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Microbial community structure and activity in a Colorado Rocky Mountain forest soil scarred by slash pile burning

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Abstract

Tree thinning and harvesting produces large amounts of slash material which are typically disposed of by burning, often resulting in severe soil heating. We measured soil chemical properties and microbial community structure and function over time to determine effects of slash pile burning in a ponderosa pine forest soil. Real time data were collected for soil temperature, heat flux, and soil moisture contents in one of two slash piles burned in April 2004. During the burn, soil temperatures reached 300 °C beneath the pile center and 175 °C beneath the pile edge. Slash pile burning increased soil pH, extractable N and P, and decreased total C levels within the first 15 cm of soil. Burning reduced soil bacterial biovolumes within the first 15 cm of soil and fungal biovolumes within the first 5 cm of soil. One month after the burn, soil microbial communities under the pile center were enriched in Gram-positive bacterial fatty acid markers compared to communities from under the pile edge and control (nonburned) soil. Fifteen months later, soil chemical properties had not returned to background levels, and microbial community structure in fire-affected soil, regardless of pile location, was distinct from communities of control soil. In fire-affected soil, concentrations of fungal fatty acid biomarkers were low and arbuscular mycorrhizal fungal biomarkers were absent, regardless of pile location. Slash pile burning also reduced fungal and bacterial respiration and resulted in large fluctuations in microbial potential N mineralization and immobilization activities. By altering soil properties important to soil conservation and plant reestablishment, slash pile burning negatively impacts forest ecosystems at localized scales.

Keywords: Slash pile burning; Soil microbial community; Ponderosa pine forest

1. Introduction

Over 100 years of fire suppression and over grazing by cattle have left profound changes to some of the ponderosa pine forests in Colorado. The forest stand structure has shifted from a wide open savanna-like structure to one with very high tree densities, which in turn has contributed to destructive forest fires such as the Hayman fire of 2002 (Graham, 2003). The current restoration program for the ponderosa pine ecosystem in the Colorado Rocky Mountains involves thinning and reintroduction of fire through prescribed burns to bring back the historical forest structure and function (Dahms and Geils, 1997). Thinning for restoration purposes, in combination with traditional harvesting practices, produces large amounts of slash material, which commonly is disposed of by burning them in large piles. Slash piling and burning is the preferred method of disposal because it can be done under a wide variety of weather conditions (Hardy et al., 1996), but disposal of slash by piling and burning presents a dilemma to forest land managers. Although it is an effective method for removal of unmarketable debris and small trees, these piles when burned often produce an extreme heat pulse into the soil, which results in severe soil heating. This raises the question of how slash pile burning impacts localized regions of forests and, depending on the size and fuel loads of the slash piles, whether such changes have ecosystem-level impacts.

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Even though slash pile burning is a preferred method of disposal among forest managers, several studies have reported profound effects to soil physical and chemical properties, such as altered clay mineralogy (Arocena and Opio, 2003) and increased pH and concentrations of base cations and sometimes extractable N and P (Arocena and Opio, 2003; Jönsson and Nihlgård, 2004; Thorpe and Timmer, 2005). Others have reported increased germination and enhanced establishment of invasive and/or non-native plant species within pile burn scars (Dickinson and Kirkpatrick, 1987; Haskins and Gehring, 2004; Korb et al., 2004, Wolfson et al., 2005). Studies which focus on soil microbial communities within burned pile scars are scarce, however. In one study, slash pile burning nearly eliminated arbuscular mycorrhizal (AM) fungal propagules and 15 months after the burn, AM fungi were still negatively affected (Korb et al., 2004). In another study, Haskins and Gehring (2004) found no effect on AM fungi 5 years after slash pile burning. However, considering that wildfires or prescribed burns are known to impact microbial biomass (Theodorou and Bowen, 1982; Villar et al., 2004), activities (Fritze et al., 1993; Pietikäinen and Fritze, 1993) and community composition (Vázquez et al., 1993; Acea and Carballas, 1996; Hamman et al., 2007), it is likely that slash pile burning will also impact soil microorganisms.

Given the limited information regarding soil biological responses to slash pile burning, we conducted a study in a ponderosa pine forest to address two research needs: (1) the immediate and short term impacts of slash pile burning on soil microbial communities under slash piles and (2) the influence of pile geometry on fire intensity and subsequent impacts to soil located under slash pile edges or centers. We formulated and tested four hypotheses. First, changes to soil chemistry and microbial communities would be larger within the first 5 cm of soil than at lower depths because of the insulating capacity of soil. Second, microbial community structure would be more affected at the center of the slash pile because of the larger heat pulse received in the soil at the center of the pile due to larger fuel density. Third, activity of surviving microorganisms under the pile edge would be enhanced immediately after the fire because of the flush of nutrients associated with soil heating events and the lower thermal input associated with less fuel loading at the edge of the pile compared to the center of the pile. Finally, once soil nutrients returned to background levels, fire effects on soil microorganisms would be similar regardless of location within the pile.

2. Materials and methods

2.1. Study site and soil sampling

The study was conducted at two locations in the Manitou Experimental Forest (MEF), centrally located in the Rocky Mountains (39°04′ North and 105°04′ West) approximately 45 km west of Colorado Springs, CO. The mean annual temperature of the forest is 5 °C, the mean annual precipitation is 40 cm, and its mean elevation is 2400 m. The study area is occupied by an overstory of mature (>150 y of age) ponderosa pine (*Pinus ponderosa*) and Douglas fir (*Pseudotsuga menziesii*) in an area of gentle, east-facing slopes. 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). More details about MEF can be found in Massman and Frank (2004) and Shepperd et al. (2006).

