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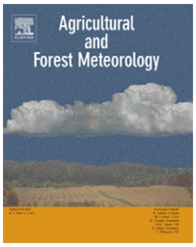

Canopy structure and atmospheric flows in relation to the $\delta^{13}C$ of respired CO_2 in a subalpine coniferous forest

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ABSTRACT

Stable isotopes provide insight into ecosystem carbon cycling, plant physiological processes, atmospheric boundary-layer dynamics, and are useful for the integration of processes over multiple scales. Of particular interest is the carbon isotope content ($\delta^{13}C$) of nocturnal ecosystem-respired CO_2 (δ_R). Recent advances in technology have made it possible to continuously examine the variation in δ_R within a forest canopy over relatively long timescales (months–years). We used tunable diode laser spectroscopy to examine δ_R at within- and below-canopy spatial locations in a Colorado subalpine forest (the Niwot Ridge AmeriFlux site). We found a systematic pattern of increased δ_R within the forest canopy ($\delta_{R\text{-}c}$) compared to that near the ground ($\delta_{R\text{-}g}$). Values of $\delta_{R\text{-}c}$ were weakly correlated with the previous day's mean maximum daytime vapor pressure deficit (VPD). Conversely, there was a negative but still weak correlation between $\delta_{R\text{-}g}$ and time-lagged (0–5 days) daily mean soil moisture. The topography and presence of sustained nightly drainage flows at the Niwot Ridge forest site suggests that, on nights with stable atmospheric conditions, there is little mixing of air near the ground with that in the canopy. Atmospheric stability was assessed using thresholds of friction velocity, stability above the canopy, and bulk Richardson number within the canopy. When we selectively calculated $\delta_{R\text{-}g}$ and $\delta_{R\text{-}c}$ by removing time periods when ground and canopy air were well mixed, we found stronger correlations between $\delta_{R\text{-}c}$ and VPD, and $\delta_{R\text{-}g}$ and soil moisture. This suggests that there may be fundamental differences in the environmental controls on δ_R at sub-canopy spatial scales. These results may help explain the wide variance observed in the correlation of δ_R with different environmental parameters in other studies.

1. Introduction

The carbon isotope ratio of carbon dioxide ($\delta^{13}C$ of CO_2) is an important tool for quantifying exchange between terrestrial ecosystems and the atmosphere (Ciais et al., 1995; Fung et al., 1997; Scholze et al., 2003). Feedbacks between ecosystem processes and climate affect the balance of photosynthesis and respiration, and the $\delta^{13}C$ of atmospheric CO_2 (Pataki et al.,

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2003; Scholze et al., 2003). Therefore, the $\delta^{13}C$ of CO_2 can provide an integrated measure of biological activity over multiple scales. On a continental scale, fluxes of carbon (C) from montane ecosystems of western North America may compose a significant portion of the total North American carbon budget (Schimel et al., 2002).

The $\delta^{13}C$ of CO_2 respired from an ecosystem (δ_R) represents the catabolism of all C substrates within the ecosystem and is directly related to the $\delta^{13}C$ of carbon inputs via photosynthesis. Stomatal conductance affects photosynthetic discrimination against ^{13}C, and consequently the $\delta^{13}C$ of fixed C (Farquhar et al., 1982). Stomatal conductance varies in response to soil moisture (Sperry et al., 2002) and atmospheric humidity (Oren et al., 1999), affecting plant gas exchange (Zhang and Nobel, 1996). As such, environmental variations have been shown to affect the $\delta^{13}C$ of photosynthetic products (Brugnoli and Farquhar, 2000; Gaudillere et al., 2002), the $\delta^{13}C$ of leaf-respired CO_2 (Duranceau et al., 1999; Ghashghaie et al., 2001; Hymus et al., 2005) and δ_R on a larger scale (Scartazza et al., 2004; Barbour et al., 2005). An aggregate correlation between δ_R and mean annual precipitation has been found across a variety of ecosystems (Pataki et al., 2003).

Correlations between δ_R and humidity and soil moisture are well documented in ecosystems. Decreased relative humidity or increased vapor pressure deficit have been correlated with δ_R and the $\delta^{13}C$ of both soil-respired (Ekblad and Högberg, 2001; Mortazavi et al., 2005), and leaf-respired (Mortazavi et al., 2005) CO_2. At the ecosystem scale, relationships between δ_R and atmospheric humidity have been observed (Bowling et al., 2002; McDowell et al., 2004a; Scartazza et al., 2004; Knohl et al., 2005; Lai et al., 2005; Mortazavi et al., 2006; Werner et al., 2006). The influence of moisture on δ_R has been found in forests (Ometto et al., 2002; Fessenden and Ehleringer, 2003; McDowell et al., 2004b; Scartazza et al., 2004; Lai et al., 2005; Mortazavi et al., 2005; Ponton et al., 2006) and grasslands (Lai et al., 2006; Ponton et al., 2006). Plant phenology (Lai et al., 2003; Zhang et al., 2006) and stand age (Fessenden and Ehleringer, 2002) also affect δ_R. Even though a mechanistic basis exists to describe moisture influences on δ_R (Bowling et al., 2002, McDowell et al., 2004a), some forests do not exhibit these relationships (Bowling et al., 2005).

Transport of CO_2 in mountain ecosystems is strongly influenced by topographically complex terrain. The katabatic flow of colder air from higher elevations to lower elevations (e.g. drainage flow) on relatively calm nights is a common phenomenon (Sturman, 1987; Yi et al., 2005). These flows typically occur when strong radiative cooling leads to the development of temperature inversions (i.e. air temperature increases with height). Cooler air created near the upward viewing surfaces (mostly at the top of the canopy) sinks to the forest floor. This air flows downhill, across the contours of slopes, and down valleys until it is no longer negatively buoyant with respect to surrounding air (of the same temperature). Horizontal advection associated with katabatic flows can make it difficult to make tower-based estimates of net fluxes of CO_2 in complex terrain (Aubinet et al., 2003; Turnipseed et al., 2003; Turnipseed et al., 2004; Yi et al., 2005), and by extension the isotopic composition of that CO_2. However, such atmospheric flow patterns may also present investigative opportunities when temporal and spatial patterns of the mixing ratio and the $\delta^{13}C$ of forest respired CO_2 are considered. For example, Yi et al. (2005) observed that during nights with high atmospheric stability, the downslope drainage flow under the tree canopy (roughly from the ground to 4–6 m height) is decoupled from that within the tree canopy (roughly from 4–6 m to top of the canopy). Yi et al. (2005) verified, via modeling and referring to tracer releases, that under stable atmospheric conditions, there exists a highly stable layer of air in the canopy. As a consequence, there is very little mixing of air below the tree canopy with that above the canopy. Thus, CO_2 respired from leaves and stems in the canopy may potentially be measured separately from CO_2 respired from the tree base, roots, and soil decomposers; this may provide unique isotopic signals for both canopy and soil respired CO_2.

Our objective was to examine patterns in the δ_R of forest air in a subalpine forest in Colorado, USA. In a previous study, Bowling et al. (2005) found no correlation between δ_R and soil moisture or atmospheric humidity. However, the nature of atmospheric flows at the site was not considered. In this study, we were interested in determining if δ_R of air in the forest canopy ($\delta_{R\text{-}c}$) was subject to different environmental controls from that below the canopy ($\delta_{R\text{-}g}$) at times when the forest air was stratified with little mixing between these layers. Since moisture may be a controlling variable and canopy air-space may often be stratified at night, we hypothesized that $\delta_{R\text{-}c}$ was controlled by the daytime vapor pressure deficit (VPD) of the air, with a short time lag (hours–days). That is, CO_2 respired by leaves was primarily derived from carbon fixed by leaves over the past several days. Any change in the isotope ratio of photosynthetic sugars in response to variation in VPD would be reflected in foliar respiration. We also hypothesized that the $\delta_{R\text{-}g}$ was dominated by soil respiration, both autotrophic (root and rhizosphere) and heterotrophic (organic matter decomposition). Approximately, 17–31% of soil respiration comes from autotrophic sources (Scott-Denton et al., 2005) in this forest. Therefore we expected $\delta_{R\text{-}g}$ to be affected by soil moisture and/or temperature (primarily through heterotrophic sources) and VPD (primarily through autotrophic sources), possibly also with some time lag. Therefore, we conducted combined observations of the vertical distribution of the $\delta^{13}C$ of atmospheric CO_2 and atmospheric stability to test the hypothesis that the correlations between the $\delta^{13}C$ of respired CO_2 and environmental factors are dependent on the relative magnitude and significance of gravitational flows as they affect atmospheric mixing within the canopy. Two sets of values of δ_R, $\delta_{R\text{-}c}$, and $\delta_{R\text{-}g}$ were calculated during time periods that included or excluded time periods of atmospheric mixing. They were compared to environmental variables to determine if any correlations emerged.

