

Wetting and drying cycles drive variations in the stable carbon isotope ratio of respired carbon dioxide in semi-arid grassland

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Abstract In semi-arid regions, where plants using both C₃ and C₄ photosynthetic pathways are common, the stable C isotope ratio ($\delta^{13}\text{C}$) of ecosystem respiration ($\delta^{13}\text{C}_R$) is strongly variable seasonally and inter-annually. Improved understanding of physiological and environmental controls over these variations will improve C cycle models that rely on the isotopic composition of atmospheric CO₂. We hypothesized that timing of precipitation events and antecedent moisture interact with activity of C₃ and C₄ grasses to determine net ecosystem CO₂ exchange (NEE) and $\delta^{13}\text{C}_R$. Field measurements included CO₂ and $\delta^{13}\text{C}$ fluxes from the whole ecosystem and from patches of different plant communities, biomass and $\delta^{13}\text{C}$ of plants and soils over the 2000 and 2001 growing seasons. NEE shifted from C source to sink in response to rainfall events, but this shift occurred after a time lag of up to 2 weeks if a dry period preceded the rainfall. The seasonal average of $\delta^{13}\text{C}_R$ was higher in 2000 (−16‰) than 2001 (20‰), probably due to

drier conditions during the 2000 growing season (79.7 mm of precipitation from April up to and including July) than in 2001 (189 mm). During moist conditions, $\delta^{13}\text{C}$ averaged −22‰ from C₃ patches, −16‰ from C₄ patches, and −19‰ from mixed C₃ and C₄ patches. However, during dry conditions the apparent spatial differences were not obvious, suggesting reduced autotrophic activity in C₄ grasses with shallow rooting depth, soon after the onset of dry conditions. Air and soil temperatures were negatively correlated with $\delta^{13}\text{C}_R$; vapor pressure deficit was a poor predictor of $\delta^{13}\text{C}_R$, in contrast to more mesic ecosystems. Responses of respiration components to precipitation pulses were explained by differences in soil moisture thresholds between C₃ and C₄ species. Stable isotopic composition of respiration in semi-arid ecosystems is more temporally and spatially variable than in mesic ecosystems owing to dynamic aspects of pulse precipitation episodes and biological drivers.

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Introduction

Understanding the size, spatial distribution and dynamics of the terrestrial C sink requires knowledge of factors controlling net ecosystem exchange of CO₂ (NEE). The stable isotope composition of atmospheric CO₂ fluxes, combined with simulation and inverse models have tremendous potential to improve understanding of NEE and its components (Keeling et al. 1995; Lloyd et al. 1996; Fung et al. 1997). Stable isotopes offer distinct labeling of flux components, thereby allowing innovative ways to

partition the land/ocean sink and photosynthesis/respiration (Ciais et al. 1995; Yakir and Wang 1996; Bowling et al. 1999; Battle et al. 2000). In particular, the stable carbon isotope ratio ($\delta^{13}\text{C}$) of ecosystem respiration ($\delta^{13}\text{C}_R$) is one of the key parameters in mass balance equations that are used to partition net ecosystem flux of CO_2 into gross photosynthetic and respiratory fluxes (Yakir and Wang 1996; Bowling et al. 1999). C assimilation sets the initial signal of $\delta^{13}\text{C}_R$, but ecosystem respiration comes from substrates ranging in age from hours to centuries. Measuring variations in $\delta^{13}\text{C}_R$ can give insight into the sources of C released from terrestrial ecosystems (e.g., Zobitz et al. 2007).

During C assimilation, ^{13}C discrimination in C_3 plants is driven primarily by stomatal conductance, which varies in response to soil water availability (Lauteri et al. 1993), vapor pressure deficit (VPD) and sunlight (Berry et al. 1997). Dry conditions reduce stomatal conductance, C_i/C_a , and hence lead to reduced discrimination against ^{13}C (Farquhar et al. 1989; Ehleringer et al. 1993). On the other hand, ^{13}C discrimination by Rubisco increases in C_4 plants responding to drying because water limitation may cause leakage of CO_2 out of bundle sheath cells (Tieszen and Boutton 1989; Buchmann et al. 1996).

Scaling from leaf to canopy level requires a mechanistic understanding of the dominant environmental factors controlling canopy-level $\delta^{13}\text{C}_R$. Assuming no fractionation occurs during respiratory processes (Lin and Ehleringer 1997; however, see Duranceau et al. 1999; Ghashghaie et al. 2001), $\delta^{13}\text{C}_R$ is controlled by the isotopic composition of plant and soil substrates. Temporal isotopic disequilibrium caused by different turnover rates of various C pools may contribute to $\delta^{13}\text{C}_R$ dynamics. Recent studies in forests have shown that factors influencing leaf-level ^{13}C discrimination, such as relative humidity and VPD, are linked to canopy-level $\delta^{13}\text{C}_R$ because changes in stomatal conductance altered the isotopic composition of recently fixed photosynthates (Ekblom and Höglberg 2001; Bowling et al. 2002; Scartazza et al. 2004; Keitel et al. 2006; Pypker et al. 2008). However, the response of $\delta^{13}\text{C}_R$ to VPD will be delayed by the time necessary for transporting recently fixed C from leaf to respiring tissue, root exudation, microbial and root turnover, and fungal transport (McDowell et al. 2004). Relationships between environmental parameters and $\delta^{13}\text{C}_R$ can be complicated in mixed C_3 and C_4 ecosystems, which have variable periods of water availability (Sala et al. 1992) and activity of C_3 and C_4 plants. Interpretation of $\delta^{13}\text{C}$ of canopy uptake and respiration in mixed C_3 – C_4 ecosystems remains a measurement and modeling challenge (Bakwin et al. 1998; Still et al. 2003).

Precipitation amount and timing regulate NEE of arid ecosystems through differential effects on ecosystem

respiration and photosynthesis (Huxman et al. 2004). The hierarchical view of precipitation pulse effects on ecological processes suggests that small pulses stimulate relatively minor ecological events, such as near-surface soil microbial communities (Schwinning and Sala 2004; Austin et al. 2004), while larger pulses are required to initiate net C assimilation (Reynolds et al. 2004; Huxman et al. 2004). Different time scales of assimilation and respiration responses to precipitation pulses should correspond to different lags in their respective contributions to precipitation-driven NEE responses (Yepez et al. 2007). Hunt et al. (2002) reported that grassland NEE changed from a C source to a C sink 3 weeks after major soil water recharge. Understanding substrate sources of ecosystem respiration may help to discern thresholds and lags in NEE associated with precipitation events.

Our first objective in this study was to investigate NEE associated with precipitation events and plant–soil C dynamics using $\delta^{13}\text{C}_R$ in shortgrass steppe. Our second objective was to evaluate the efficacy of $\delta^{13}\text{C}_R$ as a tracer for plant functional group (C_3 or C_4) activity during moist versus dry conditions. We measured ecosystem respiration and its $\delta^{13}\text{C}$ value from four different vegetation cover types, C_3 , C_4 , mixed C_3 and C_4 and bare ground patches, to evaluate when recently fixed (labile) C and relatively older C substrates were being respired. We hypothesized that: (1) precipitation events would trigger plant growth and non-zero net CO_2 exchange, with the timing of response dependent on antecedent moisture; (2) responses of plant community dynamics to precipitation would determine $\delta^{13}\text{C}_R$; and (3) labile substrates would dominate $\delta^{13}\text{C}_R$ during moist conditions when plant growth is active, and that old C substrates would dominate during dry conditions.

