

Soil Scarification and Wildfire Interactions and Effects on Microbial Communities and Carbon

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Nutrient availability is an important constraint on sustainable forest productivity, and it is crucial to understand the long-term effects of management practices, including soil scarification, on soil microbial communities because they store and cycle nutrients. In addition, because forests are subject to wildfires, it would be useful to understand potential interactive effects of wildfire and management practice on forest soil ecosystems. We studied the individual and combined effects of soil scarification and a subsequent wildfire on microbial community structure of a ponderosa pine (*Pinus ponderosa* C. Lawson) forest soil in the central Rocky Mountains. Experimental plots were scarified by rototilling in 1981, and in 2002, some of the plots were burned during a mixed-severity wildfire. In 2005, mineral soil samples (0–10-cm depth) were collected and assayed for soil chemical properties, fungal and bacterial biomass, C mineralization potential, and microbial community fatty acid composition. Compared with undisturbed soil, soil from scarified-only plots was relatively high in pH, low in total C and organic matter (OM) concentrations, low in fungal and bacterial biomass, and enriched with Gram-positive biomarkers. Regardless of scarification treatment, soil from burned plots was relatively high in pH and extractable P, low in fungal but not bacterial biomass, and enriched with Gram-negative bacterial biomarkers. Compared with scarified-only plots, scarified-plus-burned plots had greater soil C and OM concentrations. Carbon mineralization rates were not different among the plot soils. While scarification is a positive practice for aiding seedling establishment, we found long-term effects on soil C reserves and microbial communities.

Abbreviations: EL-FAME, ester-linked fatty acid methyl ester; OM, organic matter; SIR, substrate induced respiration.

Soil scarification is any technique that prepares a site to improve accessible seedbed and thus promotes growth of disseminated seed (Archibold et al., 2000). It may involve tilling, disk trenching, roller-chopping, blading, or mounding of inverted humus (Johansson, 1994), but it always entails the removal or mixing of the top organic layer of a soil with mineral soil. This is a common silvicultural practice used in parts of Canada, Europe, and the United States that has been found to significantly improve seed establishment in numerous forest tree species (Burns, 1983; Örlander et al., 1996; Karlsson and Örlander, 2000), including naturally regenerating seedlings of ponderosa pine in the Colorado Rocky Mountains (Shepperd et al., 2006). Scarification allows successful seed establishment because once the mineral soil is exposed, seedling roots can penetrate faster than if they had to pass through a thick organic layer. It can also benefit seedlings by increasing soil temperature and aeration status, and by reducing competition between

a seedling and understory vegetation for water and nutrients (Thompson, 1984; Örlander et al., 1996). It also has been shown, however, that removal or incorporation of the organic horizon from the soil surface by scarification impacts soil C dynamics, nutrient cycling, and nutrient storage (Vitousek and Matson, 1985; Rauni et al., 1992; Johansson, 1994). For example, changes brought about by scarification (e.g., warmer soil temperatures and mixing of the forest floor with mineral soil) can enhance microbial decomposition of forest floor residues, as demonstrated by Johansson (1994). While accelerated microbial activity could benefit seedlings by increasing nutrient turnover rates, it could lead to reduced levels of nutrients and soil C in the long term (Wilson and Pyatt, 1984; Johnson, 1992; Örlander et al., 1996) as soluble nutrients are leached away and soil organic matter is respired to CO₂. The latter is known as “priming” and occurs when relatively labile substrates (e.g., dissolved organic C in incorporated forest floor litter) contribute to the degradation of more resistant forms of C in soils (Bingemann et al., 1953). This effect is probably due to increased extracellular enzyme production and microbial biomass fueled by the addition of labile dissolved organic C (Shen and Bartha, 1996; Hamer and Marschner, 2002; Fontaine et al., 2004). This could explain why scarification reduced total forest soil C levels by 6 to 41% in a long-term (>20-yr) study by Örlander et al. (1996).

Few studies have examined the effects of scarification on soil microbial biomass and community structure. Short-term studies (<5 yr post-scarification) have found no effect of scarification on soil microbial biomass C (Ohtonen et al., 1992)

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or mounding practice on buried humus microbial biomass C (Smolander et al., 2000), although Staddon et al. (1997) reported reduced functional diversity of soil microbial communities 4 yr after scarification. Longer term studies are necessary, however, to ensure sustainable forest productivity. Soil microorganisms function as dynamic reservoirs of organic C and plant nutrients (Insam and Domsch, 1988; Sylvia et al., 2005; Stromberger et al., 2007), and alterations to microbial dynamics may affect soil nutrient retention and the capacity of soils to provide available nutrients to plants.

