METHODS OF SEPARATING SOIL CARBON POOLS AFFECT THE CHEMISTRY AND TURNOVER TIME OF ISOLATED FRACTIONS

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ABSTRACT. A variety of physical and chemical techniques are used to fractionate soil organic matter, but detailed comparisons of the different approaches and tests of how separation methods influence the properties of isolated organic matter pools are lacking. In this case study based on A horizon samples of 2 California coniferous forests soils, we 1) evaluate the effects of root removal and ultrasonic dispersion on the properties of the <2 g cm^{-3} light fraction and 2) compare the properties of fractions obtained by sequential density separations of ultrasonically treated soil with those obtained by density followed by acid/base hydrolysis (Trumbore et al. 1996).

A root-removal effort based on hand-picking visible roots reduced the radiocarbon content and increased the estimated turnover time of the light fraction by roughly 12%. Root-removal protocols that vary between investigators thus can potentially confound variability in carbon cycling for this fraction caused by environmental factors, such as climate. Ultrasonic dispersion did not have a clear effect on the light fraction C and N content or isotopic signature, but led to a decrease in the % C and C/N of the recovered heavy fractions, and losses of 12–19% of the total soil C to the sodium metatungstate density solution.

Sequentially isolated density fractions clearly differed in mineralogy and organic matter chemistry, but natural-abundance ^{14}C analyses indicated that distinct mineral phases did not correspond to unique C-turnover pools. Density fractions containing kaolinite group minerals alone and in combination with hydroxy-interlayered vermiculite were found to harbor both fast and slow cycling carbon. In contrast, severe chemical treatment isolated a carbon pool with the lowest overall ^{14}C content and longest inferred mean turnover time. Overall, our results show that care must be taken when relying on physical (density) separation to isolate soil fractions with different dynamics, as the details of treatment will influence the results.

INTRODUCTION

The measurement of radiocarbon in soil organic matter has greatly expanded our ability to quantitatively constrain rates of soil carbon cycling (O'Brien and Stout 1978; Goh et al. 1984; Trumbore et al. 1989; Amundson et al. 1998; Gaudinski et al. 2000; Trumbore 2000; Swanston et al. 2005). Soil organic matter is not a homogeneous pool but consists of components with turnover times ranging from years to many millennia. ^{14}C measurements of bulk soil integrate across these components and can therefore be misleading as a measure of how fast the organic matter will respond to a perturbation (Trumbore 2000). For example, while the residence time of bulk soil organic matter sampled in the 1950s may be consistent with carbon residence times of hundred to thousands of years, a time series of samples taken at the same site demonstrates rapid uptake of bomb ^{14}C produced in the early 1960s (Trumbore et al. 1996). Recognition that bulk soil ^{14}C measurements offer little insight into soil organic matter dynamics has led to a growing array of chemical and physical techniques that attempt to reliably partition bulk soil into operationally defined "fractions" with different characteristic carbon residence times (e.g. Balesdent 1987; Golchin et al. 1994; Trumbore and Zheng 1996; Shang and Tiessen 2001; Baisden et al. 2002; Masiello et al. 2004).

Soil organic matter fractionation procedures fall into broad categories such as physical methods, based on particle size, density, or degree of aggregation, and chemical methods, based on solubility in water, acids, bases, solvents, or salt solutions. Density differences have routinely been used to separate soil fractions with varying proportions of organic matter (Monnier et al. 1962; Greenland and Ford 1964; Sollins et al. 1983; Christensen 1992; Swift 1996; Shang and Tiessen 1998; Six et

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al. 2001). Organic matter in the low-density fractions (usually <1.6–2 g cm^{-3}) includes components of very different ages, ranging from slightly altered residues of roots and leaves, to charcoal fragments, to hydromorphic precipitates (humic materials). Further, depending on how fast aggregates themselves form and persist, the low-density organic matter trapped in soil aggregates should be older than that found in inter-aggregate spaces (Rasmussen et al. 2005; Ewing et al. 2006). Organic matter in the heavy fraction (usually >2 g cm^{-3}) is more directly associated with mineral surfaces, for example, via ligand exchange, cation bridging, and hydrogen bonds. These different mechanisms of soil organic matter (SOM) stabilization may in turn have different characteristic turnover times, so that the dense fraction also includes components of very different ages. Subjecting the heavy fractions to acid/base hydrolysis leaves a ^{14}C-depleted organic matter residue (Trumbore and Zheng 1996) that generally matches the century-to-millenial cycling "passive pool" of soil carbon cycling models (Parton et al. 1987; Jenkinson et al. 1990). While they do isolate a much older organic matter fraction (Paul et al. 2001, 2006), such chemical methods have been criticized, however, out of concern that they lead to random cleavage of native compounds followed by repolymerization of artificial compounds (Olk and Gregorich 2006).

The success of different operational methods in separating carbon into pools with distinct residence times varies (e.g. Trumbore and Zheng 1996; Six et al. 2000; Baisden et al. 2002; Krull et al. 2006; Sollins et al. 2006; Basile-Doelsch et al. 2007), and depends to some degree on the soil characteristics and sample pretreatment. Here, we compare the effects of root removal, sonication, and density versus chemical fractionation on the residence times of organic matter fractions obtained from A horizons of 2 coniferous forest soils sampled along an elevation gradient in the western Sierra Nevada, California, USA.