Materials and methods

Site description

This study was conducted during the 2000 and 2001 growing seasons at the USDA-ARS Central Plains Experimental Range, 56 km north-east of Fort Collins, Colorado (40°40'N, 104°45'W). This site is also part of the Shortgrass Steppe Long Term Ecological Research Site (Franklin et al. 1990). Vegetation of this region is dominated by warm-season, C_4 grasses (*Bouteloua gracilis* and *Buchloe dactyloides*), but also includes an abundance of cool-season, C_3 grasses (e.g., *Pascopyrum smithii* and *Stipa comata*). Canopy height is less than 50 cm. Belowground production comprises ~70% of net primary production (Milchunas and Lauenroth 2001). Long-term (55-year) mean annual precipitation is 320 mm, with the majority

occurring during May, June and July. Mean air temperatures are 15.6°C in summer and 0.6°C in winter with maximum July temperatures averaging 30.6°C (Lauenroth and Milchunas 1991).

Micrometeorological measurements and CO₂ flux observations

Ecosystem fluxes of CO₂ and relevant meteorological parameters were measured from a Bowen ratio energy balance (BREB) tower using an infra-red gas analyzer (model LI-6262; LiCor, Lincoln, Neb.). Air temperature was measured at two heights (1 and 2 m) above the canopy with fine wire chromel-constantan thermocouples. Soil temperatures were measured at 2-cm and 6-cm depths and were averaged across four sensors. Volumetric soil water was measured with soil moisture probes (Campbell Scientific, CS615) at 0–15 cm and corrected to the specific soil type. Micrometeorological data were stored on a 21X data logger (Campbell Scientific, Logan, Utah). The CO₂ fluxes were calculated at 20-min intervals from the following:

$$\text{CO}_2 \text{ flux} = K_c \frac{\Delta \rho_c}{\Delta z} \quad (1)$$

where $\Delta \rho_c$ is the gradient of CO₂ density (g m⁻³) and Δz is height difference of sensors (m) (Dugas 1993; Dugas et al. 1999). K_c is the turbulent diffusivity for CO₂ (assuming $K_c = K_h$, where K_h is the turbulent diffusivity for sensible heat, m² s⁻¹). The CO₂ flux was corrected for temperature and vapor density differences at the two heights (Webb et al. 1980).

Ecosystem-scale flask measurements

We measured diurnal and seasonal changes of CO₂ mixing ratio, and associated $\delta^{13}\text{C}$ values from air flasks above the canopy at 1- and 2-m heights, to estimate changes in the activities of C₃ and C₄ plants during the 2000 and 2001 growing seasons. We performed nocturnal air flask sampling on 22–23 May, 6–7 June, 24–25 July and 4–5 September for 2000, and 17–18 May, 21–22 June, 26–27 July and 7–9 September for 2001. Atmospheric flasks were collected over 15- to 30-min periods from the two heights simultaneously, using magnesium perchlorate traps to avoid contamination of the $\delta^{18}\text{O}$ values (Gemery et al. 1996). Flasks were analyzed for CO₂ using the high-precision non-dispersive infrared gas analysis system used for the NOAA global flasks (Conway et al. 1994) and for $\delta^{13}\text{C}$ using a Micromass Optima mass spectrometer at the stable isotope laboratory at the University of Colorado at Boulder (Trolier et al. 1996). We used a simple mixing model

developed by Keeling (1958, 1961) with nighttime samples to calculate the $\delta^{13}\text{C}_R$. We sampled gradients several times throughout the night and constructed a Keeling plot from pooled flask samples (typically three individuals collected over 5–8 h). Estimates of $\delta^{13}\text{C}_R$ were obtained from the y-intercept of the geometric mean linear regression (Sokal and Rohlf 1981) between $\delta^{13}\text{C}_R$ and inverse CO₂ concentrations. In this study, we presented $\delta^{13}\text{C}_R$ values estimated from significant regressions ($P < 0.01$) with R^2 ranging from 0.93 to 0.99. Uncertainties were reported as the least squares SE of the intercept.

Chamber measurements

We conducted chamber-scale measurements on the same dates as air flask sampling in 2001 to investigate the contribution of different ecosystem components to the total $\delta^{13}\text{C}$ of ecosystem respiration. We established two blocks to account for possible water gradients in the field. On each of the two field blocks, three replicate sets of closed chambers (10 cm in height, 10 cm in diameter) were placed on four different plant community treatments including the C₃ species *P. smithii*, C₄ species *B. gracilis*, mixed C₃ and C₄ species and bare ground. The 24 chambers were installed 1 month prior to experiments and were located about 30 m from the BREB tower and site of flask collections for $\delta^{13}\text{C}_R$. For CO₂ fluxes, we sampled from the headspace of each chamber with a syringe (20 ml) and then injected into pre-evacuated vials at 0, 15, and 30 min after chamber closure. To avoid any pressure decline in the soil chamber during air collections, a sterile sample hang-up bag (100 ml) was placed on an inlet open to atmospheric pressure. CO₂ concentrations were measured with a gas chromatograph (Mosier et al. 1998). Chamber sampling was performed at 8-h intervals [around noon (D), midnight (N) and early morning on the second day (E) of each diurnal set]. A storm prevented collection of nighttime samples in September.

Chamber air sampling was conducted on only one block for stable isotope analysis upon completion of the above procedure. A magnesium perchlorate trap was installed in front of the syringe to dry the air. The samples were analyzed for [CO₂] and isotope ratios to calculate Keeling plot intercepts using geometric mean regression (Pendall et al. 2003), to estimate $\delta^{13}\text{C}$ of respired CO₂ of each treatment: C₃ ($\delta^{13}\text{C}_{\text{CR-C}_3}$), C₄ ($\delta^{13}\text{C}_{\text{CR-C}_4}$), mixed C₃–C₄ grass communities ($\delta^{13}\text{C}_{\text{CR-M}}$) and bare ground ($\delta^{13}\text{C}_{\text{CR-B}}$). Data were pooled from all three replicate chambers to generate a single Keeling plot for each treatment for each of the three sampling times (D, N and E). We obtained [CO₂] ranges of 90 $\mu\text{mol mol}^{-1}$ to 1,400 $\mu\text{mol mol}^{-1}$ in chamber Keeling plots, with an average R^2 -value of 0.82.

Plant biomass

We estimated aboveground plant biomass by functional groups by clipping nine representative 0.25-m² quadrats about every month from April to October in 2000 and 2001. Quadrats were located within 30 m of the Bowen ratio energy balance tower. Green leaves, green stems, and dead material were oven dried and weighed to obtain total above ground live biomass.

$\delta^{13}\text{C}$ of leaf materials and soil organic matter

Leaf samples were collected from the dominant vegetation at the study site at the end of each air flask sampling collection in 2000. Samples were collected separately for *P. smithii* (C₃) and *B. gracilis* (C₄) in each of the two blocks at the end of each diurnal set in 2001. The leaf samples were dried for 48 h at 70°C then ground with mortar and pestle to a fine powder before analysis for $\delta^{13}\text{C}$ on a Micromass Isoprime mass spectrometer (precision <0.1‰).

Soil samples were collected at several depths (1, 5, 15 and 50 cm) at the end of each diurnal set from one pit over the growing season of 2000, and from each block (one pit per block) in 2001. Soil organic matter samples were analyzed by elemental analysis-mass spectrometry for $\delta^{13}\text{C}$ values.