In 1981, a long-term study was established to study ponderosa pine regeneration in the Colorado Front Range with the objective of comparing the effects of stand structure and soil preparation methods (with and without scarification) on the regeneration of ponderosa pine (Shepperd et al., 2006). This study concluded that scarification in combination with shelterwood overstories was the best silvicultural method for the establishment of naturally regenerating ponderosa pine seedlings. After 21 yr of monitoring, some of these original plots were burned by the Hayman wildfire of 2002, a devastating mixed-severity fire that burned >55,000 ha of Colorado's Pike National Forest. Fires are known to affect soil C and nutrient availability (Certini, 2005), microbial biomass and activity (Fritze et al., 1993; Pietikäinen and Fritze, 1993; Dumontet et al., 1996; Villar et al., 2004), and microbial community structure (Hart et al., 2005; Hamman et al., 2007), and the Hayman Fire presented an opportunity to explore potential interactive effects of wildfire and scarification on forest soil ecosystems. This could be important, because others have found that the response of soil microorganisms to a forest fire may depend on soil conditions before the fire (Choromanska and DeLuca, 2001).

The objective of this unique study was to determine the individual as well as the combined effects of soil scarification and burning on microbial community structure and activity of a forest soil. We predicted that the 1981 soil scarification treatment would have long-term impacts on soil microbial and chemical properties. Specifically, we hypothesized that scarification would reduce soil C and organic matter (OM) content in the long term as well as the biomass and activity of soil microorganisms, particularly of soil fungi, which are dependent on abundant C supplies to compete with bacteria. As a result of these changes, we predicted that the effects of a subsequent high-severity forest fire on soil microorganisms would differ between scarified and nonscarified soils.

MATERIALS AND METHODS

Site Description

Our study site is located within a larger experiment in the Manitou Experimental Forest (MEF), centrally located in the Rocky Mountains (39°4' N, 105°4' W), approximately 45 km west of Colorado Springs, CO. The mean annual temperature of the forest is 5°C, mean annual precipitation is 40 cm, and mean elevation is 2400 m (Massman and Frank, 2004). The study area is occupied by an overstory of mature ponderosa pine (>150 yr of age) in an area of gentle, east-facing slopes (Shepperd et al., 2006). The soils at MEF originated from gravelly alluvium and outwash of Pikes Peak granite and are classified as loamy, mixed Eutroboralfs or Aridic Haploborolls (Moore, 1992).

In 1981, a long-term experiment was initiated to compare the effects of seed-tree and shelterwood cutting methods, along with scarification and nonscarification of the forest floor, on ponderosa pine seedling establishment and growth. The study design and results were presented in detail in Shepperd et al. (2006). Briefly, the original design of the experiment was a split plot with replications in seven randomized blocks. Whole-plot treatments (91 by 91 m) consisted of seed-tree and shelterwood cutting methods; the shelterwood cut in 1981 left 50 mature seed-bearing trees per hectare, and the seed-tree cut left 12 trees per hectare. Cut trees were randomly skidded to designated landings with the butt ends raised to avoid excessive disturbance to plots. Logging slash was lopped and scattered according to standard timber sale contract guidelines. Split-plot treatments (31.8 by 31.8 m) were scarification and nonscarification site preparations. Slash was removed from plots to be scarified, and scarified plots were rototilled to a soil depth of 15 cm to completely incorporate understory vegetation and O horizon material into the soil with a small rubber-tired tractor that could maneuver between plots. For a natural regeneration study, split plots were parceled into 16 4-m² natural regeneration microplots.

In June 2002, the Hayman fire burned >55,000 ha of Colorado's Pike National Forest, including parts of Park, Jefferson, Douglas, and Teller counties. Fire exclusion and droughty conditions had led to dry and heavy fuel loads, and when combined with climatic and topographic conditions, the Hayman Fire became Colorado's largest wildfire in recorded history (Graham, 2003). In 1996, before the fire, some aboveground effects of scarification, such as increased grass cover and reduced sedge cover, were documented (Anna Schoettle, USDA Forest Service, personal communication, 2005). In addition, we observed thinner depths of litter in unburned, scarified plots, approximately 2 cm or less, compared with thicker litter depths on the surface of undisturbed plots, although systematic measurements of forest floor litter depth were not taken. Within 2 wk of the fire, burn severity was qualitatively assessed in the 16 natural regeneration microplots of each split plot by estimating cover percentage of scorch to vegetation, litter, and duff. For example, burning was classified as high severity if understory vegetation and organic residues were consumed down to the mineral soil in at least 80% of the microplot area, while low-severity burning resulted in microplots with unscorched duff, litter, and vegetation and no burning of mineral soil in at least 80% of the microplot area.

