



Can carbon isotopes be used to predict watershed-scale transpiration?

T. G. Pypker,¹ H. R. Barnard,² M. Hauck,² E. W. Sulzman,³ M. H. Unsworth,⁴ A. C. Mix,⁴ A. M. Kennedy,² and B. J. Bond²

Received 31 March 2008; revised 27 January 2009; accepted 11 February 2009; published 29 April 2009.

[1] The Penman-Monteith equation is often used to estimate transpiration, but an important limitation to this approach, especially for mountainous forested sites, is an accurate estimate of canopy conductance averaged over the area of interest (G_s). We propose a method for estimating watershed-scale transpiration using estimates of G_s derived from measurements of stable isotopes. To estimate G_s , we first determined the isotopic composition of ecosystem respiration ($\delta^{13}C_{ER}$) as derived from the $^{12}C:^{13}C$ ratio of respired CO_2 entrained within nocturnal cold air drainage flows exiting the base of the watershed. An isotope-derived estimate of recent canopy conductance over the entire watershed (G_{s-I}) was derived using biophysical models. To estimate daily average transpiration, we applied G_{s-I} and other measured environmental variables to the Penman-Monteith equation. The results were compared with an independent measure of transpiration using the heat dissipation method at four locations within the watershed. Considering the large number of assumptions required for both estimates of transpiration, the two estimates were remarkably similar. The relationship between the values derived by the two techniques was statistically significant (p value < 0.01), the slope of the line (slope = 1.7) was not statistically different from 1 (p value > 0.1), but the standard error was large (SE = 0.48). The results demonstrate that this technique holds promise, but the effects of potential limitations require further attention. The future research necessary to fully demonstrate the validity of this potentially promising method is discussed.

Citation: Pypker, T. G., H. R. Barnard, M. Hauck, E. W. Sulzman, M. H. Unsworth, A. C. Mix, A. M. Kennedy, and B. J. Bond (2009), Can carbon isotopes be used to predict watershed-scale transpiration?, *Water Resour. Res.*, 45, W00D35, doi:10.1029/2008WR007050.

1. Introduction

[2] Transpiration is a large component of the hydrological budget, often accounting for well over half of the water that exits annually from closed-canopied ecosystems [Hewlett, 1982]. It is therefore of great interest to hydrologists to measure and monitor rates of transpiration. In many analyses, evapotranspiration (the combination of both free evaporation from wet surfaces and transpiration) is inferred from a simple mass balance approach as difference between total precipitation and all other measured outputs. However, this approach cannot differentiate between transpiration and evaporation, and it may also be seriously in error if net changes in water storage or leakage through bedrock are not assessed. Evaporation is usually a very small component of evapotranspiration when closed canopies are dry [e.g.,

Blanken and Rouse, 1995; Schaap and Bouten, 1997]. Evapotranspiration may be estimated from microclimatology measurements by applying the Penman-Monteith equation, which elegantly incorporates the biological and physical controls over transpiration and provides a more accurate estimation of transpiration than other empirical models as it is based on sound biophysical principles [Monteith and Unsworth, 2007]. However, the Penman-Monteith equation requires an accurate estimate of canopy conductance (G_s). This term may vary greatly in response to variations in vegetation structure and function as well as microclimate. Accurate estimation of stomatal conductance is not an issue in low-stature vegetation, like grasslands, because transpiration in these systems is relatively insensitive to changes in G_s ; that is., short canopies are relatively uncoupled from the atmosphere [Jarvis and McNaughton, 1986; Monteith and Unsworth, 2007]. In tall, coniferous forest canopies, however, G_s exerts strong control over transpiration [Jarvis and McNaughton, 1986; Monteith and Unsworth, 2007], and is highly variable over daily, weekly and annual time scales [e.g., Bond and Kavanagh, 1999; Marshall and Waring, 1984]. Therefore, using a mean value for G_s derived for a particular species or vegetation type [e.g., Kelliher *et al.*, 1995] can lead to significant errors

¹School of Forest Resources and Environmental Sciences, Michigan Technological University, Houghton, Michigan, USA.

²Forest Ecosystem and Society, Oregon State University, Corvallis, Oregon, USA.

³Department of Crop and Soil Science, Oregon State University, Corvallis, Oregon, USA.

⁴College of Oceanic and Atmospheric Sciences, Oregon State University, Corvallis, Oregon, USA.

when the Penman-Monteith equation is used to estimate transpiration of forests [Mackay et al., 2007].

[3] An alternative to using the Penman-Monteith equation is to measure transpiration directly using instrumentation, or to estimate transpiration using other models. Direct approaches, such as eddy covariance techniques (which measure evapotranspiration) [Baldocchi et al., 1988; Finnigan et al., 2003; Monteith and Unsworth, 2007] and direct transpiration measurements of individual trees (heat dissipation method [Granier, 1987]) can provide excellent information, but they too, have limitations. Eddy covariance measurements generally require relatively flat, uniform terrain, which makes this approach generally untenable for hydrological studies in mountainous regions [e.g., Aubinet et al., 2003; Black et al., 1996; Lavigne et al., 1997; Paw U et al., 2004; Staebler and Fitzjarrald, 2004]. Transpiration measurements using the heat dissipation method [Granier, 1987] are time consuming, require a large amount of electrical energy, and a very large sample of plants is necessary to adequately represent variability in ecosystems with elevation gradients [Adelman et al., 2008; Lorant et al., 2008]. Indirect estimates using models such as BIOME-BGC [e.g., Coops et al., 2001; Kimball et al., 1997; Melillo et al., 1995; Running, 1994; Running and Hunt, 1993] can provide accurate estimates of both water and carbon exchange from forests, but detailed information about canopy structural and physiological properties as well as meteorological data and soil properties are needed to drive such models.

[4] New methods are emerging that may permit the monitoring of ecosystem level responses to climate variability in mountainous terrain [e.g., Finnigan, 2008; Pypker et al., 2008; Staebler and Fitzjarrald, 2004; Sun et al., 2007; Turnipseed et al., 2003; Yi et al., 2008]. These new methods are based on understanding of how airflows in three dimensions. In particular, advective, mass movement of air commonly occurs on sloping terrain (e.g., cold air drainage at night) and makes it difficult to apply eddy covariance techniques in mountainous regions [e.g., Aubinet et al., 2003; Black et al., 1996; Lavigne et al., 1997; Paw U et al., 2004; Staebler and Fitzjarrald, 2004]. In addition, new methods have been developed, and applied initially in areas with minimal topographical relief, that relate the measured ratio of ^{13}C to ^{12}C in respired CO_2 to the physiological response of forests to changing environmental conditions [e.g., Alstad et al., 2007; Bowling et al., 2002; Farquhar et al., 1989; Högberg et al., 2001; McDowell et al., 2004a; Ometto et al., 2002]. These isotopic techniques have recently been combined with our improved understanding of cold air drainage patterns to assess ecosystem sensitivity to changing environmental conditions in mountainous watersheds [Pypker et al., 2007a, 2008]. Ultimately, these newly combined techniques could be useful as tools to help hydrologists monitor how transpiration from an entire watershed responds to changing environmental conditions and consequently more accurately resolve the transpiration component of the water balance.

[5] To understand why carbon isotopes might be used to monitor transpiration, one must first understand how carbon isotopes are fractionated during photosynthesis and respiration by plants. During photosynthesis C_3 plants (almost all woody plants and most temperate herbaceous plants are C_3 plants) preferentially assimilate $^{12}\text{CO}_2$ over $^{13}\text{CO}_2$. This

fractionation is caused by differences in diffusion rates when CO_2 enters the stomata and by discrimination by the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco), which preferentially accepts $^{12}\text{CO}_2$ during the carboxylation process [Farquhar et al., 1989]. When plants are water stressed because of low soil moisture availability and/or high atmospheric vapor pressure deficit (VPD), plants often close their stomata, thereby limiting the availability of CO_2 for fixation. When CO_2 becomes limited, rubisco accepts more $^{13}\text{CO}_2$. Therefore, the stomatal conductance of a leaf is one of the two major influences that affect the ratio of ^{13}C to ^{12}C in sugars resulting from photosynthesis because stomata control the supply of CO_2 to rubisco. The other major influence is carboxylation capacity. Farquhar et al. [1989] mathematically described the relationship between carbon isotope fractionation during photosynthesis ($\Delta^{13}\text{C}$), the concentration of CO_2 within the leaf (C_i) of C_3 plants, and the concentration of CO_2 in the atmosphere (C_a):

$$\Delta^{13}\text{C} = \frac{C_i}{C_a}(b - a) + a \quad (1)$$

where a is the fractionation against ^{13}C due to the diffusion of CO_2 into the stomata and b is the net fractionation due to carboxylation. On the basis of both theoretical and empirical analysis, the values of a and b are commonly assumed to be 4.4‰ and 27.7‰, respectively [Farquhar et al., 1989]. Subsequent to carboxylation, the sugars are transported to various parts of the plant, and from the roots to the soil. Cells in growing shoots, and microorganisms in the soil, use the sugars for respiration, releasing CO_2 . Recent research suggests that a large portion of CO_2 respired from entire ecosystems (both plants and soils) is produced from sugars that were created 0–4 days earlier [Andrews et al., 1999; Bowling et al., 2002; Ekblad et al., 2005; Ekblad and Högberg, 2001; Horwath et al., 1994; Steinmann et al., 2004]. Because a large portion of the CO_2 comes from recently manufactured sugars, the isotopic signal of the respiration can be used to explore ecosystem-scale responses to plant water stress.

