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Density fractionation of forest soils: methodological questions and interpretation of incubation results and turnover time in an ecosystem context

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Abstract Soil organic matter (SOM) is often separated by physical means to simplify a complex matrix into discrete fractions. A frequent approach to isolating two or more fractions is based on differing particle densities and uses a high density liquid such as sodium polytungstate (SPT). Soil density fractions are often interpreted as organic matter pools with different carbon (C) turnover times, ranging from years to decades or centuries, and with different functional roles for C and nutrient dynamics. In this paper, we discuss the development and mechanistic basis of common density-based methods for dividing soil into distinct organic matter fractions. Further, we directly address the potential effects of dispersing soil in a high density salt solution on the recovered fractions and implications for data interpretation. Soil collected from forested sites at H. J. Andrews Experimental Forest, Oregon and Bousson Experimental Forest, Pennsylvania was separated into light and heavy fractions by floatation in a 1.6 g cm^{-3} solution of SPT. Mass balance calculations revealed that between 17% and 26% of the original bulk soil C and N content was mobilized and subsequently discarded during density fractionation for both soils. In some cases, the light isotope was preferentially mobilized during density fractionation. During a year-long incubation, mathematically recombined density fractions respired ~40% less than the bulk soil at both sites and light fraction (LF) did not always decompose more than the heavy fraction (HF). Residual amounts of tungsten (W) present even in well-rinsed fractions were enough to reduce microbial respiration by 27% compared to the control in a 90-day incubation of O_a material. However, residual W was nearly eliminated by repeated leaching over the year-long incubation, and is not likely the primary cause of the difference in respiration between summed fractions and bulk soil. Light fraction at Bousson, a deciduous site developed on Alfisols, had a radiocarbon-based mean residence time (MRT)

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of 2.7 or 89 years, depending on the interpretation of the radiocarbon model, while HF was 317 years. In contrast, both density fractions from H. J. Andrews, a coniferous site developed on andic soils, had approximately the same MRT (117 years and 93 years for LF and HF). At H. J. Andrews the organic matter lost during density separation had a short MRT (19 years) and can account for the difference in respired CO_2 between the summed fractions and the bulk soil. Recognition and consideration of the effects of the density separation procedure on the recovered fractions will help prevent misinterpretation and deepen our understanding of the specific role of the recovered organic matter fractions in the ecological context of the soil studied.

Keywords Density fractionation · Heavy fraction · Incubation · Light fraction · Mean residence time · Radiocarbon · Sodium polytungstate · Soil organic matter

Introduction

Density fractionation of soil, which divides material by floatation or sedimentation in a solution according to particle density, has been used for nearly 50 years to physically separate soil organic matter (SOM) into discrete fractions thought to have differing stability. Christensen (1992) provided a detailed review of soil physical fractionation techniques, which included both size separation and density fractionation. However, in the years since, there has been a substantial increase in the use of density fractionation methods across a variety of ecological research fields and applications. As with all methods that attempt to operationally define organic matter pools, there have been shortcomings and concerns, particularly with respect to the relationship between the conceptual organic matter pools and the actual characteristics of the resulting soil fractions. However, the development of density fractionation as a means for physically separating soil has implications for identifying mechanisms of organo-mineral interactions and quantifying pools of organic matter with different ecological roles that are relevant to SOM research. Here we summarize trends in the density fractionation literature, emphasizing the expansion of work since the introduction of sodium polytungstate (SPT) as a high density liquid, and consider the link between conceptualized organic matter fractions produced during density fractionation and the actual physical, chemical, and biological properties of the isolated fractions.

In addition to providing a brief summary of density fractionation as a method, we seek to illustrate the interaction of the method with soils from two different ecosystems and then look more closely at potential gaps in interpretation. In particular, we address the effect of density fractionation on C and N loss during separation, and discuss the implications for data interpretation with regard to isotopic and C and N cycling and mass balancing.

Development of density fractionation methodology

Density fractionation emerged initially as a means to separate both primary (Pearson and Truog 1937) and clay (Halma 1969; Francis et al. 1972) minerals. During the separation of soil minerals, it was necessary to disrupt soil aggregates and remove organic material. In the following decades, density fractionation techniques were developed with the specific goal to divide SOM into two discrete fractions (Monnier et al. 1962; Greenland and Ford 1964) that represented different stages of degradation, i.e. recent, partially decomposed organic matter versus ''thoroughly degraded'' organic matter already associated with mineral surfaces (Ford and Greenland 1968, Richter et al. 1975). A number of early studies were conducted with soil separated at ~2.0 g cm^{-3}, since most primary and secondary soil minerals are denser than 2.0 g cm^{-3}. The use of a separation density of 2.0 g cm^{-3} continued for some applications (Dalal and Mayer 1986; Trumbore and Zheng 1996); however, a lower density 1.6–1.8 g cm^{-3} eventually became more common as a way to exclude the most mineral and organo-mineral material from the LF while maximizing recovery of plant-like particulate organic matter (Ladd et al. 1977; Scheffer 1977; Young and Spycher 1979).

Many heavy liquid solutions have been used for soil fractionation, i.e. bromoform ethanol (Monnier et al. 1962), bromoform-petroleum spirit mixture (Greenland and Ford 1964), and NaI (Spycher and Young 1979; Sollins et al. 1983, Boone 1994); see Christensen (1992) for a review of methods. In the early 1980s, sodium polytungstate, $H_2Na_6O_{40}W_{12}$, (SPT, SOMETU, Berlin) was introduced as a safe, alternative high density solution (Plewinksy and Kamp 1984). Previously, heavy liquid solutions were mostly halogenated-hydrocarbons that were highly toxic to humans (Torresan 1987) while SPT is a non-corrosive, non-flammable, safe alternative (Skipp and Brownfield 1993). Sodium polytungstate is highly viscous at densities >2.7 g cm^{-3}, which can reduce the ease of use; however, with care SPT can be made to densities as high as 2.9 g cm^{-3} and used successfully. Another alternative heavy liquid was developed: Ludox^{TM}, an aqueous colloidal dispersion of silica particles in which the soil is suspended (Hassink 1995a, b; Meijboom et al. 1995; Magid et al. 1996; van den Pol-van Dasselaar 1999; Accoe et al. 2004). Ludox is highly viscous, which prevents porous particles from becoming saturated and results in floatation. The maximum density of Ludox is 1.4 g cm^{-3} (around the lower end of most organic debris) and, since saturation of the pore space does not occur, the separation density is more representative of the soil bulk density than of particle density. Thus, it was only following the widespread acceptance of SPT as a high density liquid for the separation of soil fractions, that research utilizing density fractionation began to increase substantially.

The theoretical framework underlying the density separation, including degree of organo-mineral interaction, extent of protection within aggregates, and association of SOM with different soil minerals, differs between several fractionation schemes developed and propagated throughout the literature. To divide soil into several fractions based on the degree of physical protection, or occlusion, within aggregates, Golchin et al. (1994a, b) modified the basic two-fraction method. Their modified method divides SOM into a non-protected inter-aggregate (''free'') LF separated from whole soil by floatation without sonication, a protected intra-aggregate (''occluded'') LF separated from remaining sediment by sonication and floatation, and a residual organo-mineral fraction (Golchin et al.1994b; see also Swanston et al. 2005). A more complex method follows the steps of the first, but subsequently separates the residual organo-mineral fraction by sequential fractionation into multiple fractions by increasing density in increments (Golchin et al. 1994a). Sequential fractionation of the same soil at increasing densities divides soil primarily based on mineralogy (Basile-Doelsch et al. in press); however, Arnarson and Keil (2001) and Sollins et al. (2006) also used sequential fractionation specifically to consider how organic coatings on different minerals could alter the particle density of the organo-mineral complexes (i.e., sequential density fractions), and how this might relate to C stabilization.

Many methods combine physical fractionation methods, i.e. size and density, in an attempt to target multiple, spatially explicit SOM pools that relate to stable aggregates. Based in part on earlier work by Cambardella and Elliot (1994), Golchin et al. (1994a), and Jastrow (1996), Six et al. (2000) developed a complex method in agricultural soils using a combination of size and density fractions to isolate organic matter within soil aggregates that have distinct functional roles in nutrient cycling and dominant stabilization mechanisms. Subtle modifications have been made; however, fractions generally are distinguished as either protected by organo-mineral interactions with silts and clays or by soil micro-aggregate structure, or unprotected, i.e. the light fraction and particulate organic matter not occluded in microaggregates (Six et al. 2002). Interpretation of any SOM fraction remains a function of individual soil and land use history; however, the methods introduced by Six et al. (2000) have been applied to many soils and progress has been made towards identifying soil fractions with important roles in C and nutrient cycling across ecosystem types.