2. Materials and methods

2.1. Site description

This research was conducted in a subalpine coniferous forest in the Rocky Mountains of north-central Colorado, United States, during summer and fall 2003. The Niwot Ridge AmeriFlux Tower is part of the Niwot Ridge Long Term

Ecological Research site (40.03°N, 105.55°W). Elevation at the site is 3050 m, the average annual precipitation is 800 mm, and annual temperatures average 1.5 °C. Net ecosystem carbon, water, and energy fluxes, and weather have been monitored continuously at this site since the fall of 1998 (Monson et al., 2002, 2006; Turnipseed et al., 2002). Our measurements were conducted on a 26 m tall flux tower (Monson et al., 2002). The forest is an approximately 100-year-old mixed-species subalpine forest composed of *Abies lasiocarpa* (Hook.) Nutt. (subalpine fir), *Picea engelmannii* Parry ex Engelm. (Engelmann spruce), and *Pinus contorta* Dougl. Ex Loud. (lodgepole pine). The sparse understory contains seedlings of all three tree species and scattered patches of *Vaccinium myrtillus* L. The forest slopes gently (6–7%) and uniformly from west to east. Midsummer leaf area index for the forest is $4.2\,m^2\,m^{-2}$, canopy gap fraction is 17%, canopy height is 11.4 m, displacement height is 7.6 m, and roughness length is 1.79 m (Monson et al., 2002).

2.2. $\delta^{13}C$ of respired CO_2

Measurements of $[CO_2]$ and the $\delta^{13}C$ of CO_2 in forest air were made using a tunable diode laser absorption spectrometer (TDL, model TGA100, Campbell Scientific, Inc., Logan, Utah). The operational characteristics of this particular TDL are described in detail by Bowling et al. (2003) and the calibration and performance parameters for the TDL during the present field experiment are described in detail in Bowling et al. (2005). The TDL was operational at the site between July 4 and October 12 (days 185–285), 2003. A multi-inlet sampling manifold allowed automated sampling of nine heights from the tower below (0.1, 0.5, 1, and 2 m), within (5, 7, 9, and 11 m), and above the canopy (21.5 m), as well as four calibration gases every 6 min. Air from inlets at a given height was sampled at $185\,mL\,min^{-1}$ for 20 s. Data from the last 10 s of each 20 s period were averaged to produce a measurement. This allowed the plumbing to fully flush and pressure transients (<20 Pa) to dissipate in the TDL sample cell. Water vapor was removed before measurement using a Nafion counterflow system (PD 100, Campbell Scientific, Inc., Logan, Utah). All sampling heights and calibration gases were measured every 6 min. Half-hourly estimates of $[CO_2]$ and its $\delta^{13}C$ were taken as the mean of five 6 min measurements. The precision and accuracy of these measurements was $0.15\,\mu mol\,mol^{-1}$ for $[CO_2]$ and 0.15‰ for $\delta^{13}C$ (Bowling et al., 2005).

The Keeling plot approach (Keeling, 1958) was used to determine δ_R. Ordinary least squares regressions were used to avoid bias in calculated δ_R at low $[CO_2]$ ranges that occur when using geometric mean regressions (Zobitz et al., 2006). Keeling mixing lines were established for 8.5-h nocturnal periods from 20:00 to 04:30 h. For some analyses we shortened this time period based on meteorological conditions as described below. Keeling intercepts were calculated for the whole forest (δ_R using all nine inlets from the forest floor to above canopy), subsets of the inlets that are potentially representative of processes within the vegetation canopy ($\delta_{R\text{-}c}$: 5, 7, 9, and 11 m inlets), and for soil and understory processes ($\delta_{R\text{-}g}$, 0.1, 0.5, 1, and 2 m inlets). We use δ_R, $\delta_{R\text{-}c}$, or $\delta_{R\text{-}g}$ to denote a Keeling plot intercept derived from nighttime data only. Calculated Keeling plot intercepts were removed from the dataset if they met any one of the following quality criteria: (1) a $[CO_2]$ range of less than10 ppm, (2) fewer than 9 observations, or (3) the standard error of the intercept was >1‰.

2.3. Meteorological measurements

Half-hourly mean measurements of air temperature, soil temperature, relative humidity, and soil moisture were made over the same period that isotope measurements were made. Air temperature and relative humidity were measured at 2, 8, and 21.5 m heights (HMP-35D, Campbell Scientific Inc., Logan, Utah) and were used to calculate water vapor pressure deficit (with respect to saturation) of air (VPD). Soil temperature data integrated from 0 to 10 cm depth were collected using the median of 6 different platinum resistance thermometers (STP-1, Radiation and Energy Balance Systems Inc., Seattle, Washington). Soil moisture data integrated from 0 to 15 cm depth were collected using a water content reflectometer (CS-615, Campbell Scientific Inc., Logan, Utah). All sensors were monitored using a datalogger (CR23X, Campbell Scientific Inc., Logan, Utah).

2.4. Atmospheric stability

We used three estimates of atmospheric stability to identify possible time periods when nocturnal drainage flows were stratified: friction velocity (u^*, not a true stability parameter but used here as an indicator), scaled Obukhov length atmospheric stability ($(z-d)/L$), and bulk Richardson number (Ri_b). These parameters were calculated using half-hourly measurements of wind speed/direction and temperature taken from the Niwot Ridge AmeriFlux data archive (http://urquell.colorado.edu/data_ameriflux/). Wind speed and direction were measured at 21.5 m height using a 3D sonic anemometer (CSAT-3, Campbell Scientific Inc., Logan, Utah). Friction velocity, u^*, was calculated at 21.5 m height as: $u* = \sqrt{(\overline{u'w'})}$, where u' and w' are differences from the mean horizontal and vertical wind speeds, respectively. Atmospheric stability was determined using a displacement height (d) of 7.8 m (Turnipseed et al., 2003) and calculating the Obukov length (L) according to Kaimal and Finnigan (1994). The bulk Richardson number (Ri_b), a measure of shear production to turbulent mixing of a given layer of air, was calculated after Kaimal and Finnigan (1994). Reference heights of 21.5 m and 2 m for the measurement of potential temperature and wind speed at 21.5 m were used in the calculation of Ri_b. Higher Ri_b values are indicative of more stable atmospheric conditions.

Two scenarios were developed for the purposes of estimating $\delta_{R\text{-}c}$, $\delta_{R\text{-}g}$ and the potential effects of atmospheric conditions. Under a ‘mixing’ scenario, all half-hourly time periods on a given night were used to calculate Keeling plot intercepts for δ_{R-c} and $\delta_{R\text{-}g}$. Thus, this scenario includes periods with and without nocturnal drainage flows for stable through unstable atmospheric conditions. Under a ‘no mixing’ scenario, only time periods with stable atmospheric conditions (assessed using u^*, $(z-d)/L$, and Ri_b) were used in Keeling plot calculations. Additionally, if over the course of the night, the air profile in any half hour interval was considered unstable, canopy and ground air were assumed to have mixed and no subsequent time periods were used to calculate $\delta_{R\text{-}c}$ or $\delta_{R\text{-}g}$

(even if conditions of stability were present thereafter). We applied this rule in case an unstable period resulted in mixing of within- and below-canopy air, the effects of which would persist despite the re-establishment of stable conditions later that night.

