

# Isotopic analysis of respired CO<sub>2</sub> during decomposition of separated soil organic matter pools

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Available online 8 May 2006

## Abstract

A detailed understanding of the processes that contribute to the  $\delta^{13}\text{C}$  value of respired CO<sub>2</sub> is necessary to make links between the isotopic signature of CO<sub>2</sub> efflux from the soil surface and various sources within the soil profile. We used density fractionation to divide soils from two forested sites that are a part of an ongoing detrital manipulation experiment (the Detrital Input and Removal Treatments, or DIRT project) into two soil organic matter pools, each of which contributes differently to total soil CO<sub>2</sub> efflux. In both sites, distinct biological pools resulted from density fractionation; however, our results do not always support the concept that the light fraction is readily decomposable whereas the heavy fraction is recalcitrant. In a laboratory incubation following density fractionation we found that cumulative respiration over the course of the incubation period was greater from the light fraction than from the heavy fraction for the deciduous site, while the opposite was true for the coniferous site.

Use of stable isotopes yielded insight as to the nature of the density fractions, with the heavy fraction solids from both forests isotopically enriched relative to those of the light fraction. The isotopic signature of respired CO<sub>2</sub>, however, was more complicated. During incubation of the fractions there was an initial isotopic depletion of the respired CO<sub>2</sub> compared to the substrate for both soil fractions from both forests. Over time for both fractions of both soils the respired  $\delta^{13}\text{C}$  reflected more closely the initial substrate value; however, the transition from depleted to enriched respiration relative to substrate occurs at a different stage of decomposition depending on site and substrate recalcitrance. We found a relationship between cumulative respiration during the incubation period and the duration of the transition from isotopically depleted to enriched respiration in the coniferous site but not the deciduous site. Our results suggest that a shift in microbial community or to dead microbial biomass as a substrate could be responsible for the transition in the isotopic signature of respired CO<sub>2</sub> during decomposition. It is likely that a combination of organic matter quality and isotopic discrimination by microbes, in addition to differences in microbial community composition, contribute to the isotopic signature of different organic matter fractions. It is apparent that respired  $\delta^{13}\text{C}$  CO<sub>2</sub> cannot be assumed to be a direct representation of the substrate  $\delta^{13}\text{C}$ . Detailed knowledge of the soil characteristics at a particular site is necessary to interpret relationships between the isotopic values of a substrate and respired CO<sub>2</sub>.

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**Keywords:** Alfisol; Andisol; Coniferous forest; Deciduous forest;  $\delta^{13}\text{C}$ ; Density separation; Isotopic fractionation; Organic matter; Soil respiration

## 1. Introduction

Soil organic matter (SOM) is a complex mixture of material from various sources that exists along a continuum of decomposition and stabilization in the soil profile. For simplicity and for modeling purposes, soil organic matter often is divided into several pools with different turnover times and recalcitrance. Each pool

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contributes to total efflux in different proportions depending on availability as a substrate for microbial decomposition (Parton et al., 1987; Coleman and Jenkinson, 1996; Trumbore, 1997). Many approaches have been taken to physically or chemically separate these pools in the laboratory (e.g., Strickland and Sollins, 1987; Six et al., 2000, 2001; Swanston et al., 2004). Analyses of the carbon isotopic composition of SOM pools have yielded insight into turnover rates and microbial processing (e.g., Balesdent and Mariotti, 1987; Buchmann et al., 1998; Six et al., 2001) and some progress has been made to use  $\delta^{13}\text{C}$  values of respired  $\text{CO}_2$  to identify source pools for  $\text{CO}_2$  efflux from the soil surface (Ehleringer et al., 2000). Often, these studies are in ecosystems where a shift between  $\text{C}_3$  and  $\text{C}_4$  vegetation has significantly altered the isotopic signatures of the C inputs to SOM pools. Consequent shifts of C isotopic inputs and accompanying changes in the isotopic signatures of SOM pools in these systems can help identify the sources of respiratory  $\text{CO}_2$  (e.g., Rochette and Flanagan, 1997). There is greater difficulty determining the source of respired  $\text{CO}_2$ , however, when only small variations in isotopic signatures of inputs exist, or when there is little difference in the isotopic composition between inputs and SOM pools. For these systems in particular, we need a more precise understanding of processes that control the C isotopic signature during respiration to make links between the isotopic composition of respired  $\text{CO}_2$  and its source in the soil.

The differences between the  $\delta^{13}\text{C}$  value of vegetation biomass, SOM, and respired  $\text{CO}_2$  have already been used to gain insight into biological processes that mediate C transfers among ecosystem pools (e.g., Nadelhoffer and Fry, 1988; Šantrůčková et al., 2000b; Niklaus et al., 2001). Plant litter generally has lower  $\delta^{13}\text{C}$  values than bulk soil and serves as continuous inputs into SOM in the form of both above and below ground sources (Accoe et al., 2003; Bird et al., 2003). Individual molecular components of these inputs have highly variable isotopic signatures; for example, lignin is depleted in  $^{13}\text{C}$  content by 2–6% compared to the bulk plant material and by 4–7% relative to cellulose (Benner et al., 1987), and wood cellulose is 2% enriched compared to leaf cellulose (Gleixner et al., 1993). Invertebrates have been shown to excrete frass with lower  $\delta^{13}\text{C}$  values compared to food (Šantrůčková et al., 2000a). Recent studies suggest that soil microorganisms may alter the isotopic composition of SOM during decomposition through mechanisms such as metabolic discrimination (Schmidt and Gleixner, 1998; Šantrůčková et al., 2000b), selective consumption of substrates (Macko and Estep, 1984), or preferential use of intramolecular position within substrates (Schweizer et al., 1999; Hobbie and Werner, 2004). In general, processes that control the isotopic signature of  $\text{CO}_2$  during decomposition and efflux from the soil back to the atmosphere are not well understood; indeed, whether isotopic fractionation during decomposition even occurs is currently under debate (Lin and Ehleringer, 1997; Henn and Chapela, 2000;

Šantrůčková et al., 2000b; Fernandez et al., 2003; Klumpp et al., 2005).

As decomposition of fresh plant litter progresses and the decomposition products become incorporated into the soil profile,  $\delta^{13}\text{C}$  content has been observed to increase (Buchmann et al., 1998). Multiple theories have been proposed to explain the observed trend of  $\delta^{13}\text{C}$  with depth (Ehleringer et al., 2000), including changes in the atmospheric  $\delta^{13}\text{CO}_2$  value since the Industrial Revolution, preferential feeding by microbes on isotopically light material, and metabolic fractionation during decomposition, among others. Boutton (1991) suggested that deeper SOM is older, and thus presumably more resistant to further decomposition than is surficial SOM. However, it is not clear that the observed pattern of increasing  $\delta^{13}\text{C}$  value with depth necessarily means that more labile SOM is less  $^{13}\text{C}$  enriched. In addition to incomplete understanding of processes that contribute to isotopic fractionation, we also know little about differences in the degree of fractionation during decomposition from SOM pools of different ecosystems (Ehleringer et al., 2000).