[6] Recent research has used the “Keeling plot” approach to monitor how changing environmental variables affect the ratio of ^{13}C to ^{12}C in respiration [Keeling, 1958, 1961]. The Keeling plot is a two end-member mixing model that assumes there are two sources of CO_2 in air collected in the canopy air space, ambient CO_2 and respired CO_2 [Keeling, 1958, 1961]. By using regression analysis to relate the ratio of ^{13}C to ^{12}C (y axis) to the inverse of the CO_2 concentration (x axis) of multiple air samples collected during the course of one night, one can determine the isotopic composition of the respiration by extrapolating to the y intercept. To reduce the uncertainty, this method requires the samples to have a wide range of CO_2 concentrations [Pataki et al., 2003]. When using the Keeling plot approach, the $^{13}\text{C}:^{12}\text{C}$ ratio is usually standardized by comparing the $^{13}\text{C}:^{12}\text{C}$ of the air sample (R) to a standard (Vienna Pee Dee belemnite (R_{std})) in order to express the carbon isotope ratios as $\delta^{13}\text{C}$ (‰):

$$\delta = \left(\frac{R}{R_{\text{std}}} - 1 \right) 1000 \quad (2)$$

In recent reports, we demonstrated that the very conditions that complicate many micrometeorological studies in mountainous regions may offer an opportunity for an entirely different approach for studying processes at the ecosystem scale [Pypker *et al.*, 2007a, 2007b]. In a deeply incised watershed (20 to 33° slopes) in western Oregon, we estimated that more than 90% of the respired CO₂ from the entire ecosystem can be flushed from the watershed by nocturnal cold air drainage [Pypker *et al.*, 2007b]. We also found that, on most clear nights, the range of CO₂ concentrations in drainage air over a single night was sufficient to use the Keeling plot approach to determine the carbon isotope composition of ecosystem-respired CO₂ ($\delta^{13}\text{C}_{\text{ER}}$) [Pypker *et al.*, 2007a]. Using this technique we demonstrated that changes in $\delta^{13}\text{C}_{\text{ER}}$ may be used to predict changes in G_s [Pypker *et al.*, 2008]. If this finding holds up to further scrutiny in other watersheds, and the measurement techniques are improved, it may be possible to quantify the main control on transpiration of forests in mountainous ecosystems. Furthermore, by combining the deduced G_s and locally measured meteorological data in a standard transpiration model, such as the Penman-Monteith equation [Monteith and Unsworth, 2007], it may be possible to predict watershed-scale transpiration. However, questions remain.

[7] For $\delta^{13}\text{C}_{\text{ER}}$ to be used to predict G_s using the technique outlined by Pypker *et al.* [2008] the only significant source of isotope variability must be due to fractionation during recent carbon fixation. If significant postassimilation fractionation [e.g., Badeck *et al.*, 2005; Bowling *et al.*, 2008; Duranceau *et al.*, 1999, 2001; Ghashghaie *et al.*, 2001, 2003; Hymus *et al.*, 2005; O'Leary, 1981; Prater *et al.*, 2006; Xu *et al.*, 2004] occurs in Douglas fir forests, we would be unable to predict transpiration using $\delta^{13}\text{C}_{\text{ER}}$ unless this additional term could be quantified. In addition, we assume the isotopic signal from respiration remains constant throughout the night. If these or other assumptions defined in the methods are violated, then we would be unable to predict transpiration. The goal of this paper is to determine whether $\delta^{13}\text{C}_{\text{ER}}$ has the potential to be used as a tool to estimate the transpiration of an entire watershed using the isotope-derived G_s in combination with locally measured meteorological data.

2. Materials and Methods

2.1. Study Site

[8] The study area was a 96 ha watershed (“watershed 1”), located in the H. J. Andrews Experimental Forest in the western Cascades of central Oregon, USA (44.2°N, 122.2°W) (Figure 1). The watershed is predominately covered by young Douglas fir replanted following clear-cut harvesting in the late 1960s. Smaller components of the tree basal area consist of western hemlock (*Tsuga heterophylla* (Raf.) Sarg), bigleaf maple (*Acer macrophyllum* Pursh), vine maple (*Acer circinatum* Pursh) and red alder (*Alnus rubra* Bong.); the angiosperm populations are greatest within the riparian area [Moore *et al.*, 2004]. The canopy was between 25 and 28 m tall [Moore *et al.*, 2004]. The soil has Andic properties, is seasonally reduced, and has loamy to gravelly clay loam texture [Swanson and James, 1975]. The site has wet mild winters and warm dry summers with a mean

annual precipitation of 2300 mm, about 80% of which falls during the winter months [Rothacher *et al.*, 1967]. In the warm dry summers, cold air drainage occurs on most nights [Pypker *et al.*, 2007a].

2.2. Environmental Variables

[9] A 37 m tower located at the base of the watershed was instrumented in May 2005 with ten shielded thermistors (Model 107 temperature probe, Campbell Scientific, Inc., Logan, Utah), a net radiometer (Q7 REBS, Campbell Scientific, Inc.), a shielded temperature/RH probe (HMP45c, Campbell Scientific, Inc.), eight 2-D sonic anemometers (WS425, Vaisala, Helsinki, Finland) and two 3-D sonic anemometers (81000, R. M. Young, Traverse City, Michigan). The 2-D and 3-D sonic anemometers were measured at 0.1 and 1 Hz, respectively, and values were averaged over 1 min intervals. The remaining instruments were recorded every 1 s and averaged over 15 min intervals (using CR10X and CR23X data loggers, Campbell Scientific, Inc.).

[10] Perpendicular to the axis of the valley, a transect of eight plots (four on each slope) was established in the spring of 2005 (Figure 1). At each plot, air temperature/relative humidity was measured at midcanopy (HMP45c, Campbell Scientific, Inc.) and soil moisture (Echo-20, Decagon Devices, Pullman, Washington) and soil temperature (model 107 temperature probe, Campbell Scientific, Inc.) were measured at 5, 30 and 100 cm depth. Calibration equations that were specific to HJA soils were used to convert the millivolt signal from the soil moisture sensors to volumetric water content [Czarnomski *et al.*, 2005]. Observations were recorded by a data logger (CR23X, Campbell Scientific, Inc.) every 15 s and averaged over 15 min intervals.

2.3. Sample Collection and Analysis

[11] Air samples for isotopic analysis were collected using methods outlined by Pypker *et al.* [2008]. Air samples were collected on 11 nights from 8 May to 25 October 2006 for isotope analysis using an automated air collection system [Hauck, 2006] at three heights (3, 10, 30 m above ground) on the tower (Figure 1). Air was pumped at $\sim 1.5 \text{ L min}^{-1}$ (model UNMP30KNDC, KNF Neuberger, Inc.) through tubing from one of the three heights as selected by a six position stainless steel valve (Valcon M, Valcon Instruments, Houston, Texas), then passed through a 200 cc cylinder containing magnesium perchlorate to absorb water vapor before entering a 16 position sample valve (Valcon M, Valcon Instruments). At each position on the valve, a 15 mL coiled sample loop of stainless steel was connected, so that sampled air passed through one of the 16 loops prior to exiting. The air was then directed to an infrared gas analyzer (IRGA) (LI-6252, LI-COR, Inc., Lincoln, Nebraska, USA). The valves, pumps and IRGA were controlled by a data logger (CR10X, Campbell Scientific, Inc.), which recorded the sampling time and CO₂ concentration of the air passing through the system. One hour after sunset, sampling began at 5-min intervals. To ensure a broad range of CO₂ concentrations the samples were collected only if their concentrations differed from previous samples by a value set a priori [Lai *et al.*, 2005]. If the concentration of CO₂ in the current sample loop exceeded that of past samples by >4 ppm, the valve moved to the next position and a new sampling height on the tower was selected. If the

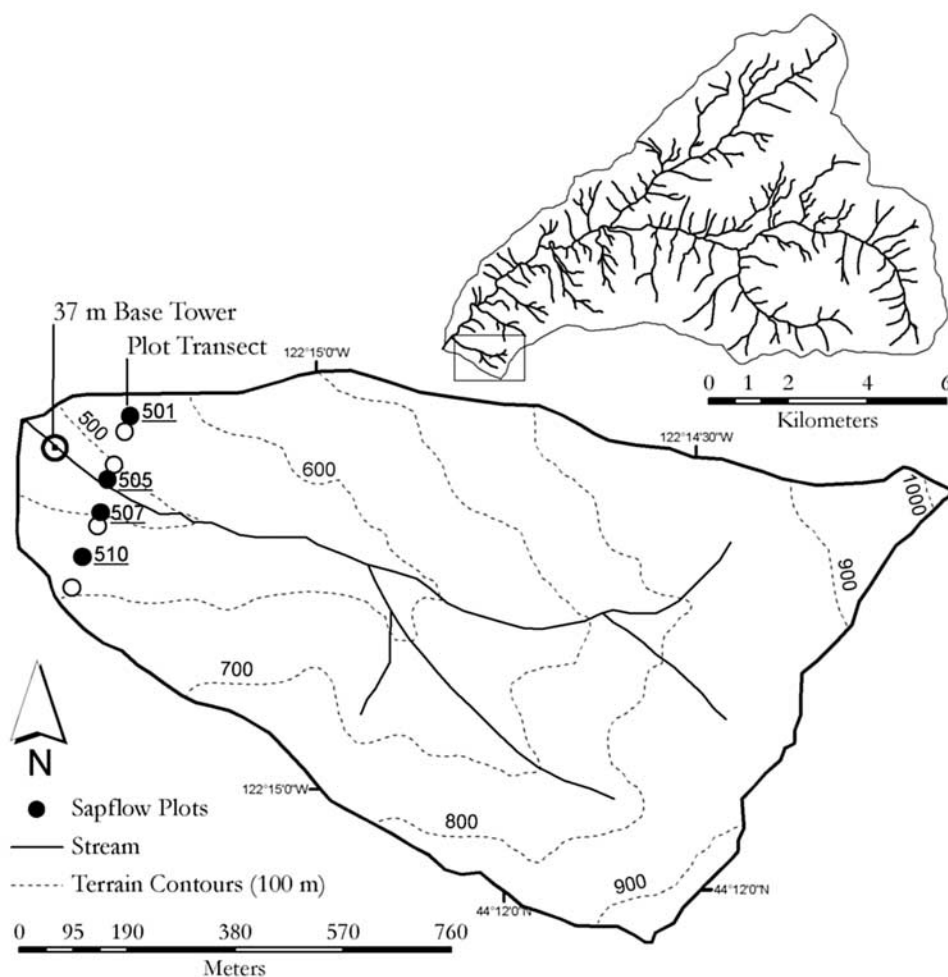


Figure 1. Map of the watershed with the locations of the 37 m base tower and the four research plots along the transect that were used in this study (501, 505, 507, and 510).

concentration difference was ≤ 4 ppm, the system continued to sample from the same tower height and sample valve position and attempted to take a sample 5 min later. This process continued until either all 15 sample loops filled (the 16th sample loop could not be used to collect a sample because it remained open to the atmosphere after the system finished collecting samples) or it was 1 h before sunrise. If the sample loops were full the system shut down. If all the sample loops were not full 1 h prior to sunrise, the system collected samples every 5 min until the remaining sample loops were filled [Hauck, 2006].