The experimental design consisted of two replications each of a control (nonburned) and a slash pile treatment. The low number of replicates reflects the time constraints of the number of slash piles we were able to safely burn in one day. Because of cost and labor issues, sensors to monitor soil temperature, heat flux, CO_2, and moisture content were installed under only one replicate of each treatment. Four 1.5-m deep pits were dug in August 2003; two pits were located under the yet-to-be-built slash pile and two pits were located in control areas where no pile was to be built. Sensors were installed in the pits at depths described below, and wires connecting the sensors were laid in trenches and attached to CR23X data loggers (Campbell Scientific, Logan, UT) located 30 m away. The pits and trenches were then carefully back filled. Thermocouples (Omega Engineering, Stamford, CT) to measure soil temperature were installed at depths of 0, 2, 5, 15, 20 and 50 cm. All thermocouple junctions were coated with epoxy (Omegabond 101) prior to insertion into the soil to insure electrical isolation. Specially designed high-temperature TDR probes (Zostrich Geotechnical, Pullman, WA) and heat flux tranducers (Thermonetics Corp., La Jolla, CA) were used at the slash pile burn site, whereas commercially available TDR probes (Campbell Scientific, Logan, UT) and heat flux transducers (Radiation and Energy Balance Systems, Seattle, WA) were used at the control site. TDR probes were installed at depths of 5 and 20 cm, and heat flux tranducers were installed at depths of 0, 2, 5, 15, and 20 cm. One slash pile was constructed over two back-filled pits so that one set of sensors would be located under the slash pile edge and another set under the slash pile center. A second replicate slash pile was constructed approximately 0.48 km south of the instrumented pile, with an area outside of the second slash pile designated as the second control. The slash piles were conical in shape (approximately 6 m × 9 m in diameter) with an approximate fuel loading of 450–600 kg m^{-2}. On April 26, 2004 the slash piles were ignited and allowed to burn under the supervision of a professionally trained fire crew until the fire had consumed the fuel. Measurements of soil temperature, heat flux, and soil moisture content were recorded before, during, and after the burn.

Soils samples were collected from the slash pile edges and centers, as well as from replicate control sites, on May 3, 2004, one week after the burn. These sites were sampled again in June 2004, approximately one month after the fire, and again at 3, 6, 12, and 15 months after the slash pile

burn. Samples were collected using a hand trowel that was rinsed in ethanol between plots to depths of 0–5 and 5–15 cm (16 soil samples total). Each sample consisted of 3–5 cores that were composited by depth (0–5 and 5–15 cm), stored in Ziploc freezer bags, and transported back to the laboratory on ice. Each composite soil sample was sieved through a 2-mm mesh screen to aid in sample homogenization.

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, NH_4–N, NO_3–N, and ammonium bicarbonate-diethylenetriaminepentaacetic 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, St. Joseph, MI) according to the protocols of Nelson and Sommers (1996). Exchangeable soil NH_4–N and NO_3–N were extracted in 2 M KCl according to Mulvaney (1996) and analyzed on a Perstorp Enviroflow flow injector (Perstorp Analytical Inc., 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).

2.2. Total bacteria and total and active fungi

Biovolumes of bacteria and fungi in soil were determined by direct microscopy techniques. Subsamples (10 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). Quantification of soil fungal hyphae was performed with the coverslip-well slide method of Lodge and Ingham (1991). Bacterial and fungal slides were observed at 1000 × and 400 × resolutions, respectively, with a Nikon Eclipse E600 epifluorescent microscope (Nikon Instruments Inc., 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 240 bacterial cells and 80 hyphal fragments (Klein and Paschke, 2000).

2.3. Microbial community structural analyses

Microbial community structure was assessed by analysis of ester-linked fatty acid methyl esters (EL-FAMEs), beginning with extraction of phospholipids 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 phospholipid material, we extracted membrane bound fatty acids using the EL-FAME method as described by Schutter and Dick (2000). Nonadecanoic acid (19:0) was added as an internal standard (20 μg), and samples were analyzed by gas chromatography (GC) analysis with an Agilent 6890 gas chromatograph (Agilent Technologies Inc., Palo Alto, CA). 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 °C and to 300 °C at a rate of 5 °C min^{-1}, with a hold at the maximum temperature for 12 min. Biomarkers of specific functional groups were assigned as follows: Gram-positive bacteria (i14:0, a15:0, i15:0, a16:0, i16:0, a17:0, i17:0, i17:1G; Zak et al., 1996; Bossio and Scow, 1998), Gram-negative bacteria (16:1ω7c, 17:0 cy, and 19:0 cy; Paul and Clark, 1996; Zak et al., 1996), and fungi (18:2ω6c and 18:2ω3c; Vestal and White, 1989; Paul and Clark, 1996; Zak et al., 1996; Bossio and Scow, 1998).

2.4. Potential C and N mineralization activities

Triplicate subsamples (12 g dry weight) of each soil were moistened to 60% field capacity and inhibitors (4 mg g^{-1} soil for streptomycin to inhibit bacteria; 15 mg g^{-1} soil for cyclohexamide to inhibit fungi) were added as required for the substrate-induced respiration inhibition assay (Anderson and Domsch, 1975; Johnson et al., 1996). Additional subsamples were also incubated in the absence of inhibitors to determine total C and N mineralization, as well as in the presence of both inhibitors. After equilibration for 16 h at 4 °C, glucose was added at a concentration of 4 mg g^{-1} soil. All substrate and inhibitor concentrations were previously determined during preliminary optimization experiments. The antibiotic concentrations we selected achieved up to 89% inhibition of bacteria and 86% inhibition of fungi. Amended soil 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 Inc., Columbia, MD). Extractable NH_4–N and NO_3–N concentrations were also determined on the samples to estimate fungal versus bacterial N mineralization activity.