^{14}C studies frequently rely on air-dried soils, for which separation of live and dead roots is not feasible. Yet in theory, only dead roots belong to the soil organic matter pool; living roots belong to the biomass pool. Prior work (Gaudinski et al. 2000, 2001) has indicated that the mean age of carbon in both live and dead fine root biomass is similar and has higher ^{14}C signature than most other components of the low-density fraction. Therefore, the ^{14}C content of the light fraction should be less affected by the separation of live and dead roots—a time-consuming and subjective procedure— than by the overall degree of root removal. A modest but consistent root-removal protocol may suffice to make light fraction residence time estimates across environmental gradients more reproducible and comparable. Accordingly, our first goal was to measure the effect of an easily achievable root-removal effort on the ^{14}C content of the <2 g cm^{-3} (light) fraction.

Our second goal was to compare the ^{14}C content and C/N ratio of soil organic matter fractions obtained by a density separation of shaken soil followed by acid/base hydrolysis of the heavy fraction (Trumbore and Zheng 1996; Trumbore et al. 1996) to fractions obtained by sequential density separations of ultrasonically treated soil (Basile-Doelsch et al. 2007). The first method (density/ hydrolysis) is designed to separate a mineral-free light fraction, a chemically labile (hydrolyzable) mineral-associated fraction, and a chemically recalcitrant (nonhydrolyzable) mineral-associated fraction. The second method (sonication/density) is designed to separate the entire light fraction (comprising both the mineral-free and mineral-occluded particulate material) and several heavy fractions characterized by distinct mineral species (e.g. kaolinite, quartz, and mica) and their associated organic matter. This comparison allows us to assess how ultrasonic dispersion energy affects the separation of light versus heavy fractions and if and how the chemical resistance of organic matter is associated with mineralogy.

SITE DESCRIPTION

Our study is based on 2 A horizon samples from granitic soils, sampled in 1992 and initially analyzed as part of an elevation gradient study on the western slope of the Sierra Nevada, California (Trumbore et al. 1996; see Table 1). The Musick soil, a well-developed Ultic Haploxeralf, is located just below the permanent winter snowline where moderate mean annual temperature and precipitation values promote chemical weathering and the accumulation of kaolinite clay and iron oxides. The Shaver soil, a less-developed Pachic Xerumbrept, is located just above the permanent winter snowline, where snowmelt leaching dominates the soil hydrology, acting to retard the accumulation of clay, but promoting the production of 2:1 layered hydroxy-Al interlayered vermiculite in addition to 1:1 layered kaolins. (Dahlgren et al. 1997).

Table 1 Characteristics of the soils used in this study.

	Musick	Shaver
Classification	Fine-loamy Ultic Haploxer-alf	Coarse-loamy Pachic Xe-rumbrept
Dominant plant species	Ponderosa pine, Incense cedar (*Libocedrus decurrens*), Manzanita (*Arctostaphylos manzanita*)	White fir (*Abies concolor*), Ponderosa pine (*Pinus ponderosa*), Sugar pine (*Pinus lambertiana*)
Elevation (m)	1240	1780
Mean annual temperature (°C)	11.7	8.9
Mean annual precipitation (cm)	94	102
Horizon and depth	A1, 0–7 cm	A2, 5–10 cm
Texture (% sand, silt, clay)	46, 34, 20 (loam)	68, 25, 7 (sandy loam)
Structure	Moderate, medium, granular	Weak, fine, granular
Roots[a]	Common very fine; few fine and coarse	Common coarse; many very fine, fine, and medium

[a]In situ observation per unit of cross-sectional area through the horizon (Schoeneberger et al. 1998).

METHODS

Two fractionation protocols were applied. The first was carried out by Tumbore et al. (1996) and the second follows the method of Basile-Doelsch et al. (2007).

1) Soil Separation by Density Followed by Acid/Base Hydrolysis (Trumbore and Zheng 1996; Trumbore et al. 1996)

Freshly collected soils were sieved to <2 mm and air-dried. Following widely accepted standards, roots that fell through the 2-mm sieve were assumed to be part of the soil organic matter pool (Nelson and Sommers 1996). One ~5-g split per sample was placed in a 50-mL polypropylene tube. The (<2 g cm^{-3}) low-density fraction was separated by adding ~35 mL 2 g cm^{-3} sodium polytungstate $(3Na_2 \cdot (WO_4 \cdot 9WO_3) \cdot H_2O)$ solution, shaking to disperse, centrifuging to settle, decanting the floating material onto a prebaked quartz fiber filter, and repeating the sequence, up to 6 times, until no additional material was retrieved. No sonication was used, only shaking to redisperse the centrifuged residue. The low-density material on the filter was rinsed with 1 L of distilled water to remove residual salts, then dried.

The heavy fraction (>2 g cm^{-3}) at the bottom of the tube was also rinsed with water, dried, weighed, and split for ^{14}C analysis and acid/base hydrolysis. For the hydrolysis procedure, tubes were filled with 0.5N HCl, shaken repeatedly, then left to sit overnight, centrifuged, decanted, and rinsed 3

times with distilled water. Base hydrolysis used 0.1N sodium hydroxide (NaOH)-sodium pyrophosphate ($Na_4P_2O_7 \cdot 10H_2O$) solution. This was added to tubes, which then were shaken (to mix), left overnight, centrifuged, and rinsed 3 times with distilled water. Finally, 6N hydrochloric acid (HCl) was added, tubes were put on a heating block to reach 95 °C, left overnight, centrifuged, and the residue was rinsed at least 3 times with distilled water (Trumbore and Zheng 1996).

The ^{14}C analyses (described below) were conducted on single subsamples of the light, heavy, and acid/base residue fractions. The % C was determined manometrically on purified CO_2. The nitrogen content of the light and heavy fractions was determined using a Fisons NA-1500 analyzer.

