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Temporal variation in δ^{13}C of ecosystem respiration in the Pacific Northwest: links to moisture stress

Abstract We measured seasonal and interannual variations in δ^{13}C values within the carbon reservoirs (leaves and soil) and CO_2 fluxes (soil and ecosystem respired CO_2) of an old growth coniferous forest in the Pacific Northwest USA with relation to local meteorological conditions. There were significant intra-annual and interannual differences in the carbon isotope ratios of CO_2 respired at both the ecosystem ($\delta^{13}C_R$) and the soil levels ($\delta^{13}C_{R\text{-soil}}$), but only limited variations in the carbon isotope ratios of carbon stocks. The $\delta^{13}C_R$ values varied by as much as 4.4‰ over a growing season, while $\delta^{13}C_{R\text{-soil}}$ values changed as much as 6.2‰. The δ^{13}C of soil organic carbon ($\delta^{13}C_{SOC}$) and needle organic carbon ($\delta^{13}C_P$) exhibited little or no significant changes over the course of this study. Carbon isotope discrimination within leaves (Δ_p) showed systematic decreases with increased canopy height, but remained fairly constant throughout the year (Δ_p=17.9‰–19.2‰ at the top of the canopy, Δ_p=19.6‰–20.9‰ at mid-canopy, Δ_p=23.3‰–25.1‰ at the canopy base). The temporal variation in the δ^{13}C of soil and ecosystem respired CO_2 was correlated (r=0.93, P<0.001) with soil moisture levels, with dry summer months having the most ^{13}C-enriched values. The dynamic seasonal changes in δ^{13}C of respired CO_2 are hypothesized to be the result of fast cycling of recently fixed carbon back to the atmosphere. One scaling consequence of the seasonal and interannual variations in $\delta^{13}C_R$ is that inversion-based carbon-cycle models dependent on observed atmospheric CO_2 concentration and isotope values may be improved by incorporating dynamic $\delta^{13}C_R$ values to interpret regional carbon sink strength.

Keywords Carbon isotopes · CO_2 · Discrimination · Ecosystem respiration · Soil respiration

Introduction

Carbon isotopes in atmospheric CO_2 have been used to partition terrestrial and oceanic carbon fluxes (Ciais et al. 1995b, 1999; Francey et al. 1995; Bakwin et al. 1998; Battle et al. 2000; Bousquette et al. 2000). $^{13}CO_2$ released from the terrestrial biosphere averages about −27‰ (Ciais et al. 1995a) while that from the oceans averages −7.4‰ (Gruber and Keeling 2001). This is because plants preferentially incorporate ^{12}C and discriminate strongly against ^{13}C during photosynthesis. Carbon isotope discrimination (Δ_p) as measured in photosynthetic tissues is 19–20‰ in C_3 plants and 4–5‰ in C_4 plants (Farquhar et al. 1989). In contrast, oceans exhibit only a slight ^{13}C fractionation during air-sea exchange, ~0.6‰ (Tans et al. 1993; Gruber et al. 1999; Gruber and Keeling 2001). This land-ocean difference in carbon isotope fractionation coupled with time series measurements of both concentration and δ^{13}C of atmospheric CO_2 around the world are used to estimate the terrestrial uptake of CO_2 for both spatial and temporal scales (Keeling et al. 1989; Tans et al. 1990; Ciais et al. 1995a, 1999; Francey et al. 1995; Keeling et al. 1995).

Spatial variations observed within δ^{13}C and concentration of atmospheric CO_2 have been attributed to relative sink strengths of land versus oceanic reservoirs, coupled to emissions of fossil fuels (Keeling et al. 1989). Temporal variations in biospheric sink strength have been linked to climate and large-scale weather changes influencing CO_2 exchanges from the natural reservoirs (Houghton et al. 1999; Bousquette et al. 2000). ^{13}C variation occurs across terrestrial ecosystems globally, which suggests that an understanding of both ^{13}C values of both stocks (canopy and soil organic matter) and fluxes

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(canopy and soil) is critical to fully understanding dynamics of the terrestrial/oceanic carbon sink (Lloyd and Farquhar 1994; Fung et al. 1997). More recent observations of the isotopic composition of CO_2 from terrestrial ecosystems indicate the potential for substantial isotopic variation in both temperate and tropical ecosystems (Bowling et al. 2002; Ometto et al. 2002; Pataki et al. 2003). If there are significant temporal differences between the carbon isotopic composition of organic matter and of CO_2 fluxes within ecosystems, then models characterizing terrestrial ecosystems based on organic matter values may not help us understand the controls over inter-annual variations in biospheric carbon sink strength. Thus, the magnitude of the oceanic versus terrestrial carbon fluxes need not temporally change, but the $\delta^{13}C$ fractionation within the terrestrial biosphere may instead be the dynamic feature.

