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Carbon dynamics during a long-term incubation of separate and recombined density fractions from seven forest soils

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Abstract

Density fractions in soils differ in their turnover rates, but direct measurement of the C dynamics in the individual density fractions is limited. In 300-day incubations of mineral soils from forests in Washington and Oregon, USA, light fractions (LF), heavy fractions (HF), whole soils (WS), and physically recombined light and heavy fractions (RF), were measured for respiration and shifts in microbial biomass. A combined fraction was calculated from the incubation results of the light and heavy fractions, and called the summed fraction (SF). Carbon concentration followed the pattern: LF > RF > HF. In accordance with this pattern, when cumulative respiration was considered per gram of substrate, the physical fractions exhibited a predictable response: LF > RF > HF. However, when expressed per gram of initial C, the respiration of the LF was not significantly different from that of the HF. These findings suggest the recalcitrance of HF is similar to that of LF and, consequently, differences in their turnover rates in WS may be due to microbial accessibility or physical protection. Whether expressed per gram of substrate or per gram of initial C, the respiration of the SF was not different from that of the WS. Within the SF, the HF was responsible for 35% of the total respiration. Lower respiration in the RF compared with WS and SF might be explained by an antagonistic interaction between the varied microbial communities that degrade LF and HF; in the heterogeneous WS, these communities may be spatially separated to a greater extent than in the laboratory substrate. Unfortunately, the microbial data were highly variable and provided little evidence to either support or refute this idea. The density separation technique appears to be a viable method for isolating different soil organic matter fractions. However, the function of these fractions should be considered more carefully in the context of accessibility and C content.

Keywords: Soil organic matter; Density fractionation; Light fraction; Heavy fraction; C mineralization; Respiration; Incubation

1. Introduction

Conceptual models that seek to describe the dynamics of soil organic C typically distinguish two or more C fractions that have different rates of biochemical and microbial degradation (Christensen, 1992; Cambardella and Elliot, 1993; Golchin et al., 1994b; Killham, 1994; Stevenson, 1994). The rates are a consequence of recalcitrance, accessibility, and interactions (Sollins et al., 1996). Recalcitrance is the inherent molecular-level resistance of a molecule to microbial or enzymatic breakdown. Accessibility is the extent to which the location of substrates controls access by microbes and enzymes. Interactions of organic substrates with organic or inorganic molecules may alter degradation rates of the substrates. In this study we have focused on the organic fractions isolated using the density fractionation technique. A fundamental question regarding this technique has yet to be sufficiently resolved; namely, are observed differences in the turnover of density fractions due primarily to interaction and accessibility or to different levels of recalcitrance?

Density fractionation physically separates soil into low- and high-density fractions, referred to as the light fraction (LF) and heavy fraction (HF) (Greenland and Ford, 1964; Sollins et al., 1999). The common conceptual model for these fractions describes the LF as a plant-like and less stable fraction with high C concentration and low ash concentration (Spycher et al., 1983; Golchin et al., 1994a; Gregorich et al., 1996), and the HF as a more stable, high-density organo-mineral fraction with much lower C concentration (Golchin et al., 1994b; Golchin et al., 1995a,b). The densities most effective at maximizing C concentration and minimizing ash concentration in the LF have typically ranged from

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Table 1
Site characteristics of seven stand management cooperative installations from which soils (0–5 cm depth) were collected

Installation	Location	Great group	Parent material	Textural class	C (g kg^{-1})	N (g kg^{-1})	C/N (g kg^{-1})
Cedar Falls (1)	47.410 N, 121.815 W	Durochrept	Glacial till	Gravelly loam	106.0	7.2	15
Deep Creek (20)	45.962 N, 123.307 W	Haplumbrept	Glacial sediments	Loam	51.0	4.5	11
Skykomish (43)	47.735 N, 121.249 W	Haplorthod	Granite	Fine sand	39.1	2.4	16
Headquarter Camp (57)	46.219 N, 122.732 W	Haplohumult	Igneous	Loam	103.0	7.2	14
Fourth Creek (65)	43.804 N, 122.381 W	ND[a]	Pumice	Loam	81.3	4.7	17
Elk Creek (89)	43.365 N, 123.868 W	Haplumbrept	Sandstone	Loam	106.0	6.5	16
Cristy Falls (103)	43.904 N, 122.301 W	ND[a]	Pumice and ash	Loam	108.0	6.0	18
Mean (SE)					84.9 (10.9)	5.5 (0.6)	15 (0.8)

[a] Great groups have not been determined at these installations.

1.6 to 2.0 g cm^{-3} (see Christensen, 1992; Sollins et al., 1999). The most common density-separation liquids have included bromoform–ethanol mixtures in early studies (Monnier et al., 1962; Richter et al., 1975), sodium iodide in later studies (Spycher et al., 1983; Janzen et al., 1992), and sodium polytungstate most recently (Golchin et al., 1994a; Sollins et al., 1999). The rate of respiration in whole soils (WS) has been shown to be well correlated with LF-C (Janzen et al., 1992), possibly indicating that LF is of primary importance in soil respiration (Alvarez et al., 2000). Additionally, the lesser stability of LF has been demonstrated in field studies investigating the effects of cultivation on the fractions (Dalal and Mayer, 1986; Skjemstad et al., 1986; Golchin et al., 1995a), although significant changes to the HF have also been demonstrated with cultivation (Boone, 1994) and season (Spycher et al., 1983).

Direct investigation of C mineralization in density fractions is limited. Whalen et al. (2000) added separated fractions to soil and incubated the mixture. They determined that respiration from the HF was negligible. Alvarez et al. (1998) and Alvarez and Alvarez (2000) incubated WS and separated density fractions from control and incubated soils. They determined that LF-C was the driving factor in soil respiration, and that HF-C was poorly related to WS respiration. We have been unable to identify studies that have isolated density fractions, without additional size fractionation, and have then incubated the isolated HF and measured C mineralization. Thus, direct comparisons of C mineralization from LF and HF are lacking.

