



Long term effects of intensive biomass harvesting and compaction on the forest soil ecosystem



Tera E. Lewandowski^a, Jodi A. Forrester^{a,*}, David J. Mladenoff^a, Erika Marin-Spiotta^b, Anthony W. D'Amato^c, Brian J. Palik^d, Randall K. Kolka^d

^a Department of Forest and Wildlife Ecology, University of Wisconsin, Madison, WI, 53706, USA

^b Department of Geography, University of Wisconsin, Madison, WI, 53706, USA

^c The Rubenstein School of Environmental and Natural Resources, University of Vermont, Burlington, VT, 05405, USA

^d U.S. Forest Service Northern Research Station, 1831 Hwy 169 E. Grand Rapids, MN, 55744, USA

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ABSTRACT

Forest soil ecosystems can be negatively affected by intensive biomass harvesting due to losses of organic inputs and soil compaction, ultimately leading to reduced forest productivity. In this research, we revisited a site from the North American Long-Term Soil Productivity study located on a sandy Spodosol within the Huron National Forest in Michigan, USA, to measure the effects of aboveground organic matter removal of different intensities (three levels: bole only; whole tree harvest; or whole tree harvest and forest floor removal) and soil compaction (2 levels: no or moderate compaction) nearly 20 years following the initial treatments. The effects of harvesting on the soil microbial community in surface and subsurface soils and on soil nutrient availability in surface soils were evaluated. Additionally, patterns of carbon and nitrogen distribution among soil organic matter pools in surface and subsurface soils were compared using a physical fractionation approach to isolate a free – light fraction of particulate organic matter external to aggregates, an occluded – light fraction, which represents particulate organic matter released from the disruption of soil aggregates, and a heavy or mineral-associated fraction. Whole-tree harvests had significantly different microbial community compositions than bole-only harvests ($P = 0.02$), a result driven by significantly lower abundance of arbuscular mycorrhizae and greater gram positive bacterial abundance in the whole-tree harvest relative to bole-only harvest conditions. Few differences in soil nutrient availability were apparent 20 years after organic matter manipulations, with the exception of reduced calcium availability where organic matter was removed. Soil compaction resulted in greater microbial biomass (0.19 versus $0.14 \mu\text{mol g}^{-1}$ soil), which may have also led to a reduced C:N ratio in the heaviest and oldest soil component and increased P availability as well. Nitrogen concentrations and stocks were greatest at the surface (0–10 cm depth) for the free and light soil fractions in bole-only removal treatments, in contrast to whole-tree harvest treatments where C and N concentrations and C stocks were greater in the subsurface soil (free - light fraction at 20–30 cm depth). The soil microbial community, soil fraction size, and soil C and N stocks differed between surface and subsurface soils, highlighting the soil forming processes at work in this Spodosol, and the importance of sampling multiple depths to address research questions. These results demonstrate the long-term effects of forest management on soil biological, physical, and chemical properties and are useful in evaluating sustainable biomass harvesting practices for comparable forests.

1. Introduction

Forest soils are an integral component of forest productivity due to their role in providing a growing environment for plants and microbes by supplying important elements, including carbon (C) and nitrogen (N). Intensive forest biomass harvesting, or the removal of all harvesting slash for use as biofuel, can alter forest soil function and be

detrimental to long-term forest productivity (Burger, 2002). High levels of organic matter removal associated with harvest, along with altered soil moisture (Stark and Firestone, 1995) and temperature (Zogg et al., 1997) can influence C and N decomposition rates by affecting microbial community structure and abundance (Chen and Xu, 2005; Hassett and Zak, 2005; Smithwick et al., 2005; Belleau et al., 2006; Geisseler et al., 2010). Harvesting equipment can also influence forest productivity by

* Corresponding author. Department of Forestry and Environmental Resources, North Carolina State University, 2800 Faucette Dr., Raleigh, NC, 27695, USA.
E-mail address: jodi_forrester@ncsu.edu (J.A. Forrester).

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increasing soil compaction, which may result in C losses from the mineral soil (Mika and Keeton, 2012) and changes to the soil microbial community (SMC) composition (Ponder and Tadros, 2002). These effects are highly site-specific (Paré et al., 2002; Thiffault et al., 2006, 2011), and attempts to generalize responses among various sites have been complicated due to climate, vegetation, and soil differences. Additional challenges to characterizing the effects of management practices on soils include the long growth cycle in forests relative to other ecological systems (Hart and Sollins, 1998), and the potential for repeated harvesting cycles that may compound consequences (Liski et al., 2001).

The North American Long-Term Soil Productivity study (LTSP) was established in the early 1990s, based on the principle that at a particular climate, the potential productivity of a site is regulated by physical, chemical, and biological soil processes, all of which are influenced by management activities (Powers et al., 2005). The LTSP study was designed to address the long term and broad scale effects of soil disturbance on forest productivity by manipulating site organic matter and soil porosity at 62 locations throughout North America (Powers et al., 2005). Initial results indicated that organic matter removal led to a reduction in soil C concentrations and nutrient availability after a decade, but had no effect on bulk soil C storage in surface layers (0–30 cm depth). Although greater heterotrophic respiration following tree harvest led to a denser soil mass per unit area, C inputs increased from fine root decomposition, leading to no absolute change in soil C mass. The effects of soil compaction were influenced by initial bulk density, with the greatest sensitivity mid-range and limited effects at low or high initial bulk densities. Management effects differed with climate; soils located in a frigid temperature regime were less resilient to soil compaction than soils in more temperate climates (Powers et al., 2005). Conclusions from the first decade of responses suggested effects may become more apparent in time, as ten years is likely not long enough to capture the trends in long-term soil C dynamics. The experimental design permits the study of many of the most important processes by which management changes forest soils - nutrient removal, compaction, and changes in organic matter content and soil water status (Worrell and Hampson, 1997).

Results from one of the earliest installations in the LTSP network of 62 sites, established in an aspen-dominated forest on a sandy Spodosol soil type, indicated that a decade after harvest, organic matter removal reduced microbial biomass and enzymatic activity but had no effect on microbial community composition (Hassett and Zak, 2005). Harvesting caused reduced Ca availability in surface soil (0–10 cm depth; Voldseth et al., 2011) and soil compaction had only transient effects on bulk density. Temporally, the effects of treatments on soil C or N were negligible at both 10 (Voldseth et al., 2011) and 15 (Kurth et al., 2014) years post-harvest, though at two different aspen-dominated LTSP sites C losses were apparent more than a decade after treatment (Kurth et al., 2014).

Detecting changes in the large stock of soil C can be difficult and may benefit from procedures or methods that move beyond measuring bulk change. Methods have been developed to separate the bulk pool into fractions that differ in their chemical and physical stability (Trumbore and Zheng, 1996). The free - light fraction (f-LF) is composed of low density, physically-uncomplexed particulate organic matter that is typically dominated by recent plant inputs and is the least decomposed. The occluded - light fraction (o-LF) represents low density organic matter released by the disruption of soil aggregates. The dense or heavy fraction (HF) isolates organic matter in close association with mineral surfaces and which is typically composed of microbially-processed organic matter (Wagai et al., 2009). Mean residence time of C associated with these fractions in surface soils ranges from annual to decadal in the f-LF (except for in the presence of charcoal or other pyrogenic organic matter) to over a century or more in the HF (Swanston et al., 2005; Kaiser et al., 2009; Sollins et al., 2009). Fractionation allows for the isolation of the fast-cycling f-LF from the

intermediate o-LF and slow cycling HF, to more accurately interpret the effect of forest management on functionally different soil organic matter.

This study combines soil microbial community analysis, nutrient analyses, and soil density fractionation to examine belowground dynamics nearly 20 years following aboveground manipulations. The long-term effects of organic matter removal and soil compaction were evaluated using the LTSP site located on a sandy Spodosol within the Huron National Forest in Michigan, USA. The study was designed to evaluate the persistence of initial responses, but also incorporate a finer-scaled approach to better assess how (or if) these processes have consequences for soil ecosystem function.

The response of the SMC to organic matter removal and soil compaction in surface (0–10 cm) and subsurface (20–30 cm) soils was measured, expecting that organic matter removal would reduce microbial biomass (Hassett and Zak, 2005), while soil compaction would affect both the overall biomass and composition of the SMC (Ponder and Tadros, 2002). Soil microbial biomass and composition was measured in spring and summer to capture microbial responses across a range of growing season temperatures. Since whole-tree harvesting in aspen stands removes a significant amount of nutrients (Alban et al., 1978; Perala and Alban, 1982), a net reduction of soil C and N (Johnson and Curtis, 2001; Jandl et al., 2007; Jones et al., 2011) and reductions in nutrient availability following organic matter removal was expected, though soil type and time since disturbance may influence the measurable response. In addition, soil compaction was expected to result in greater water holding capacity on these sandy soils (Powers et al., 2005), leading to increased aboveground productivity (Curzon et al., 2014) and more soil C and N in compacted treatments. This study investigated potential changes in C and N associated with soil organic matter pools that differed in sensitivity to environmental change due to variability in chemical composition, degree of microbial processing, and turnover time (Kaiser et al., 2009; Sollins et al., 2009). Expectations were that most of these differences would be observed in the lightest soil fraction, reflecting short-term changes in C inputs and losses.