2.5. *δ^{13}C of ecosystem components*

We sampled plant (needles, roots) and soil (organic horizon, mineral soil) components in order to determine the δ^{13}C of potential respiratory substrates. Needles were collected from three representatives of each of the three dominant tree species. We collected 3–8 needles from three relative heights (top, middle, and lower) within the canopy of each tree. Needle samples were bulked by species, dried, ground, and analyzed for δ^{13}C using an isotope ratio mass spectrometer (IRMS) (DeltaS; ThermoFinnigan, Waltham, Massachusetts). Soil cores (5 cm diameter) were collected beneath canopies of 12 representatives of each species. Cores were separated into O-horizon, typically 5–10 cm thick, and two mineral soil increments (0–10 and 10–20 cm below the termination of the O-horizon). Roots were removed by hand from each core increment and bulked for analysis. It was not possible to verify if a given root came from the tree under which it was collected. Roots and organic matter samples were dried, ground, and analyzed via IRMS.

2.6. *Statistical analysis*

Estimates of the δ^{13}C of ecosystem respired CO$_2$ were regressed against environmental parameters (VPD, soil moisture, and soil temperature) to investigate the effects of the environment on δ_R, $\delta_{R\text{-}c}$, and $\delta_{R\text{-}g}$. Micrometeorological data used for regressions were daily mean values with no averaging across days. Keeling plot intercepts (δ_R, $\delta_{R\text{-}c}$, and $\delta_{R\text{-}g}$) were calculated using nightly values of CO$_2$ mixing ratio and δ^{13}C. Isotopic data were regressed against environmental parameters that were shifted in time up to 15 days. This type of analysis has been used in previous studies investigating potential time lags in the relation between δ_R and VPD and/or soil moisture (Bowling et al., 2002; McDowell et al., 2004b; Knohl et al., 2005).

All calculations and statistical analyses were conducted using Matlab 7.0.1 software with Statistics Tool box 5.0.1 (The Mathworks, Inc., Natick, Massachusetts). Differences between mean values of $\delta_{R\text{-}c}$ and $\delta_{R\text{-}g}$, and between $\delta_{R\text{-}c} - \delta_{R\text{-}g}$ and zero were determined using a one-way analysis of variance. Significant differences are reported for $P < 0.05$. All error bars shown are the standard deviations of the mean values.

3. Results

Air temperature within the forest canopy averaged 9.3 °C from day of year 150 to 300 (Fig. 1a). After day 240 (vertical dashed line), nightly minimum air temperatures dropped below 4 °C. Sustained temperatures below 4 °C have been shown to substantially alter gas exchange in the tree species studied (Fahey, 1979; Kaufmann, 1982; DeLucia, 1986) and cold air temperature can influence δ_R in conifer forests (Bowling et al., 2002). Therefore, all subsequent analyses of relations between δ_R and environmental parameters were restricted to the time period before day 240. Air temperatures were below 0 °C intermittently on days 153–178, but no isotopic measurements were made during this time period. Soil temperature varied from −5.7 (day 300) to 21.0 °C (day 205) with a mean of 8.2 ± 4.5 °C between days 150 and 300 (Fig. 1b). VPD at 8 m (Fig. 1c) showed the greatest variation during the period between days 180 and 240 (0–2.6 kPa with a mean of 1.1 ± 0.5 kPa). Volumetric soil moisture (θ, m^3 m^{-3}) was highest early in the year (0.39 on day 151), and lowest in midsummer (0.08 on day 199) (Fig. 1d). Soil moisture was not as variable during the analysis period as compared to the entire period from day 150 to 300, and ranged from 0.08 to 0.17 m^3 m^{-3} with a mean θ of 0.11 ± 0.02 m^3 m^{-3} between days 180 and 240. The δ^{13}C of ecosystem respiration (δ_R, which includes all inlet heights) varied from −26.05 to −23.90‰ between days 185 and 285 (Fig. 1e). However, δ_R was less

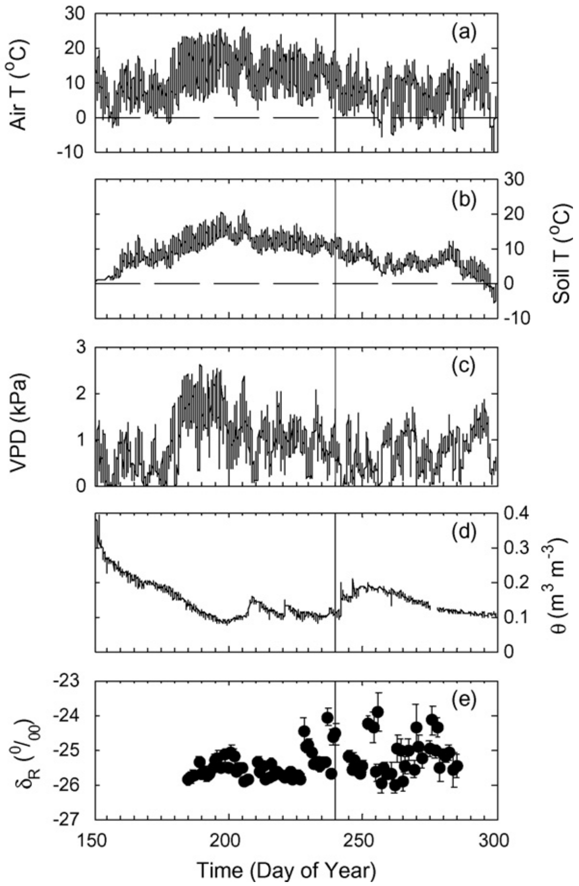

Fig. 1 – Half-hourly mean values of (a) air temperature at 8 m height, (b) soil temperature from 0 to 10 cm depth, (c) atmospheric vapor pressure deficit (VPD) at 8 m height, (d) soil moisture from 0 to 15 cm depth, and (e) nightly δ^{13}C of ecosystem respired CO$_2$ (δ_R) at the Niwot Ridge Ameriflux tower site on days 150–300, 2003. The horizontal dashed line on air and soil temperature plots denotes 0 °C. The vertical dashed line on all plots denotes day 240. Error bars represent one standard deviation of the mean (or one standard error for panel e).

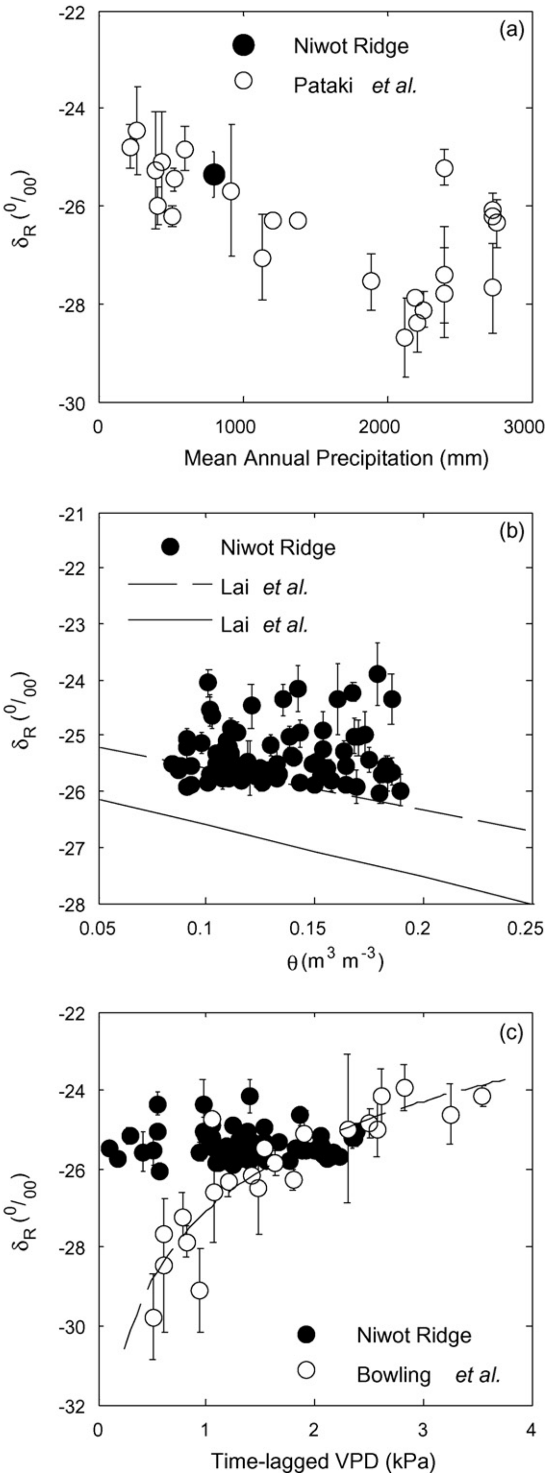

Fig. 2 – The $\delta^{13}C$ of ecosystem respired CO_2 (δ_R) compared with several environmental parameters. The seasonal (2003) mean δ_R is shown versus mean annual precipitation at Niwot Ridge compared to data from Pataki et al. (2003) in panel (a). Panel (b) shows nightly δ_R vs. mean daily soil moisture (θ, $m^3\ m^{-3}$) at Niwot Ridge and two similar regressions from Lai et al. (2005). Panel (c) shows nightly δ_R vs. mean daily maximum vapor pressure deficit (VPD) at Niwot Ridge and similar data from Bowling et al. (2002). Error bars represent one standard error of the intercept.