Other researchers have gone beyond the combination of physical fractionation methods to a coupling of chemical and physical fractionation methodology (Trumbore et al. 1989; Trumbore 1993; Trumbore and Zheng 1996). Increased

application of radiocarbon analyses with density fractionation methods revealed that the heavy fraction included a significant proportion of rapidly cycling C (Trumbore et al. 1989). Trumbore et al. (1989) and Trumbore and Zheng (1996) attempted to estimate the size of this rapidly cycling pool using an acid hydrolysis treatment (6 N HCl). This method was not uniformly successful at removing rapidly cycling C from the HF in all soil types (Trumbore 1993). However, using HCl or other chemical treatments on single or sequentially separated heavy fractions appears to be promising method for characterizing rapidly cycling C in the organo-mineral fraction.

None of these methods is monolithic: each should be applied with deliberation and carefully adapted to the given soil and research goals. To this end, the simplest, 2-pool fractionation method, which forms the basic platform for all methods of soil density fractionation, is the primary focus of this paper.

Operational considerations

Density fractions are operationally defined, such that variations in the method will directly affect the isolated fractions. An important consideration is how closely the isolated fraction agrees with the conceptual definition of the fraction. When the agreement is not close, it provides the opportunity to reconsider ecosystem processes and how the method interacts with the soil being studied. During the development of methods over the last several decades, LF material has been described in a variety of ways including partially degraded material not associated with minerals, ''active'' organic matter pool, and as a ''labile'' pool. Rapid OM loss due to disturbance (i.e. cultivation) has commonly been observed, and was conceptually attributed to a LF material that was not greatly humified or protected by minerals and was thus more susceptible to loss (Greenland and Ford 1964). The conceptual division between biologically ''active'' LF and its complement, ''passive'' HF organic matter, was complicated by several factors recognized early in the method development. For example, it was recognized that low density, amorphous minerals float at densities <2.0 g cm^{-3}, resulting in the presence of mineral material with high adsorptive surface area in the LF, particularly in allophanic soils such as Andisols (Spycher and Young 1979). Also, charcoal, which is recalcitrant, floats at low densities and is included within LF material otherwise readily degradable by soil biota (Greenland and Ford 1964; Golchin et al. 1997). Baisden et al. (2002) identified charcoal, pollen grains, and seed coats, among other chemically resistant material, that concentrated in the occluded LF as ''square pegs'', which did not match the rest of the easily-decomposable light fraction material. Given the potential complexity in interpreting density fractions, the soil type, research questions, and ecosystem of interest should be included in the active consideration of the most appropriate variation of the method, including the density at which to fractionate.

Methods

Site descriptions

Soil was collected from a coniferous site in the Cascade Range of western Oregon and from a deciduous site in western Pennsylvania. These sites 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 C in soil (the Detrital Input and Removal Treatments or DIRT project, Nadelhoffer et al. 2004).

The coniferous site is located in H. J. Andrews Experimental Forest (referred to as 'HJA' throughout this paper) within an old-growth Douglas-fir (*Pseudotsuga menziesii*) and western hemlock (*Tsuga heterophylla*) stand established approximately 500 years ago following a stand-replacing fire. The soils are a complex of several alluvial/colluvial flows of similar parent material and roughly similar age. They are intermediate between Dystrochrepts and Andisols, depending on minor variations in content of glass and amorphous minerals (Dixon 2003; Sollins et al. 2006). 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). Mean annual temperature is 8.8°C, annual rainfall is 220 cm (Sulzman et al. 2005), and N deposition is 0.2 g N m^{-2} $year^{-1}$ (Vanderbilt et al. 2003).

The deciduous site is located in Bousson Experimental Forest (referred to as 'Bousson' throughout this paper) within a nutrient-rich, mixed stand which is dominated by black cherry (*Prunus serotia*) and sugar maple (*Acer saccharum*) in the canopy and by small maple saplings in the understory and is approximately 80 year old. An extensive ground cover of maple seedlings, mayapple (*Podophyllum* sp.) and troutlily (*Erythronium* sp.) is present. The soils are fine loamy, mixed mesic Alfisols of the Cambridge B series that are moderately well drained with a bulk density of 0.52 g cm^{-3}. Soil at Bousson has mixed clay mineralogy in an intermediate to advanced stage of weathering. Clays at the surface mineral horizons are approximately 30% illite and 45% vermiculite. The remaining is kaolinite and illite interstratified with small amounts of chlorite and montmorillonite (USDA-SCS 1979). The climate is temperate with a 4-month growing season and 4 months of snow cover. Mean annual temperature is 8.3°C, annual rainfall is 105 cm, and N deposition is 10–12 g N m^{-2} $year^{-1}$. The site has a history of agriculture, however evidence in the soil profile at our site indicates plowing did not occur, and the land may have been used for grazing or wood production.

Soil collection

At HJA, soil was collected in June 2002 from three depths in the mineral soil: 0–5, 5–10, and 10–20 cm using a bucket auger. For each depth, six sub-samples were taken within each of the DIRT experimental plots and were composited in the field and mixed (one composite sample per plot) for a total sample size between 500 and 1,000 g. The deeper soil increments (5–10 and 10–20 cm) are considered more fully by Keirstead (2004), and are only briefly addressed in this work. Each composite sample of 0–5 cm soil was sieved moist to remove material >2 mm and stored at 4°C in tightly sealed bags for several weeks before and during the density fractionation procedure. Due to high clay content and stickiness, soil samples from deeper depths could not be sieved moist and were dried overnight at 80°C and then sieved to remove roots and material >2 mm.

At Bousson, soil was collected in June 2003 only from the 0 to 5 cm of the A horizon. Six subsamples were taken from each DIRT experimental plot and composited and mixed resulting in a total of ~1,000 g of soil per plot. Soil was shipped overnight in a cooler, on ice, to Oregon State University where it was sieved moist to remove material >2 mm and stored at 4°C in tightly sealed bags for several weeks during the density fractionation procedure. At both sites, 5–10 kg of 0–5 cm A-horizon soil was collected and composited from areas adjacent to the DIRT plots to use for methods validation.

Density fractionation method

Soil collected adjacent to the DIRT plots at each site was used to determine the most appropriate density of sodium polytungstate (SPT, Sometu, Sherman Oaks, CA) at which to fractionate soil from each site. Fractionation of independent bulk soil samples was conducted at a range of densities (1.2, 1.4, 1.6, and 1.8 g cm^{-3}) to determine at which density the LF contained the least amount of mineral material at the same time the HF contained the least amount of organic matter (Sollins et al. 1999). During this test for the appropriate density for each soil, LOI (proportion organic matter) was used as a proxy for organic matter content and one minus LOI (proportion ash) for mineral content. Following fractionation at each density (detailed methods below), the fractions were oven-dried overnight at 105°C to determine dry weight followed by combustion in a muffle furnace at 550°C for 4 h to determine proportion lost on ignition (LOI). A density of 1.6 g cm^{-3} was determined to be the most appropriate, i.e. organic content of the HF stopped decreasing and the mineral content in the LF started increasing, for both sites.

Moist soil was added to 1.6 g cm^{-3} density solution of SPT (1:3 soil to SPT ratio, such to ensure enough separation distance between fractions) and then separated into two fractions by aspirating the floating LF into a separate

container, leaving the HF to be collected as sediment (Strickland and Sollins 1987). Moisture contents were determined (after drying 18 h at 105°C) for each sample immediately prior to fractionation. These were used to determine how much extra SPT to add to each soil-SPT mixture to compensate for soil moisture and thereby obtain a final density of 1.6 g cm^{-3}. Bottles containing soil and SPT were shaken while lying sideways on a bench top shaker for 1 h. Following shaking, soil and debris adhering to on the cap and sides were washed into the solution with 1.6 g cm^{-3} SPT and allowed to separate gravimetrically for 24–48 h, depending on the clarity of the solution between the floating LF and the sedimentary HF. After the LF was aspirated from the surface of the SPT, the sediment was subjected to the shaking (2 min), separation, and aspiration steps, twice more.

The LF collected during the three separation cycles was combined; the remaining sediment constituted the HF. LF was rinsed thoroughly on pre-combusted (550°C) Whatman GF/F filters (0.7 μm pore size) by submerging the material at least five times with deionized water and removing the leachate with a vacuum filtration system. SPT was rinsed from the HF material by adding deionized water, shaking, and centrifuging for 15 min. Following centrifugation the supernatant was decanted, more deionized water was added to the bottle, and the HF was re-suspended before another round of centrifugation. Each bottle was centrifuged and decanted at least three times. If there was additional material liberated during centrifugation in the first rinse, when the density was ≤1.6 g cm^{-3}, the supernatant was poured through a pre-combusted Whatman GF/F filter under vacuum, rinsed thoroughly with deionized water, and collected as LF. Following fractionation, the LF and HF were air-dried and stored at room temperature until the incubation experiment began.