Reservoir carbon sink strengths are calculated using mass balance approaches where measured or modeled CO_2 flux rates are coupled to the $\delta^{13}C$ composition for a specific reservoir (Francey et al. 1995; Fung et al. 1997). Two isotope components are required to understand carbon exchange in land biota: photosynthetic carbon isotope discrimination by leaves (Δ_p) and the carbon isotope ratio of ecosystem respiration ($\delta^{13}C_R$). The isotope values of these two parameters are assumed to be constant on an annual basis, although C_3 versus C_4 biome differences have been acknowledged recently in modeling efforts (Ciais et al. 1999; Battle et al. 2000). At the same time, Δ_p is known to vary with environmental factors (Farquhar et al. 1989; Ehleringer et al. 1993), suggesting that discrimination at the ecosystem scale may also change on an annual basis in response to overall variations in soil moisture availability.

Farquhar and Richards (1984) showed that carbon isotope discrimination can be modeled as

$$\Delta_P = a + (b - a)\frac{c_i}{c_a} \qquad (1)$$

where Δ_p is the carbon isotope discrimination of a leaf or needle, a is the isotopic fractionation associated with diffusion of CO_2 (4.4‰), and b is the net fractionation associated with carboxylation (27‰, Farquhar et al. 1989). Photosynthetic discrimination is controlled by the ratio of internal to external CO_2 concentrations (c_i/c_a) and is related to the carbon isotope ratio of organic matter ($\delta^{13}C_P$) and to the $\delta^{13}C$ of atmospheric CO_2 ($\delta^{13}C_a$) as:

$$\Delta_P = \frac{\left(\delta^{13}C_a - \delta^{13}C_P\right)}{\left(1 + \delta^{13}C_P/1,000\right)} \qquad (2)$$

Variation in $\delta^{13}C_p$ is linked most strongly to changes in c_i/c_a through the discrimination term, although $\delta^{13}C_a$ can be important in the understory and lower portions of dense canopies (Buchmann et al. 1997a, 1998b, 2002). $\delta^{13}C_P$ measurements and Δ_p calculated from organic isotopes integrate carbon inputs over extended growing conditions. Depending upon a plant's growth conditions, Δ_p and $\delta^{13}C_P$ values of dominant species can vary by 3–

4‰ as different leaf cohorts are produced during a year (Farquhar et al. 1989). This leads to the question of whether variation in the $\delta^{13}C$ of ecosystem respiration ($\delta^{13}C_R$) might also differ over a similar range of values, especially in coniferous forests where there is typically only a single leaf flush per year.

The $\delta^{13}C_R$ value integrates the $\delta^{13}C$ ratio of CO_2 recently respired from the soil, leaf, and stem reservoirs within a region. Keeling (1958) modeled $\delta^{13}C_R$ in several regions in the United States using a simple 2-box mixing model as:

$$\delta^{13}Ca = M \bullet 1/[CO_2]_a + \delta^{13}C_R \qquad (3)$$

where M is $[CO_2]_{trop}.(\delta^{13}C_{trop} - \delta^{13}C_R)$, $[CO_2]_{trop}$ is the CO_2 concentration outside of the system (in the troposphere), and $\delta^{13}C_{trop}$ is the carbon isotope ratio of CO_2 in the troposphere. By measuring $\delta^{13}C$ of CO_2 as $[CO_2]$ varies within a system, $\delta^{13}C_R$ emerges as the intercept of Eq. 3. The $\delta^{13}C_R$ value varies with site conditions, such as site water content, forest age, forest species, and succession (Buchmann et al. 1998b; Fessenden and Ehleringer 2001; Bowling et al. 2002).

The Pacific Northwest in North America is dominated by extensive coniferous forest ecosystems having the potential to store large amounts of carbon (Franklin and Dyrness 1988). In the Pacific Northwest as well as in other parts of western North America, there are large seasonal changes in rainfall and consequently in soil moisture. Typically in this area, high precipitation in the late fall through spring months precedes a summer drought (Steele 1952; Franklin 1972; Hare and Mantua, 2000; Gedalof and Smith 2001). Tree growth occurs typically as a spring flush, followed by a gradual decline in needle production and herbaceous leaf-out (Franklin 1972).

In this study, we focused on understanding the dynamics of carbon isotope variation in carbon stocks and CO_2 respiration from a dominant coniferous forest ecosystem in the Pacific Northwest. We examined temporal changes in the $\delta^{13}C$ of the carbon fluxes ($\delta^{13}C_R$, $\delta^{13}C_{R-soil}$, and $\delta^{13}C_{R-leaf}$ or leaf respired CO_2) as well as $\delta^{13}C$ in organic pools ($\delta^{13}C_{SOC}$ and $\delta^{13}C_P$). We hypothesized that ecosystem water balance would influence carbon isotope ratios of all carbon pools and fluxes. We chose an old growth coniferous forest in the southern Washington (USA) where we performed measurements on a 1-month to 2-month time interval (excluding wintertime) from 1998 to 2001.

