DISSERTATION

FIRE EFFECTS ON SOIL MICROBIAL COMMUNITY STRUCTURE AND FUNCTION IN A PONDEROSA PINE ECOSYSTEM

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY AIDA E JIMENEZ ESQUILIN ENTITLED FIRE EFFECTS ON SOIL MICROBIAL COMMUNITY STRUCTURE AND FUNCTION IN A PONDEROSA PINE ECOSYSTEM BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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ABSTRACT OF DISSERTATION

FIRE EFFECTS ON SOIL MICROBIAL COMMUNITY STRUCTURE AND FUNCTION IN A PONDEROSA PINE ECOSYSTEM

Fires can cause drastic changes to the chemical and physical properties of forest soils. Depending on factors such as fire severity and intensity, these changes can be beneficial or detrimental to the soil microbial community. Because soil microbial community resurgence is crucial to the recovery of the forest ecosystem (due to the functional roles microorganisms have in soil stabilization and nutrient cycling, for example) it is important to improve our understanding of soil ecological responses to fire. Using three culture independent methods (EL-FAMEs, total microscopy counts and PCR-DGGE) to measure microbial community structure, and substrate induced respiration assays to measure microbial C and N mineralization activities, microbial response to fire was evaluated. Soil physicochemical properties were also measured. My results from microbial studies demonstrated that the rate of microbial community recovery, and thus rates of soil recovery differ depending on fire severity. Aerial hydromulching, ameliorated the effects of fire in AM fungi, but it did not hasten the recovery of soil bacteria. Also, potential microbial C and N mineralization activities had recovered in the moderate severity burn after 3 years. I also found that some negative long term-effects (21 years) of soil scarification can be ameliorated by a

pine ecosystem. However, light severity fire and high severity fire had similar detrimental effects on microbial biovolumes and C and N mineralization activities. Arbuscular mycorrhizal fungi were negatively impacted throughout the study by fire regardless of severity as measured by concentrations of the fatty acid 16:1ω5c biomarker in soil. Aerial hydromulching, a post-fire erosion control treatment, acted to maintain greater water contents in burned soil, ameliorated the effects of fire in AM fungi, but it did not hasten the recovery of soil bacteria. Also, potential microbial C and N mineralization activities had recovered in the moderate severity burn after 3 years.

I also found that some negative long term-effects (21 years) of soil scarification (e.g., reduced levels of soil C and organic matter (OM), lower biovolumes of both fungi and bacteria, and a shift in the microbial community towards one dominated by Gram-positive EL-FAME markers) can be ameliorated by a high severity fire, presumably due to the increased OM, inorganic N and extractable P associated with a fire event.

Although slash pile burning is an effective method for removal of unmarketable debris and small trees, it produces an extreme heat pulse into the soil which causes severe soil scorching. In my study on the effects of slash pile burning, I found that microbial activity had not recovered in burned soil 15 months after pile ignition. Initially, the microbial community structure was different at the edge of the pile compared to communities at the center of the pile where the fuel load and thus the heat load was larger. However, 15 months after the burn, microbial communities in burned soil were similar between the edge and center but were different from microbial communities from nonburned soil. This study showed that initial recovery dynamics were influenced by the fire intensity as shown by the differential response to location under the pile, but later the degree of recovery depended on the changes in soil properties such as nutrient content and pH of the burned soil.

There were general trends among these studies, in particular regarding the lack of fungal resiliency to fire. In all studies, fungal biovolumes and/or activity proved to be more affected by fire than bacterial biovolumes and/or activity. I tested the hypotheses that 1) the short-term negative impact on fungal populations was due to higher pH of the soil after a fire or 2) because of early competition with bacteria (which showed to be more resistant than fungi). My data suggested that elevated pH may have a negative impact on fungal biovolumes and respiration, but contrary to what was expected, in high temperature heated soil fungi were positively affected by bacterial activity. I concluded that after a high temperature heating event, the dominant interaction between the two microbial groups is not competition but commensalism or synergism.

Overall, these findings suggest that the dynamics of soil microbial recovery after a fire are not simple, and interactions exist among some of the components of the fire regime (fire severity, fire intensity, season of fire) that will affect the outcome of the soil recovery and therefore of the above ground community.

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CHAPTER 1

INTRODUCTION

Fire has played a vital role in the structure and function of natural ecosystems throughout the world. Fire is a natural process and a widespread phenomenon which occurs in almost every ecosystem. Some of the roles of fire are to 1) eliminate dead and dying plant matter, 2) recycle nutrients, 3) increase the production of native species that occur in fire dependent ecosystems, 4) influence insect and disease populations, and 5) change the structure and biological diversity of the vegetation.

In North America, fire has played a major influence on the evolution of many ecosystems, but fire suppression and over-grazing for the last 100 years has changed the natural fire regime in many of these ecosystems. The consequences of such changes to ecosystem structure and function may be detrimental in the long-term. In particular, the ponderosa pine forests of the western United States can be overstocked and many are affected by insect outbreaks and fungal diseases besides being subject to severe stand-destroying fires. These changes differentially affect above and below ground ecosystem processes. In regards to below-ground effects, fire-induced changes to soil parameters such as soil pH, soil temperature, soil structure, and soil nutrient availability have an effect on soil microbial community structure and function, and this in turn affects soil nutrient cycling and its dynamics.

However, knowledge about the effects of fire intensity and fire severity on microbial community structure and function is scarce. This dissertation is a collection of studies that address the response of the soil microbial community to fire of different severities and intensities. Fire severity is defined as the ecological effect of fire, such as the extent of burn to soil and vegetation. Fire intensity is defined as the amount of energy input to the soil, measured in watts m^{-2}. The aim of this project was to elucidate the interactions between fire severity and intensity and the soil microbiota to gain information that will aid in restoration efforts.

In Chapter 2, a review the current literature on fire as a disturbance and its effects on microorganisms is presented. The characteristics of the microbial environment after a fire and how this affects microbial biomass and activities, as well as the changes in the soil physicochemical properties due to fire, are discussed.

In Chapter 3, I present the findings of a study conducted to determine how fire severity, a component of the fire regime, affects the recovery of soil microbial community structure in a ponderosa pine forest of the central Rocky Mountains. The general objective of this study was to determine the effect of fire severity and hydromulch treatment (a commonly used emergency restoration treatment after a fire) on soil microbial communities in order to improve the current understanding of fire effects in the soil microbial communities. Furthermore, the effects of soil scarification (the shallow burial of litter into a soil) on microbial community structure and function and how these changes affect the microbial response to subsequent wildfire are discussed in Chapter 4.

In Chapter 5, I discuss the findings of an experiment designed to determine the effects of slash pile burning and fire intensity on soil microbial community structure and function as well as soil physicochemical properties such as soil temperature and moisture, pH, and nutrient content.

Chapter 6 presents the findings of two laboratory experiments designed to determine the effects of increased soil pH and bacterial competition on soil fungi. This experiment aimed to further explain the changes observed in soil fungi in studies discussed in Chapters 3, 4, and 5.

The rationale driving this project was the fundamental need to better understand the effects of fire on factors that control nutrient cycling and storage (such as soil microbial community), since an important constraint for sustainable forest productivity after a fire is nutrient availability for later succesional stages. The data obtained will improve our understanding of the effects of forest fires on soil biological and physicochemical properties of Colorado's ponderosa pine ecosystems. Furthermore, this research will improve our ability to develop new and better management practices that will sustain and maintain a broader spectrum of ecological conditions.

CHAPTER 2

LITERATURE REVIEW

Fire as a Disturbance

Fire affects over 50% of the Earth's terrestrial surface (Walker and Willig, 1999), and it is considered a primary disturbance on this planet. Carbon dating of charcoal beds suggests that as soon as there was oxygen in the atmosphere and a high enough density of terrestrial vegetation (Devonian-Carboniferous period), lightening could have ignited the biomass and produced fires. Therefore, as long as there has been terrestrial vegetation, there have been fires (Jones and Rowe, 1999; Komarek, 1973). Humans strongly control the dynamics of fire. Deliberate fire ignition by humans for hunting, cooking, landscape management, farming, forestry, and reduction of fire hazard started an anthropogenic modification of the biosphere that has interfered the natural fire cycle in many ecosystems (Pyne and Goldammer, 1997).

Fire in the Colorado Rocky Mountains: The Hayman Fire of 2002

In the Front Range of Colorado, the major forest zones include ponderosa pine savanna (approximately 5,000 and 6,500 feet above sea level), the montane forest (6,500-8,000 feet), mixed conifer forest (approximately 8,000-9000 ft) and the subalpine forest (lodgepole pine, subalpine fir, Englemann spruce and limber pine) at 9,000-11,500 feet generally. The density of ponderosa pine (*Pinus ponderosa*) generally increases with elevation through the montane forest zone. At higher elevations Douglas fir (*Pseudotsuga menziesii*) becomes increasingly important, followed by the mixed conifer area which includes ponderosa pine, Douglas-fir, lodgepole pine and limber pine. In some of these ecosystems, particularly in those at lower elevations, fire has been an important factor in ecosystem processes prior to Euro-American settlement, but intense fire suppression and over-grazing for the last 100 years has altered the natural fire cycle. Romme et al. (2003) have described three main fire regimes present in the Colorado Front Range ecosystems prior to Euro-American settlement according to the forest zones. The first fire regime encountered is a frequent low severity fire regime, characteristic of the foothills and lower montane zone. The second fire regime, a mixed severity fire regime, corresponds to the middle and upper montane zone and the mixed conifer zone. Finally, the third fire regime corresponds to the spruce-fir subalpine zone and involves infrequent high severity fires. The range of ecological processes present in the Rocky Mountain ecosystems before the impacts of Euro-American settlers is the historical range of variability and it was in this context that the severity of the Hayman fire was evaluated.