Elemental analysis and stable isotopes

Carbon concentrations of 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. Stable C and N isotope values ($\delta^{13}C$, $\delta^{15}N$) were determined on separate sub-samples for each LF, HF, and whole soil sample to ensure adequate N or C was present in the sample for accurate analysis. $\delta^{13}C$ and $\delta^{15}N$ were determined at the EPA Western Ecology Division's Integrated Stable Isotope Research Facility using an elemental analyzer (ECS 4010, Costech, Valencia, CA) coupled to a Isotope Ratio Mass Spectrometer (IRMS, Finnigan MAT Delta Plus XL, ThermoQuest Finnigan, Bremen, Germany). Some $\delta^{13}C$ 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.

A mass balance approach was used to determine if the density fractionation process caused any change in %C, %N, $\delta^{13}C$ or $\delta^{15}N$. The %C or %N was calculated for the LF and HF by multiplying the concentration data obtained from the elemental analyzer for a particular fraction by mg fraction per gram of whole soil. Since $\delta^{13}C$ and $\delta^{15}N$ are ratios and are concentration dependent for scaling, the following mass balance equation was used for their comparison:

$$A_{blk} \approx \frac{(A_{lf} \cdot [E]_{lf}) + (A_{hf} \cdot [E]_{hf})}{[E]_{lf} + [E]_{hf}},$$

where A is the isotopic value in Atom% which is the ratio of heavy to total atoms (heavy + light) multiplied by 100 and E is the proportion of initial soil recovered as LF or HF. The conversion between δ and A was made according to the following equation:

$$\delta_{sample} = \left(\frac{A_{sample}}{R_{standard}(100 - A_{sample})} - 1 \right) 1000,$$

where $R_{standard}$ is 0.0112372 for PDB, and 0.0036765 for atmospheric N_2

One year incubation of mineral soils and density fractions

Bulk soil, LF, and HF from each field site were incubated for one year. Bench-top filtration units

(Falcon Filter, Becton Dickinson Labware) were modified according to Nadelhoffer (1990) to make microlysimeter chambers. Approximately 20 g of bulk soil, 6 g of LF, or 30 g of HF material was added to a chamber. These amounts were chosen such that approximately the same amount of C was incubated. To each sample, acid-washed sand was mixed in an amount equal the weight of substrate added in order to minimize anaerobic conditions during incubation (Swanston et al. 2002). At the start of the incubation, the sand and soil mixture was re-wetted by adding 10 ml of inoculum solution prepared from fresh soil of the respective site shaken in distilled water for 1 h (1:10 soil:water). Moisture content was kept constant by adding distilled, deionized water to each chamber weekly to maintain a known weight.

CO_2 efflux from the substrates was measured for each chamber on days 3, 5, 8, 12, 17, 26, 53, 151, 267, and 361 for HJA and days 2, 5, 13, 20, 28, 64, 114, 208, 290, and 367 for BOU. Headspace was purged with CO_2-free air and sealed for approximately 240 min while respired CO_2 accumulated. A 500 μl-calibrated syringe was used to mix the headspace gas several times before extracting a sample, which was immediately injected into a 5700A Hewlett Packard gas chromatograph fitted with a Poropak R 80/100 column and thermal conductivity detector. Cumulative respiration was calculated for each substrate in SAS (SAS Institute, v. 9.1, Cary, NC) using PROC EXPAND to calculate and approximate area under the curve using the trapezoidal method. Respiration rate and cumulative respiration for the 'summed fraction' were calculated by mass weight using CO_2 efflux measured from the LF and HF for a given soil sample.

Dissolved organic carbon (DOC) was measured in soil solution leached from each chamber on days 10, 35, 101, 151, 267, and 361 of the incubation for HJA, and 30, 118, 200, 301, and 371 for Bousson. One hundred ml of deionized water was added to the upper chamber of the microlysimeter and allowed to equilibrate with the soil for 1 h. At the end of an hour, the solution was drawn through the soil and filtered through a precombusted Whatman GF/F filter (0.7 μm pore size). Solutions were kept at 4°C until analysis if within 48 h, or were otherwise were frozen at –20°C. DOC analysis was by Pt-catalyzed high-temperature combustion (Shimadzu TOC-V CSH analyzer). Cumulative DOC release for the 'summed fraction' was calculated by mass weight using DOC production measured from the LF and HF for a given soil sample.

Residual tungsten (W)

The amount of W residue in the density fractions immediately following density separation and after one year of incubation was determined by extraction of 0.5 g air dried substrate with 1 M HNO_3 (1:6 soil to acid ratio). Soil and acid were shaken on a bench top shaker for 1 h, allowed to settle for 30 min, and poured through a Whatman GF/F filter (0.7 μm pore size). Filtrate was collected and W concentration in solution was analyzed by ICP (Perkin Elmer Optima 3000DV with a diode array detector) at the Central Analytical Lab in the Crop and Soil Science department at Oregon State University. Results were used to design the short incubation experiment described below: Prior to incubation, residual W was 40.8 ± 11.5 and 28.8 ± 10.0 mg W g^{-1} HF for Bousson and HJA, respectively. The amount of residual W remaining in the LF prior to incubation was similar, 20.4 ± 2.7 and 41.5 ± 5.7 mg W g^{-1} LF for Bousson and HJA, respectively.

Three month incubation of Oa horizon material

Oa horizon material was collected from three points near the DIRT field site at H. J. Andrews in Oregon. Oi and Oe horizon material was carefully removed and the thin Oa horizon directly above the mineral A horizon was collected in a large plastic bag and thoroughly mixed for homogeneity.

Two separate experiments were designed to determine whether microbial respiration was inhibited by nutrient limitation or residual amounts of Na and W present following density fractionation. In the first experiment, Oa horizon material was shaken in SPT, Na_2SO_4, or water

and rinsed as described for the density fractionation procedure (five replicate samples). Filtrate from the control samples was collected to use as inoculum for the experiment, as for the long incubation experiment, and the recovered Oa material was air dried at room temperature. Inoculum was added to the dry substrates in order to reach 40% water content, approximately the same as field conditions.

In the second incubation experiment, various solutions were added to air-dried Oa material in the second short incubation experiment to determine whether low nutrient availability or residual salts were inhibiting microbial respiration. Six different treatments were used: one control, two nutrient treatments, two SPT treatments and a Na treatment. In one nutrient treatment, half-strength Hoagland's solution (Sigma Aldrich) was used as a general macro and micronutrient solution. In the second nutrient treatment, a 0.4151 g l^{-1} ammonium sulfate solution was used to add N to the substrate in the same concentration as the N in Hoagland's solution. In two SPT treatments, SPT solution was added such that the concentration of W corresponded to the highest and lowest residual concentration of residual W present in our density fractions for the long-term incubation experiment (58.7 and 19.0 mg W g $soil^{-1}$). For the Na treatment, sodium sulfate was added so that the final concentration of Na was the same as in the high W treatment. Treatments were randomly assigned to each microlysimeter chamber so that there were four replicate samples for each and solutions were added to the dry substrates so that 40% moisture content was attained.

Both short incubation experiments were conducted using the same microlysimeter chambers, gas sampling protocol, and calculations as previously described. Headspace gas sampling began the day following the wetting of soil with solution (day 1) and continued on days 2, 3, 5, 7, 11, 15, 21, 28, 48, and 90.

Black carbon (BC) quantification

LF material (~1.5 g) from each treatment plot was digested in 175 ml of distilled, de-ionized water, 5 g $NaOCl_2$, and 5 ml acetic acid on a rotary shaker (240 rotations per minute) for 2 h at room temperature for three digestion cycles (adapted from Simpson and Hatcher 2004). Following the final cycle, the residue was poured onto a pre-combusted Whatman GF/F (0.7 μm pore size) and rinsed thoroughly with distilled water (total rinse volume of 400 ml). The residue was then passed through a 500 μm sieve to separate fine particulate material and adhering clay and amorphous mineral particles from the larger organic matter fragments. This step allowed the larger fragments to remain mostly free of mineral coatings that could make identifying BC, which is present at our sites in the form of charcoal with visible plant morphology, more difficult in subsequent steps. Radiocarbon analysis (below) determined that the <500 μm material was modern (containing bomb-C in amounts similar to recent litter); therefore, we assumed that little BC material was lost during the size fractionation.