[12] Air samples were analyzed in the laboratory within 24 h of collection. For $\delta^{13}\text{C}$ analysis, we used a Finnigan/MAT DeltaPlus XL isotope ratio mass spectrometer interfaced to a GasBench II automated headspace sampler at the College of Oceanic and Atmospheric Sciences, Oregon State University [Hauck, 2006]. The GasBench-II is a continuous flow interface that allows injections of several aliquots of a single gas sample into a mass spectrometer for automated isotope determinations of small gas samples. The sample valve was directly plumbed into the mass spectrometer. Helium pushed the sample air out of the sample loops and into the mass spectrometer with each aliquot being 250 μL . A typical analysis consisted of three gas standards (tank CO_2 -He mixtures), five sample replicates and an

additional two gas standards for every sample loop. The CO_2 concentration [CO_2] of each sample was calculated from the peak volt area produced by the mass spectrometer analysis of each sample loop [Hauck, 2006]. To calibrate the system, each of the sampling containers was filled with a gas of a known CO_2 concentration (403 and 958 ppm; as determined by NOAA). The air was passed from the loops into the mass spectrometer in the same manner as the air samples from the field. A linear relationship between peak volt area and CO_2 concentration was generated. This CO_2 calibration was performed independently for each sampling date.

2.4. Data Analysis

[13] A two end-member mixing model (“Keeling plot”) was used to estimate $\delta^{13}\text{C}_{\text{ER}}$ [Buchmann and Ehleringer, 1998; Flanagan and Ehleringer, 1998; Keeling, 1958; 1961]. Prior to inclusion in the Keeling plot, all data points were screened for accuracy using methods outlined by Pypker *et al.* [2008]. If the standard deviation of the replicates of the $\delta^{13}\text{C}$ of the reference gas or sample gas was greater than 0.2, the point was removed. This procedure ensured that only reliable data points were included in the analysis. The $\delta^{13}\text{C}$ of the air samples from the sample loops were plotted against corresponding $1/[\text{CO}_2]$ values. We used ordinary least squares regression to relate $1/[\text{CO}_2]$ to $\delta^{13}\text{C}$

Table 1. Site Characteristics for Each of the Four Sapflow Plots^a

Plot	Stem Density (stems ha ⁻¹)	Total Basal Area (m ²)	Percent of Basal Area PSME	Mean PSME DBH ^b (cm)	Mean Measured PSME Sapwood Depth ^b (cm)	Plot LAI 2000 Measurements (m ² m ⁻²)	PSME-Only LAI (m ² m ⁻²)
501	3055	1.04	93	11.3 (0.7)	3.7 (0.3)	11.2	7.8
505	764	1.12	100	22.3 (2.0)	3.4 (0.4)	8.7	8.7
507	859	0.85	82	18.7 (1.2)	3.3 (0.5)	10.1	5.2
510	1304	1.00	96	16.8 (0.9)	3.5 (0.4)	11.1	7.5

^aProvided are the site totals and the total represented by Douglas fir (PSME) for stem density (all trees only), basal area, sapwood depth, and leaf area index (LAI).

^bStandard error is given in parentheses.

as it has been found to be the best method for Keeling plot analysis [Zobitz *et al.*, 2006]. Standard errors of the intercepts were estimated using a bootstrap method. After the Keeling plot was generated, the regression was visually checked for nonrandom residuals. If the Keeling plot had nonrandom residuals the data collected on that date were not used in the analysis as this indicated that there was likely a problem with the data set. Data from only two dates (24 April and 31 July) were rejected using this protocol.

2.5. Transpiration-Heat Dissipation Method

[14] From April to October, 2006, we used heat dissipation sensors [Granier, 1985, 1987] to measure the water flux of 10 trees per plot in four plots along the transect (Figure 1 and Table 1). The measured trees represented the range of tree diameters in each plot. In each tree, a 2 cm sensor was inserted into the xylem at the 0–2 cm depth interval at 1.4 m above ground so that the outside edge of the sensor was flush with the outer edge of the xylem. In addition, in 3 dominant trees per plot, 1 cm sensors were installed at two additional xylem depth intervals (2–3 cm and 3–4 cm) to account for radial flux profiles [Phillips *et al.*, 1996]. Sapwood depth measurements, visually inspected from increment cores, indicated that none of the sensors crossed the heartwood boundary. Measurements were recorded by a data logger (CR23X, Campbell Scientific, Inc.) every 15 s and averaged over 15 min intervals. Measurements from all pairs of probes were converted to sap flux density (g H₂O m⁻² sapwood s⁻¹) using empirical equations [Granier, 1985, 1987]. Sap flux in the inner (>2 cm depth) xylem of trees that were not equipped with inner sap flow probes was estimated from a ratio between the outer 0–2 cm flux and the inner 2–3 cm or 3–4 cm fluxes from the measured trees. We assumed that there was no change in flux between the depth of the 3–4 cm sensor and the heartwood boundary.

[15] We scaled measurements from individual sensors to mean transpired water flux per unit ground area (mm d⁻¹). First, for each plot, diameter at 1.4 m was measured for all trees. Xylem depth for each tree was calculated using a diameter to xylem depth relationship developed from over 200 tree cores taken across the watershed (equation (3) ($R^2 = 0.77$, $p < 0.01$); T. Woolley, unpublished data, 2005):

$$\text{Sapwood depth} = e^{(-1.81+1.02(\ln(\text{DBH})))} \quad (3)$$

where dbh is depth at breast height. For each tree, the total xylem area at each depth interval (0–2 cm, 2–3, and >3 cm) was calculated.

[16] The flux within each depth interval of xylem was calculated as the product of the area of that interval and the measured or predicted flux; we then summed the fluxes for each xylem depth interval to estimate total flux per tree. Last, we summed the fluxes of all the trees on each plot and divided by the ground area to estimate mean transpiration flux per unit ground area. Recent work demonstrates that trees often transpire at night when the VPD is above 600 Pa [Ewers and Oren, 2000]. Within the study watershed VPD was greater than 200 Pa on less than 30% of the sample nights (H. Barnard, unpublished data, 2006). Even on the nights with VPD greater than 200Pa, the effect on G_s is likely minimal, as past research demonstrates that nocturnal transpiration by Douglas fir trees is only 1–7% of daytime transpiration [Dawson *et al.*, 2007].

2.6. Estimates of Canopy Conductance (G_s)

[17] To test the feasibility of simulating the response of canopy average stomatal conductance to changing environmental variables using $\delta^{13}\text{C}_{\text{ER}}$ measurements, we estimated canopy average stomatal conductance by calculating the canopy average stomatal conductance necessary to produce the $\delta^{13}\text{C}_{\text{ER}}$ measured on the tower using Farquhar equations (G_{s-1}) (equations (6)–(7)) [Farquhar *et al.*, 1980, 1989].

[18] We estimated a hypothetical value for canopy average stomatal conductance (G_{s-1}) that would be expected if recent photosynthate were the only source of carbon in ecosystem respiration and fractionation during respiration was negligible. To do this, we used the methods outlined by Pypker *et al.* [2008]. In brief, we assumed that the difference between $\delta^{13}\text{C}_{\text{ER}}$ and δ_a (the isotopic ratio of the source air) is equal to photosynthetic discrimination ($\Delta^{13}\text{C}$). This assumption allowed us to estimate G_{s-1} by using a combination of biophysical models for leaf level photosynthetic isotopic discrimination (equations (4) and (5)) [Farquhar *et al.*, 1989] and carbon assimilation/internal CO₂ curves (equation (6)) [Farquhar *et al.*, 1980]. We used the following steps when estimating G_{s-1} . First, carbon discrimination ($\Delta^{13}\text{C}$) was estimated using

$$\Delta^{13}\text{C} = \frac{(\delta_a/1000 - \delta^{13}\text{C}_{\text{ER}}/1000)}{(1 + \delta^{13}\text{C}_{\text{ER}}/1000)} 1000 \quad (4)$$

where δ_a is the isotopic ratio of the source air. We assumed δ_a to be -8.2‰ . Then, the internal CO₂ concentration [C_i] was estimated using

$$C_i = \frac{(\Delta^{13}\text{C} - a)C_a}{(b - a)} \quad (5)$$

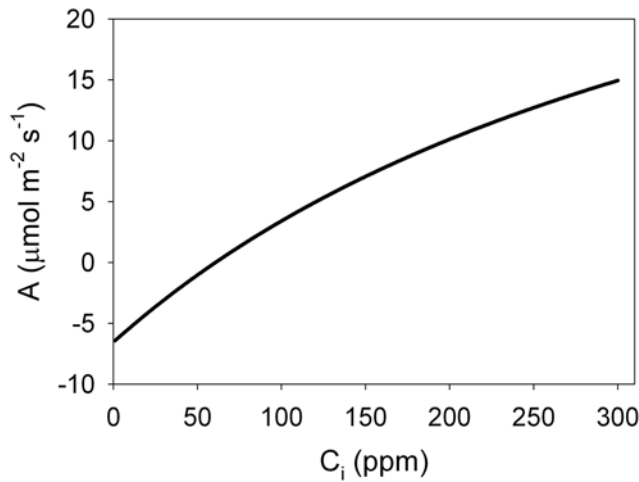


Figure 2. The assimilation rate (A) as predicted from the internal CO_2 concentration C_i . The curve was produced using equations (4)–(6). This curve was used to predict G_{s-1} using equation (7).

On the basis of the C_i values, the assimilation of CO_2 (A) was estimated using

$$A = \left(\frac{V_{\text{cmax}} C_i / 10}{(C_i / 10 + K_c (1 + O_x / K_o))} \right) \left(1 - \frac{0.5 O_x}{\tau C_i / 10} \right) - R_d \quad (6)$$

where V_{cmax} ($\mu\text{mol m}^{-2} \text{s}^{-1}$) is the maximum rate of rubisco-mediated carboxylation; K_c and K_o ($\mu\text{mol mol}^{-1}$) are the Michaelis-Menten coefficients for CO_2 and O_2 binding to rubisco, respectively; O_x ($\mu\text{mol mol}^{-1}$) is the intercellular partial pressure of oxygen; τ ($\mu\text{mol mol}^{-1}$) is the CO_2 compensation point; and R_d ($\mu\text{mol m}^{-2} \text{s}^{-1}$) is the daytime respiration rate. Values of V_{cmax} , τ and R_d ($46 \mu\text{mol m}^{-2} \text{s}^{-1}$, $27.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $42 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively) were estimated from A/C_i curves measured on Douglas fir trees of similar age at Wind River, Washington, approximately 150 km north of our site (B. J. Bond et al., unpublished data, 2002). For Douglas fir trees, V_{max} regulates photosynthesis when C_i is below 400 ppm [Ethier et al., 2006]. For our analysis, C_i ranged from 174 to 253 ppm. The variables K_c , K_o , O_x were treated as constants with assumed values of $21 \mu\text{mol mol}^{-1}$, $2.32 \mu\text{mol mol}^{-1}$ and $1.5 \mu\text{mol mol}^{-1}$, respectively. We ignored potential temperature effects on K_c , K_o , O_x and τ (Figure 2). Canopy average stomatal conductance was then estimated using

$$G_{s-1} = \frac{A}{(C_a - C_i)} 1.6 \quad (7)$$

where C_a is the atmospheric concentration of CO_2 (assumed to be $380 \mu\text{mol mol}^{-1}$), and the factor 1.6 converts from CO_2 conductance to water vapor conductance [Taiz and Zeiger, 1991].