2) Sequential Density Separation of Ultrasonically Treated Soil (Basile-Doelsch et al. 2007)

Subsamples of the archived 2-mm air-dried soils were split into four ~5-g aliquots using a precision splitter (ELE International, model CL-244, 7-mm slot riffle sampler). Roots, which may have been either live or dead at the time of sampling, were hand-picked out of each split for 10 min (after 10 min, the yield of additional fine roots decreased greatly). Roots and corresponding hand-picked soil samples were freeze-dried and weighed.

Four replicates of each soil sample were placed in 50-mL polypropylene tubes to which 30 mL of deionized water was added. Capped tubes were gently mixed by inverting 6 times and allowed to sit for at least 1 hr to fully wet the sample. After this period, the tubes were mixed using a Vortex® mixer for 10 s, immersed in an ice bath, and sonicated at 200 W for 3 min using a 350-W Branson™ sonicator with a 12.5-mm probe immersed to 3 cm depth. Tubes were then shaken at 180 rpm for 10 min and centrifuged at 20,000 g for 32 min to settle 1.05 g cm^{-3} and 0.2-µm-diameter spherical particles. The floating material, corresponding to the 0–1 g cm^{-3} fraction, was isolated by decanting.

Sequentially heavier fractions were extracted using sodium polytungstate (SPT) solution adjusted to successively higher densities between 2 and 3.2 g cm^{-3}. At each step, ~30 mL of density-adjusted solution was added to the centrifuge tubes, which were vortex-mixed to disengage the heavy fraction pellet from the bottom, shaken for 10 min, sonicated for 45 s, and centrifuged. The centrifuge time and speed was adjusted such that 0.2-µm-diameter spherical particles with a density 0.05 g cm^{-3} higher than the solution would settle to the bottom of the tube according to Stoke's law (reviewed by Christensen 1992). Following centrifugation, each floating fraction, and finally the >3.2 g cm^{-3} fraction, was decanted into a clean 50-mL tube, diluted with enough water to allow them to settle, centrifuged, and rinsed 3 times with deionized water. All fractions were freeze-dried and weighed.

Two replicates of each density fraction, as well as the root and corresponding hand-picked bulk soil samples, were used for carbon and nitrogen analysis. Samples were ground to pass through a 200-µm sieve, and stored in a dessicator for at least 24 hr prior to being loaded into tin capsules, which were weighed, and then analyzed on a Europa 20/20 continuous-flow stable isotope ratio mass spectrometer located at the Center for Stable Isotope Biogeochemistry, University of California, Berkeley. The mass spectrometer was calibrated to NIST 1547 Peach Leaves (45.5% C, 2.96% N), which, labeled as unknowns, varied between 45.0 and 46.1% C, and between 2.92 and 2.99% N for all runs.

Two replicates from each density or root fraction were combined into single samples that were analyzed for ^{14}C as described below. The agreement between % C obtained by manometric determination following sealed-tube combustion (corresponding to the Trumbore protocol) and by mass spectrometer (corresponding to the Basile protocol) was good ($C_{Sealed\ tube} = C_{Mass\ Spec} \times 0.92$; $R^2 = 0.98$, $p<0.001$, $n = 14$, residuals randomly distributed).

Radiocarbon Analyses

Subsamples for ^{14}C analyses were weighed and sealed in evacuated quartz or Vycor® tubes with 0.5 g Cu, 1 g CuO, and a strip of Ag foil (Boutton 1991) and combusted for 3 hr at 875–900 °C (Minagawa et al. 1984). The evolved CO_2 was cryogenically purified under vacuum then reduced to graphite on which the ^{14}C was determined at the Center for Accelerator Mass Spectrometry, Lawrence Livermore National Labs (CAMS). ^{14}C data are reported as Δ^{14}C:

$$\Delta^{14}C = \left(\frac{^{14}C/^{12}C_{sam} \times e^{\lambda(1950-x)}}{^{14}C/^{12}C_{std}} - 1 \right) \times 1000 \tag{1}$$

which is the fractional deviation, in parts per thousand (‰), of the ^{14}C/^{12}C ratio in the sample (corrected for the year, x, in which it was collected) from that of the internationally accepted ^{14}C dating standard (95% of the activity of the NBS oxalic acid standard in AD 1950, corrected for decay of standard, and normalized to δ^{13}C = –19‰ with respect to PDB standard) (Stuiver and Polach 1977). The ^{14}C/^{12}C of the sample is corrected to a δ^{13}C value of –25‰ to account for the assumption that plants discriminate twice as much against ^{14}C as they do against ^{13}C. Samples were either analyzed in 1993 (Trumbore protocol) or 2002 (Basile protocol). We did not correct for the ~1‰ difference in Δ^{14}C caused by radioactive decay of ^{14}C between 1993 and 2002 as this was small compared to overall measurement accuracy of ~4‰.