2. Materials and methods

2.1. Site description and experimental design

This research was conducted at the Long-Term Soil Productivity site (LTSP) within the Huron-Manistee National Forest (Tiarks et al., 1997; Page-Dumroese, 2010). This approximately 130 ha LTSP site is located on a glacial outwash plain in Michigan's Lower Peninsula (44°39' N, 83°31' W). Soils are classified as Frigid Entic Haplorthods on well-drained acidic outwash sand (SSS, NRCS, USDA). The site is dominated by trembling (*Populus tremuloides* Michx.) and bigtooth aspen (*P. grandidentata* Michx.), with red maple (*Acer rubrum* L.), red oak (*Quercus rubra* L.), black cherry (*Prunus serotina* Ehrh.), and white pine (*Pinus strobus* L.) making up lesser components of the canopy trees. The average yearly temperature is 6.8 °C and average total yearly precipitation is 74 cm (Alpena, MI weather station, 2002–2012, NOAA weather service, www.nws.noaa.gov). For further site descriptions, refer to Stone (2001), Hassett and Zak (2005), Voldseth et al. (2011), and Kurth et al. (2014).

The experimental design includes a 3 × 2 full factorial design with three levels of organic matter (OM) removal (bole-only harvest (OM₀); whole-tree harvest (OM₁); all woody vegetation harvest plus forest floor removal (OM₂)) and two levels of soil compaction (compaction associated with harvest (C₀); and light compaction to increase bulk density by 15% (C₁)). Treatments were randomly assigned and applied discretely to research plots in January–February 1992 (6 treatments replicated three times). The site was naturally regenerated to aspen. Permanent plots (50 m² plots) were established to minimize variation in vegetation and soil properties (Stone, 2001). Within each plot, measurements and samples were collected from four permanent subplot

locations arranged in the four cardinal directions away from plot center.

2.2. Field methods

Soil samples were collected during April (spring) and August (summer) 2012. Soil cores for phospholipid fatty acid (PLFA) analysis were collected from each of the four subplots at a distance of 1.5 m north of the plot center. The forest floor was removed prior to sampling, and soil cores were taken by depth to 30 cm using a 2.36 cm diameter push probe. Soil from surface (0–10 cm; predominantly A, some E horizon) and subsurface (20–30 cm; predominantly Bs, some E horizon) depths were composited from the four cores, resulting in one composite sample for each depth per plot ($n = 3$ samples per depth). PLFA soil samples were kept on ice until they could be brought back to UW-Madison, where they were stored at -20°C prior to being lyophilized (Freezemobil 12, Virtis of Gardiner, NY). Roots and stones were removed by hand from dried samples, and samples were ground in preparation for microbial lipid extraction. Soil moisture was measured adjacent to where the PLFA soil was collected to a depth of 6 cm with a calibrated TDR moisture probe attached to a HH2 Moisture Meter (Delta-T Devices, Cambridge, England). Soil temperature was measured at a depth of 10 cm using a portable probe digital long stem thermometer (model no. 15-078 k, Fisher Scientific).

Separate soil cores for organic matter fractionation were collected during the spring of 2012 at a distance of 2 m north of subplot centers using a 6.35 cm diameter core after first removing the forest floor. Soil from surface (0–10 cm) and subsurface (20–30 cm) depths were composited from the four subplot locations, resulting in one composited sample for each depth per plot ($n = 18$). Samples were air dried prior to further analysis.

Resin strips (Plant Root Simulator (PRS[™]) probes) were used to measure *in situ* exchangeable soil macronutrients (NH_4 , NO_3 , Ca, Mg, K, P) during the growing season (Western Ag. Innovations, Saskatoon, SK). A set of four anion and cation probes (8 total) were placed within each plot to assess plant available soil nutrients. A pair of probes (1 anion, 1 cation) was installed vertically into the mineral soil at each subplot concurrently with the first microbial collection during the spring (April) and then removed during the second microbial collection during the summer (August) for a total available nutrient collection period of 12 weeks (Western Ag. Innovation, PRS[™] probe Operations Manual). Following removal from the soil, the PRS probes were thoroughly washed with distilled water to remove any residual soil particles, then the four sets of anion and cation probes per plot were aggregated into one sample to account for soil heterogeneity, and shipped to Western Ag. Innovations for macronutrient extraction (Western Ag. Innovation, PRS[™] probe Operations Manual).

2.3. Lipid extraction and data processing

A two-phase, aqueous-organic, phosphate buffer-methanol-chloroform extraction, developed from a modified PLFA and fatty-acid methyl ester (FAME) method (Balsler and Firestone, 2005; Smith et al., 2015) was used to extract phospholipids from 3.5 g of lyophilized soil. Each sample was extracted twice, and then the organic phase was isolated and dried down in a RapidVap (LabConco, Kansas City, MO), saponified, subjected to alkaline methanolysis, and isolated in hexane. A Hewlett-Packard 6890 Gas Chromatograph with a flame ionization detector configured and maintained for lipid analysis according to the recommendations of MIDI (MIDI Inc., Newark DE) was used to analyze the extracted phospholipids. MIDI Sherlock microbial identification system (MIS) software (MIDI Inc., Newark DE) was used to identify peaks by comparing retention times with known standards. To quantify the amount of each lipid, peak areas were first multiplied by a response factor (Rfact), which corrects for differences in detector response across the range of chain-lengths (Christie, 1989), and is derived from the

MIDI calibration standards. Finally, lipid amounts were quantified by comparison with Rfact corrected external standards (9:0, 19:0) of known concentration.

An open source licensed Microsoft Access[®] Database was used to obtain absolute ($\mu\text{mol lipid g}^{-1}$ soil) and relative (mol%) lipid abundances. Total microbial biomass was calculated as the sum of all absolute abundances (White et al., 1979; Zelles et al., 1992; Hill et al., 1993; Balsler and Firestone, 2005). Lipids with a relative abundance (averaged over all samples) of less than 0.1 mol% were removed, while lipids with an average relative abundance greater than 0.5 mol% were kept in the dataset. If the relative abundance was between 0.1 mol% and 0.5 mol%, lipids were retained in the dataset if they were present in 10 or more samples. In this way, spurious lipids were culled from the dataset, leaving a total of 46 PLFAs in the refined dataset. With the exception of microbial biomass, relative abundance lipid data was used for all analyses. Fatty acid nomenclature is as described elsewhere (Frostegård et al., 1996; Zelles, 1997; Aanderud et al., 2008).

To better understand how microbial groups responded to compaction and organic matter removal, specific indicator lipids were classified into microbial guilds, including: arbuscular mycorrhizal fungi (AMF) (16:1 ω 5); Fungi (18:2 ω 6,9); Gram Positive bacteria (GmP) (14:0iso, 15:0anteiso, 15:0iso, 16:0iso, 17:0anteiso, 17:0iso); Gram Negative bacteria (GmN) (16:1 ω 7, 18:1 ω 7, 17:0 cyclo, 19:0 cyclo, 19:0cyc 11–12 2OH); Actinomycetes (Act.) (16:0 10 methyl, 17:0 10 methyl, 18:0 10 methyl) (Wilkinson, 1988; Vestal and White, 1989; Zelles et al., 1992; Frostegård et al., 1993, 1996; Kieft et al., 1997; Bossio et al., 1998; Olsson, 1999; Zelles, 1999). Finally, the CYC stress ratio, which is the ratio of cyclopropyl fatty acids to their monoenoic precursors (17:0cyc, 19:0cyc/16:1 ω 7c, 18:1 ω 7c) was used as an indicator of stress in the SMC (Guckert et al., 1986; Kieft et al., 1997).

2.4. Soil fractionation and C and N analysis

Whole soil samples were sequentially separated into the free light fraction (f-LF), occluded light fraction (o-LF) and heavy fraction (HF). An electrostatic attraction and density fractionation procedure was used based on the methods of Kaiser et al. (2009, 2011), as opposed to the more traditional sodium polytungstate (NaPT)-based density fractionation for multiple reasons. The NaPT method can result in high C losses (Crow et al., 2007), and can be hampered by the dispersion of organo-mineral hydrous Al and Fe compounds, which can be abundant in Spodosols.

The electrostatic attraction procedure separates physically-uncomplexed coarse organic particles > 0.25 mm. Soil (40 g) was sieved into > 1 mm, 1 mm–0.5 mm, 0.5 mm–0.25 mm, and < 0.25 mm size classes. Coarse organic particles obtained through electrostatic attraction (via a charged glass Petri dish) from each size class were composited into one sample to obtain the electrostatically isolated particulate organic matter (POM), which was oven dried at 105°C , then weighed. Physically-uncomplexed POM < 0.25 mm was isolated by first combining soil from the > 1 mm, 1 mm–0.5 mm, 0.5 mm–0.25 mm fractions with the < 0.25 mm soil fraction, and hydrated the sample. Organic particles with a density < 1 g cm³ were separated from the supernatant. The supernatant was aspirated through a 0.25 mm sieve to capture floating organic particles, which were oven dried at 105°C , then weighed. The electrostatically isolated POM and the physically uncomplexed POM were combined to create the f-LF.

The inter-aggregate occluded fraction was isolated by hydrating the remaining soil then sonicating to disperse aggregates. The floating intra-aggregate organic material was aspirated through a 0.063 mm sieve. This procedure was repeated 4–5 times, then the aspirated material was oven dried at 105°C and weighed as the o-LF. The remaining soil was isolated, oven dried at 105°C , and weighed to obtain the HF.