Charcoal pieces were manually separated from two subsets of the >500 μm residue under a dissecting microscope with a pair of fine-pointed forceps. The subsets were averaged to calculate a total recovery of BC for each sample. Since the presence of charcoal in the soil pre-dated the establishment of our experiment, all recovered BC material from each site was combined together to have enough material for accurate radiocarbon analysis.

Radiocarbon-based mean residence time (MRT)

Radiocarbon concentration was measured on the Van de Graaff FN accelerator mass spectrometer (AMS) at the Center for Accelerator Mass Spectrometry at Lawrence Livermore National Laboratory, CA. Samples were prepared for analysis by combustion of organic C to CO_2 with CuO and powdered Ag in sealed evacuated tubes and subsequent reduction of the CO_2 onto iron powder in the presence of H_2 (Vogel et al. 1984). Radiocarbon data are expressed according to Stuiver and Polach (1977) as $\Delta^{14}C$, the deviation in parts per thousand from the absolute international standard activity (^{14}C:^{12}C ratio of oxalic

acid corrected for decay since 1950). Independently measured $\delta^{13}C$ values for each fraction were used to adjust the $\Delta^{14}C$ values for mass-dependent fractionation. Since the radiocarbon concentration in the bulk soil and LF was influenced by the inclusion of BC in the soil, BC was quantified for each sample, $\Delta^{14}C$ was determined, and bulk soil and LF were mathematically adjusted to exclude the influence of the BC on the C pool size and $\Delta^{14}C$.

Mean residence times of density fractions were calculated with a time-dependent steady-state model (Trumbore et al. 1995; Gaudinski et al. 2000). Three fundamental assumptions of the model are: (1) bulk C inputs equal loss in each pool through time, though $\Delta^{14}C$ varies; (2) the $\Delta^{14}C$ of inputs to the light and HF pools are equal to that of the atmosphere in the previous year (thus, the model does not account for multi-year lags before input or transfer between pools and resulting MRTs should be considered maximum values); and (3) the $\Delta^{14}C$ of inputs to the pool attributed to OM lost during fractionation (SPT-loss) was mass-weighted between LF and HF. Three chronologies of annual atmospheric $\Delta^{14}C$ beginning in calendar year 1511 were used in the model (Stuiver et al. 1998; Hua and Barbetti 2004; Levin and Kromer 2004).

Results

Structural composition of recovered fractions

Separation of the organic debris from organo-mineral particles requires that the soil aggregate structure be disrupted to some degree, depending on the length of shaking and whether sonication was used. Scanning electron microscopy (SEM) images demonstrate the extent of disruption of the soil structure (Fig. 1A) by our basic fractionation method at both sites (Fig. 1B–D). Light fraction, particularly at the old-growth coniferous site, is composed primarily of woody debris including bark and roots, fungal fruiting bodies, and charcoal (Fig. 1B). Light fraction at the deciduous site appeared to originate mostly from deciduous leaves and fine roots (Fig. 1C). Heavy fraction from both sites consists of mineral clays and sand-size particles that had some surface organic deposits (Fig. 1D). Black C (BC) was abundant in the LF material at HJA and to a lesser extent at Bousson. Goldberg (1985) described ''woody'' and ''amorphous'' forms of BC that can result from the combustion of plant material; both were present at both sites (Fig. 1E, F).

Recovery of C and N and isotopic signature of density fractions

Light fraction accounted for less than 4% of the recovered mass at both sites (Table 1). As expected, the C:N ratio of LF was greater than HF at both sites. At Bousson, the LF C:N ratio fell in the center of the range reported by Sollins et al. (2006) (a C:N ratio of ~10–40) for a wide variety of soils that differed in mineralogy, texture, location, and land management and were all separated at 1.6 g cm^{-3}. Light fraction from HJA fell above the range of the C:N ratios reported for that density; however, was similar to LF separated at a density of 1.65 g cm^{-3} from soil collected at the same site (Sollins et al. 2006). A mass balance of recovered C and N showed that 15% of C and 9% of N was recovered as LF at HJA (Fig. 2). At Bousson, slightly less of the total C and N was present in LF than at HJA (9% for C and 5% for N). Substantial pools of C and N, however, could not be accounted for in the recovered LF or HF from both sites. We calculated that between 17% and 26% of total soil C and N was mobilized in the SPT solution and subsequently discarded during the density fractionation process in both soils (Fig. 2). The C:N ratio of the mobilized pool was 8.3 at Bousson and 38.8 at HJA, indicating potentially different sources of mobilized material between the sites.

As part of a larger investigation into changes in the stable isotopic signature of SOM pools following continuous alterations in detrital inputs to the soil (DIRT Project, see Nadelhoffer et al. 2004), isotopic analysis of the bulk soil and density separates from various depths was conducted at HJA (Keirstead 2004). Based on the mass balance of these density fractions from HJA and those fractions from the surface 0–5 cm of soil from Bousson, there is indication of a potential for preferential loss of the light isotope

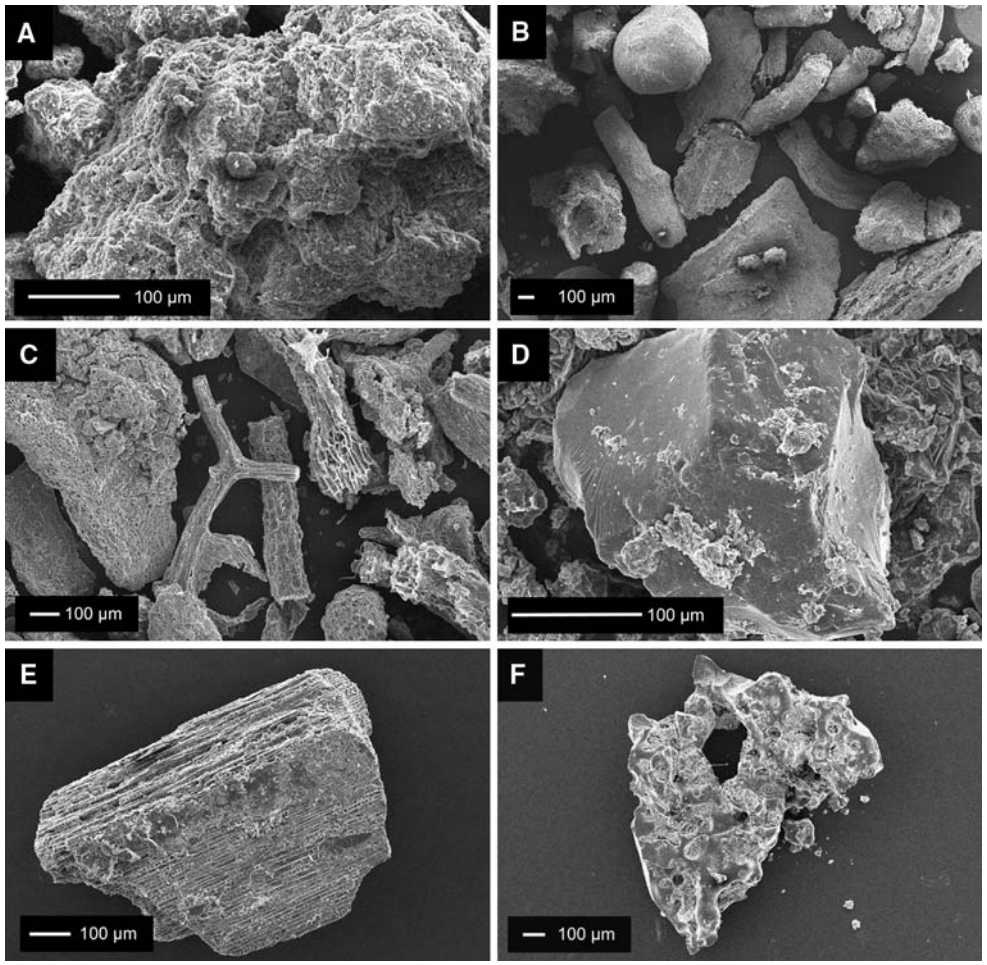

Fig. 1 SEM images of a Bousson soil aggregate (**A**), LF material from HJA (**B**), LF from Bousson (**C**), HF from Bousson (**D**) and different structures of charcoal originating from plant biomass isolated from H. J. Andrews and Bousson soils; woody (**E**) and amorphous (**F**)

Table 1 LF and HF recovery as a percent of the total mass recovered; C, N concentrations and the C:N ratio of the bulk soil and density fractions; amount of C and N of in the bulk soil and recovered density fractions normalized to the initial soil fractionated; percent of total C and N recovered (n = 3, values are means ± 1 S.E.)