In 2002, nearly 138,000 acres were burned during the Hayman fire, Colorado's largest wildfire in recorded history. The fire burned areas of Pike-San Isabel National Forest, 30 miles southwest of Denver, Colorado. The Hayman fire was ignited on the afternoon of June 8, 2002 near county road 77 and was finally contained by June 28, 2002 (Graham, 2003). The Hayman Fire mostly burned within the upper montane ponderosa pine/Douglas-fir forest; therefore, according to the definitions given above, this area corresponded to a historical mixed severity fire regime. A large forest area (44,000 acres) was burned at high severity (stand-replacing), although areas of low and moderate fire severity were also found (Romme et al., 2003). The cause for this fire was anthropogenic. Six hundred structures were burned and the cost of suppression and recovery to date is $39,100,000 (USFS, 2002). According to Romme et al. (2003), the fire history of the area comprises an average interval of 50 years between large fires between 1300 and 1800 although small fires were more frequent. Fire was actively excluded from this area between 1880 and 2002. This may have contributed to the build up of fuels and was exacerbated by the extreme fire weather conditions present in 2002 prior to the Hayman fire event. The large size and homogeneity of patches of high severity were unique in this ecosystem due to extreme weather conditions and the forest structure of the area.

Immediate soil restoration efforts for the area burned by the Hayman fire included 1) aerial hydromulching and seeding to establish vegetation cover, increase soil moisture, and prevent erosion; 2) scarification to break up the hydrophobic layer of soil as well as to prepare the forest floor for seeding, and 3) application of polyacrylamides (PAM) to aid in soil aggregation (Robichaud et al., 2004). The ecological effects of this fire are currently under study. A comprehensive summary of the effects of this fire on forest-stand structure, soil erodibility, and watershed properties can be found in Romme et al. (2003) and Robichaud et al (2004).

Slash Pile Burning in the Ponderosa Pine Ecosystem

The effects of grazing and fire suppression since the late 1880s on some ponderosa pine forests have been profound. The forest stand structure has shifted from wide-open savanna-like structure to very high tree densities, which in turn has contributed to destructive forest fires such as the Hayman fire. The current restoration program for the ponderosa pine ecosystem in the Colorado Rocky Mountains involves thinning and reintroduction of fire (prescribed burning) 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 currently 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). However, the geometry of these piles results in a fuel load that, when burned, produces an extreme heat pulse into the soil that results in severe soil scorching. The scars left on the forest floor are large, sometimes eight to ten meters in diameter, and besides being aesthetically unpleasant they can contribute to the establishment of invasive (non-native) species in the forest floor (Dickinson and Kirkpatrick, 1987). Korb et al. (2004) found that amendments of fresh soil and seeds of native species enhanced the rate of establishment for other native understory species but these treatments are not often added.

Without restoration efforts, the soil remains charred many years after burning, and vegetation density is still low. This raises the question of how these burned slash piles are impacting the ecosystem and also whether this management practice meets with restoration objectives. Even though slash pile burning is a means of disposal preferred amongst forest managers, studies considering its short and long term effects on below and above ground processes are scarce.

Microorganisms and Their Environment after a Fire

Analysis of the microbial community structure of a nondisturbed forest soil using molecular methods reveals a high bacterial diversity and a lower but stable level of fungal diversity in the upper layers of the soil horizon both which decrease with depth (Agnelli et al., 2004; Borchers and Perry, 1990). This description is valid for most forest soils, but it is also true that the structure and function of the soil microbial community of any ecosystem may change after a disturbance. The magnitude and duration of microbial community structural changes can be a factor of the soil physical and chemical alterations that occur during and after the disturbance as well as the type of disturbance.

Microbial activities are fundamental for ecosystem function. In terms of functional diversity, the microbial community performs many important soil processes including nutrient (C, N, P, S,) cycling and soil stabilization and aggregation. Microorganisms can function as plant symbionts providing plants with nutrients; for example, interactions between mycorrhizal fungi and plants improve plant nutrient uptake. Soil microbes are also important in the formation and decomposition of organic matter in soil and their contribution to N turnover in soil is crucial for both above and below ground ecosystems.

One of the earliest reports of the effects of fire on soil microbiota was by Corbet (1934) in which slash was piled up and burned after timber harvest in a tropical forest in Malaysia and culturable bacterial numbers were recorded. Bacterial numbers increased initially but returned to background levels rapidly. The author concluded that the increased bacterial numbers were due to an increase in soil pH and nutrient concentration (Corbet, 1934). Subsequent studies have reported similar as well as differing results depending on several characteristics of the system studied, including the severity and intensity of the fire, the type of fire (prescribed or wildfire), time since fire, dominant above-ground vegetation, and the method used to characterize microbial structure or function. Since then several studies (detailed below) have examined the effects of fire on soil microbial community properties, mainly culturable populations by spread-plate counting and most probable number techniques, microbial biomass C, and respiration activity.

Alghren and Alghren (1965) found that after a prescribed burn in a jack pine (*Pinus banksiana*) ecosystem, numbers of culturable microorganisms were lower in burned soil compared to nonburned soil even three years after the fire. They also found that filamentous microorganisms (streptomycetes and fungi) were more sensitive to the soil heating than bacteria. In 1982, Theodorou and Bowen reported that the greatest changes to microbial populations after a brushfire (dry-sclerophyll) in South-Australia occurred in the top 2 cm of soil. They concluded that one month after the fire, microbial counts were lower in the burned soils, but

20 months later the counts were similar in burned and unburned soil. Dunn et al. (1985) and Baath et al. (2003) reported that fungi were more sensitive to heating than bacteria in soils that were affected by both prescribed fire (in a chaparral ecosystem) and wildfire, respectively. Pietikainen and Fritze (1993) reported that prescribed fire in a coniferous forest in Finland had a more severe effect on microbial community structure than wildfire and that bacteria, particularly Gram-positive spore-forming bacteria, were stimulated by fire. According to their results, microbial numbers had not recovered to background levels three years after the fire. One particular long-term study, which involved a fire chronosequence of prescribed fires in a coniferous forest in Finland, showed that decreased microbial respiration did not recover until 12 years after the fire (Fritze et al., 1993). Using culture-based methods, Vazquez et al. (1993) determined that one month after a forest fire in a pine forest (*Pinus pinaster*) in Spain, total bacteria, acidophilic bacteria, and spore-forming bacteria were stimulated by fire whereas cyanobacteria, fungi, and algae were negatively affected. One year later, bacterial numbers decreased in the burned soil whereas fungal, algal and cyanobacterial numbers increased. In a study of microbial functional groups, Acea and Carballas (1996) found that amylolytic microorganisms predominated over cellulolytic microorganisms one month after a wildfire in a pine (*Pinus pinaster*) forest in Spain, presumably due to changes in the C quality of the burned soil.

Other studies have focused on the changes in microbial biomass C as a proxy for the microbial response to fire. Generally, these studies report a reduced microbial biomass in burned soils compared to nonburned soils and that biomass eventually recovers but not to pre-burn levels (Choromanska and DeLuca, 2002; Diaz-Ravina et al., 1996; Diaz-Ravina et al., 1992; Fenn et al., 1993; Hernandez et al., 1997; Mabuhay et al., 2003; Stendell et al., 1999). Using a different approach, Diaz-Ravina et al. (1996) measured the incorporation of leucine and thymidine residues as an indicator of bacterial activity to determine the effects of soil heating (200 °C for 1 hr). Two weeks after the heating event amino acid incorporation rates decreased 97-98 % in burned soils compared to unheated soil. Incorporation rates increased within two weeks after the heating event, although the levels in the unheated soil where still higher than the heated soil at the end of the experiment. In another study in which a chronosequence was used to determine the relationships between fire and fungi in an Alaskan boreal forest, Treseder et al. (2004) found that fungi which mineralized organic matter (ectomycorrhizal fungi) recovered faster than fungi that did not (arbuscular mycorrhizal fungi).

All of the studies mentioned above have addressed the microbial population response to fire, but few studies have linked the short and long term effects of fire on microbial populations to ecosystem functions or changes in above-ground community composition. For example, a study by Sawamoto et al. (2000) aimed to measure soil respiration in five forest soil ecosystems with different histories of fires in the Siberian Taiga. They showed that soil respiration decreased in severely burned soil compared to nonburned soil and that reduced tree (root) respiration was the main reason for the overall decrease soil respiration after a fire. Korb et al. (2004) determined that slash pile burning in a ponderosa pine forest in Arizona nearly eliminated arbuscular mycorrhizal (AM) fungal propagules, and 15 months after the burn AM fungi were still negatively affected. Also, one year after the burn, they found higher amounts of viable seeds of exotic plants in the perimeters of the slash pile compared to nonburned soil, consistent with an increase in nutrient content after a burn. This study suggested that after a fire, above ground dynamics reflected the changes occurring below ground with particular functional groups of microorganisms.

Despite these studies, knowledge of how changes to microbial communities affect ecosystem function is needed. The ecological inferences that can be drawn from these studies are also constrained by the methods used by the authors. Culturable bacteria and fungi account for less than 1 % of the soil microbial numbers, (Amann et al., 1995) and the fact that an organism may grow in laboratory medium does not imply that the particular microorganism is active in soil. Also, although microbial biomass C is useful as a general measure of microbial response to a disturbance, it does not provide any information on microbial community structure and how it is affected. Another constraint of some of the studies mentioned above is that in many cases the actual fire severity is not stated or the term fire intensity is used instead, and comparisons between studies are therefore difficult.