In the summer of 2005, we located four scarified and four non-scarified split plots within the natural regenerating shelterwood treatment that contained microplots burned at high and low severity based on the 2002 burn assessment. For the purpose of this study, we selected microplots with low burn severity as our unburned control plots, as 80% or more of the microplot area was unaffected by the fire. We sampled only four scarified split plots and four nonscarified split plots because not all of the seven split plots per site preparation treatment contained microplots with both severity "treatments"; some split plots had all microplots burned at high severity, whereas other split plots contained a combination of unburned microplots and microplots burned at low severity. Within each scarified or nonscarified split plot, there were multiple microplots burned at high severity and multiple microplots burned at low severity, but we sampled one high-severity burned microplot and one low-severity burned (unburned) microplot that were located the closest to each other to minimize potential site differences (other than fire severity) between the microplots. There was a total of four treatment combinations examined: undisturbed (nonscarified and unburned control soil), scarified but unburned

soil, nonscarified but burned soil, and scarified-plus-burned soil (16 microplots total).

Soil Sampling and Chemical Properties

In June 2005, soil samples were obtained from the top 10 cm of the soil using a hand trowel that was rinsed in ethanol between plots. Three to five samples were collected per plot and mixed together in a Ziploc freezer bag to form a composite sample. Soils were stored on ice in a cooler and transported back to the laboratory. Subsamples of field-moist soil were stored at 4°C for microscopy analysis, -80°C for ester-linked fatty acid methyl ester (EL-FAME) analysis, and oven dried to determine gravimetric water content. A 500-g portion of each composite sample was air dried and sent to the Colorado State University Soil and Plant Testing Laboratory to be tested for texture, pH, total C and N, OM, $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, and ammonium bicarbonate–diethylenetriamine pentaacetic acid (AB-DTPA)-extractable P. Soil pH was determined by the saturated paste method of Thomas (1996). Total C and N were measured using a LECO CHN-1000 automated analyzer (LECO Corp., St. Joseph, MI) according to the protocols of Nelson and Sommers (1996). Soil OM was determined by the modified Walkley–Black method (Nelson and Sommers, 1996). Exchangeable soil $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ were extracted in 2 mol L^{-1} KCl according to Mulvaney (1996) and analyzed on a Perstorp Enviroflow flow injector (Perstorp Analytical, Silver Spring, MD). The method of Barbarick and Workman (1987) was used for soil AB-DTPA-extractable P, followed by determination of constituent concentrations on an inductively coupled plasma–atomic emission spectrophotometer (Thermo Jarrell Ash Corp., Franklin, MA).

Microbial Biomass and Mineralizable Carbon

The biomasses of bacteria and fungi in soil were determined by direct microscopy techniques within 48 h of sampling. Subsamples (20-g dry weight) of soil were serially diluted in filter-sterilized water, and soil bacteria were visualized with SYBR I green fluorescent nucleotide stain solution (1 μL SYBR I mL^{-1} TE buffer, pH 7.5; Weinbauer et al., 1998) and enumerated according to the method of Bloem et al. (1995). Total and active fungal hyphae were quantified by the cover slip–well slide method of Lodge and Ingham (1991) after staining with fluorescein diacetate to detect cytoplasm-filled (active) hyphae. Bacterial and fungal slides were observed at 1000 $\times$ and 400 $\times$ resolution, respectively, with a Nikon Eclipse E600 epifluorescent microscope (Nikon Instruments, Mellville, NY) equipped with a Texas Red/UV/DTAF combination filter set and an ocular grid. Bacteria and fungi were counted in a total of 30 fields of view. Images of bacterial cells and fungal hyphae were captured with a CoolSNAP Pro_{cf} digital camera (A.G. Heinze Precision MicroOptics, Lake Forest, CA) and ImagePro Plus imaging software (Media Cybernetics, Silver Spring, MD). Biovolume conversions of bacteria and fungi were determined based on the average diameter of at least 160 cells and 48 hyphal fragments (Klein and Paschke, 2000). Fungal biomass C was estimated by multiplying biovolumes by 330 kg m^{-3} and assuming 40% C (van Veen and Paul, 1979; Frey et al., 1999). Bacterial biomass C was estimated by assuming a specific C content of 220 kg C m^{-3} (Bratbak and Dundas, 1984).

The substrate induced respiration (SIR) method was used to estimate the amount of mineralizable C associated with microbial biomass in the soil samples (Anderson and Domsch, 1978). Triplicate subsamples (12-g dry weight) of each soil were moistened to 60% field capacity and, after equilibration for 16 h, glucose was added at a

concentration of 4 mg g^{-1} soil. This substrate concentration was previously determined to yield the maximum initial respiratory response during preliminary optimization experiments (data not shown). After glucose amendment, samples were incubated at 25°C for 4 h, at which time the CO_2 evolved was measured by gas chromatography (Model GC-8A, Shimadzu Scientific Instruments, Columbia, MD).