variable between days 185 and 240 ranging from −25.92 to −24.06‰ with a mean of −25.48 ± 0.38‰. These isotope data were previously reported by Bowling et al. (2005), but that study used a regression technique which we have since abandoned (Zobitz et al., 2006). The δ_R values reported in this study differ from those of Bowling et al. (2005).

While the seasonal mean δ_R at Niwot ridge (−25.48 ± 0.38‰) falls within the range of the general relationship with mean annual precipitation (MAP) shown by Pataki et al. (2003) (Fig. 2a), there was no relation between δ_R and soil moisture (θ) nor VPD, over the 2003 growing season (Fig. 2b and c). There was no significant relation ($P > 0.05$) between δ_R and θ over the study period, though the range in θ during this time was fairly narrow (Fig. 2b). For this relation, we examined time-lags up to 15 d using 1 and 2 d mean values of θ (data shown are 1 d means with no time lag). There was also no relation between δ_R and mean maximum daily VPD (between 0930 and 1500 h) for any of the previously described combinations of time-lags or daily means (Fig. 2c). Two selected studies which demonstrated linkages between soil moisture (Lai et al., 2005) or VPD (Bowling et al., 2002) and δ_R are shown for comparison. The soil moisture patterns are shown for illustrative purposes only; we do not expect Niwot Ridge data to quantitatively follow the same patterns, since soil moisture effects are site-dependent.

When δ_R was separated into within- and below-canopy components (canopy inlets and near ground inlets respectively), there were distinct differences in the $\delta^{13}C$ of respiration over the course of the study (Fig. 3a). There was no significant difference ($P > 0.5$) between mean $\delta_{R\text{-}g}$ measured from days 185 to 300 (−25.68 ± 0.74‰) and that between days 185 and 240 (all data up to the vertical dashed line, mean of −25.92 ± 0.54). Mean $\delta_{R\text{-}c}$ was also not significantly different between the two time periods (−25.04 ± 0.83‰ between days 185 and 300, −25.15 ± 0.55‰ between days 185 and 240). There was a consistent, significant ($P < 0.05$) depletion of $\delta_{R\text{-}g}$ relative to $\delta_{R\text{-}c}$ ($\delta_{R\text{-}c} - \delta_{R\text{-}g}$) (Fig. 3b) between days 185 and 240 with a mean value of 0.81 ± 0.65‰. Though mean $\delta_{R\text{-}c} - \delta_{R\text{-}g}$ was 0.64 ± 0.94‰ between days 185 and 300, the difference was not significantly different from zero ($P > 0.05$).

By examining atmospheric stability, we identified time-periods when it was likely that mixing between within- and below-canopy airflow was minimal. It was assumed that under stable conditions, CO_2 mixing ratios of forest air would increase through the night and the $\delta^{13}C$ of that CO_2 would decrease with respiration of CO_2 into the atmosphere. The difference between $[CO_2]$ and the $\delta^{13}C$ of CO_2 measured at a given height relative to 21.5 m ($\Delta[CO_2]$ and $\Delta\delta^{13}C$ respectively) is shown in Fig. 4. $\Delta[CO_2]$ and $\Delta\delta^{13}C$ were binned by the value of a given stability parameter and assessed for buildup of gradients of CO_2 and the $\delta^{13}C$. An increase in bin-averaged $\Delta[CO_2]$ or $\Delta\delta^{13}C$ over time was indicative of stronger gradients of CO_2 and the $\delta^{13}C$ within the forest. Cutoff values (dashed vertical lines in Fig. 4) for defining stable atmospheric conditions were chosen as those values of u^*, $(z - d)/L$, and Ri_b at which CO_2 and the $\delta^{13}C$ gradients became steep in the canopy. The respective values chosen were $u^* < 0.4$ (m s^{-1}), $(z - d)/L > 0.1$ (dimensionless), and $Ri_b > 2.5$ (dimensionless). For $(z - d)/L$, and Ri_b, there appears to be a decrease in gradient size under extremely stable conditions which contrasts with the results of Burns et al. (2006), who examined gradients of

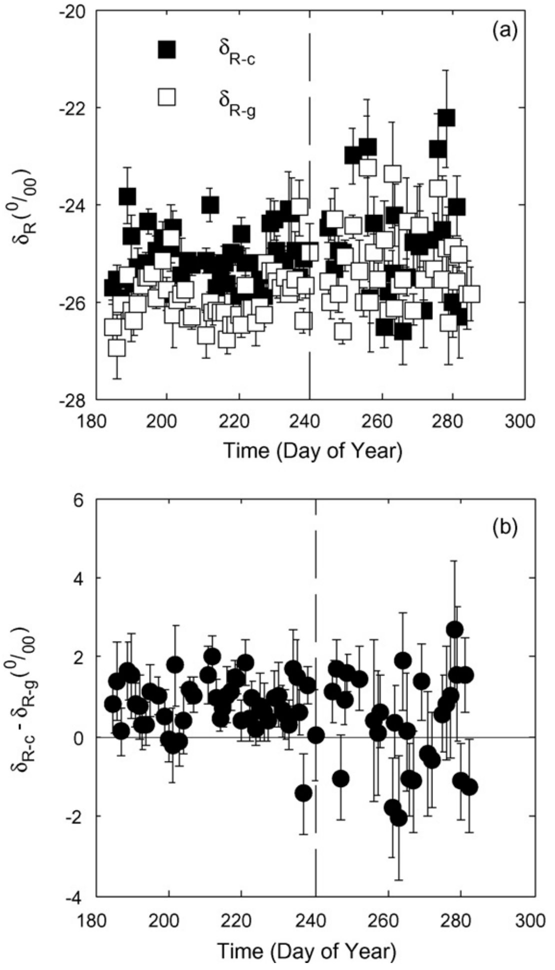

Fig. 3 – Nightly δ_R calculated separately at within-canopy ($\delta_{R\text{-}c}$, 5–11 m), and below-canopy ($\delta_{R\text{-}g}$, 0.1–2 m) locations. The top panel (a) shows values of $\delta_{R\text{-}c}$ and $\delta_{R\text{-}g}$. The bottom panel (b) shows the difference between the two ($\delta_{R\text{-}c} - \delta_{R\text{-}g}$). The vertical dashed line denotes day 240. Error bars in (a) represent standard error of the intercept, and in (b) they represent the sum of the of the relevant individual error bars in (a).

[CO_2] binned by Ri_b in the same forest as the current study but found no decrease in the [CO_2] gradient with increasing Ri_b stability. Gradient size may be decreasing with increasing stability (Ri_b or $(z - d)/L$) due to increased subcanopy drainage flow and decreasing respiration (which tends to decrease with temperature). Alternately, ecosystem respiration may be greater early in the evening when stability is weak.

When these atmospheric stability criteria were applied to all nightly half-hour time-periods between 18:00 and 06:00 h for days 185–285, a matrix of stable and unstable periods was generated (Fig. 5). Atmospheric stability parameters were considered both separately and combined, with unstable periods shown in black. The time over which Keeling plot intercepts were nominally calculated consisted of 17 half-hourly intervals between 20:00 and 04:30 h. This gave a total of 1700 half-hourly intervals over the 100 days of measurement. For the u^* case, 536 of these intervals (31.5%) had $u^* > 0.4$ and were considered unstable. For $(z - d)/L$, 537 (31.6%) of the total number of intervals were labeled as unstable. For the Ri_b case, 461 intervals (27.1%) were considered unstable. When all three meteorological parameters were considered together, 709 (41.7%) of half-hourly intervals were unstable when any one stability criteria yielded an ‘unstable’ classification.