$\delta^{13}\text{C}$ values are expressed in parts per thousand differences from the international standard Vienna-Pee Dee belemnite using the equation (Coplen 1996):

$$\delta^{13}\text{C} = \left[\left(\frac{^{13}\text{C}/^{12}\text{C}_{\text{sample}}}{^{13}\text{C}/^{12}\text{C}_{\text{standard}}} - 1 \right) \right] \times 1000 \quad (2)$$

Estimating contributions of C substrates to chamber and ecosystem respiration

We estimated relative contributions of relatively old C versus labile C substrates to C₃ and C₄ respiration chambers as follows:

$$\delta^{13}\text{C}_{\text{CR}} = f * \delta^{13}\text{C}_{\text{labile-}i} + (1 - f) * \delta^{13}\text{C}_{\text{old}} \quad (3)$$

where $\delta^{13}\text{C}_{\text{CR}}$ represents Keeling plot intercepts from each chamber, f refers to the fraction (%) of labile C substrates, i represents each chamber component such as C₃ and C₄ patches, seasonal foliar $\delta^{13}\text{C}$ values of C₃ and C₄ grasses were used for $\delta^{13}\text{C}_{\text{labile-}i}$, y-intercepts derived from Keeling plots in bare ground patches were used for $\delta^{13}\text{C}_{\text{old}}$. The labile C represented by foliar isotope values reflects assimilation within the growing season, whereas the old C end member reflects C assimilated over the past few years to decades. The SE of the intercept was used in

error propagation (below). Presumably, active roots were present below the bare ground chambers and used newly assimilated C for maintenance respiration; however, we assumed their contribution would be minor. We averaged the three times of day to generate a single value for each vegetation type on a monthly basis. Uncertainty associated with partitioning estimates was estimated using a first-order Taylor series approach (Phillips and Gregg 2001).

We compared local-scale (up to 200 m; based on a 100:1 fetch/height-above-surface ratio established by Rosenberg et al. 1983) flask results with observations from chambers (averaged over distances of ~10 m) to quantify the influence of plant community dynamics on the net flux of ¹³CO₂ added to the atmosphere at the local scale. The net flux of ¹³CO₂ has been defined as “isoflux” in the C cycle community (Bowling et al. 2001) to refer to the product of isotopic composition and CO₂ flux. We calculated nocturnal isoflux by multiplying nocturnal $\delta^{13}\text{C}_{\text{R}}$ and CO₂ flux (BREB data averaged over the nighttime period) during flask sampling ($\delta^{13}\text{C}_{\text{R}} \times F_{\text{R}}$). For the isoflux from each chamber, we multiplied each $\delta^{13}\text{C}$ of respired CO₂ including $\delta^{13}\text{C}_{\text{CR-C3}}$, $\delta^{13}\text{C}_{\text{CR-C4}}$ and $\delta^{13}\text{C}_{\text{CR-B}}$ by each CO₂ flux ($\delta^{13}\text{C}_{\text{CR-}i} \times F_i$). We defined D_i as the difference between the isofluxes from ecosystem respiration for each vegetation type, i (Eq. 4).

$$D_i = \left| \delta^{13}\text{C}_{\text{CR-}i} * F_i - \delta^{13}\text{C}_{\text{R}} * F_{\text{R}} \right| \quad (4)$$

We assumed that the contribution of the isoflux from each vegetation type to that from the total ecosystem (P_i) would be inversely proportional to D_i . Of several possible equations for estimating P_i , we applied the following:

$$P_i = \frac{(\sum_{i=1}^3 (D_i)^2) - (D_i)^2}{(n - 1) \sum_{i=1}^3 (D_i)^2} * 100 \quad (5)$$

Preliminary analysis indicated that relative P_i values of the vegetation types were consistent among the six different equations we evaluated. Thus, we used the isoflux to provide estimates of the proportional contribution of each vegetation type to the total ecosystem respiration.

Statistical analysis

We tested for differences in CO₂ fluxes from chambers using the PROC MIXED procedure (SAS version 8.0; SAS Institute, Cary, N.C.) for a design with fixed effects of block, treatment, month and time of day. Random effects were location (block), treatment × location (block), treatment × month × location (block) and residual. Block was considered as a fixed effect since the blocks encompassed a soil gradient. We performed PROC MIXED where both month and time of day were repeated measurements. Since

isotopic analyses for $\delta^{13}\text{C}$ of respired CO_2 from chambers were conducted on only one site, we tested for differences in $\delta^{13}\text{C}$ of respired CO_2 from chambers using PROC MIXED for a design with fixed effects of treatment, month and time of day. Random effects were location, treatment \times location, treatment \times month \times location and residual for testing CO_2 fluxes. Means separations were evaluated using LSMEANS at $P < 0.05$.

We conducted linear regression analyses to test if any environmental variables were coupled with $\delta^{13}\text{C}_R$ and $\delta^{13}\text{C}_{\text{CR-C}_3, \text{C}_4, \text{M}, \text{B}}$ over a range of time lags, similar to the approach described by Bowling et al. (2002) and McDowell et al. (2004). We calculated averages of a given environmental factor over 1–5 days and shifted these averages back in time by zero to 16 days for $\delta^{13}\text{C}_R$ and to 8 days for $\delta^{13}\text{C}_{\text{CR-C}_3, \text{C}_4, \text{M}, \text{B}}$. For example, a 3-day shift and a 4-day average represent the average daytime variable on the preceding 1st, 2nd, 3rd and 4th days beginning 3 days prior to air sampling. The variables expected to potentially influence the $\delta^{13}\text{C}_R$ included VPD, air temperature (T_{air}), soil temperature (T_{soil}), photosynthetically active radiation (PAR), soil water content (θ), NEE and precipitation (Ekbländ and Högberg 2001; Bowling et al. 2002; McDowell et al. 2004).

Results

Dynamics of moisture availability, CO_2 exchange and plant biomass

The growing season of 2000 was warmer and drier than 2001, especially in May and early June (Figs. 1, 2). More small (<10 mm) and moderate (10–25 mm) precipitation events in the early growing season of 2001 led to moister soils and cooler air and soil temperatures compared to 2000, except in August 2000 when a large precipitation event (>25 mm) recharged soil moisture (Figs. 1, 2). Rainfall events larger than ~ 5 mm generally improved soil water availability (Fig. 2).

CO_2 uptake by the semi-arid grassland was generally lower in 2000 than in 2001, except in later summer, reflecting trends in soil moisture availability (Fig. 2). Precipitation events resulted in peaks of CO_2 efflux (or sometimes reductions in CO_2 assimilation), followed by restoration of CO_2 uptake. The time lag between efflux and uptake was dependent on precipitation regimes. A considerable shift from pulse-driven CO_2 efflux to CO_2 uptake barely occurred with infrequent small-sized events (<5 mm). Two significant rainfall pulses in August 2000 resulted in a substantial CO_2 efflux pulse, and uptake was restored in late August to mid-September. However, prolonged dry periods (defined as times >2 weeks during

which no rainfall events >5 mm occurred) limited assimilation to only about $-1.0 \text{ g C m}^{-2} \text{ day}^{-1}$. In 2001, we measured maximum daily CO_2 assimilation rates of $-5.5 \text{ g C m}^{-2} \text{ day}^{-1}$ following rainfall pulses. The maximum time lag for NEE to switch from a source to a sink following a significant rainfall pulse (≥ 8 mm) was 12 days in 2000 compared to just 5 days in 2001 (Fig. 2).