Materials and methods

Study site

All measurements were made at the Wind River Canopy Crane Research Facility located within the Thornton T. Munger (T.T. Munger) Research Natural Area near Carson, Washington, USA (latitude 45°49′N; longitude 121°58′W, elevation: 355 m). A 74.5-m freestanding construction crane is located at this site, equipped with a suspended personnel gondola, which allowed

atmospheric and organic sampling to be conducted up to 65 m in height, a 79-m radius, and a 360° circumference around the crane for a total forest access of 2.3 ha (Dave Shaw, personal communication). The site contains an old growth stand with 400- to 450-year-old trees averaging 60 m in height and composed primarily of western hemlock (*Tsuga heterophylla*), Douglas-fir (*Pseudotsuga menziesii*), and western red cedar (*Thuja plicata*). Annual precipitation averages 2.5 m with 90% of the precipitation falling between October and May (Franklin 1972). Average annual temperature is 8.8°C with temperature ranges of 0°C (January) to 17.5°C (July) (Franklin and Dyrness 1988). Soils are composed of 2–3 m of sandy loams derived from weathered volcanic breccia, which overlies basaltic bedrock. About 2–4 cm of decomposing humus is located at the top of this mineral layer resulting from litter fall (Klopatek 2001). Within the understory, several vegetation layers exist: (1) 2–5 m tall species of mainly vine maple (*Acer circinatum*), Pacific dogwood (*Cornus nuttallii*), and western hemlock seedlings; (2) a shrub layer composed primarily of salal (*Gaultheria shallon*) and Oregon grape (*Berberis nervosa*); (3) a herb layer of twinflower (*Linnaea borealis*), vanilla leaf (*Achlys triphylla*) and red huckleberry (*Vaccinium parvifolium*); and (4) mosses of *Eurhynchium oreganum*, *Plagiothesium undulatum*, and *Dicranium fuscensens* at the surface. For more extensive species lists and information see Franklin (1972) and Franklin and Dyrness (1988).

Meteorological measurements

Micrometeorological data were measured at 30-min intervals at seven heights within the canopy and four depths in the soil and made available at http://depts.washington.edu/wrccrf/metdata/microclimate.html by several meteorological scientists stationed at the Wind River Canopy Crane Research Facility (WRCCRF).

Organic carbon measurements

Current-year foliage from sun-exposed, south-facing branches was collected from three western hemlock trees (*Tsuga heterophylla*) from the top (55 m) and middle (25 m) of the canopy. Current-year foliage from western hemlock seedlings (2 m) growing in forest gaps and understory regions (shaded) were also collected. Seven to ten clusters of needles (~120 total needles) were collected from the same branch on each sampling trip. Needles were oven dried at 80°C for 48 h immediately after collection using an oven located at the "Crane" facility. Foliage was then ground to a fine powder with a mortar and pestle.

Soil samples (30–50 g) were collected at three to five separate pits within the forest over a depth profile (litter, −0.1 cm (organic rich), −10 cm, −20 cm) for bulk litter and soil organic carbon (SOC) analyses. New pits were dug on each sampling trip. The soil was oven dried at 80°C for 48 h immediately after collection. Both coarse and fine roots were removed from dried soils using a 30 μm mesh and hand picking with tweezers and a magnifying glass. Carbonates were removed using 0.1 M HCl for 24 h at room temperature. The resulting SOC was then rinsed with deionized H_2O, dried at 70°C overnight, and then measured for $\delta^{13}C$ as described below.

Air measurements

Air was sampled from four heights within the canopy (0 m, 2 m, 24 m, and 53 m) during peak photosynthetic hours and at night when [CO_2] gradients between the top and bottom of the canopy exceeded 40 ppm. Canopy air was pulled through $^1/_4$-inch Dekoron tubing, a drying column (magnesium perchlorate), and a 100 ml glass flask with Teflon stopcocks, using a battery-operated diaphragm pump, and finally pushed through a LICOR 6200 (model LI-6200, LICOR, Lincoln, Neb., USA) for field estimates of [CO_2]. Air was captured in the 100 ml glass flasks and analyzed in

the laboratory for the $\delta^{13}C$ values of CO_2 within the flasks (Ehleringer and Cook 1998) and CO_2 concentration (Bowling et al. 2001). The precision of the measurements was ±0.03‰ for $\delta^{13}C$ and ±0.5 ppm for [CO_2].

Soil respired CO_2 (for $\delta^{13}C_{R-soil}$ measurements) was sampled from a series of 9.5 cm diameter collars (PVC rings) placed 5 cm into the ground (1–3 cm within the mineral layer) 8–24 h before actual measurements. A total of 12–15 collars were used for CO_2 efflux rates with a sub-sample of 3 collars for isotope analyses. For respiration rates, a closed system was designed incorporating a LICOR 6200 (LICOR, Lincoln, Neb., USA) equipped with a soil cuvette (model LI-6009, LICOR, Lincoln, Neb., USA) in which CO_2 was allowed to build naturally from ambient concentrations. CO_2 flux estimates were made after an initial 10-ppm CO_2 change in the chamber (total of five measurements made per collar; mean values were used in analyses). For isotope analyses, a magnesium perchlorate drying trap and a parallel array of six 100-ml glass flasks were placed into the closed loop used for respiration rates. Air samples were collected at intervals of 40–50 ppm change in chamber [CO_2] and brought back to the laboratory for isotope and concentration analyses.

Leaf respired CO_2 (for $\delta^{13}C_{R-leaf}$ measurements) was collected using the same closed loop and flask system, where 3–4 branchlets from western hemlock seedlings (total of 30 g) were clipped during the night from 2–3 m height in partial gap and deep shade microsites, placed directly into the LI-6009 cuvette head and sealed with a plastic cap. Samples were collected at intervals of 40–50 ppm change in chamber [CO_2].