	Bousson			H. J. Andrews		
% LF	2.2 ± 0.3			3.6 ± 0.6		
% HF	97.8 ± 0.3			96.4 ± 0.6		
	C (%)	N (%)	C:N	C (%)	N (%)	C:N
Bulk	6.1 ± 0.3	0.5 ± 0.0	12.5 ± 0.6	6.8 ± 1.1	0.2 ± 0.0	34.3 ± 3.6
LF	27.4 ± 4.3	1.1 ± 0.0	25.4 ± 5.4	28.7 ± 0.3	0.5 ± 0.0	60.7 ± 6.1
HF	4.7 ± 0.3	0.4 ± 0.1	13.3 ± 1.8	4.9 ± 0.8	0.2 ± 0.0	29.9 ± 1.5
	C (g C kg^{-1} soil)		N (g N kg^{-1} soil)	C (g C kg^{-1} soil)		N (g N kg^{-1} soil)
Bulk	60.8 ± 3.1		4.9 ± 0.3	67.9 ± 10.7		1.9 ± 0.1
LF	5.8 ± 0.3		0.2 ± 0.05	10.3 ± 1.8		0.2 ± 0.03
HF	44.6 ± 1.2		3.4 ± 0.1	38.4 ± 5.9		1.3 ± 0.2
LF + HF	50.4 ± 1.4		3.6 ± 0.2	48.7 ± 7.7		1.5 ± 0.2
Recovered (%)	83 ± 6		74 ± 2	72 ± 2		74 ± 6

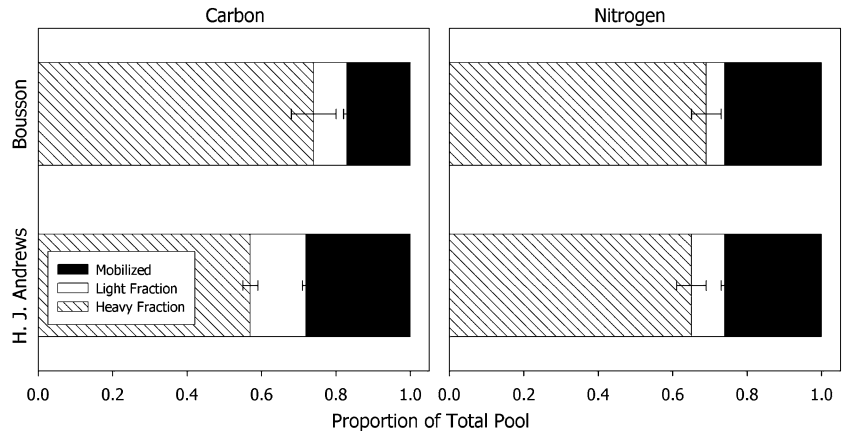

Fig. 2 C and N balance for the recovered pools (LF and HF) and the mobilized pool (SPT-soluble fraction washed away during density fractionation) for the 0–5 cm mineral soil at two forested sites, values are means ± 1 S.E

to occur during the density separation process. No difference was present in the C isotopic composition of the bulk soil and mathematically recombined density fractions from three different soil depths at HJA, or of either C or N during density fractionation of 0–5 cm mineral soil at Bousson (Fig. 3). However, at HJA we found a preferential loss of the light N isotope (^{14}N) resulting in density fractions that were isotopically heavier than the original bulk soil (an average ‰ shift of 1.3) (Fig. 3).

Respiration and DOC leaching during incubation

During a year-long incubation of bulk soil and density fractions, cumulative respiration normalized by initial substrate mass (mg CO_2 g^{-1} substrate) was greatest from the LF, followed by the bulk soil and HF for both soils (Fig. 4, upper panel). However, when cumulative respiration was normalized by the initial amount of C present in the substrate (mg CO_2 g^{-1} $C_{initial}$), bulk soil respiration from H. J. Andrews (HJA) soils was much higher than either light or heavy fraction respiration (Fig. 4, lower panel).

Initially, respiration rate from the bulk soil was an order of magnitude greater than from the summed fractions at Bousson (Fig. 5A). By day 5 of the incubation period, bulk soil respiration dropped to nearly the same rate as the summed fractions and remained for the rest of the incubation. HJA bulk soil also showed an initial flush of respiration, although not to the extent of the Bousson bulk soil. Respiration rate of the bulk soil from HJA dropped after 26 days and leveled off after 150, yet remained elevated compared to the summed fractions throughout the incubation period.

Cumulatively, bulk soil from both sites respired substantially more than the mathematically recombined LF and HF (summed fractions) (Fig. 5B). At Bousson, cumulative respiration from the LF, normalized by the initial amount of C present in the substrate (mg CO_2 g^{-1} $C_{initial}$), increased steadily during the year incubation period and ultimately was not different from bulk soil. As expected, cumulative respiration from HF was less than from LF and bulk soil. In contrast, at HJA, cumulative respiration was not different between the LF and HF but was significantly greater from bulk soil than from LF and HF. Opposite to the patterns in respiration, losses of C as DOC in leachates were significantly greater for the summed fractions than the bulk soil at both sites (Fig. 5C). Total losses of C (DOC and respiration combined) were greater for the summed fractions than for the bulk soil: bulk soil C losses were 70% of the summed fraction C losses at Bousson and 79% of the summed fractions at HJA.

Previous research with similar results from respiration studies of density fractions (i.e. Swanston et al. 2002; Magid et al. 1996) hypothesized that either residual SPT or low nutrient availability was responsible for low respiration from density fractions compared to bulk soil. We tested directly whether an inhibitory effect of residual

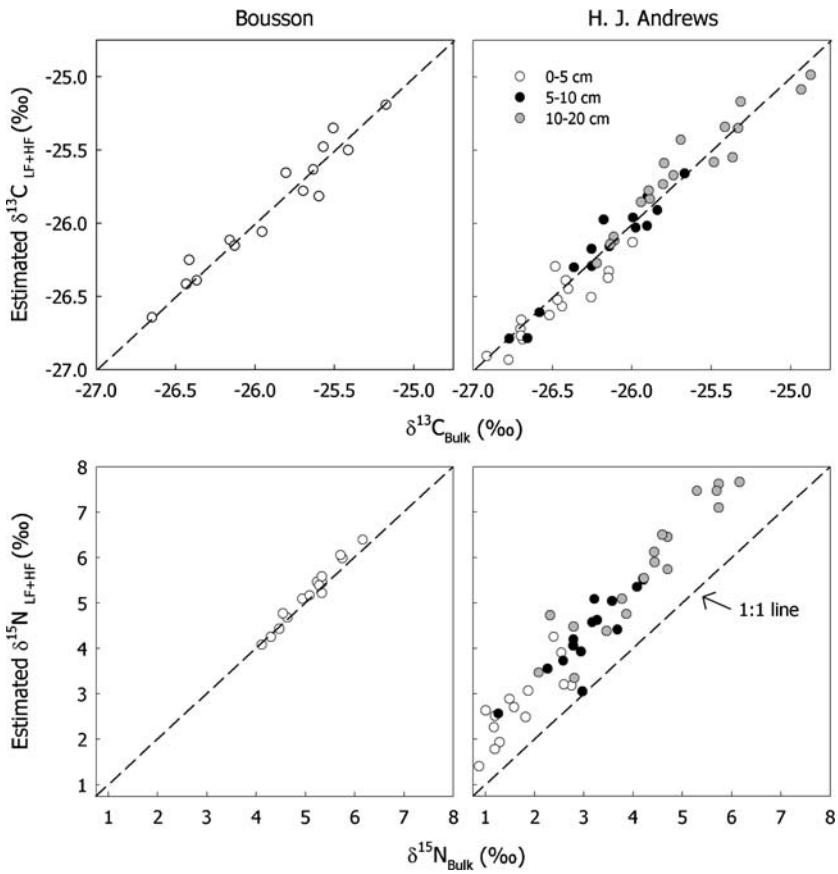

Fig. 3 Isotopic composition of the bulk soil compared to the mathematically recombined density fractions for ^{13}C (*upper panels*) and ^{15}N (*lower panels*). Each point represents one sample

SPT or a salt effect caused a decrease in respiration. Also, we tested whether a lack of nutrients limited respiration and whether the concentration of residual SPT mattered. Cumulative respiration from HJA LF deviated the most from our expectations, so we used Oa horizon material from HJA as a proxy for LF during these experiments.

In the first short incubation experiment (shaken in SPT, Na_2SO_4, or water and rinsed as for the density fractionation procedure) both the Na_2SO_4 and 1.6 g cm^{-3} sodium polytungstate (SPT) solutions significantly reduced soil respiration compared to the distilled water control ($p < 0.001$, $F = 31.80$) (Fig. 6A). Shaking and rinsing with SPT (65% of the control value) resulted in the greatest reduction in respiration. Similar results were obtained from the second short incubation experiment when W was added to O_a horizon material. Both levels of W addition, which were in concentrations that spanned the amount of residual W measured, reduced cumulative respiration compared to all other treatments ($p < 0.001$, $F = 249.04$) (Fig. 6B). Respiration from the low W treatment was reduced to 35% of the control values, and the high W treatment to 31%. In both experiments, the addition of a Na solution also significantly reduced respiration compared to the control: 85% of the control for the first incubation (Fig. 6A) and 56% of the control for the second incubation (Fig. 6B).