The three fractions isolated from each sample were analyzed using a Vario MACRO-CN elemental analyzer (Elementar Americas, Inc.) to determine % C, % N, and C:N ratio. Because there are no carbonates in

these soils at the depths sampled (NRCS Web Soil Survey; <http://websoilsurvey.nrcs.usda.gov/app/WebSoilSurvey.aspx>; accessed on May 15, 2012 SSS, NRCS, USDA), all measured C was assumed equivalent to organic C.

Percentage of bulk soil (g fraction g^{-1} bulk soil) is presented for each fraction. Between 98.1 and 99.2% of the original soil mass was recovered during the fractionation procedure. To correct for mass loss, the percentages of the individual isolated fractions were calculated based on the sum of the three recovered fractions (Marin-Spiotta et al., 2009). Concentrations of soil C (g C kg^{-1} soil) and N (g N kg^{-1} soil) for each fraction were calculated by multiplying the % C and % N by the mass of the fraction. Soil organic C and N stocks in each fraction were calculated using C and N concentrations, sampling depth thickness (10 cm), and bulk density adjusted for coarse fragment content. The bulk density dataset is reported in Slesak et al. (2017).

2.5. Statistical analyses

General linear mixed models procedures (PROC MIXED, SAS version 9.3; SAS Institute Inc., Cary, NC, USA) were used to evaluate treatment (OM removal and compaction) and sampling season effects on soil temperature and moisture, treatments effects on plant nutrient availability; treatment and depth effects on soil fraction size, C:N, and C and N stocks; and treatment, depth, and season effects on soil microbial guilds, stress ratios, and total microbial biomass. Prior to all analyses, all subplot ($n = 4$) data (temperature, moisture, plant nutrient availability) were averaged to the plot level. In all ANOVA designs, treatments, sampling season, and depth were analyzed as fixed effects and replicate plots for each treatment were analyzed as a random effect. Data were log transformed as needed for more normal distributions.

Microbial community patterns were also analyzed using Permanova and non-metric multidimensional scaling (NMDS) in PRIMER version 7 (Clarke and Gorley, 2015) with the PERMANOVA + add-on package (Anderson et al., 2008). A Bray-Curtis resemblance measure was used for all multivariate analyses. Permanova, or distance-based permutational MANOVA (Anderson, 2001) uses an ANOVA design to test the response of multivariate data to treatment factors using any resemblance measure (Anderson et al., 2008). Permanova analyses were performed with 9999 random permutations, type III sum of squares, and permutation of residuals under a reduced model. NMDS is used as a tool to aid in the visualization of the multivariate dataset in 2-dimensional space, and was set to run with 50 restarts and a minimum stress of 0.01. Pearson correlation vector overlays of microbial guilds, soil fraction size, C and N stocks, and C:N ratio are included to aid in interpretation of multivariate patterns.

3. Results

3.1. Response of the SMC biomass, guilds, and stress

Across all treatments and both seasons, total microbial biomass was greater in surface compared to subsurface soils ($P = 0.0001$) (Fig. 1). The effects of organic matter removal on SMC biomass were negligible after 20 years. Soil compaction significantly influenced total microbial biomass, but the effect differed based on soil depth and season (compaction*depth*season, p -value = 0.001). Biomass was greater in compacted ($0.19 \mu\text{mol } g^{-1}$ soil) than non-compacted soils ($0.14 \mu\text{mol } g^{-1}$ soil) at 0–10 cm depth in the spring, though in summer biomass tended to be higher in non-compacted soils.

Interactions between treatment factors significantly influenced the relative abundance of a few individual microbial guilds. GmP bacteria varied due to the interaction of OM removal with compaction and season ($P = 0.05$). In the spring, GmP bacterial abundance was greatest in the treatment with no OM removal or compaction, intermediate in treatments with OM removal only and lowest in treatments where compaction had been applied (Table 1). Trends were less consistent in

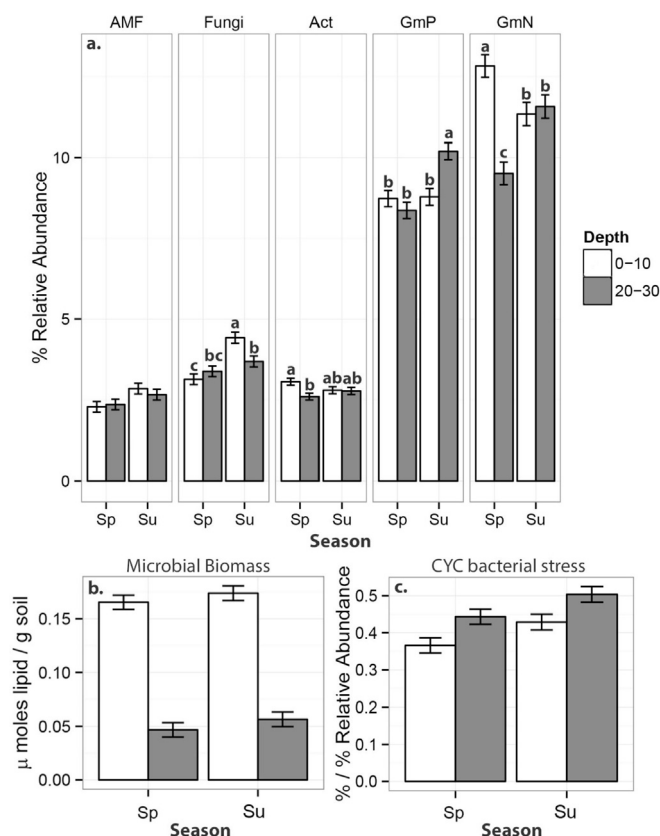


Fig. 1. Effects of sampling season (spring = sp; summer = su) and soil depth on the relative abundance of microbial groups (a), total microbial biomass (b) and the CYC bacterial stress ratio (c). Letters above bars indicate significant pairwise comparisons due to a season*depth interaction effect ($p \leq 0.05$).

the summer, but were nearly reverse with GmP abundance greater in compacted treatments and lower in the treatment with no OM removal. Organic matter removal caused a significant reduction in the relative abundance of AMF, with abundances decreasing across OM removal intensity (3%, 2.4%, and 2.2% for OM₀, OM₁ and OM₂ respectively, P values all ≤ 0.05).

Several individual guilds differed significantly between seasons or soil depth as opposed to either forest management variable (Table 1). AMF were more abundant in the summer relative to spring sampling season ($P = 0.005$). The interaction of season and soil depth explained significant differences in the abundance of fungi ($P = 0.004$), actinomycetes ($P = 0.03$), GmP ($P = 0.001$) and GmN bacteria ($P = 0.001$). In surface soils, actinomycetes and GmN bacteria were more abundant during the spring, and fungi were more abundant during the summer. In deeper soils, GmP bacteria peaked in abundance during the summer (Fig. 1a). Finally, bacterial stress was greater in deeper relative to surface soil ($P = 0.003$), and greater during the summer than the spring ($P = 0.007$) (Fig. 1c).

Results from the multivariate analyses complemented the patterns resulting from the univariate analyses. The composition of the SMC significantly differed among organic matter removal treatments ($P = 0.03$ in Permanova analysis), with distinct differences between bole only and whole tree harvests (OM₀ and OM₁) ($P = 0.02$). Composition following the more intensive OM removal (OM₂) did not differentiate significantly from either of the other removal treatments. There was no clear effect of soil compaction, but SMC structure differed significantly between sampling depths ($P = 0.0001$), season ($P = 0.0004$), and the interaction of depth with season ($P = 0.003$) (Fig. 2). The surface SMC composition differentiated from the subsurface community along axis 1 (Fig. 2), which is positively correlated

Table 1

Mean biomass ($\mu\text{mol g}^{-1}$ soil), % relative abundance of microbial groups, and bacterial stress (CYC) of soil sampled from bole-only harvests (OM_0), whole-tree harvests (OM_1), and whole-tree harvests + forest floor removal (OM_2); without (C_0) and with (C_1) soil compaction.