Numerous authors have used density fractions of SOM to represent different pools of SOM that might turnover at different rates (cf., Strickland and Sollins, 1987; Trumbore, 1997; Six et al., 2001; Baisden et al., 2002; Swanston et al., 2002). Light fraction material (LF,  $< 1.6 \text{ g cm}^{-3}$ ) is composed of partially decomposed litter debris, charcoal, and humus. Heavy fraction material (HF,  $> 1.6 \text{ g cm}^{-3}$ ) consists of mineral clays and organic material in close chemical association with mineral surfaces. Heavy fraction material typically has a lower C:N than light fraction material and is thought to contain soil organic C that is more processed and stabilized (i.e., resistant to further decay). Heavy fraction SOM is generally found to be  $^{13}\text{C}$ -enriched compared to the light fraction (Ehleringer et al., 2000; Six et al., 2001; Fernandez et al., 2003). The purpose of this study was two-fold: (1) to follow the dynamics of respired  $\delta^{13}\text{CO}_2$  during SOM decomposition and (2) to determine whether, in a soil incubation system where root contributions to soil respiration are removed from heterotrophic decomposition of SOM pools, respired  $\delta^{13}\text{CO}_2$  is a reflection of substrate  $\delta^{13}\text{C}$  in two very different forests. We expected greater cumulative respiration to occur during incubation of light fraction material than from heavy fraction material and that the isotopic signature of  $\text{CO}_2$  respired during decomposition of the light and heavy fractions would be distinct from each other and reflective of the isotopic signature of the source material of different recalcitrance for both forest soils.

## 2. Materials and methods

Soil from the 0–5 cm layer mineral A horizon was collected from two long-term experimental field sites at the H. J. Andrews Experimental Forest in the Cascade range of western Oregon in June 2002 (coniferous site) and at the Allegheny College Bousson Experimental Forest in western

Table 1  
Site descriptions

	Bousson, PA (Deciduous site)	H.J. Andrews, OR (Coniferous site)
Dominant tree spp.	Black cherry ( <i>Prunus serotina</i> ), sugar maple ( <i>Acer saccharum</i> ) <sup>a</sup>	Douglas-fir ( <i>Pseudotsuga menziesii</i> ), western hemlock ( <i>Tsuga heterophylla</i> ) <sup>b</sup>
Approximate stand age (year)	80	500
Soil order	Alfisol	Andisol
Mean annual temperature (°C)	8.3	8.8 <sup>c</sup>
Mean annual rainfall (cm)	105 <sup>a</sup>	220 <sup>c</sup>
Litterfall C (kg ha <sup>-1</sup> year <sup>-1</sup> )	2110 <sup>d</sup>	4330 <sup>c</sup>
Litterfall N (kg ha <sup>-1</sup> year <sup>-1</sup> )	31.1 <sup>d</sup>	17.5 <sup>c</sup>
Mineral soil C:N (0–5 cm)	13 <sup>c</sup>	35 <sup>f</sup>
N deposition (g N m <sup>-2</sup> year <sup>-1</sup> )	10–12	0.2 <sup>g</sup>
Mineralogy	Illite and vermiculite <sup>h</sup>	Plagioclase feldspar, quartz, smectite <sup>b</sup>

<sup>a</sup>Bowden et al. (2000).

<sup>b</sup>Sollins et al. (2006).

<sup>c</sup>Sulzman et al. (2005).

<sup>d</sup>Bowden et al. (1993).

<sup>e</sup>Unpublished data.

<sup>f</sup>Lajtha et al. (2005).

<sup>g</sup>Vanderbilt et al. (2003).

<sup>h</sup>USDA-SCS (1979).

Pennsylvania in June 2003 (deciduous site). These sites have many contrasting characteristics (e.g., vegetation type, soil mineralogy, mineral soil C:N) (Table 1) and are part of a larger on-going study to evaluate the long-term effects of changing detrital litter inputs on the accumulation and stabilization of carbon in soil (the Detrital Input and Removal Treatments or DIRT project; see Nadelhoffer et al., 2004 for a full description of the experimental design). The coniferous site is located in an old-growth Douglas-fir and western hemlock stand established approximately 500 years ago following a stand-replacing fire. The soils are andic; and coarse woody debris and a moss layer cover extensive areas of the forest floor. The climate is Mediterranean with dry summers and a wet season from October to May in which 70% of precipitation occurs (Sollins et al., 1980). The deciduous site is located in a nutrient-rich, mixed stand dominated by black cherry and sugar maple in the canopy and by small maple saplings in the understory. Extensive ground cover of maple seedlings, mayapple (*Podophyllum*) and troutlily (*Erythronium*) is present. The mixed deciduous stand is approximately 80 years old and grows on Alfisols with a fragipan present at 60 cm. The climate is temperate with a 4 month growing season and 4 months of snow cover. In this study we focused on soils from three DIRT treatments: Double Litter, in which needle or leaf input rates are doubled annually; No Inputs, which excludes both aboveground litter (via screening and sweeping) and belowground root litter (via trenching); and Control. The plots have been maintained at the coniferous site since 1997 and at the deciduous site since 1991; each detrital treatment has been applied to three replicate plots at each site which are 10 × 15 m and 3 × 3 m, respectively.

Mineral soil was collected from 6 sub-samples within each plot, then composited and mixed (one composite sample per plot). Samples were collected in June 2002 at the coniferous site and June 2003 at the deciduous site. Following collection, soils were stored at field moisture content at 4°C for up to 2 months prior to the start of the experiment while the density fractionation procedure was performed. Soil was separated into two pools by dispersion in a high-density sodium polytungstate (SPT) solution (1.6 g mL<sup>-1</sup>), in which the light fraction is collected after floating and the heavy fraction is collected as sediment (Strickland and Sollins, 1987). Following fractionation, the light and heavy fractions were air-dried and stored for an additional 1–2 months at room temperature until the incubation experiment began.

Approximately 0.5 g of light fraction material and 2 g of heavy fraction material were weighed and mixed with 3 g of acid-washed quartz sand to increase aeration during the incubation. The soil and sand mixture was transferred into 12-mL exetainer vials (Labco Ltd., UK), which were prepared with 1 cm<sup>3</sup> of glass wool in the bottom to keep the soil from becoming anaerobic; the sample occupied roughly half the volume of each vial. A microbial inoculum was prepared with fresh soil from each field site by shaking 10 g of soil with 100 mL distilled water for 1 h and filtering with Whatman GF/F filter paper. The filtrate was used to re-wet the soil to 25% water content on a mass basis. To ensure thorough wetting, the volume of inoculum was measured using a micro-syringe with a long needle that was inserted down into the soil in the exetainer. Starting from the bottom of the soil, the syringe plunger was slowly depressed and the needle raised so that the moisture would be mixed evenly with the soil. Care was taken so that no

soil was removed with the needle or otherwise. Once the soils were moist, lids were loosely placed over the top to allow gas exchange while minimizing evaporative water loss. Distilled water was added approximately every 3 days to maintain near-constant moisture content.

On days 1, 3, 5, 8, 10, 12, 17, 30, and 65 since inoculation, lids (with septa) were sealed tightly and loaded onto a Combi-PAL, auto-sampler attached to a Finnigan Gas Bench II coupled to a Delta Plus XL Continuous Flow Mass Spectrometer. The gas bench used a continuous flow of helium to flush headspace gas from each vial through a 100  $\mu$ L injection loop. The injection loop was alternately loaded and cycled 8 times through a Valco 8 port valve onto a 30 m long GC column, where CO<sub>2</sub> separated from the other soil gasses. The CO<sub>2</sub> then flowed into the mass spectrometer, where masses 44, 45 and 46 were collected simultaneously, and the ratios for <sup>13</sup>/<sup>12</sup>C in parts per thousand (‰) deviations from the defined international V-PDB standard were measured. External precision of the system for  $\delta^{13}\text{C}$  was  $\pm 0.06\text{‰}$  at 300.6 ppm and  $\pm 0.02\text{‰}$  at 9990 ppm.