In the present study, we sought to test more directly the recalcitrance of the LF and HF. Four substrates were incubated aerobically for 300 days: WS, isolated LF, isolated HF, and a physically recombined LF and HF fraction (RF); additionally, the mass-weighted sum of LF and HF was calculated as the summed fraction (SF). Using CO_2 respiration as an index of substrate recalcitrance (Stotzky, 1965; Hart et al., 1994), we addressed two basic questions: (1) do LF and HF differ in recalcitrance? (2) to what extent do LF and HF each contribute to whole-soil respiration?

2. Materials and methods

2.1. Site characteristics

Soils were collected from seven installations of the Stand Management Cooperative Nutrition Project in western Oregon and Washington, USA (Table 1; Hazard and Peterson, 1984). Although each of the installations included fertilized plots, the soils used in the present study were only from the single non-fertilized plot at each installation, summing to a total of seven distinct soil types. The overstory was primarily composed of 46–72 year old second-growth Douglas-fir (*Pseudotsuga menziesii*(Mirb.) Franco) at the time of sampling.

2.2. Collection and storage

Soils were collected in August and September, 1995 at sampling points on a 4.5×4.5 m^2 grid within each 20 m plot. At each sampling point, the O layer was removed, and a 10×10 cm^2 section of the 0–5 cm mineral soil was sampled from the side of a small soil pit. Samples from alternating grid points were immediately composited and sieved (<2 mm) to yield a homogeneous fine fraction. Samples from remaining grid points were similarly composited and sieved, resulting in two composited samples per plot. Moist subsamples of this fine fraction were removed and transported to the Forestry Sciences Laboratory in Corvallis, OR, where they were tightly sealed in plastic bags and stored frozen at −20 °C. Meijboom et al. (1995) and Magid et al. (1996) have determined that freezing does not significantly affect density fractions. Only the composited samples of the seven soils (0–5 cm) were used in this study.

2.3. Density fractionation

The density fractionation methodology was based on techniques described by Strickland and Sollins (1987) and Sollins et al. (1999), but with necessary modifications. Frozen soils were thawed in August, 1998, the two composites from each installation mixed, and analyzed for gravimetric water content. Between 400 and 700 g of soil from

Table 2
Percent recovery, C, and N of density fractions

Installation	Soil (g) (oven dry)	Recovery (%[a]	Light fraction				Heavy fraction				Recombined		
			% of recovered[b]	C (g kg^{-1})	N (g kg^{-1})	C/N (g kg^{-1})	% of recovered	C (g kg^{-1})	N (g kg^{-1})	C/N (g kg^{-1})	C (g kg^{-1})	N (g kg^{-1})	C/N (g kg^{-1})
1	417	100.7	18.3	286	13.0	22	81.7	56	4.1	14	108.0	6.3	17
20	691	100.1	8.7	258	11.0	23	91.3	33	3.8	9	47.0	4.0	12
43	704	101.6	16.9	162	6.8	24	83.1	11	1.1	10	37.0	2.0	19
57	423	98.7	18.0	280	15.0	19	82.0	52	5.1	10	97.2	7.0	14
65	423	99.0	21.0	235	11.0	22	79.0	31	2.9	11	93.2	5.0	19
89	500	99.0	17.1	264	12.0	21	82.9	53	4.6	12	104.0	6.1	17
103	366	105.1	32.6	245	10.0	24	67.4	41	4.1	10	113.0	5.8	19
Mean (SE)	503 (52)	100.6 (0.8)	19.1 (2.3)	247.2 (15.8)	11.3 (1.0)	22 (0.7)	80.9 (2.3)	39.6 (6.1)	3.7 (0.5)	11 (0.6)	85.6 (11.6)	5.2 (0.6)	17 (1.0)

[a] Percent recovery of all soil fractionated from a given installation.
[b] Percent of total solids recovered from density fractionation (LF + HF).

each installation were fractionated in order to obtain the amount of LF required for the subsequent analysis and incubation (see below) (Table 2). To fractionate the soil, approximately 100 g (dry weight equivalent) of moist soil of known mass was placed in a 600 ml tall-form beaker, followed by the addition of 250 ml of sodium polytungstate (NaPT, $Na_6[H_2W_{12}O_{40}]$, Sometu-US, Van Nuys, California) at a density of 1.80 g ml^{-1}. Distilled water was added, which, in combination with the measured soil moisture, diluted the NaPT to 1.65 ml^{-1}. The slurry was then mixed for 30 s with a Hamilton benchtop mixer on a setting of 2.5 (1800 rev min^{-1}). The blades of the mixer were rinsed over the beaker using NaPT (1.65 g ml^{-1}). The beakers were covered with plastic wrap and set aside. After 48 h, the LF floating on the supernatant was aspirated into a separate container and the remaining sediment was remixed and again allowed to settle for 48 h. Any floating LF was removed at that time and added to the previously removed LF. The separated LF was rinsed with distilled water five times on a Whatman no. 52 filter (hardened, 7 μm pore size) to remove NaPT. If particles were visible in rinse effluent, the sample was transferred to a new filter and the rinsing process reinitiated after pouring the effluent back through the sample. Rinsed LF was dried in a forced-air oven at 50 °C for 48 h. Subsamples of the oven-dried (50 °C) LF were dried at 105 °C to obtain a standard oven dry weight.

Following the second removal of the LF, the HF was flushed from the beaker into several 250 ml centrifuge bottles, mixed thoroughly with distilled H_2O and centrifuged. The non-turbid supernatant was aspirated and discarded. This process was repeated at least five times. Rinsed HF was flushed into tins and dried in a forced-air oven at 50 °C for 72 h. As with the LF, subsamples of the oven-dried (50 °C) HF were dried at 105 °C to obtain a standard oven dry weight.