Season and Depth	Treatment	Biomass($\mu\text{mol g}^{-1}$ soil)	AMF (%)	Fungi (%)	Actinomycete (%)	GmP (%)	GmN (%)	CYC (%/%)		
A. Spring	0–10 cm	OM_0C_0	0.143	2.53	3.30	2.98	9.17	14.25	0.34	
		OM_0C_1	0.158	2.29	3.00	3.07	9.49	14.34	0.38	
		OM_1C_0	0.150	2.29	2.81	3.03	8.53	12.03	0.36	
		OM_1C_1	0.240	2.13	3.40	2.55	7.67	11.02	0.38	
		OM_2C_0	0.130	2.42	2.97	3.63	9.32	13.52	0.35	
		OM_2C_1	0.172	2.03	3.33	3.10	8.19	11.78	0.39	
	20–30 cm	OM_0C_0	0.037	3.04	3.36	2.70	9.18	10.65	0.40	
		OM_0C_1	0.035	2.45	3.72	2.57	6.42	9.56	0.34	
		OM_1C_0	0.061	2.22	2.88	2.74	9.07	9.60	0.49	
		OM_1C_1	0.061	2.17	3.30	2.65	8.55	9.28	0.59	
		OM_2C_0	0.045	1.84	3.62	2.64	8.48	8.85	0.40	
		OM_2C_1	0.042	2.40	3.41	2.30	8.47	9.12	0.46	
	B. Summer	0–10 cm	OM_0C_0	0.197	3.81	4.78	2.78	8.35	11.70	0.40
			OM_0C_1	0.163	3.18	3.98	2.88	9.15	11.69	0.44
			OM_1C_0	0.185	3.08	4.06	3.11	9.23	11.76	0.43
			OM_1C_1	0.148	2.68	4.90	2.24	8.68	10.88	0.48
			OM_2C_0	0.166	2.11	4.62	2.98	8.37	10.99	0.42
			OM_2C_1	0.181	2.25	4.22	2.82	8.92	11.41	0.39
20–30 cm		OM_0C_0	0.065	3.59	3.96	2.89	10.26	11.58	0.52	
		OM_0C_1	0.038	3.09	3.85	2.92	9.70	12.40	0.55	
		OM_1C_0	0.064	1.99	2.78	2.43	10.03	10.84	0.51	
		OM_1C_1	0.065	2.56	3.83	2.83	10.45	11.24	0.48	
		OM_2C_0	0.060	1.95	3.89	2.51	10.16	11.17	0.50	
		OM_2C_1	0.044	2.80	3.85	3.09	10.56	12.04	0.46	

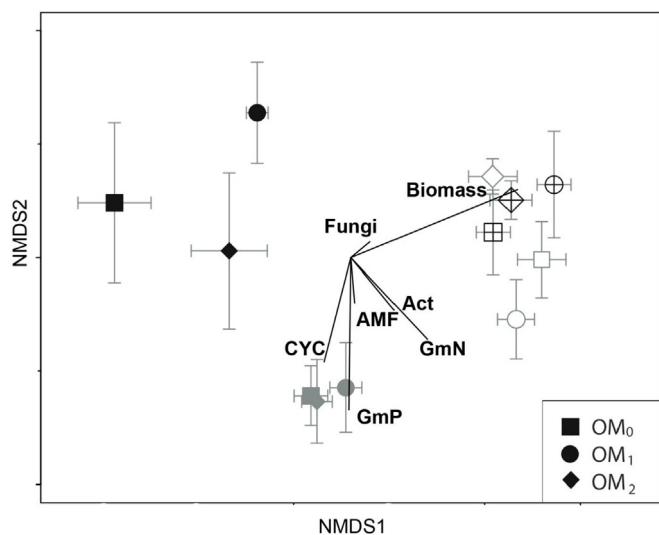


Fig. 2. NMDS illustrating the relative significance of organic matter removal treatments ($P = 0.03$) and the interaction of depth * season ($P = 0.003$) for the PERMANOVA analysis of the soil microbial community. Because compaction was not a significant factor in the model, plots ($n = 6$) are averaged by the biomass removal treatment (including C_0 and C_1 plots), to display bole-only harvests (OM_0), whole-tree harvests (OM_1), and whole-tree harvests + forest floor removal (OM_2). Bars represent standard error of the mean. Pearson correlations of microbial relative abundances and total biomass with axes 1 and 2 range from 0.1 (Fungi, axis 1) to 0.87 (Biomass, axis 1). Open Black (with cross lines) = Spring 0–10 cm; Open Grey = Summer 0–10 cm; Closed Black = Spring 20–30 cm; Closed Grey = Summer 20–30 cm.

with total microbial biomass ($r = 0.87$). Axis 2 differentiates the summer subsurface composition from the summer surface and spring surface and subsurface composition, and is most highly correlated with GmP bacteria ($r = -0.67$), the CYC bacterial stress ratio ($r = -0.46$),

and GmN bacteria ($r = -0.36$) (Fig. 2). Composition in the surface soil intensive organic matter removal (OM_2) treatment exhibited very little seasonal variation, compared to all other treatments.

During both spring and summer, the surface and subsurface SMC separated along axis 1 (Fig. 3). In the spring, total microbial biomass ($r = 0.87$), GmN bacteria ($r = 0.56$), actinomycetes ($r = 0.4$), and GmP bacteria ($r = 0.27$) were most highly correlated with surface soils, while fungi ($r = -0.48$) and AMF ($r = -0.25$) were most highly correlated with subsurface soils (Fig. 3a). In the summer, surface soils were most correlated with total microbial biomass ($r = 0.93$), fungi ($r = 0.52$), and AMF ($r = 0.16$), while subsurface soils were most correlated with GmP bacteria ($r = -0.69$), the CYC bacterial stress ratio ($r = -0.56$), and GmN bacteria ($r = -0.26$) (Fig. 3b).

3.2. Soil nutrient availability, temperature, and moisture

Concentrations of available P were greater in compacted ($11.1 \mu\text{g} (\pm 1.6 \text{ s.e.}) 10 \text{ cm}^{-2} 12 \text{ weeks}^{-1}$) than non-compacted soils ($6.1 \mu\text{g} (\pm 1.3 \text{ s.e.}) 10 \text{ cm}^{-2} 12 \text{ weeks}^{-1}$; $P = 0.05$). Available soil Ca concentrations decreased with increasing organic matter removal (from 973 ± 86 to 939 ± 179 to $675 \pm 107 \mu\text{g} 10 \text{ cm}^{-2} 12 \text{ weeks}^{-1}$ for mean + s.e. of OM_0 , OM_1 , and OM_2 respectively), though not statistically significant ($P = 0.3$). Few other significant differences in nutrients due to the organic matter and compaction manipulations were detected. Soils were warmer and drier during the summer ($19.6^\circ\text{C} \pm 0.3 \text{ s.e.}$, $6.2\% \text{ moisture} \pm 0.4 \text{ s.e.}$) than the spring sampling period ($8.2^\circ\text{C} \pm 0.3 \text{ s.e.}$, $11.6\% \text{ moisture} \pm 0.7 \text{ s.e.}$). Soil temperature and moisture differed between seasons, but did not respond to changes in organic matter removal or soil compaction treatments ($P = 0.0001$).

3.3. Soil C and N pools

Of the three fractions, the HF was the largest fraction by weight in both surface and subsurface soils, followed by the o-LF then the f-LF (Tables 2–3). When comparing the two depths, the size of the f-LF and

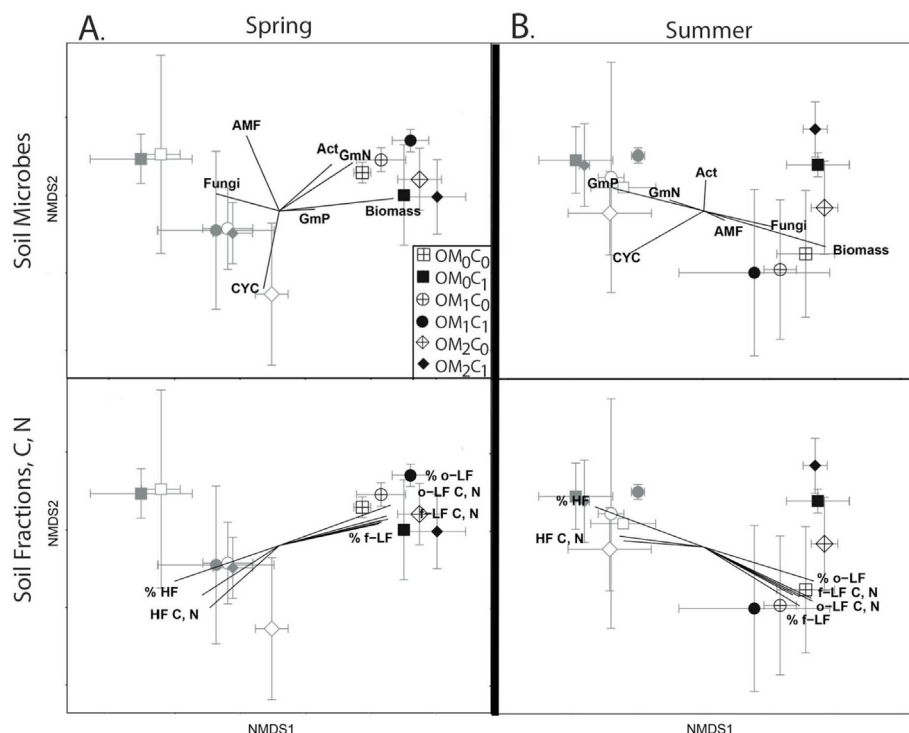


Fig. 3. NMDS of the soil microbial community illustrating the correlation of the SMC composition with microbial indices, and soil fractions and C and N stocks within the spring (A) and summer (B) sampling periods in surface (black symbols) and subsurface (grey symbols) soils. Symbols depict treatment means ($n = 3$) for combinations of bole-only harvests (OM_0), whole-tree harvests (OM_1), and whole-tree harvests + forest floor removal (OM_2); without (C_0) and with (C_1) soil compaction. Bars represent standard error of the mean. Pearson correlation vectors of microbial relative abundances and total biomass, soil fractions and C and N stocks are overlain. For microbial response variables, vectors range from 0.2 (Actinomycetes, Summer, Axis 2) to 0.9 (Biomass, Summer, Axis 1). For soil fractions C and N stocks, vectors range from -0.53 (HF C, Spring, Axis 1) to 0.85 (% o-LF, Spring, Axis 1).

o-LF pools were significantly greater in the surface soil while the HF was greater at depth ($P < 0.0001$) (Tables 2 and 3). C:N values were all significantly greater at 20–30 cm depth than 0–10 cm ($P < 0.006$); within a depth layer they followed the pattern of f-LF > o-LF > HF (Tables 2 and 3). Both C and N concentrations were significantly greater in at 0–10 than 20–30 cm sampling depth for all fractions ($P < 0.005$). Carbon and N stocks in the f-LF and o-LF were significantly greater in surface than subsurface soils ($P = 0.0001$), while C and N stocks in the HF were significantly greater in subsurface soils ($P = 0.0001$) (Tables 2 and 3).