The area under the mass 44 peak was used to calculate the concentration of CO<sub>2</sub>. A tank of compressed air was measured at 300.6 ppm CO<sub>2</sub> with a LICOR Infra-Red Gas Analyzer compared to a NOAA-calibrated CO<sub>2</sub> standard. This tank was used to calibrate the low end of the concentration calibration and a 0.99‰ (9990 ppm) tank of CO<sub>2</sub> in He was used to calibrate the high end.

In addition to the soil headspace samples, a set of exetainers with working standards (9990 ppm CO<sub>2</sub> in helium, and 300.6 ppm CO<sub>2</sub> in compressed air) was analyzed with each run for quality control. Vials were purged with CO<sub>2</sub>-free air and left sealed for 4 h while CO<sub>2</sub>

accumulated in the headspace. Laboratory tests revealed that the mass spectrometer output was linear in  $\delta^{13}\text{C}$  to  $\pm 0.06\text{‰}$  in the range 0.6–6.5 V (in which the vast majority of our samples were analyzed) and to  $\pm 0.3\text{‰}$  in the range 0.3–6.5 V (300–9990 ppm) (Fig. 1). Headspace samples that contained CO<sub>2</sub> concentrations outside this range were removed from the data set.

Carbon contents of litter material, soil, and density fractions were determined by dry micro-Dumas combustion (NA1500 C/H/N Analyzer, Carlo Erba Strumentazione, Milan) at the Stable Isotope/Soil Biology Laboratory of the University of Georgia Institute of Ecology. The  $\delta^{13}\text{C}$  of these solid samples was measured on a Finnigan MAT Delta Plus XL (Bremen, Germany) at the EPA Western Ecology Division's Integrated Stable Isotope Research Facility ( $\delta^{13}\text{C}$  precision =  $\pm 0.04\text{‰}$ ). Some samples were analyzed at the Stable Isotope Lab at the College of Oceanic and Atmospheric Science at Oregon State University using a Carlo Erba continuous flow inlet with a Finnigan MAT Delta Plus XL. Calibration at this facility was to NIST-8542 (Sucrose), and NIST 8541 (Graphite); external precision =  $\pm 0.06\text{‰}$ .

Carbon isotopic discrimination between respired CO<sub>2</sub> and the initial substrate ( $\Delta^{13}\text{C}_{(s/r)}$ ) was calculated using the following equation:

$$\Delta^{13}\text{C}_{(s/r)} = (\alpha_{s/r} - 1) = (\delta_s - \delta_r)/(1 + \delta_r), \quad (1)$$

where  $\alpha_{s/r}$  is  $R_s/R_r$ , and  $R_s$  is the <sup>13</sup>C/<sup>12</sup>C molar ratio of the substrate (s) and  $R_r$  is that of the respiration. All  $\delta$  values are expressed as absolute values; the total  $\Delta$  is multiplied by 1000 for expression in units per mil (‰). If the fractionation factor,  $\Delta$ , is positive, then the respired C is more depleted in <sup>13</sup>C than the substrate.

Comparisons between means of the detrital treatments and density fractions were made by repeated measures 2-way ANOVA in SAS v 9.2 (SAS Inc, Cary, NC) using PROC MIXED. Density fractions from each plot originated from the same bulk soil samples. Detrital treatments were classed as main plots, with density fractions treated as repeated measures (subplots). Since the deciduous and coniferous forest sites have very different characteristics, including having been experimentally manipulated for different amounts of time, no direct comparisons between the sites were made. Tukey–Kramer a priori adjustments were made for pair-wise comparisons so that experiment wise  $\alpha = 0.05$ . A comparison of pre-incubation and post-incubation soil fractions from the two sites was made with a paired Student's *t*-test. Linear regression was used to determine the nature of the relationship between cumulative respiration and isotopic zero point (SigmaPlot v 8.0).

### 3. Results

Respiration rates peaked for all soil fractions on either day 5 or 8 of the incubation and decreased in subsequent weeks. Cumulative C loss via respiration continued to increase during the 65-day incubation indicating a remaining

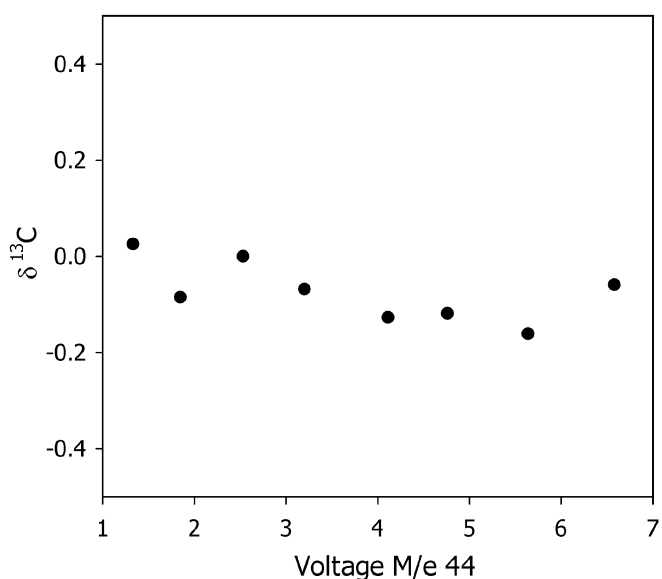


Fig. 1. Relationship of voltage to isotope ratio across the range of voltages attained during the study.  $\Delta^{13}\text{C}$  of the standard was evaluated relative to the 2.53 V level which was used for the reference gas calibration to V-PDB. Standard deviation for  $\Delta^{13}\text{C}$  across this range was  $\pm 0.06$  (‰).