Prior to fractionating the bulk soil samples, small subsamples were collected and fractionated as above, but with the additional step of sonic disruption (156 J ml^{-1} for 2 min) following mixing. This step was added to estimate the additional amount of 'occluded LF' (Golchin et al., 1994a) that would be recovered if sonication were included in the fractionation procedure. The sonication added from 0 to 0.2% to the recovery of the LF, indicating that the series of mixes alone adequately disrupted aggregation.

2.4. Substrates

Four physical substrates were analyzed: the WS, the LF, the HF, and the RF. For the LF, 2.00 g (dry weight equivalent) of substrate was mixed with 18.00 g (dry weight) of acid-washed quartz sand (650–850 μm diameter) and placed in a glass scintillation vial. Similarly, for WS and HF, 10.00 g of substrate was mixed with 10.00 g sand. The RF was created by mixing LF and HF back together in the proportions from which they were recovered from the soil (Table 2), and combining 10.00 g of the mixture with

10.00 g of sand. Actual weights were tracked to the nearest 0.001 g. Sand was added to the substrates for two reasons. The addition of sand yielded a larger sample that permitted more flexibility in the type and number of physical analyses that could be performed. In the case of the HF sand was added to create some degree of physical structure, possibly ameliorating anoxic conditions that might occur during the incubation. In the use of the sand, we assume it was inert and underwent no appreciable weight change during the course of the incubation. The WS was prepared and begun incubating within 24 h of the soil thawing.

Total initial C and N were measured on day 1 of substrate incubation (see below) and values were corrected for added sand. These analyses were carried out on a Leco CNS Analyzer (Leco, St Joseph, MI, USA) by the Central Analytical Lab (Oregon State University, Corvallis, OR, USA). In a study of the relations of site characteristics to soil organic C in western Oregon, Homann et al. (1995) considered measures of total soil C to equal soil organic C, as soils in that region are generally too acidic for carbonates to persist. Mildly acidic conditions were measured by Homann et al. (2001) in the O2 and top 5 cm of the mineral soils used in this study. They found an average pH of 5.0 in the O2 and 5.3 in the soil. We judged that carbonate concentrations would be negligible in our soils considered total C measures in this study to represent organic C.

2.5. Incubation

Substrates were incubated for 300 days at 20(±1) °C. For LF, HF, and RF, substrates and sand were placed in open 20 ml glass scintillation vials, and six vials were placed in sealed 1 l mason jars (Stotzky, 1965; Hart et al., 1994). All vials within a jar contained the same substrate from the same installation. To adequately contain the WS, three 60 ml vials were used in each of two mason jars. The multiple vials allowed periodic destructive sampling.

Each substrate was inoculated at the beginning of the incubation. The inoculation by no means ensured an identical population in each substrate, but it provided the opportunity for similar populations to arise in each substrate while ensuring that there would be a source for organisms best adapted to a given substrate. Differences that evolved between the microbial populations in the substrates could then be largely attributed to the quality of the substrate. Mineral soil (0–5 cm) used as the source of inoculum was collected from the McDonald–Dunn Research Forest within a stand similar in age and composition to those from which the incubation soils were collected. The inoculum source was stored in a slightly open cooler at room temperature and humidity. The soil was kept moist by regularly adding tap water. Inoculum was prepared by first adding the soil to distilled water in a 1 g/1.5 ml ratio of soil to water, followed by gentle shaking on a rotary shaker for 0.5 h before being allowed to settle for another 0.5 h. Finally, a 0.5 ml aliquot of supernatant from the inoculum mix was added to each vial. A fresh inoculum mix, from the original inoculum source, was created each time a new batch of substrates was ready to begin incubating.

The headspace of each jar was sampled through a septum installed in the cap of the jar. The septum in each jar was replaced frequently to maintain the integrity of the seal. Before each sample was drawn, the air in the headspace was mixed by repeatedly and vigorously drawing air from the headspace into a gas-tight syringe and plunging the air back into the jar. After mixing, a 500 μl sample was drawn and immediately injected into a GC-8A Series Shimadzu (Columbia, MD, USA) gas chromatograph fitted with a 6 ft × 2 mm (ID) Porapak Q 80/100 column. If duplicate samples did not agree to within 3%, a third sample was taken and the three samples were averaged. Samples were discarded only if they could be attributed to technician or equipment error. Once the sampling of a jar was completed, the jar was flushed with CO_2-free air and resampled to obtain a baseline CO_2 concentration. Respiration was measured in the headspace of the jars at intervals (1–21 days) sufficient to maintain a CO_2 concentration of less than 2%.

Destructive sampling of the vials occurred at seven times: days 1, 10, 30, 60, 120, 210, and 300. On each sampling date a single vial from each installation/substrate combination was removed from the jar. The substrate in the vial was removed and thoroughly mixed. The mixed substrate was then allocated to tests for active microbial biomass and gravimetric water content. Active microbial biomass was measured by direct microscopy (Soil Food Web Inc., Corvallis, OR, USA). Substrates were diluted and stained with fluorescein diacetate, allowing active bacteria and fungi to be viewed and measured with an epifluorescent microscope (Ingham and Klein, 1984; Stamatiadis et al., 1990).

2.6. Data management and analysis

SF was calculated as the mass-weighted results of the light and heavy fractions. This mathematical mixing paralleled the physical mixing of the RF.

Remaining C was calculated at the time of each respiration measurement by subtracting the cumulative respiration to that point from the initial C in the sample. The g C respired kg^{-1} remaining C day^{-1} was calculated as

$$\text{g C respired kg}^{-1}\text{ remaining C day}^{-1} = \frac{\text{g C respired}_i\text{/kg remaining C}_i}{\text{day}_i - \text{day}_{i-1}},$$

where i corresponds to the time point at which the respiration measurement was taken.