The main generalization in all of the studies summarized above is that fire has differential effects on microbial populations in soil depending on fire severity, but regardless of the fire severity, fire can affect microorganisms in soil directly by heat sterilization and indirectly by changing the biochemical and physical properties of soil.

Sterilizing Effect of Heat

Alghren and Alghren (1960) stated that the initial decrease in numbers of microorganisms commonly observed after burning is caused by the effect of heat liberated during fuel combustion. Even though most of the energy released by combustion is lost to the atmosphere, burning causes a heat input into the soil, and this heat can immediately kill or injure soil microbes. However, the temperature between the flame zone and the mineral soil is usually very different. This difference between temperatures is explained by the high insulation capacity of the soil as the organic matter conducts heat very poorly (Hillel, 1998). Fuel density and quality also influence the amount of heat that will flow into the soil during combustion (Whelan, 1995).

As mentioned earlier, bacteria are generally more tolerant to heat than fungi. The lethal temperature for bacteria has been described to be about 210 °C in dry soil and 110 °C in wet soil, while the corresponding limits for fungi are 155 °C and 100 °C (Dunn et al., 1979). Spores and other resting forms of microbes can tolerate substantially higher temperatures than cells that are undergoing active growth and metabolism. Also, microbes are more resistant to dry heat than moist heat because of the pasteurization effect of moist heat. Chroromaska and DeLuca (2001) reported that more organisms were killed in heated soils that were wetter (-0.03 MPa) compared to relatively drier soils at -1.0 and -1.5 MPa because water transmits heat faster than air. Thus, the immediate effect of a fire may be partial or complete soil sterilization depending on heat pulse or soil moisture content.

Changes in Soil Physicochemical Properties Due to Fire

Fire can modify several physical and chemical properties of a soil. Tables 2.1 and 2.2 summarize the general effects of fire on some relevant soil physical and chemical parameters and describe important consequences of such changes. However, these are general changes and the degree of severity and the duration of such changes will be highly dependant on all of the components of the fire regime such as fire intensity, fire severity, extent of fire, and season of fire (Romme et al., 2003). Also, the extent of these changes may depend on the type of fire (e.g., whether it is prescribed or a wildfire) and the general disturbance regime of the site (e.g., whether it is also grazed or harvested for timber).

Unavoidably, any fire-induced changes to environmental conditions such as pH, temperature, soil structure, and nutrient availability can affect soil microbial community structure and function, and this in turn can affect soil nutrient cycling and its dynamics. Changes to the physicochemical properties of soil due to fire may account for the long term effects of fire on below and above ground biota.

Table 2.1. Some soil physical properties that can be altered by fire and some relevant consequences.

Physical characteristics	Change(s) observed	Reference
Soil hydrophobicity	• A 40% reduction on hydraulic conductivity was reported after a forest fire. • Hydrophobicity is a function of heating and soil moisture and is transient and reported to last less than a year after the fire. *Consequence:* Increased erodibility	Robichaud, 2000. Huffman et al., 2001 and Hungerford et al., 1990.
Soil structure	• Stability of aggregates can change due to partial oxidation of cementing substances. This is more common in moderate to high severity fires. *Consequence*: decreased soil porosity	Giovannini and Lucchesi, 1997.
Soil Color	• Depending on the intensity of the fire, soil color will be darker due to charring and sometimes red due to iron oxide formation. *Consequence*: reduced albedo	Certini, 2005.
Bulk density	• May increase due to the collapse of aggregates because of the oxidation of cementing substances. *Consequence*: decreased porosity, reduced water infiltration	Certini, 2005.
Texture	• Fire may not have a direct effect on textural changes unless temperatures above 800 °C are achieved but it might have an indirect effect due to erosion.	Hungerford et al, 1990.
Temperature regime	• Darkening of soil will cause a reduced albedo effect; therefore, the soil temperature regime may change temporarily. *Consequence*: microbial community activity may change.	Hungerford et al., 1990 and Certini, 2005.

Table 2.2. Some soil chemical properties that can be altered by fire and some relevant consequences.

Chemical characteristics	Change(s) observed	Reference
pH	• Increases due to release of cations from oxidized organic matter. *Consequences*: may alter the increase the solubility of some exchangeable nutrients; may alter fungal population dynamics	Whelan, 2002; Certini, 2005.
Organic Matter	• As a consequence of organic matter oxidation, there is a partial or complete loss of organic matter depending on the temperature reached. *Consequence*: reduced exchangeable capacity	Giovannini and Lucchesi, 1997; Certini, 2005
	• Organic C changes towards the more recalcitrant form with an enrichment in aromatic components; organic C and N increased in A layer of soil. *Consequences*: reduced decomposition rates; altered soil organic C (SOC) dynamics.	Knicker et al., 2005.
	• Redistribution of organic matter in the soil profile from the forest floor has been observed for moderate severity fires. *Consequence*: altered SOC dynamics.	Wells et al.,1979.
Nutrient availability	• Oxidation of organic matter results in an increase in basic cations (Ca^{2+}, Mg^{2+}, and K^{+}) *Consequence*: increased pH and soil fertility.	Whelan, 2002; Hungerford, 1990.
	• Initially, there is an increase in inorganic N (NH_4^+) and P (PO_4^{2-}) which is a result of the partial oxidation of organic matter. These changes last only a few years after the fire. *Consequences*: increased soil fertility	Covington and Sackett, 1992; Whelan, 2002.
	• Fire increases total N content *Consequence*: Change in N mineralization dynamics	Prieto-Fernandez et al., 1993.

Summary

Most studies agree that initially, fire will reduce the microbial biomass by heat-sterilization of the soil but that microbial numbers will increase eventually due to a flush of nutrients from the oxidation of organic matter although in most cases not to pre-fire levels. However, it is also evident that this recovery will be highly dependant on the fire severity. Generally, fungi and other filamentous microorganisms will be more sensitive to fire than bacteria, and bacteria will be enriched in fire-affected soils relative to fungi. Concerning the changes in the physicochemical properties of soil, the pH and nutrient availability of the soil will increase after a fire, but this change will be transitory.

An important constraint for sustainable forest productivity after any disturbance is nutrient availability for later succesional stages, so it is important to understand the long term effects of fire on soil and on factors that control nutrient cycling and storage, including soil microbial community structure and function. Here we will address the short term effects of the Hayman fire as well as the effects of slash pile burning on the soil microbial community structure and function, which we draw on as indicators of forest soil ecosystem recovery.

References

Acea, M.J., and T. Carballas. 1996. Changes in physiological groups of microorganisms in soil following wildfire. FEMS Microbiology Ecology 20:33-39.

Agnelli, A., J. Ascher, G. Corti, M.T. Ceccherini, P. Nannipieri, and G. Pietramellara. 2004. Distribution of microbial communities in a forest soil profile investigated by microbial biomass, soil respiration and DGGE of total and extracellular DNA. Soil Biology & Biochemistry 36:859-868.

Ahlgren, I.F., and C.E. Ahlgren. 1960. Ecological effects of forest fires. Botanical Review 26:483-533.

Ahlgren, I.F., and C.E. Ahlgren. 1965. Effects of prescribed burning on soil-microorganisms in a Minnesota Jack pine forest. Ecology 46:304-310.

Amann, R.I., W. Ludwig, and K.H. Schleifer. 1995. Phylogenetic identification and in-situ detection of individual microbial-cells without cultivation. Microbiological Reviews 59:143-169.

Baath, E., and T.H. Anderson. 2003. Comparison of soil fungal/bacterial ratios in a pH gradient using physiological and PLFA-based techniques. Soil Biology & Biochemistry 35:955-963.

Borchers, J.G., and D.A. Perry, (eds.) 1990. Effects of prescribed fire on soil microorganisms., pp. 1-143-162. Oregon State University Press, Corvallis, Or.

Certini, G. 2005. Effects of fire on properties of forest soils: a review. Oecologia 143:1-10.

Choromanska, U., and T.H. DeLuca. 2001. Prescribed fire alters the impact of wildfire on soil biochemical properties in a ponderosa pine forest. Soil Science Society of America Journal 65:232-238.

Choromanska, U., and T.H. DeLuca. 2002. Microbial activity and nitrogen mineralization in forest mineral soils following heating: evaluation of post-fire effects. Soil Biology & Biochemistry 34:263-271.

Corbet, A.S. 1934. Studies on tropical soil microbiology II: The bacterial numbers in the soil of the Malay peninsula. Soil Science 38:407-416.

Covington, W. 1981. Changes in forest floor organic matter and nutrient content following clearcutting in northern hardwoods. Ecology 5:376-380.

Dahms, C.W., and B.W. Geils. 1997. An assessment of forest ecosystem health in the Southwest. General Technical Report. , *In* UFS-RMF and RESS Region., (ed.), Vol. RM-GTR-295.

Diaz-Ravina, M., A. Prieto, and E. Baath. 1996. Bacterial activity in a forest soil after soil heating and organic amendments measured by the thymidine and leucine incorporation techniques. Soil Biology & Biochemistry 28:419-426.

Diaz-Ravina, M., A. Prieto, M.J. Acea, and T. Carballas. 1992. Fumigation extraction method to estimate microbial biomass in heated soils. Soil Biology & Biochemistry 24:259-264.

Dickinson, K.J.M., and J.B. Kirkpatrick. 1987. The short-term effects of clearfelling and slash-burning on the richness, diversity and relative abundance of higher-plant species in two types of eucalypt forest on dolerite in Tasmania. Australian Journal of Botany 35:601-616.