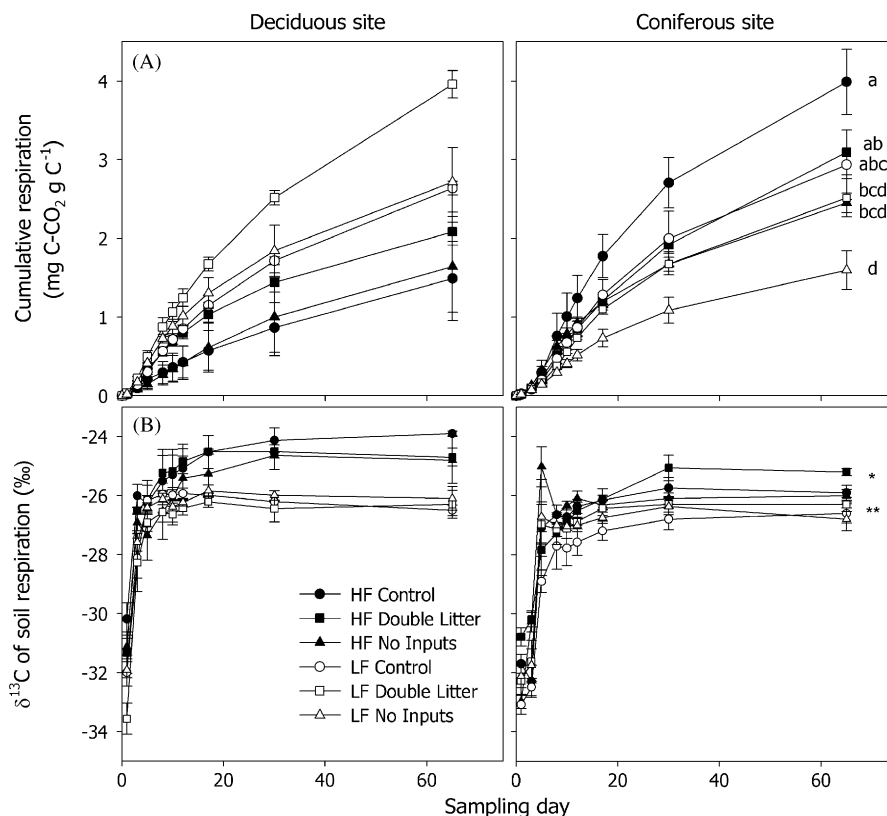


Fig. 2. (A) Cumulative  $\text{CO}_2$  efflux from incubated soil density fractions from the deciduous and coniferous DIRT sites. (B)  $\delta^{13}\text{C}$  value of respiration from incubated soil density fractions from the deciduous and coniferous sites. All values are mean  $\pm 1$  standard error,  $n = 3$ , means for values at day 65 of the incubation with different letters are significantly different. While there was no significant effect of detrital treatment on  $\delta^{13}\text{C}$   $\text{CO}_2$  values at either site, stars indicate significant differences between the density fractions ( $n = 9$ ).

supply of degradable organic substrate (Fig. 2A). Cumulative respiration from the density fractions showed the light fraction exhibited greater respiration ( $\text{mg C-CO}_2 \text{ g C initial}^{-1}$ ) when compared to its heavy fraction counterpart at the deciduous site ( $p < 0.001$ ,  $F = 51.94$ ). The opposite was true for soils of the coniferous site; respiration from heavy fraction samples was higher than all light fraction samples of the same detrital treatment ( $p < 0.001$ ,  $F = 96.90$ ). Cumulative respiration from the detrital treatments showed the greatest cumulative respiration occurred in the Control plots at the coniferous site ( $p = 0.042$ ,  $F = 5.66$ ). There was not a significant effect of detrital treatment on respiration at the deciduous site ( $p = 0.191$ ,  $F = 2.21$ ); however, for both forest types the No Inputs treatment substantially reduced 65-d cumulative respiration from both density fractions, although the reduction was greater in soils from the coniferous site. The patterns observed for light and heavy fraction respiration, as well as the detrital treatments, are consistent with those from a related 1-year incubation experiment of the same soils and density fractions where greater amounts of substrate ( $\sim 6$  g light fraction and  $\sim 25$  g heavy fraction) were incubated in chambers of greater headspace volume ( $\sim 450$  mL) than during this experiment (Crow, unpublished data). On day 64 of the 1-year

incubation of the density fractions from the deciduous soil, cumulative respiration ( $\text{mg C-CO}_2 \text{ g C initial}^{-1}$ ) from the light fraction was  $3.3 \pm 0.3$ ,  $3.9 \pm 0.4$ , and  $2.8 \pm 0.2$  from the Control, Double Litter, and No Inputs treatments respectively; heavy fraction cumulative respiration was  $0.5 \pm 0.1$ ,  $0.5 \pm 0.0$ , and  $0.4 \pm 0.0$  for the same treatments. On day 70 of the 1-year incubation of the density fractions from the coniferous soil, cumulative respiration ( $\text{mg C-CO}_2 \text{ g C initial}^{-1}$ ) from the light fraction was  $10.0 \pm 0.7$ ,  $10.6 \pm 0.6$ , and  $8.4 \pm 0.2$  from the Control, Double Litter, and No Inputs treatments respectively; heavy fraction cumulative respiration was  $12.0 \pm 2.0$ ,  $11.8 \pm 2.9$ , and  $9.5 \pm 1.1$  for the same treatments. At the end of the 1 year period, the relationship between the treatments and fractions remained generally consistent with that at days 64 and 70 for the two soils.

Isotopic composition of litter inputs varied over a wide range of  $\delta^{13}\text{C}$  values, particularly at the coniferous site; however, the detrital treatments have not yet altered the isotopic signatures of either density fraction. Two common moss species and the most common epiphytic lichen species (*Lobaria oregano*) at the coniferous site were substantially depleted in  $\delta^{13}\text{C}$  compared to the other litter inputs (Table 2), while a second common lichen species at this site, *Platismatia glauca*, was  $^{13}\text{C}$ -enriched. Other above and

Table 2  
 $\delta^{13}\text{C}$  values (‰) for dominant inputs to the forest floor and mineral soil at each study site

<i>H. J. Andrews, Coniferous site</i>	
Lichen sp. ( <i>Lobaria oregano</i> )	–33.1
Lichen sp. ( <i>Platismatia glauca</i> )	–22.9
Moss sp. ( <i>Euryhynchium oregonum</i> )	–30.8
Moss sp. ( <i>Isoetecium myosuroides</i> )	–31.4
Needles (mixed western hemlock/Doug-fir)	–26.6
Branches (mixed western hemlock/Doug-fir)	–26.4
Fine roots (<2 mm)	–27.4
<i>Bousson, Deciduous site</i>	
Senescent foliage (mixed black cherry/sugar maple)	–29.6
Branches (deciduous)	–27.2
Fine roots (<2 mm)	–26.9

Each value is the mean of analytical duplicates.

belowground detrital inputs at both sites were consistent with  $\delta^{13}\text{C}$  values typical for fresh  $\text{C}_3$  plant material. Senescent leaves of black cherry and sugar maple, dominant species at the deciduous site, were isotopically depleted relative to branches and roots (Table 2). Five years of detrital manipulation at the coniferous site and 13 years at the deciduous site have not significantly affected  $\delta^{13}\text{C}$  values of the density fractions. Unexpectedly, although there have been no fresh, isotopically depleted inputs for 13 years to the No Inputs plots at the deciduous site, light fraction from these plots had the most depleted  $\delta^{13}\text{C}$ . At both sites, all heavy fraction samples were more enriched than the light fraction samples. This enrichment was present to a greater degree at the deciduous site than at the coniferous site.

A comparison of  $\delta^{13}\text{C}$  of the substrate from the two sites before and after the incubation revealed that the heavy fraction samples from the deciduous site became  $^{13}\text{C}$  depleted ( $p = 0.014$ ) and the light fraction samples from the coniferous site became  $^{13}\text{C}$  enriched ( $p = 0.022$ ) compared to their starting values over the course of the incubation (Table 3). A comparison of the isotopic signature of the substrate and respiration ( $\Delta^{13}\text{C}_{(s/r)}$ , Eq. 1) at 2 points in time revealed that isotopic discrimination was greater for light fraction samples than for heavy fraction samples on both day 1 and 65 of the incubation. This disparity between discrimination of the density fractions was greatest at the coniferous site, where initially there was hardly any difference between heavy and light substrate  $\delta^{13}\text{C}$  values. Discrimination at day 1 was much greater than at day 65. At both sites, respiration from the heavy fraction was enriched relative to the substrate while the respiration from the light fraction was isotopically indistinguishable from the substrate or still depleted on day 65.

By day 65, there was a significant enrichment of  $\delta^{13}\text{CO}_2$  in the heavy versus light fraction samples at both sites ( $p < 0.001$  at the deciduous site,  $p = 0.014$  at the coniferous site) (Fig. 2B). Detrital treatments have not yet affected the isotopic signature of  $\text{CO}_2$  respired from the density cuts at

either site (deciduous:  $p = 0.685$ ,  $F = 0.40$ ; coniferous:  $p = 0.3514$ ,  $F = 0.40$ ). Regardless of site, density fraction, or initial substrate  $\delta^{13}\text{C}$ , the first  $\text{CO}_2$  respired was more isotopically depleted than the starting substrate (Fig. 3). However, this trend was reversed in all samples within the first 25 days, after which the respired  $\text{CO}_2$  was more  $^{13}\text{C}$ -enriched than the initial substrate. The isotopic signatures of respired  $\text{CO}_2$  from all samples stabilized in the second half of the incubation period.