For cumulative C respiration, the data at each time period were analyzed using a randomized complete block design model. The installations were blocks, while substrates were treatments. This model is subject to the standard

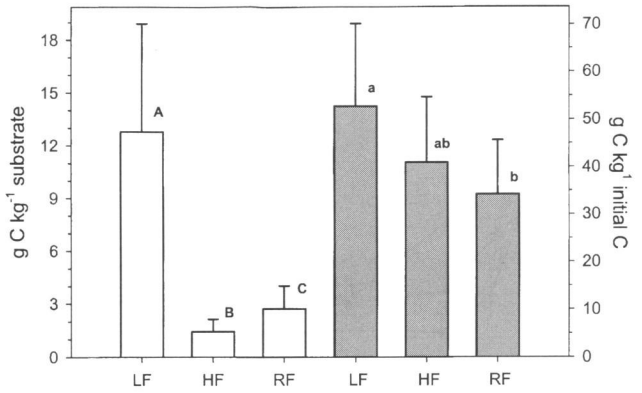

Fig. 1. Back-transformed mean cumulative C mineralization at 300 days from light fraction (LF), heavy fraction (HF), and recombined fraction (RF), relative to initial substrate mass (white bars, left axis) and initial substrate C (grey bars, right axis). Means with the same units of measurement and the same letter are not significantly different at $P < 0.05$ (FP LSD, analyses were conducted on ln-transformed data).

assumptions of the analysis of variance (ANOVA), including independence, homoscedasticity, and normality. Although duplicates of all substrates from each installation were incubated, the duplicates were only created to ensure against sample loss through accident. Prior to statistical analysis, the values for each set of duplicates were averaged, and those means were statistically analyzed. Before accepting the results of statistical tests, residuals of the models were examined for constant variance (Montgomery, 1991). If necessary, natural log transformations were used to homogenize variance. Cumulative respiration was measured using two-way analysis of variance. When an ANOVA resulted in a P-value of less than 0.05, all possible comparisons between treatments were conducted using Fisher's Protected Least Significant Difference (FP LSD). Although not as conservative as other multiple comparisons tests, FP LSD was considered appropriate in these cases because the comparisons were planned and there were never more than three treatments per test, thus minimizing compounded error (Einot and Gabriel, 1975).

Analysis of active microbial biomass data and changes in rate of C mineralization involved comparisons of data both at a time point and between time points. Although different vials were assayed for active microbial biomass each time, the same installation and substrate in each jar were being repeatedly measured through time, necessitating the use of a repeated measures ANOVA (von Ende, 1993). The repeated measures ANOVA relies on the same assumptions of the standard model, with the added dimension of constant variance through time (the sphericity condition) (Yandell, 1997). When a P-value from a repeated measures ANOVA was significant, all possible comparisons of WS, LF, HF, and RF were generated using the Turkey–Kramer HSD method. The HSD method was preferable to the FP LSD in this instance because sample sizes were sometimes unequal, not all comparisons were initially planned, and there were more comparisons than available degrees of freedom (Ramsey and Schafer, 1997).

Table 3
Mean contributions of light and heavy fractions to cumulative respiration from the summed fraction. Values in parentheses are 1 SE, $n = 7$

Contribution	Light fraction	Heavy fraction
Percent of LF + HF	19.1 (2.3)	80.9 (2.3)
Carbon (g kg^{-1} SF)	47.2 (6.2)	31.9 (4.3)
Respiration		
g C kg^{-1} initial C	32.0 (2.7)	17.8 (2.8)
g C kg^{-1} SF	2.5 (0.4)	1.4 (0.2)
Percent of total SF	64.7 (4.4)	35.3 (4.4)

All references to statistical differences between treatments are based upon a significance level of 0.05. When P-values of multiple comparisons were between 0.1 and 0.05 the values and associated t-statistics are listed in text. All statistical comparisons and all references to differences are on a dry weight (105 °C) basis. Most data manipulation, and all statistical analyses, were conducted in SAS (SAS Institute Inc., version 6.12).

3. Results

3.1. Cumulative respiration

The 300-days cumulative respiration (g C kg^{-1} initial substrate) differed substantially among LF, HF, and RF ($F_{2,12} = 142.3$, $P < 0.0001$). The LF produced several times the cumulative respiration of both the RF and the HF, while the RF was more productive than the HF (Fig. 1). When the cumulative respiration of each fraction was considered relative to initial C (g C kg^{-1} initial C, $F_{2,12} = 6.5$, $P = 0.01$), the differences between the LF, HF, and RF were much smaller (Fig. 1). The large difference in respiration between LF and HF relative to substrate mass was greatly reduced when respiration was based on initial C (Fig. 1). The difference in respiration was no longer significant, although there was still some evidence of a difference ($t_{12} = 2.1$, $P = 0.057$). The statistical difference in respiration between the LF and RF was preserved, although the difference in mean respiration was smaller. Respiration values from the HF and RF were not statistically different relative to initial C.

The LF contained six times the C of the HF (Table 2). The LF constituted only 19% of the SF mass, but it contained nearly 60% of the initial C in the SF (Table 3). Ultimately, the LF was responsible for almost 65% of the 300-day cumulative respiration from the SF.