Dunn, P.H., L.F. Debano, and G.E. Eberlein. 1979. Effects of burning on Chaparral soils 2. Soil microbes and nitrogen mineralization. Soil Science Society of America Journal 43:509-514.

Dunn, P.H., S.C. Barro, and M. Poth. 1985. Soil-moisture affects survival of microorganisms in heated chaparral soil. Soil Biology & Biochemistry 17:143-148.

Fenn, M.E., M.A. Poth, P.H. Dunn, and S.C. Barro. 1993. Microbial N and biomass, respiration and N-mineralization in soils beneath two chaparral species along a fire-induced age gradient. Soil Biology & Biochemistry 25:457-466.

Fritze, H., T. Pennanen, and J. Pietikainen. 1993. Recovery of soil microbial biomass and activity from prescribed burning. Canadian Journal of Forest Research 23:1286-1290.

Giovannini, G., S. Lucchesi, and M. Giachetti. 1990. Effects of heating on some chemical-parameters related to soil fertility and plant-growth. Soil Science 149:344-350.

Graham, R.T. 2003. Hayman fire case study: summary. Gen. Tech. Rep., *In* F. S. Department of Agriculture, Rocky Mountain Research Station, (ed.), Vol. RMRS-GTR-115.

Hardy, C.C., S.G. Conard, J.C. Regelbrugge, and D.R. Teesdale. 1996. Smoke emissions from prescribed burning of southern California Chaparral, pp. 1-8 Usda Forest Service Pacific Northwest Research Station Research Paper.

Hernandez, T., C. Garcia, and I. Reinhardt. 1997. Short-term effect of wildfire on the chemical, biochemical and microbiological properties of Mediterranean pine forest soils. Biology and Fertility of Soils 25:109-116.

Hillel, D. 1998. Environmental soil physics Academic Press, San Diego, CA.

Huffman, E.L., L.H. MacDonald, and J.D. Stednick. 2001. Strength and persistence of fire-induced soil hydrophobicity under ponderosa and lodgepole pine, Colorado Front Range. Hydrological Processes 15:2877-2892.

Hungerford, R.D., Harrington, M.G., Frandsen, W.H., Ryan, K.C.,and Niehoff, G.J. 1991. Influence of fire on factors that affect site productivity. USDA Forest Service General Technical Report No. INT-280.

Jones, T. P. & Rowe, N. P. 1999. The earliest known occurrences of charcoal in the fossil record. In: Ninth Annual V. M. Goldschmidt Conference, August 22-27, 1999, Harvard University, Cambridge, MA, USA. LPI Contribution No. 971, Lunar and Planetary Institute, Houston, Texas, USA. pp. 143-144.

Knicker, H., F.J. Gonzalez-Vila, O. Polvillo, J.A. Gonzalez, and G. Almendros. 2005. Fire-induced transformation of C- and N-forms in different organic soil fractions from a Dystric Cambisol under a Mediterranean pine forest (*Pinus pinaster*). Soil Biology & Biochemistry 37:701-718.

Komarek, E.V. 1973. Ancient fires: A quest for ecological understanding. Proceedings 13th Tall Timbers Fire Ecology Conference, Tallahasse, FL. 12: 219-241.

Korb, J.E., N.C. Johnson, and W.W. Covington. 2004. Slash pile burning effects on soil biotic and chemical properties and plant establishment: Recommendations for amelioration. Restoration Ecology 12:52-62.

Mabuhay, J.A., N. Nakagoshi, and T. Horikoshi. 2003. Microbial biomass and abundance after forest fire in pine forests in Japan. Ecological Research 18:431-441.

Pietikainen, J., and H. Fritze. 1993. Microbial biomass and activity in the humus layer following burning - short-term effects of two different fires. Canadian Journal of Forest Research 23:1275-1285.

Prieto-Fernandez, A., M.C. Villar, M. Carballas, and T. Carballas. 1993. Short term effects of a wildfire on the nitrogen status and its mineralization kinetics in an Atlantic forest soil. Soil Biology & Biochemistry 25:1657-1664.

Pyne, S.J., and J.G. Goldammer. 1997. The culture of fire: An introduction to anthropogenic fire history. . In: Clark, J. S., Cachier, H., Goldammer, J. G. & Stocks, B.(eds). Sediment Records of Biomass Burning and Global Change. NATO ASI Series Springer-Verlag, Berlin, Heidelberg. 51:71-114.

Robichaud, P., L. MacDonald, J. Freeouf, D. Neary, D. Martin, and L. Ashmun. 2004. Postfire rehabilitation of the Hayman fire.USDA Forest Service Gen. Tech. Rep. RMRS-GTR-114. 2003.

Robichaud, P.R., and R.D. Hungerford. 2000. Water repellency by laboratory burning of four northern Rocky Mountain forest soils. Journal of Hydrology. 231:207-219.

Romme, W., T.T. Veblen, M.R. Kaufmann, R. Sherriff, and C.M. Regan. 2003. Ecological effects of the Hayman fire. USDA Forest Service Gen. Tech. Rep. RMRS-GTR-114.

Sawamoto, T., R. Hatano, T. Yajima, K. Takahashi, and A.P. Isaev. 2000. Soil respiration in Siberian Taiga ecosystems with different histories of forest fire. Soil Science and Plant Nutrition 46:31-42.

Stendell, E.R., T.R. Horton, and T.D. Bruns. 1999. Early effects of prescribed fire on the structure of the ectomycorrhizal fungus community in a Sierra Nevada Ponderosa pine forest. Mycological Research 103:1353-1359.

Theodorou, C., and G.D. Bowen. 1982. Effects of a bushfire on the microbiology of a South-Australian low open (Dry Sclerophyll) forest soil. Australian Forest Research 12:317-327.

Treseder, K.K., M.C. Mack, and A. Cross. 2004. Relationships among fires, fungi, and soil dynamics in Alaskan boreal forests. Ecological Applications 14:1826-1838.

United States Forest Service. 2002. Hayman fire assistance website. http://www.fs.fed.us/r2/psicc/hayres/. Accessed December 13, 2004.

Vazquez, F.J., M.J. Acea, and T. Carballas. 1993. Soil microbial-populations after wildfire. FEMS Microbiology Ecology 13:93-103.

Walker, L.R., and R. Willig. 1999. Ecosystems of disturbed ground, p. xii, 868, *In* L. Walker, ed. Ecosystems of the world ; 16., 1st ed. Elsevier, Amsterdam ; New York.

Wells, C.G., C.F. Campbell, L.F. Debano, R.L. Lewis, E.C. Frederiksen, R.C. Franklin, and P.H. Dunn. 1979. Effects offire on soil: A state-of-knowledge review. USDA For. Serv. General Tech. Rep. , *In* D. Washington, (ed.), Vol. 7. USFS.

Whelan, R.J. 1995. The ecology of fire. Cambridge University Press, Cambridge ; New York.

CHAPTER 3

IMPACT OF FIRE SEVERITY AND AERIAL HYDROMULCHING ON THE RECOVERY OF SOIL MICROBIAL COMMUNITY STRUCTURE IN A PONDEROSA PINE FOREST

Abstract

Because of their interactions with plant roots and their roles in nutrient cycling and soil stabilization, the recovery of soil microbial community structure and function after a wildfire may be important to above ground community recovery in forest ecosystems. Using two culture-independent methods (EL-FAMEs and PCR-DGGE), we were able to monitor over the course of three growing seasons the recovery of the soil microbial community in response to the Hayman fire of 2002, which was the largest fire in Colorado's recorded history. Soil physicochemical properties were also monitored. The main objective of this study was to determine the effects of fire severity and post-fire aerial hydromulching on the recovery of microbial community composition and some soil chemical parameters. Burned soils were drier and higher in pH than nonburned soils, and the moderate severity burn soil often contained elevated concentrations of plant nutrients compared to nonburned soils. Changes in microbial community structure in fire-affected soil varied with fire severity and with season. Soil bacterial and fungal biovolumes were more tolerant of a

moderate severity fire than a light or high severity, which may correspond to the fact that frequent low-to-moderate severity fires are common in this ecosystem. Arbuscular mycorrhizal fungi were found to be negatively impacted throughout the study by fire as measured by concentrations of the fatty acid 16:1ω5c biomarker in soil. Although aerial hydromulching acted to maintain greater water contents in burned soil, it did not accelerate the recovery of soil bacteria. However, concentrations of fungal and AM fungal EL-FAME biomarkers were greater in burned soil that had been hydromulched compared to nontreated burned soil, presumably due to increased soil moisture content and the presence of triticale (*Triticale hexaploide*) which germinated from seeds contained in the hydromulch. Overall, soil fungal and bacterial populations appeared to have recovered from fire within 2 years of the disturbance, based on resilience calculations, although not to nonburned soil levels. With regards to microbial function, potential microbial C and N mineralization activities had recovered in the moderate severity burn after 3 years, but not in soil burned at light or high severity. Fire severity should be taken into account when designing soil restoration programs in fire-affected ecosystems because rates of microbial community recovery, and thus rates of soil recovery, will differ depending on fire severity.