CO_2 concentrations of air in all flasks were reanalyzed in the laboratory using a compressible volume (bellows) system equipped with an IRGA (model LI-6262) (Bowling et al. 2001).

Isotope analyses and calculations

We measured carbon isotope ratios using a Finnigan Delta S isotope ratio mass spectrometer equipped with an elemental analyzer (for organic samples) and a Finnigan MAT 252 isotope ratio mass spectrometer equipped with a PreCon gas-condensing device (for gas samples). The mass spectrometers were operated in a continuous flow mode. For organic analyses, we analyzed about 2 mg of dried, ground tissues per analysis. For atmospheric gas samples, the typical injection volume was 200–400 µl atmospheric air, depending on total carbon dioxide concentration within the sample. All carbon isotope ratios are expressed in delta notation (δ), presented relative to the Pee Dee Belemnite standard, and are corrected for $\delta^{17}O$. N_2O interference did not occur because the PreCon includes a gas chromatography column to separate CO_2 and N_2O. Overall precision of the carbon isotope measurements was typically better than ±0.11‰ for organic carbon and ±0.03‰ for carbon in gas samples.

The ratio of intercellular to ambient CO_2 (c_i/c_a) was calculated using the carbon isotope ratio of leaf or needle material ($\delta^{13}C_p$) and atmospheric CO_2 ($\delta^{13}C_a$ from mid-day measurements) using Eqs. 1 and 2.

The $\delta^{13}C$ value of respiration ($\delta^{13}C_R$ for ecosystem respiration and $\delta^{13}C_{R-soil}$ for soil respired CO_2) was calculated using Eq. 3. Geometric mean (GM) regressions were used on $\delta^{13}C$ versus 1/[CO_2] plots of CO_2 collected at night from the canopy ($\delta^{13}C_R$) and from the cuvette ($\delta^{13}C_{R-soil}$ and $\delta^{13}C_{R-leaf}$). Keeling plots were typically constructed from 15–20 individual flask samples. Flasks $\delta^{13}C$ values of CO_2 exceeding greater than three standard deviations away from predicted regression values were excluded from the regression (routine outlier test). Uncertainties are reported as the standard error of the intercept.
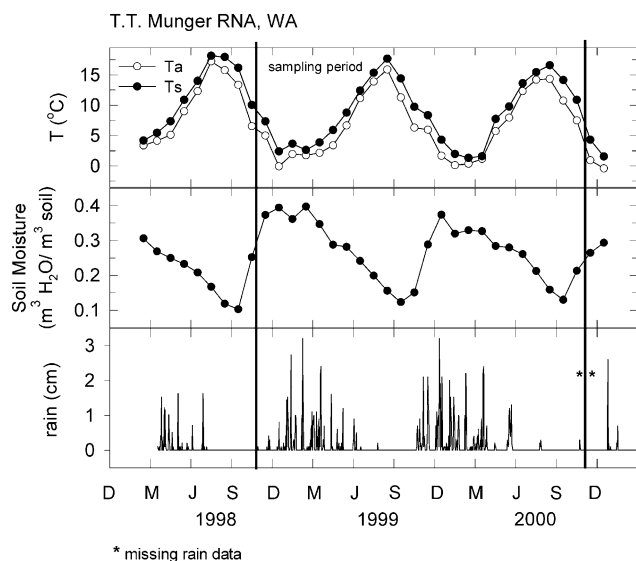
Fig. 1 Meteorological variation of soil (T_s) and air temperature (T_a), soil water content, and precipitation events, measured at the Wind River Canopy Crane Research Facility (WRCCRF) tower located 30 m west of the air-sampling site. Data were collected daily at 30-min intervals and available on the web at: http://depts.washington.edu/wrccrf/metdata/microclimate.html. The figure is partitioned to highlight the years when this study was conducted (October 1998 through October 2000)

Results

Local weather and meteorology

There were significant interannual and interseasonal fluctuations in air temperature, soil moisture, and precipitation over the 3 years of sampling (Fig. 1). A seasonal pattern was clear: winters were cold and wet and summers were warm and dry in this location. About 90% of the precipitation fell between October and May, with the majority of water falling between December and February. Little if no precipitation fell between July and September. Soil water content rose from October until April, and was lowest in August and September, even below the detection limit of the 40-cm moisture probe (which was 0.2 m^3 H$_2$O/m^3 soil). Soil and air temperatures exhibited similar patterns with peaks in summer and troughs in winter.

