Evidence for Losses From Strongly Bound SOM Pools After Clear Cutting in a Northern Hardwood Forest

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Abstract: Forest soils in the northeastern United States store considerable amounts of carbon (C). With the increasing utilization of biomass as a "C-neutral" form of energy in the United States, these forests are susceptible to clear cutting and large losses of soil organic matter (SOM) to the atmosphere as carbon dioxide (CO_2). The relative stability versus susceptibility of SOM to degradation can be approximated, in part, through the strength of organo-mineral interactions, that is, the strength of binding between SOM and mineral surfaces in the soil. This study investigated differences in SOM organo-mineral binding between northern hardwood forest stands with varying clear-cutting histories in Bartlett Experimental Forest in Bartlett, New Hampshire. Sequential chemical extractions were performed to quantify SOM storage in organo-mineral pools of various binding strength. In this case study, soils from Mature forest stands stored significantly more SOM in strongly mineral-bound and stable C pools than soils from Cut stands did. Differences in the relative distribution of C in organo-mineral pools in Mature and Cut forests may inform our understanding of SOM bioavailability, microbial decomposition, and CO_2 production in ecosystems after clear cutting. These findings should contribute to discussions on long-term SOM stability in northeastern U.S. soils.

Key Words: Clear cutting, climate change, organo-mineral binding, soil organic matter

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Alternative energy sources are needed to stabilize and decrease carbon dioxide (CO_2) emissions. The use of biomass to generate heat and electricity has been proposed as a more carbon (C)–neutral form of energy (Cherubini and Strømman, 2011). However, the C neutrality of biomass has been questioned (Johnson, 2009; Cherubini et al., 2011; Searchinger, 2010). Cherubini et al. (2011) assert that before CO_2 released from burning biomass can be recaptured by forest regrowth, the CO_2 molecules will spend time in the atmosphere and contribute to global warming for years or decades. Searchinger (2010) maintains that using biofuels does not reduce C emissions unless current plant growth is increased. If a forest or agricultural land was to sequester C under natural conditions, then no "additional" C is captured from the atmosphere. Schulze et al. (2012) modeled a hypothetical switch to biomass for 20% of the current global primary energy supply; the resulting deforestation from this hypothetical scenario produced younger forests, depleted biomass pools, and did not achieve the main objective of decreasing greenhouse gas emissions.

Forest harvest has been shown to cause major changes in soil C pools. Soil is the world's largest terrestrial C pool (Davidson and Janssens, 2006; Jobbágy and Jackson, 2000; Schlesinger and Bernhardt, 2013). There is approximately three times as much C in soils than in aboveground biomass and twice as much as in the atmosphere (Eswaran et al., 1993). Globally, forest ecosystems are predicted to contain 1150 Pg C, with more than two-thirds of that C stored in soils (Dixon et al., 1994). In northern hardwood forests in the United States, mineral soil C pools in particular store up to 50% of total ecosystem C (Fahey et al., 2005). Land-use change is cited as one of the major causes of soil C release (Jobbágy and Jackson, 2000). However, not enough is known about the effects of land-use change on mineral soil C pools to accurately characterize soil C release and sequestration for use in C accounting models (Paul et al., 2003; Falloon and Smith, 2003; Sanderman and Baldock, 2010).

In the scientific community, there has been recent interest to further investigate and understand soil C dynamics in forested ecosystems after logging. These topics are of particular importance in the northeastern United States because there is great potential for the use of biomass as part of a diversified renewable energy portfolio (Buchholz and Canham, 2011). Several studies have observed soil C release from certain horizons in temperate forest soils after harvest (Grand and Lavkulich, 2012; Nave et al., 2010). Studies specific to northern New England have observed C decreases in mineral soil horizons after clear cutting (Zummo and Friedland, 2011; Petrenko and Friedland, 2014).

Impending climate change further complicates the issue of soil C release. Some studies predict that warmer climates will enhance forest growth, leading to larger net primary productivity and increased C sequestration (IPCC, 2007). Yet several studies also link warming to increased soil microbial respiration, a major pathway by which soil C is returned to the atmosphere (Schlesinger and Andrew, 2000; Rustad et al., 2001). In order to make informed forest management decisions regarding the longer-term C neutrality of biomass as an energy source, the lability of soil C after forest harvest must be considered.

