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Contribution of new photosynthetic assimilates to respiration by perennial grasses and shrubs: residence times and allocation patterns

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Summary

• Quantification of the fate of carbon (C) used by plant metabolism is necessary to improve predictions of terrestrial ecosystem respiration and its sources.
• Here, a dual isotope (^{13}C and ^{14}C) pulse-label was used to determine the allocation of new C to different respiratory pathways in the early and late growing seasons for two plant functional types, perennial grasses and shrubs, in the Owens Valley, CA, USA.
• Allocation differences between plant types exceeded seasonal allocation variation. Grasses respired 71 and 64% and shrubs respired 22 and 17% of the label below-ground in the early and late growing seasons, respectively. Across seasons and plant types, ~48–61% of the label recovered was respired in 24 h, ~68–84% in 6 d, and ~16–33% in 6–36 d after labeling.
• Three C pools were identified for plant metabolism: a *fast pool* with mean residence times (MRTs) of ~0.5 and ~1 d below- and above-ground, respectively; an *intermediate pool* with MRTs of 19.9 and 18.9 d; and a *storage pool* detected in new leaf early growing season respiration > 9 months after assimilation. Differences in allocation to *fast* vs *intermediate C pools* resulted in the mean age of C respired by shrubs being shorter (3.8–4.5 d) than that of the grasses (4.8–8.2 d).

Key words: accelerator mass spectrometry, allocation, autotrophic respiration, Owens Valley, plant metabolism, pulse-chase label, ^{13}C, ^{14}C.

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Introduction

Recent literature has highlighted the need to better understand plant carbon (C) allocation patterns in order to accurately predict terrestrial ecosystem respiration sources (Trumbore, 2006). The allocation of recently assimilated C (henceforth 'new' C) to below- vs above-ground plant components and to growth vs respiration are key uncertainties in global terrestrial ecosystem C models (Friedlingstein *et al.*, 1999). How plants allocate C determines how long that C may remain in the plant or soil before returning to the atmosphere by respiration. The C allocated to constructing plant tissues (e.g. stems, leaves, and roots) will typically reside in the plant and soil longer than C used for plant metabolism (e.g. growth and maintenance respiration). Growth and maintenance respiration accounts for a large percentage of assimilated C, from ~30 to 70% depending on the ecosystem (Amthor, 2000). Determination of this ecosystem C use efficiency (the fraction of photosynthetic uptake respired by plant metabolism) has been particularly challenging because below-ground respiration (soil respiration) is the sum of plant and microbial sources that are methodologically difficult to quantify individually (Hanson *et al.*, 2000; Norby & Jackson, 2000; Trumbore, 2006). Additionally, not much is known about the sources of C that support plant metabolism, but there is clear evidence that both new and stored (nonstructural carbohydrate) C sources contribute (Dickson, 1991; Ekblad & Högberg, 2001; Högberg *et al.*, 2001; Bhupinderpal-Singh *et al.*, 2003; Körner, 2003; Czimczik *et al.*, 2006; Keel *et al.*, 2006; Schuur & Trumbore, 2006; Taneva *et al.*, 2006).

Plant biomass is commonly used to describe plant allocation patterns. Jackson *et al.* (1996) demonstrated that plant functional types follow universal biomass distributions. Both shrubs and grasses generally posses high biomass root to shoot ratios ($B_{R:S}$) of ~4.5 and 3.7, respectively, and trees have typically lower $B_{R:S}$ of < 1. The $B_{R:S}$ is a direct indicator of C used to build structural tissues, but it does not necessarily represent the C spent on metabolic respiration (Litton *et al.*, 2007). The turnover of different plant components is also essential for understanding how much C is allocated to the growth and maintenance of tissues. For example, while minirhizotron studies have shown that there is a dynamic fine-root population with lifetimes of weeks to months, there is also evidence that the majority of tree root biomass is long-lived (i.e. years) (Gaudinski *et al.*, 2001; Matamala *et al.*, 2003). Thus, the allocation of C below-ground to support the growth and maintenance of roots that live for many years will differ from that of roots that are replaced frequently (Trumbore *et al.*, 2006). Yet, few studies have demonstrated how the partitioning of C in respiration ($R_{R:S}$) or net primary production ($P_{R:S}$) relates to that of biomass.

Within the plant, the allocation of C is thought to be determined by source–sink interactions (Wardlaw, 1990; Dickson, 1991; Farrar & Jones, 2000). At the whole-plant level, sources (e.g. mature photosynthetically active leaves) supply new C, and sinks (e.g. developing leaves, shoots, roots, and other plant organs) compete for new C. The flow of C to sinks depends on the strength of the sink, which is determined by the size of sink tissue (or organ), growth rate, metabolic activity, and respiration rate. Thus, sink demands should vary with environmental conditions, plant functional type, plant age and phenology (Dickson, 1991).

Nearly all of our knowledge of how plants allocate newly assimilated C comes from pulse-chase labeling studies conducted in controlled laboratory and glasshouse settings (Isebrands & Dickson, 1991). The few studies that have been performed under field conditions used radiocarbon (^{14}C) pulse-chase labeling techniques at high concentrations that are no longer feasible today, because of increased regulation of radioactivity in the environment (Dahlman & Kucera, 1968; Caldwell & Camp, 1974; Milchunas *et al.*, 1985, 1992; Isebrands & Dickson, 1991). Additionally, only a handful of these directly quantified the C allocated to plant metabolism (Warembourg & Paul, 1973, 1977; Horwath *et al.*, 1994).

This study was designed to look at plant functional and seasonal differences in the respiration of newly assimilated C in perennial grasses and shrubs under field conditions. Understanding the fundamental C cycling patterns in grasses and shrubs is of interest because of the prevalence of vegetation shifts from herbaceous to woody species in the arid and semiarid western USA, and globally (Schlesinger *et al.*, 1990; Jackson *et al.*, 2000; Asner *et al.*, 2003; Elmore *et al.*, 2003). Observed modifications to the C budget associated with a shift from grass to shrub vegetation include increases in standing biomass, altered root and leaf litter inputs, changes in spatial distributions of organic matter and nutrients, and potential shifts in soil C storage (Jackson *et al.*, 2000). Yet, there is little data that demonstrates how such vegetation shifts affect the residence time of C used for plant metabolism, or may alter the partitioning of new C to respiration below- vs above-ground on both short and longer time scales.