Introduction

Fires can cause drastic changes to the chemical and physical properties of forest soils (Whelan, 1995). Depending on fire severity, these changes can be beneficial or detrimental to the soil microbial community (Hungerford et al., 1990;

Romme et al., 2003). Soil microbial community resurgence is crucial to the recovery of the forest ecosystem as a whole due to the functional roles microorganisms play in soil stabilization and plant nutrient cycling. Although the effect of wild and prescribed fires in soil microbial communities and microbial activities have been studied before, little is known about the composition of the microbial community immediately after the fire and how it fluctuates for the first few years. In addition, the methods used so far to assess soil microbial community structure after fire events have some limitations. For example, Widden and Parkinson (1975) studied the effects of fire on soil fungi with the spread plate method to culture fungi in selective media enriched with burnt soil. They found that the growth of some pathogenic fungi such as *Candida destructans* was not inhibited by the compounds present in burnt litter extract, but the growth of nonpathogenic fungi such as *Trichoderma* and *Penicillum* were inhibited. Vazquez et al. (1993) found that heterotrophic bacteria were stimulated by wildfire in a pine (*Pinus pinaster*) forest while cyanobacteria, algae, and fungi were negatively affected. In 1996, Acea and Carballas re-examined the changes to soil microbes involved in C and N cycles after a wildfire in the same forest type using MPN technique and enriched selective media. They found that cellulolytic microorganisms decreased in burned soil and that changes were more drastic one month after the fire. Also, ammonifiers were favored by fire and the changes lasted even a year after the fire. In another study by Theodorou and Bowen (1982), culturing was done on soils of different depths using selective media to quantify the amounts of total bacteria, Gram-negative bacteria, *Bacillus* spp., *Pseudomonas* spp., actinomycetes, and fungi in soil affected by different severity burns in a dry sclerophyll forest in southern Australia. They found a reduction in all microbial population numbers, although most changes due to fire occurred in the top 2 cm of soil.

Although the findings of these studies parallel and generally agree, the methods used in the above mentioned studies only allowed for the assessment of the culturable species in the soil which accounts for less than 1% of the total soil microbial community. Other studies done in coniferous forests have only assessed C and N mineralization and immobilization rates in soil, providing only information about how microbial community function but not structure changed with fire (Fritze et al., 1993; Prieto-Fernandez et al., 2004). Studies that assess the changes in microbial community structure using culture-independent methods are few. For example, Fritze et al. (1993) used ergoesterol concentrations in soil to assess changes in fungal biomass due to fire in a coniferous forest and found that ergosterol levels initially decreased but then recovered with time; however, only the changes in the fungal population component of the microbial community was evaluated using this biomarker.

Another important question that needs to be addressed is how different fire severities affect microorganisms and their recovery rates after a fire. Fire severity is defined by the ecological ramifications of the fire (e.g., tree mortality, percent litter consumption, and depth of soil charring) and is related to intensity of the fire as determined by fuel loadings and fuel type, among other things (Hungerford et al., 1990; Robichaud et al., 2004; Romme et al., 2003; Whelan, 1995). Because fire severity differentially affects plants and soils, we expect that different fire severities will have different effects on the soil biota and their resistance and resilience to fire. Understanding the effects of severity is relevant because in the montane and mixed conifer zone of the Colorado Rocky Mountains (~7000-8500 ft), mixed severity fires are common and within the historical range of variability (Romme et al., 2003). A mixed severity fire regime is defined as a combination of frequent low severity fires and infrequent high severity fires with fire intervals from 10-100 years and that burn at variable severity patches (Romme et al. 2003).

The objective of this study was to describe the microbial community structure and its short term (3 year) recovery after a wildfire of mixed severity using culture-independent methods, specifically ester-linked fatty acid methyl ester (EL-FAME) analysis of soil lipids and denaturing gradient gel electrophoresis (DGGE) of bacterial DNA extracted and amplified from soil. These methods do not rely on culturing of microorganisms and target a broader number of populations in soil, offering more detailed information than in the studies previously discussed. A second objective was to determine if soil recovery could be accelerated by a Burned Area Emergency Rehabilitation (BAER) practice, specifically aerial hydromulching. With aerial hydromulching, a hydromulch mixture of water, mulch, fertilizer, seed, and tackifier is applied to burned areas via helicopter or plane to reduce erosion and speed revegetation. Whether this practice will enhance the recovery of soil chemical and biological properties compared to non-treated burned soil is unknown. By examining recovery under mixed fire severities and with or without a rehabilitation treatment, we aim to improve the current understanding of microbial ecology in fire-affected soil.

Methods

Site description

The Hayman fire, the largest Colorado fire in recorded history, burned approximately 138,000 acres of forest land including some parts of Manitou Experimental Forest (MEF) where the first study site is located. The Hayman fire was ignited on the afternoon of June 8 near county road 77 and was finally contained by June 28, 2002. MEF is located in the montane zone (6,500 to 8,000 feet) and the overstory is composed of ponderosa pine (*Pinus ponderosa*) and Douglas fir (*Pseudotsuga menziesii*). The understory is composed of grasses, forbs, and shrubs (including some non-native invasives). The average annual precipitation at MEF is 40 cm. 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). The soils specifically within our MEF study area were classified as loams and sandy loams according to the methods of Gee and Bauder (1986).

Within MEF we located three areas that were affected by the Hayman fire with different degrees of severity: light severity burn, moderate severity burn, and high severity burn. The different severity areas were defined according to the BAER and the US Forest Service classifications (Romme et al., 2003). The light severity fire burned the aboveground litter but the mineral soil was not affected by the fire. Some trees were burned but the fire was not stand-replacing. In the moderate severity burn, the litter and some small trees were consumed by the fire. Between 0 and 2.5 cm of the soil was scorched by the fire. The high severity burn was lethal and stand-replacing and it burned the top 5 cm of the soil. For comparative purposes we also sampled an area of the MEF that was not affected by the fire. The extent of the area sampled for each fire severity was approximately 90 meters in length.

To determine the effects of hydromulch treatment in a soil affected by high severity burn, we chose a second study site in which this treatment was implemented shortly after the fire. The second study site was located approximately 25 km north of MEF on a part of the forest that was affected by high severity burn (as described above). Although the vegetation composition and the soil description were similar to MEF, this site had steeper slopes compared to the experimental forest site. At this location, aerial hydromulch was used as an emergency rehabilitation response to prevent soil erosion after the fire. The hydromulch, composed of a cellulose-based mulch and triticale seeds, was applied over 600 ha at an application rate of 2.4 Mg ha^{-1}. Within the second study site, we located a nonburned soil area, an area of soil affected by high severity burn without the hydromulch treatment, and an area of soil affected by high severity burn with aerial hydromulch treatment. All areas sampled for this study were approximately 100 meters in length.

Soil sampling and experimental design

At the first study site, samples were obtained from 3 locations within the light, moderate, and high severity burned areas as well as from nonburned soil. Soil samples were collected from the top 10 cm of the A horizon aseptically using a hand trowel that was rinsed in ethanol between plots. Three to five samples were collected per sampling location and mixed together in a Ziploc freezer bag to form a composite sample. Soils were stored on ice in a cooler, and transported to back to the laboratory for analyses. Sampling occurred over the course of 3 growing seasons at the following time points: 20 days, 10, 13, 16, 22, 26, 28 and 36 months post-fire.

At the hydromulch study site, samples were collected with a trowel as described above from the nonburned soil, the burned soil with hydromulch treatment, and the burned soil without the hydromulch treatment. Four composite samples were collected from each of the three treatments: two composite samples from a north-facing slope and two from a south-facing slope. Monitoring occurred over the course of 2 growing seasons, with sampling events at 3, 11, 14, 16, 22, and 25 months after the Hayman fire. The soil samples were processed as described above and the same analyses were performed.

Soil chemical analyses

Air-dried soil samples were analyzed for pH, total C and N, NH_4-N, NO_3-N, and ammonium bicarbonate-diethylenetriaminepentaacetic acid (AB-DTPA)-extractable elements. 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 metals, followed by determination of constituent concentrations on an inductively coupled plasma-atomic emission spectrophotometer (Thermo Jarrell Ash Corp., Franklin, MA). For the first sampling date at MEF, there was not enough soil collected to conduct soil chemical analyses on each replicate sample, so soils from the three sampling locations within each burn treatment were combined (n=1).

Microbial community structure analysis by EL-FAMEs

Microbial community structure was assessed by analysis of ester-linked fatty acid methyl esters (EL-FAMEs), beginning with extraction of phospholipids from 4 grams 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). After addition of 20 μg nonadecanoic acid (19:0) as an internal standard, samples were analyzed by gas chromatography (GC) analysis with an Agilent 6890 gas chromatograph (Agilent Technologies, Inc., Palo Alto, CA) by the University of Delaware. The GC capillary column was an Ultra 2 Agilent #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 (FID) was achieved at a temperature of

250°C using a carrier gas of hydrogen 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; all functions of the GC were under the control of the computer and this method. 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 according to Sullivan et al. (2006). This was done for all samples collected for the duration of this study.

Microbial community structure by 16S rDNA amplification and DGGE

Total soil microbial community DNA was extracted using the Fast Spin DNA kit (Qbiogene, Inc., Carlsbad, CA) as directed by the manufacturer. Bacterial 16S rDNA was amplified using eubacterial primers 338F and 518R (Nakatsu et al., 2000) by polymerase chain reaction. The 50 µl reaction mixture contained 10 ng of template DNA, 0.2 mM dNTPs, 2.5mM $MgCl_2$, 0.08µg/µl bovine serum albumin, 5% dimethylsulfoxide, 2.5µM of each primer, and 1 unit of Taq polymerase. The amplification was done using an iCycler Thermal Cycler (Bio-Rad Laboratories, Hercules, CA) and consisted of a 9 min hot start at 94 °C followed by 30 cycles of denaturing step at 94 °C for 30 sec, primer annealing step at 60 °C for and primer extension step at 72 ° C for 30 sec. A final extension cycle was performed for 7 minutes at 72 ° C. Amplification was confirmed by visualization of product in a 1% agarose gel.