Many previous studies have attributed soil C stabilization to the chemical properties of the soil. These studies emphasized the importance of separating the supposedly nondegradable humic substances from the nonhumic substances and chemically characterizing the structure of these humic organic compounds (e.g., see Dai et al., 2002; Ussiri and Johnson, 2007). Recently, the importance of these methods has been called into question. Investigators from different disciplines have found that factors such as organo-mineral associations have a stronger effect on soil organic matter (SOM) bioavailability. Organo-mineral association is the strength with which SOM binds to mineral complexes in the soil. Soil C is more resistant to microbial degradation when the strength of the organo-mineral association exceeds that of the microbial enzyme active sites (Dungait et al., 2012). Thus, the strength with which

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TABLE 1. Average Soil Acidity and Texture Data for All Stands Included in the Study—Previously Reported in Petrenko and Friedland (2014) and Vario et al. (2014)

Stand Age	Soil pH	% Sand	% Silt	% Clay
5 y	5.0	67	27	6
55 y	5.1	70	26	4
Mature, undisturbed	5.1	67	28	5

SOM is bound to mineral surfaces in the soil may serve as an approximate measure for bioavailability of soil C.

Sequential chemical extractions have been suggested as a way to measure the strength with which SOM binds to mineral complexes. Combining extraction techniques intended for different fractions of the C pool, Lopez-Sangil and Rovira (2013) proposed methods for the quantification of different organo-mineral fractions according to the strength of their bonds. Extraction solvents are intended to interrupt intermolecular forces binding the organic matter to mineral complexes and solubilize the SOM (Lopez-Sangil and Rovira, 2013; Oste et al., 2002). The method relies on the premise that SOM that is more strongly bound to minerals is less available for microbes to respire, and SOM that is more weakly bound to minerals is more available for microbes to respire and will result in production of CO_2 from the soil (Lopez-Sangil and Rovira, 2013).

Our study investigated changes in SOM organo-mineral binding in soils after clear cutting at Bartlett Experimental Forest (BEF), New Hampshire. Sequential chemical extractions were performed to quantify SOM storage in different organo-mineral fractions of varying binding strength. It was hypothesized that (i) soils from Cut stands would have greater amounts and greater proportion of C in less tightly bound organo-mineral fractions, and (ii) soils from Mature stands would have greater amounts and greater proportion of C in more tightly bound, stable fractions.

MATERIALS AND METHODS

Study Sites

This study utilized soils from BEF, a research forest in Bartlett, New Hampshire. At BEF, three different-aged forest stands were selected for analysis: 5 years, 55 years, and an undisturbed, old-growth stand. In this study, "stand" refers to an area of BEF of specified age, such as the 5-year stand. Time since last harvest (age) and locations of the different forest stands were determined from knowledge from research forest managers. All stands were low-elevation (304 m) northern hardwoods, composed of *Betula papyrifera* Marsh., *Betula alleghaniensis* Britton, *Populus* species, *Fagus grandifolia* Ehrh., and *Acer saccharum* Marsh. (Vario et al., 2014). All soils analyzed were well-drained Spodosols (Leak, 1991; Zummo and Friedland, 2011). Both disturbed stands, the 5 years and 55 years, were classified as "Cut," and the recently undisturbed, old-growth stand was classified as "Mature."

Soil Collection

Within each forest stand, we selected three microsites for soil excavation. Microsites were at least 10 m apart from each other, free of recently formed pit and mound topography, at least 1 m in distance from the nearest tree, and had ground slope of less than 10 degrees (Vario et al., 2014). Except for the Mature stand, all soils were collected using a gas-powered auger (Earthquake 9800B; Ardisam, Cumberland, WI) with a diamond-tipped, 9.5-cm-diameter drill bit and extension tube (Tools Direct Premium Red Diamond Drill Bit; Tools Direct of North America, Inc., Kennesaw, GA). Soil from the Mature stand was collected from 0.5-m^2, quantitative soil pits. A quantitative pit is a prismatic hole dug in discrete depth increments to collect a precisely known quantity of soil. Cores and pits were excavated incrementally in the following order: organic soil horizons; and mineral soil depth increments: 0 to 10, 10 to 20, 20 to 30, 30 to 45, and 45 to 60 cm. Only mineral soils from depth increments 0 to 10, 10 to 20, 20 to 30, and 30 to 45 cm were analyzed in this study.