There were three primary goals here: to determine the fraction of newly assimilated C respired below- vs above-ground; to understand how that partitioning varied seasonally; and to quantify the speed at which new C was respired by (cycled through) the ecosystem. It was hypothesized that the intrinsic differences in plant functional types, such as woody vs herbaceous tissues, growth rates, and rooting depth would result in different overall respiration partitioning between below- and above-ground plant components. It was also hypothesized that seasonal differences in the environment and plant phenology would exhibit control on respiration partitioning and speed of cycling of new assimilates.

In order to address our research goals we used a dual (^{13}C and ^{14}C) isotope pulse-chase labeling approach. This technique allowed allocation patterns to be safely observed in the field under ambient CO_2 concentrations, and with less disturbance to the plants and soil in comparison with other methods of tracking plant contributions to below-ground respiration, such as continuous labeling, trenching and girdling (Hanson *et al.*, 2000). The use of both C isotopes permitted us to quantify C used for respiration on different time scales. The ^{13}C label for the short-term (hours to days) response was used to take advantage of its greater ease and lower cost of analysis. The ^{14}C label measured by accelerator mass spectrometry (AMS) was used to detect the small quantities of label in CO_2 respired weeks to months following the label application. For a more thorough discussion of the pros and cons of using ^{13}C and ^{14}C labels under field conditions, see Carbone *et al.* (2007).

Materials and Methods

Field site description

This study took place near the city of Bishop (37°60′N, 118°60′W), in the Owens Valley, California, USA. The Owens Valley is located in eastern California between the Sierra Nevada and the White-Inyo Mountain ranges at approx. 1250 m elevation. The climate is like that of the nearby Great Basin desert, with average temperatures ranging from 11 : 6°C (daytime high:nighttime low) in winter to 37 : 14°C in summer. The average annual precipitation is ~150 mm, with 75% falling in the winter months between November and March. Runoff from the snow pack in the Sierra Nevada and White-Inyo Mountains flows into the valley in the spring and summer, resulting in a high groundwater table (Hollett *et al.*, 1991) and providing an additional water supply for plants. The vegetation in the valley floor is dominated by phreatophytes (groundwater-using plants) typical of the Great Basin region.

Two representative sites were selected that differed in dominant vegetation. The first (grass site) was an alkali meadow plant community, with the perennial grasses *Distichlis spicata* (L.) E. Greene (inland saltgrass) and *Juncus balticus* Willd. (wirerush), and a water table that remained relatively shallow (~1–2 m depth) during the period of observation. The second (shrub site) was a phreatophytic scrub plant community comprised of the winter deciduous perennial shrubs *Ericameria nauseosa* (Pall. ex Pursh) (rubber rabbitbrush, formerly *Chrysothamnus naseosus*) and *Sarcobatus vermiculatus* (Hook.) Torrey (greasewood). *Atriplex lentiformis* ssp. *torreyi* (saltbush) was also present at this site, but not represented in the experimental plots. Shrubs located in the experimental plots were classified as adults (based on size and yearly flowering) according to criteria used in Toft & Fraizer (2003). The water table depth at the shrub site was greater than at the grass site, ranging from ~1.7 to 2.5 m depth during the period of observation. Together, alkali meadow and phreatophytic scrub plant communities make up ~23.6 and 29.9% of land cover in the Owens Valley, respectively (Elmore *et al.*, 2003).

The grasses and shrubs have similar phenologies. Growth begins in the spring (April). Maximum leaf area is reached by early summer (June), and flowering occurs in mid to late summer (July–August). Changes in the $\delta^{18}O$ of stem water show that the more shallow-rooted grasses lose contact with the groundwater in late summer, resulting in earlier senescence (D. Pataki, unpublished data). By contrast, the deeper-rooting shrubs maintain access to groundwater throughout the growing season (D. Pataki, unpublished data), and remain physiologically active until temperatures drop below freezing in autumn (Donovan *et al.*, 1996).

Experimental set-up

Paired control and label plots, each ~14 m^2, were established at both sites. Two pulse-chase labeling campaigns took place over the course of the experiment, using the same label plots. The late growing season label was applied in late July 2005. The early growing season label was applied in early May 2006. Annual precipitation (water year) and groundwater table were comparable and above average for both 2005 and 2006 (Inyo County Water Department, pers. comm.). Air temperature, air relative humidity, soil temperature and moisture (at multiple depths) were continually measured and recorded on a CR10X data logger in both plots and sites (Campbell Scientific, Logan, UT, USA).