PCR products were resolved using denaturing gradient gel electrophoresis (DGGE). DGGE was performed in a DCode Detection System (Bio-Rad

Laboratories, Hercules, CA) according to the manufacturer's instructions with modifications to a previously described method by Nakatsu et al. (2000). An 8% (w/v) polyacrylamide was used with a denaturing gradient ranging from 45 to 55% (100% denaturant is 7M urea and 40% formamide). Electrophoresis was carried out at 130 V for three hours. The electrophoresis buffer (1X TAE) was kept at 60°C for the duration of the run. Gels were stained with ethidium bromide (10 mg ml^{-1}) for 20 minutes and destained using 1X TAE buffer for 30 minutes. Gels were visualized under UV light and photographed (AlphaImager 2200 and AlphaEase Software, San Leandro, CA). Gels were scored visually for the number of dominant bands in each sample which corresponds to the number of dominant species in the bacterial community. The score was used as bacterial species richness (S).

Single-point measurements of microbial properties

In 2005, 3 years after the fire, fungal and bacterial biovolumes, fungal and bacterial C and N mineralization activities, and concentrations of glomalin (a glycoprotein produced and excreted into soil by arbuscular mycorrhizal fungi) were determined to assess the recovery of microbial community size and activity following the Hayman fire. 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 according to Klein and Paschke (2000) based on the average diameter of ~150 bacterial cells and 50 hyphal fragments.

For C and N mineralization, triplicate subsamples (12 g dry weight) of each soil were moistened to 60 % field capacity and inhibitors (4mg g^{-1} soil for streptomycin to inhibit bacteria; 15mg g^{-1} soil for cyclohexamide to inhibit fungi) were added as required for the substrate-induced respiration (SIR) 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. After equilibration for 16 hrs 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. After this, samples were incubated at 25 °C for 4 hours at which time the CO_2 evolved was measured by gas chromatography (model GC-8A, Shimadzu Scientific Instruments, 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.

To determine glomalin levels we used the method of Wright and Upadhyaya (1996) which uses total extractable protein as an indicator of glomalin concentration. Briefly, soil protein was serially extracted from 1 g soil (dry weight) in 2 mL of 50 mM citrate (pH 8.0) for 90 min at 121 ° C, following by centrifuging to collect supernatant. A total of 7-to-9 extractions were performed until the supernatant was straw-colored. Total protein was quantified using the Bradford protein assay on a model 680 microplate reader (Bio-Rad Laboratories, Hercules, CA).

Statistical analyses

Both experiments were analyzed as repeated measures designs. Statistical analyses were performed using the SAS statistical Package version 9.1 (SAS Institute Inc, Cary, NC). The PROC MIXED option with autoregressive covariance structure was used to conduct repeated measures analysis of variance (RM ANOVA) tests. One-way ANOVA was done on specific microbial or soil chemical data and mean comparisons were performed using a Fisher-protected LSD (α=0.1). Multivariate analyses, specifically principal components analysis (PCA) were performed on 2002 and 2005 microbial EL-FAME data using the PC-ORD statistical package (MjM Software, Gleneden Beach, OR, 1999). Prior to PCA, EL-FAMEs data were normalized as relative mol% and then log transformed to satisfy the assumption of normality.

Results

Soil water content and chemical properties

We observed several changes in soil properties soon after the Hayman fire (Table 3.1). Most notably, burned soils were drier than nonburned soils, and burned soils were elevated in pH and some soil nutrients (inorganic N, extractable P, and Mn). Moreover, total C and N, and extractable NH_4-N, K, Mn, and Zn concentrations were highest in the soil affected by the moderate severity burn. Extractable Cu was highest in nonburned soil and decreased with increasing fire severity (Table 3.1).

Three years after the Hayman fire, the soil water content and some chemical properties at MEF were still differentially affected by fire severity (Table 3.2). The moderate severity and high severity soils were still drier and higher in pH than nonburned soil. The moderate severity soil remained enriched in extractable P and Zn compared to the other soils, and total C was greatest in moderate and light severity burned soils. Concentrations of total N, inorganic N, Mn, and Cu did not vary significantly among the burned and nonburned soils. Soil N levels in burned soil had returned to background levels by fall 2004 (data not shown).

Microbial community structure by EL-FAMEs

Using EL-FAMEs we were able to follow the changes in soil microbial community structure after the Hayman fire in a ponderosa pine forest.

Table 3.1. Soil water content and chemical characteristics at MEF immediately after the Hayman fire (summer 2002; n=1).

Burn Severity	θv	pH	C	N	Inorganic N	Texture
			------mg cm^{-3}------			
Nonburned	0.070	5.4	28.5	1.83	0.008	Sandy Loam
Light	0.058	5.4	26.4	1.57	0.006	Sandy Loam
Moderate	0.044	5.8	50.2	2.92	0.021	Loam
High	0.035	6.7	26.4	1.33	0.015	Loamy Sand
Burn Severity	**P**	**K**	**Z**	**Fe**	**Mn**	**Cu**
	------μg cm^{-3}------					
Nonburned	0.64	148	4.99	82.6	29.1	0.83
Light	2.43	150	6.21	87.1	36.6	0.30
Moderate	9.47	210	15.6	84.5	66.1	0.05
High	17.9	151	3.46	95.0	57.1	0.03

Table 3.2. Soil water content and chemical characteristics at MEF three years after the fire (summer 2005; n=3). Mean comparisons are among treatments (within columns) and mean values followed by different letters are significantly different at α=0.10.

Burn Severity	θv	pH	C	N	Inorganic N	Texture
			---------------mg per cm^{-3}	----------------	----------------	
Nonburned	0.120 b	5.4 c	31.1 b	1.92 a	0.009 a	Loam
Light	0.102 b	5.3 c	64.0 a	2.14 a	0.011 a	Loam
Moderate	0.165 a	6.1 a	69.4 a	2.27 a	0.013 a	Loam
High	0.142 a	5.8 b	30.4 b	1.57 a	0.013 a	Loam

Burn Severity	P	K	Zn	Fe	Mn	Cu
	---------------------------------------μg pr cm^{-3}--					
Nonburned	2.35 c	430 a	3.99 c	73.4 b	12.1 a	1.46 a
Light	4.91 bc	358 a	14.4 b	129 a	24.7 a	2.01 a
Moderate	12.2 a	354 a	23.3 a	112 a	29.3 a	1.47 a
High	9.69 ab	132 b	5.08 c	107 a	20.5 a	1.88 a

EL-FAME data were classified into 3 major groups: bacterial populations (sum of 17:1 iso G, 15:0 iso/anteiso, 16:0 iso, 17:0 iso/anteiso, 16:1ω7c, 17:0 cy and 19:0 cy markers), fungal populations (sum of 18:2ω6, 9c and 18:3ω6c), and arbuscular mycorrhizal (AM) fungi (16:1ω5c). Repeated measures analysis of variance revealed that changes in the mean amounts of EL-FAMEs markers for all groups studied had similar and significant trends over time and also significant fire severity effects. However, the fire effects were not dependent on seasonality. As shown in Fig. 3.1, for the first year after the fire the amount of bacterial markers was generally higher in the nonburned soil compared to the soil affected by fire. The general trend in the soil (burned or nonburned) was a decrease in bacterial markers over time in 2003 (summer, spring, and fall), followed by a increase in 2004 that remained stable until summer of 2005. Also, except for summer 2004, the amount of bacterial markers in the soil affected by high severity burn was always lower than in the nonburned soil. Interestingly, there was an increase (although not statistically significant) in the amounts of bacterial markers in the moderate severity burned soil every summer throughout the course of this study. Changes observed in bacterial EL-FAME markers due to fire severity were dependent on seasonality (RM ANOVA $P=0.0003$ for the interaction). For fungal EL-FAME biomarkers, the initial pattern among soils was different than that of the bacterial markers where the amount of fungal biomarkers, particularly of AM fungi, were low in the burned soil (Fig. 3.2a and 3.2b) regardless of fire severity until the summer of 2004. (RM ANOVA $P=0.0032$).