There were differences in 300-days cumulative respiration between the WS, SF, and RF when considered relative to substrate mass ($F_{2,12} = 6.63$, $P = 0.01$) or initial C ($F_{2,12} = 6.01$, $P = 0.01$). Multiple comparisons demonstrated that cumulative respiration from the RF was

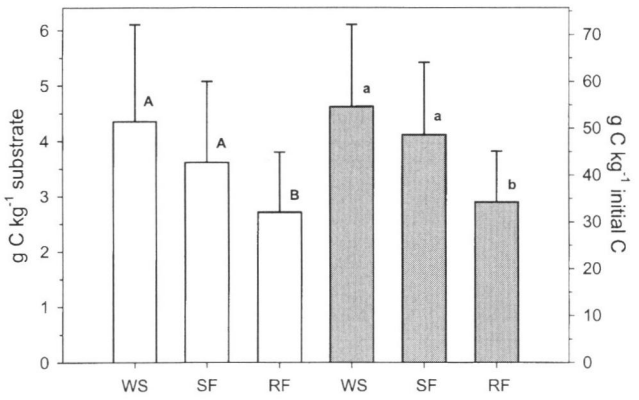

Fig. 2. Back-transformed mean cumulative C mineralization at 300 days from whole soil (WS), summed fraction (SF), and recombined fraction (RF), relative to initial substrate mass (white bars, left axis) and initial substrate C (grey bars, right axis). Means with the same units of measurement and the same letter are not significantly different at $P < 0.05$ (FP LSD, analyses were conducted on ln-transformed data).

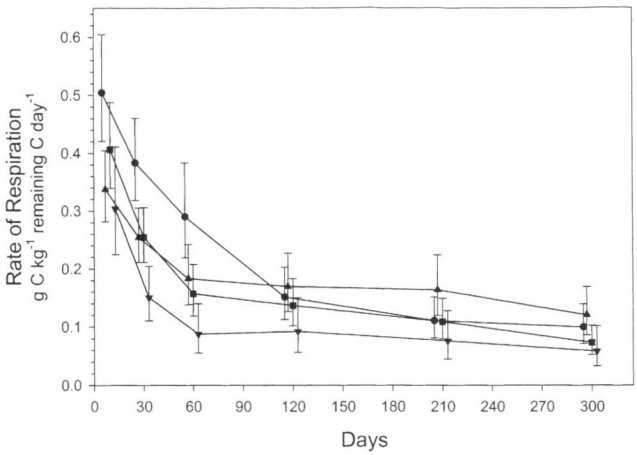

Fig. 3. Rate of respiration through time in whole soil (●), recombined fraction (▼), light fraction (▲),and heavy fraction (■). Analyses were conducted on ln-transformed data. The data presented here are back-transformed means and 95% confidence intervals. Symbols have been offset in time to avoid overlapping bars.

significantly lower than that from the WS and SF, which were not different from each other; this pattern held whether the data were based on substrate or initial C (Fig. 2).

3.2. Rate of respiration

A repeated measures ANOVA revealed a significant time by substrate interaction in the rate of respiration (g C kg^{-1} remaining C day^{-1}), indicating that the rate of respiration changed differently through time for the substrates ($F_{15,70} = 5.23$, $P < 0.0001$). The rate of respiration from the WS was higher than that from the RF for the first 60 days, but was no longer different by 120 days (Fig. 3). The rate of respiration from the LF and HF were not significantly different at any time during the incubation. The rates of respiration from the LF and HF were not different from that of the RF at day 10, but RF respiration rapidly dropped and was lower than both fractions by day 30. This difference persisted with the LF until at least day 60, but was no longer evident by day 120. There were no significant differences detected in the rates of respiration between substrates beyond 120 days.

3.3. Active biomass

Active fungal biomass (mg biomass kg^{-1} C) was marked by high variability that resulted in few clear trends. There was only weak evidence of a time by substrate interaction in active fungal biomass ($F_{18,66} = 1.68$, $P = 0.066$). On the first day of the incubation, the WS was the only substrate that consistently contained active fungal biomass; in general, no active fungal biomass was detected in the other substrates at day 1 (Table 4). By day 10, however, active fungal biomass had sharply increased in the fractions, and there was some evidence of a difference between substrates ($F_{3,11} = 3.07$, $P = 0.057$). Namely, HF contained more active fungal biomass than the WS. There were no other significant differences between substrates at any time period.

Active bacterial biomass (mg biomass kg^{-1} C) was also characterized by high variability (Table 5) and few statistical differences, although there was very strong evidence of a time by substrate interaction ($F_{18,66} = 3.00$, $P < 0.001$). The LF and HF, while not different from each other,

Table 4
Mean active fungal biomass (mg kg^{-1} C) in whole soil (WS), recombined fraction (RF), light fraction (LF), and heavy fraction (HF). Values in parentheses are 1 SE, $n = 7$

Day	Whole soil	Recombined fraction	Light fraction	Heavy fraction
1	37.2 (20.0)	0.6[a] (0.6)	0	3.8[a] (3.8)
10	27.0[b] (9.7)	73.3 (29.1)	170.4 (94.7)	160.0 (69.0)
30	99.7 (42.7)	170.3 (61.0)	26.2 (25.5)	120.9 (91.7)
60	120.1 (40.4)	93.2 (53.5)	100.0 (27.6)	325.2 (71.4)
120	179.3 (53.7)	58.9 (18.8)	62.7 (38.9)	127.5 (43.8)
210	24.9 (13.1)	4.4[b] (3.1)	45.1 (16.1)	27.0 (14.3)
300	24.4[b] (22.4)	42.9 (27.8)	44.3 (17.7)	11.1 (5.4)

[a] RF sample from installation 1contained 27.1 mg kg^{-1}; HF sample from installation 57 contained 4.2 mg kg^{-1}; no fungal biomass was detected in RF or HF samples from other installations on day 1.

[b] WS: $n = 6$ at 10 days, $n = 4$ at 300 days; RF: $n = 4$ at 210 days.