Labeling procedure

A modified version of the field labeling procedure described in Carbone *et al.* (2007) was used. Briefly, a dual isotope (^{13}C and ^{14}C) label was applied by enclosing the vegetation in a ~24-m^3 translucent polyethylene dome (described by Arnone & Obrist, 2003) over a period of ~45–60 min. The dome was sealed to the ground by placing the PVC base on top of a closed-cell foam gasket, and weighting with 12 cinder blocks. If needed, fine sand was used to complete the dome sealing to the ground surface. Dilute HCl was injected with a syringe into a mason jar with isotopically enriched $NaHCO_3$ solution to produce CO_2 with a label signature of ~20 and ~200 times background abundance for ^{13}C and ^{14}C, respectively. The enriched isotope solution contained ~1 g of ^{13}C and ~300 Bq of ^{14}C. The radioactivity in the label solution was considered 'exempt quantities' (specific activity < 296 Bq ml^{-1} liquid; California Code of Regulations (California Department of Health Services)). The labeled CO_2 was circulated (1 l min^{-1}) into the dome, where six fans were used to mix air throughout the labeling period. The CO_2 concentrations in the dome were continuously monitored with a LI-800 Infrared Gas Analyzer (IRGA) (Li-Cor, Lincoln, NE, USA). Immediately following the label addition, CO_2 concentrations increased by no more than 20 ppm and then decreased as a result of net photosynthetic uptake of CO_2 by vegetation. During the early growing season labeling, an additional unlabeled ~1.4 g C was acidified and released to prevent CO_2 concentrations from dropping below ambient concentrations. The CO_2 concentrations within the labeling dome were kept within the range of 320–420 ppm during all labeling applications. To lessen the environmental effects of the dome, the length of the labeling period was minimized, and labeling took place at mid-morning when plants were most photosynthetically active (Kajji *et al.*, 1993). Nonetheless, air temperatures within the dome were elevated 8–10°C above ambient temperatures, but they never exceeded daily maximum air temperatures for the given season. Condensation of water observed on the dome walls suggested that air relative humidity reached saturation by the end of the labeling periods (while those outside the tent did not exceed 35%). Multiple samples to monitor the ^{13}C and ^{14}C content of CO_2 in the dome air were taken throughout the labeling period and used together with the change in CO_2 concentration to quantify the rate of uptake of the label by plants.

CO_2 measurements

Within each plot, measurements of soil respiration (below-ground respiration) and leaf and stem respiration (above-ground respiration) were made for the duration of the early and late growing season labeling campaigns. Soil respiration measurements were conducted with automated soil respiration chambers developed and built at UC Irvine based on the design of Goulden & Crill (1997). Eight chambers (internal diameter 25 cm, height 21 cm, volume ~11 l) were installed at each site, four in each plot. Soil respiration was measured in each chamber every ~1.5 h and continually at the site for the entire measurement period. At the grass site, living above-ground vegetation was removed (by clipping grass at the soil surface) from some of the chambers to separate below- and above-ground respiration sources. Chamber bases were not

inserted into the soil but, instead, a sand ring diffusion barrier was used to seal the chamber to the ground. Thus, roots freely extended underneath all chambers, and the clipped chambers were representative of below-ground respiration.

Above-ground leaf respiration was measured with branch bags made of opaque polyethylene sheet. The bags were placed over the vegetation, and the opening was lined with closed-cell foam and sealed with compression fittings. The CO_2 flux was measured over ~5 min by connecting tubing in the bag with the LI-800 IRGA and a pump (1 l min^{-1}). Four bags were used in each plot.

Isotope sampling and processing

Within each plot, CO_2 was collected to determine the isotopic content (^{13}C and ^{14}C) of soil respiration and leaf respiration 24 h before labeling (background measurements), and frequently after labeling (chase measurements). The dual isotope label approach was designed to maximize the resolution of sampling (with ^{13}C measurements), and to capture the slower and smaller cycling pools (with ^{14}C), while minimizing the time and cost associated with ^{14}C analyses. The chase measurement sample intervals for ^{13}C were 4 h, 1, 2, 3 and 6 d after labeling. Sample intervals for ^{14}C were 1, 3, 13 and 36–50 d after labeling.

Samples for ^{13}C content in soil respiration and leaf respiration were collected from the chambers and branch bags by syringe. The CO_2 was allowed to accumulate to ~1000 ppm, and then two 60-ml syringe samples were extracted. The first sample was used to measure the CO_2 concentration with the LI-800 IRGA. The second was flushed through a 12-ml exitainer with a septum cap and returned for ^{13}C analyses on a Thermo Electron Gas Bench II coupled with a Delta Plus IRMS (Thermo Scientific, Waltham, MA, USA) at UC Irvine. All ^{13}C samples were analyzed less than 5 d after collection to minimize storage effects. Temporal variability in the $\delta^{13}C$ signature of respiration, including that attributable to isotopic fractionation during dark respiration and photorespiration, was not quantified (Ghashghaie *et al.*, 2003; Prater *et al.*, 2006) but was likely small compared with our large label signal over the 6-d time period, and spatial heterogeneity.

Samples for ^{14}C content in soil respiration and leaf respiration were collected from the chambers and branch bags by molecular sieve traps. Samples were allowed to accumulate CO_2, and the concentration was measured. Sample air was then pumped from the chamber or bag (1 l min^{-1}) through a $MgClO_4$ drying column to an activated molecular sieve 13× trap which quantitatively removed CO_2. The air was then returned to the chamber or bag. Samples were returned to UC Irvine, where the CO_2 was extracted from the molecular sieve, purified on a vacuum line, and converted to graphite (Xu *et al.*, 2007). The ^{14}C content of the graphite was measured using accelerator mass spectrometry (NEC 0.5MV 1.5SDH-2 AMS system; National Electrostatics Corp., Middleton, WI, USA) at the W. M. Keck-CCAMS facility of UC Irvine (Southon *et al.*, 2004).

The radiocarbon data ($\Delta^{14}C$) are reported in per mil (‰), the deviation (in parts per thousand) of the ratio of ^{14}C : ^{12}C in a sample divided by that of a standard of fixed isotopic composition (0.95 times the ^{14}C : ^{12}C ratio of oxalic acid I standard; decay corrected to 1950). Measurements were corrected for the effects of mass-dependent isotope fractionation by correcting to a common $\delta^{13}C$ value (–25‰) and assuming that ^{14}C is fractionated twice as much as ^{13}C (Stuiver & Polach, 1977). Measurements taken early in the chase period (1 and 3 d after labeling) had elevated $\delta^{13}C$ values as a consequence of the use of the dual isotope labeling method, and therefore the $\delta^{13}C$ of the sample was not the correct measure of isotopic fractionation. In these cases, the sample ^{14}C content was corrected for isotopic fractionation with average background $\delta^{13}C$ values following the method described by Torn & Southon (2001).