[19] Pypker et al. [2008] compared G_{s-1} to estimates of midday G_s produced from the transpiration measurements (heat dissipation method) measured 0–9 days earlier in 2006. The lag analysis was conducted because the estimates

of G_{s-1} represent an integrated signal of carbon fixed over a period of days [Andrews et al., 1999; Ekblad et al., 2005; Ekblad and Högberg, 2001; Horwath et al., 1994; McDowell et al., 2004b; Steinmann et al., 2004]. Pypker et al. [2008] found a significant correlation between stomatal conductance measured 3–5 days earlier derived from the heat dissipation method and G_{s-1} . It is hypothesized that lag in the response of $\delta^{13}\text{C}_{\text{ER}}$ to changes in VPD and G_s corresponds to the time required for the sugars to move from the leaves to the growing parts of the tree (e.g., roots).

2.7. Estimates of Transpiration From Isotope Measurements

[20] Transpiration for the watershed was estimated by inserting G_{s-1} and the average weather conditions measured 3–5 days earlier into the Penman-Monteith [Monteith and Unsworth, 2007]. The Penman-Monteith equation was run on a 15 min time step for each of four plots (501, 505, 507, and 510; see Figure 1) and the total transpiration was calculated for each day. The totals from each plot were then averaged, thereby providing an estimate of transpiration for the entire watershed. To estimate the 3–5 day lagged transpiration from the watershed we used

$$E = \frac{\Delta(R_n - G) + \rho c_p \{VPD\} / r_a}{\lambda(\Delta + \gamma(1 + r_a G_{s-1}))} \quad (8)$$

where E is transpiration ($\text{kg m}^{-2} \text{s}^{-1}$), Δ is the rate change of saturation vapor pressure with temperature (Pa K^{-1}), R_n is net radiation measured in the tower at the base of the watershed (W m^{-2}), G is the ground heat flux (W m^{-2}), ρ is the density of air (kg m^{-3}), c_p is the heat capacity of air ($\text{J kg}^{-1} \text{K}^{-1}$), r_a is the aerodynamic resistance of the canopy (s m^{-1}), λ is the latent heat of evaporation (J kg^{-1}), and VPD as measured in each plot (Pa). Relative to E and the sensible heat flux, G is usually small in forested ecosystems [Oke, 1992]. We assumed G was only 10% of R_n . The aerodynamic resistance (r_a) was estimated using

$$r_a = \frac{\{\ln[(z - d)/z_o]\}^2}{k^2 u(z)} \quad (9)$$

where z is the height of the canopy (m), d is the height of the zone of zero displacement (m), k is von Karman's constant (0.41), z_o is the roughness length (m) and $u(z)$ is the wind speed at 33 m on the base tower (m s^{-1}). In using equations (8) and (9) we assumed that $d = 0.65 h$, where h is canopy height ($h = 28 \text{ m}$), $z_o = 0.1 h$, the relative humidity and temperature measured at canopy height at four plots in the watershed (Figure 1) were representative for the entire watershed and atmosphere was neutrally stable [Campbell and Norman, 1998; Monteith and Unsworth, 2007]. The average isotope-derived estimates of evapotranspiration for each plot with sap flow measurements were compared to the average transpiration measurements from the four plots for dates when both methods provided data ($n = 10$). Two dates with isotopic measurements were not included in the analysis because one or more of the sites measuring transpiration using the heat dissipation method were not in operation.

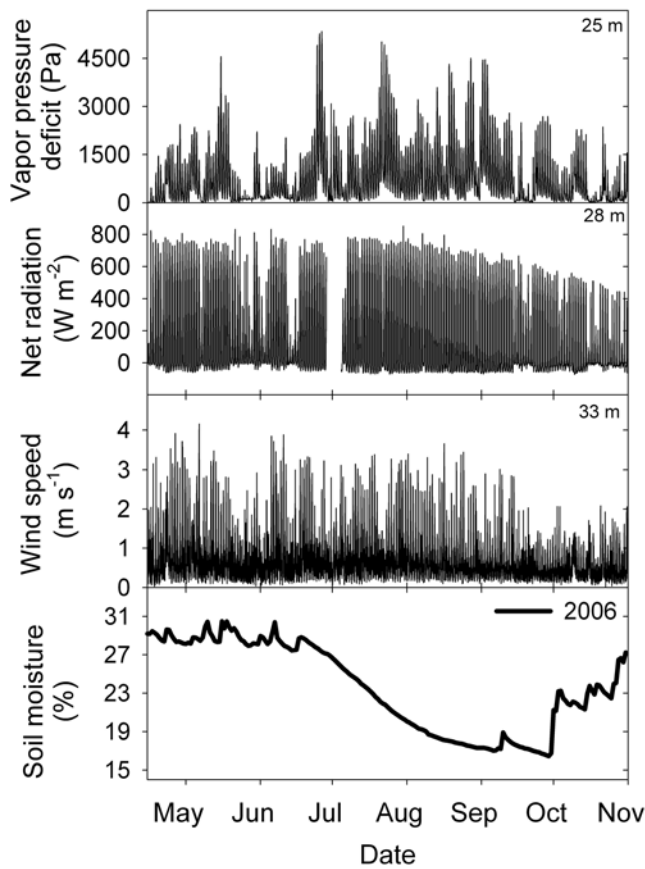


Figure 3. Canopy average vapor pressure deficit in the four research plots, net radiation (28 m), wind speed (33 m), and average volumetric soil moisture (100 cm depth) as measured in the plots (VPD and soil moisture) or at the base tower (net radiation and wind speed). Tick marks on the x axis represent the first of the month.

2.8. Statistics

[21] All statistical analyses were performed in S-PLUS (S-PLUS[®] 8.0 for Windows, Insightful Corp., Palo Alto, California).

3. Results

3.1. Environmental Variables

[22] The weather in 2006 followed a common pattern for the Pacific Northwest; the summer was dry, with increasing atmospheric vapor pressure deficit during the summer months (Figure 3). The soil moisture declined throughout the summer as there was very little rain during the summer at the site. The wind speeds near the top of the forest canopy were usually below 1 m s^{-1} , but occasionally exceeded 3 m s^{-1} .

3.2. Seasonal Changes in $\delta^{13}\text{C}$ of Ecosystem Respiration ($\delta^{13}\text{C}_{\text{ER}}$) and Transpiration

[23] The $\delta^{13}\text{C}_{\text{ER}}$ ranged between -23.4 ± 0.8 to -27.6 ± 0.5 ‰ from 8 May to 25 October 2006 (Figure 4). In the spring, $\delta^{13}\text{C}_{\text{ER}}$ remained relatively unchanged until mid-July. After this time, changes in VPD and soil moisture resulted in an increase in the $\delta^{13}\text{C}_{\text{ER}}$ because the shifts in environmental variables likely reduced canopy average

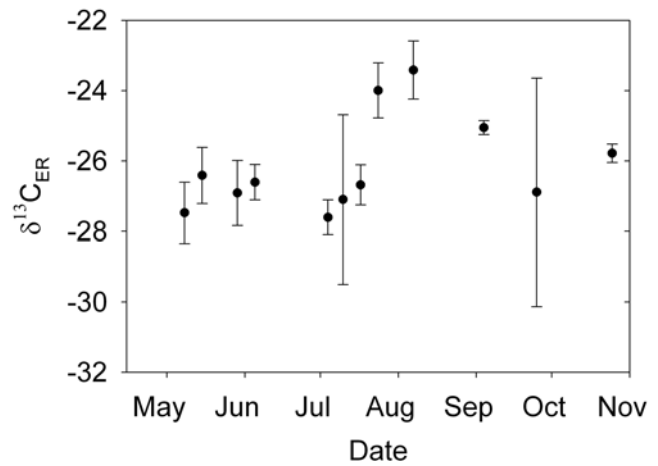


Figure 4. The $\delta^{13}\text{C}$ of ecosystem respiration ($\delta^{13}\text{C}_{\text{ER}}$) from air samples collected at the base of a 96 ha watershed dominated by ~ 40 year old Douglas fir trees. Tick marks on the x axis represent the first of the month.

stomatal conductance [Pypker *et al.*, 2008]. When the VPD and G_s measured and modeled 5 days earlier are compared to changes in $\delta^{13}\text{C}_{\text{ER}}$, there is a significant correlation [Pypker *et al.*, 2008].

[24] Average transpiration, as estimated using the heat dissipation method, was highly variable both seasonally and daily. Within a single season the average transpiration rates ranged from 0.1 (SE = 0.03) to 1.7 (SE = 0.17) mm d^{-1} , with the highest values typically occurring in late June to mid-July (Figure 5). After late July average transpiration steadily declined. Spatially, transpiration also was variable, as the plots near the top of the slopes (501 and 510) typically had greater transpiration rates relative to those located near the bottom of the deeply incised valley (plots 505 and 507). Not surprisingly, plot 507 had the lowest transpiration rates as it was near the bottom of the valley and was on a north facing slope (Figure 1).

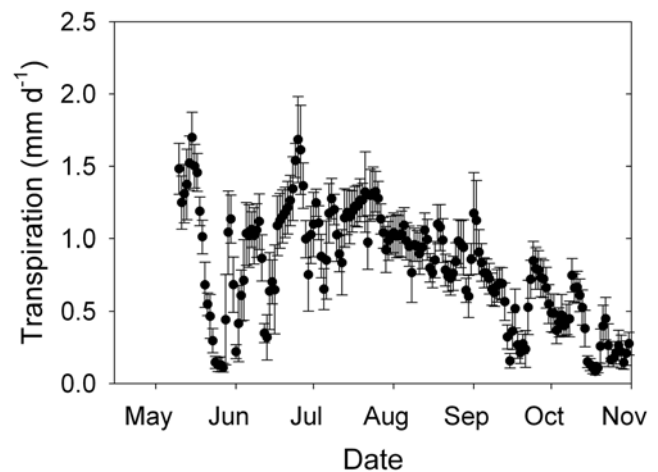


Figure 5. Transpiration from Douglas fir as measured using the heat dissipation method at four plots (Figure 1) in a 96 ha watershed. The tick marks on the x axis represent the first of the month.