El Niña/La Niña transitions were also observed in this data set. During the El Niño of 1998, total precipitation was 24% less than the long-term average. In contrast, a La Niña event occurred in 1999 and precipitation was 33% greater than the long-term average. Annual soil water content was ~13% lower in 1998 versus 2000 and ~5% higher in 1999 versus 2000. The soil water content was at its lowest during the month of September for both 1999 and 2000, yet the low was 1 month earlier in 1998. Average annual temperatures did not show the same trends as the water cycle. Mean annual temperatures (T) were highest in 1998 (air T=8.3°C, soil T=10.4°C),

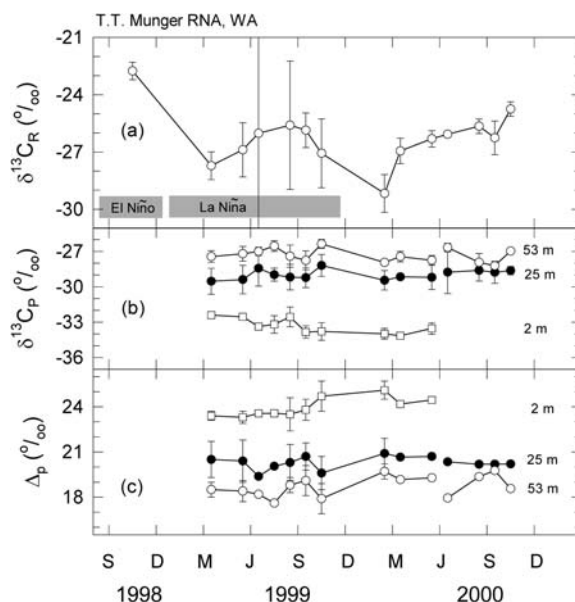**Fig. 2a–c** Monthly measurements of ecosystem respiration ($\delta^{13}C_R$), bulk organic tissue from current-year needles ($\delta^{13}C_P$), and carbon discrimination (Δ_p) are shown. Three years of data are reported: 1998 (an El Niño year), 1999 (a La Niña year), and 2000 (typical meteorological conditions). *Error bars* indicate standard error. **a** $\delta^{13}C_R$ from June 1999 was calculated from only 6 samples causing a high standard error (±6.7‰); all other $\delta^{13}C_R$ values were calculated from 15–20 samples. **b** Foliar samples from current year needle cohorts were collected at the top (55 m), middle (25 m), and bottom (2 m) of the canopy from *Tsuga heterophylla* trees and seedlings. **c** Using Eq. 2, discrimination at the leaf (Δ_p) was calculated from current-year *Tsuga heterophylla* needles ($\delta^{13}C_P$), and canopy CO_2 ($\delta^{13}C_a$ only measured in 1999) collected during peak photosynthetic times (1320–1640) at the top (5 m), middle (25 m), and bottom (2 m) of the canopy

followed by 1999 (air T=6.8°C, soil T=8.9°C), then 2000 was the coldest (air T=6.2°C, soil T=8.2°C).

Carbon isotope ratios of respired CO_2 and organic pools

Ecosystem respiration ($\delta^{13}C_R$) showed significant temporal variation over the three years of analysis (Fig. 2a). An overlying seasonal pattern was seen in $\delta^{13}C_R$ where steady ^{13}C enrichment occurred as the growing season progressed. $\delta^{13}C_R$ increased 2.4‰ between late winter (February) and mid-fall (September). On the same time scale, ecosystem water stress increased between July and September as the majority of annual precipitation fell between October and April, with little to no rain falling after June (Fig. 1). $\delta^{13}C_R$ also varied annually, ranging from −22.8‰ (October 1998, El Niño) to −27.1‰ (October 1999, La Niña), with an average climatic year yielding a value of −24.8‰ (October 2000). These differences correlated with variation in the soil moisture availability (r=0.63 to precipitation, P=0.03), where drought conditions occurred in 1998 (low precipitation), unusually large precipitation totals occurred in 1999, and normal levels of precipitation (~2 m total) fell in 2000 (Fig. 1).
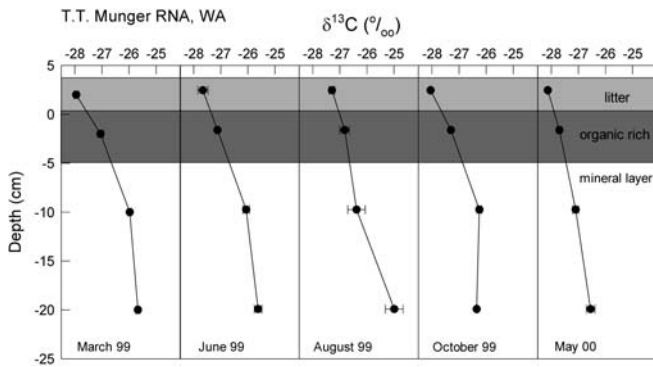
Fig. 3 Depth profiles of carbon isotope ratios of bulk soil organic carbon ($\delta^{13}C_{SOC}$) are shown over the course of the growing season in 1999 and in spring of 2000. Recently fallen and intact litter was observed on the top 1 cm of the soil, with 2–5 cm of decomposing litter/humus in the organic rich layer and >20 cm of mineral soil interlaid with fine and sub-course roots. Symbols represent averages for $\delta^{13}C_{SOC}$ (n=3–5 different soil pits) with *error bars* corresponding to standard error

Fig. 4 a Carbon isotope ratios of soil respired CO_2 ($\delta^{13}C_{R\text{-soil}}$) were collected at multiple soil collars (n=2–3) in 2000. **b** Soil flux rates in 1999 and 2000 are averaged from 12–15 collars and compared to published values measured from the same area (Klopatek 2001). *Circled symbols* correspond to respiration rates measured from the same collars used in the Klopatek 2001 study. *Error bars* represent standard error