Data analyses and definition of terms

The $^{13,14}C$ contents of soil respiration and leaf respiration were combined with CO_2 flux measurements to estimate the total amount of $^{13,14}C$ respired during the period from 4 h to 36 d following the application of the label (Carbone *et al.*, 2007). We assumed that $^{13,14}C$ concentrations varied linearly between observations. We estimated errors in the allocation of C over the measurement period following the labeling by propagating errors (±1 standard deviation) from isotope measurements, and using the mean of the continuous CO_2 flux measurements over time. Continuous CO_2 fluxes were not available for the leaf respiration in the shrub ecosystem, and empirically derived exponential temperature relationships based on branch bag CO_2 flux measurements and hourly air temperatures were used to estimate fluxes. Differences in the photosynthetic assimilation of the label on different dates and between vegetation types meant that we did not make comparisons based on the absolute amount of label respired and how it was allocated. Instead, we compared the proportional allocation of the total label recovered.

The total label recovered (TLR) was defined as the calculated sum of the label respired ($^{13,14}C$ g) from below- and above-ground between 4 h to 36 d after the label was applied. This quantity was defined as 100% for each labeling. The below- and above-ground components were then partitioned into percentage of TLR over two different time periods, using ^{13}C to calculate allocation partitioning from 4 h to 6 d, and ^{14}C for 6 to 36 d after labeling.

The fraction of respiration from label (FRL) for below- and above-ground components was defined with the same equations as described in Carbone *et al.* (2007), where an isotope mass balance approach was used to partition the fraction of respiration coming from the label with time for both ^{13}C and ^{14}C measurements. For example, the equation used for ^{13}C was:

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Table 1 Mean (24 h) environmental conditions and background $\delta^{13}C$ signatures of CO_2 respired above- and below-ground during the time of measurements, in the early and late growing seasons

	Grass		Shrub	
	Early season	Late season	Early season	Late season
Air temperature (°C)	18.8	27.1	19.0	27.7
Air relative humidity (%)	30.8	35.7	28.8	30.6
Volumetric soil water content at 20 cm (%)	59.5	17.2	18.3	8.4
Depth to water table (m)	1.1	1.8	1.6	>2.0
Below-ground $\delta^{13}C$ background (‰)*	–16.8 ± 1.3	–17.7 ± 1.2	–19.9 ± 0.7	–15.4 ± 1.2
Above-ground $\delta^{13}C$ background (‰)*	–22.3 ± 1.2	–21.3 ± 1.2	–22.5 ± 1.1	–20.6 ± 0.5

*Values are mean ± 1 standard deviation.

$$FRL = (\delta^{13}C_S - \delta^{13}C_B)/(\delta^{13}C_L - \delta^{13}C_B) \quad \text{Eqn 1}$$

(FRL, the fraction of respiration from the label; $\delta^{13}C_S$, the measured sample respiration signature; $\delta^{13}C_B$, the background respiration signature (prelabel); $\delta^{13}C_L$, the label signature.) $\delta^{13}C_L$ was determined as the measured mean of $\delta^{13}C$ of CO_2 assimilated during the ~1 h of label application.

The mean residence time (MRT) of the label in the below- and above-ground components was calculated by fitting exponential decay functions to the FRL. The MRT represents the time required for the amount of label in respiration to be reduced to 1/e times its initial value. As a single decay function would not fit all data points, we calculated separate MRTs for the first 6 d (^{13}C data) and 6–36 d (^{14}C data) after labeling.

The mean age (MA) of (autotrophically) respired C was calculated for below-ground, above-ground, and total ecosystem respiration by combining the percentage of TLR and the MRT for each component over the two defined time periods. For example, the following equation was used for the below-ground component:

$$MA_B = \frac{(TLR_{B(0-6)} \times MRT_{B(0-6)} + TLR_{B(6-36)} \times MRT_{B(6-36)})}{TLR_B} \quad \text{Eqn 2}$$

(MA_B, the mean age of respired C from below-ground; $TLR_{B(0-6)}$, $TLR_{B(6-36)}$, and TLR_B, the percentages of total label in respiration from the below-ground component for the first 6 d, for 6–36 d, and in total (0–36 d), respectively; $MRT_{B(0-6)}$ and $MRT_{B(6-36)}$, the corresponding mean residence times of the label below-ground over the same defined time periods.) For total ecosystem respiration, the mean age of respired C was calculated as a weighted sum of the below- and above-ground components. While we define MA as 'autotrophic', this measurement invariably incorporates respiration from root-associated microbes and the decomposition of very short-lived fine roots.

Results

Environmental conditions

Mean (24-h) air temperatures, air relative humidity, volumetric soil moisture content at 20 cm, and depth to ground water are shown in Table 1 for both measurement periods (early and late growing seasons) and sites. In general, the early growing season was cooler, with greater surface soil moisture, and shallower water tables. The late growing season was much warmer, with lower surface soil moisture, and deeper water tables.

Raw isotopic values

Raw $\delta^{13}C$ (4 h to 6 d) and $\Delta^{14}C$ (1 to 36–50 d) respiration signatures are presented in Fig. 1. Labeled respiration $\delta^{13}C$ values were larger than background values (Table 1), even on day 6 (see legend to Fig. 1). Background $\delta^{13}C$ values show the influence of both C_3 and C_4 plant contributions.

Label partitioning

The majority of the TLR was respired in the first 6 d after labeling, with clear differences in allocation between functional types (Fig. 2, Table 2). In the grass ecosystem, a greater proportion of the TLR was respired below-ground; 71 and 64% in the early and late growing seasons, respectively. In contrast, the shrub ecosystem respired a larger proportion of the TLR above-ground; 78 and 83% in the early and late growing seasons, respectively.