When $\delta_{R\text{-}c}$ and $\delta_{R\text{-}g}$ were calculated separately for either: (1) all time periods (i.e. including periods when mixing between canopy and ground inlets was likely), or for (2) only those times when no mixing had occurred, there was an effect on the correlation between $\delta_{R\text{-}c}$ and $\delta_{R\text{-}g}$ and environmental parameters (Fig. 6). Under the ‘mixing’ regime (including periods considered unstable), there was a Pearson correlation coefficient of 0.35 between $\delta_{R\text{-}c}$ and daily mean maximum VPD with no time lag. However, this correlation was not statistically significant ($P > 0.05$) (Fig. 6a). This held true for VPD lagged up to 15 days (e.g. tonight's $\delta_{R\text{-}c}$ compared to VPD from 0 to 15 days ago). When only time periods with stable atmospheric conditions were used to calculate $\delta_{R\text{-}c}$ (‘no mixing’ regime), there was a significant ($P < 0.05$) Pearson correlation coefficient of 0.82 between $\delta_{R\text{-}c}$ and VPD with no time lag. This means that a given night's $\delta_{R\text{-}c}$ was correlated with mean maximum VPD during the day prior. Conversely, $\delta_{R\text{-}g}$ appeared to be negatively correlated to soil moisture (θ) under both ‘mixing’ and ‘no mixing’ regimes ($P < 0.05$), but the relation was stronger under the ‘no mixing’ regime (Fig. 6b). The correlation of $\delta_{R\text{-}g}$ to θ under the ‘mixing’ regime ranged from −0.32 to −0.49 and spread across θ time-lagged from 0 to 6 days. Correlation coefficients for the ‘no mixing’ regime were stronger (more negatively correlated) over roughly the same days (0–5) and ranged from −0.46 to −0.76. There was an inverse relationship (maximum PCC of 0.75) between $\delta_{R\text{-}g}$ and soil temperature with the same time lagged response as that for θ (data not shown). Correlations between $\delta_{R\text{-}c}$ and θ, and $\delta_{R\text{-}g}$ and VPD were calculated, but the results were not significant ($P > 0.05$, data not shown).

The actual relation between $\delta_{R\text{-}c}$ and VPD, or $\delta_{R\text{-}g}$ and θ is shown when both $\delta_{R\text{-}c}$ and $\delta_{R\text{-}g}$ are plotted against the appropriately time-lagged environmental variable (Fig. 7). In this case, both VPD and θ are shown with 0 d time lags, and $\delta_{R\text{-}c}$ and $\delta_{R\text{-}g}$ are for the ‘no mixing’ regime. The $\delta_{R\text{-}c}$ ranged from -25.95 ± 0.22 to -24.57 ± 0.33‰ over a range of VPD from 1.3 to 2.4 kPa (Fig. 7a). Data from a similar δ_R versus VPD relationship from Bowling et al. (2002) are shown for comparison. The Niwot Ridge data follow closely the fit reported by Bowling et al. (2002). It must be noted however, that the δ_R displayed for Bowling et al. (2002) are for whole-ecosystem respired CO_2, not just canopy air ($\delta_{R\text{-}c}$), as in the present study. Values of $\delta_{R\text{-}c}$ were higher than the $\delta^{13}C$ values of potential substrates, ranging from 0.7 to 2.1‰ higher than the combined $\delta^{13}C$ of biomass of upper canopy (sunlit) needles, and 2.8–4.2‰ higher than that of lower canopy (shaded) needle biomass (Table 1). The negative relation between $\delta_{R\text{-}g}$ and θ is shown in Fig. 7b. Here, $\delta_{R\text{-}g}$ varied between -27.95 ± 1.03 and -24.40 ± 0.69‰ over a range of θ from 0.09 to 0.15 $m^3\ m^{-3}$. Two comparative relations from Lai et al. (2005) are shown as well. Comparing $\delta_{R\text{-}g}$ to potential respiratory substrates, the mean $\delta_{R\text{-}g}$ (-26.18 ± 0.9‰) was 0.3‰ higher than bulked root $\delta^{13}C$, 0.2‰ lower than the soil O-horizon $\delta^{13}C$, and 1.1‰ lower than the 0–10 cm layer mineral soil organic matter $\delta^{13}C$ (Table 1).

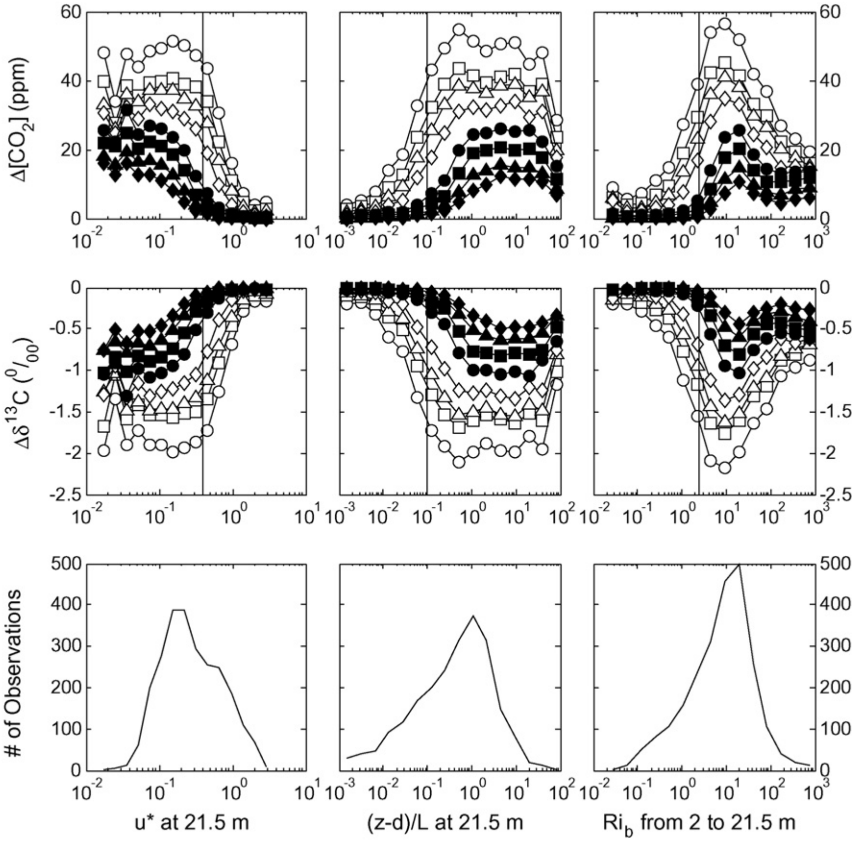

Fig. 4 – Difference in CO$_2$ (Δ [CO$_2$], ppm) and the δ^{13}C (Δ δ^{13}C, ‰) of CO$_2$ in the forest air relative to above canopy (21.5 m height) for three separate estimates of atmospheric stability (u*, $(z - d)/L$, and Ri_b). Closed symbols are canopy level inlets (11 m = diamonds, 9 m = triangles, 7 m = squares, and 5 m = circles). Open symbols are near ground inlets (2 m = diamonds, 1 m = triangles, 0.5 m = squares, and 0.1 m = circles). Stability estimates are binned on a logarithmic scale. The vertical dashed lines denote threshold values for stable atmospheric conditions based on gradient development in the canopy. These arbitrary threshold values were selected at 0.4, 0.1, and 2.5 for u*, $(z - d)/L$, and Ri_b respectively. Bottom panels show the distribution of the number of observations for each of the bins.