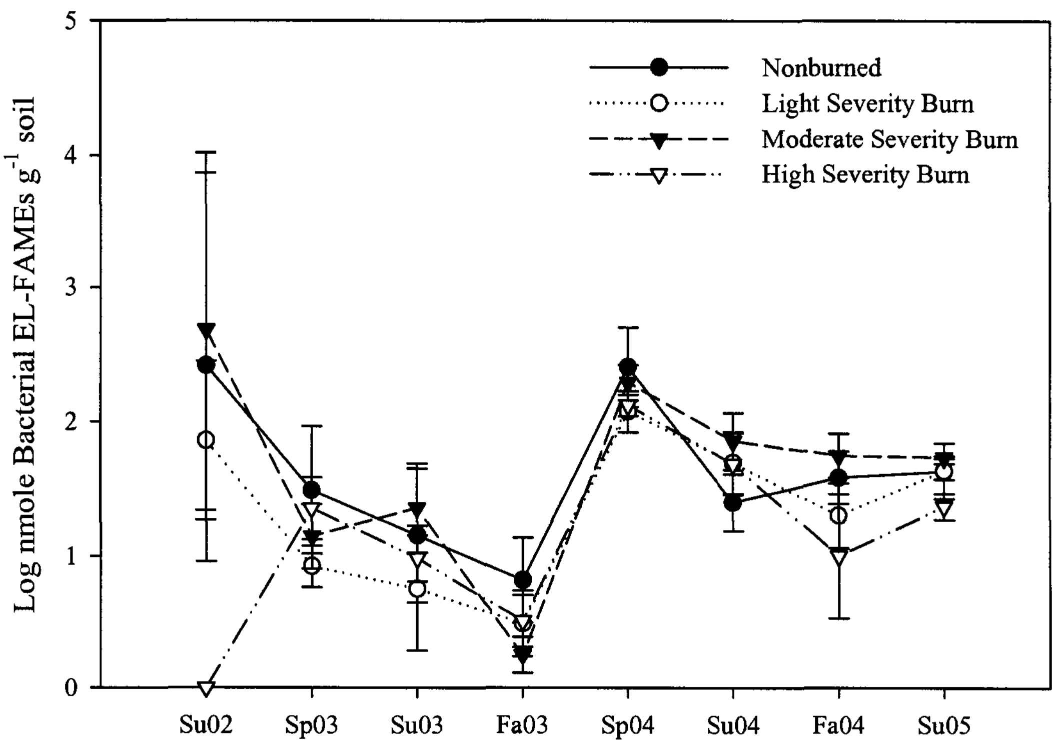
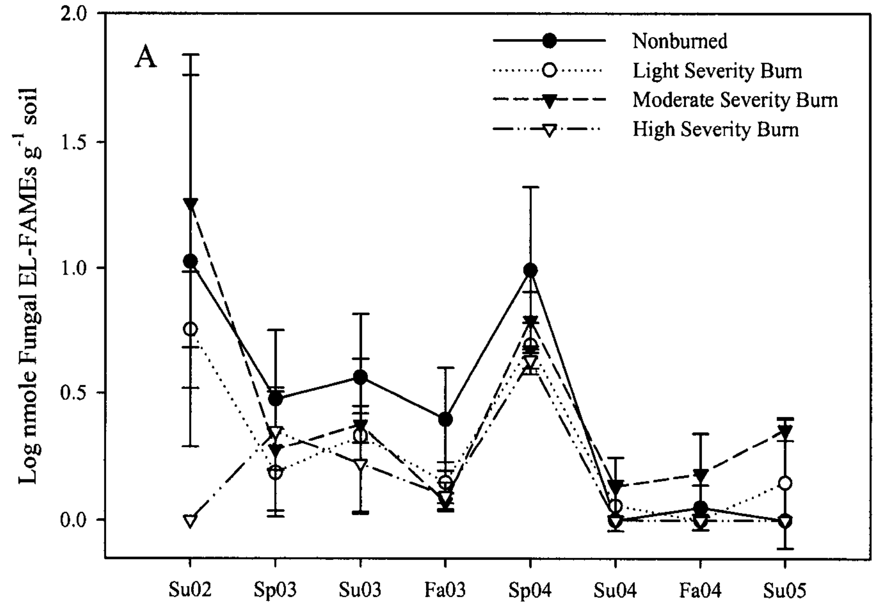


Figure 3.1. Repeated measures analysis of bacterial EL-FAME markers in burned and nonburned forest soil. There was a significant fire severity effect (*P=0.07*) and a significant time (season) effect (*P<0.0001*). Su=summer, Sp=spring, and Fa=fall. Standard deviation bars (± 2) are shown.

No fungal markers were detected in the nonburned soil in summer and fall of 2004 and in the summer of 2005. Fungal markers but not AM fungi were higher in the moderate severity burn treatment after summer 2004.

We used EL-FAME data from summer 2002 to characterize microbial community structure by PCA. Because no EL-FAMEs were detected in the high severity burn soil at this point, this treatment was excluded from the analysis. Results are shown in Fig. 3.3. Microbial communities are separated along PCs 1 and 2 according to burn severity. Both principal components explained a total of 76.7% of the variability in the data. Microbial EL-FAMEs with large, positive eigenvalues (and therefore associated with communities to the right of PC 1) included biomarkers for fungi (18:2ω6 and 18:3ω6c), whereas the heaviest loadings for communities to the left of PC 1 were EL-FAMEs of bacterial origin, mostly Gram-positive (15:0 iso/anteiso, 16:0 iso) and also a fungal marker (18:3ω6c).

In the summer of 2005, principal component analysis revealed that microbial community structure was still affected significantly by the Hayman fire in the area where the fire burned at high severity. As shown in Fig. 3.4, microbial communities nonburned soil and in soil affected by the light and moderate severity fire were separated to the right of PC 1, and microbial communities in soil affected by the high severity fire were separated to the left of PC 1. Communities to the right of PC 1 were enriched with the Gram-negative marker 16:1 2OH, Gram-positive markers 14:0 iso and 17:0 iso, and with the fungal marker 18:3ω6c.



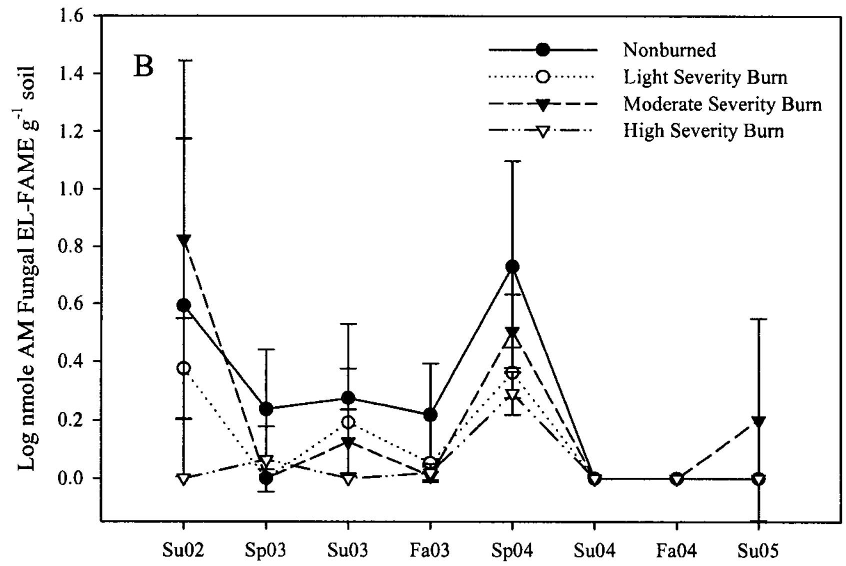


Figure 3.2. Repeated measures analysis of EL-FAME markers for fungi (A) and arbuscular mycorrhizal fungi (B) in burned and nonburned forest soil. There was a significant fire severity effect (*P=0.018*) and a significant time (season) effect for fungi (*P<0.0001*) and a significant fire severity effect (*P=0.049*) and a significant time (season) effect (*P<0.0001*) for AM fungi. Su=summer, Sp=spring, and Fa=fall. Standard deviation bars (± 2) are shown.

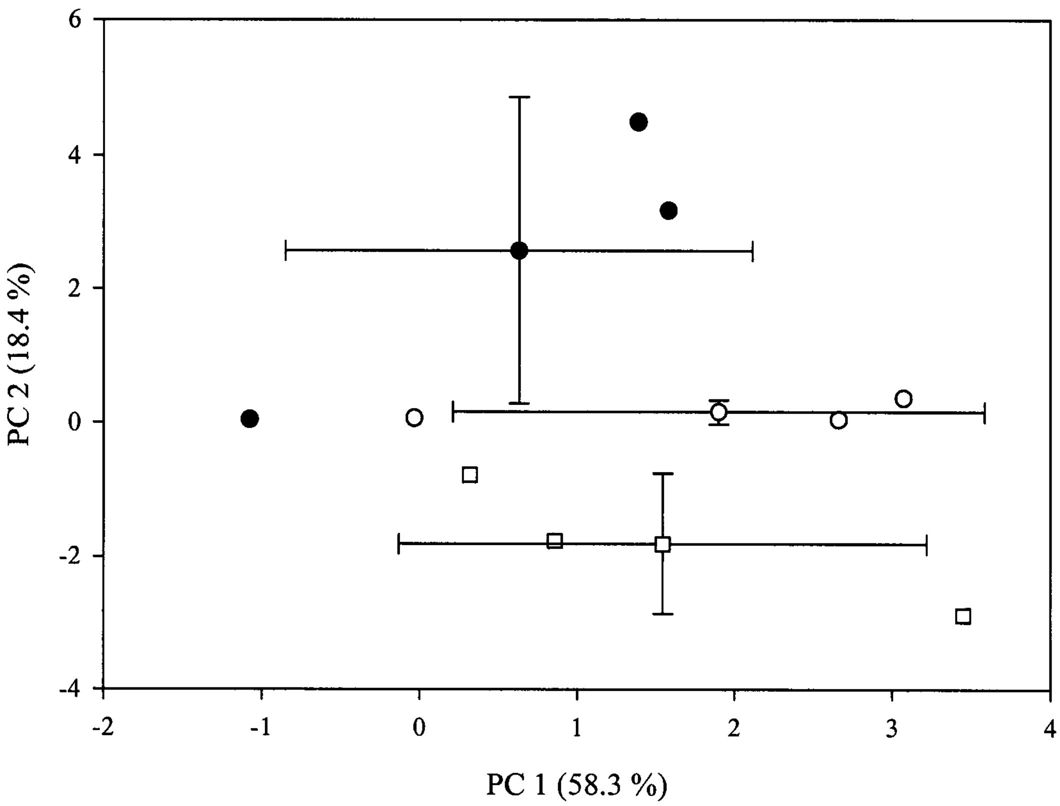


Figure 3.3. PCA of EL-FAME data of burned and nonburned soil shortly after the Hayman fire (summer 2002). Soil burned at high severity was excluded from the analysis due to absence of EL-FAMEs. Shown are the individual data points as well as the mean value, along with standard deviation bars (± 2). The percent variability explained by each PC is shown in parentheses.