Seasonal differences in allocation were detectable, but were small in comparison to plant functional differences. Both sites allocated more TLR below-ground in the early growing season, and more above-ground in the late growing season. The grass ecosystem exhibited a greater seasonal effect than the shrub ecosystem. A greater proportion of labeled C respired in the 6–36-d period was allocated below-ground by the grass in the late growing season. In contrast, in the shrub ecosystem,

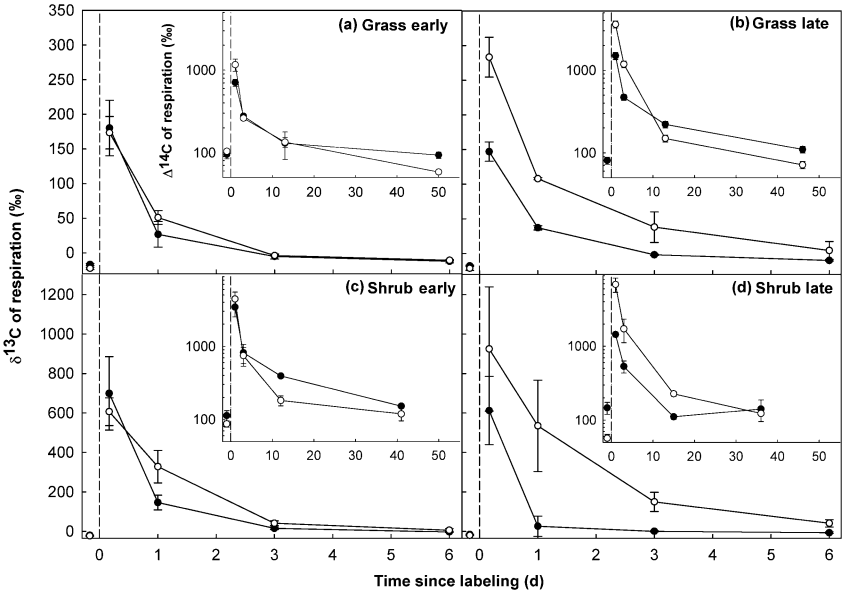

Fig. 1 Raw isotopic values for below-ground (closed circles) and above-ground respiration (open circles) for (a) the grass early growing season, (b) the grass late growing season, (c) the shrub early growing season and (d) the shrub late growing season. For primary plots, *y*-axes are $\delta^{13}C$ values (‰). For inset plots, *y*-axes are $\Delta^{14}C$ values (‰, in log scale). All *x*-axes are time elapsed since labeling (in days). Labeling occurred at time = 0, marked with a dashed vertical line. Background values are shown for reference before labeling. Error bars represent ± 1 standard deviation in field isotope measurements ($n = 4$). Differences in $\delta^{13}C$ on day 6 are difficult to see on the figure but for grasses below- and above-ground respiration was enriched by 4.7–16.8‰ compared with background values (given in Table 1). For the shrubs, day 6 below- and above-ground respiration enrichment ranged 8.3–60‰ above background values.

Table 2 Percentage allocation of the total label recovered in respiration (TLR) for below-ground and above-ground respiration for three time periods: 0–36 d (total), 0–6 d, and 6–36 d after labeling

		TLR (%)								
		0–36 d			0–6 d			6–36 d		
Site	Season	Below	Above	Total	Below	Above	Total	Below	Above	Total
Grass	Early	74 ± 3	26 ± 3	100	57 ± 5	21 ± 5	78	16 ± 4[a]	6 ± 4[b]	22
	Late	61 ± 2	39 ± 2	100	36 ± 2	32 ± 2	68	25 ± 2[a]	7 ± 2[b]	33
Shrub	Early	22 ± 2[a]	78 ± 2[a]	100	16 ± 2[b]	68 ± 2[b]	84	6 ± 2[a]	10 ± 2[a]	16
	Late	17 ± 3[a]	83 ± 3[a]	100	15 ± 5[b]	65 ± 5[b]	80	2 ± 2[a]	17 ± 2[a]	19

Values are mean ± 1 standard deviation. Differences were statistically significant ($P < 0.01$) between seasons, except where indicated with [a]($P < 0.1$) and [b](not significant). Calculations were based on ^{13}C for 0–6 d and ^{14}C for 6 to 36 d; partitioning on days with both ^{13}C and ^{14}C data available (days 1 and 3) was in agreement.

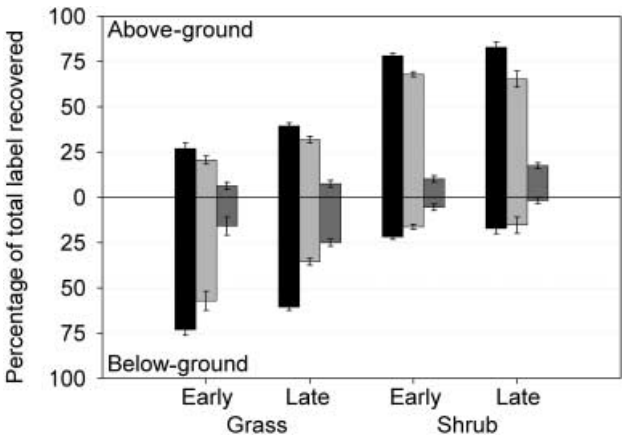

Fig. 2 Percentage allocation of the total label recovered in respiration for below-ground (below 0 on *y*-axis) and above-ground (above 0 on *y*-axis) respiration for three time periods: 0–36 d (black), 0–6 d (light gray), and 6–36 d (dark gray) after labeling.

the majority of the below-ground component was respired during the first 6 d in the late growing season.

Label timing

Between 48 and 61% of the TLR was respired within the first 24 h after labeling during both seasons and by both ecosystems. Between 68 and 84% of the TLR was respired within the first 6 d, and 16–33% was respired in the time period from 6 to 36 d after labeling. A time lag of less than 4 h was observed before labeled C appeared in respiration, with peak values occurring in the first sampling period for all below- and above-ground components.

Mean residence times

The MRTs for the label to be respired below- and above-ground are shown for the two time periods in Table 3. For the first 6 d,

Table 3 Mean residence times (MRTs) for the label in below- and above-ground respiration for 0–6 d and 6–36 d after labeling.