Although there appears to be a salt and W effect on respiration during this short incubation experiment, the long-term incubation differed in that the density fractions were leached many times during the incubation period. As a result, residual W from both soils was greatly reduced by the end of the incubation period. The greatest amount of residual W left in any fraction was 1.2 ± 0.1 mg W g^{-1} soil, representing a 94.3–98.3% decrease for all substrates.

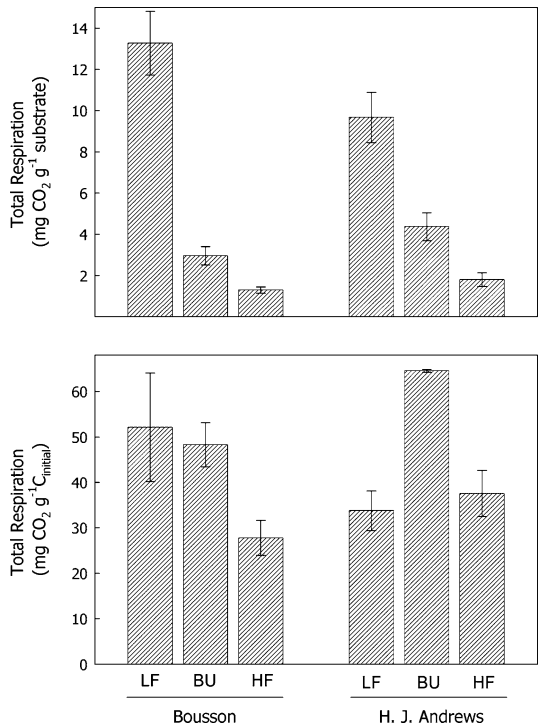

Fig. 4 Cumulative C respired during a year-long incubation of density fractions and bulk soil normalized to the amount of substrate (*upper panel*) or the amount of C (*lower panel*) incubated. Bars are means ± 1 S.E

The mean C:N ratio of LF was high (61.0 ± 6.1) at HJA; however, the addition of nutrient solutions to HJA Oa material did not result in increased respiration during the short incubation experiments. In fact, both the Hoagland's solution and the N solution significantly reduced respiration compared to the control ($p < 0.001$, $F = 249.04$) (Fig. 6B).

Mean residence time of density fractions and mobilized organic matter

The amount of black carbon isolated from LF was variable between plots at both sites (Table 2). At HJA, the present-day forest developed following a stand replacing fire approximately 500 years ago. Accordingly, up to 1.2% of total soil C was from BC in the form of charcoal, which had a ^{14}C age of 770 years BP (±45 years, analytical error). The fire history is not known at Bousson, but up to 1.9% of total soil C was from charcoal, which had a ^{14}C age of 630 years BP (±40 years analytical error). The radiocarbon content of the charcoal was removed mathematically by mass balance from the values of the light fraction and bulk soil used in the model to estimate MRT. Generally, all soils and density fractions had some incorporation of atmospheric bomb carbon (Table 2).

The nature of the bomb curve is such that there are often two possible solutions to fractions containing a significant amount of bomb carbon, reflecting the increasing side of the bomb curve and the decreasing side respectively. Such is the case at Bousson, where two solutions for the MRT of the LF and SPT-soluble fraction were possible (Table 3). Regardless of which solution for the LF is considered however, the average radiocarbon-based MRT of the LF was short (2.7 ± 0.7 or 89 ± 12 years) compared to the HF (323 ± 24 years) at Bousson (Table 3). In contrast, LF and HF MRT were similar to each other at HJA (LF: 117 ± 24 years; HF: 93 ± 12 years). In addition to the density fractions, the MRT of the organic matter mobilized during fractionation was estimated by difference. At Bousson, the MRT of the SPT-mobilized fraction was 134 ± 46 years associated with the shorter MRT solution for LF, or 27 ± 12 years associated with the longer MRT solution for LF. At HJA the SPT-mobilized fraction had a MRT shorter than both solutions at Bousson, 19 ± 11 years.

Discussion

Environmental influence on density fractions

The LF and HF historically were considered to be 'active' and 'passive', although ample evidence to the contrary has emerged over time. Once separated and no longer interacting with one another within the intact soil matrix, the LF and HF do not cycle C and N as they would in the bulk soil (Sollins et al. 1984; Boone 1994; Swanston et al. 2002, 2004; Bhupinderpal-Singh 2005; Crow et al. 2006), further complicating the interpretation of their ecological roles. Rovira and Vallejo (2003) determined that the least chemically recalcitrant OM in Mediterranean calcareous forest soils was protected within aggregates in the ''occluded'' LF and the HF (using the Golchin et al. 1994a, b

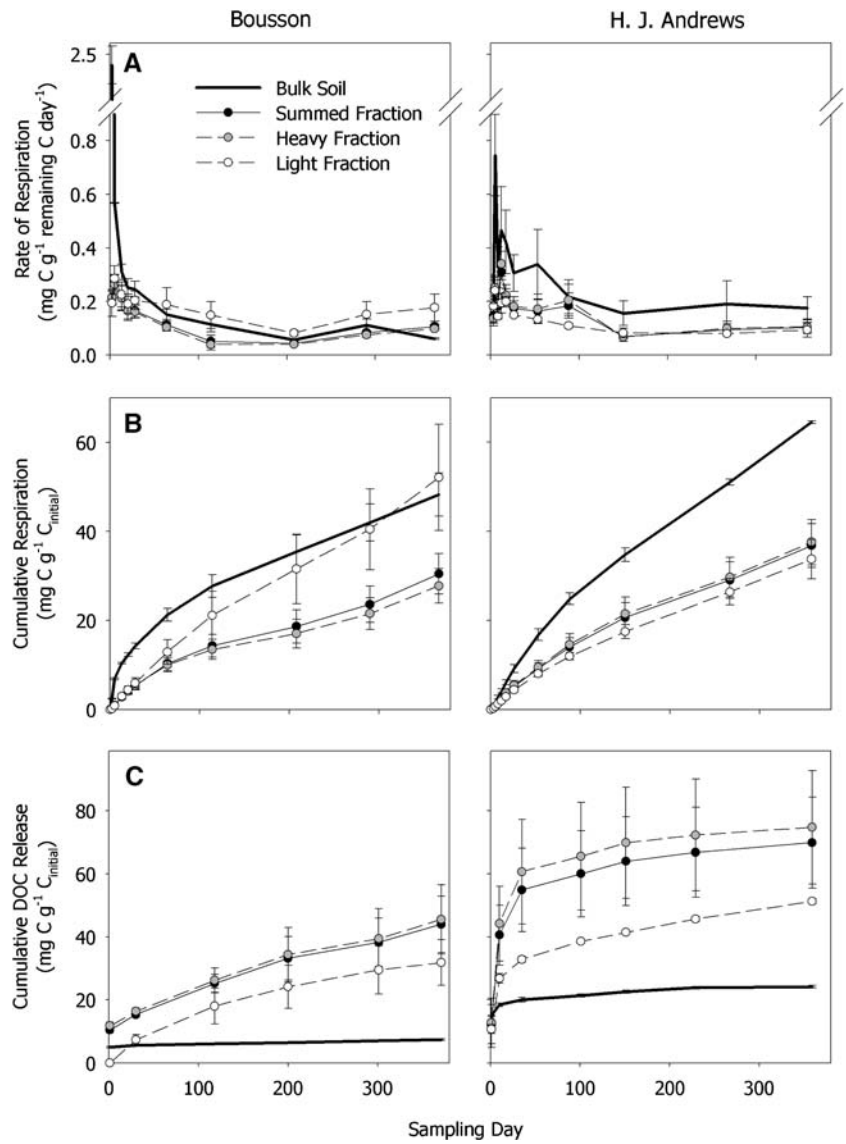

Fig. 5 Rate of respiration (**A**) cumulative respired CO_2 (**B**) and DOC released (**C**) during a year-long incubation of bulk soil, density fractions, and mathematically summed LF and HF (summed fraction) from two forested sites, values are means ± one S.E

method). The free-LF material (separated at 1.6 g cm^{-3}), although composed of recognizable plant material, was of an intermediate chemical recalcitrance and not always the most fresh and non-decomposed material (Rovira and Vallejo 2003). Swanston et al. (2005) found the heavy fraction to be initially more responsive to recent atmospheric enriched-radiocarbon inputs (EBIS project, Oakridge, TN) than the ''occluded'' LF. In some soils, HF is less resistant to decomposition than the LF (Crow et al. 2006).

