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ORIGINAL PAPER

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# Distribution and fate of $^{13}$C-labeled root and straw residues from ryegrass and crimson clover in soil under western Oregon field conditions

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**Abstract** Annual ryegrass (*Lolium multiflorum* Lam.) and crimson clover (*Trifolium incarnatum* L.) were pulse-labeled with $^{13}C\text{-}CO_2$ in the field between the initiation of late winter growth (mid-February) and through flowering and seed formation (late May). Straw was harvested after seed maturation (July), and soil containing $^{13}$C-labeled roots and root-derived C was left in the field until September. $^{13}$C-enriched and $^{13}$C-unenriched straw residues of each species were mixed in factorial combinations with soil containing either $^{13}$C-enriched or $^{13}$C-unenriched root-derived C and incubated in the field for 10 months. The contributions of C derived from straw, roots, and soil were measured in soil microbial biomass C, respired C, and soil C on five occasions after residue incorporation (September, October, November, April, and June). At straw incorporation (September), 25–30% of soil microbial biomass C was derived from root C in both ryegrass and clover treatments, and this value was sustained in the ryegrass treatment from September to April but declined in the clover treatment. By October, between 20 and 30% of soil microbial biomass C was derived from straw, with the percentage contribution from clover straw generally exceeding that from ryegrass straw throughout the incubation. By June, ryegrass root-derived C contributed 5.5% of the soil C pool, which was significantly greater than the contributions from any of the three other residue types (about 1.5%). This work has provided a framework for more studies of finer scale that should focus on the interactions between residue quality, soil organic matter C, and specific members of the soil microbial community.

**Keywords** $^{13}$C labeling · Annual ryegrass · Crimson clover · Straw and root C decomposition · Soil C

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## Introduction

Despite the wealth of information available on the subject of residue decomposition (Berg and McClaugherty 2003; Cadisch and Giller 1997; Swift et al. 1979), several aspects need further study. For example, the factors that control the fate and distribution of root-derived C in the field are complex, with several reports indicating that the fate of root C is influenced by (1) the type of plant species (De Neergaard and Gorissen 2004; Herman et al. 1977; Malpassi et al. 2000; Muller et al. 1988; Xu and Juma 1995), (2) the stage of plant development (Butler et al. 2004; Swinnen et al. 1995), (3) the presence of rhizodeposits (Mayer et al. 2004), (4) root disturbance (Martin 1989; Van Ginkel and Gorissen 1998), (5) size of root fragments (Bending and Turner 1999; Bolger et al. 2003), (6) the nitrogen status of the soil (Cheng and Coleman 1990; Liljeroth et al. 1994; Van der Krift et al. 2001b; Van Ginkel and Gorissen 1998), and (7) the presence or absence of living plant roots (Bottner et al. 1999; Dormaar 1990; Loya et al. 2004; Van der Krift et al. 2001a). Furthermore, many of the above studies were focused upon the fate of root and straw C inputs from grasses and cereals, and only a few reports have compared the fates of straw- and root-derived C of different qualities from grasses and legumes under the same field conditions (De Neergaard and Gorissen 2004; Lucero et al. 2002). Although several reports have shown that litter/residue decomposition occurs

during winter months under cold soil temperatures and/or flooded soil conditions (Beare et al. 2002; Bird et al. 2001, 2002; Bossio et al. 1999; Cheshire et al. 1999; Clein and Schimel 1995; Devevre and Horwath 2000; Hobbie and Chapin 1996; Lipson et al. 1999, 2002; Van Scholl et al. 1997), to our knowledge, no studies have compared the fates of root and straw residue C of a grass and a legume, and their impact on soil C, from the onset of fall rains through the winter and into the following growing season.

Many of the papers mentioned above involved the use of radioactive $^{14}C$ as a tracer. Although the use of $^{14}C$ under field conditions has been curtailed in recent years, the stable isotope $^{13}C$ offers an alternate means to follow the fate of plant-derived C in field studies (Ostle et al. 2000; Lu et al. 2003; Malosso et al. 2004; Puget and Drinkwater 2001; Waldrop and Firestone 2004).

The purpose of our study was to trace the movement of $^{13}C$ from field-labeled straw and root residues into the microbial, soil, and respiratory pools under field conditions and to determine if C from straw and root residues of both a field-grown legume and a grass with different residue quality might result in differential transfer and accumulations within these pools under diverse western Oregon field conditions. Our experimental approach involved production of $^{13}C$-labeled crimson clover (*T. incarnatum* L.) and annual ryegrass (*Lolium multiflorum* Lam.) under field conditions. Labeling was commenced in mid-February to ensure that all phases of plant growth and C allocation were captured. To ensure that an authentic $^{13}C$-labeled residue was used, straw and roots were allowed to mature in the field throughout the summer until initiation of the experiment in September. Soil and residues were sampled on five occasions between September 2002 and June 2003 to include the full range of environmental conditions associated with fall, winter, and spring field conditions of western Oregon.