		MRT (d)			
		Below-ground		Above-ground	
Site	Season	0–6 d	6–36 d	0–6 d	6–36 d
Grass	Early	0.48 ± 0.04	20.4 ± 6.2	0.99 ± 0.04	20.5 ± 2.5
	Late	0.73 ± 0.05	26.2 ± 2.5	0.60 ± 0.23	17.5 ± 1.3
Shrub	Early	0.55 ± 0.07	22.1 ± 0.9	1.54 ± 0.20	20.7 ± 2.2
	Late	0.39 ± 0.14	12.1 ± 2.5	0.95 ± 0.15	16.7 ± 0.5
Mean		0.54 ± 0.14	19.9 ± 6.4	1.00 ± 0.39	18.9 ± 2.0

Values are mean ± 1 standard deviation.

Table 4 Mean age of autotrophically respired C for below-ground, above-ground, and total ecosystem components

		Mean age (d)		
Site	Season	Below-ground	Above-ground	Total ecosystem
Grass	Early	4.8	5.3	5.0
	Late	11.2	3.6	8.2
Shrub	Early	6.4	4.0	4.5
	Late	1.8	4.2	3.8

the labeled C cycled rapidly, with MRTs of < 1 d in all cases, except for the early growing season above-ground component at the shrub site. For this time period, the MRTs were generally shorter below-ground (0.54 d) than above-ground (1.0 d). Much slower cycling of labeled C was observed for the time period of 6–36 d after labeling, with little difference in MRTs between the below-ground (19.9 d) and above-ground (18.9 d) components. The longest MRT of 26.2 d was observed in the late growing season below-ground at the grass site.

Mean age of autotrophically respired C

Overall, the MA of C respired by shrubs was shorter than that of the grasses (Table 4). In the early growing season, the MAs of respired C from the grass and shrub ecosystems were similar; 4.5 and 5.0 d, respectively. By contrast, the MAs deviated in the late growing season, with the grass ecosystem MA increasing to 8.2 d, and the shrub MA decreasing to 3.8 d. These differences were largely attributable to the differential allocation to the more slowly cycling C pool in the below-ground components, where the MA of below-ground respired C from the grasses increased to 11.2 d, and that from the shrubs decreased to 1.8 d.

Contribution of the label to instantaneous respiration

The FLR is shown in Fig. 3. Both sites initially (for 0–6 d, when the majority of label was respired) showed greater contribution of the label to respiration above- than below-ground for both the early and late growing seasons. Both sites also showed greater disparity between below- and above-ground respiration label contributions in the late growing season, and less difference in the early growing season. The average above- to below-ground ratios of label in respiration were 1.4 and 2 for the grasses in the early and late growing seasons, respectively. In the shrubs, ratios averaged 1.4 and 6.2 in the early and late growing seasons, respectively.

Storage of the label

At both sites, background leaf respiration $\Delta^{14}C$ signatures before the first labeling campaign in the late growing season (2005) were similar to atmospheric air samples, which would be expected if leaves were respiring current-year photosynthate. Control plot early growing season (2006) leaf respiration background measurements were also in the air $\Delta^{14}C$ range. However, the early growing season label plot leaf respiration had significantly ($P < 0.01$) higher $\Delta^{14}C$ values, reflecting a measurable contribution from the label assimilated over 9 months before (Fig. 4). The appearance of stored labeled C was not detected in soil respiration measurements, which displayed much greater seasonal and spatial variation attributable to differential contributions of soil organic matter decomposition and root respiration.

Discussion

Plant functional type allocation – different patterns

Distinct patterns were observed in the respiration of new assimilates in these two plant functional types. In the grass

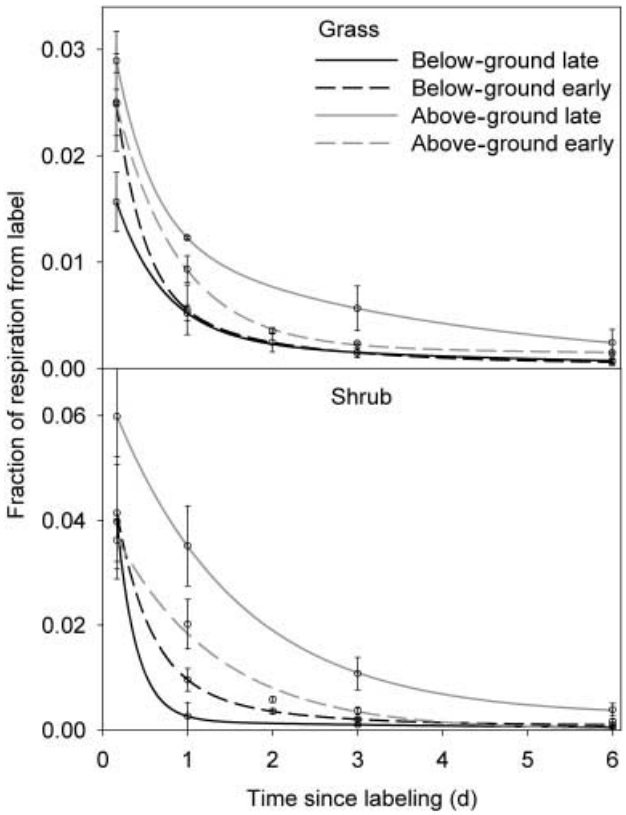

Fig. 3 The fraction of respiration from label over the first 6 d after labeling. Top, grass site; bottom, shrub site; dashed black line, below-ground early growing season; solid black line, below-ground late growing season; dashed gray line, above-ground early growing season; solid gray line, above-ground late growing season. Error bars represent ± 1 standard deviation in field isotope measurements ($n = 4$).

