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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 ($\delta^{13}C_{R\text{-}soil}$), 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 $\delta^{13}C_R$, $\delta^{13}C_{R\text{-}soil}$, $\delta^{13}C_P$ 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 levels), $\delta^{13}C_{SOC}$ (throughout the soil column), $\delta^{13}C_{R\text{-}soil}$ (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 $\delta^{13}C_{R\text{-}soil}$ and the difference between $\delta^{13}C_R$ and $\delta^{13}C_{R\text{-}soil}$ were more ^{13}C enriched in older forests than in young forest: $\delta^{13}C_R - \delta^{13}C_{R\text{-}soil}$ = 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 $\delta^{13}C_R$: $\delta^{13}C_R - \delta^{13}C_P$ = 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 $\delta^{13}C_{R\text{-}soil}$ 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. 1997*a*, 1997*b*, 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 (Δ_{leaf}) during photosynthesis. Farquhar and Richards (1984) showed that the Δ_{leaf} of C_3 plants can be modeled as:

$$\Delta_{leaf} = a + (b - a)(c_i/c_a), \quad (1)$$

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 Δ_{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 Δ_{leaf} is related to the carbon isotope ratios of organic tissues ($\delta^{13}C_P$) and atmospheric CO_2 ($\delta^{13}C_a$):

$$\Delta_{leaf} = (\delta^{13}C_a - \delta^{13}C_P) / (1 - \delta^{13}C_P/1000). \quad (2)$$

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. 1997*b*, 1998, Bowling et al. 2001*c*). 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. 2001*c*, 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. 1997*a*, 1997*b*). These

variations in $\delta^{13}C_P$ occur mainly as a result of vertical changes in photon flux density (PFD) and to a lesser amount from changes in $\delta^{13}C_a$ (Farquhar et al. 1989, Ehleringer et al. 1993, Brooks et al. 1997, Buchmann et al. 1997*b*). Increased water stress and reduced hydraulic conductance can further increase $\delta^{13}C_P$ 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 $\delta^{13}C$ of CO_2 diffusing from leaves and roots reflects, in part, the carbon isotope ratio of the assimilated carbon ($\delta^{13}C_P$, Lin and Ehleringer 1997) and the isotope ratio of the most recently fixed carbohydrate (Bowling et al. 2001*b*, Ekblad and Högberg 2001). Yet, it should be noted that species-specific enrichment in $\delta^{13}C$ has been observed between sucrose stores and respired CO_2 (Duranceau et al. 1999, 2001, Ghashghaie et al. 2001).

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

The $\delta^{13}C$ of ecosystem respiration ($\delta^{13}C_R$) 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 CO_2 from the biosphere imparts an isotopic signal to the atmosphere. Values of $\delta^{13}C_R$ can be constant if pool components show limited turnover or dynamic if short-term carbon pools respond rapidly to changes in environmental conditions. Although $\delta^{13}C_R$ represents a nighttime measurement of how ecosystems are functioning (Flanagan and Ehleringer 1997), daytime atmospheric measurements of $\delta^{13}C_a$ can be used to determine photosynthetic carbon isotope discrimination at the ecosystem scale (Lloyd et al. 1996, Yakir and Sternberg 2000, Bowling et al. 2001*c*).

Our interest in understanding how stand age might influence $\delta^{13}C_R$ was prompted by recent data sets suggesting that $\delta^{13}C_P$ increases with stand age. To assess how physiological and stand-age components might influence these results, we examined effects of stand age on $\delta^{13}C$ patterns on both interseasonal and interannual bases. We measured $\delta^{13}C$ of different carbon pools and of respired CO_2 in 20-, 40- and 450-year-old temperate forests composed primarily of Douglas-fir (*Pseudotsuga menzesii* (Mirb.) Franco) and western hemlock (*Tsuga heterophylla* (Raf.) Sarg) located within 3 km of each other in southern Washington, USA (Shaw et al. 2001). We measured $\delta^{13}C$ of needle tissues ($\delta^{13}C_P$), soil organic carbon ($\delta^{13}C_{SOC}$), soil respired CO_2 ($\delta^{13}C_{R\text{-}soil}$) and ecosystem respired CO_2 ($\delta^{13}C_R$) during the dry season (September 1999 and 2000) and the wet season (just after snow-melt, May 2000), to examine meteorological and forest-age influences on carbon reservoirs in this temperate coniferous biome.

