

SOIL ORGANIC MATTER FRACTIONS IN EXPERIMENTAL FORESTED WATERSHEDS

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Abstract. Recent concerns about climate change and atmospheric greenhouse gas concentrations have demonstrated the importance of understanding ecosystem C source/sink relationships. Soil organic matter fractionation was carried out in three paired, forested watershed sites where one of each watershed pair represented a different ecosystem perturbation. The perturbations were 8 years of experimental N amendments at the Bear Brook Watershed in Maine (BBWM), a 50 year old intense wildfire and subsequent regeneration at Acadia National Park (ANP), and a 17 year old whole-tree harvest at the Weymouth Point Watershed (WPW). At each site, mineral soils were sampled by uniform depth increments. Mineral soil (< 2 mm) was separated into light, occluded light, and heavy density fractions by floatation in NaI solution (1.7 g cm^{-3}). Mineral soil (< 2 mm) was also separated into particle-size fractions of sand (2.0 to 0.05 mm), silt (0.05 to 0.002 mm), and clay (< 0.002 mm) by wet sieving and centrifugation. Whole soils, and density and particle-size fractions were analyzed for total C and N. Both fractionation schemes showed that all soil organic matter fractions had lower C/N ratios as a result of N enrichment at BBWM. At ANP, soil organic matter fractions generally had lower C/N associated with the wildfire and subsequent shift from softwood to hardwood regeneration. Few significant whole soil and soil organic matter fraction differences were associated with the whole-tree harvest. Within watershed pairs, both density and particle-size fractionation techniques usually indicated similar responses. Soil organic matter fractionation results indicated that there were no consistent shifts in fraction distributions in response to perturbation that were consistent across all paired watershed study sites.

Keywords: ecosystem perturbation, forest soils, soil organic matter fractions

1. Introduction

The threat of climate change in response to or exacerbated by increased greenhouse gas emissions has provided an impetus to discern ecosystem C source/sink relationships. Globally, soil organic matter (SOM) contains approximately $1500 \text{ to } 1600 \times 10^{15} \text{ g C}$, which is more C than in the atmosphere ($750 \times 10^{15} \text{ g}$) or terrestrial vegetation ($500 \text{ to } 800 \times 10^{15} \text{ g}$) (Post *et al.*, 1990). Terrestrial C balances may be influenced by perturbations, such as N deposition, fire, and harvesting. The mechanisms through which these perturbations may impact soil C cycling operate on various time scales and include biotic controls, which influence the amount of C



added to soils by plants and the resistance to decay, and physical controls, including the area and chemistry of surfaces for SOM stabilization, and the availability of oxidants to decomposers (Trumbore, 1997).

Elevated emissions of nitrogen oxides and ammonia are primarily due to the combustion of fossil fuels, the manufacture and use of fertilizers, livestock waste, and the burning of biomass (Galloway *et al.*, 1995). Increased N deposition may increase SOM decomposition rates in N-limited systems, decreasing soil C pools (Gill and Lavender, 1983; Hunt *et al.*, 1988; Fenn, 1991; McNulty *et al.*, 1991; Conn and Day, 1996; Downs *et al.*, 1996), or may decrease SOM decomposition rates, increasing soil C pools (Martikainen *et al.*, 1989; Nohrstedt *et al.*, 1989; Prescott, 1995). Berg (2000) suggested that increased concentrations of soil N may initially stimulate SOM decomposition and may subsequently retard decomposition in the later stages, with the effect on total soil C reservoirs unknown.

The potential for a warmer climate with altered precipitation patterns and lightning frequency, in response to or exacerbated by increased greenhouse gas emissions, may change wildfire frequency. For example, fire frequency in Canada is predicted to increase by 50% if the concentration of atmospheric CO₂ doubles (Flannigan and Van Wagner, 1991). Increased fire occurrence may act as a positive feedback to climate change by reducing terrestrial C and N reservoirs and increasing atmospheric CO₂ and NO_x concentrations. In addition, wildfire could be an important factor in determining the long-term sequestration of C and N in forest soils.

With increasing population and a concomitant increase in the demand for forest products, there has also been a concern over the potential effects of forest harvesting on soil C storage. In a review of the literature, Johnson (1992) found no change in average soil C content with forest harvesting, although individual sites showed net losses and/or gains depending on residue management. Whole-tree harvests commonly result in lower soil C through increased rates of decomposition due to increased soil temperature (Ovington, 1968; Witkamp, 1971; Marks and Bormann, 1972; Edwards and Ross-Todd, 1983; Mroz *et al.*, 1985), and initial decreases in leaf and wood litter inputs due to overstory removal. Covington (1981) found that the mass of SOM in the forest floor decreased by over 50% 15 years following a whole-tree harvest, suggesting that harvesting may have long-term impacts on soil C pools.

The influence of ecosystem perturbations such as N deposition, fire, and harvesting on total soil C pools, particularly in the forest floor, are more commonly studied than the influence on soil C sequestration. Understanding the influence of these perturbations on total soil C pools, does not implicitly express the influence on soil C stability. Despite the complex structure of SOM and the numerous factors that determine SOM decay rates, several widely accepted approaches are used to partition SOM into operationally defined soil fractions. However, no single method yet exists to definitively separate SOM into components with distinct turnover rates.

Nevertheless, a few methods, most commonly used with agricultural soils, show promise for partitioning SOM into biologically meaningful fractions.

Chemical fractionation methods have been traditionally used to evaluate SOM (Stevenson and Elliott, 1989), although soil chemical extracts are not directly related to C turnover (Duxbury *et al.*, 1989). Physical fractionation techniques are generally less destructive than chemical techniques and are thought to relate more directly to the *in situ* structure and function of SOM than chemical extracts (Christensen, 1992). Particle-size fractionation is based on the observation that C in the sand fraction is generally more labile than C in clay and silt size fractions (Tiessen and Stewart, 1983; Christensen, 1992). The association of SOM with clay and silt particles is a dominant mechanism for physical protection (Theng, 1979), and is important in determining the stability of SOM. Baldock *et al.* (1992), using CP/MAS ^{13}C NMR spectroscopy, found that the extent of decomposition follows a continuum with decreasing particle-size. Fresh plant residues were associated with larger size fractions, while the most humified and microbially synthesized components were associated with the clay fraction. Density fractionation is based on the observation that during the humification process, SOM becomes intimately associated with the mineral portion of the soil (Barrios *et al.*, 1996). Thus, more humified particles are associated with the mineral fraction, collectively known as the heavy fraction. The non-complexed light fraction, an intermediate between plant material and humified SOM, has a relatively rapid turnover rate (Gregorich *et al.*, 1995; 1996). Appreciable amounts of microbial and microfaunal debris, including fungal hyphae and spores, are found in the light fraction (Molloy and Speir, 1977; Spycher *et al.*, 1983). The light fraction is more sensitive to management and residue inputs than total SOM (Dalal and Mayer, 1987; Skjemstad *et al.*, 1988; Janzen *et al.*, 1992). The occluded light fraction, an intermediate between light and heavy fractions, represents the light fraction material protected within aggregates (Golchin *et al.*, 1994).