Within each microsite, we extracted three soil cores and bulked the depth increments to achieve a sample mass more similar to quantitative soil pits. In the cored stands, this resulted in nine independent and subsequently three pooled soil cores per forest stand. In the BEF Mature stand, one pit was dug per microsite. The data presented here represent 18 deep soil cores and three quantitative soil pits, which were pooled to nine samples, three Mature and six Cut, per depth interval. Additional field methods and results of bulk density, sample transport, texture, and acidity analyses are reported in Vario et al. (2014) and Petrenko and Friedland (2014). Results of these measures are listed in Tables 1 and 2.

Sequential Organic Matter Extractions

Extraction methods were based on the procedure of Lopez-Sangil and Rovira (2013). Air-dried samples, passed through a 2-mm sieve, were bulked into 0- to 20-cm and 20- to 45-cm depths by adding approximately 5 g of soil from each depth fraction, for a total of 10 g of soil to be extracted. Bulked soils were put into 50-mL Falcon vials, two 0.625-in-diameter glass marbles were added, and the vials were filled to volume with deionized water. The tubes, containing the soil, water, and marbles, were shaken on an automated shaker for 1 h to disperse any macroaggregates in the soil slurry. Samples were sonified in an ice bath using a Branson Sonifier 450 (Emerson Industrial Automation, St. Louis, MO) at 60% power for 5 min per sample to disperse remaining aggregates in the soil and separate out particulate matter. Dispersed soil slurries were wet sieved through a 53-μm mesh to remove larger particulates from the samples. The particulate matter appeared largely sandy (mineral) and was excluded from further analyses.

The fraction of less than 53 μm was transferred to 1-L polyethylene bottles with additional deionized water, and the bottles were filled to a total volume of 1 L with deionized water. To each

TABLE 2. Average Bulk Density and Bulk Soil C Concentration Data by Depth Increment for All Stands Included in the Study—Previously Reported in Vario et al. (2014)

Age, y	Depth, cm	Bulk Density 0–45 cm, g/cm^3	Bulk Soil C Concentration, mg C/10-g Soil
5	0–10	0.52	570.6
5	10–20	0.51	361.1
5	20–30	0.54	183.0
5	30–45	0.52	98.1
55	0–10	0.57	477.3
55	10–20	0.57	249.3
55	20–30	0.45	244.1
55	30–45	0.51	113.1
Mature	0–10	0.43	684.6
Mature	10–20	0.37	591.2
Mature	20–30	0.43	286.7
Mature	30–45	0.47	190.4

of the 1-L polyethylene bottles, 4 mL of flocculant solution (saturated aluminum potassium sulfate) was added. The bottles were placed in the refrigerator for 48 h to allow for thorough flocculation. The flocculated solutions were decanted. Bottles were then rinsed with deionized water, and the resulting soil slurries were transferred to clean 50-mL Falcon vials. Falcon vials were centrifuged and decanted, and more slurry was added until the Nalgene bottles were rinsed clean. After transfer, a soil pellet remained in the bottom of the Falcon vial, ready for extraction.

Targeted organo-mineral pools and extraction order are shown in the following list. The most loosely bound, most bioavailable SOM was extracted first, and more recalcitrant SOM was removed with each subsequent extraction:

(i) Water soluble—Potassium sulfate (0.5 *M* K_2SO_4) extracts any water-soluble and therefore highly bioavailable organic compounds.
(ii) Weakly mineral bound—Sodium tetraborate (0.1 *M* $Na_2B_4O_7$, pH adjusted to 9.7) disrupts electrostatic interactions, such as hydrogen bonding or cationic bridges with clay particles, between SOM and the mineral matrix. This weakly bound SOM is susceptible to detachment from the SOM-mineral adsorption site and is thus easily accessible to microbes and microbial respiration.
(iii) Cation bound—Sodium pyrophosphate (0.1 *M* $Na_4P_2O_7$, pH adjusted to 10.2) is a chelator and is used to extract SOM precipitated by metallic cations in the soil (Ca, Mg, Fe, Al). However, sodium pyrophosphate does not interrupt the Al or Fe oxides targeted in later extractions.
(iv) Strongly mineral bound—Sodium hydroxide (0.1 *M* NaOH) disrupts strong associations between SOM and mineral surfaces.
(v) Iron oxide bound—Sodium dithionite treatment and extraction (0.1 *M* Na_2SO_4, pH adjusted to 8.0, followed by 0.1 *M* NaOH extraction) reduces amorphous or crystalline Fe^{-} oxides and hydroxides, and the following sodium hydroxide extraction solubilizes and extracts any SOM loosened by the reduction of these oxide or hydroxide structures (Lopez-Sangil and Rovira, 2013).