Materials and methods

Study site

Canopy CO_2, soil CO_2, and bulk needle and soil organic carbon were collected for $\delta^{13}C$ 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 CO_2 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

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 $\delta^{13}C_P$ 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 H_2O and dried at 70 °C overnight, followed by measurement of $\delta^{13}C_{SOC}$.

Air measurements To measure $\delta^{13}C_a$ for use in estimating $\delta^{13}C_R$, canopy air was sampled after dark when CO_2 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 CO_2 concentration ($[CO_2]$) measured with an LI-6200 (Li-Cor, Lincoln, NE) infrared gas analyzer, and then isolated in 100-ml glass flasks for laboratory analyses of $\delta^{13}C$ (Ehleringer and Cook 1998) and additional $[CO_2]$ measurements (Bowling et al. 2001*a*). For c_i/c_a calculations, $\delta^{13}C_a$ was measured at each canopy height during the midday period when PFD was highest (Table 1).

Soil CO_2 efflux (respired CO_2 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 CO_2 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 $[CO_2]$ 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 CO_2 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 $[CO_2]$ measurements see Bowling et al. 2001*a*).

Isotope analyses

We measured $\delta^{13}C$ 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 CO_2 and with an elemental analyzer operating in continuous-flow mode for analyses of organic materials. Carbon isotope ratios are expressed as:

$$\delta^{13}C = (R_{sample}/R_{standard} - 1)\,1000‰, \quad (3)$$

where $\delta^{13}C$ is carbon isotope ratio expressed in ‰, R_{sample} is $^{13}C/^{12}C$ ratio in the sample and $R_{standard}$ is $^{13}C/^{12}C$ ratio of the Pee Dee Belemnite (PDB) standard. Overall, long-term precision of the $\delta^{13}C$ measurements was ± 0.11‰ for $\delta^{13}C_P$ and ± 0.03‰ for $\delta^{13}C_a$.

The c_i/c_a ratio was calculated based on $\delta^{13}C_P$ and $\delta^{13}C_a$ (Equations 1 and 2). The $\delta^{13}C$ of ecosystem respiration was calculated with a simple mixing model (Keeling 1958, 1961):

$$\delta^{13}C_a = M\frac{1}{[CO_2]_a} + \delta^{13}C_R, \quad (4)$$

where M is $([CO_2]_{trop}(\delta^{13}C_{trop} - \delta^{13}C_R))$, $[CO_2]_{trop}$ is $[CO_2]$ above the forest boundary layer, $\delta^{13}C_{trop}$ is isotope ratio of CO_2 above the forest boundary layer and $\delta^{13}C_R$ is the carbon isotope ratio of ecosystem respiration. A linear relationship exists between $\delta^{13}C_a$ and $1/[CO_2]_a$, with a slope, M, and intercept value of $\delta^{13}C_R$. We used this linear extrapolation of $[CO_2]$ and $\delta^{13}C_a$ to obtain $\delta^{13}C_R$. We also used this approach to determine the carbon isotope ratio of respired CO_2 ($\delta^{13}C_{R\text{-}soil}$).

Results

Carbon isotope ratios of needles and SOC

Forest age and $\delta^{13}C_P$ of needle tissues of Douglas-fir were positively related (mid- and upper-canopy positions in Table 1). This difference in $\delta^{13}C_P$ among needles from different-aged forests was greater during dry periods than during the wetter period of the year. For example, differences in $\delta^{13}C_P$ of foliage between the old-growth and young forest stands ($\delta^{13}C_{P(450\text{-}year\text{-}old)} - \delta^{13}C_{P(20\text{-}year\text{-}old)}$) 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 ($\delta^{13}C_a$) , and the carbon isotope ratio of needles ($\delta^{13}C_P$) at different positions in dominant trees across stands of different ages. Also shown are the calculated[1] values for carbon isotope discrimination (Δ_{leaf}), the ratio of intercellular to ambient carbon dioxide (c_i/c_a) and the intercellular carbon dioxide concentration (c_i). NR = not recorded.