#### 4. Discussion

Due to more fresh plant material and low mineral interactions of the organic matter in the light fraction, we expected these substrates to exhibit greater cumulative respiration than the heavy fraction. Similarly, we expected soils from the input-addition treatment (Double Litter) to have the greatest cumulative respiration, as they have received additional inputs of the most labile organic matter (fresh litter) compared to soils from control or input removal plots. The soils from the deciduous site did in fact show increased respiration in response to the Double Litter treatment while those from the coniferous site did not (Fig. 2A). The deciduous site is characterized by a lower soil C:N than the coniferous site (Table 1), which has a large amount of background C from the legacy of coarse woody debris inputs to the forest floor, and productivity and microbial activity is not generally limited by nitrogen (Bowden et al., 2000). Doubling leaf litter at the deciduous site adds fresh, labile carbon that may contribute to an increase in the rate of decomposition from the light fraction organic matter pool. We found no increase in respiration in response to the Double Litter treatment in the heavy fraction, even though the influence was strong in the light fraction at the deciduous site. A lack of similar influence of doubling litter inputs on the heavy fraction may be due to low interaction between fresh organic inputs and mineral surfaces, rapid decomposition of litter and little or no movement into deeper soil horizons, or to a very strong chemical recalcitrance of the C entering the mineral fraction possibly as a result of chronic high N deposition in the region (Fog, 1988; Bowden et al., 2000; Swanston et al., 2004). Possibly, over a longer period of time, DOC transported to deeper soil will accumulate enough to be detectable against the background soil C. Studies have suggested that decomposed litter already present as O-horizon material, as opposed to fresh litter, is the primary source of DOC leached into the A-horizon (Froberg et al., 2003; Yano et al., 2005). In this case, there will be a lag time between litter manipulation and resulting changes in organic matter pool size and dynamics (Yano et al., 2005) and the length of the lag time will be dependant on site characteristics which influence decomposition rates as well as the sorption capacity of a particular soil.

Density fractions from the coniferous site yielded different patterns of respiration during incubation than those observed at the deciduous site (Fig. 2A, B).

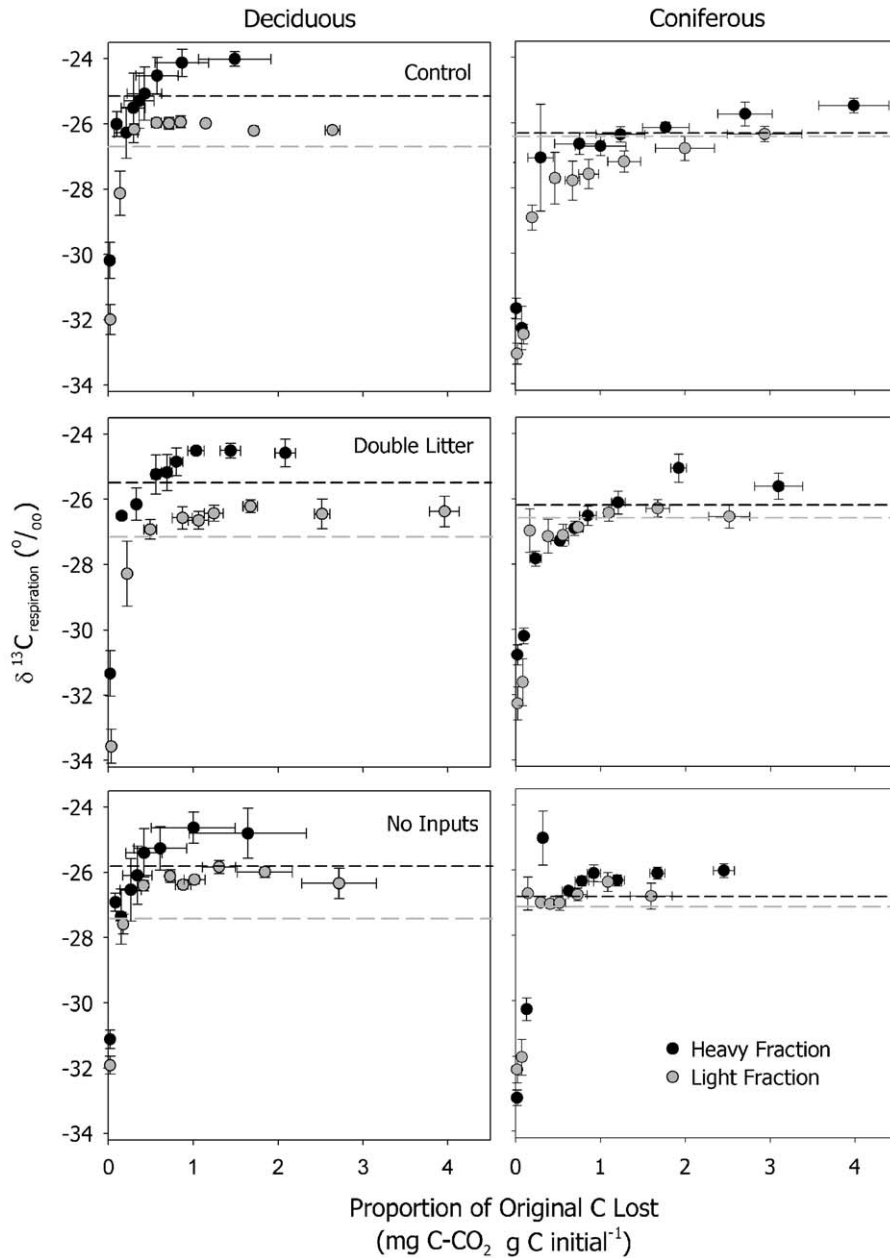


Fig. 3.  $\delta^{13}\text{C}$  CO<sub>2</sub> signature during the incubation period as a function of the amount of original C loss (mg C-CO<sub>2</sub> g C initial<sup>-1</sup>). Initial isotopic signature of the substrate is referenced for both light fraction (gray dotted line) and heavy fraction (black dotted line). Values are means  $\pm$  1 standard error along both axes.

Incubation of the light fraction at the coniferous site resulted in lower rates of respiration than expected. Soil at this site is characterized by a legacy of woody debris, charcoal inputs from periodic fires, and low atmospheric N deposition, conditions that have contributed to high soil C:N and potential N-limitation of microbial activity (Myrold et al., 1989). The low degradability of these components contributes to the chemical recalcitrance of this fraction, which likely resulted in low cumulative respiration. Conversely, incubation of the heavy fraction at the coniferous site produced more respired CO<sub>2</sub> than expected, possibly due to a rapid-turnover pool within the

heavy fraction that may have been made freshly-available for microbial degradation during the density fractionation procedure. Swanston et al. (2002) incubated light and heavy fractions of several soils from western Oregon and Washington, and also found no difference in the cumulative respiration at of density fractions at 60 days. Yano et al. (2005) found root litter to be the dominant producer of dissolved organic matter at this site. The removal of root inputs, both as fine root litter and root exudation, caused a substantial decrease in respiration in the No Inputs treatment for both light and heavy fractions even after only 5 years of manipulation.