Turnover Time Calculation

Carbon turnover times were calculated using a time-dependent steady-state box model described in detail elsewhere (Trumbore 1988; Gaudinski et al. 2000; Torn et al. 2002; Wang and Hsieh 2002). For each year, y:

$$(C_y) \times (^{14}C_y) = (C_{y-1}) \times (^{14}C_{y-1}) \times (1-k-\lambda) + I \times (^{14}Catm_{(y-lag)}) \tag{2}$$

where, C is the carbon inventory of a soil fraction (mass); ^{14}C is the ^{14}C value of a soil fraction (‰); k is the first-order decomposition constant for homogeneous C pools (Rodhe 1992) (year^{-1}); λ is the ^{14}C decay constant (1.21E-4 year^{-1}); I is the annual carbon input (mass year^{-1}); $^{14}Catm$ is the ^{14}C value of the atmosphere (Levin and Hesshaimer 2000); and lag is the average number of years that atmospheric carbon is retained in plant tissue before becoming part of the soil organic matter pool. At steady state, $C_y = C_{y-1}$ and $I = kC$, so Equation 2 can be divided by C_y to obtain:

$$^{14}C_y = (^{14}Catm_{(y-lag)}) \times k + (^{14}C_{y-1} \times (1-k-\lambda)) \tag{3}$$

The decomposition constant, k, or its inverse, the turnover time, T, is obtained by matching the modeled and measured ^{14}C values for the year in which the soil was sampled.

Mineralogy

One replicate from each mineral-rich fraction (including the acid/base hydrolysis residue) was ground to <100 μm and subjected to powder X-ray diffraction (Rigaku Geigerflex, Cu Kα). Diffraction intensity was recorded every 0.05° for 2.5 seconds between 2 theta 3° and 65° by Theta software. The diffraction data of Brindley and Brown (1984) were used to identify mineral species.

RESULTS

Root Removal

Fine roots (<2 mm diameter) represented 7–11% of the light fraction carbon and had ^{14}C values that were 100–200‰ higher than the remaining material (Table 3; and see Table 2 for a list of abbreviations used in all tables). For the Musick soil, turnover times were estimated to be 10–12 yr for roots versus 55–59 yr for the remaining light fraction. For the Shaver soil, the turnover times were 38–42 yr for roots and 88–104 yr for the remaining light fraction. Root removal increased estimated turnover time of the light fraction carbon by ~12%; i.e. ~6 yr for the Musick and ~11 yr for the Shaver soil.

Table 2 List of abbreviations and subscripts used in Tables 3–5.

Abbreviations:	Mt	proportion of total mass
	Ct	proportion of total carbon
Subscripts:	bulk	bulk soil
	root	removed roots
	LF	<2 g cm^{-3} light fraction
	HF	>2 g cm^{-3} heavy fraction
	hyd	hydrolyzable fraction
	res	acid/base residue fraction
	lost	dissolved lost fraction

Table 3 The effect of root removal on the proportion of total carbon (Ct), Δ^{14}C content, and ^{14}C-derived turnover times of the <2 g cm^{-3} light fraction.

Soil	Fraction	Ct[a]		Δ^{14}C (‰)		Turnover time (yr)[b]	
		mean	s.e.		Error[c]	mid	range
Musick	Roots	0.07	0.02	354	5	11	1
	Hand-picked light fraction	0.93	0.11	137	4	57	2
	Whole light fraction	1	0.11	152	4	51	2
Shaver	Roots	0.11	0.03	185	5	40	2
	Hand-picked light fraction	0.89	0.10	84	4	96	8
	Whole light fraction	1	0.10	95	4	85	7

[a]Ct = proportion total carbon.

[b]To fit the Musick ^{14}C data to our model, it was necessary to assume an 11-yr lag time (Equations 2 and 3). The turnover times of the Shaver root and light fraction were not sensitive to lag time; the values shown span the results for lag times from 5 to 11 yr. The turnover times of the whole light fraction were derived from the corresponding ^{14}C values, which were calculated as the Ct-weighted ^{14}C values of the root and hand-picked light fractions. The range of turnover times is based on the ^{14}C error.

[c]Analytical error reported by CAMS.

Density/Hydrolysis Fractionation (Trumbore Protocol)

The % C values of the light fractions (Table 4) were lower than expected due to the presence of quartz fiber filter scrapings, which also led to a high proportion of the total mass in the low-density fraction, particularly for the Shaver soil. This did not affect the ^{14}C values, however. The light fractions were enriched by 56‰ (Musick) and 43‰ (Shaver) relative to the bulk soil, whereas the acid/base residue fractions, which contained 14% (Shaver) to 25% (Musick) of the heavy fraction carbon, were noticeably ^{14}C-depleted. X-ray diffraction (XRD) data show that quartz is the principal mineral phase in the acid/base residue, which also includes hornblende and feldspars (Figure 1).

Table 4 Results of the separation by density followed by acid/base hydrolysis (density/hydrolysis fractionation).[a]

Soil	Fraction	Mt	C (%)	Ct	$\Delta^{14}C$
Musick	Measured bulk soil		3.9		108
	Light fraction	0.11	15.7	0.46	164
	Heavy fraction[b]	0.89	2.4	0.54	24
	Hydrolyzate[c]	0.23	6.8	0.41	44
	Residue	0.65	0.8	0.13	−40
Shaver	Measured bulk soil		1.5		66
	Light fraction	0.25	3.8	0.63	109
	Heavy fraction[a]	0.75	0.7	0.37	73
	Hydrolyzate[b]	0.14	3.4	0.32	99
	Residue	0.61	0.1	0.05	−87

[a]Mt = proportion total mass and Ct = proportion total carbon.

[b]The Mt_{HF}, Ct_{HF}, and Ct_{LF} values were calculated based on the measured % C values of the bulk, LF, and HF fractions and by assuming $Mt_{HF} + Mt_{LF} = 1$.