Microbial Community Structure

Microbial community structure was assessed by analysis of ester-linked fatty acid methyl esters (EL-FAMES), beginning with extraction of lipids from 4 g of each soil sample using a 1:2:0.8 mixture of chloroform/methanol/phosphate buffer (pH = 7.4) as described by Bossio and Scow (1998). From 0.5 mL of total lipid material, we extracted membrane-bound fatty acids using the EL-FAME method as described by Schutter and Dick (2000). After addition of 20 μg nonadecanoic acid (19:0) as an internal standard, samples were analyzed by gas chromatography (GC) with an Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA) by the University of Delaware. The GC capillary column was an Ultra 2 Agilent no. 1909 1B-102 crosslinked 5% phenyl methyl silicone, 25 m long, with an internal diameter of 0.2 mm and film thickness of 0.33 μm . Flame ionization detection was achieved at a temperature of 250°C using a carrier gas of H_2 at a flow rate of 0.8 mL min^{-1} . Samples were run using the Microbial ID (Newark, DE) Eukaryote methods and peak naming table. To clean the column between samples, oven temperature ramped from 170 to 300°C at a rate of 5°C min^{-1} , with a hold at the maximum temperature for 12 min. The following biomarkers were assigned to Gram-positive bacteria: i14:0, a15:0, i15:0, a16:0, i16:0, a17:0, i17:0, and i17:1G (Zak et al., 1996; Bossio and Scow, 1998); Gram-negative bacteria: 16:1 ω 7c, 17:0 cy, and 19:0 cy (Paul and Clark, 1996, p. 49; Zak et al., 1996); and fungi: 18:1 ω 9c, 18:2 ω 6c, and 18:3 ω 6c (Vestal and White, 1989; Paul and Clark, 1996, p. 49; Zak et al., 1996; Bossio and Scow, 1998; Bååth, 2003; Högborg et al., 2007).

Statistical Analyses

Statistical analyses were performed with the PROC MIXED model of the SAS Statistical Package Version 9.1 (SAS Institute, Cary, NC). Because individual blocks did not necessarily contain all four treatment combinations (thus our replicates were not distributed among the same four blocks), the experiment was analyzed as a complete randomized two-way factorial design, with two levels of scarification and two levels of fire disturbance. The EL-FAME data were log transformed before analysis to satisfy the assumption of normality and equality of variances. Analysis of variance tests were performed on univariate data (microbial biomass, EL-FAME concentrations of summed biomarkers, C mineralization rates, and soil chemical data), and mean comparisons were performed using Fisher's protected LSD ($\alpha = 0.10$). Correlations among microbial parameters were tested by Pearson's correlation analysis in SAS. Principal components analysis (PCA) was performed using the correlation matrix on relative mole percent EL-FAME data.

RESULTS

Soil Chemistry

Results from the soil chemical analyses are shown in Table 1. Soil pH was significantly greater in disturbed soil; in undisturbed soil, the pH was 5.4, whereas the pH was ~ 6.0 in soils that were scarified, burned, or both scarified and burned. Soil

Table 1. June 2005 chemical properties of a ponderosa pine forest soil (0- to 10-cm depth) subjected to scarification in 1981, a high-severity fire in 2002, or both.

Treatment	pH	Total C	Total N	Organic matter	NH ₄ -N	NO ₃ -N	AB-DTPA† extractable P
		g kg ⁻¹			mg kg ⁻¹		
Undisturbed	5.4 (0.06) b‡	44 (0.48) a	2 (0.03)	72 (0.49) a	1.0 (0.15)	1.7 (0.31)	3.5 (0.49) b
Scarified	6.1 (0.11) a	24 (0.67) b	1 (0.04)	48 (0.99) b	0.4 (0.14)	2.4 (0.61)	5.9 (2.41) ab
Burned	6.0 (0.08) a	45 (0.72) a	2 (0.02)	69 (0.45) a	3.8 (2.30)	4.4 (2.66)	11.2 (2.43) a
Scarified and burned	5.8 (0.19) a	50 (1.37) a	3 (0.05)	67 (0.61) a	3.8 (2.37)	4.8 (1.61)	9.6 (2.58) a

† Ammonium bicarbonate–diethylenetriamine pentaacetic acid.

‡ Standard errors (±1) are shown in parentheses, and within columns, means followed by different letters are significantly different ($\alpha = 0.10$).

from scarified-only plots was significantly lower in total C and OM concentrations, whereas extractable soil P concentrations were greater in burned plots regardless of site preparation treatment. No significant differences were observed in total soil N and extractable NH₄-N and NO₃-N levels in response to scarification or burning, although the general trends in burned plots followed that of P, where values were elevated in burned plots.