## Materials and methods

### Field site and experimental setup

Field plots were located on a Woodburn silty clay loam (Aquultic Argixerolls) located at the Hyslop Agricultural Field Laboratory, Oregon State University, Corvallis, OR, USA (44°38′N, 123°12′W). The climate is characterized by cool, wet winters, and dry, warm summers with about 100 cm of annual precipitation. At the field site, the soil has a long history of being conventionally tilled and cropped to rotations that include small grains, forage legumes, and grasses and is relatively low in organic matter (1.3% organic C). The experimental design was a randomized complete block with four replicates per treatment. Each 30×5 m replicated block was seeded with either annual ryegrass (*L. multiflorum* Lam.) or crimson clover (*Trifolium incarnatum* L.). Plots were planted in September 2001, and seeds germinated in response to fall rain. The herbicide, clethodim, was applied to clover plots in late February to control annual bluegrass.

### $^{13}C$ enrichment of plant biomass

Isotopic enrichment of the plant shoots and roots was accomplished by six repeated pulse labelings of subplots (0.6 $m^{-2}$) throughout the growing season (mid-Feb to May 2002) using a 320-L Plexiglas chamber (0.6×0.6×0.75 m) as described in detail elsewhere (McMahon et al. 2005). $^{13}CO_2$ was generated from 99% $^{13}C$-enriched $NaHCO_3$ (Cambridge Isotope Labs., Andover, MA, USA) and injected directly into the Plexiglas chamber. After the $CO_2$ in the chamber dropped to 150 µmol $mol^{-1}$ of air, more $^{13}CO_2$ was injected, and the process was repeated until a total of 600 mg of $^{13}C$ had been injected into each chamber. Subsequently, an identical quantity of tank $CO_2$

**Table 1** Percentage of total C and the $\delta^{13}C$ contents of chemically fractionated straw carbon pools

| | Total straw | | Water soluble | | | Lipid | | | Cellulose/hemicellulose[a] | | | Lignin | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ($\delta^{13}C$-PDB natural abundance) | ($\delta^{13}C$-PDB with label) | (%[b]) | ($\delta^{13}C$-PDB natural abundance) | ($\delta^{13}C$-PDB with label) | (%[b]) | ($\delta^{13}C$-PDB natural abundance) | ($\delta^{13}C$-PDB with label) | (%[b]) | ($\delta^{13}C$-PDB natural abundance) | ($\delta^{13}C$-PDB with label) | (%[b]) | ($\delta^{13}C$-PDB natural abundance) | ($\delta^{13}C$-PDB with label) |
| Annual rye-grass | −29.4* (0.25) | 90.6 (8.3) | 29.8* (0.44) | −29.8* (0.45) | 85.4 (13) | 1.74 (0.27) | −35.1* (0.62) | 118.3 (17) | 59.8 (1.2) | −29.0 | 92.4 | 8.61* (0.46) | −30.2 (1.6) | 91.48 (9.4) |
| Crim-son clover | −25.6 (0.12) | 107 (8.6) | 24.4 (1.3) | −26.9 (0.02) | 73.9 (11) | 2.01 (0.12) | −32.2 (0.52) | 90.4 (8.9) | 66.6 (2.6) | −24.7 | 121 | 6.88 (0.41) | −28.6 (0.32) | 94.1 (4.3) |

Asterisks indicate the occurrence of a statistically significant difference between plant species ($\alpha$=0.05). Numbers in parentheses are standard error of the mean ($n$=4)

[a]The difference between the total plant C and the sum of that obtained from the three measured fractions was used to derive the cellulose plus hemicellulose C

[b]Percentage of total straw C found in each of the designated fractions

(99% $CO_2$; $\delta^{13}$C-PDB=−36.5‰) was injected to ensure that most of the $^{13}CO_2$ respired by the plants was re-incorporated. Because each labeling event required about 2 h per plot, we expect that there was a minimal influence of the chamber and enriched $CO_2$ conditions on plant residue composition (Table 1). Plants were harvested in July 2002 after seed maturation, and yield and $^{13}$C content were measured. Seed heads were removed, and straw was chopped into pieces less than 5 cm and homogenized. At maturity, the $\delta^{13}$C-PDB values of ryegrass and clover straw were +91 and +108‰, respectively. Roots from ryegrass and clover were labeled to similar degrees ($\delta^{13}$C=+31‰). Biomass yields of ryegrass roots and shoots were 210 and 920 g $m^{-2}$, respectively. Standing biomass yields of clover roots and shoots were 200 and 800 g $m^{-2}$, respectively. The C/N ratios of clover and ryegrass roots were 36 (%N=1.25) and 75 (%N=0.60), respectively, and those of clover and ryegrass straw were 44 (%N=1.00) and 125 (%N=0.36), respectively.