[c]By definition, $Mt_{hyd} = Mt_{HF} − Mt_{res}$ and $Ct_{hyd} = Ct_{HF} − Ct_{res}$. Thus, % C_{hyd} and $^{14}C_{hyd}$ were calculated as weighted differences of the corresponding values for heavy and residue fractions.
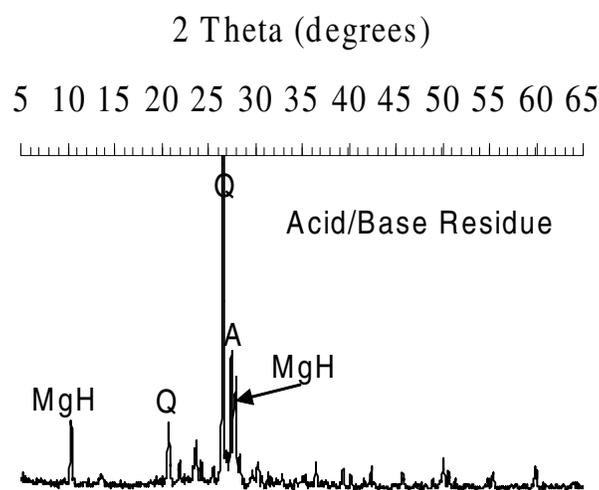

Figure 1 Powder X-ray diffraction spectrum for the acid/base residue of the Shaver soil (Musick spectrum is identical). Diagnostic peaks for quartz (Q), anorthite (A), and magnesiohornblende (MgH) are labeled.

Sonication/Density Fractionation (Basile Protocol)

The mineral-free <2 g cm^{-3} light fractions accounted for less than 10% of the soil mass but had 41% (Shaver) to 59% (Musick) of the soil carbon, which was ^{14}C-enriched by 20‰ (Shaver) to 40‰ (Musick) relative to the bulk soil (Table 5). The XRD data show that the principal mineral phases found in the 2–25, 2.5–2.6, 2.6–2.7, 2.7–3.2, and >3.2 g cm^{-3} fractions of the Musick soil were the kaolin-type clays (halloysite and kaolinite), kaolin-type clays plus feldspars, quartz, mica, and hornblende, respectively. In addition to halloysite and kaolinite, the Shaver 2.2–2.5 g cm^{-3} fraction contained vermiculite and gibbsite (Figure 2). The % C, C/N, and ^{14}C values generally tended to decrease with density. The 2–2.2 g cm^{-3} fractions, with higher % C but lower C/N ratios than the

Table 5 Results of the density separation following ultrasonic treatment (sonication/density fractionation).[a]

Soil	Fraction/density (g cm⁻³)	Dominant mineral	Mt mean	Mt s.e.	C (%) mean	C (%) s.e.	C/N mean	C/N s.e.	Ct mean	Ct s.e.	¹⁴C Δ¹⁴C	¹⁴C Δ¹⁴C
Musick	roots		0.01	0.00	30.1	5.8	42.4	11.0	0.04	0.01	354	
	root-free bulk soil		0.99	0.00	4.0	0.5	22.8	2.8	0.96	0.16	97	
	0–1 g cm⁻³	(Mineral-free)	0.00	0.00	31.4	0.0	17.2		0.01			137
	1–2 g cm⁻³	(Mineral-free)	0.06	0.00	38.7	0.0	29.6	0.8	0.58	0.07		
	2–2.2 g cm⁻³	Kaolins	0.03	0.00	12.5	0.4	14.6	0.5	0.11	0.01	64	
	2.2–2.5 g cm⁻³	Kaolins	0.17	0.01	2.1	0.1	10.5	0.4	0.09	0.01	68	
	2.5–2.6 g cm⁻³	Feldspars	0.18	0.01	0.6	0.0	11.3	0.3	0.03	0.00	38	14
	2.6–2.7 g cm⁻³	Quartz	0.35	0.01	0.1	0.0	9.4	0.7	0.01	0.00		
	2.7–3.2 g cm⁻³	Micas	0.13	0.01	0.3	0.1	7.3	1.5	0.01	0.00	−48	
	>3.2 g cm⁻³	Hornblende	0.05	0.01	0.2	0.0	9.9	0.6	0.00	0.00		
	calculated bulk soil		0.99	0.02	3.5	0.1	20.3	0.8	0.83	0.07	113	
Shaver	roots		0.00	0.00	27.8	5.5	47.3	10.2	0.05	0.01	185	
	root-free bulk soil		1.00	0.00	1.4	0.0	22.4	1.3	0.93	0.02	60	
	0–1 g cm⁻³	(Mineral-free)	0.00	0.00	72.5	6.6	43.4	6.1	0.11	0.02		84
	1–2 g cm⁻³	(Mineral-free)	0.02	0.00	22.4	2.8	32.8	5.3	0.30	0.04		
	2–2.2 g cm⁻³	Kaolins	0.02	0.01	9.9	0.1	17.8	0.5	0.11	0.00	39	
	2.2–2.5 g cm⁻³	Kaolins, gibbsite, vermiculite	0.14	0.00	1.4	0.1	11.7	1.1	0.13	0.01	49	
	2.5–2.6 g cm⁻³	Feldspars	0.17	0.00	0.4	0.0	12.0	0.5	0.04	0.00	45	47
	2.6–2.7 g cm⁻³	Quartz	0.41	0.00	0.1	0.0	12.5	0.6	0.03	0.00		
	2.7–3.2 g cm⁻³	Micas	0.17	0.00	0.2	0.0	6.8	0.3	0.02	0.00	44	
	>3.2 g cm⁻³	Hornblende	0.08	0.00	0.1	0.0	12.3	0.8	0.01	0.00		
	calculated bulk soil		1.01	0.01	1.1	0.1	19.5	2.0	0.74	0.05	67	