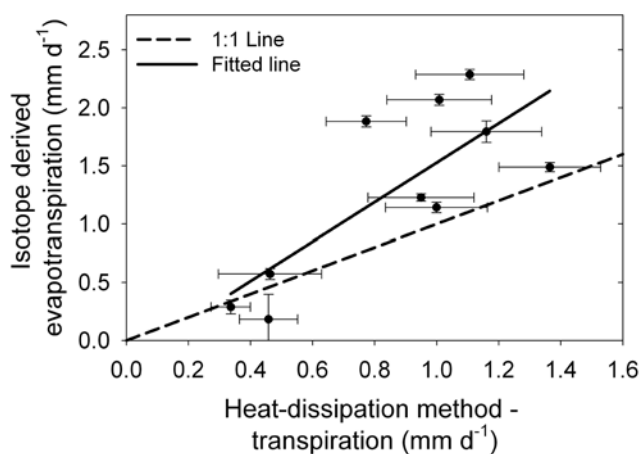


Figure 6. Comparison of average transpiration using measurements (heat dissipation method) from four plots along a transect and modeled estimates of evapotranspiration using the Penman-Monteith equation. The Penman-Monteith equation was parameterized using VPD averaged at 15 min intervals from measurements in the four plots and net radiation and wind speed as measured on the 37 m base tower. The error bars represent the average of the sap flow measurements (horizontal) and the isotope derived estimates (vertical) of transpiration and evapotranspiration, respectively. The fitted linear regression is significant (slope = 1.7; p value < 0.01; SE = 0.48; R^2 < 0.60), but the slope is not statistically different from a slope of 1 (p value > 0.10).

3.3. Estimates of Canopy Average Transpiration Using the Penman-Monteith Equation

[25] Average daily transpiration, as estimated by the heat dissipation method, was similar to the mean transpiration predicted by the Penman-Monteith equation in combination with the isotope derived G_{s-I} (Figure 6). The slope of the fitted line relating the heat dissipation method derived and isotope derived transpiration was significant (slope = 1.69; SE = 0.48; p value < 0.01; R^2 = 0.60); the slope was not statistically different from a slope of 1 (p value > 0.1) and the intercept was not statistically different from zero (intercept = -0.17; SE = 0.45; p value > 0.7). The lack of statistical difference between the regression slope and the 1:1 line was likely due, in part, to the large standard error. The isotope-derived transpiration measurements were considerably greater than the sap flow measurements at transpiration rates above 0.8 mm d⁻¹.

4. Discussion

4.1. Contrasting the Two Methods for Estimating Transpiration

[26] Measured transpiration in this forest was more variable than the observed changes in $\delta^{13}C_{ER}$. During the growing season the sap flow, as measured by the heat dissipation method, varied considerably in the late spring and early summer, yet the $\delta^{13}C_{ER}$ did not. This is possibly because stomatal conductance is only one variable that controls transpiration from a forest; other significant drivers include vapor pressure deficit, net radiation and aerodynamic resistance (equation (8)). Therefore, it is possible for

stomatal conductance (and $d^{13}C_{ER}$) to mildly vary, while transpiration experiences large changes.

[27] The significant correlation between the two measurement methods suggests that measurements of $\delta^{13}C_{ER}$ can be used in combination with biophysical models to estimate transpiration. The $\delta^{13}C_{ER}$ derived estimates of transpiration were usually greater than the average heat dissipation method measurements although the difference between the slope of the fitted line and the 1:1 line was not statistically different. The lack of statistical difference resulted because estimated slope had a large standard error (SE = 0.48). The results demonstrate that there is a correlation between the two results, but it is likely that confounding factors do not allow them to agree at high transpiration rates. The offset between the two methods may result from many factors as there are errors in each method.

[28] The difference in the transpiration estimated by the two methods could result from different estimates of canopy average stomatal conductance. When comparing G_{s-I} with estimates of G_s from the heat dissipation method, Pypker *et al.* [2008] found the two were significantly correlated (p value < 0.05; R^2 > 0.51) but G_{s-I} was on average 0.00024 m s⁻¹ greater (for values ranging between 0.0012 and 0.0036 m s⁻¹). When applied to the Penman-Monteith equation, this results in greater estimates of transpiration.

[29] The differences between the estimates of G_s and G_{s-I} could occur because the heat dissipation method provides a canopy average estimate of G_s for the dominant tree type, Douglas fir, whereas the isotope method provides a flux weighted estimate of G_{s-I} . In this paper we assume that G_{s-I} represented average canopy conductance, but in actuality, it is weighted to the locations where photosynthesis is fixing the most carbon. These locations in the canopy with greater carbon fixation have subsequently greater stomatal conductance relative to the estimates from the heat dissipation method. Hence, at high transpiration rates, it is likely that estimates of G_{s-I} will be greater than G_s .

[30] The differences between the two methods could be further increased because the Penman-Monteith equation estimates transpiration from the soil and understory up to the top of the forest canopy, whereas the heat dissipation method only provides estimates for the dominant trees. The understory can supply approximately 10 to 20% of whole canopy transpiration [Black and Kelliher, 1989]. Therefore, the Penman-Monteith equation may exceed the transpiration estimates of only overstory trees, particularly when the soils are relatively moist.

[31] The difference in transpiration rates could also occur because of errors associated with the heat dissipation method caused by scaling from only four locations in the watershed. With only four plots, one plot could have unreasonable influence on the average. For example, one of the four plots was located in a shady region near the base of a north facing slope (plot 507). Its inclusion in the analysis weakened the relationship between the two methods used to derive transpiration. If the plot is removed, the relationship between the two methods for estimating transpiration is improved (slope = 1.4; SE = 0.39, p value < 0.01, R^2 = 0.61 and intercept = 0.001; SE = 0.39; p value > 0.95) (data not shown). However, more work is needed to determine if this plot is representative of areas of extremely low transpiration rates that occur elsewhere in this water-

shed. For this reason this plot remained in the analysis. In short, both methods for estimating transpiration have many sources of errors and uncertainty that needs further attention in future research needs. Discussion of some needed research follows.

4.2. Review of Research Needs

[32] Using the Keeling plot approach, studies have shown that the $^{13}\text{C}_{\text{ER}}$ becomes enriched with increasing VPD [Alstad et al., 2007; Bowling et al., 2002; Knohl and Buchmann, 2005; Mortazavi et al., 2005], decreased precipitation [Ometto et al., 2002], decreased soil moisture content [Lai et al., 2005; Ponton et al., 2006], and increased soil temperature [McDowell et al., 2004b]. All of these environmental conditions are associated with reduced stomatal conductance, which in turn should result in decreased isotopic discrimination, i.e., relative enrichment of ^{13}C [Farquhar et al., 1989; Madhavan et al., 1991]. Our recent work suggests that observations of $\delta^{13}\text{C}_{\text{ER}}$ can be used to predict the average canopy stomatal conductance (G_s) of an entire watershed, thereby opening the door to modeling how ecosystem-scale transpiration will respond to changing environmental variables [Pypker et al., 2008]. Questions still remain as to the validity of this method for predicting transpiration.

[33] The use of the linear Farquhar model currently requires many assumptions that can lead to inaccurate estimates of G_s [Seibt et al., 2008]. For example, the equations we used do account for internal transfer of CO_2 and photorespiration [Farquhar and Sharkey, 1982; Ghashghaie et al., 2003; Wingate et al., 2007], and this can lead to inaccurate estimates of C_i [Seibt et al., 2008]. To apply our simple method for estimating transpiration, we needed to make numerous assumptions as outlined in our methods. Each of these assumptions must be reviewed, and its effect quantified, if this technique is to be used to estimate transpiration in complex terrain. For example, we assumed that the $\delta^{13}\text{C}_{\text{ER}}$ signal was constant throughout the night. However, recent research has shown that the signal can shift as the evening progresses [e.g., Hymus et al., 2005; Prater et al., 2006; Werner et al., 2006]. These shifts could result from changing temperatures in combination with changing substrates consumed during respiration [Kodama et al., 2008]. In our watershed the temperature is variable both spatially and temporally [Pypker et al., 2007b]. Two key assumptions that are central to this paper were that fractionation after assimilation was negligible and the carbon respired at night was recently fixed. These two assumptions must be better explored if this technique is to be applicable.

[34] The significance of fractionation that occurs after assimilation needs to be assessed and other possible sources of fractionation should be explored. Past research suggest that the difference between the $\delta^{13}\text{C}$ of sucrose in the leaf and respired CO_2 can range between ± 0 –10‰ [e.g., Duranceau et al., 1999; Duranceau et al., 2001; Ghashghaie et al., 2003; Ghashghaie et al., 2001; Hymus et al., 2005; O'Leary, 1981; Prater et al., 2006; Xu et al., 2004]. For example, a shift in the $\delta^{13}\text{C}_{\text{ER}}$ may result because plants might switch the substrate they are metabolizing (e.g., from glucose to lipids) and alter the proportion of sucrose being converted to CO_2 and lipids during respiration [Ghashghaie et al., 2003]. These shifts will alter the isotopic ratio of respired CO_2 , because, as the portion of respired CO_2 that is derived from

lipids increases, the ^{13}C content of the respiration decreases. Research demonstrates that the difference found between the $\delta^{13}\text{C}$ of the sucrose in the leaf and the respired CO_2 can change over the course of an evening if a plant changes the substrate being metabolized [e.g., Barbour et al., 2007; Mortazavi et al., 2006]. Differences between the $\delta^{13}\text{C}$ of ecosystem respired air and the bulk leaf is believed to be common, resulting in the ecosystem respired air to be on average 1.7‰ enriched (greater ^{13}C) relative to the bulk leaf [Bowling et al., 2008]. While it is accepted that postassimilation fractionation occurs, it is not known whether size of this fractionation is significant in all species and in all environments [Bowling et al., 2008]. For example, past research demonstrates that difference between the $\delta^{13}\text{C}$ of ecosystem respiration and the sugars in the leaf is highly variable; enrichment of the respired air relative to the bulk leaf can range from approximately -0.75 ‰ to 4.5 ‰ [Bowling et al., 2008].