Table 5
Mean active bacterial biomass (mg kg^{-1} C) in whole soil (WS), recombined fraction (RF), light fraction (LF), and heavy fraction (HF). Values in parentheses are 1 SE, $n = 7$

Day	Whole soil	Recombined fraction	Light fraction	Heavy fraction
1	179.9 (42.0)	30.1 (10.2)	68.3 (9.9)	93.9 (34.0)
10	170.5[a] (44.8)	110.0 (43.2)	511.1 (118.7)	147.3 (56.6)
30	193.6 (35.2)	193.4 (53.9)	455.3 (150.8)	269.4 (78.1)
60	105.6 (25.5)	236.3 (57.1)	608.7 (144.9)	588.6 (211.3)
120	197.5 (30.2)	413.7 (142.6)	1352.5 (330.3)	1025.3 (358.1)
210	90.3 (30.3)	79.5[a] (6.2)	439.8 (159.3)	513.0 (194.0)
300	34.0[a] (17.9)	399.4 (249.5)	515.5 (137.3)	78.1 (45.9)

[a] WS: $n = 6$ at 10 days, $n = 4$ at 300 days; RF: $n = 4$ at 210 days.

supported more active bacterial biomass by day 60 than the WS. This difference had greatly increased by day 120, and included the RF, as well. There were no other significant differences between substrates at any time period.

4. Discussion

There is ample evidence, from a variety of approaches, that LF typically has faster rates of turnover than HF. Golchin et al. (1995b) collected soils from a chronosequence of forest, pasture, and deforested pasture. The ^{13}C signatures in the LF and HF of the soils were used to estimate C accumulation and loss from the fractions in the deforested pastures. After 83 years, only 3% of the original forest-C remained in the free LF in the deforested pasture, while 19–32% of the original forest-C remained in the heavier fractions. Compton and Boone (2000) measured natural abundance ^{15}N in LF and HF collected from permanent woodlots and reforested sites that were formerly pastures or cultivated. They suggested that higher C/N ratios and lower $\delta^{15}N$ in the LF indicated organic matter of a more recent origin, and that converging $\delta^{15}N$ in the pastures and cultivated sites indicated greater mixing and humus degradation. Alvarez et al. (1998) incubated whole soils for 160 days, then fractionated the incubated soils to measure changes in the C content of the LF and HF. They determined that the LF lost five times more C than the HF. Our results are consistent with these previous findings, when respiration was considered per gram of initial substrate. The LF lost over five times more C than the HF during 300 days of incubation (Fig. 1).

When expressed per gram of initial C, however, the C mineralization of the HF and LF were similar (Fig. 1). This suggests that the recalcitrance of these fractions is similar, and that the difference in turn-over rates that occurs in whole soils is caused by different degrees of accessibility. A strong influence of physical soil characteristics on density fraction stability has been suggested by several studies, many using ^{13}C NMR techniques. Skjemstad et al. (1986) separated LF and HF from a chronosequence of cultivated Vertisols. They reported that the C content of the LF, but not the HF, was reduced by cultivation. Considering changes in particle density, clay content, and ^{13}C NMR spectra, they concluded that the higher stability of HF was not attributable to the recalcitrance of HF-C, but instead to inaccessibility or interaction. Golchin et al. (1994b) analyzed LF and HF from several different soil types and consistently found high levels of carbohydrates and no phenolic compounds in the HF. The nature of the C compounds, and low C/N ratios, indicated that the C was more likely protected than recalcitrant. Golchin et al. (1995a,b) noted strong *O*-alkyl signals in the HF of an Oxisol. They suggested that the HF-C was protected through complexation with clays, not by recalcitrance. They also reported that while HF-C appeared to be well protected in undisturbed soils, it rapidly declined with soil disturbance. Dalal and Mayer (1986) studied loss of organic C from the density fractions of six cultivated soils. They concluded that HF-C was protected from microbial attack by its interaction with clay. Sørensen (1983) and Franzluebbers (1999) noted increased respiration as aggregates were destroyed. In the present study, we likely increased the accessibility, especially to C in the HF fraction, by largely destroying soil aggregation during the fractionation process.

Although the rates of respiration were not different at any time period, it is nonetheless tempting to examine Fig. 3 for different trends in the respiration rates of the LF and HF through time. A larger number of samples may have allowed for a significant separation of the mean rates. However, the timing of these differences would still raise the question of biological significance. Soils in these forested sites can be expected to have frequent input of particulate and dissolved OM (Jandl and Sollins, 1997; Wagai, 1999; Prescott et al., 2000) that would contribute to the LF and HF (Spycher et al., 1983; Chotte et al., 1998). This frequent replenishment of less recalcitrant C would probably prevent a separation of rates of respiration for an extended period of time. Thus, even if the differences between the means were statistically significant, it seems unlikely that they would be biologically significant. Surely the LF is the source of a disproportionate amount of heterotrophic soil respiration (Table 3), but that contribution may be more related to the high C content of LF than to higher recalcitrance of the HF.

The C concentration of the LF may be ten times that of the HF in some soils (Swanston and Myrold, 1997), and was six times higher in this study. Thus, even though the LF is typically less than 15% of the soil weight, it may easily contain 65% of the soil C content (Christensen, 1992). If C were mineralized from the two fractions at similar rates, the LF might still be expected to contribute more C to soil respiration, as was the case in the SF (Table 3). The SF, however, may under represent the proportional contribution of LF to WS respiration, as much of the HF-C in the WS may interact with minerals (Young and Spycher, 1979) or be inaccessible (Golchin et al., 1994b).

The RF-C should have been more accessible than C in the WS because of the destruction of aggregates during the fractionation process, but the RF had a consistently lower cumulative respiration. Additionally, the rate of respiration in the RF was lower for up to 120 days. The density fractionation procedure may have resulted in loss of dissolved organic C, toxic levels of Na and tungsten contamination from NaPT (Magid et al., 1996), and reduction of the viable microbial population (Compton and Boone, in press; Tables 4 and 5). All of these factors may have contributed to reduced respiration in each of the fractions, perhaps offsetting what would otherwise be enhanced respiration via increased accessibility. However, if these were the sole causes of reduced respiration in the RF, then the cumulative respiration of the SF should have been similar to that of the RF. The lower values of the RF seem to indicate that there was a negative interaction between the LF and HF when the soil structure was removed.