Organic matter removal affected C concentrations and stocks in the f-LF at 20–30 cm depth ($P = 0.07$ and $P = 0.04$, respectively) (Table 3),

resulting in greater C with increasing levels of organic matter removal ($OM_1 > OM_0$) (Table 3). Nitrogen concentrations in the f-LF were affected by organic matter removal at both sampling depths ($P = 0.06$) (Tables 2 and 3). In surface soils, N concentrations were reduced with increasing levels of organic matter removal ($OM_0 > OM_2$) (Table 2), while in subsurface soils, N concentrations were greater with increased organic matter removal ($OM_1 > OM_0$) (Table 3). Soil N stocks were lower in treatments with increased organic matter removal in the f-LF at 0–10 cm depth ($OM_0 > OM_2$) ($P = 0.03$) (Table 2). Compacted soils had a lower C:N ratio (15.0) than non-compacted soils (16.11) averaged over both sampling depths in the HF ($P = 0.02$). Organic matter removal and compaction treatments had no effects on the mass (% of bulk

Table 2

Mean (standard error) mass recovery, C/N, carbon (C) and nitrogen (N) concentrations ($g\ kg^{-1}$ soil), and C and N stocks ($Mg\ ha^{-1}$) from 0 to 10 cm depth. Soil was sampled from bole-only harvests (OM_0), whole-tree harvests (OM_1), and whole-tree harvests + forest floor removal (OM_2); without (C_0) and with (C_1) soil compaction. Different letters indicate statistically significant differences within soil fraction type for test of the interaction of biomass removal*depth ($P \leq 0.1$).

Density fraction		% of bulk soil	C:N	g C kg^{-1} soil	g N kg^{-1} soil	C Stocks($Mg\ ha^{-1}$)	N Stocks($Mg\ ha^{-1}$)	
0–10 cm	f-LF	OM_0C_0	3.4 (0.6)	23.5 (1.4)	6.67 (0.9)	0.29 (0.05) a	19.18 (2.9)	0.83 (0.16) a
		OM_0C_1	2.3 (0.4)	25.5 (2.6)	4.18 (0.42)	0.17 (0.03) a	13.83 (1.2)	0.56 (0.09) a
		OM_1C_0	2.5 (0.8)	28.1 (3.8)	6.97 (2.82)	0.23 (0.06) ab	19.13 (8.1)	0.64 (0.19) ab
		OM_1C_1	2.2 (0.5)	22.1 (0.5)	4.55 (1.03)	0.21 (0.05) ab	14.21 (2.6)	0.65 (0.13) ab
		OM_2C_0	1.9 (0.7)	26.7 (1.3)	4.24 (1.46)	0.16 (0.05) b	12.60 (4.4)	0.47 (0.15) b
		OM_2C_1	1.7 (0.4)	26.0 (1.0)	3.18 (0.79)	0.12 (0.03) b	10.40 (2.1)	0.41 (0.09) b
20–30 cm	o-LF	OM_0C_0	4.6 (0.4)	19.2 (0.8)	8.83 (1.22)	0.46 (0.07)	25.25 (3.4)	1.32 (0.18)
		OM_0C_1	5.4 (0.6)	19.7 (0.9)	8.39 (0.27)	0.43 (0.01)	27.98 (1.9)	1.42 (0.05)
		OM_1C_0	5.9 (1.1)	20.3 (1.5)	11.57 (1.09)	0.57 (0.04)	31.36 (4.8)	1.54 (0.20)
		OM_1C_1	4.5 (0.7)	18.9 (0.6)	8.05 (1.10)	0.43 (0.06)	25.39 (2.9)	1.34 (0.14)
		OM_2C_0	3.9 (0.8)	20.8 (1.0)	7.35 (2.39)	0.35 (0.12)	21.92 (7.3)	1.05 (0.36)
		OM_2C_1	4.1 (0.9)	21.8 (1.3)	7.67 (2.08)	0.36 (0.1)	25.41 (6.7)	1.18 (0.32)
0–10 cm	HF	OM_0C_0	92.0 (0.5)	14.35 (0.37)	5.34 (0.93)	0.37 (0.06)	15.27 (2.5)	1.06 (0.15)
		OM_0C_1	92.3 (0.2)	14.73 (0.62)	4.67 (0.22)	0.32 (0.02)	15.56 (1.2)	1.06 (0.07)
		OM_1C_0	91.6 (1.9)	15.83 (0.58)	5.56 (0.72)	0.36 (0.06)	14.74 (1.6)	0.94 (0.13)
		OM_1C_1	93.3 (1.2)	14.52 (0.55)	6.38 (0.94)	0.44 (0.05)	20.08 (2.4)	1.37 (0.12)
		OM_2C_0	94.2 (1.3)	15.52 (1.02)	6.67 (1.88)	0.42 (0.10)	19.92 (5.9)	1.25 (0.31)
		OM_2C_1	94.2 (1.4)	14.82 (0.19)	5.20 (0.23)	0.35 (0.02)	17.53 (1.2)	1.18 (0.07)

Table 3

Mean (standard error) mass recovery, C:N, carbon (C) and nitrogen (N) concentrations (g kg^{-1} soil), and C and N stocks (Mg ha^{-1}) from 20 to 30 cm depth. Soil was sampled from bole-only harvests (OM_0), whole-tree harvests (OM_1), and whole-tree harvests + forest floor removal (OM_2); without (C_0) and with (C_1) soil compaction. Different letters indicate statistically significant differences within soil fraction type for test of the interaction of biomass removal*depth ($P \leq 0.1$).

Density fraction 20–30 cm	% of bulk soil	C:N	g C kg^{-1} soil	g N kg^{-1} soil	C Stocks(Mg ha^{-1})	N Stocks(Mg ha^{-1})	
f-LF	OM_0C_0	0.1 (0.01)	41.5 (1.4)	0.27 (0.04) b	0.006 (0.001) b	1.59 (0.3) b	0.04 (0.007)
	OM_0C_1	0.09 (0.002)	40.1 (2.8)	0.17 (0.01) b	0.004 (0.0001) b	1.20 (0.1) b	0.03 (0.001)
	OM_1C_0	0.2 (0.09)	44.4 (4.6)	0.65 (0.27) a	0.014 (0.01) a	4.00 (1.6) a	0.09 (0.04)
	OM_1C_1	0.2 (0.02)	45.3 (5.9)	0.32 (0.05) a	0.007 (0.001) a	2.24 (0.3) a	0.05 (0.004)
	OM_2C_0	0.2 (0.03)	45.4 (0.7)	0.38 (0.11) ab	0.008 (0.002) ab	2.43 (0.7) ab	0.05 (0.02)
	OM_2C_1	0.2 (0.08)	44.5 (2.5)	0.36 (0.15) ab	0.008 (0.003) ab	2.55 (1.1) ab	0.06 (0.02)
o-LF	OM_0C_0	0.3 (0.10)	33.0 (3.6)	0.69 (0.22)	0.023 (0.010)	4.01 (1.1)	0.13 (0.05)
	OM_0C_1	0.2 (0.09)	34.4 (0.9)	0.38 (0.13)	0.011 (0.004)	2.66 (0.9)	0.08 (0.02)
	OM_1C_0	0.4 (0.10)	39.2 (4.6)	0.82 (0.27)	0.021 (0.010)	4.97 (1.6)	0.13 (0.05)
	OM_1C_1	0.2 (0.04)	38.2 (3.9)	0.42 (0.02)	0.011 (0.001)	3.02 (0.2)	0.08 (0.01)
	OM_2C_0	0.3 (0.05)	35.9 (2.2)	0.67 (0.16)	0.018 (0.004)	4.22 (1.0)	0.11 (0.02)
	OM_2C_1	0.3 (0.07)	39.3 (3.8)	0.67 (0.27)	0.017 (0.010)	4.75 (2.0)	0.12 (0.04)
HF	OM_0C_0	99.6 (0.10)	16.92 (0.52)	4.83 (0.69)	0.29 (0.050)	28.33 (2.9)	1.69 (0.22)
	OM_0C_1	99.7 (0.09)	15.70 (0.15)	3.63 (0.47)	0.23 (0.030)	25.57 (3.0)	1.63 (0.17)
	OM_1C_0	99.4 (0.20)	17.52 (1.18)	4.94 (0.88)	0.28 (0.030)	30.25 (5.4)	1.70 (0.20)
	OM_1C_1	99.6 (0.05)	15.02 (0.09)	3.70 (0.25)	0.25 (0.020)	26.31 (1.8)	1.75 (0.13)
	OM_2C_0	99.6 (0.08)	16.56 (0.16)	3.85 (0.04)	0.23 (0.002)	24.13 (0.9)	1.46 (0.06)
	OM_2C_1	99.5 (0.20)	15.22 (1.57)	4.07 (0.77)	0.26 (0.030)	28.61 (5.4)	1.85 (0.16)

soil) of the fractionated soil pools (Tables 2 and 3).