## Incorporation and incubation of $^{13}$C-labeled residues

On 1 September 2002, soil was excavated from 0- to 20-cm depth of each labeled plot and also from equal size plots of unlabeled plants. To eliminate source variability, straw residues and excavated soil from each of the four labeled replicate plots were mixed thoroughly. Appropriate amounts of straw that approximated the crop yields (about 950 g $m^{-2}$) were thoroughly mixed with the excavated soil samples so that they contained both roots and shoots of one plant species. In a plot of land immediately adjacent to the 2001–2002 field site, four blocks of crimson clover and annual ryegrass were seeded. In each of the four blocks of each species, three subplots (0.6×0.6 m) were excavated to receive the following treatments:

1. Excavated soil containing root-derived C/root residue and straw residue of ambient $\delta^{13}$C
2. $^{13}$C-enriched straw and excavated soil containing unlabeled root-derived C
3. Excavated soil containing $^{13}$C-enriched root-derived C/root residue and unlabeled straw

Soil plus residue treatments were mixed into the plots on 1 September 2002. At time intervals, soil and residues from each treatment were sampled (0- to 20-cm depth) with a 5-cm-diameter PVC core at three randomly chosen points within each plot. The three samples within a plot were homogenized together, residues removed by hand, and soil sieved to pass a 5-mm mesh.

## Analyses

### *Residue analysis*

Straw was chemically separated into water-soluble, lipid, cellulose, and lignin fractions (Table 1). Water-soluble C was determined by extracting 1 g (air-dried) residue with 50 mL Millipure water. Lipid extraction was conducted using the procedure of White and Ringelberg (1998). The lower chloroform phase was removed, and particulates were removed by passing through a Whatman no. 1 filter, dried under ultrahigh-purity $N_2$, and stored at −10°C. Cellulose and lignin fractions were determined by hydrolysis with 24 N $H_2SO_4$ as described by Horwath and Elliot (1996). The $\delta^{13}$C and total C contents of soluble C, lipid C, and lignin C were measured on a continuous flow Europa 20/20 Isotope Ratio Mass Spectrometer (Europa Scientific, Cheshire, England). The remaining unaccounted for mass and $^{13}$C was attributed to cellulose.

### *Determination of microbial biomass, respiratory, and soil C and mineral N levels*

Microbial biomass C was estimated on 20- to 25-g portions of moist soil by the chloroform fumigation extraction method using a $k_c$ of 0.45 (Vance et al. 1987) and extracted and analyzed for $^{13}$C according to the method of Bruulsema and Duxbury (1996). One set of samples for estimation of microbial biomass was fumigated for 28–36 h with chloroform, and another set of nonfumigated controls was stored at 4°C. After mixing on an orbital shaker (300 rpm) for 1 h, soil extracts (0.05 M $K_2SO_4$) were allowed to settle for 20 min, passed through a Whatman no. 1 filter, and analyzed. Subsamples of the solutions were transferred to acetone-rinsed tin squares (Microanalysis, Manchester, UK), dried at 65°C, and processed for total C and $^{13}$C analysis. Additional aliquots of the $K_2SO_4$ extracts from unfumigated soil samples were used for determination of $NH_4^+$-N and $NO_3^-$-N with an autoanalyzer.

With the expectation that the fumigated (microbial+ soluble C) and unfumigated (soluble C) samples represent their respective pools and were homogeneously mixed, we calculated $\delta^{13}$C of microbial C using

$$\delta^{13}C = \frac{\delta_{Fum} - (\delta_{unfum} * P_{unfum})}{(1 - P_{unfum})} \quad (1)$$

where $\delta_{Fum}$ represents the $\delta^{13}$C of the C extracted from the fumigated sample, $\delta_{Unfum}$ represents the $\delta^{13}$C of the C extracted from the unfumigated sample, and $P_{Unfum}$ represents the proportion of C in the unfumigated compared with the fumigated sample. The $\delta^{13}$C and C content of the fumigated and unfumigated samples were measured by isotope ratio mass spectrometry (IRMS). Once the $\delta^{13}$C of the microbial C was calculated, a mixing model was used to derive the fraction ($F$) of residue C that was found in the soil microbial pool using

$$F = \frac{\delta_m - \delta_c}{\delta_r - \delta_c} \quad (2)$$

where $\delta_m$ is the isotopic signature for microbial C in $^{13}$C-enriched residue-treated soil, $\delta_c$ is the isotopic signature of the microbial C in unenriched residue-treated soil, and $\delta_r$ is

the isotopic signature of the residue (root-derived C or straw C). By multiplying the fractions of microbial C (*F*) derived from a residue source by the total microbial C pool, values for root-derived C and straw C in the microbial biomass were obtained. The contributions of soil C to both microbial biomass and respired C were calculated by subtracting the root and straw contributions from the total.

Microbial respiration was measured on freshly sampled soil (less than 2 days) containing residues. Field moist soil (20–25 g) was added to 200-mL mason jars. Jars were fitted with lids that contained a sampling port made of rubber butyl and sealed with silicone gel. Samples were incubated in the air-tight jars at 22°C for 20–32 h. $CO_2$ concentrations were measured by mass spectrometry to determine total and $\delta^{13}C$ of $CO_2$. The amount of $CO_2$ evolved was subtracted from a mason jar containing no soil and 2 mL of water. Respiration rates were calculated as micrograms of $CO_2$-C per gram of soil per hour.