Soils at the coniferous site derive from volcanic parent material and have strong andic properties, such as high amorphous Al hydroxide and aluminosilicate content (oxalate-extractable Al = 1.1%) and pH in 1 M NaF (10.7) (Yano et al., 2004). Andic soil characteristics likely contribute to high sorption of fresh inputs to the mineral fraction at the coniferous site and help account for the apparent differences in the recalcitrant nature of the heavy fraction C between sites. In addition, clay content of the mineral soil is lower at the deciduous (2%) than the coniferous site (13%) (Bowden et al., 2000; Dixon, 2003) giving less surface area for sorption to occur. Lajtha et al. (2005) demonstrated the potential for sorption of fresh DOC inputs to mineral surfaces at the coniferous site as a mechanism for long-term stabilization of soil organic C. Our data supported those findings: the coniferous heavy fraction contained significant amounts of labile C that became available for microbial degradation once soil structure was disrupted during density fractionation. However, in andic soils C stabilized by sorption to mineral surfaces may not ultimately increase SOM storage if the system is disturbed and soil aggregate structure is lost.

Many authors have observed a pattern of  $\delta^{13}\text{C}$  enrichment of SOM with depth in the soil profile (e.g., Nadelhoffer and Fry, 1988; Boutton, 1991; Trumbore et al., 1995; Boutton et al., 1998; Bowling et al., 2002), consistent with the observation that as decomposition of fresh plant litter progresses and the decomposition products become incorporated into the soil profile,  $^{13}\text{C}$  content increases (Buchmann et al., 1997; Buchmann et al.,

1998). Thus, we expected heavy fraction and samples from No Inputs plots to be isotopically enriched relative to light fraction and to samples from the Double Litter treatment. Indeed, heavy fraction samples were isotopically enriched relative to light fraction samples at both sites; however, the  $\delta^{13}\text{C}$  values of the density fractions are more similar to each other at the coniferous site than at the deciduous site (Table 2 and Fig. 3, dashed lines). The similarity of isotopic values for the density fractions at the coniferous site could be further evidence of the close association between inputs and mineral soil through sorption of dissolved organic matter. No change occurred yet in the isotopic value from the input removal treatments compared to control at either site despite the fact that the treatments have been in place for 5 and 13 years for the coniferous and deciduous sites respectively (Table 3). A large background C content, particularly at the coniferous site, may mask any subtle changes in isotopic signature resulting from our experimental treatments (see also Table 4).

All of our substrates lost between 0.14% and 0.40% of the initial C content through respiration as a result of a combination of mineralogy, nutrient status, and SOM quality. Regardless of these characteristics or the initial  $\delta^{13}\text{C}$  of the substrate, the  $\delta^{13}\text{C}$   $\text{CO}_2$  of every sample shifted from depleted  $^{13}\text{C}_{(s/r)}$  to enriched within the first 30 days of incubation (Figs. 2B and 3). Many similar studies with incubations of chemically separated SOM fractions and plant material have also documented the same shift in respiratory  $^{13}\text{CO}_2$  from depleted to enriched relative to the initial substrate (e.g., Mary et al., 1992; Schweizer et al.,

Table 3  
 $\delta^{13}\text{C}$  values of soil fractions on days 1 and 65 of the incubation for the two forested sites

Site	Density	Substrate $\delta^{13}\text{C}$ (‰)		$\Delta^{13}\text{C}_{(s/r)}$ (‰)	
		Day 1	Day 65	Day 1	Day 65
Deciduous	Light fraction	$-26.8 \pm 0.3$	$-26.2 \pm 0.3$	$5.4 \pm 0.3$	$0.0 \pm 0.3$
	Heavy fraction	$-25.3^* \pm 0.2$	$-26.9^* \pm 0.3$	$5.0 \pm 0.6$	$-3.0 \pm 0.2$
Coniferous	Light fraction	$-26.9^* \pm 0.3$	$-25.3^* \pm 0.1$	$6.4 \pm 0.2$	$1.1 \pm 0.2$
	Heavy fraction	$-26.6 \pm 0.2$	$-27.0 \pm 0.3$	$5.2 \pm 0.5$	$-1.5 \pm 0.1$

$\Delta^{13}\text{C}_{(s/r)}$  values represent discrimination against the heavy isotope in the conversion of substrate to product (see text). Data are for Control plots only,  $n = 3$ ; values are means  $\pm$  one standard error. Substrate  $\delta^{13}\text{C}$  mean values on day 1 and 65 were compared with paired t-tests for each site and density cut, significant differences are indicated with\*.

Table 4  
 $\delta^{13}\text{C}$  values of the initial substrate, mean  $\pm$  one standard error

Site	Density fraction	Double litter	Control	No inputs
Deciduous	Light	$-27.2 \pm 0.1^{\text{cd}}$	$-26.8 \pm 0.3^{\text{bcd}}$	$-27.5 \pm 0.5^{\text{d}}$
	Heavy	$-25.6 \pm 0.2^{\text{ab}}$	$-25.3 \pm 0.2^{\text{a}}$	$-25.8 \pm 0.2^{\text{abc}}$
Coniferous	Light*	$-26.7 \pm 0.2$	$-26.9 \pm 0.3$	$-27.1 \pm 0.1$
	Heavy*	$-26.3 \pm 0.1$	$-26.6 \pm 0.2$	$-26.6 \pm 0.1$

Means were compared within each site for differences due to detrital treatment and density fraction; means that are significantly different are indicated with different letters.

\*No significant differences between detrital treatments were present at the coniferous site; stars indicate a significant difference between the overall mean  $\delta^{13}\text{C}$  values for light and heavy fraction.



1999; Plante and McGill, 2002; Fernandez et al., 2003; Fernandez and Cadisch, 2003). Fernandez et al. (2003) observed that the transition between depleted and enriched  $\delta^{13}\text{C}_{\text{CO}_2}$  respired from various plant materials did not occur either simultaneously or at the same degree of decomposition of the original material. To equalize the transition time to account for differences between the substrates in respiration rates and in amount of total C initially present, a quantification of the transition period was calculated by Fernandez et al. (2003) as a measure of the degree of decomposition that had occurred when  $\Delta^{13}\text{C}_{(s/r)} = 0$  (Eq. (1)). Likewise, we calculated this transition period as the proportion of initial C loss from the density fraction via respiration at the point when  $\Delta^{13}\text{C}_{(s/r)} = 0$  (i.e., where there is no difference between the isotopic composition of the substrate and respired  $\text{CO}_2$ ). This estimate was made for each sample using a non-linear regression (three parameter, exponential decay). Similar to the findings of Fernandez et al. (2003), the proportion of decomposition that had

occurred when  $\Delta^{13}\text{C}_{(s/r)} = 0$  for the density fractions from the coniferous site were variable (Fig. 4). However at the deciduous site the transition period was not different between the light and heavy fraction substrates (Fig. 4).