Foliage carbon isotope ratios from current-year cohorts of *Tsuga heterophylla* canopy trees and seedlings did not change significantly over this 3-year period (Fig. 2b) (ANOVA, P=0.24). Though temporal variation in $\delta^{13}C_P$ was small (and not significant, P=0.24), a distinct height-dependent pattern was maintained regardless of season or year. Foliar $\delta^{13}C_P$ values were more ^{13}C enriched with increased height (2‰ between top and mid-canopy heights and 3.5‰ between mid- to below-canopy heights). Patterns of leaf level discrimination (Δ_p, Fig. 2c) corresponded to foliar $\delta^{13}C_P$ (inherent in the calculation, see Eq. 2) where little variation existed within the season, though spatial variation could be seen in the current 1999-year cohort (average Δ_p at canopy top=18.7‰, mid canopy=20.4‰, understory=24.0‰).

The isotopic composition of the organic rich SOC (top 10 cm) did not change over the course of a season (Fig. 3). However, $\delta^{13}C_{SOC}$ increased with increasing depth in the soil column (as much as 2.2‰, with the largest ^{13}C enrichment between the decomposing litter layer and the mineral interface). Spatial variation in $\delta^{13}C_{SOC}$ among soil pits was small (±0.02–0.1‰) except for August 1999 samples (±0.3‰), which might be related to microsite $\delta^{13}C_{SOC}$ sampling variation and/or soil heterogeneity.

Temporal changes in the carbon isotope ratio of soil respired CO_2 ($\delta^{13}C_{R\text{-soil}}$) and soil CO_2 efflux rate followed a consistent seasonal pattern (Fig. 4a, b). Carbon isotope ratios of soil respired CO_2 ($\delta^{13}C_{R\text{-soil}}$) became enriched in ^{13}C over the growing season with values in May 2000 of −26.6‰ and in October 2000 of −20.4‰. Flux rates were lowest in the winter (1.4 μmol m^{-2}s^{-1} in February 2000) and highest at the peak of the growing season (9.1 μmol m^{-2}s^{-1} in June 2000). The soil CO_2 efflux rates were equal to or slightly higher than published values measured at the T.T. Munger RNA (Klopatek 2001).

Though ecosystem and soil respiration followed similar patterns to precipitation events (with ^{13}C-depeted
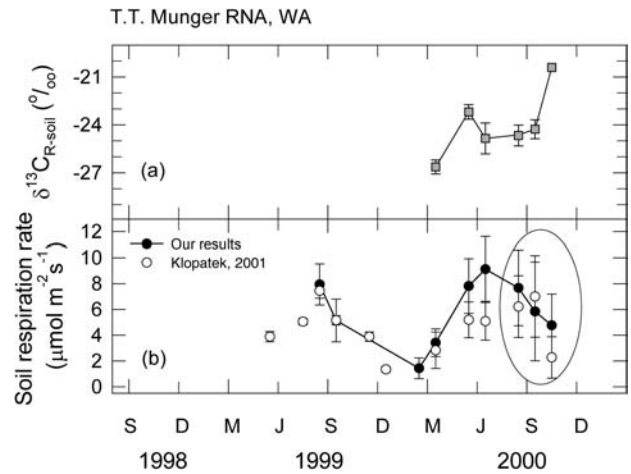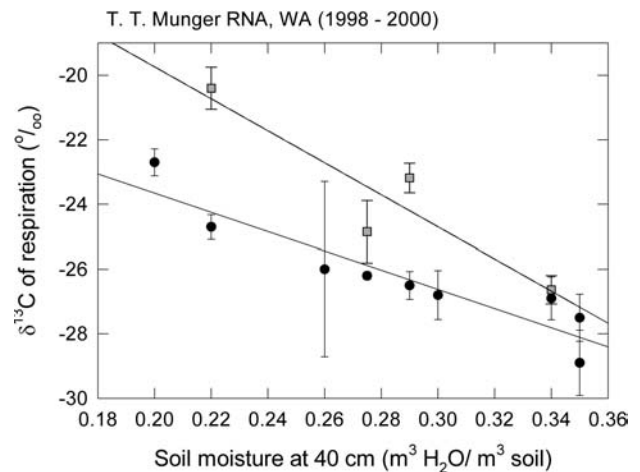

Fig. 5 Carbon isotope ratios of ecosystem respiration (*closed circles* $\delta^{13}C_R$) and soil respiration (*squares* $\delta^{13}C_{R\text{-soil}}$) are compared to soil moisture measured at 40 cm on site. Measurements taken between October and June in years 1998 to 2000 are shown in this graph. The detection limit of the 40 cm moisture probe used in this study was 0.2 m^3 H_2O/m^3 soil (see: http://depts.washington.edu/wrccrf/metdata/microclimate.html). Measurements between July and September fell outside of the detectable limits of this probe and therefore were not used in this regression analysis. This analysis shows statistically significant covariation between soil moisture and respiration (r=0.93 for $\delta^{13}C_R$ and $\delta^{13}C_{R\text{-soil}}$) but a different correlation between soil moisture and ecosystem respiration (Y=−29.7X−17.7) versus soil respiration (Y=−49.5X−9.8)

values corresponding to precipitation events, Figs. 1, 2a, 4a), $\delta^{13}C_R$ and $\delta^{13}C_{R\text{-soil}}$ were most highly correlated with soil moisture levels (r=0.93, P<0.001 Fig. 5). Within the measurement limits of the moisture probe at 40 cm depth, the isotopic composition of soil respiration covaried with local soil moisture; dry periods corresponded to ^{13}C-