The C content of soil and its $\delta^{13}C$ were determined by IRMS. To remove residues, 50-g portions of soil were mixed on an orbital shaker in 100 mL of deionized water (300 rpm for 1 h), allowed to settle for 20 min, and centrifuged at 11,000×*g* for 15 min (4°C). The eluate containing the detrital organic matter with a density less than 1 g cm$^{-3}$ was decanted off. The process of removing residue pieces was repeated, and the soil was dried for 2 days at 65°C. Subsequently, we refer to "soil C" with the explicit intention of differentiating it from residue and detrital forms of C that are intimately associated with the mineral and/or stable aggregate fraction of the soil.

### Statistical analysis

Total C pools from soil, microbial, soluble, and straw C were measured in the three plots of each treatment block and averaged before statistical analysis. (SAS Institute, 1996). Except for the day of straw incorporation, the $\delta^{13}C$ of soil C pools derived from enriched shoots were always significantly greater than those from the unenriched samples. For straw- and root-derived C, model class statements were treatment (clover roots, clover shoots, ryegrass roots, and ryegrass shoots) and replication; replication (block) was analyzed as the random effect and time as the repeated measure. For total pool sizes, all factors were defined similarly, except that the model class statement of treatment was used for clover and ryegrass. Though straw and roots were mixed in early September, $^{13}C$ derived from roots was deposited into the soil well before that of straw, and thus, we did not conduct statistical tests which compared repeated measurements between root- and shoot-derived C pools. We did consider statistical differences at the end of the study to compare contributions from the four treatments to microbial and soil pools. Least significant difference tests were used to determine where significant differences occurred. All correlations were based on a standard linear model. All results were considered significant at $p<0.05$ unless noted otherwise.

## Results

### Residue chemistry and quantity

Water-soluble C made up a considerable percentage of the residue C in both ryegrass and clover straw residues ranging between 24.4 and 29.8% (Table 1). Similarly, the percentage of plant biomass composed of lignin and water-soluble C was significantly greater in ryegrass than in clover straw. The cellulose/hemicellulose fraction was not included in statistical tests between treatments because it was derived by subtraction from the measured fractions. Because lignin and water-soluble C were greater in ryegrass, it is logical to conclude that the percentage of cellulose was greater in clover. While there was variability in the $\delta^{13}C$ signature between the different chemically defined plant fractions, there was no significant difference in the isotopic signatures of the same specific chemical fraction from ryegrass and clover straws.

Although similar amounts of ryegrass and clover straw were incorporated into soil, the amount of ryegrass residue that was recovered from the soil on each sampling date was always significantly greater (about twofold) than the amount of clover residue (Table 2). Because residues and soil were mixed to a known depth (20 cm) and soil bulk density was measured (1.30±0.05 g cm$^{-3}$), the losses of both water-soluble and water-insoluble C were calculated and compared on a per gram of soil basis. For example, during the September–October interval, the soluble C components of both residues declined virtually to zero. In the case of clover, approximately equal losses of both water-soluble (0.40 mg C g$^{-1}$ soil) and non-water-soluble material (0.44 mg C g$^{-1}$ soil) occurred, whereas the total weight loss from ryegrass residue (0.3 mg C g$^{-1}$ soil) was somewhat less than the water-soluble C content of the straw (0.5 mg C g$^{-1}$ soil). Between October and November, a greater loss of ryegrass residue C occurred than between September and October (0.7 mg C g$^{-1}$ soil vs 0.3 mg C g$^{-1}$ soil), whereas the loss in weight of clover residue C (0.4 mg C g$^{-1}$ soil) was similar to the loss of non-water-soluble material that occurred between September and October. During November–June, the weights of ryegrass and clover residues remained relatively constant, representing about 42 and 25%, respectively, of the initial ryegrass and clover residue dry weights (Table 2).

### Dynamics of inorganic N

The soil contained low levels of $NH_4^+$-N and $NO_3^-$-N at the time of residue incorporation, and, in the case of the ryegrass treatment, the combined concentrations of mineral N remained less than 4 μg N g$^{-1}$ soil throughout the incubation (Fig. 1). For clover-amended soil, however, immobilization of $NO_3^-$-N during September–October was followed by a transient increase in $NO_3^-$-N between October and November. Assuming a microbial biomass C/N ranging between 5:1 and 10:1, the increase in soil microbial biomass C that occurred during September–

**Table 2** Mass of straw (dry weight) and straw-derived soluble C recovered from plots on specific sampling dates[a]

| | September 2002 | | October 2002 | | November 2002 | | June 2003 | |
|---|---|---|---|---|---|---|---|---|
| | (g straw plot$^{-1}$) | (mg soluble C g$^{-1}$ straw C) | (g straw plot$^{-1}$) | (mg soluble C g$^{-1}$ straw C) | (g straw plot$^{-1}$) | (mg soluble C g$^{-1}$ straw C) | (g straw plot$^{-1}$) | (mg soluble C g$^{-1}$ straw C) |
| Annual ryegrass | 365 | 298* (4.4) | 310* (42) | 2.1 (0.40) | 160* (16) | 0.39 (0.04) | 155* (34) | 0.04 (0.01) |
| Crimson clover | 340 | 244 (13) | 162 (33) | 1.8 (0.32) | 83 (21) | 0.35 (0.03) | 89 (30) | 0.04 (0.02) |