Generally, the free-LF responds most strongly to changes in C inputs and environment, but characterizations of the ''occluded'' LF and the HF (isolated as a single fraction) are much less consistent. These fractions seem to vary most strongly with differences in soil structure and mineralogy, respectively. However, the nature of the C inputs cannot be discounted when considering the relationship of the light and heavy fraction to each other in the context of ecosystem function. When we examined the LF and HF isolated from the deciduous forest at Bousson, the results generally reflected the early views of these fractions in respect to rates of C cycling. Incubation results and ^{14}C-based MRT each

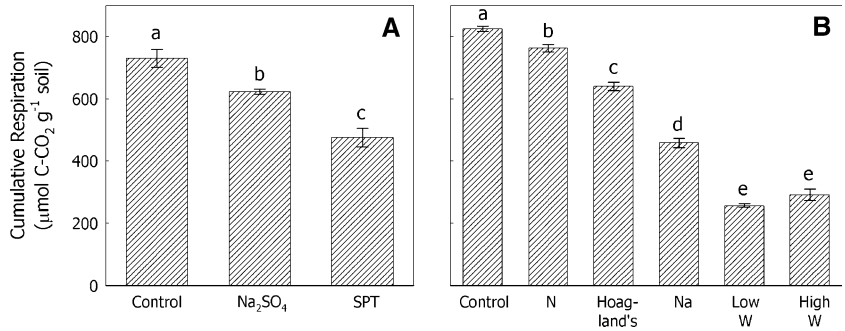

Fig. 6 Cumulative respiration from O_a material from the H. J. Andrews field site during a 90-day incubation following (**A**) shaking and rinsing in distilled water (Control), Na_2SO_4, or SPT solutions and (**B**) adding distilled water (Control), nutrients (N and Hoagland's solution), salt (Na), or tungsten solutions of two concentrations (Low W and High W). The values are means ± 1 S.E, $n = 5$ for (**A**)and $n = 4$ for (**B**)

Table 2 Isotopic data for individual soils and density fractions used for estimation of the radiocarbon-based MRTs of SOM pools

Site	Soil fraction (replicate)	$\delta^{13}C$ (‰)	CAMS #	$\Delta^{14}C$ (‰) ± analytical error	% of total C present as BC
Bousson	Bulk (1)	–25.2	102770	26.7 ± 4.4	1.9
	Bulk (2)	–25.6	102771	17.7 ± 4.0	0.4
	Bulk (3)	–25.4	102769	9.3 ± 4.0	0
	Light fraction (1)	–26.1	102763	45.8 ± 4.1	19.8
	Light fraction (2)	–27.1	102767	82.3 ± 4.2	3.6
	Light fraction (3)	–27.1	105378	90.1 ± 4.7	0
	Heavy fraction (1)	–25.2	102765	2.7 ± 3.5	n.a.
	Heavy fraction (2)	–25.7	105385	8.4 ± 3.9	n.a.
	Heavy fraction (3)	–25.1	102773	–5.5 ± 3.9	n.a.
H. J. Andrews	Bulk (1)	–26.4	97207	100.1 ± 4.4	0.6
	Bulk (2)	–26.2	97208	54.8 ± 4.2	1.2
	Bulk (3)	–27.0	114322	101.6 ± 4.3	0.3
	Light fraction (1)	–26.8	97276	113.8 ± 4.2	3.7
	Light fraction (2)	–26.4	97277	43.7 ± 3.6	8.2
	Light fraction (3)	–27.4	97278	62.8 ± 3.6	2.2
	Heavy fraction (1)	–26.5	97209	99.0 ± 4.4	n.a.
	Heavy fraction (2)	–26.4	97210	77.4 ± 4.3	n.a.
	Heavy fraction (3)	–27.0	97211	116.8 ± 4.4	n.a.

Percent of total C present as BC for bulk soil was calculated by the mass balance from the light fraction BC amounts

indicated that the LF-C turned over at faster rate than the HF-C. However, the incubation and MRT results for the fractions separated from the mature coniferous forest at HJA reflected the recalcitrant nature of the woody and charcoal inputs, as well as the high reactivity and surface area of the dominant mineralogy typically associated with andic soils. The incubation and ^{14}C results from HJA fractions were again consistent, each indicating that C cycles through these fractions at a similar rate, if not more slowly through the LF.

Organic matter mobilized

A substantial amount of C and N was mobilized and subsequently discarded during the density separation process. Soil from HJA displayed an isotopic discrimination of N during the density fractionation, with a preferential loss of the light isotope, associated with the mobilized OM. We did not find a similar preferential mobilization of the light isotope of C at either site nor of the light N isotope at Bousson. It is not clear why the isotopic fractionation of N occurred in the HJA soil alone

Table 3 Radiocarbon-based estimates of MRTs in years for the light fraction, heavy fraction and soluble SOM pools

		Light fraction		Heavy fraction	SPT-mobilized	
Site	Replicate	MRT (range)		MRT (range)	MRT (range)	
Bousson	1	1.5 (1.5–1.5)	65 (115–59)	312 (328–297)	43 (50–36)	4.5 (1.0–5.0)
	2	3.0 (2.3–3.7)	104 (109–99)	288 (305–273)	172 (184–161)	35 (36–33)
	3	3.7 (3.0–4.5)	99 (104–94)	369 (369–331)	186 (224–164)	42 (52–36)
H. J. Andrews	1	70 (87–66)		90 (94–85)	18 (0.5–18)	
	2	149 (157–142)		115 (121–109)	38 (40–36)	
	3	131 (138–125)		73 (77–70)	0.5 (0.5–0.5)	
Bousson	Mean ± S.E.	2.7 ± 0.7 or 89 ± 12		323 ± 24	134 ± 46 or 27 ± 12	
H. J. Andrews	Mean ± S.E.	117 ± 24		93 ± 12	19 ± 11	

Both possible solutions for MRT are presented when applicable. The range of error for the estimated MRT, calculated using the minimum and maximum fraction modern (F) values possible for each fraction due to analytical error in the steady-state model, are also reported

and not Bousson. One possibility is that mineral-bound NH_4^+ was present at HJA and exchanged in solution with the tungsten ions. Given that the same separation procedures were used on both soils, the isotopic fractionation was likely soil dependent rather than method dependent. Ignoring this potential for isotopic discrimination may lead to misinterpretation of N fluxes, and C and N interactions. Conversely, focus on this fractionation may reveal patterns that are otherwise obscured.

Overall, both of our soils demonstrated a reduction in cumulative respiration between the bulk soil and recombined density fractions (summed fraction) following the one-year incubation. At Bousson, a large initial flush of respiration from bulk soil established the difference in the cumulative C loss curve, which then persisted for the duration of the incubation period. Without the initial flush of respiration from the bulk soil, cumulative respiration would have been similar between bulk soil and summed fractions. Conversely, HJA bulk soil and summed fractions had different rates of respiration continuously during the one-year incubation and, as a result, cumulative C loss continued to diverge during the incubation.

At Bousson, the mobilization of a labile C pool associated with the large initial flush of respiration from bulk soil could account for the decrease in respired C from the summed fraction. If the shorter LF MRT solution for Bousson is considered, then the mobilized organic matter pool (calculated by mass balance) was much longer at Bousson than at HJA (134 years compared to 19 years). Such a long MRT of this pool at Bousson indicates that the mobilized C was not primarily from fresh detrital inputs. The low C:N ratio of the SPT-mobilized pool (8.1 ± 2.7) also supports this idea, since the C:N ratio typically decreases with degree of decomposition. However, the longer LF MRT solution for Bousson was associated with a shorter mobilized organic matter MRT (27 years). In this case, the results are similar for the soil from HJA, the MRT of the mobilized C was substantially shorter than that for either density fraction. The C:N ratio of the SPT-mobilized pool also was substantially greater at HJA than at Bousson (41.8 ± 10.2) perhaps indicating a less degraded substrate was removed during density fractionation. On the other hand, greater rates of N deposition at Bousson may also contribute to higher concentrations of soluble N and a similar pattern in the C:N ratio of the soluble fraction between sites.