[a]The dominant mineral in each fraction was determined based on the X-ray diffraction patterns. Mt = proportion total mass and Ct = proportion total carbon. Ct in each density fraction, i, is $Ct_{(ix)} = \%C_{(ix)} \times Mt_{(ix)} \div \%C_{bulk(avg)}$, where x is the laboratory replicate for i. Where reported, the standard errors (s.e.) for Mt and % C are the absolute standard errors of the mean of laboratory replicates ($n = 4$ for mass fractions and $n = 2$ for % C, N, and δ^{13}C). Note that different subsamples were used for the bulk soil, versus root and density fractions. Standard errors for C/N and Ct are calculated using Gaussian error propagation. The 0–1 and 1–2 g cm⁻³ fractions as well as the >2.5 g cm⁻³ were composited for ¹⁴C analysis, where $n = 1$.

bulk soil, reflected a transition from mineral-free to mineral-associated organic matter. Most of the mineral-associated carbon was found in the 2–2.5 g cm^{-3} secondary mineral fractions. The quartz-dominated fractions (2.6–2.7 g cm^{-3}) had the lowest % C values and 35–41% of the total soil mass. The mica-dominated fractions (2.7–3.2 g cm^{-3}) had the lowest C/N ratios and, in the case of the Musick soil, a ^{14}C content that was depleted by –145‰ relative to the bulk soil. None of the density fractions captured a sizable homogeneous pool of ^{14}C-depleted carbon, however; the mica-dominated Musick fraction only accounted for 1% of the total carbon. Among the mineral fractions, the ^{14}C content varied more for the Musick than the Shaver soil; the range between maximum and minimum Δ^{14}C values was 185‰ for the former but only 45‰ for the latter.
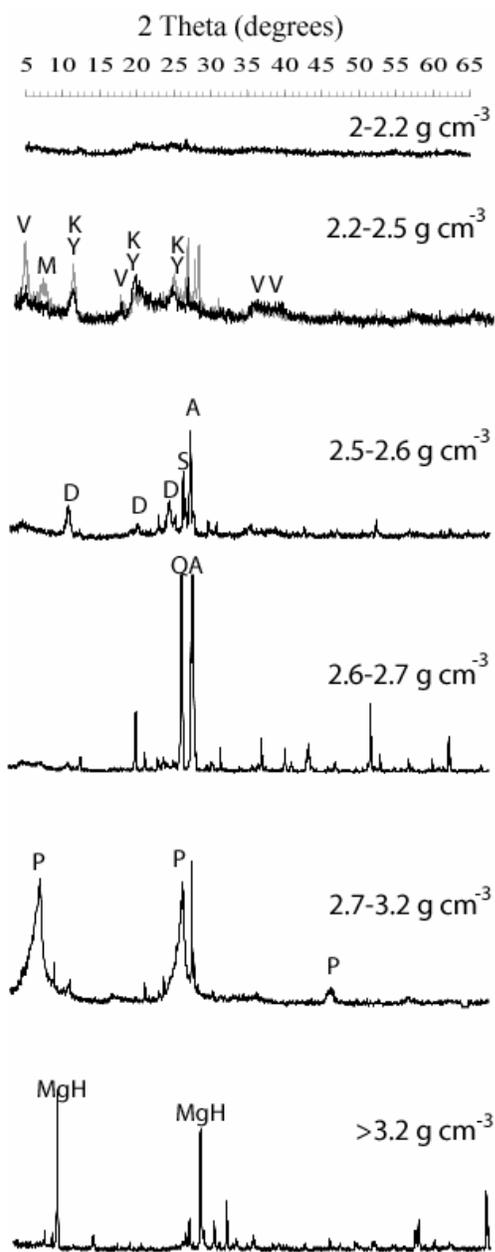
Figure 2 Powder X-ray diffraction spectra for density fractions, labeled in g cm^{-3}. Spectra are shown for the Musick soil only because spectra were identical for both soils with the exception of the 2.2–2.5 g cm^{-3} fraction, where the Shaver soil spectrum is in gray. Diagnostic peaks for halloysite (Y), kaolinite (K), vermiculite (V), quartz (Q), anorthite (A), sanidine (S), dickite (D), phlogipite (P), and magnesiohornblende (MgH) are labeled.

Density Separation Preceded by Shaking (Trumbore Protocol) vs. Sonication (Basile Protocol)

The results for light versus heavy fractions obtained after shaking versus sonication are summarized in Table 6. For the purposes of this comparison, all the light fraction values include roots (see details in Table 6 footnote).

Table 6 Comparison of the light vs. heavy fraction split obtained by shaking vs. sonication for bulk soil (roots included), light (<2 g cm^{-3}), and heavy (>2 g cm^{-3}) fractions.

	Musick			Shaver		
Fraction	C (%)	C/N	Δ^{14}C (‰)	C (%)	C/N	Δ^{14}C (‰)
Bulk with roots[a]	3.9	21	108	1.5	23	66
	Density/hydrolysis separation (shaken up to 6 times)					
Light fraction with roots	15.7	30	164	3.8	31	109
Heavy fraction	2.4	22	24	0.7	19	73
	Sonication/density separation (1300 J sonication per g soil solution)					
Light fraction with roots[b]	37.9	30	152	26.0	36	95
Heavy fraction[c]	1.1	12	56	0.5	13	45
Dissolved fraction[d]		26	−16		53	35

[a]The Musick soil % C_{bulk} and C/N_{bulk} values were obtained from direct measurement of unpicked bulk samples ($n = 4$). The Shaver % C_{bulk} and C/N_{bulk} as well as the ^{14}C values for both soils are weighted sums of the separate root and hand-picked bulk samples ($n = 2$ for % C and N, $n = 1$ for ^{14}C).