Fernandez et al. (2003) found a positive correlation between the percentage of decomposition at the transition from depleted to enriched respired  $\text{CO}_2$  and several measures of C quality of the plant litter, including a modeled estimate of labile C and an acid-detergent extractable fraction (sugars, starch, hemicellulose, lipids, proteins and nucleic acids). We also found a relationship between the cumulative loss of C over the course of the entire incubation period (an indication of the overall decomposability of the organic matter present in the substrate) and the proportion of decomposition that had occurred when  $\Delta^{13}\text{C}_{(s/r)} = 0$  for the substrates at the coniferous site. Although the total cumulative C loss during the incubation period varied within the same range for the substrates from both sites, there was no relationship

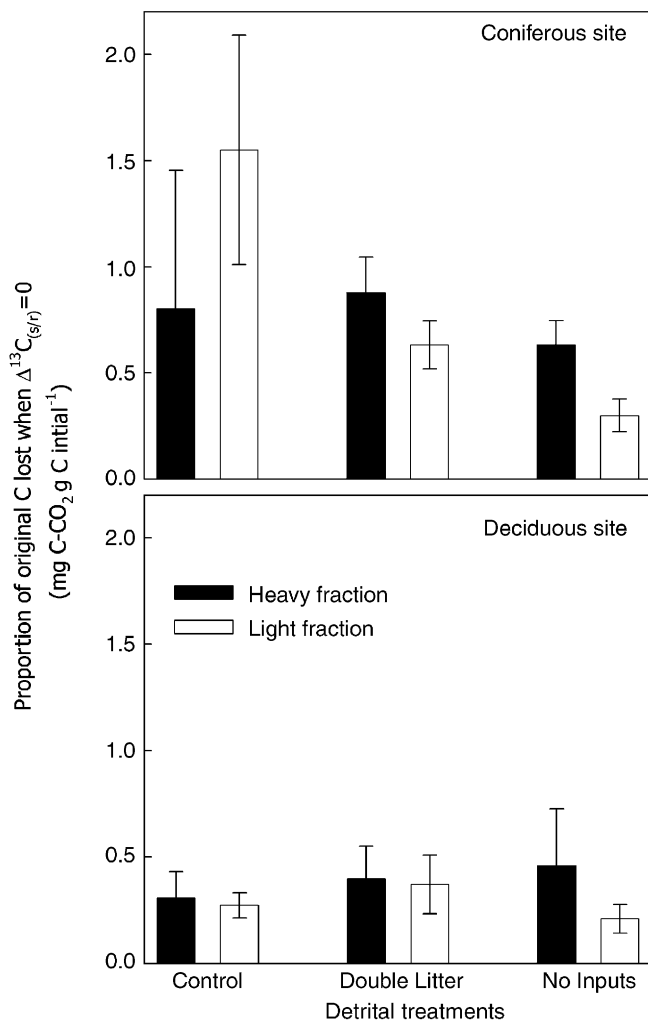


Fig. 4. The transition period between isotopically depleted and enriched respiration, represented as amount of original C loss from the density fractions  $\Delta^{13}\text{C}_{(s/r)} = 0$  for each detrital treatment at both sites (see text for explanation of calculation). Bars are means ( $n = 3$ )  $\pm 1$  standard error.

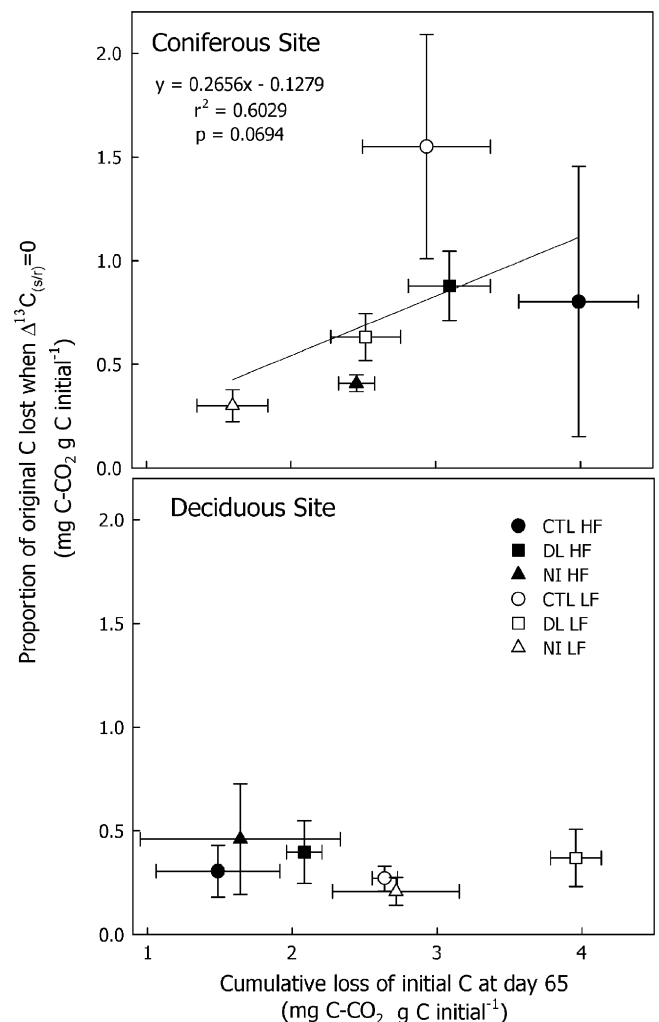


Fig. 5. The relationship between the amount of original C lost when  $\Delta^{13}\text{C}_{(s/r)} = 0$  and the overall lability (measured as cumulative C loss during 65 days of incubation) of the soil fractions at both study sites.

between cumulative C loss and the transition period from depleted to enriched respired CO<sub>2</sub> in the substrates from the deciduous site (Fig. 5).

A possible explanation for the presence of a relationship between measures of lability and the transition from depleted to enriched respired CO<sub>2</sub> is that more labile C pools are isotopically lighter than less labile pools and are utilized first by the microbial community. However, Fernandez et al. (2003) did not find a depleted  $\delta^{13}\text{C}$  in the acid-detergent extractable C pool compared to the whole plant material. This indicates that the initial depletion of respired CO<sub>2</sub> compared to the substrate was not a direct result of isotopic discrimination of chemically defined labile material. Plant storage compounds, including starch and sugars, are typically enriched compared to mean plant biomass (Gleixner et al., 1993), while more recalcitrant structural molecules such as lignin are depleted (Benner et al., 1987). Schweizer et al. (1999) also found no relationship between the isotopically depleted CO<sub>2</sub> phase and the  $\delta^{13}\text{C}$  of several labile C pools (including non-acid-detergent fiber and cellulose), further supporting the idea that labile C pools are not necessarily isotopically depleted and that substrate pool switching from depleted to enriched is not solely driving respired CO<sub>2</sub> isotopic signature during decomposition.

While chemically defined labile C pools may not be isotopically depleted, it is still possible that certain detrital inputs with fast turnover times are isotopically depleted and decomposition of these inputs contribute to the early phase of depleted respiration. An extensive ground cover of bryophytes is present at the coniferous site and epiphytic lichens are ubiquitous in the forest canopy. Thus, inputs of lichens and mosses contribute substantially to above-ground litter inputs at this site. A common lichen species and both common moss species analyzed have isotopic values between  $-33.1$  and  $-31.4\%$  (Table 2) which is substantially more isotopically depleted than either soil density fraction. The epiphytic lichen species *Lobaria* is quickly shredded by herbivores and detritivores once on the forest floor and then decomposed further by small arthropods and microbes. Mean turnover time for *Lobaria* is 7 months (McCune and Daly, 1994). At the deciduous site, senescent leaves also have a low isotopic signature ( $-29.6\%$ ) and have high concentrations of soluble sugars and cellulose (Nadelhoffer et al., 2004), which contribute to a fast turnover rate (50–60% mass loss over 1 year for mixed black cherry and sugar maple foliage) (Bowden, unpublished data). Particularly at the coniferous site, where high annual rainfall constantly carries soluble material from the forest floor to the mineral horizons and properties of the mineral soil promote sorption of dissolved organic matter (as discussed above), decomposition of detrital inputs with fast turnover times and depleted isotopic signature early in the incubation may contribute the early depleted stage of the respiration. However, at both sites, there is a disparity of the isotopic signature between the lichens, mosses, and deciduous leaves and the

respective light fraction material. This isotopic evidence further supports Yano et al. (2005) conclusion that most decomposition of these materials occurs before entering the mineral horizon and may not exert a great influence on A-horizon soil. Therefore, decomposition of these isotopically light inputs is not likely to be wholly responsible for the depleted phase of respiration.