Asterisks indicate the occurrence of a statistically significant difference between plant species during that month ($\alpha$=0.05). Numbers in parentheses are standard errors of the mean ($n$=4)
[a]Data for April 2002 were not collected

October (about 125 μg C g$^{-1}$ soil in both treatments) would immobilize between 12.5 and 25 μg N g$^{-1}$ soil. It was calculated earlier that 0.84 mg of clover C g$^{-1}$ soil was consumed between September and October and, with a C/N ratio similar to the residue average (44:1), would contain sufficient N (about 20 μg N g$^{-1}$ soil) to meet the soil microbial growth needs. In the case of ryegrass, however, it was calculated that 0.3 mg of soluble ryegrass straw C g$^{-1}$ soil was consumed during September–October, containing only 2.4 μg N g$^{-1}$ soil. Obviously, a substantial supplement of soil N and/or root-derived N was required to support the soil microbial biomass growth that occurred in the ryegrass treatment during this period.

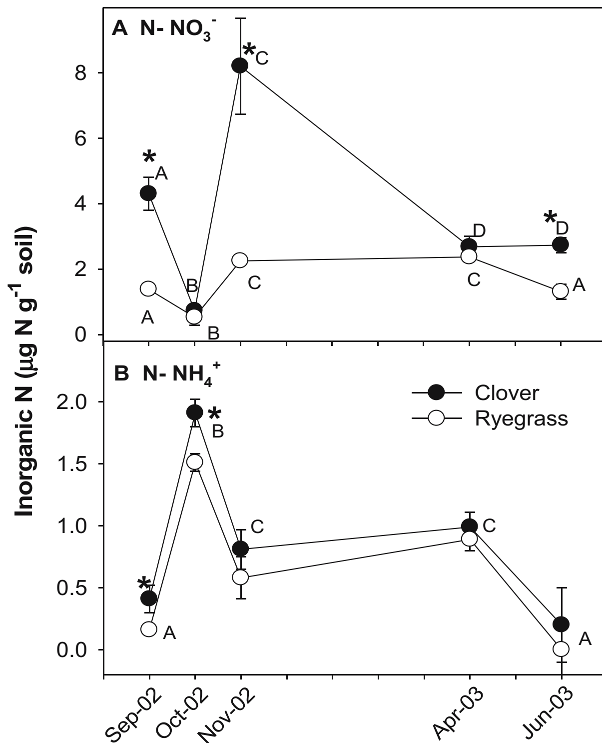


**Fig. 1** Dynamics of (**a**) $NO_3^-$-N and (**b**) $NH_4$-N (μg g soil$^{-1}$) during the decomposition of crimson clover (●) and ryegrass (○) residues under field conditions. Data points and variation are presented as averages±one standard error ($n$=4). Significant differences between sample dates are noted by *different upper case letters*. *Asterisks* denote differences between treatments on the same sample date ($\alpha$=0.05)

## Contributions of soil, root, and shoot residues to soil microbial C

Between September and October, a significant twofold increase in microbial C occurred in response to both types of residues (Fig. 2). In the case of ryegrass, the increase in total microbial C was associated primarily with equal contributions from soil and straw C (about 50 μg C g soil$^{-1}$) and a smaller contribution from root-derived C. In the case of clover, the microbial biomass C increase was derived primarily from straw, with root and soil C making smaller contributions (Fig. 2). The increase in soil microbial biomass C in October did not persist and declined to September values between October and

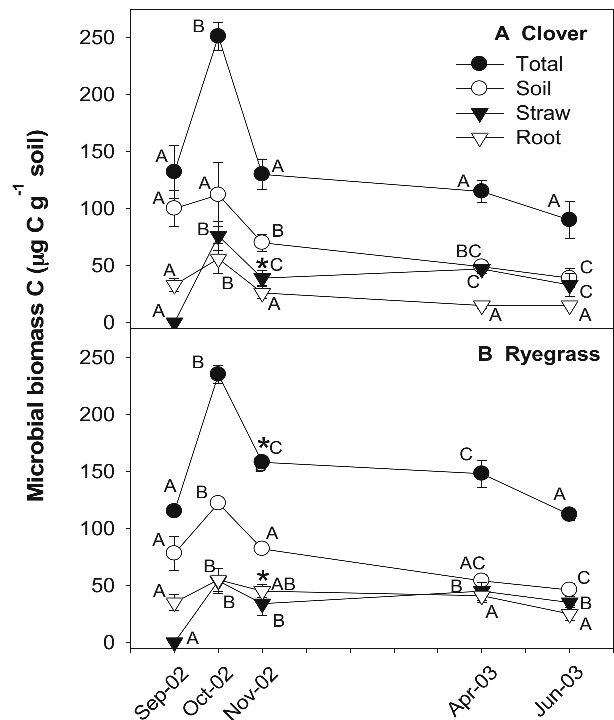


**Fig. 2** Dynamics of the C contributions from straw (▾), root (▿), and soil (○) to total soil microbial biomass C (●) during the decomposition of clover (**a**) and ryegrass (**b**) residues under field conditions. Data points and variation are presented as averages±one standard error ($n$=4). Significant differences between sample dates are noted by *different upper case letters*. *Asterisks* denote differences between treatments on the same sample date ($\alpha$=0.05)

November. During the winter period (November–April), microbial C values remained steady in both treatments, but by the end of the study in June, microbial C in the ryegrass treatment showed a relatively small, albeit significant decrease.