2.5. Statistical analyses

Statistical analyses were performed using the SAS statistical Package version 9.1 (SAS Institute Inc., Cary, NC). Repeated measures analysis of variance was conducted with the PROC MIXED option on all data for each sampling time using the autoregressive covariance structure and time after fire (season), depth, and treatment (pile edge, pile center, or control) as class variables ($\alpha = 0.10$). Principal components analysis (PCA) was performed using the correlation matrix on EL-FAME data that was log transformed and relativized as mol%.

3. Results

3.1. Soil temperature, heat flux, and moisture content during the burn

Changes in soil temperature recorded during the burn are shown in Fig. 1A. There was a lag in temperature increase between the center and edge of the pile that was most likely due to the geometry of the pile, as the fuel density was lower at the edges of the pile compared to the center of the pile. During the burn, soil temperatures reached 300 °C beneath the pile center and 175 °C beneath the pile edge. Surface soil (0–5 cm) at the edge of the pile experienced temperatures above 100 °C for around 10 h, whereas surface soil at the center of the pile experienced above-100 °C temperatures for over 17 h and over 8 h at the lower depth (5–15 cm). The corresponding fluxes of heat into soil are shown in Fig. 1B, with large fluxes occurring between 4 and 14 h in the edge soil and not until 16 h for soil under the slash pile center. Compared to surface soils, soil at 5–15 cm depths experienced only small heat fluxes.

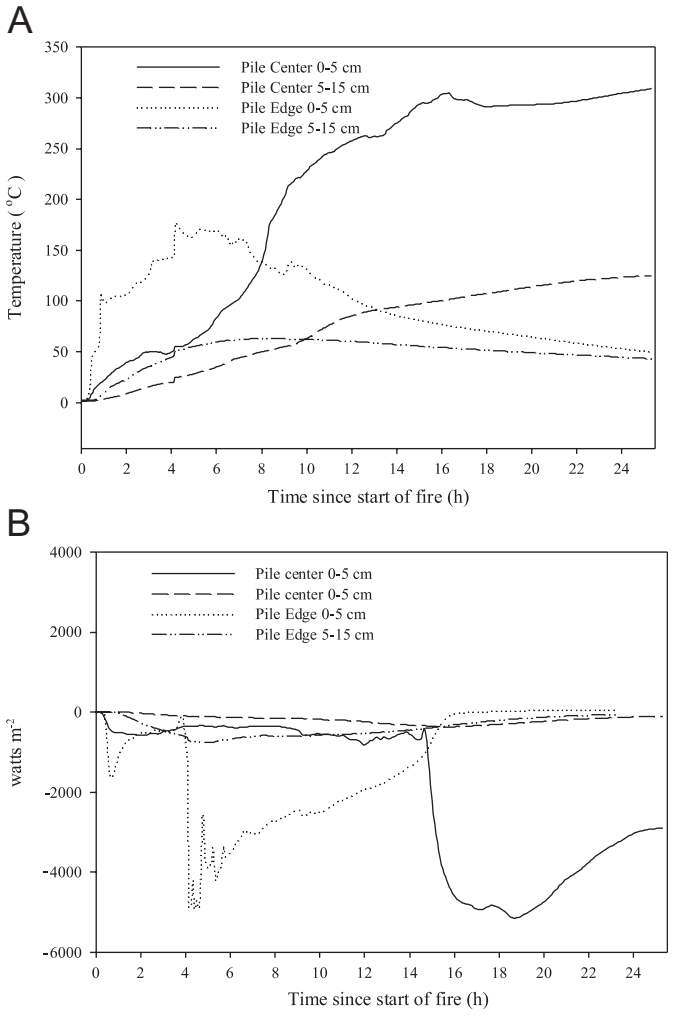

Fig. 1. Soil temperature (A) and heat flux (B) measured at 0–5 and 5–15 cm depths under the edge and the center of a slash pile as it burned on April 26, 2004. Heat flux data (B) do not include correction for the difference between thermal conductivity of soil and the heat flux plates (Philip, 1961; Massman and Frank, 2004).

Volumetric water content in the soil at the pile edge (0–5 cm depth) was ~0.22 until 18 h after the fire started, when it then dropped to 0.20. At the lower depth of the pile edge, the water content remained constant at 0.15 until 18 h after the fire started and then it slowly increased to 0.16, presumably due to water vapor movement from the surface layer and further condensation at the lower depth. At the center of the pile, the water content at 0–5 cm depth increased from ~0.16 to 0.21 during the first 20 h and then rapidly dropped to ~0.01.

3.2. Soil chemical properties

No statistically significant differences were found between the two soil depths (0–5 and 5–15 cm) for the soil chemical properties measured, so data were averaged over depth. Initial (one week) and final (15 month) values of soil pH, total C, and extractable N and P after the slash pile burn are shown in Table 1. Soil pH in control soil was typically around 5.5, and throughout the study, control soil pH was significantly lower than in fire-affected soil, where the pH ranged from 6.5 to 7.8. At the end of this study, summer of 2005, the pH of fire-affected soil was still above 6.5.