Differences in seasonality of moisture differentially affected aboveground biomass for C_3 and C_4 plants. Biomass of C_4 plants was up to 3 times greater than that of C_3 plants during 2000, whereas the cooler, wetter conditions of 2001 favored C_3 production until late in the growing season. In 2000, aboveground live biomass averaged $10.2 \pm 3.4 \text{ g m}^{-2}$ for C_3 plants, less than half of the $23.7 \pm 6.4 \text{ g m}^{-2}$ observed for C_4 plants. In 2001, live biomass of C_3 and C_4 plants was similar, averaging $32.0 \pm 19.2 \text{ g m}^{-2}$ and $29.2 \pm 16.2 \text{ g m}^{-2}$, respectively (Fig. 3a).

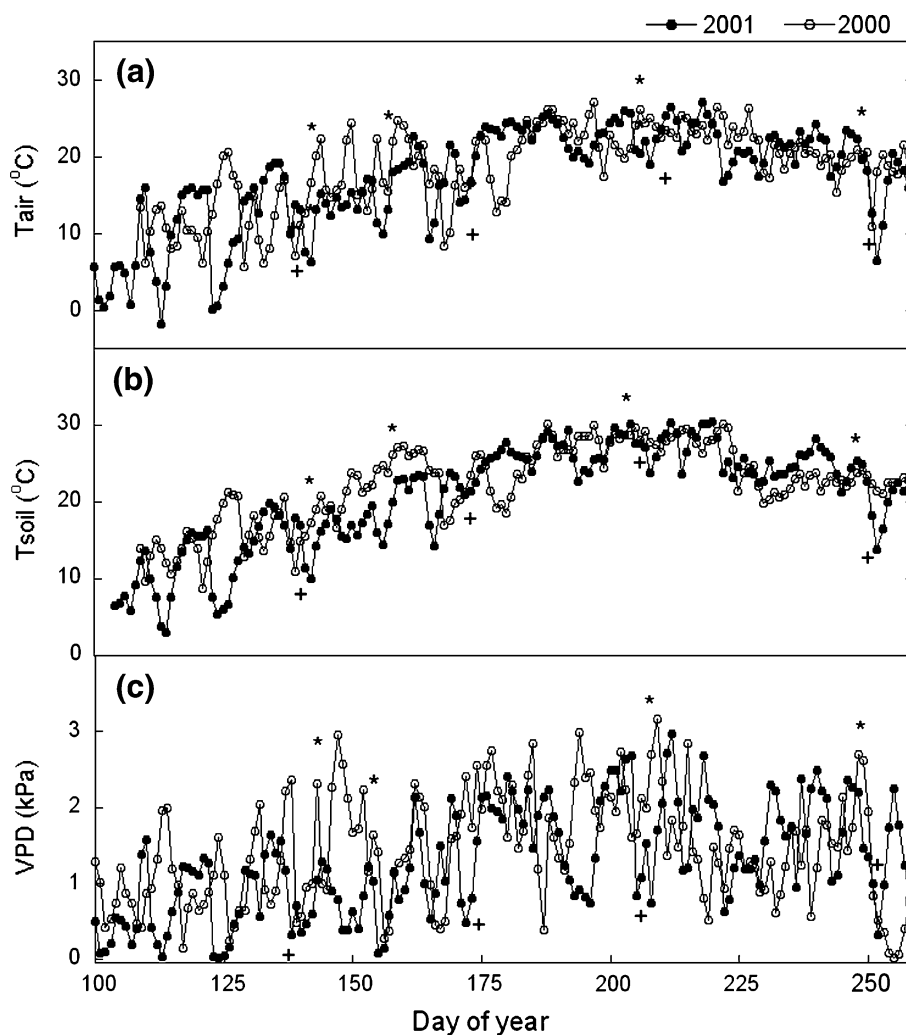
CO_2 fluxes from chambers (measured only in 2001) averaged across the four vegetation patches and three times were 1.6, 3.1, 1.1 and $0.9 \text{ g CO}_2 \text{ C m}^{-2} \text{ day}^{-1}$ in May, June, July and September, respectively ($F = 31.08$, $P < 0.01$) (Table 1). Average CO_2 efflux was correlated with total aboveground plant live biomass ($R^2 = 0.6$; Fig. 3b). Bare ground chambers had significantly lower efflux than vegetated chambers in the early growing seasons ($P < 0.05$). More CO_2 was emitted during daylight periods than during nighttime in May and June ($P < 0.01$). Diurnal differences in CO_2 respiration disappeared in July.

Temporal variations in $\delta^{13}\text{C}_R$ and leaf and soil organic materials

Substantial seasonal variations were observed in the $\delta^{13}\text{C}$ of ecosystem respiration measured from nighttime air flasks over 2 years (Fig. 4a). The $\delta^{13}\text{C}_R$ values were about -15% in early summer of both years, and decreased by as much as 5.21% and 7.13% through the growing seasons of 2000 and 2001, respectively. $\delta^{13}\text{C}_R$ averaged $-16.2 \pm 0.9\%$ in 2000 and $-19.9 \pm 0.7\%$ in 2001.

Temporal variations in $\delta^{13}\text{C}_R$, with lower values during the moister growing season of 2001 than in 2000, may be partly explained by lower values in C_3 leaf tissue and in soil organic C in the 1-cm depth soil layer (Fig. 4b, c). The C_3 foliage $\delta^{13}\text{C}$ values averaged -24.7% in 2000 and -26.4 in 2001 (ANOVA, $F = 7.79$, $P = 0.04$), and soil organic C was -15.9% in 2000 and -17.1% in 2001 (ANOVA, $F = 6.30$, $P = 0.04$). $\delta^{13}\text{C}$ of C_4 plant leaf materials remained constant at -14.1% (ANOVA, $F = 0.31$, $P = 0.6$). C_3 leaves had lower $\delta^{13}\text{C}$ values in early (-26.3%) compared to late growing seasons (-24.8%) (ANOVA, $F = 17.75$, $P < 0.01$). We also

Fig. 1 Variation of **a** air temperature (T_{air}), **b** soil temperature (T_{soil}) and **c** vapor pressure deficit (VPD) over 2 consecutive years (April 2000 up to and including December 2001), measured at the Bowen ratio energy balance (BREB) tower located at the flask sampling site. Data were collected daily at 20-min intervals and calculated as averaged daily values. *Asterisks* represent data collection periods for 2000, *crosses* represent data collection periods for 2001



observed a consistent increase in the $\delta^{13}\text{C}$ of soil organic C with an increase in soil depth (Fig. 4c) (Nadelhoffer and Fry 1988; Balesdent et al. 1993).