This negative interaction could have been physical, microbial, or a combination of the two. The physical interaction could have been as simple as reduced aeration in the RF. Alternatively, microbial communities that degrade LF and HF may have different ideal compositions of species. To the extent that the fractions are separated in space (Ladd et al., 1993; Golchin et al., 1994b), the communities may also be separated (Chotte et al., 1998; Guggenberger et al., 1999). Removing soil structure and mixing the fractions into a uniform matrix may have reduced the overall efficiency of the resulting community, perhaps by engendering competition between organisms that are typically more segregated in the WS.

Measurements of active bacterial and fungal biomass were intended to provide a simple picture of these interactions and their evolution through time. Unfortunately, these values proved to be extremely variable, and revealed few statistically significant trends. Future studies that incubate density or size fractions may wish to employ some type of fatty acid analysis (Vestal and White, 1989) to determine the structures of the communities degrading the fractions. Both the presence and abundance of different types of organisms could be compared between fractions and the whole soil. Differences between fractions and similarities to the whole soil may shed light on the relative importance and function of the fractions in the whole soil. Use of the same methodologies in a RF would yield important information on the interaction of these communities with each other and with soil structure.

5. Conclusions

Considering cumulative respiration and rates of respiration through time, we did not find convincing evidence of a difference in the recalcitrance of LF and HF in these forest soils. The LF, however, appeared to dominate soil respiration largely because of its high C content. If the respiration of the SF approximates that of the WS, then the HF contributed up to a third of the C mineralized during the 300-day incubation and should also be considered as an important component of soil respiration.

The density separation technique appears to be a viable method for isolating soil organic matter fractions that differ in chemistry and in C concentration. Yet these fractions should be considered more carefully in the context of their respective C content and accessibility within the soil matrix. Variation in soil structure, texture, and proportions of density fractions in the soil will alter the respiration from the fractions, perhaps yielding confusing results if these factors are not taken into account.

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References

Alvarez, R., Alvarez, C.R., 2000. Soil organic matter pools and their associations with carbon mineralization kinetics. Soil Science Society of America Journal 64, 184–189.

Alvarez, C.R., Alvarez, R., Grigera, M.S., Lavado, R.S., 1998. Associations between organic matter fractions and the active soil microbial biomass. Soil Biology and Biochemistry 30, 767–773.

Boone, R.D., 1994. Light-fraction soil organic matter: origin and contribution to net nitrogen mineralization. Soil Biology and Biochemistry 26, 1459–1468.

Cambardella, C.A., Elliot, E.T., 1993. Methods for physical separation and characterization of soil organic matter fractions. Geoderma 56, 449–457.

Chotte, J.L., Ladd, J.N., Amato, M., 1998. Sites of microbial assimilation, and turnover of soluble and particulate ^{14}C-Labelled substrates decomposing in a clay soil. Soil Biology and Biochemistry 30, 205–218.

Christensen, B.T., 1992. Physical fractionation of soil and organic matter in primary particle size and density separates. Advances in Soil Science 20, 1–90.

Compton, J.E., Boone, R.D., 2000. Long-term impacts of agriculture on soil carbon and nitrogen in New England forests. Ecology 81, 2314–2330.

Compton, J.E., Boone, R.D. Soil nitrogen transformations and the role of light fraction organic matter. Soil Biology and Biochemistry, in press.

Dalal, R.C., Mayer, R.J., 1986. Long-term trends in fertility of soils under continuous cultivation and cereal cropping in Southern Queensland. IV. Loss of organic carbon from different density functions. Australian Journal of Soil Research 24, 301–309.

Einot, I., Gabriel, K.R., 1975. A study of the powers of several methods of multiple comparisons. Journal of the American Statistical Association 70, 351.

von Ende, C.N., 1993. Repeated-measures analysis: growth and other time-dependent measures. In: Scheiner, S.M., Gurevitch, J. (Eds.). Design and Analysis of Ecological Experiments. Chapman & Hall, New York, pp. 112–136.

Franzluebbers, A.J., 1999. Potential C and N mineralization and microbial biomass from intact and increasingly disturbed soils of varying texture. Soil Biology and Biochemistry 31, 1083–1090.

Golchin, A., Oades, J.M., Skjemstad, J.O., Clarke, P., 1994a. Study of free and occluded particulate organic matter in soils by solid-state ^{13}C NMR spectroscopy and scanning electron microscopy. Australian Journal of Soil Research 32, 285–309.

Golchin, A., Oades, J.M., Skjemstad, J.O., Clarke, P., 1994b. Soil structure and carbon cycling. Australian Journal of Soil Research 32, 1043–1068.

Golchin, A., Clarke, P., Oades, J.M., Skjemstad, J.O., 1995a. The effects of cultivation on the composition of organic matter and structural stability of soils. Australian Journal of Soil Research 33, 975–993.

Golchin, A., Oades, J.M., Skjemstad, J.O., Clarke, P., 1995b. Structural and dynamic properties of soil organic matter as reflected by ^{13}C natural abundance, pyrolysis mass spectrometry and solid-state ^{13}C NMR spectroscopy in density fractions of an Oxisol under forest and pasture. Australian Journal of Soil Research 33, 59–76.

Greenland, D.J., Ford, G.W., 1964. Separation of partially humified organic materials by ultrasonic dispersion. Eighth International Congress of Soil Science, Transactions 3, 137–148.

Gregorich, E.G., Monreal, C.M., Schnitzer, M., Schulten, H.R., 1996. Transformation of plant residues into soil organic matter: chemical characterization of plant tissue, isolated soil fractions, and whole soils. Soil Science 161, 680–693.

Guggenberger, G., Elliott, E.T., Frey, S.D., Six, J., Paustian, K., 1999. Microbial contributions to the aggregation of a cultivated grassland soil amended with starch. Soil Biology and Biochemistry 31, 407–419.

Hart, S.C., Nason, G.E., Myrold, D.D., Perry, D.A., 1994. Dynamics of gross nitrogen transformations in an old-growth forest soil: the carbon connection. Ecology 75, 880–891.