Total soil C content was always greater in the control soil compared to fire-affected soil under both pile locations, except 1 week after the burn, when there was a significantly greater amount of C in the soil at the edge of burned pile than in the control soil (Table 1). No differences were observed between soil depths nor were there any detectable changes in soil C over time. Total soil N concentrations, which averaged $1.5\,g\,kg^{-1}$ soil, was not significantly affected by slash pile burning.

Concentrations of extractable inorganic N and P were significantly greater in fire-affected soil compared to control soil for the duration of this study. There was one exception to this trend for extractable P in late October 2004 (6 month sampling time), when the soil was frozen at the time of sampling and there were no significant differences in extractable P among the soils (data not shown). For both inorganic N and P, the changes observed were dependent on location under the pile and time after fire but not on depth. Changes in soil nutrients (C, inorganic N, and extractable P) due to burning were still significant 15 months after the burn (Table 1).

3.3. Microbial biovolumes

As an index of microbial biomass, we measured total bacterial and total and active fungal biovolumes using microscopy techniques. As shown in Fig. 2A, the immediate effect of slash pile burning was to significantly reduce bacterial biovolumes from $10^8\,\mu m^3\,g^{-1}$ in control soil to $10^7\,\mu m^3\,g^{-1}$ soil at the pile edge and $10^6\,\mu m^3\,g^{-1}$ soil at the pile center (RM ANOVA $P<0.0001$). There was no

Table 1
pH, total C, and extractable N and P from nonburned (control) soil and soil under the edge and center of two burned slash piles (0–15 cm depth)

Location	pH		Total C		NH_4–N + NO_3–N		AB-DTPA ext. P	
	Initial	Final	Initial ($g\,kg^{-1}$)	Final ($g\,kg^{-1}$)	Initial ($mg\,kg^{-1}$)	Final ($mg\,kg^{-1}$)	Initial ($mg\,kg^{-1}$)	Final ($mg\,kg^{-1}$)
Nonburned	5.76b	5.53b	16.6b	30.5a	6.3c	25.9b	2.2b	2.9c
Pile edge	6.35a	6.52a	21.1a	16.5b	37.7b	66.4a	7.0b	28.1b
Pile center	6.38a	6.74a	12.2c	17.5b	61.9a	87.4a	38.8a	38.4a

Properties were determined one week (initial) and fifteen months (final) after slash pile burning in a ponderosa pine ecosystem.
Within a column, means followed by different letters are significantly different at $\alpha = 0.10$.

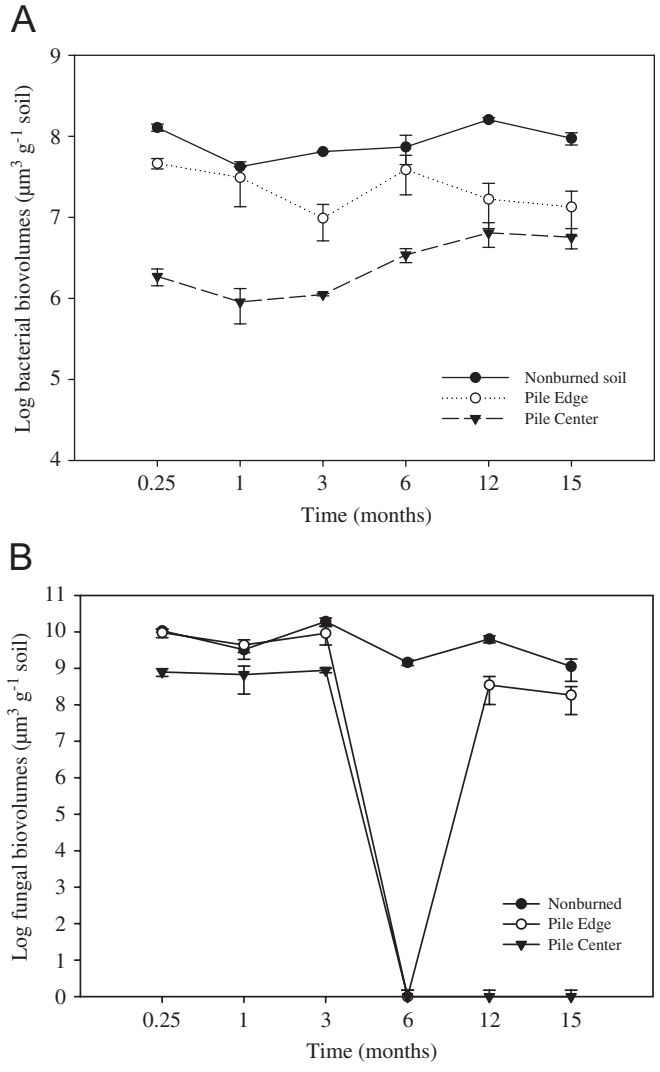

Fig. 2. Biovolumes of total bacteria (0–15 cm soil depth) (A) and fungi (0–5 cm soil depth) (B) in nonburned, control soil and soil under the edge and center of burned slash piles ($n = 2$). Standard error bars are shown.

significant effect of time or of depth, and values shown in Fig. 2A are averaged across the 0–5 and 5–15 cm depths. Fifteen months after the burn, bacterial biovolumes had not recovered to control levels at either location within the pile, and throughout the study, amounts of bacterial biovolumes at the edge of the pile were always greater than the amounts at the center of the pile.