Table 1 Carbon isotope signatures of respiring reservoirs. Carbon isotope ratios of ecosystem respiration ($\delta^{13}C_R$; $n=18$–20), soil respiration ($\delta^{13}C_{R\text{-soil}}$; $n=6$), and leaf respiration ($\delta^{13}C_{R\text{-leaf}}$; $n=5$–6) over the growing season in 2000. $\delta^{13}C_R$ and $\delta^{13}C_{R\text{-leaf}}$ were measured at night, while $\delta^{13}C_{R\text{-soil}}$ was measured at mid-day. *Error bars* indicate standard error

Date	$\delta^{13}C_R$ (‰)	$\delta^{13}C_{R\text{-soil}}$ (‰)	$\delta^{13}C_{R\text{-leaf}}$ (‰) Sun	$\delta^{13}C_{R\text{-leaf}}$ (‰) Shade
23/3/2000	−26.9	−26.9		
15/5/2000	−26.3	−24.2		
21/6/2000	−26.1	−24.3		
15/8/2000	−25.6	−24.7		
8/9/2000	−26.3	−24.1	−28.8	−31.0
15/10/2000	−24.8	−20.1	−28.6	−30.9

enriched $\delta^{13}C_R$ and $\delta^{13}C_{R\text{-soil}}$ values. During the summer months (June–September), soil moisture values decreased below detectable limits. During these periods, both $\delta^{13}C_R$ and $\delta^{13}C_{R\text{-soil}}$ values were ^{13}C-depeted relative to the values extrapolated based on the modeled regression analysis in Fig. 5. This late-summer result suggests that these trees might have accessed deeper soil water (lower than 40 cm) and soil respiration might have occurred from lower in the soil column during this time and therefore were not as moisture-limited as predicted by the extrapolation of the regression model in Fig. 5. Correlation analyses were also conducted on vapor pressure deficit vpd and either $\delta^{13}C_R$ or $\delta^{13}C_{R\text{-soil}}$ with limited to no correlation observed between factors (data not shown).

Based on a few observations at the end of this study, ecosystem respiration ($\delta^{13}C_R$) was composed of relatively ^{13}C-enriched CO_2 respired from the soil ($\delta^{13}C_{R\text{-soil}}$) and relatively ^{13}C-depleted CO_2 respired from the leaves ($\delta^{13}C_{R\text{-leaf}}$, measured from a cuvette) (Table 1). The carbon isotope ratio of CO_2 respired by gap-exposed leaves (−28.5‰) and shade leaves (−30.5‰) were more ^{13}C-enriched than expected based on organic value observations in Fig. 2. This is likely to have been a result of water stress late in the summer causing lower stomatal conductance and therefore respiration of carbon that had been fixed at lower c_i/c_a values.

Overall, the results from this study showed temporal changes in the CO_2 respired at the ecosystem ($\delta^{13}C_R$) and the soil ($\delta^{13}C_{R\text{-soil}}$) levels, with noteworthy correlations with soil moisture levels. Yet at the same time, the isotopic composition organic pools of carbon ($\delta^{13}C_P$, and $\delta^{13}C_{SOC}$) showed almost a 3-fold smaller seasonal change. This is believed to result from differences in timescales of integration, where organic carbon pools integrate discrimination variation over the course of a season, while carbon fluxes show immediate carbon isotope discrimination from a rapid turnover respiring reservoir.

Discussion

Presently, the terrestrial sink strength for atmospheric CO_2 is estimated from atmospheric CO_2 measurements and carbon isotope discrimination (Δ_p) within organic tissues associated with different latitudes (Ciais et al. 1995a; Fung et al. 1997; Battle et al. 2000). These models use Δ_p to scale from the leaf up to the region to estimate regional $\delta^{13}CO_2$ of the atmosphere (Ciais et al. 1995a; Battle et al. 2000). Temporal variation in global Δ_p values has not been incorporated into models (Battle et al. 2000), but has been shown to vary with space (C_3 vs C_4, Buchmann et al. 1998a), species composition (deciduous vs coniferous, Brooks et al. 1997), and environmental conditions (light, Ehleringer et al. 1986; Buchmann et al. 1997b; water-limitation, Ryan and Yoder, 1997). Δ_p is isotopically linked to the $\delta^{13}C$ of respired CO_2 because autotrophic respiration is a non-fractionating process (Lin and Ehleringer 1997) and reflects the carbon isotope ratio of organic material ($\delta^{13}C_P$, see Eq. 2, though see Duranceau et al. 1999, 2001). Therefore, Δ_p has been used in modeling efforts to represent the $\delta^{13}C$ of CO_2 respired from a terrestrial ecosystem (Ciais et al. 1995a; Fung et al. 1997; Battle et al. 2000).