The mobilization and loss of a fast-cycling C pool may have contributed substantially to the difference in respiration from the summed fractions compared to the bulk soil over the course of the incubation at both sites. Conceptually, if 28% of bulk soil C had not been lost during fractionation of the HJA soil and ~5% (1/MRT) of this pool had been respired during the year incubation period (equation below), then an additional 30.95 mg $C\text{-}CO_2$ g^{-1} $C_{initial}$ would have been added to the cumulative C lost through

respiration. This represents an amount approximately equal to the gap between respiration from bulk soil and summed fractions.

$$\text{Estimated total } CO_2 = \frac{CO_2 \text{ respired}_{SF} + \left[\left(\frac{1}{MRT}\right) * (\text{C lost})\right]}{[C]_{BU}},$$

where estimated total CO_2 (C-CO_2 g^{-1} $C_{initial}$) is the total respiration expected if the organic matter mobilized in SPT during density fractionation had not been lost, and CO_2 $respired_{SF}$ is the cumulative mg C respired g^{-1} summed LF + HF soil, C lost is the amount of C (mg g^{-1} soil) lost during density fractionation, and $[C]_{BU}$ is g C g^{-1} bulk soil.

Based on this estimate, summed fraction respiration at HJA would increase from an average of 57% of bulk soil respiration to 105% of bulk soil. By the same calculation, if 17% of C had not been lost during fractionation and using MRT of 27 years, summed fraction respiration at Bousson would only increase from 63% of bulk soil to 88% of bulk soil, the alternate solution accounting for even less. At HJA, the organic matter mobilized during density fractionation may have been a more labile organic matter pool originating from fresher inputs than at Bousson, which, in the bulk soil, was more available for microbial decomposition throughout the incubation period. This disparity between sites also indicates that factors other than just the loss of SPT-mobilized OM likely influenced respiration from the fractions, particularly at Bousson.

Light fraction composition

Several factors beyond the SPT-soluble C rinsed during the density fractionation procedure, including the presence of black carbon and woody material that is resistant to decay and some mineral-bound organic matter, influenced the apparent decomposition of LF during incubation. In 1964, Greenland and Ford described LF material as irregularly shaped plant fragments, small amounts of charcoal, and partially degraded plant material. Nonetheless, they measured the ash content of the LF at ~50%, resulting from the presence of plant phytoliths, amorphous clay minerals, and quartz that had also floated during fractionation (Greenland and Ford 1964). Similarly, ash content of the LF we recovered at Bousson was 42.5% and at HJA was 31.0%, indicating considerable mineral content in the LF at both sites. Organic coatings on the mineral particles in the LF may have had different chemistry and MRTs than the remainder of the LF material (Sollins et al. 2006).

The appropriate method for the quantification of BC in forest soils depends on the form present, which can exist along a combustion continuum ranging from slightly burned biomass, to charcoal, to highly condensed soot (Hedges et al. 2000; and reviewed by Masiello 2004). Schmidt et al. (2001) found a 500-fold difference in BC content for the same sample quantified by several methods, and the issue of methodology is still being debated in the literature (Schmidt et al. 2001; Skjemstad et al. 2002; Masiello 2004; Simpson and Hatcher 2004; Krull et al. 2006; Knicker this volume). The presence of BC alters both the apparent rate of respiration during incubation, particularly when using units of CO_2 g^{-1} total C since BC has a high C concentration, and the radiocarbon-based MRT estimates, thus needs to be quantified in some way.

Inhibition of microbial respiration

We initially hypothesized that some form of microbial inhibition contributed to the decrease in respiration from the summed density fractions relative to the bulk soil. Although the C:N ratio of the LF is large (61.0 ± 6.1 at HJA), nutrient limitation was not likely to be a factor controlling the reduction of respiration from the LF.

Charcoal and woody debris, both characterized by a high C:N ratio, are generally resistant to decay and thus would not strongly affect the nutrient demands of microbes. In fact, during incubation of Oa material, respiration was reduced in both nutrient addition treatments. Soil at the HJA DIRT site receives a large amount of woody inputs and has greater lignin content than the Bousson site (S. E. Crow unpublished data). It is possible that the reduction in respiration when nutrients were added to the Oa substrate from HJA was due to deactivation of the phenol-oxidase enzyme (used by microbes for lignin degradation) as a result of excess soluble N (Sinsabaugh et al. 2002; Waldrop et al. 2004) or a shift in allocation of metabolites from microbial respiration to microbial biomass or storage in the presence of available nutrients.

In the initial stages of the short incubation, residual Na and W reduced respiration from the substrates. This indicates that both Na and W present in the SPT solution influenced the ability of the microbial community to utilize the substrate, as previously suggested by Magid et al. (1996), Compton and Boone (2002), and Swanston et al. (2002). Yet, residual W from both soils was greatly reduced by the end of the incubation period, during which time the substrates had been leached repeatedly. The most residual W left in any fraction was 1.2 ± 0.1 mg W g^{-1} soil, representing a 94.3–98.3% decrease for all substrates. Thus, at some point during the incubation, the inhibition of respiration by residual W was most likely reduced or eliminated.

During a similar long-term incubation of whole soil and density fractions, Swanston et al. (2002) found initially that microbial biomass was greater in the bulk soil than in the density fractions even though all substrates had been inoculated. By day 10 however, the LF and HF had over five times the active microbial biomass of the bulk soil, although the rate of respiration (mg CO_2 g^{-1} $C_{remaining}$ day^{-1}) from the density fractions remained approximately half of the whole soil (Swanston et al. 2002). The disparity in respiration rate between the bulk soil and the fractions had disappeared by 120 days. It seems that although W is initially inhibitory, it may not have a lasting effect on the rate of respiration per unit C, though not necessarily per unit biomass. The inhibition of respiration related to Na and W toxicity in our soils was probably not strong enough to account for the substantial reduction of cumulative respiration from the summed density fractions.

Concluding remarks

Widely used models over the past several decades, including BIOME-BGC and CENTURY, have represented the heterogeneity of SOM as discrete conceptual pools of various sizes and turnover times (Jenkinson and Raynor 1977; Running and Coughlin 1988; Parton et al. 1994). Ultimately, the conceptual SOM pools in models need to be constrained by measurable pools that can be monitored in an ecosystem and used to validate model predictions. Without such validation, it is not clear how well these models will represent ecosystem response to changing conditions when used prognostically. Increasingly, there are attempts to integrate soil physical fractions or constraints that were derived from those fractions, into process models (Six et al. 2002) and estimates of carbon MRTs (Gaudinski et al. 2000; Baisden et al. 2002a; Torn et al. 2005).

Complex process models incorporate numerous interactions between ecosystem variables. Although progress has been made, the ability to quantitatively separate and determine turnover rates of various SOM pools for input into these process models has not been fully attained. The interactions among variables, particularly with reference to SOM stabilization/destabilization mechanisms, are inextricably integrated within the OM fractions produced by methods like density fractionation. No single method can viably isolate fractions to reasonably represent every variable and interaction. Indeed, some commonly used physical fractionation schemes rapidly generate an unmanageable number of fractions from a modeling perspective, and these fractions will nonetheless generally include more than one major OM pool. Applying several methods independently to the same soil may help

identify mechanisms, yet will not result in the desired exclusive and discrete fractions. Further, the inherent limitations of these methods should be taken into account when attempting to integrate these fractions into process models, with the understanding that the method alone will introduce artifacts. Failing to consider these artifacts can lead to misinterpretation of the role that those fractions may play in ecosystem processes. This misinterpretation can be exacerbated when measurements are extrapolated to a changing environment. Conversely, using density fractionation with a clear understanding of its operational limitations, and associated limitations on interpretation can provide valuable insights into ecosystem processes.

Density fractionation is a powerful step to include in methods for quantitatively isolating SOM into fractions with relevance to soil function. Particularly as the use of density fractionation has expanded since the introduction of SPT, the implications of dispersing soil in SPT should be addressed directly. A modification of the method, such as a pre-leaching with potassium sulfate, may minimize subsequently unmeasured C loss during the density fractionation. Alternatively, a common suggestion is to use other physical fractionation methods that do not include a density separation, such as the isolation of POM (Cambardella and Elliot 1992) that shares some characteristics with LF (Magid et al. 1996). However, most of these methods include shaking soil in a dispersant such as sodium hexametaphosphate, which also has been shown to result in losses of up to 19% of organic C during separation (Chan 2001; Moran et al. 2005). Those researchers using density fractionation may benefit by simply using its limitations to their advantage in the interpretation of results. Specifically, even when direct measurements of the mobilized OM are not made, its properties should be calculated by difference and interpreted as an additional organic matter fraction. Organic matter mobilized during dispersion in SPT will be a function of many aspects of a soil, including the chemical nature of detrital inputs, soil structure, mineralogy, and land use history. Ultimately, direct investigation into the source of the SPT-soluble material is needed in order to properly interpret this pool, and the recovered organic matter should be carefully interpreted within the context of the system ecology and with recognition of method-induced artifacts.

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