Fungal biovolumes in control soil were generally 100-fold greater than bacterial biovolumes in control soil, but the burning of slash piles caused fungal biovolumes to fall dramatically. Burning resulted in a significant reduction of total fungal biomass at the surface (0–5 cm) from to 10^{10} to $10^{8}\,\mu m^3\,g^{-1}$ soil initially, and to nearly nondetectable values 6 months after the fire (Fig. 2B). Changes to fungal biovolumes depended on depth and time (RM ANOVA $P = 0.0014$). At the lower depth, fungal biovolumes were not significantly affected by slash pile burning.

3.4. Microbial community structure

Principal components analysis of EL-FAME data collected one month after the burn revealed that microbial community structure was significantly affected by slash pile burning at both depths sampled. Results of combined soil depths are shown in Fig. 3. One month after the slash pile burn, microbial communities in the control soil and microbial communities in the soil at the edge of the pile were separated from communities in the soil at the center of the pile along PC 1 and PC 2 (Fig. 3A). Principal components 1 and 2 accounted for 71.5% of the variability in the EL-FAME data set. Communities to the right of PC 1 (pile edge and most of the control soil communities) were enriched with the Gram-negative bacterial markers 16:1 ω7, 17:0 cy and 19:0 cy, fungal markers 18:2ω6c and 18:3ω6c, and the AM fungal marker 16:1ω5c. Communities to the left of PC 1 (pile center) were enriched with Gram-positive bacterial markers a15:0, i15:0, i16:0, a17:0, and i17:0. Principal Component 2 separated the soil at the center of the pile further from the other treatments; the EL-FAME with the greatest positive loading on PC 2 was the Gram-positive bacterial marker i17:0. Separations among communities were significant along PC 1 ($P = 0.08$) and PC 2 ($P = 0.03$).

Fifteen months after the burn, PCA showed a distinct separation of microbial communities by treatment (Fig. 3B). In contrast to initial microbial community patterns, the communities from soil under the edge of the pile now clustered together with communities from the soil under the pile center. Two principal components accounted for 56% of the variability in the data. Microbial communities on the positive side of PC 1 (fire-affected soil) were enriched with the Gram-positive bacterial markers a15:0,

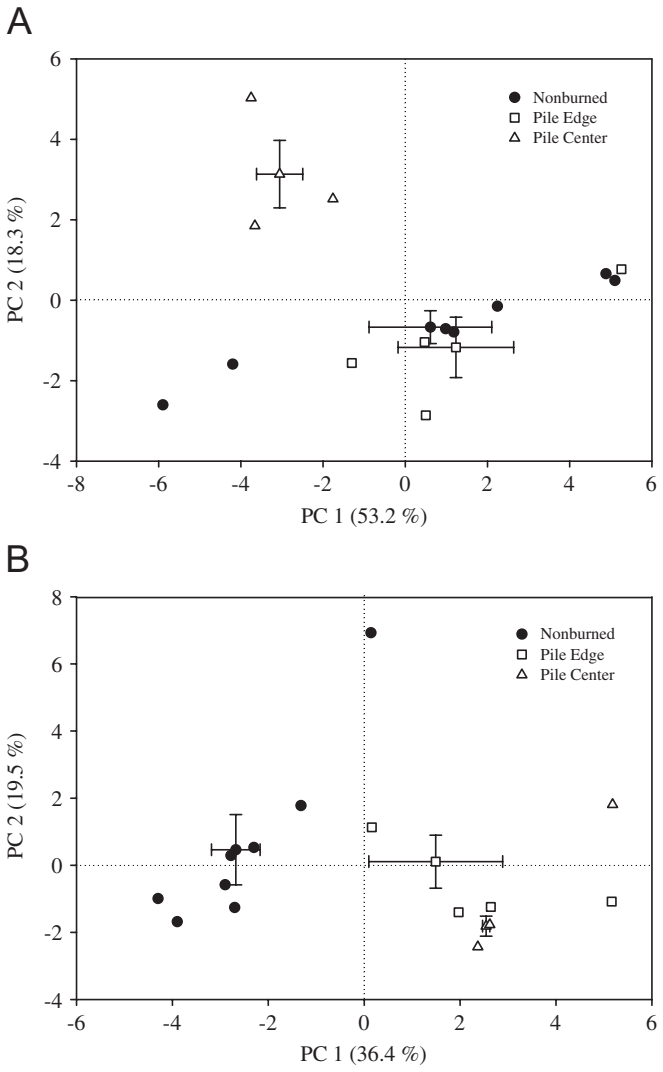

Fig. 3. Principal components analysis of microbial community structure in nonburned, control soil and soil under the edge and center of burned slash piles (0–15 cm depth) one month (A) and 15 months (B) after slash pile burning ($n = 2$). Individual data points as well as the average PC scores for each treatment are shown along with standard error bars. The percent variability explained by each PC is shown in parentheses.

i15:0, and a17:0 and Gram-negative markers bacterial 17:0 and 19:0 cy. No fungal markers were dominant in the communities on the positive side of PC 1. Microbial communities to the left of PC 1 were enriched with the fungal markers 18:2ω6c and 18:3ω6c, the AM fungal marker 16:1ω5c, and Gram-negative bacterial markers 16:1ω7c and 16:1 2OH. Microbial communities were not separated further by PC 2, but separations on PC 1 were significant ($P = 0.007$).