Temporal variations in $\delta^{13}\text{C}$ of respired CO_2 from chambers

We observed significant linear relationships between $\delta^{13}\text{C}$ and $1/[\text{CO}_2]$ from the respiration chambers (Table 2; average $R^2 = 0.82$). In May 2001, $\delta^{13}\text{C}_{\text{CR-C}_3, \text{C}_4, \text{M}, \text{B}}$ were similar, averaging $-22.1 \pm 2.1\text{‰}$ (Table 2; $P > 0.05$) even though high CO_2 respiration rates occurred in C_3 patches (Table 1). However, in June 2001, $\delta^{13}\text{C}_{\text{CR-C}_3}$ was lower (-21.7‰) than $\delta^{13}\text{C}_{\text{CR-C}_4}$ (-16.0‰) and $\delta^{13}\text{C}_{\text{CR-M}}$ (-19.0‰), suggesting that labile substrates via root respiration were contributing significantly to ecosystem respiration ($P < 0.05$). $\delta^{13}\text{C}_{\text{CR-M}}$ values were almost always intermediate between values from C_3 and C_4 patches, but in July they were not different from C_3 patches. Bare ground $\delta^{13}\text{C}_{\text{CR}}$ values tended to be similar to C_3 patches. $\delta^{13}\text{C}_{\text{CR}}$ averaged over all patches was generally

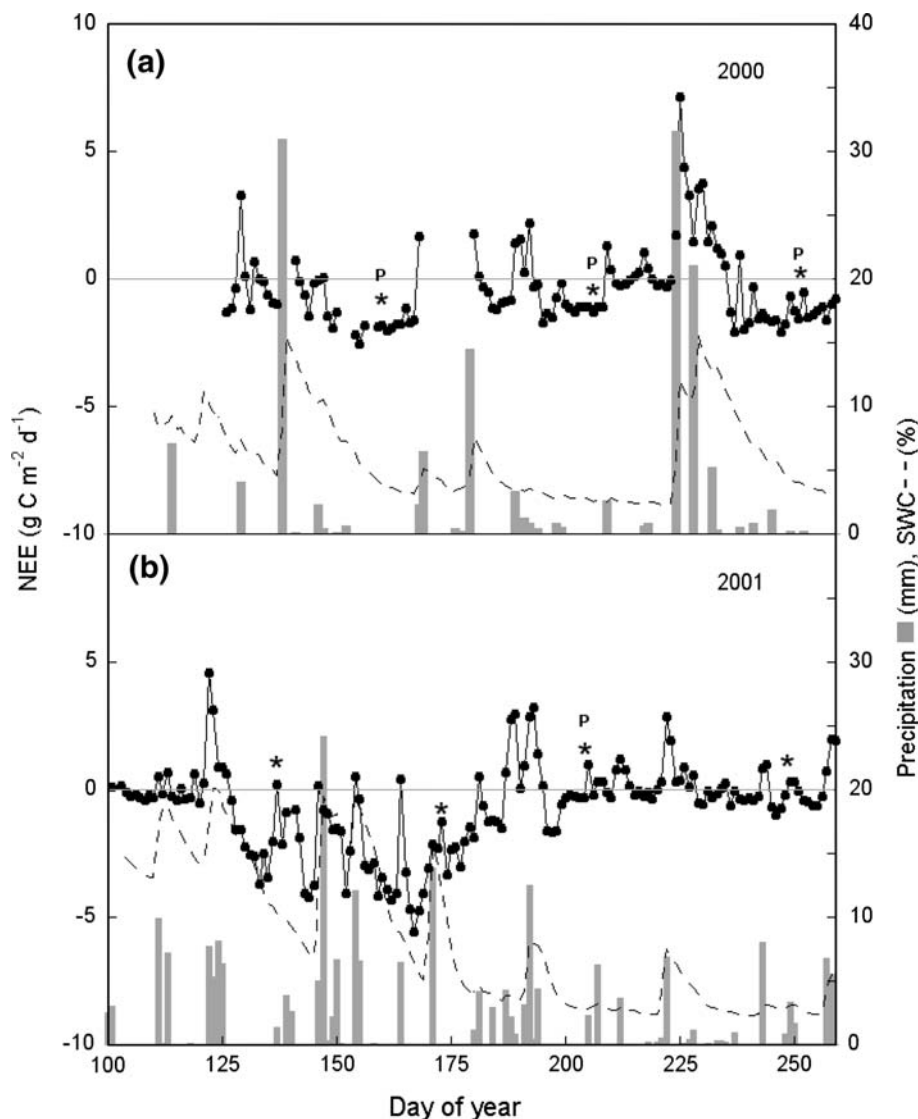
lower during daylight (-20.4‰ and -21.1‰) than during nighttime measurements (-18.5‰ and -19.2‰) in June and July, respectively ($P < 0.05$).

Contributions of C substrates to chamber and ecosystem respiration

We incorporated the observed seasonal variations in labile and old end-member $\delta^{13}\text{C}$ values into estimates of their contributions to ecosystem respiration, by assuming that leaf $\delta^{13}\text{C}$ values represented labile C substrates (Fig. 4), and that $\delta^{13}\text{C}_{\text{CR-B}}$ represented old C (Table 2) for each sampling date. In C_3 patches, labile C substrates contributed 23% of soil respiration in May, 13% in June and none in July (Table 3). In C_4 patches, the highest labile C contribution to soil respiration was shown in June (65%). Large uncertainties associated with partitioning demonstrate that the two-part mixing model is not always useful in this ecosystem.

The chamber isofluxes were all more positive than the local-scale (BREB) isoflux except from the C_4 patch in

Fig. 2 Variations of precipitation (gray bars), soil moisture content (SWC; dashed lines) and net ecosystem CO₂ exchange (NEE) (points and solid lines; negative values are associated with C uptake and positive values with net emission) over two growing seasons, **a** 2000 and **b** 2001, measured at the BREB tower and air sampling site. Solid horizontal line NEE = 0, dotted horizontal line SWC = 5%. Data were collected daily at 20-min intervals. Asterisks represent data collection periods for 2000, crosses represent data collection periods for 2001. P Sampling dates during prolonged dry conditions, defined as periods >2 weeks during which no rainfall events >5 mm occurred



September (Table 4). The contribution to local isoflux (P_i) from C₃ patches remained near 50% through the growing season; P_i from C₄ patches increased from around 37% to 50% in September, at the expense of contribution from bare ground. The $\delta^{13}\text{C}_{\text{R-B}}$ was close to that of C₃ patches (Table 2), but, since they had low CO₂ fluxes, their P_i contributions were low compared to vegetated patches (Table 4).

Correlations of $\delta^{13}\text{C}$ of respiration with environmental and biological drivers

$\delta^{13}\text{C}_{\text{R}}$ measured over two growing seasons was most strongly negatively correlated with air and soil temperature, suggesting that warmer conditions promoted respiration of more ¹³C-depleted substrates (Table 5). The negative correlation between $\delta^{13}\text{C}_{\text{R}}$ and 6 weeks prior cumulative precipitation suggests that C₃ plant activity, reflected in low $\delta^{13}\text{C}_{\text{R}}$ values, requires greater soil moisture