The pH of each extraction solution was adjusted using 6 N hydrochloric acid and NaOH pellets. The pH of extraction solutions was monitored using ThermoScientific Orion Star A111 pH meter (ThermoFisher Scientific Inc., Waltham, MA).

For each extraction, 30 mL of extraction solution was added to each Falcon vial, and the vials were shaken overnight (approximately 16 h) on a horizontal, automated shaker. After shaking overnight, the vials were centrifuged for 20 min at 3500 revolutions per minute (~1000 g), and the supernatant was decanted and saved as the extraction sample. This process was repeated with the same extraction solution, but with the successive extraction shakes lasting only 1 h. Subsequent supernatants were combined with their corresponding first- and second-round supernatants. The number of successive extractions was determined by supernatant appearance. Once the supernatant became completely clear after centrifugation, the extraction process was repeated with the next extraction solution in the sequence outlined previously. There were approximately two to 15 rounds per extraction solution per sample. The sodium dithionite treatment had a different protocol: only 20 mL of sodium dithionite solution was added to each vial, which was shaken overnight. Vials were centrifuged and decanted, but the successive two shakes and decants used 30 mL of deionized water to "rinse" the soil of the sodium dithionite (Lopez-Sangil and Rovira, 2013).

All extractions were stored at 4°C until filtration through Whatman 47-mm, glass microfiber filters (GE Healthcare Life Sciences, Pittsburgh, PA) (1.5 μm). A 40-mL aliquot of each filtered sample was kept for analysis. Extractions were diluted to achieve a 0- to 40-ppm C range. Dilutions were analyzed on a Shimadzu TOC-5000A Total Organic Carbon Analyzer (Shimadzu, Columbia, MD), for determination of C concentration. The instrument was recalibrated daily. For approximately every 12 samples, one analytical blank and two analytical replicates were measured to ensure instrument performance. All measured replicates and blanks were within ±10% of known values. The solid soil pellet that remained after the sequential extractions was rinsed with deionized water, dried, and massed, and its percent C was determined using a Carlo-Erba NA 1500 Element Analyzer (CE Elantech, Inc., Lakewood, NJ). The C that remained in the pellet was classified as "stable C."

Statistical Analyses

The amount and the proportion of SOM extracted from the organo-mineral pools were compared for Mature and Cut stands in two depth categories: 0- to 20-cm and 20- to 45-cm. For each chemical extraction, the amount C extracted was normalized to 10 g of sample. The total C extracted was calculated by summing the normalized amount C extracted from each chemical extraction for every sample. The total C extracted for the entire sample profile (0–45 cm) was calculated by summing the total C extracted for each 0- to 20-cm sample with its corresponding sample in the 20- to 45-cm depth. For each organo-mineral pool, the proportion C extracted was calculated by dividing the amount C extracted (mg) by the total C extracted (g) for that same sample. For statistical analyses, the amount C and the proportion C extracted were natural log transformed to meet the normal distribution assumption for one-way analysis of variance (ANOVA) and *t* tests. One-way ANOVA and Tukey honestly significant difference tests were performed to ensure the 5- and 55-year stands were not significantly different from one another ($\alpha = 0.05$).

Differences between means for total C extracted for Mature and Cut stands for the entire sampled profile (0- to 45-cm depth) and the different depth categories were determined by one-tailed *t*-tests, allowing for unequal variances ($\alpha = 0.05$).