The primary objective of this study was to apply density and particle-size fractionation techniques to the evaluation of soil C as influenced by experimentally elevated N deposition, wildfire, and whole-tree harvesting. The second objective of this study was to compare these two methods for their efficacy in evaluating the influence of these ecosystem perturbations on soil C fractions and the implications for C cycling.

2. Materials and Methods

2.1. SITE DESCRIPTION

The Bear Brook Watershed in Maine (BBWM)

The BBWM is located in eastern Maine (44°52'N, 44°52'W), 50 km from the Gulf of Maine, on the upper 265 to 475 m of the southeast slope of Lead Mountain. The

average slope from the top of the watershed to the weirs is 31%. The West Bear watershed has been treated bimonthly with ammonium sulfate $[(\text{NH}_4)_2\text{SO}_4]$ since November 1989 as part of a whole-watershed manipulation experiment designed to investigate the effects of atmospheric deposition of N and S. Granular $(\text{NH}_4)_2\text{SO}_4$ has been aerially applied at approximately $28.8 \text{ kg S ha}^{-1} \text{ yr}^{-1}$ and $25.2 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. The treated watershed, West Bear (NIT-TRT, 10.2 ha), is adjacent to the reference watershed, East Bear (NIT-REF, 10.7 ha). The upper reaches of the watersheds are predominately pure red spruce (*Picea rubens* Sarg.) stands, and the lower ~60% of the watersheds are mixed northern hardwood stands, dominated by American beech (*Fagus grandifolia* Ehrh.), sugar maple (*Acer saccharum* Marsh), and red maple (*Acer rubrum* L.). Hardwood stands are 40 to 50 years old, reflecting previous harvesting practices, while softwood stands on the upper slope are 80 to 100 years old. Soils are predominantly Typic and Lithic Haplorthods formed from dense basal till. Additional site characteristics and biogeochemical data on soils and soil solutions from this study site may be found in Fernandez *et al.* (1999), Kahl *et al.* (1999), and Norton *et al.* (1999).

Acadia National Park (ANP)

During the dry season of 1947, wildfires consumed thousands of hectares throughout northern New England. Approximately 6,956 hectares burned on Mount Desert Island, located on the coast of eastern Maine ($44^\circ 23' \text{N}$, $69^\circ 15' \text{W}$), that included >4,000 ha of ANP. The Canon Brook watershed (BRN-TRT) is located on the eastern slope of Cadillac Mountain between 152 to 457 m elevation in an area of ANP that burned in 1947. The southern tributary of the Canon Brook stream is located in the BRN-TRT watershed. Pioneer species such as paper birch (*Betula papyrifera* Marsh) and striped maple (*Acer pennsylvanicum* L.) currently dominate the BRN-TRT watershed. Prior to European settlement in the late 1700s, fires burned infrequently (approximately every 500 years) in coastal Maine forests (Patterson *et al.*, 1983). However, large fires occurred on Cadillac mountain in 1889 and in 1896 or 1889 (Moore and Taylor, 1927). The Hadlock Brook watershed (BRN-REF) lies on the southeastern slope of Sargent mountain at an elevation of 152 to 396 m, and is used as a reference watershed. Hadlock Brook drains an area that escaped the 1947 fire and red spruce is the dominant canopy species. Both watersheds have similar soils, predominantly coarse-loamy, mixed, frigid, Aquic Haplorthods. The watersheds are approximately 4.5 km apart and sampling sites had slopes of 20–30%.

Weymouth Point Watershed (WPW)

The WPW is located on commercial spruce-fir forest land in northern Maine ($49^\circ 57' \text{N}$, $69^\circ 19' \text{W}$) at an elevation of 287 to 315 m. In 1981, a whole-tree harvest was conducted, removing approximately 90% of the 232 Mg ha^{-1} of available biomass from a 48 ha watershed (WTH-TRT) (Smith *et al.*, 1986). An adjacent watershed (73 ha) was not harvested and serves as the reference watershed (WTH-

REF). Vegetation on WTH-REF consists of a two-aged red spruce (*Picea rubens* Sarg.) and balsam fir (*Abies balsamea* [L.] Mill.) forest that developed from the 1913 to 1919 spruce budworm (*Choristoneura fumiferana* Clem.) epidemic. Regeneration on the WTH-TRT watershed is predominantly red spruce and balsam fir, which were approximately two to three meters in height at the time of sampling. Soils in the WPW are coarse-loamy, mixed, frigid Aquic Haplorthods and Aeric Haplaquepts of the Chesuncook catena formed from dense basal till. Drainage class differs significantly across the gently sloping landscape due to classic pit and mound topography, ranging from moderately well drained (WPW-MWD), which accounts for 25% of the total WPW area, to very poorly drained (WPW-VPD), which accounts for 34% of the total WPW area.

2.2. SOIL SAMPLING

Soil sampling depth increments included the forest floor, the upper 5 cm of the B horizon, and the 5–25 cm increment of the B horizon. A horizons were not present. The E horizon was thin, discontinuous, and typically is low in C or N content in the soils at our sites. The chemical characterization of E horizons also often reflects admixtures of overlying O or underlying B horizon material incorporated during sampling. For these reasons, E horizons were sampled, but chemical analyses were not conducted on these samples. Forest floors were quantitatively sampled at each site with a 0.71 × 0.71 m frame (Fernandez *et al.*, 1993). Mineral soil was also quantitatively sampled at BBWM, but bulk pit face samples of mineral increments were collected at ANP and WPW. Mineral soils from all sites were sieved (6 mm) and homogenized in the field, and subsamples were air-dried, then sieved through 2 mm mesh sieves in the laboratory to isolate fine earth fractions (< 2 mm). Forest floor samples were collected in their entirety and taken to the laboratory for analysis.