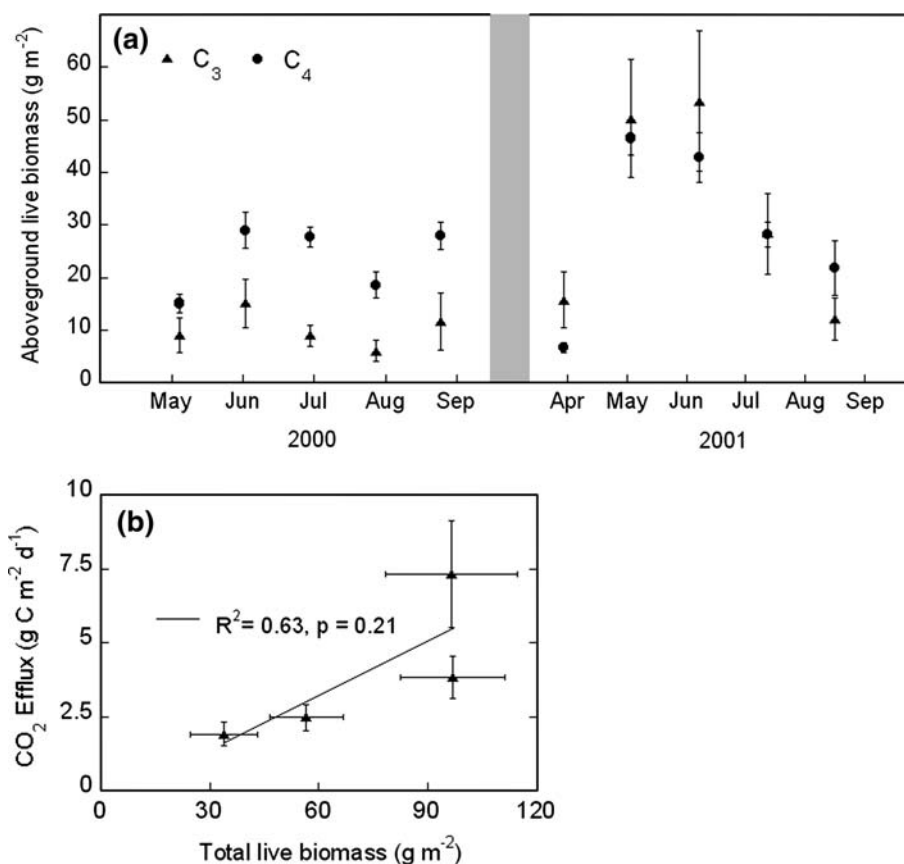
input (or possibly deeper soil moisture) than does C₄ plant activity. Negative correlations with VPD and positive correlations with soil moisture were opposite to expectations based on water-stress-induced stomatal closure and reduced ¹³C discrimination (Table 5).

$\delta^{13}\text{C}_{\text{CR-C3}}$ was positively correlated with VPD, air and soil temperatures, NEE and PAR with lags ranging from 2 to 8 days (Table 5). High $\delta^{13}\text{C}_{\text{CR-C3}}$ occurred in connection with conditions causing plant water stress and stomatal closure. The response of $\delta^{13}\text{C}_{\text{CR-C4}}$ to VPD was opposite to that of C₃ chambers; $\delta^{13}\text{C}_{\text{CR-C4}}$ was negatively correlated with VPD, soil temperature and PAR.

Discussion

We found that the variability of $\delta^{13}\text{C}_{\text{R}}$ in semi-arid grassland can be explained by its relation with temporal

Fig. 3 **a** Plant aboveground live biomass classified by functional group over the growing seasons of 2000 and 2001, **b** correlation between average CO₂ efflux from vegetated chambers and total aboveground plant live biomass in 2001



dynamics among precipitation pulses, antecedent soil moisture and activity of plant functional groups. Differences in $\delta^{13}\text{C}_R$ between pure C₃ and C₄ patches were observed only in moist conditions of an active growing season. In contrast to more mesic ecosystems, $\delta^{13}\text{C}_R$ was better correlated with temperature than with VPD and soil moisture (Bowling et al. 2002; Fessenden and Ehleringer 2003).

Pulse precipitation and plant species composition drive seasonal variations in $\delta^{13}\text{C}_R$

Variations in $\delta^{13}\text{C}_R$ were driven by the relative contributions from labile and old C substrates from C₃ and C₄ plants in our C₃–C₄ ecosystem, assuming no fractionation occurs during dark respiration and microbial respiration (Lin and Ehleringer 1997; but see discussion below). The timing of transfer of labile (recently fixed) C to $\delta^{13}\text{C}_R$ was dependent on the local precipitation regime and the intensity of soil water stress. If water is not limiting, respiration of labile C may be detected a few days after C assimilation occurs (Bowling et al. 2002). However, in our semi-arid ecosystem, the detection of $\delta^{13}\text{C}$ signatures of labile C in $\delta^{13}\text{C}_R$ was lagged by up to 6 weeks (Table 5) during prolonged water stress.

Plant community composition can shift in response to growing season rainfall patterns in grasslands (Kuchler 1974; Epstein et al. 1999). C₃ grasses are more competitive during years with moist, mild springs and dry summers; on the other hand, in years with dry springs and wet summers, C₄ species are favored (Monson et al. 1983). In 2000, a dry spring was associated with lower C₃/C₄ live biomass ratios compared to 2001, when more moisture allowed for superior growth of C₃ species. Plant community dynamics, coupled with water relations, were likely responsible for inter-annual variations in $\delta^{13}\text{C}_R$. However, $\delta^{13}\text{C}_R$ was not always coupled to vegetation composition at our site, especially during cool early seasons and prolonged dry conditions; $\delta^{13}\text{C}_R$ varied as much as 7‰ despite similarities in C₃/C₄ composition in May and June 2001. The relatively high $\delta^{13}\text{C}_R$ values early in the growing seasons (–15‰) likely reflect a greater importance of older C rather than strong C₄ activity as shown by individual patch results (Table 3), but we cannot rule out the possible autotrophic contribution of C₄ plants due to their growth in May (Fig. 3). Lagged response of $\delta^{13}\text{C}_R$ to precipitation inputs suggests that C assimilation and growth of the dominant, deep-rooted C₃ grass, *P. smithii*, is limited by deep (>20-cm) infiltration of soil moisture (Nelson et al. 2004), requiring several precipitation pulses especially

Table 1 CO₂ fluxes (g C m⁻² day⁻¹) from C₃, C₄, mixed C₃ and C₄ (M) and bare ground (B) chambers over the growing season of 2001. Different letters within each column represent treatment differences at

that time. Different letters following times of day represent diurnal differences ($P < 0.05$). SEs in parentheses ($n = 3$). D Daytime, N nighttime, E dawn

	May			June			July			September
	D a'	N b'	E ab'	D a'	N b'	E b'	D a'	N a'	E a'	D
C ₃	2.5 a (0.2)	1.8 a (0.4)	2.6 a (0.5)	6.0 a (1.5)	2.3 a (0.5)	2.6 a (0.5)	1.3 a (0.2)	1.1 a (0.1)	1.1 a (0.2)	0.8 a (0.1)
C ₄	2.0 ab (0.3)	1.2 ab (0.3)	1.4 b (0.4)	6.2 a (1.7)	2.6 a (0.7)	2.2 a (0.5)	1.6 a (0.3)	1.1 a (0.3)	1.2 a (0.2)	1.1 a (0.3)
M	1.3 bc (0.5)	1.4 ab (0.2)	1.6 b (0.2)	5.1 a (1.5)	2.7 a (0.7)	2.4 a (0.3)	1.6 a (0.7)	1.2 a (0.2)	0.7 a (0.1)	1.0 a (0.3)
B	0.9 c (0.1)	0.6 bc (0.1)	1.0 b (0.1)	2.2 b (0.4)	1.7 b (0.4)	1.3 b (0.2)	0.4 b (0.1)	0.7 b (0.1)	0.8 a (0.2)	0.5 a (0.1)