3.4. Interrelationship of SMC composition with soil C and N fractions and microbial indices

During both the spring and summer sampling periods, % f-LF and % o-LF, and C and N stocks within these fractions were most correlated with the surface SMC ($r = 0.68$ – 0.84), while %HF and HF C and N stocks were most correlated with the subsurface SMC ($r = 0.53$ – 0.85) (Fig. 3a and b).

4. Discussion

4.1. Effects of changes in the quantity of organic matter inputs to soil

Previous research at this site 10 and 15 years post-treatment found no effects of organic matter removal on forest floor or mineral soil N (Voldseth et al., 2011; Kurth et al., 2014). However, separation of the bulk soil into fractions indicated long-term changes in N dynamics that might otherwise have been overlooked in coarse assessments of soil pools. The physically-uncomplexed, fastest cycling soil fractions were the most sensitive to intensive organic matter removal treatment (OM_2), reflecting potential changes in plant litter inputs or decomposition rates. Because the forest floor is a large N reservoir (Powers et al., 2005), its removal decreases inputs into the mineral soil N pool, resulting in reduced N stocks in surface soil following whole tree harvest and forest floor removal compared to soils following less intensive management (bole only harvests). Slesak et al. (2017) also reported a significant decrease in soil C twenty years after the removal of the forest floor. Results from their comparison of the temporal response across other aspen-dominated LTSP sites highlight that effects of organic matter removal are not universal and instead certain soils are more susceptible than others.

C and N concentrations and C stocks in the f-LF at 20–30 cm depth were greater in the whole-tree harvest treatment (OM_1) than in the bole-only harvest treatment (OM_0). These patterns may be a result of differences in litter inputs among treatments. Because stem density, standing biomass, and stem diameter growth were all found to decrease with increasing organic matter removal at 15 years following harvest (Curzon et al., 2014), greater light levels likely promoted higher

understory biomass. Whole-tree harvest treatments tended to have more shrub biomass than bole-only harvest and whole-tree harvest with forest floor removal treatments (OM_2) (Curzon et al., 2014). It is possible that greater C and N concentrations and C stocks in the whole-tree harvest than the bole-only harvest may be due to differences in the quantity of organic inputs from the ground layer herbaceous species.

At 10 years post-harvest, previous research at this site found no effects of organic matter removal on microbial guilds (Hassett and Zak, 2005). These results could be explained by similarities in overstory tree establishment among treatments, or by environmental conditions at the time of sampling. Two decades post-harvest, a significant difference was detected between the SMC composition in bole-only harvest and whole-tree harvest treatments, and greater AMF abundance in bole-only harvest compared with whole-tree harvest and whole-tree harvest with forest floor removal treatments. AMF are symbiotic fungi that associate with grasses and herbaceous plants and some tree species (Sylvia, 2005), such as red maples, which are a co-dominant tree species at this site. Greater AMF abundance in bole-only harvest is most likely due to a greater abundance of red maple in bole-only harvest (0.78 Mg ha^{-1} ; 3.6% of total woody biomass) than whole-tree harvest (0.5 Mg ha^{-1} ; 2.1% of total woody biomass) and whole-tree harvest with forest floor removal (0.21 Mg ha^{-1} ; 1.3% of total woody biomass) treatments (M. Curzon, personal communication). GmP bacterial abundance was greater in the compacted whole-tree harvest treatment (OM_1C_1) than in the compacted bole-only harvest treatment (OM_0C_1) at a depth of 20–30 cm. GmP bacteria generally decompose “older”, more complex C structures (Kramer and Gleixner, 2008), which can accumulate with a reduction in fresh C inputs under a whole-tree harvest than under treatments such as a bole-only harvest, that receives fresh C inputs via slash. Finally, we note that in the most intensive organic matter removal treatment (OM_2), there is a reduction in the variability of the SMC composition both within and between seasons. This pattern has implications for the resiliency of the SMC biodiversity to recover following intensive harvesting practices.

There is concern that forest harvesting with increasing amounts of organic matter removal will lead to major site-level nutrient reductions in aspen stands, including losses of Ca and Mg in the tree bole and bark, and N, P, and K in the branches and foliage (Alban et al., 1978; Perala and Alban, 1982). Ten years post-harvest at this site, total soil Ca concentrations were significantly reduced in treatments with greater

biomass removal (Voldseth et al., 2011). Soil Ca levels appear to be recovering 20 years post harvest, possibly due to a lagged response following vegetation regrowth, but successive harvest rotations, or reduced length of time during harvest, could lead to soil Ca deficiencies and reduced soil productivity. Slesak et al. (2017) found a pattern of increasing soil Ca in the less intense treatments after 20 years, but where forest floor was removed no significant change in Ca had occurred.

4.2. Interrelated effects of compaction on sandy soils

Powers et al. (2005) concluded that compaction on sandy soils increased aboveground site productivity due to improved water holding capacity. Results at this site 10 years post-harvest corroborate this idea by indicating that compaction increased aspen biomass compared to the non-compacted, bole-only harvest (Voldseth et al., 2011). A modest trend of increasing stem density with compaction was also identified at 15 years post-harvest (Curzon et al., 2014).

In this study, it was hypothesized that the greater microbial biomass, reduced C:N ratio in the HF, and greater available P are interrelated outcomes resulting from compaction of sandy soils as well. An increase in soil water holding capacity would have a positive effect on total microbial biomass by creating a more hospitable environment for the SMC, while an increase in aboveground productivity would support a larger microbial population through greater rhizodeposition and organic matter inputs. The reduction of the C:N ratio in the HF of compacted treatments could be due to a combination of the increase in aboveground productivity and greater microbial biomass, resulting in more microbial decomposition in compacted soils. Finally, compacted soils had almost double the amount of available P compared to non-compacted soils. Soil P tends to have low availability in many soils because it is readily adsorbed to soil minerals, and is only made available through the mineralization activities of soil microbes. The greater microbial biomass in compacted soils most likely led to an increase in P availability due to higher microbial mineralization of adsorbed soil P. These results reflect the idea that soil management practices can result in long-term changes in physical, chemical, and biological soil properties.

4.3. Depth effects reflect soil forming processes

Contrary to our expectations, subsurface C:N ratios for all three pools were significantly greater than the paired surface pools (Table 2). Typically the soil C:N ratio decreases with increasing depth, indicating that soil organic matter in the subsoil is primarily derived from microbial by-products and is low in plant material, which has greater C:N ratios (Bååth and Wallander, 2003; Rumpel and Kögel-Knabner, 2011). These results are most likely due to podsolization, which is the soil forming process in Spodosols where soluble organic compounds from surface soils are eluviated into deeper soil horizons (Buol et al., 1997). Spodosols have been shown to have greater total C stocks than other soil orders, which are driven by high C concentrations at depth in mineral soils, specifically the podzolic B horizon (Shaw et al., 2008). Results from Nave et al. (2010) suggest that harvesting on Spodosols can accelerate podsolization, leading to greater accumulation of recent organic C compounds in subsurface mineral soil. Ussiri and Johnson (2007) found that total C increased in the B_s soil horizon 15 years after clearcutting while soil N remained the same. Therefore the greater C:N ratios in subsurface soil in this dataset implies the eluviation of less decomposed organic C compounds into the subsurface horizons.

However, this theory of “younger”, plant-derived C in subsurface horizons is contradicted by the patterns observed in our analysis of the soil microbial population. Microbial biomass was expected to be higher in areas with greater amounts of less decomposed C. However, microbial biomass in subsurface soil was less than half that in surface soils during both sampling periods (Fig. 1b; Fig 2). With the exception of

GmP bacteria, all of the microbial guilds were more abundant in surface than subsurface soil (Fig. 1b). This is likely due to larger f-LF and o-LF pools supplying readily available C and N sources for microbial decomposition in surface soils (Blume et al., 2002; Taylor et al., 2002). For the same reasons, the CYC ratio, which is a measure of resource stress in GmN bacteria (Guckert et al., 1986; Kieft et al., 1997; Bossio et al., 1998), was greater at depth than in the surface soil. GmP bacteria, which are able to decompose “older”, more highly decomposed C sources (Kramer and Gleixner, 2008), were the only microbial group that was more abundant at depth (Fig. 1a). These results are inconsistent with greater soil C:N ratios at depth from the accumulation of fresh, plant inputs from the organic horizon. Therefore, it is proposed that the subsoil HF is an average of younger and old C originating both from eluviation from surface horizons and microbial decomposition processes *in situ*. A greater C:N ratio in the subsoil relative to the surface HF indicates the presence of fresh plant inputs, but it is possible that the greater size of the HF in subsurface soil contributes a proportionally large amount of highly decomposed C as well, explaining the reduced microbial biomass, but greater abundance of GmP bacteria at depth.