[35] The correlation between the isotope-derived transpiration and the transpiration estimated using the heat dissipation method suggests that fractionation that occurs after assimilation might be negligible in this Douglas fir forest during the summer of 2006. If fractionation after assimilation in this forest was indeed substantial, the estimates of G_{s-1} and transpiration using $\delta^{13}\text{C}_{\text{ER}}$ would not be expected to be close to the values on the basis of direct transpiration measurements (heat dissipation method). If there was a small shift in the isotopic ratio from respiration relative to the isotopic ratio of the photosynthate, equations (4)–(7) would not accurately predict the correct G_s . Modeling, field and laboratory experiments suggest that there is indeed an offset between isotopic composition of respiration and photosynthate [see reviews by Bowling et al., 2008; Ghashghaie et al., 2003]. Hence, it is important that future research quantify the effect of postassimilation fractionation on the isotopic ratio of respiration. However, in the current study, the two estimates of transpiration were not very different, which suggests that there was either little fractionation after assimilation in this forest or there were other processes (e.g., offsetting fractionation shifts in the sources of ecosystem respiration) that were offsetting. In the future, the magnitude of fractionation after assimilation for Douglas fir forests must be determined if stable isotopes are to be reliably used to estimate watershed-scale transpiration.

[36] In applying this method we assumed that all of the respired carbon was recently fixed. Recent research suggests that this may be a reasonable assumption for some forests. For example, Högberg et al. [2008] and Högberg and Read [2006] both demonstrated that greater than 50% of the belowground respiration was derived from recent photosynthate. Other researchers report that transfer of photosynthate from leaves to the roots was very rapid, with substantial portion of the recently fixed carbon exiting the soil in 1–4 days [Andrews et al., 1999; Ekblad et al., 2005; Ekblad and Högberg, 2001; Horwath et al., 1994; Steinmann et al., 2004]. On the ecosystem scale, the significant correlation between changes in $\delta^{13}\text{C}_{\text{ER}}$ and environmental variables further supports the assumption that a substantial portion of the respired CO_2 is from recent photosynthate [e.g., Alstad et al., 2007; Bowling et al., 2002; Knohl and Buchmann, 2005; Lai et al., 2005; McDowell et al., 2004b; Mortazavi et al., 2005; Ometto et al., 2002; Ponton et al., 2006]. If recent

photosynthate did not represent a substantial portion of ecosystem respiration, then the $\delta^{13}\text{C}_{\text{ER}}$ would not be sensitive to environmental changes.

4.3. Other Requirements for Using Carbon Isotopes to Derive Canopy Average Stomatal Conductance

[37] To successfully use seasonal changes in $\delta^{13}\text{C}_{\text{ER}}$ to estimate ecosystem transpiration one must know the size and stability of the source area for CO_2 . In the study watershed, the cold air drainage patterns are ideal for monitoring seasonal changes in $\delta^{13}\text{C}_{\text{ER}}$ [Pypker et al., 2007b]. Nocturnal cold air drainage in this watershed is persistent and occurs on greater than 80% of summer nights [Pypker et al., 2007a]. Because a strong inversion forms above the canopy, respired CO_2 is effectively trapped within the watershed [Pypker et al., 2007b]. Below the inversion layer, the air is well mixed, resulting in an integrated $\delta^{13}\text{C}$ signal. Over the course of an evening the range of CO_2 concentrations is sufficient to use the Keeling plot approach to estimate $\delta^{13}\text{C}_{\text{ER}}$ [Pypker et al., 2007a]. Trace gas experiments have indicated that the source area probably encompasses the entire watershed [Pypker et al., 2007b]. However, other watersheds may not have a well-defined source area, suitable range in CO_2 concentrations or be as decoupled from the synoptic flow. Researchers should determine if the wind patterns and range of CO_2 concentrations in their watershed are appropriate prior to attempting this method.

[38] Despite some challenges in using this method, the use of stable isotopes to derive watershed-scale transpiration has some benefits over direct transpiration measurements using the heat dissipation method. It can be challenging and labor intensive to measure direct transpiration from a sufficient number of trees to accurately estimate transpiration in a mountainous watershed. Douglas fir is conservative when using water, regulating its stomata to prevent leaf water potential from dropping below -2.1 MPa [Bond and Kavanagh, 1999; McDowell et al., 2002]. Thus, the stomatal conductance of Douglas fir is nonlinearly correlated to changes in vapor pressure deficit and soil moisture availability [Bond and Kavanagh, 1999]. In mountainous regions, the microclimate is highly variable because changes in elevation, aspect and soil depth will directly affect incident radiation, VPD and soil moisture [Geiger, 1965]. This variability requires researchers to monitor a large number of trees to accurately represent transpiration in the watershed. In contrast, the air sampled at the base of the watershed requires only one measurement location and provides an integrated signal of the CO_2 respired from the entire watershed [Pypker et al., 2007b]. More frequent sampling of atmospheric isotopes is now possible with portable tunable diode lasers [Bowling et al., 2003]. These systems could provide values of $\delta^{13}\text{C}_{\text{ER}}$ for each night with suitable weather, thereby providing a dynamic estimate of G_s . Such an advance would help us to understand how the different ecosystem processes affect the $\delta^{13}\text{C}_{\text{ER}}$ signal.

4.4. Conclusions

[39] The observed shifts in $\delta^{13}\text{C}_{\text{ER}}$ during the summer resulted from recent changes in discrimination during photosynthesis. The measurement of these shifts allowed realistic estimates of G_s [Pypker et al., 2008] and transpiration using $\delta^{13}\text{C}_{\text{ER}}$ in combination with biophysical and hydrological models. However, isotope derived estimates of

transpiration were consistently greater than the sap flow estimates. This indicates that further research is needed to quantify the sources of the respired CO_2 within the watershed and the effect of all of the assumptions on the $\delta^{13}\text{C}_{\text{ER}}$ signal.

[40] **Acknowledgments.** We thank J. Moreau, T. Cryer, A. Padilla, J. Mandrick, and C. Czarnomski for their help with site construction, fieldwork, and data collection; A. Schauer for help with data logger programming; and C. Tarasoff for her helpful comments on the manuscript. Last, we wish to thank the anonymous reviewers for their very helpful comments. Funding for this project was provided by the National Science Foundation (grants DEB-0132737 and DEB-0416060).

References

- Adelman, J. D., B. E. Ewers, and D. S. MacKay (2008), Use of temporal patterns in vapor pressure deficit to explain spatial autocorrelation dynamics in transpiration, *Tree Physiol.*, *28*, 647–658, doi:10.1093/treephys/28.4.647.
- Alstad, K., C.-T. Lai, L. B. Flanagan, and J. R. Ehleringer (2007), Environmental controls on the carbon isotope composition of ecosystem-respired CO_2 in contrasting forest ecosystems in Canada and the USA, *Tree Physiol.*, *27*, 1361–1374, doi:10.1093/treephys/27.10.1361.
- Andrews, J. A., K. G. Harrison, R. Matamala, and W. H. Schlesinger (1999), Separation of root respiration from total soil respiration using carbon-13 labeling during free-air carbon dioxide enrichment (FACE), *Soil Sci. Soc. Am. J.*, *63*, 1429–1435.
- Aubinet, M., B. Heinesch, and M. Yemaux (2003), Horizontal and vertical CO_2 advection in a sloping forest, *Boundary Layer Meteorol.*, *108*, 397–417, doi:10.1023/A:1024168428135.
- Badeck, F.-W., G. Tcherkez, S. Nogués, C. Piel, and J. Ghashghaie (2005), Post-photosynthetic fractionation of stable carbon isotopes between plant organs—A widespread phenomenon, *Rapid Commun. Mass Spectrom.*, *19*, 1381–1391, doi:10.1002/rcm.1912.
- Baldocchi, D. D., B. B. Hicks, and T. P. Meyers (1988), Measuring biosphere-atmosphere exchanges of biologically related gases with micrometeorological methods, *Ecology*, *69*, 1331–1340, doi:10.2307/1941631.
- Barbour, M. M., N. G. McDowell, G. Tcherkez, C. P. Bickford, and D. T. Hanson (2007), A new measurement technique reveals rapid post-illumination changes in the carbon isotope composition of leaf-respired CO_2 , *Plant Cell Environ.*, *30*, 469–482, doi:10.1111/j.1365-3040.2007.01634.x.
- Black, T. A., and F. M. Kelliher (1989), Processes controlling understory evapotranspiration, *Philos. Trans. R. Soc. London, Ser. B*, *324*, 207–231, doi:10.1098/rstb.1989.0045.
- Black, T. A., et al. (1996), Annual cycles of water vapour and carbon dioxide fluxes in and above a boreal aspen forest, *Global Change Biol.*, *2*, 219–229, doi:10.1111/j.1365-2486.1996.tb00074.x.
- Blanken, P. D., and W. R. Rouse (1995), Modelling evaporation from a high subarctic willow-birch forest, *Int. J. Climatol.*, *15*, 97–106, doi:10.1002/joc.3370150110.
- Bond, B. J., and K. L. Kavanagh (1999), Stomatal behavior of four woody species in relation to leaf-specific hydraulic conductance and threshold water potential, *Tree Physiol.*, *19*, 502–510.
- Bowling, D. R., N. G. McDowell, B. J. Bond, B. E. Law, and J. R. Ehleringer (2002), ^{13}C content of ecosystem respiration is linked to precipitation and vapor pressure deficit, *Oecologia*, *131*, 113–124, doi:10.1007/s00442-001-0851-y.
- Bowling, D. R., S. D. Sargent, B. D. Tanner, and J. R. Ehleringer (2003), Tunable diode laser absorption spectroscopy for stable isotope studies of ecosystem-atmosphere CO_2 exchange, *Agric. For. Meteorol.*, *118*, 1–19, doi:10.1016/S0168-1923(03)00074-1.
- Bowling, D. R., D. E. Pataki, and J. T. Randerson (2008), Carbon isotopes in terrestrial ecosystem pools and CO_2 fluxes, *New Phytol.*, *178*, 24–40, doi:10.1111/j.1469-8137.2007.02342.x.
- Buchmann, N., and J. A. Ehleringer (1998), CO_2 concentration profiles, and carbon and oxygen isotopes in C_3 and C_4 crop canopies, *Agric. For. Meteorol.*, *89*, 45–58, doi:10.1016/S0168-1923(97)00059-2.
- Campbell, G. S., and J. M. Norman (1998), *An Introduction to Environmental Biophysics*, 2nd ed., 286 pp., Springer, New York.
- Coops, N. C., R. H. Waring, S. R. Brown, and S. W. Running (2001), Comparisons of predictions of net primary production and seasonal patterns in water use derived with two forest growth models in southwestern Oregon, *Ecol. Modell.*, *142*, 61–81, doi:10.1016/S0304-3800(01)00279-4.
- Czarnomski, N. M., G. W. Moore, T. G. Pypker, J. Licata, and B. J. Bond (2005), Precision and accuracy of three alternative soil water content instruments in two forest soils of the Pacific Northwest, *Can. J. For. Res.*, *35*, 1867–1876, doi:10.1139/x05-121.