3.5. Potential C and N mineralization activities

Potential mineralization of carbon in the form of glucose to CO_2 was measured for both bacteria and fungi. Fungal and bacterial contributions to N mineralization potential were also measured, but patterns of N mineralization by fungi and bacteria were similar and thus the data are not shown. As shown in Fig. 4A, bacterial respiration was low in the control site during the first six months after the burn, an apparent seasonality effect that was also evident in fire-affected soil (RM AOV $P = 0.07$). Nonetheless, bacterial respiration increased in control soil in 2005 but not in fire-affected soil. These changes in bacterial respiration among treatments were significantly different (RM AOV $P = 0.0087$), and there was a time after fire effect as all values followed similar trends over time (RM AOV $P = 0.075$). However, the treatment effect was not dependent on time after the fire as evidenced by the nonsignificant time by treatment interaction (RM AOV $P = 0.89$).

Fungal respiration was not influenced by time (RM AOV $P = 0.59$) or depth (RM AOV $P = 0.81$), but it was negatively influenced by fire. As shown in Fig. 4B, fungal respiration was reduced by slash pile burning immediately after the fire; 15 months after the fire fungal respiration was

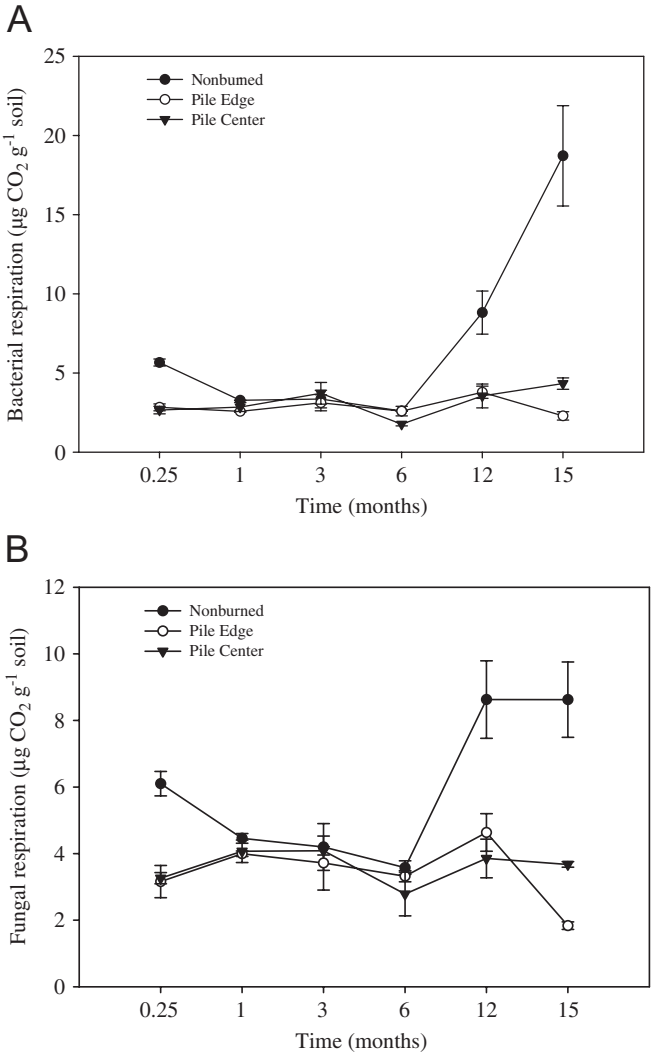

Fig. 4. Bacterial (A) and fungal (B) respiration in nonburned, control soil and soil under the edge and center of burned slash piles (0–15 cm depth) as determined by a 4-h substrate induced respiration assay. Standard error bars are shown ($n = 2$).

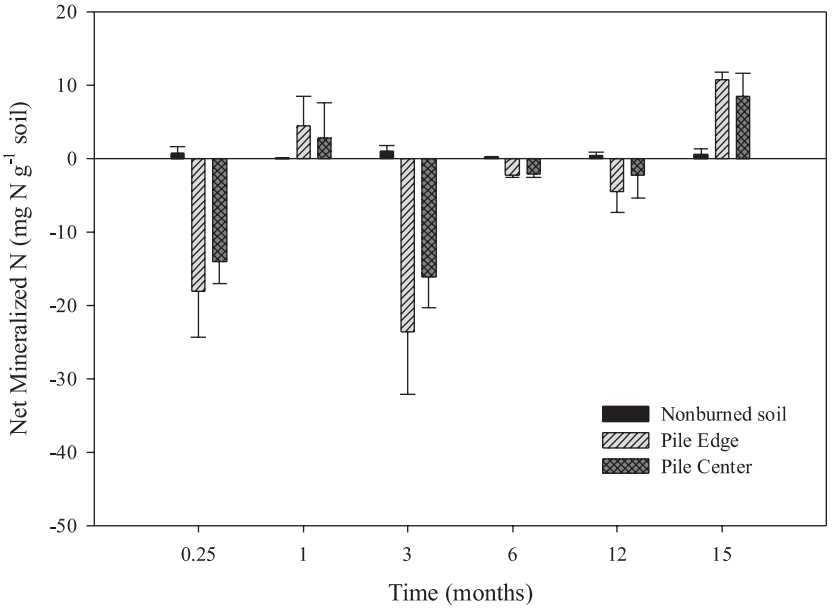

Fig. 5. Potential N mineralization in nonburned, control soil and soil under the edge and center of burned slash piles (0–15 cm depth) as determined by a 4-h incubation assay. Standard error bars are shown ($n = 2$).

still low (RM AOV $P = 0.006$). Regardless of pile location or depth, fungal respiration was lower in fire-affected soil compared to control soil for the duration of this study.