Hazard, J.W., Peterson, C.E., 1984. Objectives and analytical methods of the Regional Forest Nutrition Project. Institute of Forest Resources, Contribution No. 53, College of Forest Resources, University of Washington, Seattle.

Homann, P.S., Sollins, P., Chappell, H.N., Stangenberger, A.G., 1995. Soil organic carbon in a mountainous, forested region: relation to site characteristics. Soil Science Society of America Journal 59, 1468–1475.

Homann, P.S., Caldwell, B.A., Chappell, H.N., Sollins, P., Swanston, C.W., 2001. Residual effects of urea fertilization on Douglas-fir soil C and N properties a decade after termination of urea fertilization. Canadian Journal of Forest Research 31, 2225–2236.

Ingham, E.R., Klein, D.A., 1984. Soil fungi: measurement of hyphal length. Soil Biology and Biochemistry 16, 279–280.

Jandl, R., Sollins, P., 1997. Water-extractable soil carbon in relation to the belowground carbon cycle. Biology and Fertility of Soils 25, 196–201.

Janzen, H.H., Campbell, C.A., Brandt, S.A., Lafond, G.P., Townley-Smith, L., 1992. Light-fraction organic matter in soils from long-term crop rotations. Soil Science Society of America Journal 56, 1799–1806.

Killham, K., 1994. Soil Ecology. Cambridge University Press, Cambridge 242 p.

Ladd, J.N., Foster, R.C., Skjemstad, J.O., 1993. Soil structure: carbon and nitrogen metabolism. Geoderma 56, 401–434.

Magid, J., Gorissen, A., Giller, K.E., 1996. In search of the elusive active fraction of soil organic matter: three size-density fractionation methods for tracing the fate of homogeneously ^{14}C-labelled plant materials. Soil Biology and Biochemistry 28, 89–99.

Meijboom, F., Hassink, J., van Noordwijk, M., 1995. Density fractionation of soil organic matter using silica suspensions. Soil Biology and Biochemistry 27, 1109–1111.

Monnier, G., Turc, L., Jeanson-Luusinang, C., 1962. Une méthod de fractionnement densimétrique par centrifugation des matières organiques du sol. Annales Agronomiques 13, 55–63.

Montgomery, D.C., 1991. Design and Analysis of Experiments. Third ed. Wiley, New York 649 p.

Prescott, C.E., Chappell, H.N., Vesterdal, L., 2000. Nitrogen turnover in forest floors of coastal Douglas-fir at sites differing in soil nitrogen capital. Ecology 81, 1878–1886.

Ramsey, F.L., Schafer, D.W., 1997. The Statistical Sleuth: a Course in Methods of Data Analysis. Duxbury Press, New York 742 pp.

Richter, M., Mizuno, I., Aranguez, S., Uriate, S., 1975. Densimetric fractionation of soil organo-mineral complexes. Journal of Soil Science 26, 112–123.

Skjemstad, J.O., Dalal, R.C., Barron, P.F., 1986. Spectroscopic investigations of cultivation effects on organic matter of Vertisols. Soil Science Society of America Journal 50, 354–359.

Sollins, P., Homann, P., Caldwell, B.A., 1996. Stabilization and destabilization of soil organic matter: mechanisms and controls. Geoderma 74, 65–105.

Sollins, P., Glassman, C., Paul, E.A., Swanston, C., Lajtha, K., Heil, J.W., Elliott, E.T., 1999. Soil carbon and nitrogen: pools and fractions. In: Robertson, G.P., Bledsoe, C.S., Coleman, D.C., Sollins, P. (Eds.). Standard Soil Methods for Long-Term Ecological Research. Oxford University Press, New York, pp. 89–105.

Sørensen, L.H., 1983. The influence of stress treatments on the microbial biomass and the rate of decomposition of humified material in soils containing different amounts of clay. Plant and Soil 75, 107–119.

Spycher, G., Sollins, P., Rose, S., 1983. Carbon and nitrogen in the light fraction of a forest soil: vertical distribution and seasonal patterns. Soil Science 135, 79–87.

Stamatiadis, S., Doran, J.W., Ingham, E.R., 1990. Use of staining to separate fungal and bacterial activity in soil. Soil Biology and Biochemistry 22, 81–88.

Stevenson, F.J., 1994. Humus Chemistry: Genesis, Composition, Reactions. Second ed. Wiley, New York 496 p.

Stotzky, G., 1965. Microbial respiration. In: Black, C.A. (Ed.). Methods of Soil Analysis. Part 2. Agronomy Monographs, vol. 9. American Society of Agronomy, Madison, WI, pp. 1550–1572.

Strickland, T.C., Sollins, P., 1987. Improved method for separating light- and heavy-fraction organic material from soil. Soil Science Society of America Journal 51, 1390–1393.

Swanston, C.W., Myrold, D.D., 1997. Incorporation of nitrogen from decomposing red alder leaves into plants and soil of a recent clearcut in Oregon. Canadian Journal of Forest Research 27, 1496–1502.

Vestal, J.R., White, D.C., 1989. Lipid analysis in microbial ecology: quantitative approaches to the study of microbial communities. Bioscience 39, 535–541.

Wagai, R., 1999. Dynamics of water-soluble carbon in forest soils of

contrasting fertility. MS Thesis, Oregon State University, Corvallis, OR, USA.

Whalen, J.K., Bottomley, P.J., Myrold, D.D., 2000. Carbon and nitrogen mineralization from light- and heavy-fraction additions to soil. Soil Biology and Biochemistry 32, 1345–1352.

Yandell, B.S., 1997. Practical Data Analysis for Designed Experiments. Chapman & Hall, New York pp. 383–408.

Young, J.L., Spycher, G., 1979. Water dispersible soil organic-mineral particles. 1. Carbon and nitrogen distribution. Soil Science Society of America Journal 43, 324–328.