- Dawson, T. E., S. O. O. Burgess, K. P. Tu, R. S. Oliveira, J. B. Fisher, L. S. Santiago, K. S. Simonin, and A. R. Ambrose (2007), Nighttime transpiration in woody plants from contrasting ecosystems, *Tree Physiol.*, *27*, 561–575.
- Duranceau, M., J. Ghashghaie, F. Badeck, E. Deleens, and G. Cornic (1999), $\delta^{13}\text{C}$ of CO_2 respired in the dark in relation to $\delta^{13}\text{C}$ of leaf carbohydrates in *Phaseolus vulgaris* L. under progressive drought, *Plant Cell Environ.*, *22*, 515–523, doi:10.1046/j.1365-3040.1999.00420.x.
- Duranceau, M., J. Ghashghaie, and E. Brugnoli (2001), Carbon isotope discrimination during photosynthesis and dark respiration in intact leaves of *Nicotiana sylvestris*: Comparisons between wild type and mitochondrial mutant plants, *Aust. J. Plant Physiol.*, *28*, 65–71.
- Eklblad, A., and P. Höglberg (2001), Natural abundance of ^{13}C in CO_2 respired from forest soils reveals speed of link between tree photosynthesis and root respiration, *Oecologia*, *127*, 305–308, doi:10.1007/s004420100667.
- Eklblad, A., B. Boström, A. Holm, and D. Comstedt (2005), Forest soil respiration rate and $\delta^{13}\text{C}$ is regulated by recent above ground weather conditions, *Oecologia*, *143*, 136–142, doi:10.1007/s00442-004-1776-z.
- Ethier, G. J., N. J. Livingston, D. L. Harrison, T. A. Black, and J. A. Moran (2006), Low stomatal and internal conductance to CO_2 versus rubisco deactivation as determinants of the photosynthetic decline of ageing evergreen leaves, *Plant Cell Environ.*, *29*, 2168–2184, doi:10.1111/j.1365-3040.2006.01590.x.
- Ewers, B. E., and R. Oren (2000), Analyses of assumptions and errors in the calculation of stomatal conductance from sap flux measurements, *Tree Physiol.*, *20*, 579–589.
- Farquhar, G. D., and T. D. Sharkey (1982), Stomatal conductance and photosynthesis, *Annu. Rev. Plant Physiol.*, *33*, 317–345, doi:10.1146/annurev.pp.33.060182.001533.
- Farquhar, G. D., S. von Caemmerer, and J. A. Berry (1980), A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species, *Planta*, *149*, 78–90, doi:10.1007/BF00386231.
- Farquhar, G. D., J. R. Ehleringer, and K. T. Hubick (1989), Carbon isotope discrimination and photosynthesis, *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, *40*, 503–537, doi:10.1146/annurev.pp.40.060189.002443.
- Finnigan, J. (2008), An introduction to flux measurements in difficult conditions, *Ecol. Appl.*, *18*, 1340–1350, doi:10.1890/07-2105.1.
- Finnigan, J. J., R. Clement, Y. Malhi, R. Leuning, and H. A. Cleugh (2003), A re-evaluation of long-term flux measurement techniques part 1: Averaging and coordinate rotation, *Boundary Layer Meteorol.*, *107*, 1–48, doi:10.1023/A:1021554900225.
- Flanagan, L. B., and J. A. Ehleringer (1998), Ecosystem-atmosphere CO_2 exchange: Interpreting signals of change using stable isotope ratios, *Trends Ecol. Evol.*, *13*, 10–14, doi:10.1016/S0169-5347(97)01275-5.
- Geiger, R. (1965), *The Climate Near the Ground*, Harvard Univ. Press, Cambridge, Mass.
- Ghashghaie, J., M. Duranceau, F.-W. Badeck, G. Cornic, M.-T. Adeline, and E. Deleens (2001), $\delta^{13}\text{C}$ of CO_2 respired in the dark in relation to $\delta^{13}\text{C}$ of leaf metabolites: Comparison between *Nicotiana sylvestris* and *Helianthus annuus* under drought, *Plant Cell Environ.*, *24*, 505–515, doi:10.1046/j.1365-3040.2001.00699.x.
- Ghashghaie, J., F.-W. Badeck, G. Lanigan, S. Nogués, G. Tcherkez, E. Deléens, G. Cornic, and H. Griffiths (2003), Carbon isotope fractionation during dark respiration and photorespiration in C_3 plants, *Phytochem. Rev.*, *2*, 145–161, doi:10.1023/B:PHYT.0000004326.00711.ca.
- Granier, A. (1985), Une nouvelle méthode pour la mesure du flux de sève brute dans le tronc des arbres, *Ann. Sci. For.*, *42*, 193–200, doi:10.1051/forest:19850204.
- Granier, A. (1987), Evaluation of transpiration in a Douglas-fir stand by means of sap flow measurements, *Tree Physiol.*, *3*, 309–320.
- Hauck, M. J. (2006), Isotopic composition of respired CO_2 in a small watershed: development and testing of an automated sampling system and analysis of first year data, M.S. thesis, 104 pp., Oreg. State Univ., Corvallis.
- Hewlett, J. D. (1982), *Principles of Forest Hydrology*, 183 pp., Univ. of Ga. Press, Athens, Ga.
- Höglberg, P., and D. J. Read (2006), Towards a more plant physiological perspective on soil ecology, *Trends Ecol. Evol.*, *21*, 548–554, doi:10.1016/j.tree.2006.06.004.
- Höglberg, P., A. Nordgren, N. Buchmann, A. F. S. Taylor, A. Eklblad, M. N. Höglberg, G. Nyberg, M. Ottosson-Löfvenius, and D. J. Read (2001), Large-scale forest girdling shows that current photosynthesis drives soil respiration, *Nature*, *411*, 789–792, doi:10.1038/35081058.
- Höglberg, P., et al. (2008), High temporal resolution tracing of photosynthate carbon from the tree canopy to forest soil microorganisms, *New Phytol.*, *177*, 220–228.
- Horwath, W. R., K. S. Pregitzer, and E. A. Paul (1994), ^{14}C allocation in tree-soil systems, *Tree Physiol.*, *14*, 1163–1176.
- Hymus, G. J., K. Maseyk, R. Valentini, and D. Yakir (2005), Large daily variation in ^{13}C -enrichment of leaf-respired CO_2 in two *Quercus* forest canopies, *New Phytol.*, *167*, 377–384, doi:10.1111/j.1469-8137.2005.01475.x.
- Jarvis, P. G., and K. G. McNaughton (1986), Stomatal control of transpiration—Scaling up from leaf to region, *Adv. Ecol. Res.*, *15*, 1–49, doi:10.1016/S0065-2504(08)60119-1.
- Keeling, C. D. (1958), The concentration and isotopic abundances of atmospheric carbon dioxide in rural areas, *Geochim. Cosmochim. Acta*, *13*, 322–334, doi:10.1016/0016-7037(58)90033-4.
- Keeling, C. D. (1961), The concentration and isotopic abundances of carbon dioxide in rural and marine air, *Geochim. Cosmochim. Acta*, *24*, 277–298, doi:10.1016/0016-7037(61)90023-0.
- Kelliher, F. M., R. Leuning, M. R. Raupach, and E.-D. Schulze (1995), Maximum conductances for evaporation from global vegetation types, *Agric. For. Meteorol.*, *73*, 1–16, doi:10.1016/0168-1923(94)02178-M.
- Kimball, J. S., M. A. White, and S. W. Running (1997), BIOME-BGC simulations of stand hydrologic processes for BOREAS, *J. Geophys. Res.*, *102*, 29,043–29,051, doi:10.1029/97JD02235.
- Knohl, A., and N. Buchmann (2005), Partitioning the net CO_2 flux of a deciduous forest into respiration and assimilation using stable carbon isotopes, *Global Biogeochem. Cycles*, *19*, GB4008, doi:10.1029/2004GB002301.
- Kodama, N., et al. (2008), Temporal dynamics of the carbon isotope composition in a *Pinus sylvestris* stand: From newly assimilated organic carbon to respired carbon dioxide, *Oecologia*, *156*, 737–750, doi:10.1007/s00442-008-1030-1.
- Lai, C.-T., J. R. Ehleringer, A. J. Schauer, P. P. Tans, D. Y. Hollinger, K. T. Paw U, J. W. Munger, and S. C. Wofsy (2005), Canopy-scale $\delta^{13}\text{C}$ of photosynthetic and respiratory CO_2 fluxes: Observations in forest biomes across the United States, *Global Change Biol.*, *11*, 633–643, doi:10.1111/j.1365-2486.2005.00931.x.
- Lavigne, M. B., et al. (1997), Comparing nocturnal eddy covariance measurements to estimates of ecosystem respiration made by scaling chamber measurements at six coniferous boreal sites, *J. Geophys. Res.*, *102*, 28,977–28,985, doi:10.1029/97JD01173.
- Lorant, M. M., D. S. Mackay, B. E. Ewers, J. D. Adelman, and E. L. Kruger (2008), Environmental drivers of spatial variation in whole-tree transpiration in an aspen-dominated upland-to-wetland forest gradient, *Water Resour. Res.*, *44*, W02441, doi:10.1029/2007WR006272.
- Mackay, D. S., B. E. Ewers, B. D. Cook, and K. J. Davis (2007), Environmental drivers of evapotranspiration in a shrub wetland and an upland forest in northern Wisconsin, *Water Resour. Res.*, *43*, W03442, doi:10.1029/2006WR005149.
- Madhavan, S., I. Treichel, and M. H. O’Leary (1991), Effects of relative humidity on carbon isotope fractionation in plants, *Bot. Acta*, *104*, 292–294.
- Marshall, J. D., and R. H. Waring (1984), Conifers and broadleaf species: Stomatal sensitivity differs in western Oregon, *Can. J. For. Res.*, *14*, 905–908, doi:10.1139/x84-161.
- McDowell, N. G., N. Phillips, C. Lunch, B. J. Bond, and M. G. Ryan (2002), An investigation of hydraulic limitation and compensation in large, old Douglas-fir trees, *Tree Physiol.*, *22*, 763–774.
- McDowell, N. G., D. R. Bowling, B. J. Bond, J. Irvine, B. E. Law, P. Anthoni, and J. R. Ehleringer (2004a), Response of the carbon isotopic content of ecosystem, leaf, and soil respiration to meteorological and physiological driving factors in a *Pinus ponderosa* ecosystem, *Global Biogeochem. Cycles*, *18*, GB1013, doi:10.1029/2003GB002049.
- McDowell, N. G., D. R. Bowling, A. Schauer, J. Irvine, B. J. Bond, B. E. Law, and J. R. Ehleringer (2004b), Associations between carbon isotope ratios of ecosystem respiration, water availability and canopy conductance, *Global Change Biol.*, *10*, 1767–1784, doi:10.1111/j.1365-2486.2004.00837.x.
- Melillo, J. M., et al. (1995), Vegetation ecosystem modeling and analysis project - comparing biogeography and biogeochemistry models in a continental-scale study of terrestrial ecosystem responses to climate change and CO_2 doubling, *Global Biogeochem. Cycles*, *9*, 407–437, doi:10.1029/95GB02746.
- Monteith, J. L., and M. H. Unsworth (2007), *Principles of Environmental Physics*, 3rd ed., 418 pp., Elsevier, New York.
- Moore, G. W., B. J. Bond, J. A. Jones, N. Phillips, and F. C. Meinzer (2004), Structural and compositional controls on transpiration in a 40- and 450-year-old riparian forest in western Oregon, USA, *Tree Physiol.*, *24*, 481–491.
- Mortazavi, B., J. P. Chanton, J. L. Prater, A. C. Oishi, R. Oren, and G. Katul (2005), Temporal variability in ^{13}C of respired CO_2 in a pine and hard-