By October, soil microbial C was composed of 20–30% C derived from straw residues (Fig. 3). Generally speaking, the percentage of microbial C derived from clover straw C was consistently greater than for ryegrass on all sampling occasions. Though there was a trend for a progressive increase in the percent of microbial C derived from straw between November and June, only the amount contributed by ryegrass straw changed significantly (Fig. 3a). At the time of straw incorporation, 25–30% of microbial biomass C was derived from root C and the remainder from soil C in both clover and ryegrass treatments (Fig. 3). Even though the percentage contribution of ryegrass root-derived C to microbial biomass C changed little between September and April, the percentage contribution of clover root C declined progressively over this period. The percentage of microbial C derived from ryegrass roots was consistently greater than that derived from clover roots throughout the incubation and was significantly greater in November and April (Fig. 3b).

## Contributions of soil, root, and shoot residues to soil respiration

Total respiration increased between September and October in the ryegrass treatment and between September and November in the clover treatment (Fig. 4). Subsequently, respiration declined throughout the remainder of the incubation; in June, the contribution of residue C to respiration was almost negligible. In November, there was a significantly higher contribution of clover straw C to respiration than from ryegrass straw C. In the case of ryegrass, the contribution of soil C to respiration remained relatively constant throughout the study and generally made a larger contribution than straw C to total respiration. In the case of clover, however, respiration of soil-derived C declined markedly between the September and November samples, and soil C contributed significantly less than straw C to respiration in the November sample.

## Contributions of root and shoot residues to soil carbon

Following straw incorporation into soil, the contribution of clover straw to the soil C pool increased more rapidly than did ryegrass between September and November (Fig. 5a).

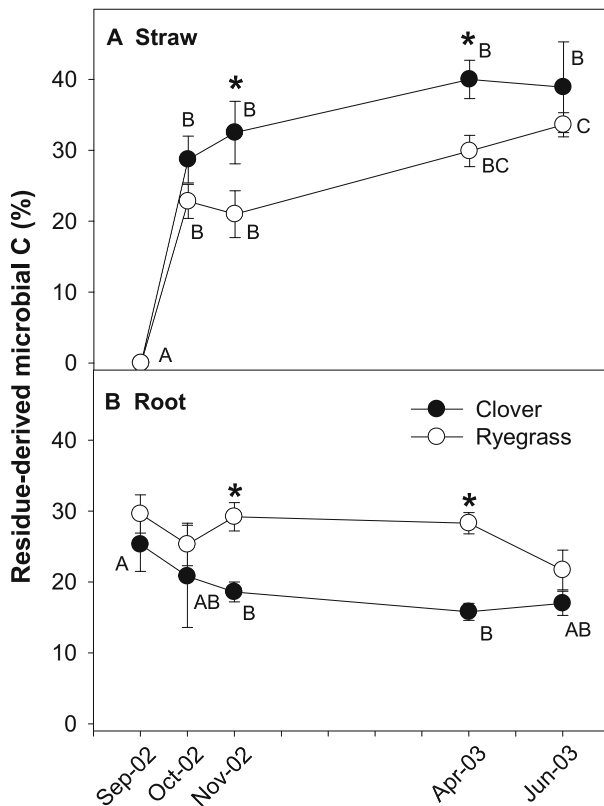


**Fig. 3** Comparison of the percentage of microbial biomass C that is derived from straw (**a**) and root (**b**) of clover (●) and ryegrass (○) residues during decomposition under field conditions. Data points and variation are presented as averages±one standard error ($n$=4). Significant differences between sample dates are noted by *different upper case letters*. *Asterisks* denote differences between treatments on the same sample date ($\alpha$=0.05)