Microbial discrimination against isotopically heavy C molecules in the most labile C pool is yet another possible explanation for the transition from isotopically depleted to enriched respired CO<sub>2</sub>. With more complex substrates made of material with different isotopic signatures all contributing differently to CO<sub>2</sub> efflux, this process is difficult to quantify (Schweizer et al., 1999). However, in simple one-substrate and one-species aerobic incubations, there is evidence for the selective use of isotopically light C molecules from a uniformly labeled glucose substrate (Blair et al., 1985). Species-specific discrimination patterns, while still under debate, may also make the interpretation of these data complicated. Fernandez and Cadisch (2003) found that two species of white rot fungi discriminated against  $^{13}\text{C}$  to different degrees, and that the degree of discrimination changed over the course of a 76-days incubation. In addition, Henn and Chapela (2000) found species-specific isotopic fractionation during sugar uptake that was mediated by the intra-molecular distribution of C atoms and by micro-environmental conditions in three species of basidiomycetes.

As decomposition continues and pools of readily degradable organic matter are depleted, alterations in microbial community can occur. Plante and McGill (2002) found that the labile portion of a particulate organic matter amendment to agricultural soils was decomposed and respired after 2 weeks of incubation. At approximately the same time,  $^{13}\text{CO}_2$  also made the transition from depleted to enriched. Our results from the coniferous site, like those of Fernandez et al. (2003), showed a relationship between substrate C quality and the duration of the transition from depleted to enriched respiration. These patterns suggest that a shift in the microbial community following consumption of easily decomposed C pools occurs, and may contribute to the change from depleted to enriched respiration. As the readily available pool of substrate is depleted, the colonizing microbial community may become the food for subsequent populations; however, our data did not allow us to directly test this hypothesis.

Recent studies have documented consistent enrichment of  $\delta^{13}\text{C}$  values between food sources and fungal biomass (Gleixner et al., 1993; Hobbie et al., 1999; Höglberg et al., 1999; Kohzu et al., 1999). Early research using stable isotopes to study trophic levels demonstrated that heterotrophs are on average 1‰ enriched in  $^{13}\text{C}$  relative to their diet (DeNiro and Epstein, 1977). Enrichment occurs during transport from foliage to roots and during fungal chitin bio-synthesis (Hobbie et al., 1999). Gleixner et al. (1993) proposed that fractionation during glycolysis causes an intramolecular enrichment of the glucose molecules at the C3 and C4 position, contributing to this biomass

enrichment. Saprotrophs are more enriched than mycorrhizae (Hobbie et al., 1999; Högberg et al., 1999) and further enrichment may be due to selective use of isotopically enriched C molecules such as starches, as is common for mycorrhizal species. Kohzu et al. (1999) found enrichment factors of 1.4 for ectomycorrhizal fungi/wood and 3.5 for wood decaying fungal species/wood for 115 species across diverse tropical ecosystems in Japan and Malaysia (Kohzu et al., 1999). In cases where the decomposer population is dominated by fungi, as it is at our coniferous site (Brandt et al., in review *Oecologia*) alteration in the species composition or increased reliance on dead biomass for food would eventually lead to an overall enrichment of respired CO<sub>2</sub> during decomposition.

On average, the transition from isotopically depleted to enriched respiration at the deciduous site ( $0.33 \pm 0.06$  mg C-CO<sub>2</sub> g<sup>-1</sup> C initial,  $n = 18$ ) was shorter than that at the coniferous site ( $0.76 \pm 0.16$  mg C-CO<sub>2</sub> g<sup>-1</sup> C initial,  $n = 18$ ) ( $p = 0.0150$ ), indicating that different mechanisms may have controlled the shift from isotopically depleted to enriched respiration. It is likely that a combination of selective use of isotopically light, readily available organic inputs and a shift in microbial community, including the fast development of a fungal-dominated microbial community, contributed to the initial depletion of respired CO<sub>2</sub> compared to the substrate  $\delta^{13}\text{C}$ . This concept is supported by our results from the density fractions from the coniferous forest showing a relationship between substrate quality and duration of the depleted phase. The variable transition times from depleted to enriched respiration were directly related to the lability of the organic matter present, regardless of the detrital treatment or density fraction, suggesting that shifts in the microbial community occurred as the easily degraded organic matter pools were used. In contrast, the transition between isotopically depleted and enriched respiration came uniformly quickly for all substrates at the deciduous site. The reasons for the difference in pattern between the sites are not easily discernable; however, microbial community composition may have driven differences in respiratory isotopic pattern between the substrates at the coniferous and deciduous sites. Using phospholipid fatty acid analysis to identify relative abundances of functional groups within the microbial communities at the two study sites, Brant et al. (in review *Oecologia*) found distinct communities, with fungi dominant at the coniferous site and Gram negative bacteria dominant at the deciduous forest site. Because the incubated soils were inoculated with soil slurry from each respective site, it also is possible that the mechanisms causing isotopic shifts during decomposition are related to the differences in dominant members and shifts in community composition between the sites.

## 5. Conclusion

Large scale C-balance models recognize that above- and belowground ecosystem components contribute to total

CO<sub>2</sub> efflux and that the relative contributions of these components depend on pool sizes and retention time of C within those pools. The variations in isotope discrimination between substrate and respiration in our sites suggests that detailed knowledge of factors influencing the differences in <sup>13</sup>C signatures of SOM and respired CO<sub>2</sub> are needed to properly constrain large-scale C balances that link terrestrial  $\delta^{13}\text{C}$  values and  $\delta^{13}\text{C}$  CO<sub>2</sub> efflux (Ehleringer et al., 2000). While respired  $\delta^{13}\text{C}$  reflected more closely the initial substrate value by the end of our incubation, it is apparent that respired  $\delta^{13}\text{C}$  should not be assumed to be a direct reflection of the pool  $\delta^{13}\text{C}$ . The relationship between respired  $\delta^{13}\text{C}$  and the  $\delta^{13}\text{C}$  of the substrate is complex, and further knowledge of stable isotope composition of specific compounds or classes of compounds and microbial communities during decomposition is needed to understand processes which together determine the isotopic composition of soil organic matter pools and respired CO<sub>2</sub> from forest soils.

## Acknowledgments

We thank Sarah Beldin, Katie Delaney, Heath Keirstead, Dave Dinette, and Tim Filley for help in the sample preparation and isotopic analysis and Nick Baldauf for collection of Bousson soils. Chris Swanston and Phil Sollins gave helpful comments on early drafts of the manuscript, for this we are very grateful. We also thank Paul Rygiel for first suggesting the microbial connection following an early presentation of the data. Funding was provided by NSF DEB-0087081 and USDA NRICGP 97-35101-4256 awarded to Kate Lajtha and Bruce Caldwell, and by Oregon State University Research funds to Elizabeth Sulzman through the Crop and Soil Science Department.

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