO2 respired from the whole ecosystem, including both aboveground (leaf and stems) and belowground (roots) sources. Consistent with observed differences in δ13C of soil carbon, we observed that δ13C increased with forest age as well as paralleled seasonal shifts associated with soil drought (Figure 6).

Integrated over time, δ13C is not fractionated during respiration (Lin and Ehleringer 1997), and therefore the carbon that is incorporated during photosynthesis (to a first approximation the same as δ13C) should be of similar value to the CO2 that is later respired by needles, stems and roots. However, because of temporal offsets in carbon sequestration and carbon respiration as well as the possibility of transport-dependent time lags between shoot and root, it is easy to imagine a situation where the δ13C of ecosystem respiration is not identical with that of component fluxes. It is thus noteworthy that consistent with observed differences in δ13C values of different-aged forests, we observed that δ13C was also increased with forest age and paralleled the seasonal shifts associated with soil drought (Figure 6). Ekblad and Högberg (2001) and Bowling et al. (2001b) have recently shown strong correlations between δ13C respired from the soil and ecosystem and the local vapor pressure deficit and precipitation. They attributed this association to short-term changes in Δleaf of the local trees responding to...
the regional climatic and water stress conditions. Varying $\Delta_{\text{leaf}}$ values are then reflected in the respired CO$_2$ from rapidly cycling carbon pools effluxed from either soils or shoots.

If $\delta^{13}C_R$ has direct linkages with leaf-level and soil-level processes, then we can begin to provide mechanistic links between ecophysiological and ecosystem processes. That linkage opens the possibility for isotope flux measurement to be used to understand short-term ecosystem-level changes associated with, for example, drought and humidity stresses, or age- or size-dependent hydraulic constraints. Scaling between ecophysiological and regional levels is important if we are to understand how ecosystem-scale processes respond to climatic fluctuations. Furthermore, an appreciation of processes at the ecosystem scale is of direct relevance to regional models, where the objective is to better understand biosphere–atmosphere processes that influence regional gas exchange and carbon balance. Our study strongly suggests that both environmental and stand-age factors play major roles in the $^{13}$CO$_2$ released from and taken up by terrestrial ecosystems. The inclusion of these biological and environmental factors will improve our understanding of regional carbon cycles, especially as we better appreciate the need to integrate the mosaic of forest ages across regional landscapes.

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References