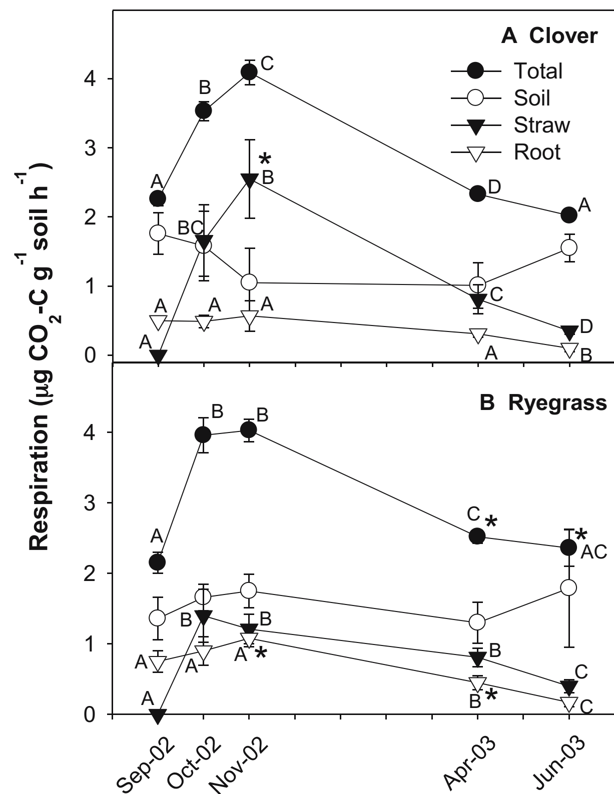


**Fig. 4** Dynamics of the C contributions from straw (▾), root (▿), and soil (○) to the total respiratory rate of $CO_2$ (●) during the decomposition of clover (**a**) and ryegrass (**b**) residues under field conditions. Data points and variation are presented as averages±one standard error ($n$=4). Significant differences between sample dates are noted by *different upper case letters*. *Asterisks* denote differences between treatments on the same sample date ($\alpha$=0.05)

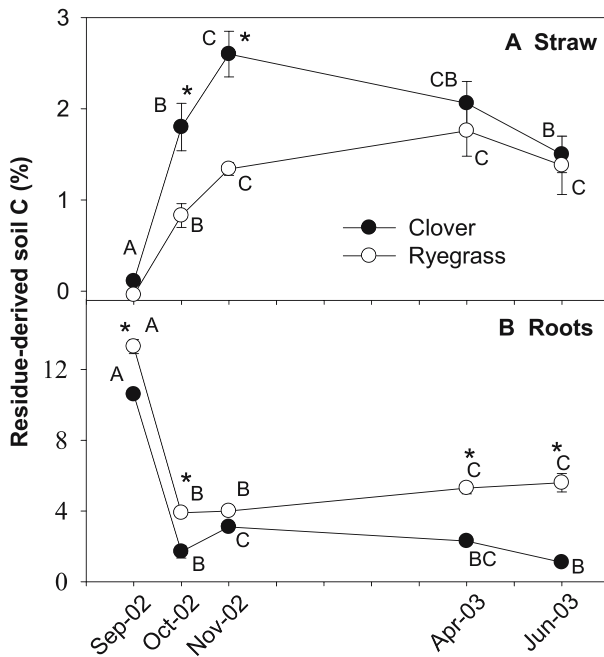


**Fig. 5** Percentage of soil C that is derived from straw (**a**) and root (**b**) of clover (●) and ryegrass (○) during the decomposition of plant residues under field conditions. Data points and variation are presented as averages±one standard error ($n$=4). Significant differences between sample dates are noted by *different upper case letters*. *Asterisks* denote differences between treatments on the same sample date ($\alpha$=0.05)

These differences disappeared by April as a result of the ryegrass contribution increasing through the winter months, while that of clover declined. In June, both ryegrass and clover straw contributions represented about 1.5% of soil C. In the case of root-derived C, both ryegrass and clover roots made large contributions to the soil C pool, representing 13 and 11% of the total soil C pools, respectively (Fig. 5b). Most of this pool of C disappeared quickly between September and October. During November–June, however, the percentage of ryegrass root C in this pool increased slightly, albeit significantly, to represent about 5.5% of total soil C, whereas the contribution of clover root-derived C fell to a value similar to that of the straw residues (1–1.5%).

## Discussion

Despite the extensive amount of information already available on residue decomposition in the literature, research with isotopically labeled residues continues to reveal new insights into this subject. Although our investigation was conducted in the Mediterranean climate of western Oregon, the results have implications for soil C flow and storage in other regions of the temperate world, where summers are dominated by drought, and winters are relatively mild. Moreover, some of the results might apply to an even broader range of agroecosystems where root C inputs are important for maintaining soil organic matter levels. We highlight the dynamics and accumulation of root-derived C in soil that occurred under both an annual legume and a grass during the growing and dry summer seasons, contrast the different temporal behaviors of ryegrass and clover straw decomposition that occurred during the fall months, and compare the temporal dynamics of ryegrass and clover root-derived C in soil during the winter months. In addition, we distinguish the different effects of ryegrass and clover on the contributions of soil C to microbial biomass and to total respiration in the early period of decomposition in the fall.

At the initiation of straw incorporation in the late summer (September), both clover and ryegrass root-derived C had already made a substantial contribution to soil microbial biomass (26–29% of the total biomass C). It is possible that the high $^{13}C$ content of the soil microbial biomass is a consequence of microbes assimilating labeled C directly from roots senescing during the summer months or represents microbial attack on $^{13}C$ that had entered the SOM pool or some remnants of the microbial population labeled by rhizodepositions much earlier during active root growth. In any case, the data indicate that future studies should be directed at a finer-scale dissection of the flow of this root-derived C through the microbial community during different stages of the plant life cycle.