Differences between means for Mature and Cut forests for both the amount C and proportion C data for each chemical extraction were determined by one-tailed *t* tests, allowing for unequal variances ($\alpha = 0.05$). Water-soluble, weakly mineral-bound, and cation-bound pools were considered less tightly bound C pools; strongly mineral-bound, iron oxide-bound, and stable C pools were considered more tightly bound pools (Lopez-Sangil and Rovira, 2013). Statistical analyses were performed using JMP 11 (JMP version 11; SAS Institute, Inc, Cary, NC). All figures were made using GraphPad Prism version 6.0 (GraphPad Software Inc, La Jolla, CA).

RESULTS

The 5- and 55-year sites were not statistically significantly different from one another for either the amount C or the proportion C data for any organo-mineral pool in either depth category and thus were combined for all further analyses (see Tables 1 and 2, Supplemental Digital Content 1 [http://links.lww.com/SS/A36], and figures, Supplemental Digital Content 2 [http://links.lww.com/SS/A37] and 3 [http://links.lww.com/SS/A38], which display ANOVA and Tukey honestly significant difference test results).

For the entire soil profile (0–45 cm), significantly more C was extracted from Mature forest soil profiles ($P = 0.022$) (Fig. 1). Significantly more C was extracted from Mature stands in the 0- to 20-cm depth ($P = 0.020$), and the results were close to significant in the 20- to 45-cm depth ($P = 0.057$) (Fig. 2).

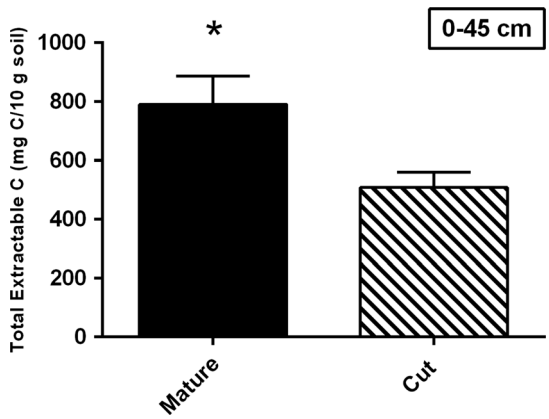

FIGURE 1. Normalized mean mass of total C extracted from entire soil profile for BEF. Error bars represent ±1 SEM. Asterisks denote statistically significant differences between Mature and Cut stands. From Mature stands, 790 ± 100 mg C was extracted, and from Cut stands, 510 ± 50 mg. Significantly more C was extracted from Mature forest soil profiles ($P = 0.022$).

At both the 0- to 20-cm and 20- to 45-cm depths, significantly more C was extracted from the strongly mineral-bound organo-mineral pool from Mature forest soils than from Cut forest soils. At the 20- to 45-cm depth, significantly more C was extracted from the stable C pool, and significantly less C was extracted from the water-soluble pool from Mature forest soils than from Cut forest soils (Fig. 3). Mean amount C extracted and results of these multiple *t* tests are reported in Tables 1 and 2 in Supplemental Digital Content 4 (http://links.lww.com/SS/A39).

At both the 0- to 20-cm and 20- to 45-cm depths, the proportion C extracted from the strongly mineral-bound pool was significantly larger, and the proportion C extracted from the water-soluble and weakly mineral-bound pools was significantly smaller for Mature stands than for Uncut stands. At only the 0- to 20-cm depth, the proportion C extracted from cation-bound pools was significantly smaller for Mature stands than for Cut stands. At the 20- to 45-cm depth, the proportion C extracted from the stable C pool was noticeably larger for Mature stands than for Cut stands, but results were not significant ($P = 0.056$) (Fig. 4).

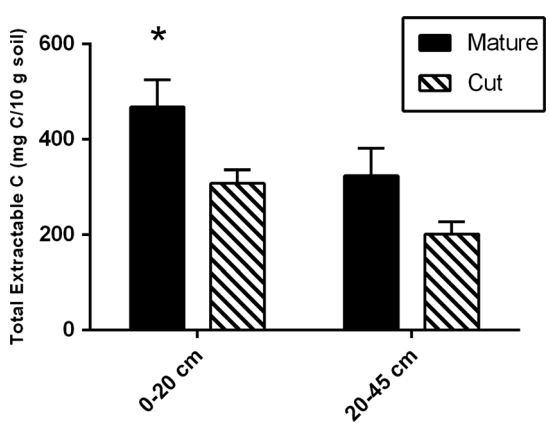

FIGURE 2. Mean mass of total C extracted per depth, normalized to a 10-g soil sample, for BEF. Error bars represent ±1 SEM. Asterisks denote statistically significant differences between Mature and Cut stands. In the 0- to 20-cm depth, 470 ± 60 mg C was extracted from Mature sites and 310 ± 30 mg C from Cut sites. In the 20- to 45-cm depth, 320 ± 60 mg C was extracted from Mature sites and 200 ± 30 mg C from Cut sites. Significantly more C was extracted from Mature stands in the 0- to 20-cm depth ($P = 0.020$), and results were close to significant in the 20- to 45-cm depth ($P = 0.057$).