As shown in Fig. 5, potential net nitrogen mineralization (combined for bacteria and fungi) occurred in control soil at low rates. In fire-affected soils, potential N dynamics fluctuated between net mineralization and net immobilization, but trends were similar in soils from under the edge and the center of the pile for the duration of the study. Except for the 1-month sampling date, potential net N immobilization occurred in fire-affected soil at every sampling time for the first year after the burn. During summer of 2005, 15 months after the burn, greater rates of net mineralization occurred in fire-affected soil compared to control soil. Overall, potential N mineralization was affected by fire (RM ANOVA $P = 0.0004$), by time after the fire (RM ANOVA $P < 0.0001$), and by depth (RM ANOVA $P = 0.0845$).

4. Discussion

Slash pile burning is a very effective slash disposal method, but as this study has shown, it can produce soil temperatures high enough to cause plant root death (40–70 °C), soil water loss (60–100 °C), seed death (50–120 °C), bacterial and fungal death (50–160 °C), and the destructive distillation of organic matter (200–315 °C) (Hungerford et al., 1991; Neary et al., 2005). In our study, soil (0–5 cm depth) underneath the slash pile experienced temperatures as high as 175 or 300 °C, depending on the location under the pile. We attribute immediate changes in soil chemical and microbiological properties to these high temperatures, although these effects may have been confounded by the presence of the slash pile itself, which was on the soil surface for 9 months prior to burning. Given the lethal temperature is approximately 110 °C for bacteria and 100 °C for fungi in moist soil (Dunn et al., 1979), there is evidence that partial if not total sterilization occurred within the first 5 cm of soil under the slash pile. This is supported by the reduction of microbial biovolumes we measured in the 0–5 cm soils sampled, although it does not explain the reduction of bacterial but not fungal biovolumes in the 5–15 cm depths. Others have reported reduced microbial biomass in soils burned by prescribed or wild fires (Theodorou and Bowen, 1982; Fritze et al., 1993; Pietikäinen and Fritze, 1993; Vázquez et al., 1993; Acea and Carballas, 1996; Villar et al., 2004; De Marco et al., 2005; Hart et al., 2005). In the present study, fungal biovolumes under the slash pile center were especially sensitive, and by the end of the study, the ratio of fungal-to-bacterial biovolumes was greatly reduced in this fire-affected soil relative to the control. Moreover, microbial biovolumes in soil from the pile center were smaller than under the pile edge throughout the study, indicating longer term effects of fire intensity on this microbial parameter.

Death of microorganisms, plant roots, and the oxidation of organic matter in soil under the slash piles presumably caused total soil C levels to fall, except one week after the burn, when total C was greater in soil under the edge of the pile compared to the center or outside the pile. This was probably due to partially consumed plant biomass that leached to the soil after the burn (Knicker et al., 2005), but the overall reduction in total C corresponds to the lower microbial and plant root biomass of fire-affected soils. Furthermore, soil temperatures were high enough to oxidize organic matter. This would have released basic cations into soil, causing soil pH to increase, and also would have resulted in the mineralization of organic-bound

P and N. In the present study, we measured increased soil pH and extractable N and P in soil under burned slash piles, and these effects persisted through the duration of the study. Our findings agree with those from previous slash pile burn studies for soil pH (Arocena and Opio, 2003) as well as extractable N (Jönsson and Nihlgård, 2004; Thorpe and Timmer, 2005) and P (Thorpe and Timmer, 2005). In the present study, total N was unaffected likely because soil temperatures were not high enough to cause N volatilization (300–500 + °C; Hungerford et al., 1991).

Changes in soil chemical properties, specifically pH, may partially explain the decline in fungal biovolumes and EL-FAME biomarkers we measured in response to slash pile burning. We conducted correlation analyses between fungal biomarkers and environmental variables to elucidate possible environmental controls on fungal recovery, at least in the monitored site where we had continuous soil temperature and moisture data (Table 2). Initially, fungal EL-FAME concentrations were significantly and negatively correlated with soil pH (up to one month after the burn) and temperature (one week after the burn), indicating that initial fungal recovery may be linked to degree of soil heating as well as post-fire changes in soil pH. Later, fungal EL-biomarkers were positively correlated with soil N content and negatively correlated with soil moisture content. Because the nonburned control soils were drier than soils under the burned slash pile, fungal recovery did not seem to be limited by water availability. These data may be useful for formulating hypotheses as to why fungi are generally more sensitive to fire or soil heating events, as several studies have shown (Dunn et al., 1985; Vázquez et al., 1993; D'Ascoli et al., 2005; Guerrero et al., 2005; Hamman et al., 2007). While the exact mechanism(s) has yet to be tested, greater fungal sensitivity to fire and slower recovery compared to bacteria have been attributed to physiological factors, such as lower tolerance of fungal propagules (relative to bacteria) to soil heating (Bollen, 1969; Dunn et al., 1985; Guerrero et al., 2005, Pietikäinen et al., 2005), fire-induced pH increases in soil (Adedeji, 1983; Penalva and Herbert, 2002), subsequent soil moisture and temperature fluctuations under reduced plant cover (Rutigliano et al., 2002; D'Ascoli et al., 2005), as well as changes in organic substrate quantity and quality and/or the presence of fungal-inhibiting chemicals in burned soil (Widden and Parkinson, 1975; Vázquez et al., 1993).