The quantity of root-derived C found in the soil (September 2002) was equivalent to 1 mg root C $g^{-1}$ soil (about 200–250 g C $m^{-2}$ per 20-cm depth) and was similar to the root biomass yields found at the end of the growing season for both plant species. These values are similar to other measurements that have been made of below ground C inputs from grasses and cereals (Kuzyakov et al. 2001; Saggar et al. 1997). This observation establishes that root productivity and rhizodeposits from an annual clover species can contribute as substantially as grasses to soil C as reported recently for the perennial white clover, *Trifolium repens* (De Neergaard and Gorissen 2004).

Although we did not examine in detail the physical location of the root-derived C in soil, our data can be compared with a recent laboratory study by Gale and Cambardella (2000), which showed that approximately one half of root C from $^{13}C$-labeled oat roots was found in the small particulate organic matter and silt plus clay fractions of soil after 90 days of incubation. Subsequently, it was shown that much of the root-derived C had accumulated in soil microaggregates (53–250 µm; Gale et al. 2000a,b). In our study, most of this C pool disappeared between September and October, and since only a small amount of it appeared in microbial biomass C, the pool probably represents material that was easily catabolized, but inefficiently assimilated, possibly as a result of N limitation to microbial growth (Bottner et al. 1998; Henriksen and Breland 1999). Moreover, we cannot overlook the possibility that much of the microbial growth was not measured because it was intimately associated with the root residues. Indeed, Berg et al. (1987) showed a 15-fold increase in the amount of root-associated fungal mycelia on red clover roots decomposing in buried bags. A recent $^{13}C$ study also showed a low conversion of root-derived C into soil biomass C (Puget and Drinkwater 2001). In that case,

however, root residues from a green manure of hairy vetch (*Vicia villosa*) underwent decomposition under a summer crop of irrigated corn, where it was possible that much of the microbial biomass C was derived from unenriched rhizodeposits originating from the corn plants (Balesdent and Balabane 1996; Liljeroth et al. 1994).

As mentioned in the introduction, interest has been shown in residue/litter decomposition under winter conditions in both agricultural and native ecosystems throughout the Northern Hemisphere. Three observations from our study suggest that C flow from ryegrass residues was affected by winter conditions:

1. A significant increase was observed in the percentage of root-derived ryegrass C in the soil.
2. A significant decline in the contribution of root-derived C to respiration occurred over the winter months.
3. A substantial increase was measured in the contribution of ryegrass straw C to soil microbial biomass C.

It is tempting to speculate that the increase in ryegrass root-derived C in the soil might be the result of higher humification activity in the ryegrass treatment promoted by a combination of the low substrate N in the residual root C pool and low availability of N in the vicinity of the pool. It is well recognized that N limitation promotes expression of lignin-degrading enzymes which are implicated in the humification process (Carreiro et al. 2000; DeForest et al. 2004; Saiya-Cork et al. 2002). Certainly, mineral N was extremely low in the ryegrass treatment throughout the study, whereas in November, a peak of mineral N was observed in the legume treatment which presumably raised N availability for the microbial community. Further work is needed to address the chemical composition of the ryegrass root-derived soil C pool during the winter, its relationship to C processing and humification, and the nature of the microbial community associated with it.

Finally, we need to discuss the link between residue source, N availability, and the contributions of soil to microbial biomass and respiratory C in the ryegrass vs clover treatments between September and November. Our results from the ryegrass treatment superficially resemble recent observations by Waldrop and Firestone (2004), who reported that $^{13}C$ from labeled pine needle residue added to a grassland soil was not only incorporated into microbial biomass but also stimulated respiration of SOM-C and its incorporation into microbial phospholipid fatty acids. Processing of SOM-C was much less extensive when the same residue was added to a more fertile soil recovered from beneath oak trees immediately adjacent to the grassy areas. Although C input-induced mineralization of SOM has often been attributed to low N availability, the subject is extremely complex and controversial (Bottner et al. 1999; Cheng and Coleman 1990; Dormaar 1990; Fontaine et al. 2003; Kuzyakov et al. 2001; Li and Yagi 2004; Liljeroth et al. 1994; Loya et al. 2004; Van Ginkel and Gorissen 1998). Unfortunately, we have no other explanation for why the ryegrass residue treatment should promote both a transient assimilation of soil C into microbial biomass and sustained respiration of soil C. The data serve to emphasize the need for more studies to analyze the interactions between root residue C, soil C, and microbial communities at finer temporal and spatial scales.

## Conclusion

We observed that residue type influenced the flow of C within microbial, respiratory, and soil pools. In particular, the results confirmed the importance of rhizodeposition as a major source of microbially available substrate that represented about 12% of the late summer soil C pool. Furthermore, we substantiated that root residue type was an important factor influencing the accrual of C in soil over 1-year. Although the exact nature of the factors responsible for C retention remains somewhat elusive, our results will guide future studies that dissect the temporal/substrate quality/microbial biomass interactions most responsible for controlling the processing and accumulation of residue C in soil.

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