DISCUSSION

Overall, the findings at BEF were consistent with prior studies that show that Spodosols in Mature forests contain more mineral soil C than Spodosols in Cut forests (Diochon et al. 2009; Vario et al., 2014). In general, Mature and Cut stands contained similar amounts of C in each organo-mineral pool. However, Mature stands stored significantly more C in strongly mineral-bound and stable C pools than Cut stands did. Both the strongly mineral-bound and stable C fractions represent more tightly bound, less bioavailable organo-mineral pools. Therefore, lower amounts of C in these pools were unexpected. The differences in the amount C for these pools are not derived solely from differing amounts of total C in Mature and Cut forest soils. Significant differences in the proportion C extracted for the water-soluble, weakly mineral-bound, and strongly mineral-bound C pools suggest there are differences in the distribution and therefore structure of soil C pools for Mature and Cut forest stands. The differences in both the amount C and proportion C data suggest that clear cutting a Mature forest would result in decreases in SOM, especially from the strongly mineral-bound and stable C pools.

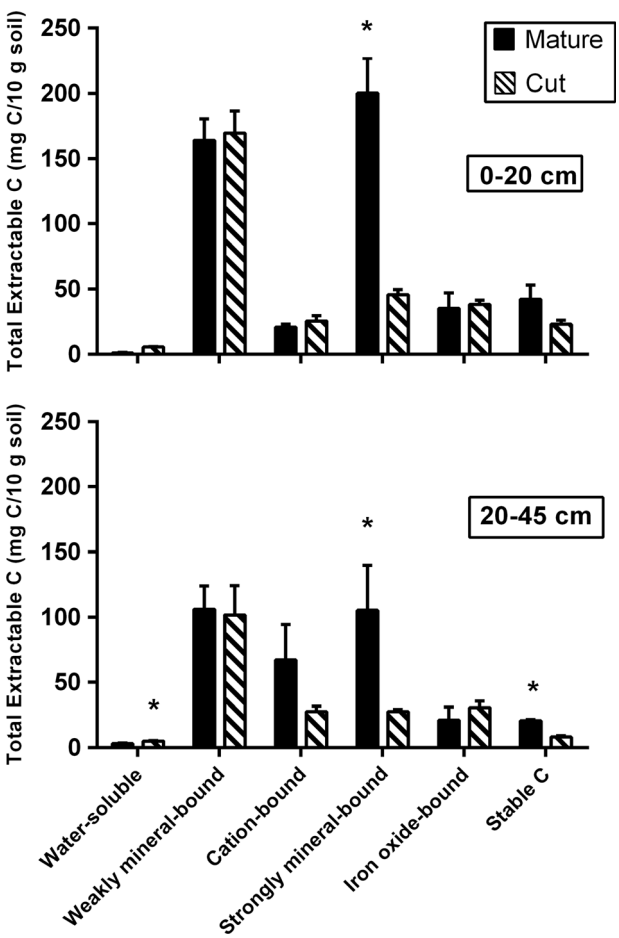

FIGURE 3. Mean extracted mass of C in organo-mineral pools of varying stability, normalized to a 10-g soil sample for BEF. Carbon fractions increase in chemical stability from left to right on the *x* axis. Error bars represent ±1 SEM. Asterisks denote statistically significant differences between Mature and Cut stands.