The Bear Brook Watershed in Maine (BBWM)

Sampling at BBWM focused on capturing potential contrasts between NIT-TRT and NIT-REF watersheds within two dominant forest types after eight years of N additions. The contrast of forest stand types at BBWM also allowed potential differences between hardwood and softwood forest types to be evaluated. Within each watershed, three soil pits were excavated in hardwood stands and three in softwood stands within the Tunbridge soil series. Soil samples were collected June through August of 1998, and watershed and forest types were equally represented during each sampling month.

Acadia National Park (ANP)

Sampling at ANP was intended to capture potential differences in BRN-TRT and BRN-REF watersheds 50 years after a wildfire. Soil sampling at ANP was limited to two stands to minimize destructive sampling within Acadia National Park as per

our sampling permit. One stand was selected in each of the BRN-TRT and BRN-REF watersheds at similar elevations (approx. 275 m). A transect was established in each stand with 12 equidistant sampling points spaced at four meter intervals. At each sampling point, a 15 × 15 cm soil sample was excavated from the Dixfield soil series. Three adjacent sampling points were bulked and homogenized in the field to create four samples per transect. Sampling at BRN-TRT was conducted in September 1998 prior to leaf fall, and BRN-REF was sampled in October 1998.

Weymouth Point Watershed (WPW)

Sampling at WPW focused on capturing potential contrasts between WTH-TRT and WTH-REF watersheds within two soil drainage classes 17 years after the harvest. Sixteen study plots (10 × 10 m) were selected from the 27 plots described in the experimental design of Briggs *et al.* (1999). The selected study plots were stratified by two soil drainage classes, MWD and VPD. For this study, soil pits were excavated adjacent to the four existing plots in each of MWD and VPD soil drainage classes in each watershed. Samples were collected in June and July of 1998, and watersheds and drainage classes were equally represented during each sampling month.

2.3. PARTICLE-SIZE FRACTIONATION

Air-dried mineral soils were partitioned into three size fractions, using the method outlined by Amelung *et al.* (1998). Fine earth (30g, < 2mm) was ultrasonically treated at 60 J mL⁻¹ with a probe-type sonifier (Branson[®] 450) in a soil:water ratio of 1:5 to disperse microaggregates. The probe tip was placed in the center of the sample and was submerged 1.5 cm into the solution. Sand-sized (2.0 to 0.05 mm) material (henceforth, sand) was removed by wet sieving. Ultrasonic dispersion was applied at 440 J mL⁻¹ to the < 0.05 mm fraction with a soil:water ratio of 1:10. An ice bath was used during ultrasonic dispersion. Centrifugation was used to separate the silt-sized (0.05 to 0.002 mm) fraction (henceforth, silt) from the clay-sized (< 0.002 mm) fraction (henceforth, clay). All particle size fractions were dried at 40 °C, and were ground with a mortar and pestle for chemical analysis.

2.4. DENSITY FRACTIONATION

Air-dried mineral soils were partitioned into three density fractions (Golchin *et al.*, 1994), using NaI at a density of 1.7 g cm⁻³ (Gregorich and Ellert, 1993). Fine earth soil (20 g, <2 mm) was mixed with 200 mL of NaI, and was allowed to sit for 24 hours. The light fraction (LF), less dense than the NaI solution, floated to the top of the solution. The LF was removed by suction using vacuum filtration (Strickland and Sollins, 1987) and glass-fiber filter paper (Whatman GF/A). This procedure was repeated when necessary to remove the entire LF. The remaining soil was washed into a 125 mL beaker with NaI, and the volume was

adjusted to 80 mL. Samples were ultrasonically dispersed with a probe-type sonifier (Branson® 450) for five minutes at half power to release LF material trapped within microaggregates. The light fraction released, therefore, is referred to as the occluded light fraction (OL). An ice bath was used during the ultrasonic dispersion process (Christensen, 1992). The probe tip was positioned in the center of the beaker and was submerged 1.5 cm into the suspension. The sample was allowed to sit for 24 hours, after which OL was removed by vacuum filtration. Both the LF and OL were rinsed with at least 75 mL of 0.01 M CaCl₂ and 75 mL deionized water. The remaining soil was designated the heavy fraction (HF), and was rinsed with at least 75 mL of 0.01 M CaCl₂ and 150 mL of deionized water using centrifugation and vacuum filtration. All fractions were dried at 60 °C and were ground with a mortar and pestle for chemical analysis.

2.5. LABORATORY ANALYSIS

Percent air-dry moisture was measured for fine earth soils and coarse organic soils (>6 mm) (Robarge and Fernandez, 1986). Fine earth soil pH was measured using 0.01M CaCl₂ (Hendershot *et al.*, 1993), and SOM by loss-on-ignition at 450 °C for 12 hours. Total C and N were measured on whole soils and particle and density fractions using a LECO® CN 2000 (St. Joseph, MI) analyzer employing the Dumas method of combustion at 1350 °C in a pure O₂ environment.

2.6. STATISTICAL ANALYSIS

All analyses were carried out using the Statistical Analysis System (SAS Institute, Inc., 1988), with an alpha level of 0.05. Because the data did not meet the assumptions of normality and equality of variance, a rank transformation was used (Conover, 1971; Zar, 1984). A general linear model (GLM) was applied to the ranked data for each site to analyze the differences among main effects (watersheds, forest types, and drainage class) and interactions.

3. Results and Discussion

3.1. FOREST SOIL ORGANIC MATTER FRACTIONS

Particle-size fractionation results were consistent with the general sandy loam texture of these forest soils (Figure 1). Sand was over half, and clay was less than 10%, of the soil mass. The heavy density fraction represented the majority of the soil mass. (Figure 1). The LF averaged was less than 5% of the total soil mass, and the OL fraction was less than 1%. The small amount of OL recovered was likely a result of the dominant presence of sand, which has low aggregative ability. The relative contribution of these three density fractions to whole soil C concentrations corresponded to fraction percentages (Figure 1).