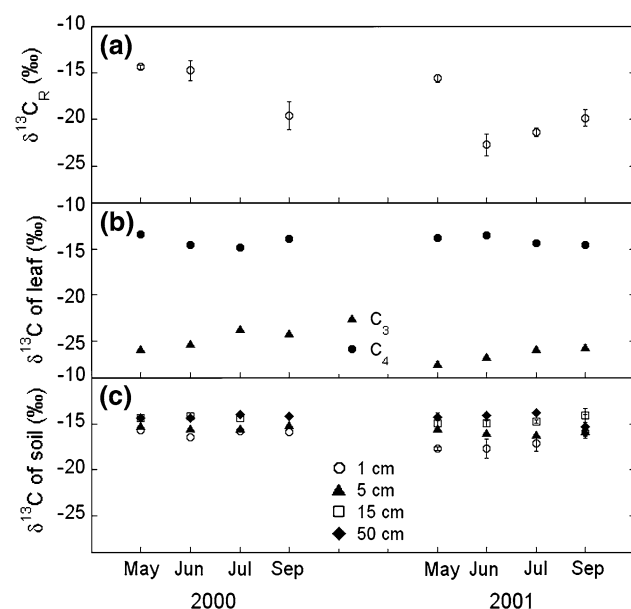


Fig. 4 Measurements of **a** $\delta^{13}\text{C}$ of ecosystem respiration ($\delta^{13}\text{C}_R$), **b** bulk organic tissue from C₃ and C₄ plants, and **c** soil organic matter from four different depths (1, 5, 15 and 50 cm) over two growing seasons (2000 and 2001). Error bars indicate SE

during the warm summer months. There is a possibility that C₃-C substrates accumulated during the dry year 2000 may have been abruptly respired following two significant rainfall pulses (>20 mm) in August, leading to the low $\delta^{13}\text{C}_R$ value in September.

The hierarchical view of precipitation pulse patterns and their effect on ecosystems suggests that brief, small pulses only stimulate the surface-dwelling soil microbes, while it takes longer pulse duration and deeper infiltration to affect C assimilation in higher plants (Schwinning and Sala 2004; Austin et al. 2004; Reynolds et al. 2004). This is presumably due to plant acclimation and the growth of new roots and leaves (Ogle and Reynolds 2004). Our NEE data

demonstrate that assimilation had a delayed response to precipitation pulses compared to respiration (Fig. 2), especially when soils were dry. Frequent, small (<5 mm) rainfall events in June 2001 promoted rapid restoration of net assimilation, when soil moisture was >5%. However, occasional 7-mm pulses were not enough to switch NEE from a net C source to a sink (Fig. 2b; after day-of-year 200), when soil moisture was <5%. In 2000, NEE shifted from a large C source to a small sink, but only after a lag of nearly 2 weeks after large rainfall pulses in August (Fig. 2a). This is consistent with findings by Yopez et al. (2007) that net C losses were associated with monsoon precipitation pulses, and that net ecosystem C uptake lagged precipitation inputs by several weeks in the Sonoran desert. A lagged time response of CO₂ flux to precipitation events has been reported in many other ecosystem types (Flanagan et al. 2002; Monson et al. 2002; Hunt et al. 2002). A longer delay in assimilation even in response to large pulses in late season may be explained by a lower photosynthetic capacity of relatively old leaves (Huxman et al. 2004). The lagged response of vegetation to rainfall pulses was supported by the correlation between $\delta^{13}\text{C}_R$ and 6 weeks' prior cumulative precipitation. Our results suggest that active biosphere-atmosphere ¹³CO₂ exchanges occurred after precipitation events of 5–7 mm, with soil moisture >5%; the influence of labile C on $\delta^{13}\text{C}_R$ was never observed below those thresholds at our semi-arid grassland site.

The negative correlation between $\delta^{13}\text{C}_R$ and soil (air) temperature resulted from the general decrease in $\delta^{13}\text{C}_R$ over both growing seasons as temperatures warmed, possibly associated with a strong isoflux from C₃ ecosystem components. The lack of correlation between $\delta^{13}\text{C}_R$ and VPD could be explained by the over-riding influences of changing proportions of recently fixed and relatively old C from C₃ and C₄ sources, in contrast to forest ecosystems (Bowling et al. 2002).

Table 2 C isotope ratios (‰) of respired CO₂ from C₃, C₄, M and B components in 2001. Different letters in D columns represent treatment differences for each diurnal set. Different letters following times of day represent diurnal differences. There was no time of day effect by treatment interaction for δ¹³C of respired CO₂ (δ¹³C_{CR})

	May			June			July			September
	D a'	N a'	E a'	D a'	N b'	E ab'	D a'	N b'	E b'	D
C ₃	-26.0 a (4.8)	-20.5 (0.6)†	-22.9 (1.0)*	-22.2 a (1.8)*	-21.7 (0.4)*	-21.2 (1.72)†	-21.2 a (1.29)*	-20.6 (0.8)*	-20.1 (1.7)†	-20.0 (1.8)†
C ₄	-24.0 a (1.7)*	-23.8 (1.0)*	-20.8 (1.4)*	-16.0 c (0.9)	-15.4 (0.6)†	-16.5 (0.8)*	-19.1 b (1.39)*	-16.0 (0.6)*	-17.8 (1.8)	-15.6 (0.9)†
M	-18.5 a (2.2)	-19.8 (1.3)*	-22.0 (2.0)*	-20.6 b (1.4)*	-17.7 (2.4)	-18.8 (1.2)*	-20.8 a (5.8)	-19.3 (0.9)†	-19.3 (0.6)*	-26.1 (3.4)
B	-23.5 a (2.8)†	-21.8 (1.5)*	-20.9 (2.2)†	-22.7 ab (4.1)	-19.3 (1.7)†	-19.9 (1.4)*	-23.4 c (2.1)†	-20.9 (1.9)†	-22.3 (1.6)*	-19.1 (2.1)†

† $P < 0.1$, * $P < 0.05$

Table 3 Contribution (%) of “old” versus “new” (recently fixed) C substrates using two-part mixing model for C₃ and C₄ patches only. Combined errors from all steps of the partitioning (Phillips and Gregg 2001) are shown in parentheses. Out-of-bound fraction values resulted in negative (or greater than 100) values

Patch type	Substrate	May	June	July
C ₃	Old	77 (75)	87 (75)	150 (108)
	New	23 (75)	13 (75)	-50 (108)
C ₄	Old	110 (29)	35 (12)	40 (12)
	New	-10 (29)	65 (12)	60 (12)

Plant community patches alter δ¹³C values of respired CO₂

Our isotopic data suggest that the C₃ and C₄ species we studied had different responses to precipitation pulses. In C₃ chambers, labile C was readily distinguished during relatively moist and cool conditions, but disappeared during prolonged dry conditions (Tables 2, 3). Diurnal variations in CO₂ efflux were observed in May and June, but not July (Table 1), supporting the contribution of autotrophic respiration during moist conditions. However, in C₄ patches in July, a small rainfall pulse that occurred between daytime and nighttime sampling appeared to lead to efflux of root (and root-associated microbe) respiration in a few hours, as indicated by labile δ¹³C (Table 2). *B. gracilis* is shallow rooted (Nelson et al. 2004) and has been shown to increase leaf water status within hours of a 5-mm

analyses because data were pooled from all three replicate chambers to generate a single Keeling plot for each of the three sampling times (D, N, E). SEs in parentheses. Geometric mean regressions were performed to calculate intercepts. For other abbreviations, see Table 1

Table 4 Nocturnal isofluxes (‰ g C m⁻² day⁻¹) from C₃, C₄, bare ground chambers and local-scale air flasks, and % contributions of the net flux of ¹³CO₂ from each patch type to total nocturnal ecosystem respiration (P_i) in 2001

	C ₃		C ₄		Bare ground		Local scale
	Isoflux	P _i	Isoflux	P _i	Isoflux	P _i	Isoflux
May	-36.0	42	-29.0	37	-13.2	21	-60.7
June	-51.0	50	-40.7	38	-32.0	12	-52.2
July	-21.9	47	-17.0	34	-13.8	19	-25.7
September	-16.0	49	-17.1	50	-9.7	1	-16.9

rain event (Sala et al. 1982). Our results demonstrated that autotrophic respiration from shallow-rooted plants can respond quickly to small rainfall pulses (Schwinning and Sala 2004). As expected, δ¹³C_{CR-C3} increased and δ¹³C_{CR-C4} decreased in response to increasing VPD and air temperature (Table 5). This effect was also noted in the leaf tissue samples over the growing season (Fig. 4). The opposing C₃ and C₄ responses to high VPD and temperature probably contributed to the similar δ¹³C_{CR} values during dry conditions.