Our findings provide insight into potential soil effects of sustainable biomass harvesting practices; however, due to the complexity of the factors influencing soil forming processes, they are primarily applicable to forests grown on Spodosols with similar tree species and climate. In future research, more detailed information on the actual age of C in different pools in surface and subsurface soils would improve understanding of C cycling and storage mechanisms in Spodosols and the relationship with soil microbes.

5. Conclusions

The large-scale, Long-Term Soil Productivity experiment was established on the understanding that forest management affects site organic matter and soil porosity regardless of silvicultural practice or harvest intensity. The range of possible responses have played out because these factors were directly manipulated on a network of sites across the continent. Now, nearly two decades after the experiments were initiated, important hypotheses as to whether impacts are irreversible or dissipate over time can be addressed. While in this study we found lasting effects of organic matter removal on the soil microbial community, and soil C and N cycling, these patterns are not universal.

The large amount of variability observed in soil C and N and the SMC between surface and subsurface soils highlights the soil forming processes at work in this Spodosol, and the importance of considering soil type when assessing management effects on soil properties and their consequence for forest productivity. Management practices that prioritize the retention of organic matter may be the best option to mitigate negative impacts of harvesting on long term productivity in this instance.

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References

- Aanderud, Z.T., Shuldman, M.I., Drenovsky, R.E., Richards, J.H., 2008. Shrub-interspace dynamics alter relationships between microbial community composition and belowground ecosystem characteristics. *Soil Biology and Biochemistry* 40, 2206–2216. <https://doi.org/10.1016/j.soilbio.2008.04.008>.
- Alban, D.H., Perala, D.A., Schlaegel, B.E., 1978. Biomass and nutrient distribution in aspen, pine, and spruce stands on the same soil type in Minnesota. *Canadian Journal of Forest Research* 8, 290–299. <https://doi.org/10.1139/x78-044>.
- Anderson, M., Gorley, R., Clarke, R., 2008. *PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods*. PRIMER-E Ltd, Plymouth, UK.
- Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology* 26, 32–46.
- Bååth, E., Wallander, H., 2003. Soil and rhizosphere microorganisms have the same Q10 for respiration in a model system. *Global Change Biology* 1788–1791. <https://doi.org/10.1046/j.1529-8817.2003.00692.x>.
- Balser, T.C., Firestone, M.K., 2005. Linking microbial community composition and soil processes in a California annual grassland and mixed-conifer forest. *Biogeochemistry* 73, 395–415. <https://doi.org/10.1007/s10533-004-0372-y>.
- Belleau, A., Brais, S., Paré, D., 2006. Soil nutrient dynamics after harvesting and slash treatments in boreal aspen stands. *Soil Science Society of America Journal* 70, 1189–1199. <https://doi.org/10.2136/sssaj2005.0186>.
- Blume, E., Bischoff, M., Reichert, J.M., Moorman, T., Konopka, A., Turco, R.F., 2002. Surface and subsurface microbial biomass, community structure and metabolic activity as a function of soil depth and season. *Applied Soil Ecology* 20, 171–181. [https://doi.org/10.1016/S0929-1393\(02\)00025-2](https://doi.org/10.1016/S0929-1393(02)00025-2).
- Bossio, D.A., Scow, K.M., Gunapala, N., Graham, K.J., 1998. Determinants of soil microbial communities: effects of agricultural management, season, and soil type on phospholipid fatty acid profiles. *Microbial Ecology* 36, 1–12.
- Buol, S., Hole, F., McCracken, R., Southard, R., 1997. *Soil Genesis and Classification*, fourth ed. Iowa State University Press, Ames, IA.
- Burger, J., 2002. Soil and long-term site productivity values. In: Richardson, J., Bjorheden, R., Hakkila, P., Lowe, A., Smith, C. (Eds.), *Bioenergy from Sustainable Forestry: Guiding Principles and Practices*. Kluwer Academic Publishers, Dordrecht, pp. 165–189.
- Chen, C., Xu, Z., 2005. Soil carbon and nitrogen pools and microbial properties in a 6-year-old slash pine plantation of subtropical Australia: impacts of harvest residue management. *Forest Ecology and Management* 206, 237–247. <https://doi.org/10.1016/j.foreco.2004.11.005>.
- Christie, W.W., 1989. *Gas Chromatography and Lipids*, third ed. The Oily Press Ltd, Bridgewater, Somerset.
- Clarke, K., Gorley, R., 2015. *PRIMER V7: User Manual/Tutorial*. PRIMER-E Ltd, Plymouth, UK.
- Crow, S.E., Swanston, C.W., Lajtha, K., Brooks, J.R., Keirstead, H., 2007. Density fractionation of forest soils: methodological questions and interpretation of incubation results and turnover time in an ecosystem context. *Biogeochemistry* 85, 69–90. <https://doi.org/10.1007/s10533-007-9100-8>.
- Curzon, M.T., D'Amato, A.W., Palik, B.J., 2014. Harvest residue removal and soil compaction impact forest productivity and recovery: potential implications for bioenergy harvests. *Forest Ecology and Management* 329, 99–107. <https://doi.org/10.1016/j.foreco.2014.05.056>.
- Frostegård, Å., Tunlid, A., Bååth, E., 1993. Phospholipid fatty acid composition, biomass, and activity of microbial communities from two soil types experimentally exposed to different heavy metals. *Applied and Environmental Microbiology* 59, 3605–3617.
- Frostegård, Å., Tunlid, A., Bååth, E., 1996. Changes in microbial community structure during long-term incubation in two soils experimentally contaminated with metals. *Soil Biology and Biochemistry* 28, 55–63.
- Geisseler, D., Horwath, W.R., Joergensen, R.G., Ludwig, B., 2010. Pathways of nitrogen utilization by soil microorganisms – a review. *Soil Biology and Biochemistry* 42, 2058–2067. <https://doi.org/10.1016/j.soilbio.2010.08.021>.
- Guckert, J.B., Hood, M.A., White, D.C., 1986. Phospholipid ester-linked fatty acid profile changes during nutrient deprivation of *Vibrio cholerae*: increases in the trans/cis ratio and proportions of cyclopropyl fatty acids. *Applied and Environmental Microbiology* 52, 794–801.
- Hart, C., Sollins, P., 1998. Soil carbon and nitrogen pools and processes in an old-growth conifer forest 13 years after trenching. *Canadian Journal of Forest Research* 28 (8), 1261–1265.
- Hassett, J., Zak, D., 2005. Aspen harvest intensity decreases microbial biomass, extracellular enzyme activity, and soil nitrogen cycling. *Soil Science Society of America Journal* 69, 227–235.
- Hill, T., McPherson, E., Harris, J., Birch, P., 1993. Microbial biomass estimated by phospholipid phosphate in soils with diverse microbial communities. *Soil Biology and Biochemistry* 25, 1779–1786. [https://doi.org/10.1016/0038-0717\(93\)90183-C](https://doi.org/10.1016/0038-0717(93)90183-C).
- Jandl, R., Lindner, M., Vesterdal, L., Bauwens, B., Baritz, R., Hagedorn, F., Johnson, D., Minkinen, K., Byrne, K., 2007. How strongly can forest management influence soil carbon sequestration? *Geoderma* 137, 253–268. <https://doi.org/10.1016/j.geoderma.2006.09.003>.
- Johnson, D., Curtis, P., 2001. Effects of forest management on soil C and N storage: meta analysis. *Forest Ecology and Management* 140, 227–238. [https://doi.org/10.1016/S0378-1127\(00\)00282-6](https://doi.org/10.1016/S0378-1127(00)00282-6).
- Jones, H., Beets, P., Kimberley, M., Garrett, L., 2011. Harvest residue management and fertilisation effects on soil carbon and nitrogen in a 15-year-old *Pinus radiata* plantation forest. *Forest Ecology and Management* 262, 339–347. <https://doi.org/10.1016/j.foreco.2011.03.040>.
- Kaiser, M., Ellerbrock, R.H., Sommer, M., 2009. Separation of coarse organic particles from bulk surface soil samples by electrostatic attraction. *Soil Science Society of America Journal* 73, 2118–2130. <https://doi.org/10.2136/sssaj2009.0046>.
- Kaiser, M., Walter, K., Ellerbrock, R.H., Sommer, M., 2011. Effects of land use and mineral characteristics on the organic carbon content, and the amount and composition of N-phyrophenolate-soluble organic matter, in subsurface soils. *European Journal of Soil Science* 62, 226–236. <https://doi.org/10.1111/j.1365-2389.2010.01340.x>.
- Kieft, T.L., Wilch, E., O'Connor, K., Ringelberg, D.B., White, D.C., 1997. Survival and phospholipid fatty acid profiles of surface and subsurface bacteria in natural sediment microcosms. *Applied and Environmental Microbiology* 63, 1531–1542.
- Kramer, C., Gleixner, G., 2008. Soil organic matter in soil depth profiles: distinct carbon preferences of microbial groups during carbon transformation. *Soil Biology and Biochemistry* 40, 425–433. <https://doi.org/10.1016/j.soilbio.2007.09.016>.
- Kurth, V.J., D'Amato, A.W., Palik, B.J., Bradford, J.B., 2014. Fifteen-Year patterns of soil carbon and nitrogen following biomass harvesting. *Soil Science Society of America Journal* 78, 624–633. <https://doi.org/10.2136/sssaj2013.08.0360>.
- Liski, J., Pussinen, A., Pingoud, K., Mäkipää, R., Karjalainen, T., 2001. Which rotation length is favourable to carbon sequestration? *Canadian Journal of Forest Research* 31 (11), 2004–2013. <https://doi.org/10.1139/x01-140>.
- Marin-Spiotta, E., Silver, W., Swanston, C., Ostertag, R., 2009. Soil organic matter dynamics during 80 years of reforestation of tropical pastures. *Global Change Biology* 15, 1584–1597. <https://doi.org/10.1111/j.1365-2486.2008.01805.x>.
- Mika, A.M., Keeton, W.S., 2012. Factors contributing to carbon fluxes from bioenergy harvests in the U.S. Northeast: an analysis using field data. *Glob. Chang. Biol. Bioenergy* 5, 290–305. <https://doi.org/10.1111/j.1757-1707.2012.01183.x>.
- Nave, L., Vance, E., Swanston, C., Curtis, P., 2010. Harvest impacts on soil carbon storage in temperate forests. *Forest Ecology and Management* 259, 857–866. <https://doi.org/10.1016/j.foreco.2009.12.009>.
- Olsson, P.A., 1999. Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soil. *FEMS Microbiology Ecology* 29, 303–310. <https://doi.org/10.1111/j.1574-6941.1999.tb00621.x>.
- Page-Dumroese, D.S., 2010. *the North-American long-term soil productivity Study: concepts and literature*. USDA For. Serv. Proc. 43–60.
- Paré, D., Rochon, P., Brais, S., 2002. Assessing the geochemical balance of managed boreal forests. *Ecological Indicators* 1, 293–311. [https://doi.org/10.1016/S1470-160X\(02\)00026-2](https://doi.org/10.1016/S1470-160X(02)00026-2).
- Perala, D.A., Alban, D.H., 1982. Biomass, nutrient distribution and litterfall in *Populus*, *Pinus* and *Picea* stands on two different soils in Minnesota. *Plant and Soil* 64, 177–192.
- Ponder, F., Tados, M., 2002. Phospholipid fatty acids in forest soil four years after organic matter removal and soil compaction. *Applied Soil Ecology* 19, 173–182. [https://doi.org/10.1016/S0929-1393\(01\)00182-2](https://doi.org/10.1016/S0929-1393(01)00182-2).
- Powers, R., Scott, D., Sanchez, F., Voldseth, R., Page-Dumroese, D., Elioif, J., Stone, D., 2005. The North American long-term soil productivity experiment: findings from the first decade of research. *Forest Ecology and Management* 220, 31–50. <https://doi.org/10.1016/j.foreco.2005.08.003>.
- Rumpel, C., Kögel-Knabner, I., 2011. Deep soil organic matter—a key but poorly understood component of terrestrial C cycle. *Plant and Soil* 338, 143–158. <https://doi.org/10.1007/s11104-010-0391-5>.
- Shaw, C., Banfield, E., Kurz, W., 2008. Stratifying soils into pedogenically similar categories for modeling forest soil carbon. *Canadian Journal of Soil Science* 88, 501–516.
- Slesak, R.A., Palik, B.J., D'Amato, A.W., Kurth, V.J., 2017. Changes in soil physical and chemical properties following organic matter removal and compaction: 20-year response of the aspen Lake-States Long Term Soil Productivity installations. *Forest Ecology and Management* 392, 68–77.
- Smith, P., Marin-Spiotta, E., Balser, T., 2015. Successional and seasonal variations in soil and litter microbial community structure and function during tropical post-agricultural forest regeneration: a multiyear study. *Global Change Biology* 21, 3532–3547.
- Smithwick, E.A.H., Turner, M.G., Metzger, K.L., Balser, T.C., 2005. Variation in NH₄⁺ mineralization and microbial communities with stand age in lodgepole pine (*Pinus contorta*) forests, Yellowstone National Park (USA). *Soil Biology and Biochemistry* 37, 1546–1559. <https://doi.org/10.1016/j.soilbio.2005.01.016>.
- Sollins, P., Kramer, M., Swanston, C., Lajtha, K., Filley, T., Aufdenkampe, A., Wagai, R., Bowden, R., 2009. Sequential density fractionation across soils of contrasting mineralogy: evidence for both microbial- and mineral-controlled soil organic matter stabilization. *Biogeochemistry* 96, 209–231. <https://doi.org/10.1007/s10533-009-9359-z>.
- Stark, M., J., Firestone, K., M., 1995. Mechanisms for soil-moisture effects on activity of nitrifying bacteria. *Applied and Environmental Microbiology* 61, 218–221.
- Stone, D., 2001. *Sustaining aspen productivity in the lake states*. In: Shepperd, W.D., Binkley, D., Bartos, D.L., Stohlgren, T.J., Eskew, L.C. (Eds.), *Sustaining Aspen in Western Landscapes: Symposium Proceedings*. RMRS-P-18. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, pp. 47–59.
- Swanston, C., Torn, M., Hanson, P., Southon, J., Garten, C., Hanlon, E., Ganio, L., 2005. Initial characterization of processes of soil carbon stabilization using forest stand-level radiocarbon enrichment. *Geoderma* 128, 52–62. <https://doi.org/10.1016/j.geoderma.2004.12.015>.
- Sylvia, D., 2005. Mycorrhizal symbiosis. In: Sylvia, D., Fuhrmann, J., Hartel, P., Zuberer, D. (Eds.), *Principles and Applications of Soil Microbiology*. Pearson Education Inc., Upper Saddle River, NJ, pp. 263–282.
- Taylor, J., Wilson, B., Mills, M.S., Burns, R.G., 2002. Comparison of microbial numbers and enzymatic activities in surface soils and subsoils using various techniques. *Soil Biology and Biochemistry* 34, 387–401.
- Thiffault, E., Hannam, K., Paré, D., 2011. Effects of forest biomass harvesting on soil productivity in boreal and temperate forests—a review. *Environmental Reviews* 19, 278–309. <https://doi.org/10.1139/A11-009>.