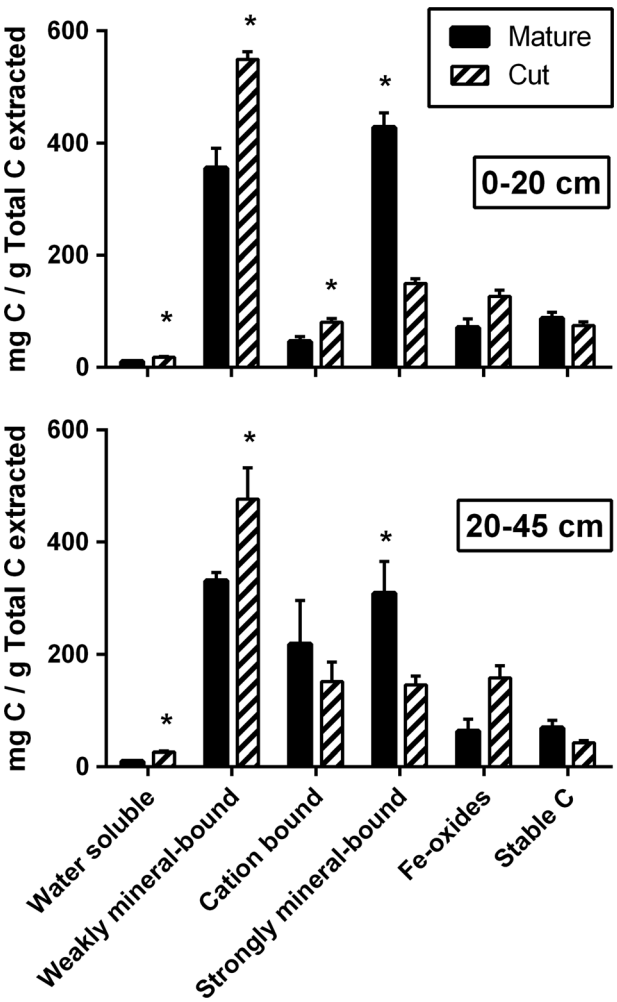

FIGURE 4. Mean proportion C extracted in fractions of varying organo-mineral stability for samples from BEF. Organo-mineral pools increase in chemical stability from left to right on the *x* axis. Error bars represent ±1 SEM. Asterisks denote statistically significant differences between Mature and Cut stands.

If the observed differences between Mature and Cut stands are indicative of SOM loss, this loss may be driven by physical disruption of the soil profile, during the process of clear cutting and biomass removal, and/or changes in microbial decomposition. Zummo and Friedland (2011) found a negative relationship between degree of physical disturbance of forest soils at BEF and amount of mineral soil C. The physical disturbance of logging may disrupt soil aggregates, reducing the physical protection of SOM (Besnard et al., 1996; Balesdent et al., 2000).

Alternatively, clear cutting may provide fresh organic matter inputs to the mineral soil, in the forms of slash debris and increased downward dissolved organic carbon flux (Grand and Lavkulich, 2012; Piirainen et al., 2002). These inputs may stimulate microbial decomposition of more stable SOM by causing increases in soil microbial activity and population (Bolan et al., 2011; Fontaine et al., 2003; Fontaine et al., 2007). If increased microbial respiration from the decomposition of fresh organic matter inputs was the principal cause of the lower observed C stores, no differences in the proportion C for any of the organo-mineral pools would be expected. Decreases in the amount C for all organo-mineral pools, including the more loosely bound C pools, would be expected. However, the fresh C inputs from slash could be replenishing these more weakly bound organo-mineral pools, making changes in the amount C for these same pools undetectable.

CONCLUSIONS

This study investigated differences in the amount and proportion of SOM in various organo-mineral pools between Mature and Cut stands at BEF. Sequential chemical extractions were used to investigate variation in organo-mineral binding in these forest soils. Analysis of samples from BEF showed statistically significant differences in the amount C stored between Mature and Cut stands in the strongly mineral-bound C pool at both the 0- to 20-cm and 20- to 45-cm depths and in the stable C pool at the 20- to 45-cm depth. Similarly, Mature stands stored a significantly larger proportion of C extracted in the strongly mineral-bound C pool for both the 0- to 20-cm and the 20- to 45-cm depths and a notably larger proportion of C in the stable C pool at the 20- to 45-cm depth. These differences indicate that losses of SOM may occur in strongly mineral-bound and stable C pools after clear cutting. If this small study at BEF is representative of other northern hardwood forests, then our findings suggest that the relative strength of organo-mineral interactions may not prevent SOM decline after clear cutting.

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