Date	Height	Age (year)	Sample	c_a (ppm)	$\delta^{13}C_a$ (‰)	$\delta^{13}C_P$ (‰)	Δ_{leaf} (‰)	c_i/c_a	c_i (ppm)
Sept. 20, 1999	Top	450	*Pseudotsuga menziesii*	373	–8.62	–26.7 ± 0.7	18.5	0.62	233
		40	*Pseudotsuga menziesii*	368	–8.16	–28.1 ± 0.6	20.5	0.71	262
		20	*Pseudotsuga menziesii*	375	–8.57	–29.3 ± 0.6	21.4	0.75	281
	Middle	450	*Pseudotsuga menziesii*	369	–8.48	–27.7 ± 0.5	19.8	0.68	251
		40	*Pseudotsuga menziesii*	364	–8.02	NR	NR	NR	NR
		20	*Pseudotsuga menziesii*	367	–8.27	–30.9 ± 0.5	23.3	0.84	307
	Bottom (seedlings)	450	*Tsuga heterophylla*	402	–10.03	–33.9 ± 0.4	24.6	0.90	360
		40	*Tsuga heterophylla*	382	–8.83	–33.2 ± 0.3	25.2	0.92	351
		20	*Tsuga heterophylla*	388	–9.21	–33.2 ± 0.8	24.8	0.90	350
May 15, 2000	Top	450	*Pseudotsuga menziesii*	365	–8.28	–26.9 ± 0.4	19.1	0.65	238
		40	*Pseudotsuga menziesii*	376	–8.62	–29.6 ± 0.7	21.7	0.76	287
		20	*Pseudotsuga menziesii*	374	–8.52	–30.6 ± 0.5	22.8	0.81	304
	Middle	450	*Pseudotsuga menziesii*	365	–8.74	–27.7 ± 0.7	19.5	0.67	245
		40	*Pseudotsuga menziesii*	374	–8.60	NR	NR	NR	NR
		20	*Pseudotsuga menziesii*	377	–8.92	–31.2 ± 0.6	23.0	0.82	310
	Bottom (seedlings)	450	*Tsuga heterophylla*	379	–9.42	–33.5 ± 0.5	25.0	0.91	345
		40	*Tsuga heterophylla*	383	–9.01	–33.2 ± 0.3	25.0	0.91	350
		20	*Tsuga heterophylla*	389	–9.03	–32.8 ± 0.4	24.5	0.89	346
Sept. 20, 2000	Top	450	*Pseudotsuga menziesii*	371	–8.45	–25.6 ± 0.7	17.6	0.58	216
		40	*Pseudotsuga menziesii*	367	–8.26	–28.4 ± 0.4	20.8	0.72	266
		20	*Pseudotsuga menziesii*	374	–8.87	–28.5 ± 1.5	20.2	0.70	262
	Middle	450	*Pseudotsuga menziesii*	370	–8.69	–26.9 ± 0.5	18.7	0.63	234
		40	*Pseudotsuga menziesii*	370	–8.38	NR	NR	NR	NR
		20	*Pseudotsuga menziesii*	370	–8.45	–30.7 ± 0.4	22.9	0.82	303
	Bottom (seedlings)	450	*Tsuga heterophylla*	419	–10.70	–32.5 ± 0.7	22.5	0.80	355
		40	*Tsuga heterophylla*	378	–8.67	–31.9 ± 0.1	23.9	0.86	327
		20	*Tsuga heterophylla*	374	–8.73	–31.5 ± 0.4	23.5	0.85	316

[1] Values of current-year foliage $\delta^{13}C_P$ (whole needles) and $\delta^{13}C_a$ were used to calculate Δ_{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 Δ_{leaf}, c_i/c_a and c_i, once propagated, will reflect those for $\delta^{13}C_P$. Values for c_i were determined based on atmospheric [CO_2] taken at midday from each height where vegetation was sampled. Values for c_a 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 $\delta^{13}C_P$ ($\delta^{13}C_{P(40\text{-year-old})} - \delta^{13}C_{P(20\text{-year-old})}$) 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_a, because $\delta^{13}C_a$ values at the canopy tops were similar at the three sites. Calculated c_i/c_a and Δ_{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 ($\delta^{13}C_{P(canopy\ top)} - \delta^{13}C_{P(canopy\ bottom)} = 6.9$ ‰), followed by the 40-year-old forest ($\delta^{13}C_{P(canopy\ top)} - \delta^{13}C_{P(canopy\ bottom)} = 5.2$‰), and the 20-year-old forest ($\delta^{13}C_{P(canopy\ top)} - \delta^{13}C_{P(canopy\ bottom)} = 3.7$ ‰). Calculated c_i/c_a and Δ_{leaf} were lowest at the top of the canopy and highest in the understory canopy with the greatest difference in Δ_{leaf} with canopy position ($\Delta_{leaf(canopy\ top)} - \Delta_{leaf(canopy\ bottom)}$) 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