4. Discussion

The mixing ratio and the δ^{13}C of CO$_2$ were measured at multiple heights in the forest, and these data were used to examine atmospheric and environmental effects on the δ^{13}C of respired CO$_2$ (δ_R). When all sampling inlets were used in the analysis to calculate δ_R, there was no apparent relation with VPD or soil moisture (Fig. 2b and c). However, there is a local layer of high atmospheric stability within the canopy at Niwot Ridge that separates two distinct atmospheric zones under stable nighttime atmospheric conditions, one in the upper canopy and one nearer the ground, with little mixing between them (Yi et al., 2005). After separating sampling inlets vertically into within- and below-canopy domains (i.e. canopy and ground sampling inlets) and calculating $\delta_{R\text{-}c}$ and $\delta_{R\text{-}g}$, significant correlations between $\delta_{R\text{-}c}$ and VPD, and $\delta_{R\text{-}g}$ and soil

Table 1 – Stable carbon isotope content (δ^{13}C, ‰) of plant biomass (needles, roots), and soil organic matter (O-horizon, mineral soil)

Location	Subalpine Fir	Lodgepole Pine	Engelmann Spruce	All Species
Needles (top)	−27.51	−26.68	−26.26	−26.66 (0.38)
Needles (mid)	−28.70	N/A	−27.51	−28.10 (0.84)
Needles (lower)	−29.26	−28.86	−28.16	−28.76 (0.55)
Roots	−26.70 (0.21)	−26.51 (0.37)	−26.29 (0.32)	−26.50 (0.33)
O-horizon	−26.06 (0.19)	−26.04 (0.26)	−25.74 (0.16)	−25.95 (0.24)
Mineral soil (0–10 cm)	−25.40 (0.14)	−25.30 (0.24)	−25.10 (0.19)	−25.27 (0.22)
Mineral soil (10–20 cm)	−25.02 (0.05)	−24.90 (0.27)	−24.79 (0.23)	−24.91 (0.21)

Mean values for all three tree species combined are also shown. Values in parentheses are the standard deviations of the means (with the exception of needles, please see Section 2 for a full explanation).

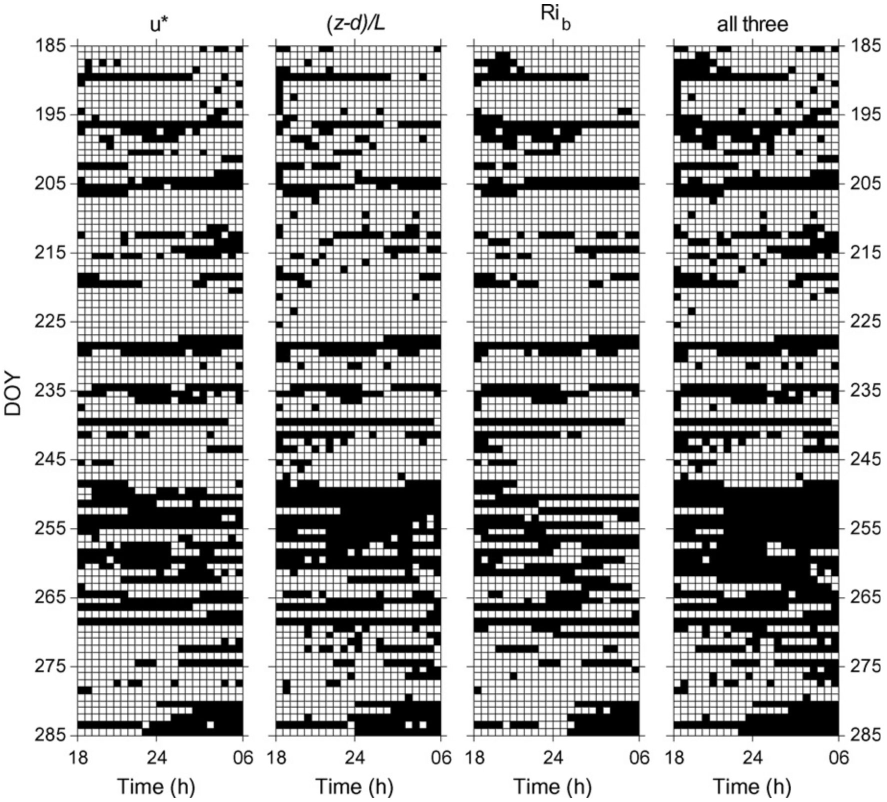

Fig. 5 – Matrices showing half hourly time periods (for days 185–285, 2003) where atmospheric conditions were considered to be unstable (filled squares) given three estimates of atmospheric (u^*, $(z-d)/L$, and Ri_b). The fourth panel shows time periods of unstable conditions as determined by any of the three stability estimates.

moisture were observed (Figs. 6 and 7). The δ_{R-c} relationship with VPD was positive with no time lag, and the δ_{R-g} relationship with θ was negative with a lag of 0–6 days. Additionally, these correlations were strongest when time periods of stable atmospheric conditions were considered for the calculation of Keeling plot intercepts (Fig. 6). These results support our hypotheses that (1) δ_{R-c} is more sensitive to VPD than to soil moisture at Niwot Ridge, with a relatively short time lag (hours), and δ_{R-g} is more sensitive to soil moisture than VPD, with a longer time lag (hours to several days).

Photosynthetic carbon isotope discrimination (Δ) is directly linked to the ratio of intercellular to ambient CO_2 (c_i/c_a) in C_3 plants (Farquhar et al., 1982) and controls the $\delta^{13}C$ of sugars that are produced through photosynthesis. Ultimately, fixed carbon will be respired back to the atmosphere, either directly by foliage, stems, roots, or rhizospheric symbionts, or after litter deposition and incorporation into soil organic matter. All respiring components in an ecosystem contribute to "total" ecosystem respiration, and hence, δ_R is dependent on a variety of physical and biological processes. Thus, δ_R is an integrated measure of whole ecosystem respiratory processes (Flanagan et al., 1996; Buchmann et al., 1998). A fairly robust relation between ecosystem δ_R and mean annual precipitation has emerged in recent years (Pataki et al., 2003). The mean seasonal δ_R (whole forest) from Niwot Ridge in 2003 is consistent with that predicted by this general relation on the basis of mean annual precipitation (Fig. 2), suggesting that δ_R at Niwot Ridge and other sites is strongly related to ecosystem water balance. However, the initial lack of correlation between δ_R and environmental parameters at Niwot Ridge (Fig. 2b and c) is not consistent with the hypothesis that ecosystem carbon cycle processes are dependent on moisture with resulting changes in δ_R. Cold temperatures are a potentially confounding factor that may explain the lack of correlation of δ_R with environmental factors. Cold air temperatures can affect the sensitivity of stomatal conductance to atmospheric humidity, potentially altering δ_R. Freezing night-time air temperatures have been shown to alter the dependence of the $\delta^{13}C$ of ecosystem respiration on VPD in conifer forests (Bowling et al., 2002; McDowell et al., 2004a). Cold soil (Dykstra, 1974; DeLucia, 1986; Day et al., 1989) and air temperatures (Fahey, 1979; Kaufmann, 1982) can also reduce stomatal conductance and photosynthesis of the three conifer tree species present at Niwot Ridge. For these reasons, we chose a cutoff point for our isotope analysis of air temperatures $<4\,^{\circ}C$ (day 240), which coincides with observed changes in δ_R (Fig. 1) and $\delta_{R-c} - \delta_{R-g}$ (Fig. 3).