[b]Light fraction values are weighted sums of the root, 0–1, and 1–2 g cm^{-3} fractions.

[c]Heavy fraction values are weighted sums of the recovered mineral fractions and do not include losses.

[d]The lost fraction is the difference between the root, light, and heavy fractions and the bulk soil with roots.

Despite inconsistencies in the light fraction % C values (caused by the presence of quartz fiber scrapings in the combusted sample), C/N and ^{14}C values obtained using the 2 fractionation methods were in close agreement. Both approaches resulted in light fractions with C/N ratios of ~30. The light fraction of the sonicated Shaver sample had a higher C/N ratio than that of the shaken sample, but the difference falls within 1 standard error of the mean (determined by Gaussian error propagation) of laboratory replicates. The light fractions of the sonicated soils had slightly lower ^{14}C values than those of the shaken soils ($\Delta\Delta^{14}$C ~12‰ for Musick and ~14‰ for Shaver).

The heavy fractions of the sonicated samples had C/N ratios of ~12, whereas the heavy fractions of the shaken samples had C/N ratios of ~20—very close to the bulk soil C/N values (Table 6). Sonication was associated with the dissolution of 12 ± 8% of the whole soil carbon for Musick and 19 ± 5% of the whole soil carbon for Shaver. Mass balance calculations indicate that the dissolved fraction had relatively high C/N ratios (53 for Musick and 26 for Shaver) and depleted Δ^{14}C values. The difference between the Δ^{14}C values of the heavy fraction and dissolved fraction was substantially greater in the case of Musick ($\Delta\Delta^{14}$C ~ −72‰) than in the case of Shaver ($\Delta\Delta^{14}$C ~ −10‰). This generally agrees with the observation that for the Musick soil, the heavy fraction obtained after sonication is ^{14}C-enriched relative to that obtained after shaking ($\Delta\Delta^{14}$C = 32‰), while the reverse is true for Shaver ($\Delta\Delta^{14}$C = −28‰) (Table 6).

Charcoal

We found that charcoal was unevenly distributed in the initial splits of the Shaver soil: One of the 0–1 g cm^{-3} replicates had a C/N ratio of 52 (Table 5), and the ^{14}C of the heavy fraction obtained with the density/hydrolysis method was higher than the ^{14}C of the bulk sample (Table 6).

DISCUSSION

Effects of Root Removal

The size and ^{14}C-derived turnover times of the Musick root fraction (7% of the light fraction carbon and ~11 yr, respectively) are comparable to those obtained by Gaudinski et al. (2000), who carefully quantified root dynamics in eastern US hardwood forest soils. Differences between the ^{14}C values of the Musick and Shaver root fractions illustrate that this fraction can have highly variable dynamics, however. Our results suggest that the modest root-removal effort increased the ^{14}C-derived turnover times of the Musick and Shaver light fractions by ~12% (equivalent to ~6 yr for Musick and ~11 yr for Shaver). Assuming this is a robust methodological effect (i.e. validated by more soils and replication), it is on par with measurable environmental effects such as a 1 °C increase in mean annual temperature (as deduced from Trumbore et al. 1996).

Effect of Sonication

We used sonication to disaggregate minerals so they could be separated according to differences in specific gravity. Sonication releases organic matter from the mineral matrix and the ionic strength of the SPT solution promotes its dissolution. Sonication may have led to a slight increase in the ^{14}C-derived average age of the light fractions, an effect that would be due to liberation of mineral-protected carbon from inside aggregates (likely for the Musick soil) or loosely bound to minerals. Dissolution of organic matter in the SPT solution led to a marked decrease in the C/N of the heavy fractions (Table 6), an effect that supports the notion that N-rich compounds form the most stable organomineral bonds (Sollins et al. 2006; Kleber et al. 2007).

We recovered 41–59% bulk C in the light fraction of our sonicated samples. In comparable California coniferous forest soil samples, Rasmussen et al. (2005) recovered ~40% bulk C in the <1.65 g/cc fractions prior to sonication (1500 J g soil^{-1}), but ~60% bulk C in the <1.65 g/cc fractions following sonication. The proportion of C recovered in the light fraction is highly variable (see reviews by Sollins et al. [2006] and Basile-Doelsch et al. [2007]). For example, Baisden et al. (2002) recovered 21–56% bulk C in the <1.85 g/cc fraction of sonicated A horizon California grassland soils, while Sollins et al. (1983) recovered between 28–63% bulk C in the <1.65 g/cc fraction of 0–30 cm unsonicated soil samples from a mudflow chronosequence in northern California from which roots had been removed.

Christensen (1992) reported that 1000 J of sonication energy per g of soil solution caused 25% of the soil carbon to be transferred from the silt fraction to the clay fraction. Using ultrasonic dispersion energy of 400 J per g of solution, Shang and Tiessen (2001) noted that carbon losses increase with the density of the SPT solution to a maximum of 10% of the total soil carbon at their highest density (2.4 g cm^{-3}). Following the method of Baisden et al. (2002), we sonicated between each density separation, applying a total of ~1300 J of ultrasonic dispersion energy per g of soil solution. This treatment successfully segregated mineral phases, but solubilized 17–26% of the bulk carbon in the SPT solution (Table 5). The amount dissolved is higher than the ~11% loss reported by Baisden et al. (2002), yet similar to the 17–28% reported by Crow et al. (2007), who did not sonicate the soils but allowed them to separate gravimetrically (by standing for ~4 days in a low-density SPT solution).