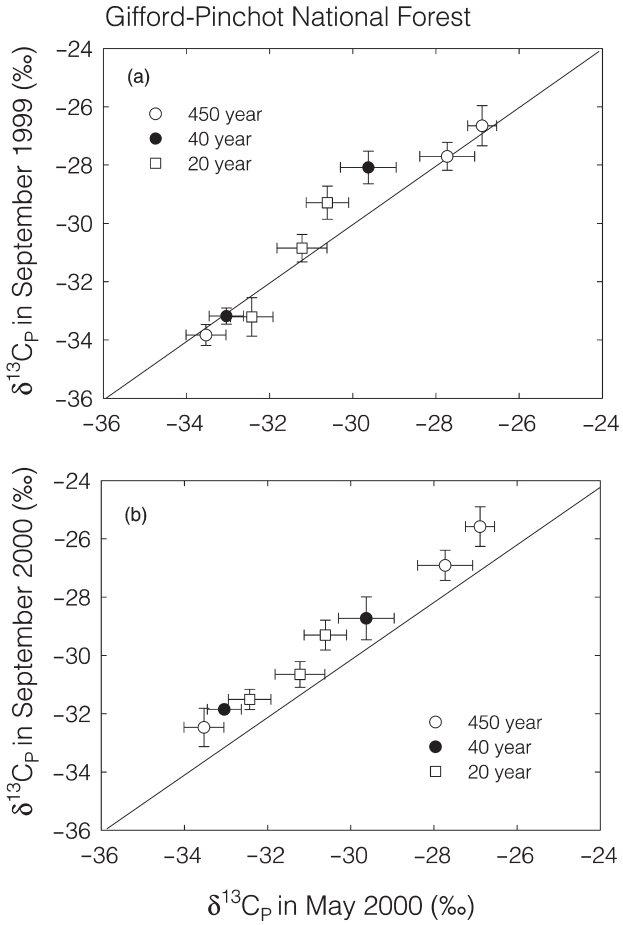

Figure 1. (a) Comparison of $\delta^{13}C_P$ from current-year needles of Douglas-fir and western hemlock collected from canopy top, mid-canopy, and understory positions within each forest over the wintertime (September 1999 to May 2000). (b) Comparison of $\delta^{13}C_P$ from current-year needles of Douglas-fir and western hemlock collected from canopy top, mid-canopy, and understory positions within each forest over the growing season (May 2000 to September 2000; bud break occurred 2 weeks after the May 2000 sampling). Error bars indicate standard error.

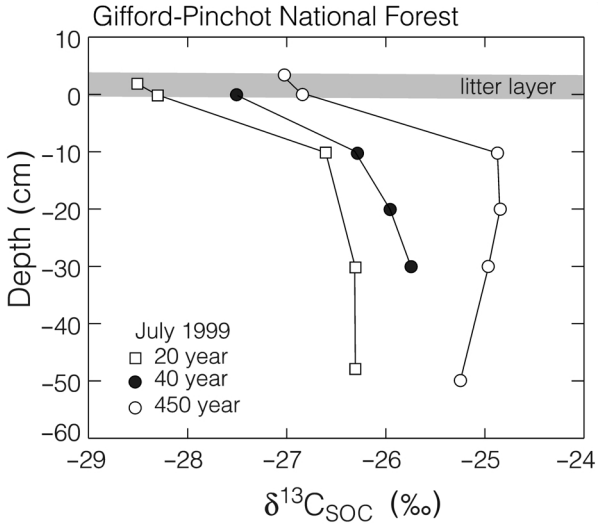

Figure 2. Depth profiles of carbon isotope ratios from bulk soil organic material ($\delta^{13}C_{SOC}$). Measurements were made at the three forests in July 1999. There was a 5-cm litter layer above the mineral soil. Though not shown, profiles of $\delta^{13}C_{SOC}$ did not change over time or space within one forest stand (J.E. Fessenden and J.R. Ehleringer, unpublished observations).