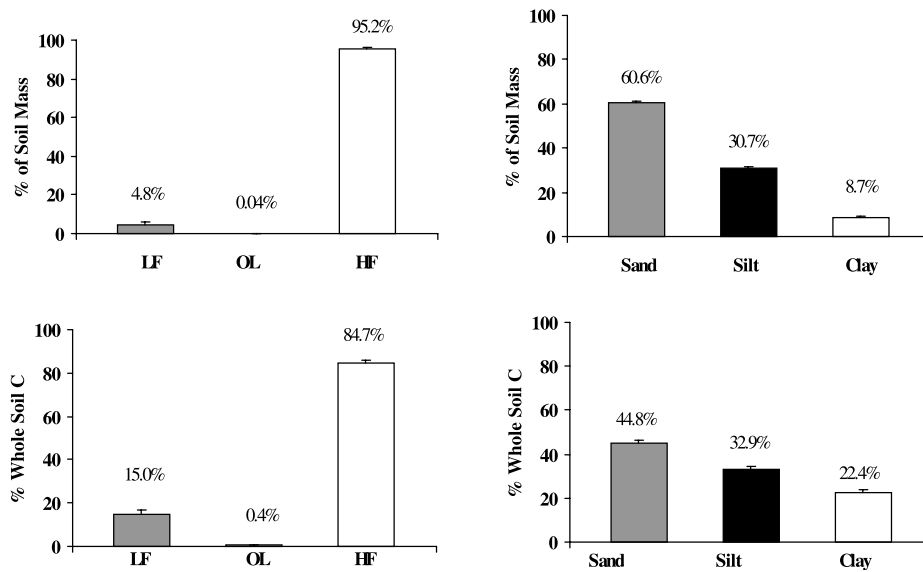


Figure 1. Mean percentages (+ SE) of density and particle-size fractions, and the relative contribution to whole soil C concentrations (+ SE).

BBWM data were used to demonstrate depth trends in soil C fractions because quantitative investigations of all soil depth increments were conducted at this site (Figure 2). The relative contribution of sand and LF to whole soil C and N concentrations generally decreased with soil depth. Depth trends for the particle-size analyses demonstrated the dominance of sand-sized separates in these forest soils throughout the soil profile. Sand C and N contributed more than 50% to whole soil C and N concentrations in the upper 5 cm of the B horizon and decreased with depth to a low of 36 and 33%, respectively, in the C horizon. The SOM adsorbed to silt-size separates increased with depth, and clay remained relatively constant. In the upper 5 cm of the B horizon, light fraction C and N were approximately 20% of whole soil C and N concentrations and decreased to 5% in the C horizon. Soil organic matter adsorbed onto mineral particles in the HF increased with depth. The OL remained constant with depth. Spycher *et al.* (1983) reported similar depth trends for HF and LF in Oregon Douglas-fir forest soils. Both fractionation schemes demonstrated the importance of adsorbed SOM in deep soil layers and non-complexed SOM in shallower surface soils.

3.2. N ENRICHMENT

At BBWM, particle-size and density fractions generally had higher mean N concentrations in NIT-TRT than NIT-REF, consistent with the experimental N enrichment in the NIT-TRT watershed (Table I). Although, density fraction N concentrations were not significantly different between watersheds, particle-size sand and

TABLE I

Whole soil, soil organic matter (SOM), density fraction (light fraction – LF, occluded light – OL, and heavy fraction – HF), and particle-size fraction (sand, silt, and clay) means by depth from the Bear Brook Watershed in Maine (BBWM). Means marked with an asterisk (*) are significantly different ($p < 0.05$) between $(\text{NH}_4)_2\text{SO}_4$ treated (NIT-TRT) and reference (NIT-REF) watersheds

Watershed		Whole Soil	SOM	Density fractions			Particle-size fractions		
				LF	OL	BF	Sand	Silt	Clay
% of Soil Mass									
NIT-TR	0–5 cm	–	16.8	7.3	0.05	92.7	68.6	26.6	4.8*
	5–25 cm	–	12.3	2.3	0.04	97.7	70.1	27.2	2.8*
	25 cm–C	–	8.9	0.9	0.03	99.0	65.5	32.2	2.2*
	C Horizon	–	7.4	0.6	0.05	99.4	63.7	34.0	2.3
NIT-REF	0–5 cm	–	15.2	10.1	0.03	89.9	69.5	23.9	6.5
	5–25 cm	–	11.3	2.6	0.04	97.4	69.2	26.0	4.8
	25 cm–C	–	7.2	0.7	0.04	99.2	66.6	28.0	5.4
	C Horizon	–	5.0	0.4	0.02	99.6	69.2	27.3	4.9
% N									
NIT-TR	0–5 cm	0.38*	–	0.94	1.36	0.33	0.30*	0.37*	1.40
	5–25 cm	0.23	–	0.79	0.76	0.22	0.16	0.32	0.99
	25 cm–C	0.14	–	0.63	0.80	0.13	0.07	0.25	0.73*
	C Horizon	0.15	–	0.63	0.95	0.14	0.07	0.23	0.75
NIT-REF	0–5 cm	0.29	–	0.69	1.04	0.24	0.22	0.25	1.00
	5–25 cm	0.21	–	0.71	0.80	0.17	0.13	0.22	0.68
	25 cm–C	0.14	–	0.63	0.90	0.13	0.07	0.18	0.47
	C Horizon	0.09	–	0.64	0.86	0.09	0.05	0.14	0.55
% C									
NIT-TR	0–5 cm	7.8*	–	23.3	28.0	6.7	6.8	8.0	19.2
	5–25 cm	5.0	–	24.3	29.6*	4.5	3.6	6.7	15.7
	25 cm–C	2.9	–	23.0	38.0	2.6	1.6	4.8	12.3
	C Horizon	3.0	–	20.4	28.9	2.8	1.6	4.6	12.6
NIT-REF	0–5 cm	7.1	–	21.1	40.6	5.5	6.0	6.6	17.9
	5–25 cm	4.5	–	24.6	38.4	3.9	3.4	5.1	11.4
	25 cm–C	2.7	–	25.8	40.2	2.7	1.7	4.0	7.8
	C Horizon	1.9	–	24.7	37.4	1.9	1.2	3.0	8.9
C/N									
NIT-TR	0–5 cm	20.5	–	24.9*	20.6	20.0*	22.6	21.5	18.0
	5–25 cm	21.6	–	30.7*	38.9	20.8*	22.9*	20.9*	17.1
	25 cm–C	20.2	–	36.8	47.6	19.3	22.5	19.1*	16.7
	C Horizon	19.6*	–	32.8	30.3	19.9*	21.8*	19.8*	15.9
NIT-REF	0–5 cm	25.2	–	30.6	39.3	23.5	27.3	26.4	14.0
	5–25 cm	23.9	–	35.0	48.3	22.4	25.7	23.3	16.3
	25 cm–C	21.4	–	40.7	46.3	20.8	25.0	21.1	17.1
	C Horizon	20.5	–	39.7	43.7	19.8	22.1	19.9	17.0