The organic matter losses reported by Crow et al. (2007) had C/N ratios that were similar to their bulk soils (but lower than their light fraction). Our results follow this trend for Shaver, but not for Musick (with a dissolved fraction C/N ratio ~53). In addition, our ^{14}C results indicate that the lost fractions correspond to relatively old carbon, especially for Musick. In the Musick soil, where Fe and Al oxides promote moderate degrees of aggregation, the dissolved material probably corresponded to a mixture of colloidal particles (smaller than the 0.2 um diameter used to determine centrifugation/settling time according to Stoke's law) and intra-aggregate and oxyhydroxide bound organic matter that passed through the quartz fiber filters (e.g. Golchin et al. 1997). In the coarse-textured and weakly aggregated Shaver soil, OM losses may be more directly related to the ability of the acidic SPT solution to solubilize organic substances.

Chemical Versus Density Separation of the Heavy Fraction

The acid/base hydrolysis isolated a distinctly old carbon pool, which we failed to obtain by density alone. The only sonication/density fractions with enough carbon to account for this carbon pool are the 2–2.5 g cm^{-3} secondary mineral fractions, which, due to their high ^{14}C values ($\Delta^{14}C_{(2-2.5 \text{ g cm}^{-3})}$ − $\Delta^{14}C_{(residue)}$ = +106‰ for Musick and +131‰ for Shaver), are inferred to contain a mix of old and young carbon. Additionally, despite distinct levels of organic matter associated with the 2–2.2 and 2–2.5 g cm^{-3} fractions, their ^{14}C values were indistinguishable. This corresponds with observations by both Baisden et al. (2002) and Jastrow (2003), who found distinct density fractions associated with similar turnover times, and suggests that fast and slow cycling carbon pools are intimately associated (Swanston et al. 2005), either because sites available for organic matter sorption on mineral surfaces are not saturated (Oades 1995) or because organic molecules are arranged onto minerals via tightly bound stable inner layers as well as loosely bound dynamic outer layers (Kleber et al. 2007).

According to the X-ray diffraction data, the acid/base residue fraction is made up of quartz, hornblende, and anorthite. The ^{14}C values in the density-separated quartz (2.6–2.7 g cm^{-3}), hornblende (>3.2 g cm^{-3}), or feldspar-dominated (2.5–2.6 g cm^{-3}) fractions are, however, too high to match those of the acid/base residue. Therefore, it appears that the acid/base residue fraction contains chemically recalcitrant organic matter from throughout the soil mass, rather than organic matter protected by chemically resistant minerals, as has sometimes been assumed. One reason for very low ^{14}C values in the acid/base residue is that acid/base hydrolysis dissolves Fe and Al oxyhydroxides, which tend to layer onto kaolin surfaces (Sposito 1989) and in A horizons tend to complex fairly recent organic matter (Masiello et al. 2004). Dissolving this layer of higher-turnover organomineral (oxide) associations would leave behind the smaller proportion of organic matter that adheres directly to the kaolin surfaces.

The ^{14}C values associated with kaolins, feldspars, and micas were distinct in the Musick soil, but not in the Shaver soil. This may be a function of differences in the nature of the mineral surfaces or in the nature of the organic matter and its ability to sorb to mineral particles. Compared to the Shaver soil, the Musick soil may, for example, have more Fe and/or Al oxyhydroxide kaolin coatings associated with young carbon. In the Shaver soil, intense leaching at snowmelt rapidly transforms micas into vermiculite, which has much greater specific surface area (Douglas 1989; Dahlgren et al. 1997), but may not sequester organic matter for as long as the Musick's long-lived micas, which can intercalate organic matter for extended periods of time.

SUMMARY AND CONCLUSIONS

The low-density fraction can be quite heterogeneous and include contributions from different kinds of organic material from roots, with relatively fast turnover, to charcoal, which can persist for millennia. Because the ^{14}C of the root fraction can be quite different from the rest of the low-density fraction, roots should be separated before calculating the turnover time of the remaining light fraction. The effect of our simple, operationally defined live+dead root removal was comparable to that based on quantitative live root removal; this indicates that such an effort can help to quantify soil organic matter dynamics when removal of the live root (non-soil component) is not feasible. Time lags associated with roots and leaf litter inputs will also directly affect the ^{14}C-derived dynamics of the low-density fraction. Therefore, researchers need to make sure they describe how the sample is treated and what assumptions are used in estimating turnover time.

The degree to which sonication augments the light fraction or promotes the dissolution of organic matter in the SPT solution depends in part on the amount of organic matter trapped in soil aggregates and weakly bound to minerals and in part on the details of the methodology. In this study, sonication had a minor effect on the ^{14}C value and C/N of the light fraction, but promoted the solubilization of a substantial portion of the soil organic matter in the SPT solution and led to dramatic reductions in the total C, C/N, and ^{14}C values of the heavy fractions.

Sonication enabled the separation of unique mineral classes along a continuum of increasing density. This approach may thus be useful in detailed assessments of intact organomineral phases. The various minerals were not, however, associated with unique carbon pools; none of the mineral fractions corresponded to a carbon pool that was both as old and as large as the acid/base residue. Overall, the density/hydrolysis fractionations (Trumbore protocol) isolated soil fractions with more distinct ^{14}C signatures than the sonication/density fractionations (Basile protocol). Combining data from both fractionation approaches and X-ray diffraction, we learned that in these soils the turnover rate of the slowest cycling carbon is governed by chemical recalcitrance of the organic matter, not by the nature of the minerals with which it is associated.

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