Our data showed limited temporal variation in Δ_p at specific locations within the canopy (change of 1‰ for 1999, Fig. 2c), although there were also acclimatory location-dependent adjustments in Δ_p with canopy position. Δ_p differences with canopy height are consistent with observations from other studies (e.g., Buchmann et al. 1997b) and can be explained by light reduction within the canopy (Ehleringer et al. 1986, 1993; Farquhar et al 1989; Panek 1996; Buchmann et al. 1997b; Ryan and Yoder 1997). Δ_p varied little over the growing season due to the consistent carbon isotope ratio of $\delta^{13}C_P$ at each canopy position (e.g., Fig. 2b). Only a slight change in $\delta^{13}C_P$ was observed at the start of the growing season during leaf elongation (June 2000 only). From this result, we suspect that the $\delta^{13}C_P$ is largely determined during bud break and needle elongation from a flush of stored carbon (from the previous growing season) and recently fixed CO_2. The carbon assimilated during the remainder of the growing season appeared to have limited influence on the carbon isotope ratio of the needle tissues. Avenues for recently fixed carbon during this period likely include roots, stems, storage, and exudate formation. Both mini-rhizotron root studies and stem diameter studies have detected significant root growth and bole diameter increase during the summer months (Dave Shaw, personal communication).

Because Δ_p, $\delta^{13}C_P$, and $\delta^{13}C_{SOC}$ values did not appreciably vary over the course of the growing season (Figs. 2, 3), ecosystem respiration ($\delta^{13}C_R$) was also expected to remain isotopically invariant as it represents

CO_2 derived from autotrophic and heterotrophic components of the system. Yet, $\delta^{13}C_R$ values varied by as much as 4.4‰ on seasonal and 3.1‰ on annual time scales (noteworthy 1998–1999 El Niño/La Niña transition, Fig. 2a). Clearly one must distinguish among the isotopic composition of fast versus slow-turnover carbon pools. Soil respiration ($\delta^{13}C_{R\text{-soil}}$) showed an even larger 6.2‰ change over the course of the season (Fig. 4a). These results suggest that the $\delta^{13}C$ of respired fluxes may be more dynamic than previously assumed and need not reflect the $\delta^{13}C_P$, and Δ_p associated with the leaf organic matter.

Recent work by Bowling et al. (2002) and Ekblad and Högberg (2001) has shown correlations with $\delta^{13}C$ of respiration (ecosystem and soil respiration, respectively) with local vpd. In these studies, relative humidity accounted for more than 80% of the observed variations in $\delta^{13}C$ of the respiring reservoir (ecosystem or soil). The vpd values were positively correlated with $\delta^{13}C_R$ after a lag of 4–7 days (Bowling et al. 2002) indicating a relatively rapid response of $\delta^{13}C_R$ to the local meteorological conditions. In the present study, we did not detect as great a correlation of $\delta^{13}C_R$ with relative humidity as with soil moisture. The $\delta^{13}CO_2$ respired in this ecosystem appeared to be most significantly correlated with soil moisture levels on the day of the measurements (Fig. 5), with greater sensitivity observed in the soil respired reservoir ($\delta^{13}C_{R\text{-soil}}$). Possible reasons for this difference are that heterotrophic respiration may be a stronger component to this ecosystem and that soil moisture and vpd share a common impact on stomatal dynamics in these forest trees.

Regardless of mechanism, it is clear that the $\delta^{13}C$ of ecosystem respiration was influenced by soil water availability (either vpd or soil moisture) and that transitions in $\delta^{13}C_R$ can be experienced over periods of months (this study) or days (Bowling et al. 2002).

From this study and others (Flanagan et al. 1996, 1999; Buchmann et al. 1997a, 1997b; Bowling et al. 2002; Ometto et al. 2002; Pataki et al. 2003), it is clear that both $\delta^{13}C_{R\text{-soil}}$ and $\delta^{13}C_R$ are dynamic, reflecting recent environmental changes and the proportions of respiratory sources contributing to those fluxes. Environmental factors such as vapor pressure deficit and available soil moisture can influence $\delta^{13}C_{R\text{-soil}}$ and $\delta^{13}C_R$ on a short-term basis. This is an important consideration for interpreting bi-monthly flask measurements made by the global CO_2 flask sampling networks. Some of the variability observed within the $^{13}CO_2$ released from an ecosystem at any given latitude could impact the observed values and ultimately affect our interpretation of those tropospheric observations. Measurements of $\delta^{13}C_R$ made on a weekly basis may also be more indicative of integrated ecosystem gas exchange responses, which could contribute to our interpretations of eddy covariance observations. Clearly the temporal dynamics of $\delta^{13}C_R$ observed in this study suggest that interpretation of ecosystem function based on $\delta^{13}C_p$ values alone may not provide sufficient information on the ^{13}C dynamics that influence isotopic exchange between the biosphere and the atmosphere. Modeling efforts utilizing both $\delta^{13}C_R$ and regional flask data may better capture the changing nature of terrestrial ecosystem gas exchange impacts on the isotopic composition of atmospheric CO_2.

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