Table 2
Pearson correlation coefficients among fungal EL-FAME biomarkers and selected environmental variables measured in soil (0–5 and 5–15 cm depths) under a burned slash pile within a ponderosa pine ecosystem ($n = 8$)

Time (months)	pH	N	Temperature	Moisture
0.25	−0.84*	0.15	−0.63**	0.33
1	−0.59**	−0.24	−0.26	−0.48
3	−0.09	−0.09	0.11	−0.28
12	0.41	0.64**	−0.07	−0.75*
15	0.29	−0.34	−0.18	−0.77*

No fungal markers where detected in any of the samples for the 6 month after the burn period.
*Significant at $\alpha = 0.05$.
**Significant at $\alpha = 0.10$.

Along with fungi, there were other microbial groups affected by slash pile burning, and these effects initially were dependent on fire intensity. One month after the burn, the control soil and the soil at the edge of the pile were enriched with EL-FAME biomarkers for Gram-negative bacteria, fungi, and AM fungi one month after the slash pile burn. Others have found a stimulatory effect of low and moderate severity fires on fungi (Certini, 2005) and AM fungi (Korb et al., 2003). In the present study, enhanced nutrient availability likely explain the initial enrichment of Gram-negative bacteria and fungi in burned pile edge soil, but since all plants were killed, the increase in the AM fungal marker may be due to outgrowth of extraradical hyphae from dying roots. In contrast, the soil at the center of the pile was depleted with fungal biomarkers and enriched with Gram-positive bacterial markers. This is expected as some Gram-positive bacteria can form endospores capable of withstanding extremely high temperatures (100 °C moist heat; Russell, 2003), and if they survived the burn, the endospores presumably germinated in response to the flush of soil nutrients, thus explaining the apparent increase in the EL-FAME markers. However, fire intensity did not seem to have longer-term effects on microbial community EL-FAME structure, because microbial communities were similar between edge and center pile soils fifteen months after the burn. Longer-term changes to microbial community structure and activity, and to fungi in particular, were probably due to long lasting effects of lack of vegetation, higher soil pH, altered C and nutrient availability, or other soil physicochemical properties which we were not able to measure.

Similarly, fire intensity did not have differential effects on potential C and N mineralization activities, because activities in pile edge and center soils were equally affected by slash pile burning. Microbial respiration was low in fire-affected soils compared to control soil in May 2004, April 2005, and July 2005. In contrast, bacterial and fungal respiration did not differ and was low in all soils (control and fire-affected) in June, late July, and early November 2004. Soils were frozen at the November sampling time, which may explain the low levels of activity, whereas the June and late July 2004 sampling dates correspond to periods of very wet soil conditions. During the months of May, June and July 2004, the MEF received 16.2 cm of rain, nearly half of its annual precipitation (34.3 cm). Despite ideal conditions during the incubation assay, the activity of aerobic heterotrophs (specifically glucose utilizers) may still have been impacted by non-ideal field conditions. These inconsistencies may also reflect methodological issues, such as incomplete inhibition of targeted organisms by the antibiotics used in the assay. While we did achieve a high level of inhibition at times, over the

course of the study, the antibiotic doses averaged 68.7% inhibition of bacteria and 66.2% for fungi. Incomplete inhibition of fungi by the SIR method may also explain why we measured some fungal respiration in the fire-affected soils, despite severe reductions in fungal biovolumes and EL-FAME biomarkers, especially under the pile center. Despite methodological problems, however, the potential C mineralization results from the SIR assay are consistent with the findings that soil bacterial and fungal biovolumes were reduced after slash pile burning.

Overall, most of our hypotheses were not supported by our data. Our first hypothesis is partially rejected because soil chemistry, bacterial biovolumes, and microbial community structure in the 0–5 and 5–15 cm soil depths were equally affected by slash pile burning, despite depth differences in soil temperature and heat flux which occurred during the burn. Fungal biovolumes, however, were reduced by slash pile burning within the first 5 cm of soil but not in the deeper depth. Our second hypothesis is accepted because there were differential effects of slash pile burning on microbial community biovolumes and initially on structure depending on the location under the pile (edge or center). Contrary to our third hypothesis, microbial activity was not enhanced under the burned pile edge, but potential C mineralization and sometimes N mineralization activities were low regardless of the location within the pile. Finally, there were no differences at the end of the study in microbial community structure and activity between burned pile edge and center soils as we hypothesized, but soil nutrient levels had not yet returned to background levels as we hypothesized.

In summary, this study has shown that slash pile burning changes soil characteristics at discrete locations within a managed forest. Although our study was conducted on a limited scale, normal forest thinning practices results in the construction and burning of multiple piles in a patchy distribution throughout the forest ecosystem. Future studies should investigate the ecosystem-level consequences of these disturbed soil patches. We also recommend, as have Korb et al. (2004), that future studies focus on identifying means to mitigate detrimental effects of slash pile burning. One example may be to excavate top soil prior to slash pile construction and burning, then replacing top soil after burning and reseeding with native plant species. A second research need is to determine the sustainability of alternative slash disposal options, such as slash mastication and dispersal of wood chips as a mulch (Massman et al., 2006) and the burning of lopped and scattered slash.

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Erratum

Erratum to "Microbial community structure and activity in a Colorado Rocky Mountain forest soil scarred by slash pile burning" [Soil Biol. Biochem. 39(5) (2007), 1111–1120]

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In Section *2.3. Microbial community structural analyses*, p. 1113, the word phospholipids should be replaced with lipids. Also, fungal biomarker 18:2ω3c should be replaced with 18:3ω6c.

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