- wood forest subject to similar climatic conditions, *Oecologia*, *142*, 57–69, doi:10.1007/s00442-004-1692-2.
- Mortazavi, B., J. P. Chanton, and M. C. Smith (2006), Influence of ^{13}C -enriched foliage respired CO_2 on $\delta^{13}\text{C}$ of ecosystem-respired CO_2 , *Global Biogeochem. Cycles*, *20*, GB3029, doi:10.1029/2005GB002650.
- O'Leary, M. H. (1981), Carbon isotope fractionation in plants, *Phytochemistry*, *20*, 553–567, doi:10.1016/0031-9422(81)85134-5.
- Oke, T. R. (1992), *Boundary Layer Climates*, 2nd ed., 435 pp., Routledge, New York.
- Ometto, J. P. H. B., L. B. Flanagan, L. A. Martinelli, M. Z. Moreira, N. Higuchi, and J. R. Ehleringer (2002), Carbon isotope discrimination in forest and pasture ecosystems of the Amazon Basin, Brazil, *Global Biogeochem. Cycles*, *16*(4), 1109, doi:10.1029/2001GB001462.
- Pataki, D. E., J. R. Ehleringer, L. B. Flanagan, D. Yakir, D. R. Bowling, C. J. Still, N. Buchmann, J. O. Kaplan, and J. A. Berry (2003), The application and interpretation of Keeling plots in terrestrial carbon cycle research, *Global Biogeochem. Cycles*, *17*(1), 1022, doi:10.1029/2001GB001850.
- Paw U, K. T., et al. (2004), Carbon dioxide exchange between an old-growth forest and the atmosphere, *Ecosystems*, *7*, 513–524.
- Phillips, N., R. Oren, and R. Zimmermann (1996), Radial patterns of xylem sap flow in non-diffuse and ring-porous tree species, *Plant Cell Environ.*, *19*, 983–990, doi:10.1111/j.1365-3040.1996.tb00463.x.
- Ponton, S., L. B. Flanagan, K. P. Alstad, B. G. Johnson, K. I. Morgenstern, N. Kljun, T. A. Black, and A. G. Barr (2006), Comparison of ecosystem water-use efficiency among Douglas-fir forest, aspen forest and grassland using eddy covariance and carbon isotope techniques, *Global Change Biol.*, *12*, 294–310, doi:10.1111/j.1365-2486.2005.01103.x.
- Prater, J. L., B. Mortazavi, and J. P. Chanton (2006), Diurnal variation of the $\delta^{13}\text{C}$ of pine needle respired CO_2 evolved in darkness, *Plant Cell Environ.*, *29*, 202–211, doi:10.1111/j.1365-3040.2005.01413.x.
- Pypker, T. G., M. H. Unsworth, A. C. Mix, W. Rugh, T. Ocheltree, K. Alstad, and B. J. Bond (2007a), Using nocturnal cold air drainage flow to monitor ecosystem processes in complex terrain, *Ecol. Appl.*, *17*, 702–714, doi:10.1890/05-1906.
- Pypker, T. G., M. H. Unsworth, B. Lamb, E. Allwine, S. Edburg, E. Sulzman, A. C. Mix, and B. J. Bond (2007b), Cold air drainage in a forested valley: Investigating the feasibility of monitoring ecosystem metabolism, *Agric. For. Meteorol.*, *145*, 149–166, doi:10.1016/j.agrformet.2007.04.016.
- Pypker, T. G., et al. (2008), Toward using $\delta^{13}\text{C}$ of ecosystem respiration to monitor canopy physiology in complex terrain, *Oecologia*, *158*, 399–410, doi:10.1007/s00442-008-1154-3.
- Rothacher, J., C. T. Dyrness, and R. L. Fredriksen (1967), Hydrologic and related characteristics of three small watersheds in the Oregon cascades, Pacific Northwest Forest and Range Experiment Station, pp. 1–54, For. Serv., U. S. Dep. of Agric., Portland, Oreg.
- Running, S. W. (1994), Testing forest-BGC ecosystem process simulations across a climatic gradient in Oregon, *Ecol. Appl.*, *4*, 238–247, doi:10.2307/1941930.
- Running, S. W., and E. R. Hunt, Jr. (1993), Generalization of a forest ecosystem process model for other biomes, BIOME-BGC, and an application for global-scale models, in *Scaling Physiological Processes: Leaf to Globe*, edited by J. R. Ehleringer and C. B. Field, pp. 141–158, Academic, San Diego, Calif.
- Schaap, M. G., and W. Bouten (1997), Forest floor evaporation in a dense Douglas-fir stand, *J. Hydrol.*, *193*, 97–113, doi:10.1016/S0022-1694(96)03201-5.
- Seibt, U., A. Rajabi, H. Griffiths, and J. A. Berry (2008), Carbon isotopes and water use efficiency: Sense and sensitivity, *Oecologia*, *155*, 441–454, doi:10.1007/s00442-007-0932-7.
- Staebler, R. M., and D. R. Fitzjarrald (2004), Observing subcanopy CO_2 advection, *Agric. For. Meteorol.*, *122*, 139–156, doi:10.1016/j.agrformet.2003.09.011.
- Steinmann, K., R. T. W. Siegwolf, M. Saurer, and C. Körner (2004), Carbon fluxes to the soil in a mature temperate forest assessed by ^{13}C isotope tracing, *Oecologia*, *141*, 489–501, doi:10.1007/s00442-004-1674-4.
- Sun, J., S. P. Burns, A. C. Delany, S. P. Oncley, A. A. Turnipseed, B. B. Stephens, D. H. Lenschow, M. A. LeMone, R. K. Monson, and D. E. Anderson (2007), CO_2 transport over complex terrain, *Agric. For. Meteorol.*, *145*, 1–21, doi:10.1016/j.agrformet.2007.02.007.
- Swanson, F. J., and M. E. James (1975), Geology and geomorphology of the H. J. Andrews Experimental Forest, Western Cascades, Oregon, *Res. Pap. PNW-188*, 14 pp., Pac. Northwest For. and Range Exp. Stn., For. Serv., U. S. Dep. of Agric., Portland, Oreg.
- Taiz, L., and E. Zeiger (1991), *Plant Physiology*, 2nd ed., 565 pp., Benjamin Cummings, New York.
- Turnipseed, A. A., D. E. Anderson, P. D. Blanken, W. M. Baugh, and R. K. Monso (2003), Airflows and turbulent flux measurements in mountainous terrain: Part 1. Canopy and local effects, *Agric. For. Meteorol.*, *119*, 1–21, doi:10.1016/S0168-1923(03)00136-9.
- Werner, C., S. Unger, J. S. Pereira, R. Maia, T. S. David, C. Kurz-Besson, J. S. David, and C. Máguas (2006), Importance of short-term dynamic in carbon isotope ratios of ecosystem respiration ($^{13}\text{C}_R$) in a Mediterranean oak woodland and linkage to environmental factors, *New Phytol.*, *172*, 330–346, doi:10.1111/j.1469-8137.2006.01836.x.
- Wingate, L., U. Seibt, J. B. Moncrieff, P. G. Jarvis, and J. Lloyd (2007), Variations in ^{13}C discrimination during CO_2 exchange by *Picea sitchensis* branches in the field, *Plant Cell Environ.*, *30*, 600–616, doi:10.1111/j.1365-3040.2007.01647.x.
- Xu, C.-Y., G. Lin, K. L. Griffin, and R. N. Sambrotto (2004), Leaf respiratory CO_2 is ^{13}C -enriched relative to leaf organic components in five species of C_3 plants, *New Phytol.*, *163*, 499–505, doi:10.1111/j.1469-8137.2004.01153.x.
- Yi, C., D. E. Anderson, A. A. Turnipseed, S. P. Burns, J. P. Sparks, D. I. Stannard, and R. K. Monson (2008), The contribution of advective fluxes to net ecosystem exchange in high-elevation, subalpine forest, *Ecol. Appl.*, *18*, 1379–1390, doi:10.1890/06-0908.1.
- Zobitz, J. M., J. P. Keener, H. Schnyder, and D. R. Bowling (2006), Sensitivity analysis and quantification of uncertainty for isotopic mixing relationships in carbon cycle research, *Agric. For. Meteorol.*, *136*, 56–75, doi:10.1016/j.agrformet.2006.01.003.

H. R. Barnard, B. J. Bond, M. Hauck, and A. M. Kennedy, Forest Ecosystem and Society, Oregon State University, Corvallis, OR 97331-5752, USA.

A. C. Mix and M. H. Unsworth, College of Oceanic and Atmospheric Sciences, Oregon State University, Corvallis, OR 97331, USA.

T. G. Pypker, School of Forest Resources and Environmental Sciences, Michigan Technological University, Houghton, MI 49931, USA. (tgpypker@mtu.edu)

E. W. Sulzman, Department of Crop and Soil Science, Oregon State University, Corvallis, OR 97331-5752, USA.