Microbial Biomass and Mineralizable Carbon

Total and active fungal biomass was low in plots affected by scarification or burning (Fig. 1A). In burned or scarified

plots, fungal biomass was 60 to 70% lower than the undisturbed treatment. Burning of scarified soil did not significantly alter fungal biomass any more than did scarification alone. Soil bacterial biomass was approximately 50% lower in scarified-only plots than in undisturbed plots (Fig. 1B), whereas there was no difference in soil bacterial biomass among burned and undisturbed plots 3 yr after the high-severity fire. Overall, the ratio of total fungal/bacterial biomass was lower in disturbed soil than in undisturbed soil, regardless of disturbance type (Table 2). Fungal/bacterial biomass ratios ranged from 19.2:1 in undisturbed plots to significantly lower values of ~10:1 or 9.3:1 in disturbed plots. In addition, the ratio of active/total fungal biomass was significantly lower in burned soil than the control or scarified-only soils.

Despite the differences in fungal and bacterial biomass among the plot treatments, there were no significant differences in SIR mineralizable C among the treatments. Average mineralization rates ranged from 2.0 mg CO₂-C kg⁻¹ soil h⁻¹ in burned plots (with or without scarification treatment) to 2.2 and 2.8 mg CO₂-C kg⁻¹ soil h⁻¹ in control and scarified-only plots, respectively. Moreover, SIR rates did not correlate with other microbial parameters, including fungal or bacterial biomass or EL-FAME concentrations summed for fungal or bacteria biomarkers (Table 3).

Microbial Community Structure

Microbial community structure was assessed by PCA of EL-FAMES extracted from soil lipids (Fig. 2). Principal component 1 (PC 1) separated microbial communities of disturbed soil from microbial communities of undisturbed soil. The difference between these communities was statistically significant by ANOVA of community coordinates along PC 1 ($P = 0.0035$). Separation of disturbed soil microbial communities by disturbance type was provided by PC 2, which distinguished

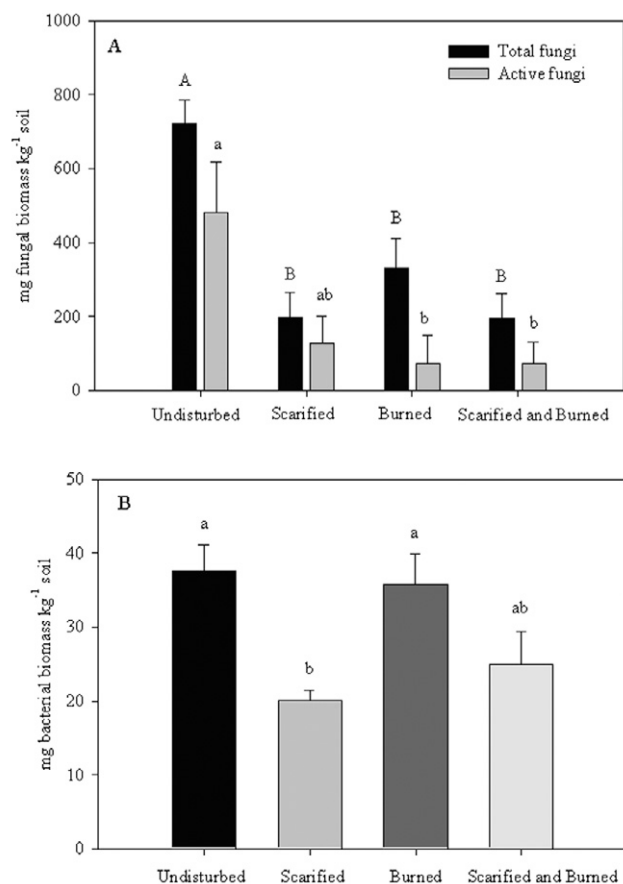


Fig. 1. Mean biomass of (A) total and active fungi and (B) total bacteria in a ponderosa pine forest soil affected by scarification in 1981, a high-severity surface fire in 2002, or both. Mineral soil samples (0–10-cm depth) were collected and analyzed by direct microscopy in June 2005. Standard error bars (±1) are shown. Means labeled with different letters are significantly different ($\alpha = 0.10$); total fungal biomass means are separated by uppercase letters and active fungal biomass means are separated by lowercase letters.

Table 2. Ratios of total fungal/bacterial biomass and active/total fungal biomass in a ponderosa pine forest soil affected by scarification in 1981, a high-severity fire in 2002, or both. Mineral soil samples (0–10-cm depth) were collected and analyzed by direct microscopy in June 2005.