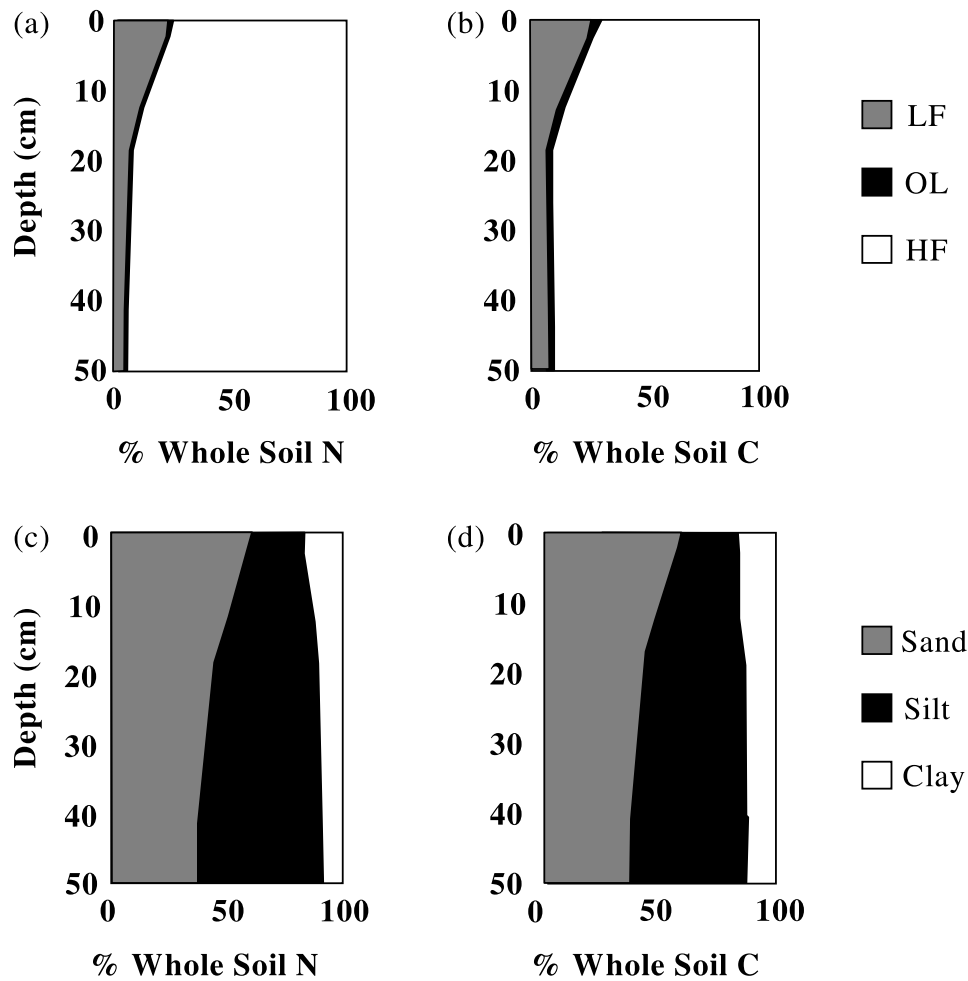


Figure 2. The relative contribution of density and particle-size fractions to whole soil N and C concentrations by depth at BBWM.

silt N concentrations in the upper 5 cm of the B horizon were significantly greater in NIT-TRT soils than NIT-REF soils. Clay N was also significantly greater in the lowest B horizon depth increment of NIT-TRT compared to NIT-REF. Despite significantly higher whole soil C concentrations in the upper 5 cm of NIT-TRT soil compared to NIT-REF, particle-size fraction C concentrations were not significantly different between these watersheds. Similarly, density fraction C concentrations were not different between BBWM watersheds besides OL C in the 5 to 25 cm increment of the B horizon, which was significantly lower in NIT-TRT than NIT-REF. Given that the OL fraction was only 0.04% of the soil mass and was not consistent with whole soil C concentrations, it is not important to soil C sequestration effects in these watersheds. These particle-size and density fraction

TABLE II

Whole soil, soil organic matter (SOM), density fraction (light fraction – LF, occluded light – OL, and heavy fraction – HF), and particle-size fraction (sand, silt, and clay) means by depth from the Bear Brook Watershed in Maine (BBWM). Means marked with an asterisk (*) are significantly different ($p < 0.05$) between soils under softwood and hardwood stands

Forest type	Whole Soil	SOM	Density fractions			Particle-size fractions		
			LF	OL	BF	Sand	Silt	Clay
% of Soil Mass								
Softwood	–	14.6*	3.1*	0.03*	96.9*	68.8	27.5	3.6
Hardwood	–	9.0	1.8	0.05	98.2	70.5	25.6	4.0
% N								
Softwood	0.26*	–	0.74*	1.08	0.25*	0.19*	0.34	0.93
Hardwood	0.16	–	0.76	0.62	0.15	0.11	0.21	0.67
% C								
Softwood	5.9*	–	23.0	38.6	5.3*	4.6*	7.3	14.6
Hardwood	3.7	–	26.9	32.5	3.2	2.6	4.6	11.4
C/N								
Softwood	22.7*	–	31.5*	36.7*	21.8	24.9	21.6	16.5
Hardwood	23.0	–	35.8	53.6	21.6	24.0	22.7	17.1

C and N data suggest that no one fraction accounts for the majority of whole soil C and N concentration differences between watersheds, and the variability of C and N concentrations was greater within fractions than whole soils.