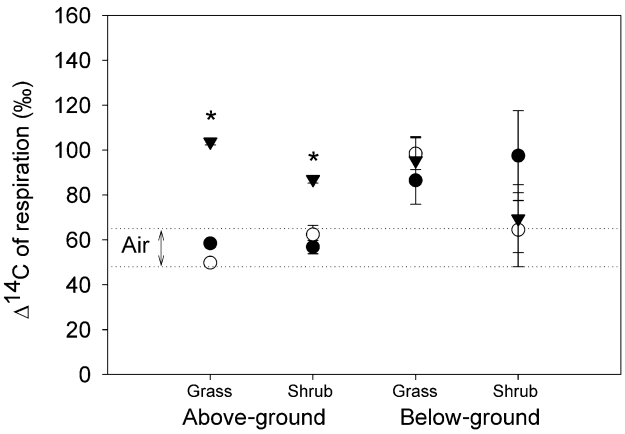

Fig. 4 Background (before labeling) respiration $\Delta^{14}C$ signatures for both above- and below-ground. Closed circles, control and label plots in the late growing season of 2005; open circles, control plot in the early growing season of 2006; triangles, label plot in the early growing season of 2006. There were significant differences between control and label plot above-ground respiration in 2006 (*, $P < 0.01$). Error bars represent ± 1 standard deviation in field isotope measurements ($n = 4$); in some cases symbols are larger than error bars. Dashed lines represent the range of air $\Delta^{14}C$ signatures taken over the entire field campaign ($n = 32$).

ecosystem, the majority of the new C was allocated to respiration below-ground. In contrast, a greater majority of new C was allocated to above-ground respiration in the shrub ecosystem. While there were some discernible seasonal differences, these general patterns appear to dominate C entering these ecosystems for at least the early and late portions of the growing season.

The major energetic costs below-ground are the growth of new roots and the maintenance of existing roots (Dobrowolski *et al.*, 1990). Based on typical $B_{R:S}$ values observed in these plants, we expected both grasses and shrubs to invest heavily below-ground. Caldwell & Camp (1974) reported $B_{R:S}$ as high as 11 and estimated that ~75% of net primary productivity went below-ground in co-occurring Great Basin shrubs. While the grasses allocated the majority of new C to below-ground respiration, in the shrub ecosystem, the corresponding amount of new C was not being respired below-ground.

Two factors could explain why the observed below-ground allocation by the shrubs was lower than might be expected, given that the $B_{R:S}$ should be similar to (or greater than) that of the grasses. First, high water tables and above-average winter precipitation may have allowed a significant reduction in below-ground allocation to biomass by shrubs in the Owens Valley and/or in the seasons in which we made our measurements. The tap-rooted shrubs would not invest more in root biomass than necessary because of energetic costs (Toft, 1995). Tap roots (responsible for water uptake) are estimated to represent only 3–4% of shrub root biomass, with the remainder in fine roots primarily in upper soil layers (Caldwell & Camp, 1974). It is unknown whether these shrubs access nutrients from the groundwater, as opposed to surface soil layers, but it is possible. Together, the availability of water and nutrients could potentially explain the reduced allocation to below-ground respiration sources in the shrub ecosystem.

Alternatively, if we assume that the plant functional types have typical biomass distributions (i.e. high $B_{R:S}$), then the shrubs exhibited fundamentally less demand for new C below-ground. Thus, it is more likely the $B_{R:S}$ is not representative of the partitioning of C used for respiration or net primary production. We propose that differences in the root lifetimes and maintenance costs between plant functional types resulted in the large disparity observed in the proportion of new C allocated below-ground. A review by Gill & Jackson (2000) estimated annual root turnover to be faster in perennial grasses (53% yr^{-1}) than in shrubs (34% yr^{-1}). Thus, a similar root biomass would be associated with ~55% more below-ground C allocated just to annual root growth in grasses. Caldwell & Camp (1974) estimated annual root turnover to be 17–30% yr^{-1} in Great Basin cold desert shrubs, which could imply even less below-ground allocation needed to maintain root biomass.

It has been suggested that plants growing in nutrient-poor environments might increase root lifespan to avoid nutrient loss (Ryser, 1996). Soil nitrogen content was also over 10 times lower in the shrub ecosystem (D. Pataki, unpublished data). Additionally, there is strong evidence that root maintenance respiration is minimized in cold desert shrubs (Dobrowolski *et al.*, 1990). Thus, longer shrub root lifetimes (i.e. less new root growth respiration) and lower specific root respiration may also explain the observed allocation and differences between the grasses and shrubs in this study.

In addition to small below-ground C sinks, the shrubs had greater above-ground C costs. The construction of woody biomass during the growing season and maintenance over the entire year requires continuous C investment. The grasses have no above-ground maintenance costs outside of the growing season because the leaves senesce. Additionally, the grasses are rhizomatous, and thus most of the leaf growth respiration takes place below-ground. These plant functional differences in above-ground demands for C may have contributed to either one or a combination of our above explanations.

Below-ground respiration of the label may have been underestimated in both the grasses and the shrubs. To minimize disturbance to the plants and soils, the plots were not trenched. Therefore, loss of labeled C to transport outside the plot by roots may have occurred. In addition, the transport of unlabeled C by roots into the plot was also possible, causing additional dilution of the label. Because samples were not taken outside the plots, this is a source of error we cannot quantify. However, we believe that this error was likely small, because samples of soil respiration and soil pore space CO_2 (data not shown) taken near the edge of the label plots were not statistically different from those taken from the center of the plots.

Seasonal allocation – similar patterns and evidence of storage

Variations in seasonal allocation patterns were smaller than the plant functional differences in allocation. Both the grasses and the shrubs allocated more new C to respiration below-ground in the early growing season, when root growth has been shown to be greatest in upper soil layers in co-occurring Great Basin grasses and shrubs (Fernandez & Caldwell, 1975; Peek *et al.*, 2005). Additionally, Great Basin shrubs have been shown to regulate root respiration capacity, with the maximum occurring in spring and the minimum in late summer (Holthausen & Caldwell, 1980). In the late growing season, proportionally more new C was allocated to respiration above-ground. This coincided with greater above-ground biomass, vegetative flowering, and high daily air temperatures, all of which raise metabolic C costs above-ground.