Treatment	Fungal/bacterial biovolume ratio	Active/total fungal ratio
Undisturbed	19.2 (0.3) a†	0.67 (0.20) a
Scarified	10.5 (3.8) b	0.67 (0.33) a
Burned	9.3 (1.9) b	0.25 (0.25) b
Scarified and burned	10.1 (4.4) b	0.39 (0.31) b

† Standard errors (±1) are shown in parentheses. Within columns, means followed by different letters are significantly different ($\alpha = 0.10$).

between communities of scarified-only plots vs. burned-only and scarified-plus-burned plots. Community structure was significantly different between these treatment groups along PC 2 ($P = 0.0210$). Soil communities to the left of PC 1 (undisturbed soil) were enriched in fungal markers (18:3 ω 6c and 18:1 ω 9c) compared with soil samples on the right of PC 1. Analysis of variance tests conducted on individual EL-FAMES showed that the fungal biomarker 18:1 ω 9c was significantly affected by burn severity ($P = 0.0004$); relative amounts of 18:1 ω 9c were 1.58% in unburned soil vs. 0.13% in high-severity burned soil. Based on eigenvector values, communities positioned on the positive side of PC 2 (scarified-only plots) contained elevated amounts of Gram-positive bacterial EL-FAME markers i15:0, a15:0, i16:0, i17:0, and a17:0, as well as EL-FAME 16:1 2OH. Of these EL-FAMES, all but i17:0 and 16:1 2OH had a statistically significant site preparation $\times$ burn severity interaction, with significantly greater relative amounts of these EL-FAMES occurring in scarified-only plots than in scarified-plus-burned plots (data not shown). Communities with negative positions on PC 2 (burned-only and scarified-plus-burned plots) were enriched in 17:1 ω 7c and Gram-negative bacterial EL-FAME markers 17:0 cy and 19:0 cy. For these EL-FAMES, significantly greater amounts of 17:1 ω 7c and 19:0 cy were recovered from scarified-plus-burned plots than scarified-only plots (3.28 vs. 2.45% for 17:1 ω 7c and 8.65 vs. 6.40% for 19:0 cy). There were significantly greater amounts of 17:0 cy from burned-only (4.32%) than from undisturbed plots (2.62%).

Correlation analysis revealed positive relationships among summed fungal EL-FAME biomarker concentration, total fungal biomass, and active fungal biomass (Table 3). Summed bacterial EL-FAME biomarker concentrations were also positively correlated with fungal EL-FAME biomarkers and biomass but not bacterial biomass.

DISCUSSION

Scarification studies are often difficult to compare due to differences in scarification method used, time since scarification, climate, and forest and soil types. Furthermore, few studies have examined scarification impacts on soils, making it even more difficult to generalize effects. Our study demonstrated, however, that forest soil scarification can have long-term (>20-yr) impacts on soil chemical and biological properties, at least for the particular scarification method used and ponderosa pine forest studied. Similar to the long-term study of Örlander et al. (1996), we detected lower concentrations of total C and OM in scarified soil than in undisturbed soil. Other nutrients, however, were not significantly different between scarified and nonscarified soils. Ring (1996) also reported no effect of scarification by disk trenching on soil inorganic N concentrations 4 yr after scarification, Lundmark and Nömmik (1984) and Nohrstedt (2000) found increased inorganic N levels in buried humus but not necessarily soil, while Örlander et al. (1996) found that scarification lowered or did not affect concentra-

Table 3. Pearson correlation coefficients (r values) between selected microbial parameters measured in June 2005 in a ponderosa pine forest soil (0–10-cm depth) subjected to scarification in 1981, a high-severity fire in 2002, or both ($n = 15$).

	Total fungal biomass	Active fungal biomass	Fungal EL-FAMES†	Total bacterial biomass	Bacterial EL-FAMES
Active fungal biomass	0.65**				
Fungal EL-FAMES	0.68**	0.68**			
Total bacterial biomass	0.55*	0.32	0.17		
Bacterial EL-FAMES	0.63*	0.72**	0.60*	0.12	
SIR‡	0.28	–0.07	–0.11	0.22	–0.08

* Significant at the $P \leq 0.05$ level.

** Significant at the $P \leq 0.01$ level.

† Ester-linked fatty acid methyl esters.

‡ Substrate-induced respiration.

tions of macronutrients in surface soils but increased concentrations of P, Mg, and S at deeper depths (60–100-cm depths). Interestingly, in the present study, soil pH in scarified plots was nearly one unit greater than in undisturbed plots, a result that differs from those of Munson et al. (1993), Burgess et al. (1995), and Ring (1996), who found no effects of scarification on soil pH. Given the lack of predisturbance data, we are not able to explain this pattern completely, although lower fungal biomass in scarified soil than in undisturbed soil leads us to speculate whether heterotrophic nitrification activity (which generates acidity) was also lower in the scarified soil.