Particle-size and density fraction C/N ratios reflected higher N concentrations in NIT-TRT soils than NIT-REF soils, particularly in the B horizon (Table I). Chichester (1969) found that particle-size C/N ratios were inversely related to N mineralization under water-logged conditions. If this also holds true for more mesic upland forest soils, then lower particle-size fraction C/N ratios in NIT-TRT soils could result in higher N mineralization rates than NIT-REF. Sollins *et al.* (1984) showed that N mineralization was negatively correlated with LF C/N ratios ($R^2 = 0.74$) and HF C/N ratios were positively correlated to net N mineralization ($R^2 = 0.85$). They suggested that LF decomposition is more likely N limited and HF decomposition is more likely C limited. Significantly lower LF C/N ratios in surface NIT-TRT soils compared to NIT-REF soils, and numerically lower percentages of LF in NIT-TRT soils, may suggest that LF decomposition rates in NIT-TRT soils had been stimulated by the N enrichment. However, Sollins *et al.* (1984) suggested that the C/N ratio does not control HF mineralization. Therefore, lower HF C/N ratios in NIT-TRT soils compared to NIT-REF may not directly influence C and N sequestration in the HF. Given the relative importance of HF in total C, further investigation is needed to define controls on HF mineralization rates.

3.3. FOREST TYPE

Few interactions between forest type and N enrichment were found. Table II shows the results for the differences between forest types at BBWM. In general, whole soil SOM, C, and N concentrations and particle-size and density fraction C and N concentrations were higher in soils under softwood stands than hardwoods stands. This reflects slower decomposition rates in soils supporting softwoods compared to hardwoods due to poorer litter quality (higher C and lower N) in the softwood stands, and litter quality differences are probably more important than litterfall rates in explaining these forest type differences (Parker *et al.*, 2001). Light fraction C and N did not correspond to the whole soil in the upper B horizon, and LF percentages were higher under softwood stands compared to hardwood stands, particularly in the upper B horizon (Table II). Boone (1994) similarly reported that LF percentages were greater in softwood stands (white pine) than in hardwood stands (pure maple stands) in Massachusetts. Light fraction likely increases as decomposition declines and as residue inputs increase (Christensen, 1992; Janzen *et al.*, 1992). Therefore, LF decomposition rates are slower in softwood soils compared to hardwood soils, resulting in a greater accumulation of LF materials. Using spectroscopic techniques, Amalfitano *et al.* (1995) showed that the composition of forest LF materials and litter were similar. Thus, litter quality differences between these two forest types may control the amount of LF in these soils and LF C and N concentrations.

3.4. WILDFIRE

Fifty years after the wildfire at ANP, the particle-size analysis showed that BRN-TRT soils had significantly higher sand percentages and lower clay and silt percentages in the upper 5 cm of the mineral soil than BRN-REF soils (Table III). Although, increased erosion following wildfire (Díaz-Fierros *et al.*, 1987; McNabb and Swanson, 1990; Ulery and Graham, 1993) could have reduced the presence of fine soil particles in the uppermost mineral soil of BRN-TRT, these differences may also simply reflect the natural heterogeneity in soil texture in these ANP watersheds. The density fractionation showed significantly higher (LF) and lower (HF) in the upper 5 cm of the B horizon of BRN-TRT than BRN-REF. Light fraction, expressed as a proportion of SOM rather than whole soil mass, was also significantly greater in the upper 5 cm of BRN-TRT (52%) than BRN-REF (22%). Higher LF percentages in upper B horizon in BRN-TRT may be related to antecedent environmental conditions, vegetation, and wildfire. At ANP we found a trend towards higher LF in the BRN-TRT watershed where hardwoods dominated. At BBWM we found higher concentrations of LF in soils supporting softwoods compared to hardwoods. This may suggest that the differences between soil LF at ANP are attributable to either the wildfire or antecedent conditions rather than a vegetation type effect. The wildfire and concomitant shift in vegetation type at BRN-TRT was associated with significantly higher pH values in the upper 5 cm of BRN-TRT

TABLE III

Whole soil, soil organic matter (SOM), density fraction (light fraction – LF, occluded light – OL, and heavy fraction – HF), and particle-size fraction (sand, silt, and clay) means by depth from Acadia National Park (ANP). Means marked with an asterisk (*) are significantly different ($p < 0.05$) between burned (BRN-TRT) and reference (BRN-REF) watersheds

Watershed	Whole Soil	SOM	Density fractions			Particle-size fractions			
			LF	OL	BF	Sand	Silt	Clay	
% of Soil Mass									
BRN-TRT	0–5 cm	–	16.5*	8.5*	0.04	91.5*	71.9*	23.4*	4.7
	5–25 cm	–	14.2	3.8	0.08	96.1	69.4	24.0	6.6
BRN-REF	0–5 cm	–	9.2	2.0	0.04	97.9	63.1	28.0	7.3
	5–25 cm	–	12.7	1.4	0.17	98.4	56.5	31.9	11.7
% N									
BRN-TRT	0–5 cm	0.39*	–	1.01*	0.99	0.33*	0.31*	0.40*	1.02
	5–25 cm	0.30*	–	0.91*	0.83	0.28*	0.22	0.37	0.75
BRN-REF	0–5 cm	0.17	–	0.82	0.78	0.17	0.12	0.11	0.62
	5–25 cm	0.24	–	0.72	0.90	0.23	0.24	0.31	0.84
% C									
BRN-TRT	0–5 cm	7.3*	–	25.3*	46.3	5.9	6.6*	6.9*	13.6
	5–25 cm	5.8	–	26.6	38.3	5.1	4.9	6.3	11.3
BRN-REF	0–5 cm	4.1	–	31.3	43.7	3.8	3.5	2.7	11.0
	5–25 cm	5.4	–	26.7	41.1	5.1	5.7	6.3	12.7
C/N									
BRN-TRT	0–5 cm	19.0*	–	25.3	27.7	17.8*	21.7	18.4*	13.6
	5–25 cm	19.4	–	29.3	21.0	18.5*	21.9	17.4	15.3
BRN-REF	0–5 cm	24.2	–	38.8	56.9	22.7	30.0	23.9	17.8
	5–25 cm	22.7	–	37.2	45.8	21.7	24.7	21.2	15.8