While the two plant functional types exhibited similar seasonal patterns, a greater seasonal effect was observed in the grass ecosystem. Although surface soil moisture markedly decreased over the growing season, continued access to groundwater by the shrubs may explain the relatively small variation in allocation observed between the early and late growing seasons. Differences in the potential rooting depth of plant functional types (grasses are more shallowly rooted than shrubs) and depth to water (the water table becomes deeper over the growing season) between the early and late growing seasons most likely explain the larger seasonal effect observed in the grasses vs the shrubs. Additionally, the grasses were phenologically more advanced (nearer to senescence) than the shrubs at the time of the late growing season labeling campaign.

Stored C from the late growing season labeling campaign contributed to above-ground respiration in the following early growing season, > 9 months later. We estimated the stored C used for leaf respiration by assuming that the ^{14}C content in leaf respiration was constant for the initial stages of new leaf growth in the spring. Based on growth observations in the field, we assumed that the duration of respiration with this ^{14}C signature was 60–90 d for the grass leaves and 45–75 d for the shrub leaves, and redefined TLR (100%) to include this amount. The result suggests that 4–6 and 2–3% of TLR and 9–13 and 3–4% of the above-ground respiration from the late growing season labeling was stored and remobilized in the following early growing season in the grass and shrub ecosystems, respectively. This is consistent with the findings of other studies which demonstrated that stored C can be used for new shoot growth in the spring and for maintenance in the dormant season (Lippu & Puttonen, 1989; Dickson, 1991; Pregizter, 2003). Thus, the size of this stored pool may be underestimated, particularly if it contributed to dormant season maintenance respiration, as a result of a lack of winter measurements in stem respiration in the shrubs and below-ground respiration at both sites.

Speed of cycling

New C cycled quickly within and through these ecosystems, with approximately half of the new C respired within the first day. Translocation of C from leaves to roots and into soil respiration occurred before we made our initial measurement, 4 h after assimilation. This time lag is significantly shorter than the ~3–5 d observed in trees, and comparable to previous observations in grasses (Warembourg & Paul, 1973; Horwath *et al.*, 1994; Ekblad & Högberg, 2001; Bowling *et al.*, 2002; Carbone *et al.*, 2007). We would expect this time lag to be even shorter for the grasses, because it may depend on path length and plant size (Farrar & Jones, 2000). Because our first sampling point was 4 h after labeling, we may have underestimated the most rapidly cycling C in these ecosystems.

The rate of loss of labeled C from the leaf (or root) depends on pool size, respiratory losses, assimilation, and rate of C export in phloem (rate of C import in phloem) (Wardlaw, 1990). Undoubtedly these factors varied between seasons, plant functional types, and even plant species, making it

difficult to compare seasons or attribute differences in the rates of cycling to specific plant physiological mechanisms. Yet, there were general patterns in the MRTs of new C. Surprisingly, the MRTs were similar between plant functional types, in spite of the allocation patterns which were very different. Most respired new C cycled quickly (i.e. ~1–6 d) with MRTs consistently shorter below-ground than those for above-ground respiration; half a day and a day, respectively, for both grasses and shrubs. However, there were also significant detectable pools of new C with much longer MRTs, ranging from many days to months, and perhaps years. The differential allocation by grasses (larger) and shrubs (smaller) to a more slowly cycling pool was noticeable below-ground in the late growing season (Fig. 2, Table 2).

Sources of plant respired C

Our data show that there were potentially three C pools fueling plant respiration in this ecosystem at any given time: the *fast pool*, composed of assimilation of the current day; an *intermediate pool*, which integrated assimilation during the growing season with MRTs of tens of days; and a *storage pool*, which was mobilized when necessary, such as during initial leaf growth in the spring, with MRTs of months to years. The integration of the *fast* and *intermediate pools* resulted in the MA of respired C from both plant types being several days, with longer MAs in the grasses than the shrubs. Mobilization of the *stored C pool* affects the MA of respired C from these ecosystems. If we conservatively estimated a MRT of 60 d for this storage pool, and included it in our MA calculation, the MA for the total ecosystem respiration increased by 30–55%. In the early growing season, when this stored pool was easily detected, the MA of respired C increased from 5.0 to 7.7 d. In the shrub ecosystem, it increased from 4.5 to 5.9 d.

Thus, including the contribution of the *storage pool*, the MA of respired C from these ecosystems was still only days. While the MA of respired C from trees will differ from that of grasses and shrubs, our data concur with the findings of forest studies that correlated changes in the $\delta^{13}C$ of assimilation (associated with stomatal response to relative air humidity) to that of soil respiration with a lag of several days (Ekblad & Högberg, 2001; Bowling *et al.*, 2002). However, in the Owens Valley ecosystems, a deviation in the $\delta^{13}C$ of assimilation would result in a much more rapid (< 0.5–1 d) change in the $\delta^{13}C$ of autotrophic respiration, and the observed change would be diluted by a longer term average signal to a greater (grasses) or lesser (shrubs) degree. These results also directly support the findings of Tang *et al.* (2005), where soil respiration was linked to photosynthetic uptake with two different time lags, several hours and 5–6 d. Our measured MA of respired C from grasses and shrubs most likely differs from that observed in trees because transport distances from leaves to roots are longer and stored nonstructural C pools may be greater in large-stature vegetation.

In conclusion, there were significant longer lived C pools used for plant metabolism in these ecosystems, but the majority of new C respired cycled quickly through the plants and soils. The contribution of these pools to respiration was different for below- and above-ground sources. It could be that this 'buffering' of below-ground respiration by longer lived C pools that inhibits directly relating above-ground processes such as photosynthesis to soil respiration. Additional research is required to better understand the roles of these different C pools in respiration, and would enhance our ability to predict terrestrial ecosystem respiration fluxes. Furthermore, the ecological importance, size, and residence times of these longer lived pools remain poorly understood (Körner, 2003).

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