Underestimation of net ecosystem CO_2 fluxes under stable atmospheric conditions is an acknowledged problem in eddy covariance studies (Aubinet et al., 2003, 2005; Baldocchi, 2003; Froelich and Schmid, 2006). In sloping terrain, nocturnal drainage flows can lead to the lateral advection of CO_2 that affects the local carbon budget. The vertical structure of the downslope drainage flows at this site, by which flows higher

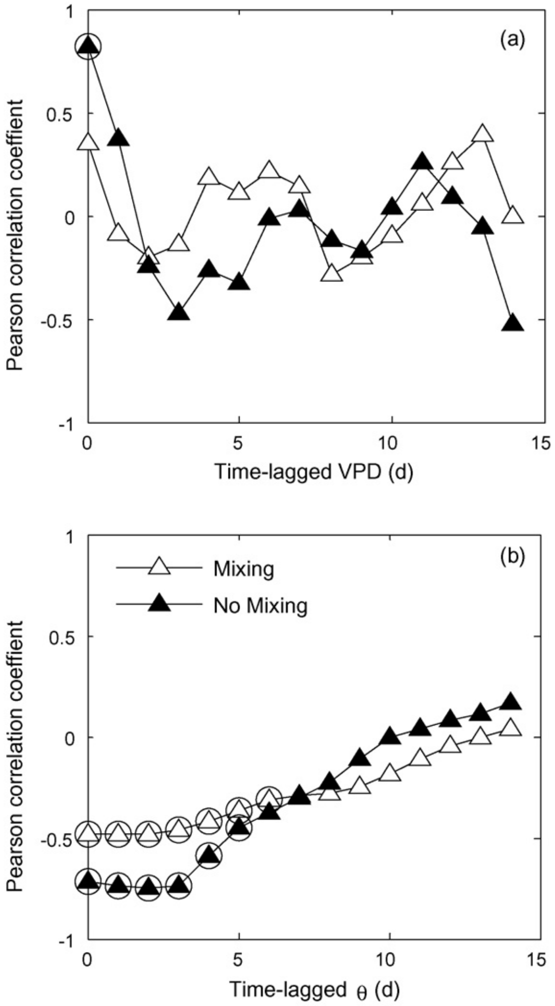

Fig. 6 – Pearson correlation coefficient for (a) $\delta_{R\text{-}c}$ and time-lagged mean maximum VPD, and (b) $\delta_{R\text{-}g}$ and time-lagged mean daily soil moisture (θ) for days 185–240. The time lag represents estimates of VPD and θ from 0 to 15 days prior to a given estimate of $\delta_{R\text{-}c}$ or $\delta_{R\text{-}g}$. Mean values of VPD and θ are single day means. Correlations with $\delta_{R\text{-}c}$ and $\delta_{R\text{-}g}$ are shown for both 'mixing' (open symbols) and 'low mixing' (closed symbols) scenarios. Circled points denote significant ($P < 0.05$) correlations.

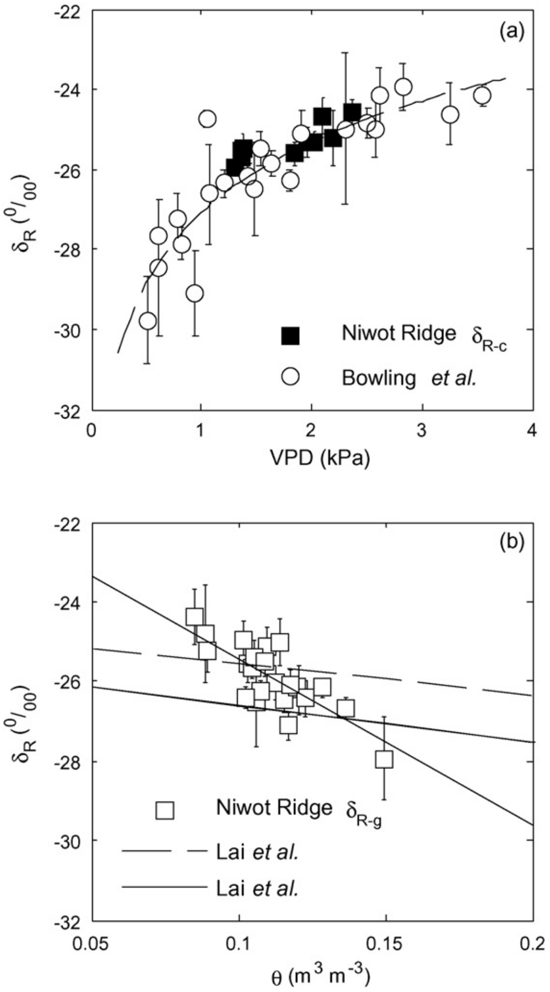

Fig. 7 – Relationship between (a) $\delta_{R\text{-}c}$ and VPD (0 days time lagged), and (b) $\delta_{R\text{-}g}$ and soil moisture (0 days time lagged). The upper panel (a) has data from Bowling et al. (2002) plotted for comparison (δ_R). The lower panel (b) has two regression relationships from Lai et al. (2005) plotted for comparison. Error bars represent standard error of the intercept.

and lower in the canopy are physically separated (Yi et al., 2005; Sun et al., 2007), provides an opportunity to independently partition the contributions of respiration from different vertical domains in the ecosystem (e.g. upper canopy from lower canopy plus soil) to the local carbon budget. We observed a consistently greater $\delta_{R\text{-}c}$ relative to $\delta_{R\text{-}g}$ between days 185 and 240 (Fig. 3). This pattern was insensitive to whether a 'mixing' or 'no mixing' scenario was considered for calculating Keeling plot intercepts (data not shown). This suggests that there may be a distinction between the δ^{13}C of the source of respiration in the air layer higher in the tree canopy compared to that nearer the ground. Enrichment of the δ^{13}C of respired CO_2 with height has been observed in other conifer ecosystems (Ogee et al., 2003; Mortazavi et al., 2006), but there is at least one exception to this pattern (Tu and Dawson, 2005). As is commonly observed, the δ^{13}C of needle biomass increases with height in this forest (Table 1). While $\delta_{R\text{-}c}$ may be dominated by foliage respiration, $\delta_{R\text{-}g}$ consists of a mix of autotrophic (root) and heterotrophic (rhizosphere and organic matter-derived microbial) respiration, and understory foliar respiration (although the understory at Niwot Ridge is fairly sparse). Observed $\delta_{R\text{-}g}$ spanned the observed range for potential respiratory substrates (roots and soil organic matter) at the Niwot Ridge forest (Table 1). Differences between autotrophic and heterotrophic carbon sources may fully account for the observed $\delta_{R\text{-}c} - \delta_{R\text{-}g}$, and studies of the δ^{13}C values of respiration from different plant tissues are consistent with our observed patterns. At the whole-plant scale, the δ^{13}C of shoot respired CO_2 has been shown to be enriched relative to root respired CO_2 (Klumpp et al., 2005; Schnyder and Lattanzi, 2005). This is likely due to differences in the δ^{13}C of carbon substrates being respired (Ghashghaie et al., 2003), and/or carbon isotope fractionation associated with metabolism (Tcherkez and Farquhar, 2005). Fractionation with respiration, as well as the isotopic composition of respired substrates is not well characterized at the ecosystem-scale. For example, we observed that mean $\delta_{\text{R-c}}$ was greater than $\delta_{\text{R-g}}$, yet needle δ^{13}C was lower than root and organic matter δ^{13}C (Table 1). A detailed examination of the δ^{13}C of carbon substrates (sugars, lipids, etc.) in leaves and roots and their respiratory CO_2, and that of bulk materials to δ_R in these ecosystems is needed to determine the mechanisms behind these observed differences in δ_R with height.

The VPD of forest air can act as a shorter-term (hours–days) influence on δ_R in comparison to soil moisture. Our results show that the carbon isotopic content of respired CO_2 in the tree canopy ($\delta_{\text{R-c}}$, 5–11 m) was correlated to VPD on the day immediately preceding our nocturnal measurements (Fig. 6). Additionally, there was no significant correlation between $\delta_{\text{R-c}}$ and VPD until unstable time periods were removed from the analysis of $\delta_{\text{R-c}}$. This may be due to the fact that gradients of CO_2 and the δ^{13}C of CO_2 were smaller within the tree canopy than closer to the ground (Fig. 4) and a smaller CO_2 range will increase the uncertainty in the calculated Keeling plot intercept value, and therefore affect any estimates of correlation with environmental variables. However, Mortazavi et al. (2005) directly measured the isotope ratio of respiration from foliage and the soil using chambers, and found the linkage to VPD in the foliar respiration was stronger than in the soil respiration. The correlations observed in our study were between VPD and $\delta_{\text{R-c}}$ only, and were not a direct measure of the δ^{13}C of foliar respiration. Even when foliar and soil processes have not been considered separately, correlations of δ_R with VPD have been observed in European beech (Scartazza et al., 2004; Knohl et al., 2005), oak savanna (Werner et al., 2006), mixed broadleaf forests (Mortazavi et al., 2005), and conifer forests (Bowling et al., 2002; McDowell et al., 2004a,b; Mortazavi et al., 2005; Aranibar et al., 2006). In all of these studies, the relation exhibited a time lag with δ_R correlated to VPD shifted by 1–10 days. However, light intensity, temperature, soil moisture and VPD are all correlated and have very similar effects on carbon isotope discrimination. As a result it is very difficult to determine the primary role of any one of these environmental factors singularly controlling the response of carbon isotope discrimination. A multivariate analysis of a wide range of environmental factors is necessary to examine the combined influences of temperature, VPD, soil moisture, and light (McDowell et al., 2004b; Alstad et al., 2007).