soils (pH = 4.5) than BRN-REF soils (pH = 3.6). Microbial biomass concentrations decline with decreasing soil pH and exchangeable base cations (Joergensen *et al.*, 1995; Raubuch and Beese, 1995; Anderson and Joergensen, 1997; Bauhus *et al.*, 1998). Because the LF may contain appreciable amounts of microbial biomass (Molloy and Speir, 1977), elevated percentages of LF in BRN-TRT may reflect a greater microbial biomass in BRN-TRT soils than BRN-REF soils. In addition, SOM and charcoal were likely deposited in the surface soils of BRN-TRT following the wildfire. It is unknown if SOM incorporated into the mineral soil at the time of the fire would be evident in the LF 50 years later, and the study reported here did not include a quantification of charcoal in soils. Macroscopic charcoal fragments were not evident in the LF, although charcoal has been shown elsewhere to be a component of LF material using an electron microscope (Spycher *et al.*,

1983). Balesdent *et al.* (1988) found that approximately 40% of prairie-derived LF remained 97 years after conversion from prairie to timothy grass. Fernández *et al.* (1997) also suggested that fire increases the stability of SOM, noting an increase in humin, SOM bound to Fe and Al, and relative amounts of lignin and lipids. It is unknown which density fraction might reflect these changes and how long they would persist through time.

Particle-size and density fraction C and N concentrations and C/N ratios paralleled whole soil C and N concentrations, particularly in the upper B horizon (Table III). Parker *et al.* (2001) suggested that higher whole soil C and N in the upper B horizon of BRN-TRT may have been due to the wildfire, contrasting vegetation type, and antecedent conditions. However, LF C concentrations were significantly lower in BRN-TRT than BRN-REF, despite significantly higher whole soil C concentrations in the upper 5 cm of the B horizon in BRN-TRT. Light fraction C concentrations were significantly lower in BRN-TRT than BRN-REF, suggesting that charcoal may not have been a dominant component of the LF in BRN-TRT soils. Thus, lower LF C concentrations in the upper 5 cm of BRN-TRT soils may reflect differences in LF inputs from hardwood stands in BRN-TRT compared to softwood inputs in BRN-REF, or greater decomposition of LF C under hardwood stands than softwood stands.

Interestingly, sand generally followed the response of the whole soil, whereas LF did not (Table III). Light fraction C concentrations were significantly lower in the upper 5 cm of BRN-TRT soils than BRN-REF soils, and whole soil C concentrations were significantly higher in BRN-TRT soils. Light fraction SOM contains most of the macroorganic matter, which is the SOM associated with the sand-sized fraction (Gregorich *et al.*, 1995). However, light fractions are also present in all particle-size fractions (Turchenek and Oades, 1979). Light fraction and macroorganic matter may not be equivalent, because non-complexed SOM has a different chemical composition than SOM adsorbed to sand grains (Zhang *et al.*, 1988). Our findings suggest that LF and sand fractions are not equivalent C fractions in these soils.

3.5. WHOLE-TREE HARVEST

The relative proportions of fractions were not significantly different between WTH-TRT and WTH-REF (Table IV). Density and particle-size C and N concentrations were not significantly different between WTH-TRT and WTH-REF, consistent with whole soil C and N. Heavy fraction C/N ratios were significantly lower in the 5 to 25 cm depth increment of WTH-TRT than WTH-REF, paralleling whole soil C/N ratios. The heavy fraction represented nearly the whole soil mass in the 5 to 25 cm increment of WTH-TRT and WTH-REF soils. This underscores the role of adsorbed SOM in deep soil layers and the need to better understand HF dynamics. Clay C/N ratios were also significantly lower in both depth increments of the mineral soil in WTH-TRT compared to WTH-REF. Clay fractions represented a much

TABLE IV

Whole soil, soil organic matter (SOM), density fraction (light fraction – LF, occluded light – OL, and heavy fraction – HF), and particle-size fraction (sand, silt, and clay) means by depth from the Weymouth Point Watershed (WPW). Means marked with an asterisk (*) are significantly different ($p < 0.05$) between whole-tree harvested (WTH-TRT) and reference (WTH-REF) watersheds.

Watershed		Whole Soil	SOM	Density fractions			Particle-size fractions		
				LF	OL	BF	Sand	Silt	Clay
% of Soil Mass									
TH-TRT	0–5 cm	–	11.6	4.3	0.04*	95.7	45.1	37.2	17.7
	5–25 cm	–	7.3	2.8	0.02	97.2	46.5	38.5	15.0
WTH-REF	0–5 cm	–	18.9	22.9	0.02	77.1	51.7	32.9	15.4
	5–25 cm	–	6.6	1.2	0.02	98.8	45.7	37.4	16.9
% N									
TH-TRT	0–5 cm	0.25	–	0.62	11.05	0.23	0.21	0.15	0.60
	5–25 cm	0.15	–	0.79	0.84	0.13	0.11	0.11	0.40
WTH-REF	0–5 cm	0.50	–	1.39	1.34	0.17	0.66	0.18	0.70
	5–25 cm	0.14	–	0.80	1.22	0.14	0.08	0.10	0.43
% C									
TH-TRT	0–5 cm	5.5	–	23.4	42.5	4.6	5.8	3.4	8.2
	5–25 cm	2.9	–	23.6	41.6	2.5	2.8	2.4	6.3
WTH-REF	0–5 cm	10.9	–	30.1	34.8	3.8	14.4	4.5	12.9
	5–25 cm	3.5	–	23.6	34.3	3.3	1.9	2.5	9.6
C/N									
TH-TRT	0–5 cm	22.6	–	34.7	41.0	20.3	28.5	23.3	14.7
	5–25 cm	19.0*	–	30.5	41.6	19.2*	22.7	20.3	14.3*
WTH-REF	0–5 cm	22.5	–	22.7	28.6	21.7	26.0	24.8	18.0
	5–25 cm	24.5	–	30.5	30.9	23.2	23.9	24.4	21.9

smaller percentage of the soil mass than HF. Borchers and Perry (1992) conducted a soil particle-size analyses in Oregon and found that relative concentrations of clay fraction C and N were generally lower in clearcut soils than control soils (0 to 15 cm). Soils at their study site had similar clay percentages (6.4 to 15.2%) to those at WPW. This may imply that C associated with the clay fraction is relatively labile. Others have suggested that a portion of the clay fraction may be relatively labile (Tiessen and Stewart, 1983; Feller and Beare, 1997). Ultrasonic dispersion and DOC production/deposition may also redistribute labile C compounds to the clay fraction (Christensen, 1992; Collins *et al.*, 1997). Therefore, it is difficult to discern the ecological significance of lower clay C/N ratios in WTH-TRT soils compared to WTH-REF soils.