The mean δ¹³C_{R-B} was approximately 3–4‰ lower than the δ¹³C of soil organic matter at 1-cm soil depth. Discrepancies between δ¹³C of soil organic matter and of soil respiratory flux have been commonly reported in C₃ and C₄ ecosystems (Buchmann and Ehleringer 1998; Ehleringer et al. 2000; Still et al. 2003). This may reflect the long-term enrichment in δ¹³C values associated with decomposition (Nadelhoffer and Fry 1988; Balesdent et al. 1993), shifts in the abundance of C₃ and C₄ vegetation (Pendall et al. 2003) and/or changing δ¹³C of atmospheric CO₂ (Friedli et al. 1987).

Table 5 Correlation coefficients (r) from linear regression analysis of $\delta^{13}\text{C}_R$ and $\delta^{13}\text{C}_{\text{CR-C3, C4, M, B}}$ versus environmental variables. First and second numbers *in parentheses* are number of days lagged and number of days average, respectively; for precipitation, first number is number of weeks lagged. *VPD* Vapor pressure deficit, T_{soil} soil

	VPD	T_{soil}	T_{air}	θ	NEE	PAR	Precipitation
$\delta^{13}\text{C}_R$	-0.47† (5, 1)	-0.58* (9, 3)	-0.73* (11, 2)	+0.51† (3, 2)	+0.5* (8, 4)	+0.38* (9, 1)	-0.39† (6)
$\delta^{13}\text{C}_{\text{CR-C3}}$	+0.82* (5, 5)	+0.81* (6, 5)	+0.89* (4, 5)	-0.82* (8, 1)	+0.7* (2, 1)	+0.69* (8, 5)	
$\delta^{13}\text{C}_{\text{CR-C4}}$	-0.85† (7, 1)	-0.96* (0, 2)	NS	NS	NS	-0.87* (5, 4)	
$\delta^{13}\text{C}_{\text{CR-M}}$	NS	NS	NS	NS	-0.78* (0, 3)	+0.64† (0, 5)	
$\delta^{13}\text{C}_{\text{CR-B}}$	NS	NS	NS	NS	NS	-0.6* (8, 5)	

† $P = 0.1$, * $P = 0.05$, NS no significant relation ($P > 0.1$)

Detecting C sources for respiration using $\delta^{13}\text{C}$

The two-source isotope partitioning approach is valid when the $\delta^{13}\text{C}_R$ value is intermediate between labile and stable end members. However, several factors can lead to the respiration value falling outside the end-member values; in these cases, “out-of-bound” partitioning estimates are generated. We observed out-of-bound conditions from chamber respiration in two cases (July for C_3 chambers, May for C_4 chambers; Table 3). The propagated error estimates show that although there was a large degree of uncertainty, the respiration was dominated by old substrates in both cases. Uncertainty was highest in partitioning calculations for the C_3 chambers, because the $\delta^{13}\text{C}$ values of labile and stable end-members were close to the $\delta^{13}\text{C}_{\text{CR-C3}}$ value.

Out-of-bound conditions (and large uncertainties associated with partitioning) may occur when one or both end-member isotopic values are too close to $\delta^{13}\text{C}_R$, relative to the inherent isotopic variability (e.g., 2–3‰), if inappropriate end-member signatures are assigned, or if fractionation occurs during respiration. Using $\delta^{13}\text{C}$ of sucrose or carbohydrates extracted from leaves or roots would be more appropriate than bulk tissue for $\delta^{13}\text{C}_{\text{labile-}i}$ because they are known substrates for respiration, and represent the isotopic signature of recent photosynthetic products (Brugnoli and Farquhar 2000). $\delta^{13}\text{C}$ of leaf sucrose was more positive relative to $\delta^{13}\text{C}$ of leaf organic matter by ~ 2 and $\sim 1.5\%$ in *Nicotiana sylvestris* and *Helianthus annuus* (Ghashghaie et al. 2001), but this effect was not so significant in other C_3 species (Xu et al. 2004). The amount of ^{13}C enrichment in leaf sucrose relative to leaf organic tissue was larger in dehydrated plants due to drought-induced decreases in stomatal conductance and

temperature, T_{air} soil temperature, θ soil water content, *NEE* net ecosystem CO_2 exchange, *PAR* photosynthetically active radiation, + positive correlation, – negative correlation; for other abbreviations, see Table 1

C_i/C_a (Ghashghaie et al. 2001). Incorporating species-specific $\delta^{13}\text{C}$ of sucrose as the labile end-member, varying with wetting and drying cycles, may improve applicability of our partitioning approach.

Isotope partitioning would also be improved by explicit measurement of potential fractionation during respiration or decomposition. Accumulated evidence has demonstrated that apparent fractionation occurs during dark respiration leading to ^{13}C enrichment in respiratory CO_2 in C_3 plants (Ghashghaie et al. 2001). This fractionation varies with environmental or physiological factors (Tcherkez et al. 2003) but differently among plant species (Ghashghaie et al. 2001). It is uncertain the degree to which enrichment in ^{13}C of respired CO_2 during respiration actually influenced $\delta^{13}\text{C}_R$ at our mixed C_3 and C_4 ecosystem.

Our observations of $\delta^{13}\text{C}_R$ and $\delta^{13}\text{C}_{\text{CR}}$ reflected flux-weighted ^{13}C signatures from different plant communities and soil components, albeit with a large degree of uncertainty, especially during low-flux conditions. The relative contribution of labile C associated with plant functional type (C_3 versus C_4 plants) was estimated as a distance from the total isoflux (D), and corresponded to seasonal trends in C_3/C_4 biomass (Table 4; Fig. 3), indicating that labile C from C_3 and C_4 plants can be observed in soil respiration. Isotope partitioning thus contributed to an understanding of how precipitation pulses affected different ecosystem components and their contributions to respiration fluxes.

Conclusion

Variations in $\delta^{13}\text{C}_R$ were used as a tracer of C cycling, to evaluate how NEE responses to pulses of rainfall might transfer the $\delta^{13}\text{C}$ signal from labile C to ecosystem respiration. The response of NEE to pulse precipitation events

was not immediate; it took almost 2 weeks to shift from C source to C sink when soil moisture was <5%. Considerations of NEE dynamics responding to local precipitation events and plant community composition helped explain the observed variations of $\delta^{13}\text{C}_R$. Flux-weighted $\delta^{13}\text{C}$ of respiration from plant functional groups and bare ground patches can be used to partition the relative contributions of each component to total net flux of $^{13}\text{CO}_2$ at ecosystem level during relatively moist conditions. Incorporating dynamic aspects of labile/old C sources and C_3/C_4 plant communities varying with local precipitation pulses should improve regional- and global-scale C cycle models.

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