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 ~2‰ 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‰ 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‰ enrichment in $\delta^{13}C_{SOC}$ was observed between the 20- and 40-year-old forests and a 0.98‰ 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 $\delta^{13}C$ 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 ± 0.2‰ (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‰ 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 ($(\delta^{13}C_{R(450\text{-year-old})} - \delta^{13}C_{R(20\text{-year-old})}) = 1.3$‰ for September 1999, 0.8‰ for May 2000 and 0.9‰ for September 2000).

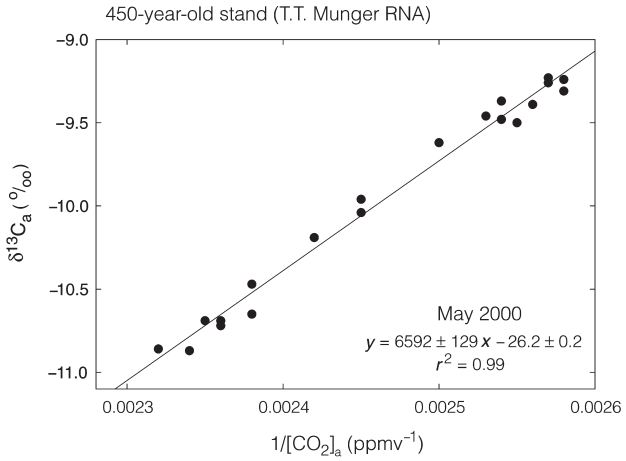

Figure 3. Keeling plot of canopy CO_2 collected at the 450-year-old forest in the T.T. Munger Research Natural Area in May 2000. Canopy CO_2 was collected at four heights, above the canopy (53 m) in the middle of the canopy (24 m), at the top of the seedlings (2 m) and at the ground surface (0 m). All samples were collected 1 h after sunset.

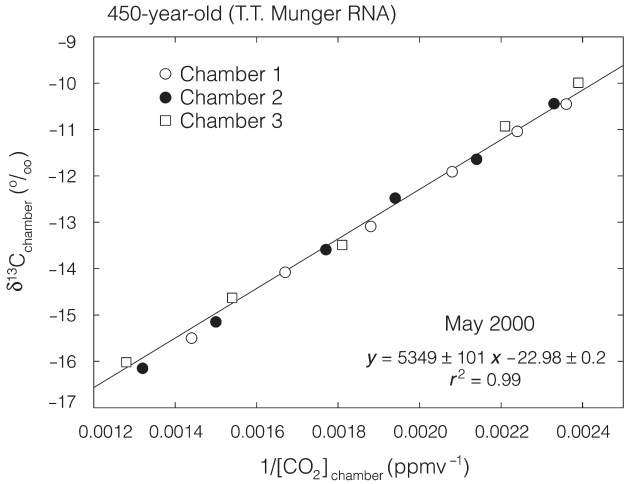

Figure 5. Keeling plot of soil respired CO_2 from chambers at three locations in the 450-year-old site in May 2000. Six samples were collected from each chamber with a 50 ppm CO_2 change between successive samples. Measurements were made at noon when soil temperatures averaged 10 °C.

Based on the same mixing model approach, we calculated $\delta^{13}C$ of CO_2 effluxed from the soils ($\delta^{13}C_{R\text{-}soil}$). Within each forest, variability was low and replicate measurements of $\delta^{13}C_{R\text{-}soil}$ were statistically indistinguishable (e.g., May 2000 from the 450-year-old forest, Figure 5). In contrast, $\delta^{13}C_{R\text{-}soil}$ of CO_2 effluxing from soil differed from that of the entire ecosystem. For example, in May 2000, $\delta^{13}C_{R\text{-}soil}$ in the 450-year-old stand was –23.0‰, whereas $\delta^{13}C_R$ was –26.2‰. This pattern of $\delta^{13}C_{R\text{-}soil}$ increasing with stand age held true for all forest stands (Figure 6). The extent of the enrichment in $\delta^{13}C_{R\text{-}soil}$ relative to $\delta^{13}C_R$ appeared to depend on season, especially in the 450-year-old forest (5.2‰ in May 2000 and 2.5‰ in September).