TABLE V

Whole soil, soil organic matter (SOM), density fraction (light fraction – LF, occluded light – OL, and heavy fraction – HF), and particle-size fraction (sand, silt, and clay) means by depth from the Weymouth Point Watershed (WPW). Means marked with an asterisk (*) are significantly different ($p < 0.05$) between the very poorly drained (VPD) and moderately well drained (MWD) soils

Drainage		Whole Soil	SOM	Density fractions			Particle-size fractions		
				LF	OL	BF	Sand	Silt	Clay
% of Soil Mass									
VPD	0–5 cm	–	19.9	22.2*	0.02	77.8	49.1	32.1	18.8
	5–25 cm	–	5.0	1.0	0.04	98.9	45.3	35.7	19.0*
MWD	0–5 cm	–	10.6	5.0	0.02	97.0	47.8	37.9	14.3
	5–25 cm	–	8.9	3.0	0.02	95.0	46.8	40.2	13.0
% N									
VPD	0–5 cm	0.49*	–	1.39	1.24*	0.22*	0.65*	0.17	0.65*
	5–25 cm	0.12	–	0.85*	1.16	0.11	0.08	0.07*	0.39
MWD	0–5 cm	0.20	–	0.60	1.06	0.18	0.13	0.16	0.58
	5–25 cm	0.17	–	0.77	0.76	0.16	0.12	0.15	0.51
% C									
VPD	0–5 cm	10.4*	–	30.6	40.7*	4.6	14.6*	4.1	10.5*
	5–25 cm	2.8	–	28.1	39.0	2.6	2.0	1.7*	7.8
MWD	0–5 cm	4.5	–	19.0	37.8	3.8	3.5	3.7	10.0
	5–25 cm	3.8	–	22.6	36.3	3.3	2.9	3.3	8.5
C/N									
VPD	0–5 cm	22.2*	–	23.2	36.2	20.1	26.9*	24.0	15.7*
	5–25 cm	21.5*	–	35.2	38.1	20.5	22.2	22.7	18.8
MWD	0–5 cm	23.2	–	31.8	47.2	22.0	27.7	24.0	17.5
	5–25 cm	22.0	–	30.5	40.5	21.0	24.6	21.9	17.3

3.6. SOIL DRAINAGE CLASS

We did not find significant interactions between soil drainage class and harvesting treatments at WPW. Differences between soil drainage classes are provided in Table V. As expected, clay percentages were greater in VPD compared to MWD, and were significantly greater in the 5 to 25 cm increment of the B horizon. Light fraction percentages were significantly greater in the upper 5 cm of VPD soils than MWD soils. Maximum and minimum LF percentages for all sites occurred in the VPD soils at WPW, suggesting a high degree of heterogeneity for LF due to its relatively small mass and the nature of VPD conditions. It is likely that anaerobic conditions in VPD soils reduce SOM humification and decomposition rates (Parker *et al.*, 2001), and thus decrease downward movement of LF material within the

mineral soil. Furthermore, VPD soil conditions may encourage a shallow rooting depth, reducing LF inputs in deeper soil layers. Whole soil C and N similarly suggest a decrease in the downward movement of SOM in VPD soils. Whole soil C, N, and SOM concentrations in the VPD-WPW soil were numerically higher in the upper 5 cm of the B horizon and lower in the 5 to 25 cm increment compared to MWD-WPW soils. Likewise, LF C concentrations were significantly higher in the upper 5 cm of the B horizon and silt N concentrations were significantly lower in the lower B horizon of VPD soil than MWD.

4. Conclusions

Within watershed pairs, both density and particle-size fractionation schemes suggested similar responses to ecosystem perturbations. Both fractionation schemes at BBWM showed that all soil organic matter fractions had lower C/N ratios as a result of N enrichment. Soil organic matter fractionation did not support the hypothesis, as suggested by Berg (2000), that N deposition would stimulate the decomposition of labile SOM and hinder the decomposition of recalcitrant SOM. There were some differences in the density fractionation data from the N enrichment site to warrant further investigation of this hypothesis. Contrasting forest types at BBWM showed a lack of response to N enrichment. The density fractionation showed greater percentages of LF in soils under softwood stands compared to hardwood stands at BBWM. It is likely that C quality is important in determining the lability of the LF and thus more LF in softwood soils may not equate to more labile C in softwood soils. The contrast between hardwoods and softwoods in density fraction percentages were opposite each other at ANP and BBWM. This further reinforced the importance of C quality factors beyond operationally defined fractions in determining the lability of SOM. At ANP, particle-size and density fraction C and N concentrations and C/N ratios generally paralleled whole soil C and N concentrations. The only exception was the LF C in the upper 5 cm of the B horizon, which was significantly lower in the burned watershed compared to the reference. This suggested that charcoal, a recalcitrant form of C, may not have been an important component of the LF from the burned watershed. Therefore, differences between whole soil and LF C may reflect the wildfire and subsequent hardwood regeneration or antecedent conditions. At the WPW, whole-tree harvesting did not have a significant effect on SOM fractions. However, contrasting soil drainage classes at WPW did influence the quantity and quality of SOM fractions, particularly density fractions, and underscored the importance of further exploring the influence of soil drainage on soil C sequestration.

This study showed that particle-size and density fractions can be useful to understand changes in forest SOM that result from ecosystem perturbation. The particle-size analysis approach was more difficult to interpret with a paired-watershed design, due to antecedent textural differences between watersheds. Particle-size techniques

would be best applied to a single site and used to document changes over time. Density fractions appear more successful at partitioning non-complexed SOM from adsorbed SOM, and thus these fractions provide important ecological insights on decomposition processes. This study demonstrated the need to improve our ability to measure meaningful changes in SOM composition that can be pragmatically related to C sequestration objectives in future forest management.

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