- Thiffault, E., Paré, D., Bélanger, N., Munson, A., Marquis, F., 2006. Harvesting intensity at clear-felling in the boreal forest. *Soil Science Society of America Journal* 70, 691. <https://doi.org/10.2136/sssaj2005.0155>.
- Tiarks, A.E., Buford, M.A., Powers, R.F., Ragus, J.F., Page-dumroese, D.S., Ponder, F., Stone, D.M., 1997. North American long-term soil productivity research program. In: *Proceedings of the National Silviculture Workshop, Warren, PA. General Technical Report NE-238*. 19-22 May 1997. USDA Forest Service, Northeastern Forest Experiment Station, Radnor, PA, pp. 140–147.
- Trumbore E., S., Zheng H., S., 1996. Comparison of fractionation methods for soil organic matter 14C analysis. *Radiocarbon* 38 (2), 219–229.
- Ussiri, D.A., Johnson, C.E., 2007. Organic matter composition and dynamics in a northern hardwood forest ecosystem 15 years after clear-cutting. *Forest Ecology and Management* 240, 131–142. <https://doi.org/10.1016/j.foreco.2006.12.017>.
- Vestal, J., White, D., 1989. Lipid analysis in microbial ecology. *BioScience* 39, 535–541.
- Voldseth, R., Palik, B., Elioff, J., 2011. Ten-year results from the long-term soil productivity study in aspen ecosystems of the northern Great Lakes region. In: *Res. Pap. NRS-17*. U.S. for. Serv. North. Res. Stn., Newtown Square, PA.
- Wagai, R., Mayer, L., Kitayama, K., 2009. Nature of the “occluded” low-density fraction in soil organic matter studies: a critical review. *Soil Science & Plant Nutrition* 55, 13–25. <https://doi.org/10.1111/j.1747-0765.2008.00356.x>.
- White, D.C., Davis, W.M., Nickels, J.S., King, J.D., Bobbie, R.J., 1979. Determination of the sedimentary microbial biomass by extractable lipid phosphate. *Oecologia* 40, 51–62. <https://doi.org/10.1007/BF00388810>.
- Wilkinson, S.G., 1988. Gram negative bacteria. In: Ratledge, C., Wilkinson, S.G. (Eds.), *Microbial Lipids*. Academic Press, London, pp. 299–488.
- Worrell, R., Hampson, A., 1997. The influence of some forest operations on the sustainable management of forest soils— a review. *Forestry: An International Journal of Forest Research* 70 (1), 61–85. <https://doi.org/10.1093/forestry/70.1.61>.
- Zelles, L., 1997. Phospholipid fatty acid profiles in selected members of soil microbial communities. *Chemosphere* 35, 275–294.
- Zelles, L., 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. *Biology and Fertility of Soils* 29, 111–129. <https://doi.org/10.1007/s003740050533>.
- Zelles, L., Bai, Q.Y., Beck, T., Beese, F., 1992. Signature fatty-acids in phospholipids and lipopolysaccharides as indicators of microbial biomass and community structure in agricultural soils. *Soil Biology and Biochemistry* 24, 317–323.
- Zogg P., G., Zak R., D., Ringelberg B., D., MacDonald W., M., Pregitzer S., K., White C., D., et al., 1997. Compositional and functional shifts in microbial communities due to soil warming. *Soil Science Society of America Journal* 61, 475–481.