The lack of a time lag in the observed correlation suggests that a substantial quantity of recent photosynthate (C fixed several hours to 1 day ago) is respired by needles and/or branches. A mechanistic link between observed changes in δ_R and leaf-level ecophysiological processes was described by Bowling et al. (2002), who found that when δ_R was used to estimate total canopy conductance (stomatal + boundary layer), a realistic relationship between conductance and time-lagged VPD emerged. This relationship was revealed for four different conifer forests that spanned a 2500 mm range in mean annual precipitation. Our results of $\delta_{\text{R-c}}$ versus VPD fall along roughly the same regression line as that reported by Bowling et al. (2002) for the range of values of VPD measured in our study and suggest that $\delta_{\text{R-c}}$ is varying in response to VPD induced changes in stomatal conductance and photosynthetic capacity. Mechanistic studies are needed that examine the direct links between plant stomatal function and the δ^{13}C of ecosystem respiration.

Soil moisture appears to be important in determining the δ^{13}C of respired CO_2 near the ground (0.1–2 m) in this subalpine forest. A negative correlation between soil moisture and $\delta_{\text{R-g}}$ was observed regardless of whether separation of atmospheric flows was considered. However, the correlation was stronger under stable atmospheric conditions when within- and below-canopy drainage flows were minimally mixing (Fig. 6). This significant relation was maintained when $\delta_{\text{R-g}}$ was compared to soil moisture with no time lag (e.g. most recent night's $\delta_{\text{R-g}}$ compared to day's soil moisture) to a time lag of 6 days. The broad peak in this relation may be due to the fact that changes in soil moisture occur relatively slowly compared to atmospheric humidity. It may also be due to the fact that the soil moisture only ranged from 0.08 to 0.17 $m^3\,m^{-3}$ (with a standard deviation of 0.01 $m^3\,m^{-3}$). In other studies, soil moisture has been shown to exert a seasonal or monthly influence on δ_R. Negative correlations between δ_R and soil moisture have been reported in Brazilian tropical forest (Ometto et al., 2002), at sites dominated by European beech (Scartazza et al., 2004), mixed grass prairie and aspen (Ponton et al., 2006), loblolly pine (Mortazavi et al., 2005), mixed boreal conifers (Lai et al., 2005), and Douglas-fir (Fessenden and Ehleringer, 2003; McDowell et al., 2004b; Ponton et al., 2006). In contrast, a ponderosa pine forest, whose trees had access to groundwater, did not show this pattern (McDowell et al., 2004b).

In our study, $\delta_{\text{R-g}}$ is likely heavily influenced by soil respiration, especially during those time periods where air near the ground (0.1–2 m) is stratified and decoupled from that in the rest of the canopy (5–11 m). Maximum rates of soil respiration of 6 μmol $m^{-2}\,s^{-1}$ were observed from soils at this site in 2003 (Scott-Denton et al., 2005). The δ^{13}C of CO_2 respired from soils comes from a combination of autotrophic and heterotrophic sources. At this study site, between 17 and 31% of total soil respiration came from autotrophic sources in 2003 (Scott-Denton et al., 2005). In comparison, these values for soil respiration are actually greater than modeled estimates of total ecosystem respiration (approximately 5 μmol $m^{-2}\,s^{-1}$) (Yi et al., 2004). This disparity likely arises from the fact that estimates of soil respiration (Scott-Denton et al., 2005) were point measurements taken over a relatively short time period, and total ecosystem respiration estimates (Yi et al., 2004) were the result of a modeling study over larger temporal and spatial scales. Autotrophic sources include root and stem respiration, with mycorrhizal and rhizosphere respiration likely to reflect root respiration in isotopic content. As previously mentioned, the δ^{13}C of leaf sugars is subject to environmental and biochemical control. After production, these sugars are then transported to roots and stems to be used as energy sources, thereby providing a link between the δ^{13}C of soil/ecosystem respired CO_2 and canopy processes. Studies examining the relation between soil respired δ^{13}C (as opposed to ecosystem respired CO_2) and humidity have found significant correlations (Ekblad and Högberg, 2001; McDowell et al., 2004a; Mortazavi et al., 2005) with time lags ranging from 3 to 5 days. Though not statistically significant, we also observed correlations between δ_R, $\delta_{R\text{-}g}$, and VPD (0.54 for both δ_R and $\delta_{R\text{-}g}$) with a time lag of 7 days. Heterotrophic sources of respiration include organisms such as bacteria, fungi, and soil invertebrates feeding on substrates that vary in their age and lability from root exudates to humic acids (Kuzyakov, 2006). The large range in potential substrates equates to a large range in potential $\delta^{13}C$ values, and hence the $\delta^{13}C$ of CO_2 respired from these different substrates (Ehleringer et al., 2000). Thus, isotopic linkages between soil respired CO_2 and current environmental conditions may be difficult to find. It has been suggested that the $\delta^{13}C$ of heterotrophic respired CO_2 will be relatively insensitive to climate on the scale of days (Ponton et al., 2006). However, it has been shown that soil heterotrophic respiration rate, and the $\delta^{13}C$ of respired CO_2 can vary with temperature (Andrews et al., 2000), and presumably soil moisture since the two co-vary. In agreement with Andrews et al. (2000) and McDowell et al. (2004b), we observed a positive correlation between $\delta_{R\text{-}g}$ and soil temperature (data not shown) that parallels the relationship between $\delta_{R\text{-}g}$ and soil moisture. From a biological perspective, this may be indicative of changes in the activity, fractionation with respiration, or composition of the heterotrophic community, or the relative fluxes from the autotrophic and heterotrophic sources. Alternatively, it may simply be a function of covariance between soil moisture and temperature.

These findings contribute to our understanding of the controls of climate on ecosystem respiration, and have the potential to enhance future work in several ways. First, such findings may be useful to studies that attempt to partition net ecosystem exchange (NEE) into photosynthetic and respiratory components (Bowling et al., 2001; Lai et al., 2003; Ogee et al., 2003; Knohl and Buchmann, 2005; Zobitz et al., 2007) in which values of δ_R can have a large influence on partitioning results. Second, an understanding of the controls on the isotopic content of δ_R provides an independent perspective to evaluate measurements of NEE and evapotranspiration (ET). Changes in ecosystem-scale water use efficiency (WUE) might result from relative changes in the gross fluxes of photosynthesis and respiration, or from water stress and resulting stomatal response. If changes in δ_R are tied to changes in photosynthesis and stomatal conductance, then investigations of δ_R provide a fully independent means to verify the flux-based estimates of WUE (Ponton et al., 2006). Understanding the complexities of the factors that influence δ_R is needed to realistically make a comparison to eddy-flux based estimates of WUE.

In summary, we initially observed very low correlation between whole-forest respired $\delta^{13}C$ (δ_R) and environmental drivers at Niwot Ridge. The presence of nocturnal drainage flows coupled with the aerodynamic properties of the forest canopy led to time periods where airflow was stratified on nights with stable atmospheric conditions. When we separated our calculation of δ_R in the same manner ($\delta_{R\text{-}c}$ and $\delta_{R\text{-}g}$) and confined our analyses to time periods with stable atmospheric conditions, correlations with environmental variables emerged that were consistent with observations from other forests. Observed $\delta_{R\text{-}c}$ was significantly correlated with VPD with no time lag and $\delta_{R\text{-}g}$ was significantly correlated with soil moisture. Both correlations held only in the absence of freezing temperatures. Now, rather than being faced with the puzzle of explaining how δ_R does not appear to be related to environment, we have results consistent with findings of mechanistic and process-based studies of how the $\delta^{13}C$ of ecosystem respiration varies due to leaf-level ecophysiology.

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