Lower total C and OM contents may explain why both fungal and bacterial biomass were ~ 70 and 50% lower, respectively, in scarified than in nonscarified plots. Few others studies have examined microbial responses to forest soil scarification, and our results are the first we know of that suggest a negative impact. Ohtonen et al. (1992) reported no effects of scarification on soil microbial biomass 4 yr after scarification was conducted on a clear-cut in Ontario. Microbial biomass was measured by the chloroform fumigation–extraction method.

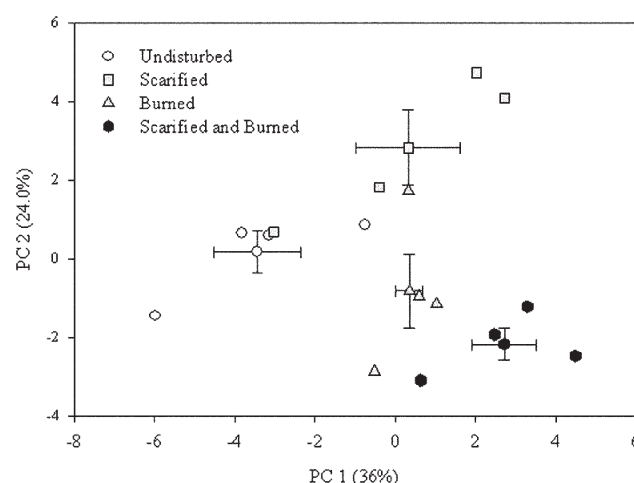


Fig. 2. Principal components (PC) analysis of the microbial community structure based on ester-linked fatty acid methyl ester (EL-FAME) profiles in a ponderosa pine forest soil that was scarified in 1981, burned in a high-severity surface burn in 2002, or both. Mineral soil samples (0–10-cm depth) were collected and analyzed in June 2005. Individual samples as well as the average PC scores for each treatment are shown along with standard error bars (± 1). The amount of variability explained by each PC axis is shown in parentheses.

Ohtonen et al. (1992) also examined bacterial and fungal biovolumes by direct microscopy, but only for bacteria and fungi associated with litter placed in litter bags, and not microorganisms in mineral soil. Similarly, Smolander et al. (2000) used the chloroform fumigation–extraction method to estimate microbial biomass associated with humus buried in soil by mounding and found no effect; however, microbial biomass of the mineral soil in mounded plots was not measured.

In the present study, broad differences in microbial community structure in response to both scarification and fire were revealed by direct microscopy. Overall, communities were strongly dominated by fungi, which represented 95% of the total microbial biomass in undisturbed soil based on ratios of total fungal/bacterial biomass. Relative amounts of fungi were ~5% lower in plots that had been scarified, burned, or both. Greater resolution of microbial community structural differences, particularly within bacterial groups, was achieved by EL-FAME analysis. Based on biomarker EL-FAMES, the bacterial community of scarified soil was favored by Gram-positive bacteria, compared with the bacterial communities of undisturbed and burned soils. In contrast, there were relatively more Gram-negative bacteria in burned-alone or scarified-plus-burned plots. Many Gram-positive bacteria are known *k* strategists and are selected for oligotrophic environments (Yao et al., 2000), and this is consistent with the fact that there was less total C and OM in scarified soil. In contrast, many Gram-negative bacteria are *r* strategists, which favor copiotrophic (higher nutrient) environments (Andrews and Harris, 1986), and relatively more Gram-negative bacteria in fire-affected scarified soil corresponds with the greater total C and OM in this soil compared with scarified-only soil.

In contrast to direct microscopy and EL-FAME methods, the SIR method did not detect changes in microbial biomass in response to scarification or burning. Our SIR values are comparable to those obtained from an unfertilized agricultural soil studied by Enwall et al. (2007), are lower but within the same order of magnitude of values reported by Dehlin et al. (2006) for a boreal forest, and are several orders of magnitude lower than values obtained from forest soils by Wallenstein et al. (2006), even though our glucose amendment rate was similar to those used in the other studies. The SIR method measures the biomass of microorganisms that rapidly respond to and mineralize glucose (or other labile C substrate), and it is possible that such microorganisms represented only a small portion of the total microbial community in the present study. In contrast, Williams and Rice (2007) found good agreement between SIR and microbial phospholipid fatty acid patterns in a grassland soil irrigated to enhance water availability. In addition, we observed good and positive correlations between fungal biomass as measured by direct microscopy and EL-FAME biomarkers. Moreover, bacterial biomass and EL-FAME biomarker concentrations were positively correlated with fungal biomass and EL-FAME biomarkers, which agrees with the observations that both fungi and bacteria biomass were relatively high in undisturbed plots and lower in scarified and scarified-plus-burned plots. Surprisingly, bacterial EL-FAME biomarker concentrations and total bacterial biomass were not correlated. This may be due to the fact that bacteria altogether only made up a small portion of the microbial community, and

thus numbers may have been near the limit of detection with the dilution series we used for our microscopy method.