Discussion

The $\delta^{13}C$ values of needle and leaf tissues ($\delta^{13}C_P$) reflect long-term patterns in c_i/c_a (Farquhar et al. 1989) and to a lesser extent the influence of $\delta^{13}C_a$ (Brooks et al. 1997, Buchmann et al. 1997*b*). Several environmental factors influence c_i/c_a and therefore $\delta^{13}C_P$ in a predictable fashion (Farquhar et al.1989, Ehleringer et al. 1993). Increasing PFD, decreasing humidity, increasing soil compaction, and decreasing soil water availability all increase $\delta^{13}C_P$ (Ehleringer et al. 1986, 1993, Sprugel 1990, Brooks et al. 1991, 1996, 1997, Flanagan et al. 1996, 1999, Buchmann et al. 1997*b*, 1998). In addition, internal plant characters, such as increased nutritional status (N content) and decreased hydraulic conductance, also increase $\delta^{13}C_P$ (Ehleringer et al. 1993, Waring and Silvester 1994, Yoder et al. 1994, Panek and Waring 1995, 1997, Panek 1996,

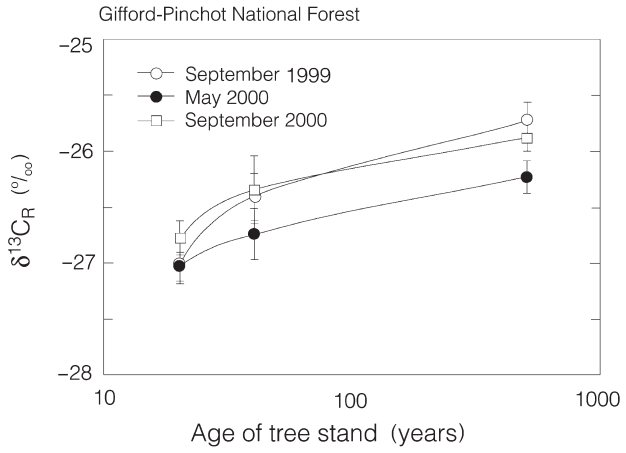

Figure 4. Age-dependent variation of the isotope ratio of ecosystem respiration ($\delta^{13}C_R$) for the three forests. A comparison is made between water-limited conditions, September 1999 and 2000, and water-saturated conditions, May 2000. Error bars = standard error.

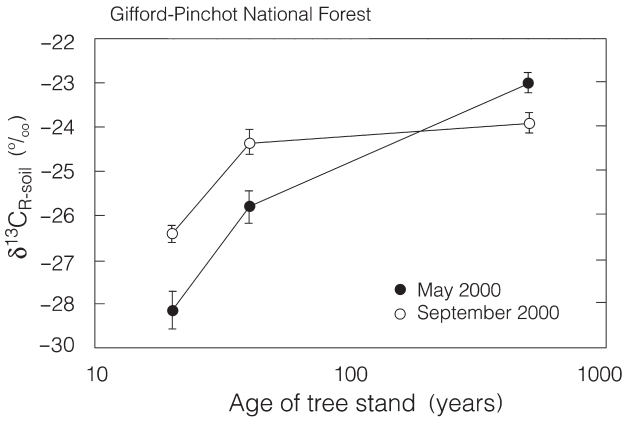

Figure 6. Isotope ratios of soil respired CO_2 for the three forest stands. Each value is the mean of 18 measurements taken at three locations in each forest. Error bars = standard error.