Three years after the Hayman fire, we observed higher soil pH, greater amounts of extractable P, and slightly greater concentrations of inorganic N in the high-severity burned plots. Others have reported an increase in soil pH and available soil nutrients immediately following a fire event (Fyles et al., 1991; Pietikäinen and Fritze, 1993; Prieto-Fernandez et al., 1998, 2004; Certini, 2005). Soil pH increases after a fire due to the release of basic cations (Mg^{2+} , K^+ , Ca^{2+}) from oxidized organic matter and because of volatilization of soil organic acids, and these changes may last several years after a fire (Certini, 2005). In the present study, we also observed lower fungal biomass, no difference in bacterial biomass, and thus lower ratios of total fungal/bacterial biomass in soil from burned plots. Similarly, Dunn et al. (1985), Vázquez et al. (1993), D'Ascoli et al. (2005), and Guerrero et al. (2005) reported greater sensitivities of soil fungi to a fire or soil heating event than bacteria. While total fungal biomass was relatively low in scarified and burned plots compared with undisturbed plots, the effects of the fire alone appeared more detrimental to fungi as evidenced by low ratios of active/total fungal hyphae in burned soil. Whereas scarification alone did not affect the ratio of active/total fungal biomass, the high-severity fire reduced the proportion of fungal biomass that was active.

An interesting observation was that total soil C concentration in scarified-only plots was nearly half the level measured in undisturbed, burned-only, and scarified-plus-burned plots. Örländer et al. (1996) previously measured lower total C in scarified vs. control soils in Swedish Scots pine (*Pinus sylvestris* L.) forests, where multiple scarification methods were used 20 to 70 yr before soil sampling. One study site in particular was scarified by plowing with an agricultural tractor, and 23 yr later, total C was 41% lower in the scarified plots than the control plots. This agrees with the difference we measured between scarified-only and undisturbed plots in the present study. With regard to fire, we found no difference in soil C between undisturbed and burned-only plots, and this is in agreement with results from a previous study conducted 1 yr after the Hayman fire on nonscarified areas of the same forest, where Hamman et al. (2007) found no effects of high-severity fire on total soil C concentration. In the present study, we measured nearly twice the soil C in scarified-plus-burned plots than in scarified-only plots, however, which could be interpreted as (i) the Hayman Fire increased soil C levels in scarified but not in nonscarified soil, or (ii) there were pretreatment differences in soil C between the microplots of these treatment combinations. Fires can have mixed effects on soil C, and there have been cases where fire-affected soils actually accrued rather than lost C. Knicker et al. (2005) measured twice as much total organic C in fire-affected soil (0–15-cm depth) as in unaffected soil after a wildfire in a Mediterranean pine forest. Czimczik et al. (2003) also reported a 25% increase of the organic C stocks of a Siberian Scots pine forest floor 2 d after a surface fire. Knicker et al. (2005) explained that severe fires typically reduce soil C concentrations due to complete combustion of vegetation and organic matter in surface soils. Less-severe fires that only partially destroy vegetation, however, produce considerable amounts of charred residue on the soil surface,

which then moves downward into the mineral soil to effectively increase soil C levels. We observed this pattern in a previous study, when we measured greater C levels in soil under the edge of a slash pile after the slash pile was burned, but lower C levels in soil under the center of the slash pile where the slash and underlying vegetation were completely combusted (Jiménez Esquilín et al., 2007). In the present study, this fire severity–degree of combustion explanation is logical assuming that scarified plots contained less surface litter and organic residues than nonscarified plots, and that the decreased fuel loads then caused the Hayman Fire to burn less severely and combust the vegetation and surface litter less completely.

Our data support the hypothesis that soil scarification can have long-term reductions in forest soil C and OM as well as fungal and bacterial biomass. More long-term studies are needed, however, to determine if these findings extrapolate to other forests and with other scarification methods, because although scarification is a common practice, scarification by rototilling is not. In contrast to what we predicted, we did not observe many differences, other than total C and OM, between burned-only and scarified-plus-burned plots that could be attributed to a scarification–burn interaction, and the scarification treatment did not alter the impact of the high-severity fire on soil microbial communities.

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