Ryan and Yoder 1997). Our $\delta^{13}C_P$ observations 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 $\delta^{13}C_P$ observed with height ($\delta^{13}C_P$ 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 ^{13}C 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 $\delta^{13}C_P$ as indications of partial stomatal closure and decreased c_i/c_a, 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 $\delta^{13}C_P$ in older trees on ecosystem-scale processes are also evident in the soil organic carbon profiles. Litter and upper soil layers reflect the elevated $\delta^{13}C_P$ inputs from older trees, so shifts in upper soil $\delta^{13}C_{SOC}$ values paralleling the observed differences in $\delta^{13}C_P$ 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 $\delta^{13}C_P$ and $\delta^{13}C_{SOC}$, because rapid SOC turnover is implied. Several hypotheses could account for these observations: (1) rapid turnover of carbon may exist in the upper soil layers of these young forests, (Gaudinski et al. 2000, M. Harmon, Oregon State University, Corvallis, OR, personal communication); (2) new carbon inputs may be swamping out the previous $\delta^{13}C_{SOC}$ signal from the original forest; (3) clear-cutting followed by fire clearing may have volatilized or homogenized old carbon stocks existing in these areas (Simard et al. 2001); or (4) a change in the logging practices (from horse to mechanical logging: an inherent problem with chronosequence studies, Yanai et al. 2000). Increasing $\delta^{13}C_{SOC}$ is expected with increased soil depth, because C turns over rapidly near the soil surface from microbes and invertebrates. In these undisturbed soils, $\delta^{13}C_{SOC}$ tended to increase through time associated with decomposition in all forests, irrespective of forest age. There did not appear to be any carryover of C in deeper soils of the young forest that was consistent with $\delta^{13}C_{SOC}$ measured at depth in the 450-year-old forest. The mechanisms by which $\delta^{13}C_{SOC}$ becomes enriched in C_3 forests are still not well understood, but both environmental and biological factors are thought to play a role (Balesdent and Mariotti 1996, Boutton 1996, Ehleringer et al. 2000).

Our study is the first to demonstrate that $\delta^{13}C_P$ patterns observed in coniferous trees of different ages can also be detected in air at the canopy scale (i.e., $\delta^{13}C_R$). Because air turbulence is the major factor governing CO_2 transport within canopies, the $\delta^{13}C_P - \delta^{13}C_R$ pattern suggests that the carbon isotope ratio of fluxes from leaves directly scale to the ecosystem scale. The positive association between $\delta^{13}C_P$ and $\delta^{13}C_R$ need not necessarily occur if understory vegetation plays a proportionally greater role in C balance as canopies develop. That is, because understory vegetation exhibits the most negative $\delta^{13}C_P$ (e.g., Table 1, Farquhar et al. 1989), the depleted $\delta^{13}C$ content of respiration from these elements could potentially offset the more enriched $\delta^{13}C$ effluxing from older trees such that $\delta^{13}C_R$ remained constant as tree canopies aged. Because $\delta^{13}C_R$ is observed to increase with stand age, our tentative conclusion is that gas exchange by understory elements does not make a significant enough contribution to canopy gas exchange to influence the isotope ratio of CO_2 released by these coniferous canopies.

Both $\delta^{13}C_P$ and $\delta^{13}C_{SOC}$ influence the ($\delta^{13}C_a$ through addition of respired CO_2. In turn, it is now clear that these biosphere ^{13}C signals influence the $\delta^{13}C$ values of regional and tropospheric CO_2 ($\delta^{13}C_{trop}$) (Francey et al. 1995, Schimel 1995, Bakwin et al. 1998). By using Keeling plot analyses, we can determine the $\delta^{13}C$ of CO_2 respired from the whole ecosystem, including both aboveground (leaf and stems ~$\delta^{13}C_P$) and belowground (root ~$\delta^{13}C_P$ and heterotrophic ~$\delta^{13}C_{SOC}$) sources. Consistent with observed differences in $\delta^{13}C_P$ as forests aged, we observed that $\delta^{13}C_R$ increased with forest age as well as paralleled the seasonal shifts associated with soil drought (Figure 6).

Integrated over time, $\delta^{13}C$ 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 $\delta^{13}C_P$) should be of similar value to the CO_2 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 $\delta^{13}C$ of ecosystem respiration is not identical with that of component fluxes. It is thus noteworthy that consistent with observed differences in $\delta^{13}C_R$ values of different-aged forests, we observed that the $\delta^{13}C_{R\text{-}soil}$ also increased with forest age and paralleled the seasonal shifts associated with soil drought (Figure 6). Ekblad and Högberg (2001) and Bowling et al. (2001*b*) have recently shown strong correlations between $\delta^{13}C$ respired from the soil and ecosystem and the local vapor pressure deficit and precipitation. They attributed this association to short-term changes in Δ_{leaf} of the local trees responding to

the regional climatic and water stress conditions. Varying Δ_{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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