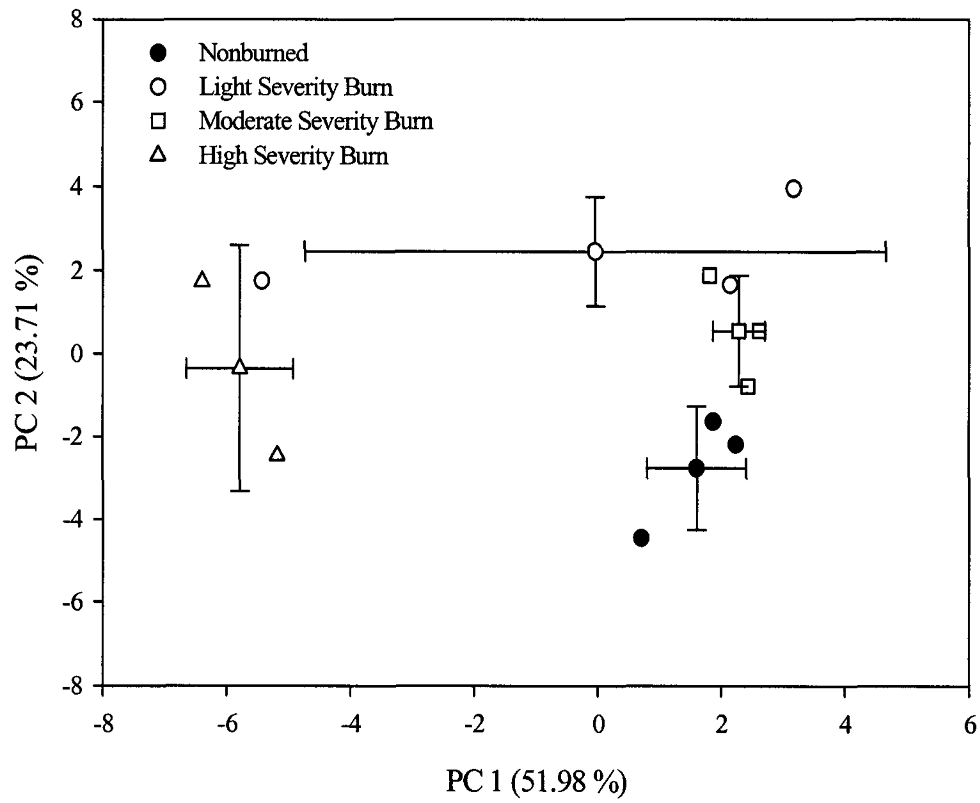


Figure 3.4. PCA of EL-FAME data of burned and nonburned three years after the Hayman fire (summer 2005). Shown are the individual data points as well as the mean value, with standard deviation bars (± 2). The percent variability explained by each PC is shown in parentheses

Communities to the left of PC 1 were enriched with Gram-positive bacterial markers 15:0 iso/anteiso, 16:0 iso, and 17:0 iso/anteiso, Gram-negative markers 17/19:0 cy and sulfur reducing bacterial marker 17:1ω7c. Principal component 2 separated the light and moderate severity soil communities further from the nonburned and high severity soil communities. The light and moderate severity treatments were enriched with the fungal marker 18:3ω6c.

Bacterial community structure by 16S rDNA PCR and DGGE

The bacterial community of fire-affected soil at MEF was further analyzed using molecular tools. As shown in Fig. 3.5, the soil affected by light severity and moderate severity fire (summer 2002) initially showed an increase in species richness (defined as number of bands present in a lane) compared to the nonburned soil and the high severity burned soil; the number of dominant bands ranged from 7 in the light and moderate severity burned soil compared to 4 and 5 in the nonburned and high severity soil, respectively. One year after the fire (summer 2003), species richness in the high severity burned soil was still lower than in the light severity and moderate severity soil (5 bands), but it was higher than in the nonburned soil (2 bands). Species richness in moderately burned soils was also significantly greater than in the nonburned soils in 2003.

Microbial community resistance and resilience

Resistance, defined as the amount of change initially caused by the fire, and resilience, defined as the speed with which a system returns to its pre-

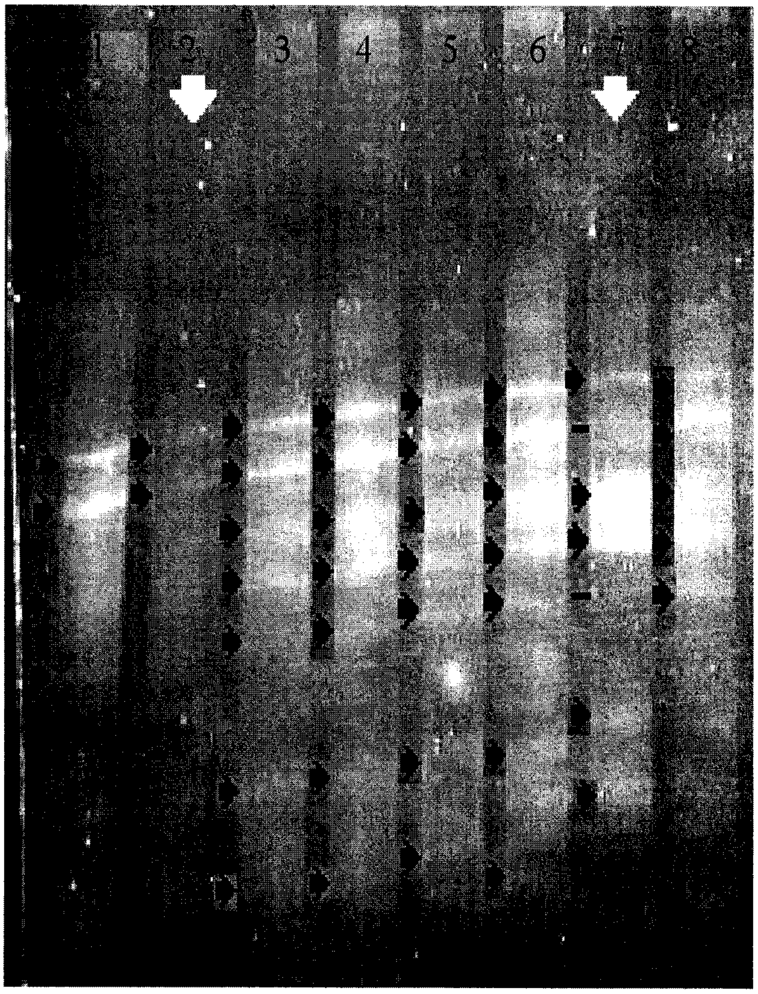


Figure 3.5. Molecular analysis of bacterial communities from burned and nonburned forest soil by PCR-DGGE. Lane 1: Nonburned soil (summer 2002). Lane 2: Nonburned (summer 2003). Lane 3: Light severity burn (summer 2002). Lane 4: Light severity burn (summer 2003). Lane 5: Moderate severity burn (summer 2002). Lane 6: Moderate severity burn (summer 2003). Lane 7: High severity burn (summer 2002). Lane 8: High Severity burn (summer 2003). Black arrows: Dominant species in all treatments. White arrows: less smearing and band intensity. Minus signs: bands absent in lane 7 but present in all other lanes.

disturbance levels, was determined for bacteria and fungi using EL-FAME markers. Resistance and resilience indices were calculated as described by Orwin and Wardle (2004) using EL-FAME markers to quantify the stability of the microbial community in the soil affected by the fire. For resistance calculations, we used the nonburned samples from summer 2002 as the control (C_0) and the burned samples at the same sampling time were used as perturbed soil (P_0). Summer 2002 samples were also set to be the end of the disturbance for the resiliency calculations. Soil samples on consecutive sampling dates (after summer 2002) where use as the control soil (Cx) and perturbed soil (Px) in resilience calculations. For example,

$$\text{Resistance}=1-[(2\,|D_0|)/(C_0+|D_0|)],$$

where D_0 was P_0-C_0 and

$$\text{Resilience}=[(2\,|D_0|)/(|P_0|+|D_x|)]-1,$$

where D_x was P_x-C_x. For the resistance index, a value of 1 means no change due to disturbance and a value of less than 1 implies proportionally more change. A value of 1 for resilience index means full recovery and a value of less than 1 means slower rates of recovery. Table 3.3 shows the resistance and resilience indices calculated every summer after the fire until the last sampling event. Resilience indices were calculated for spring and fall sampling dates, but I found it difficult to interpret the results due to seasonal influences on microbial community EL-FAME structure. Bacteria were initially more resistant to the moderate severity fire than fire of light or high severity; however, two years after the fire (summer

2004) the bacterial community was less resilient to the moderate severity fire disturbance. In the soil affected by light severity or high severity fire, the bacterial communities were less resistant but more resilient. The fungal populations were not resistant to the fire disturbances, regardless of severity, but they demonstrated some resiliency one year after the fire. Resiliency was especially high in summer 2003 for the AM fungi in soils burned by light and moderate severity fire, although this trend did not continue into the other summers.

Table 3.3. Resistance and resiliency indices for EL-FAME data over all summers sampled after the fire.

EL-FAME Group	Burn Severity	Resistance Index 2002	----------Resilience Indices---------- 2003	2004	2005
Bacteria					
	Light	0.13 b	0.42 a	0.21 a	0.19 b
	Moderate	0.38 a	0.54 a	-0.38 b	0.45 a
	High	0.00 c	0.65 a	0.30 a	0.37 a
Fungi					
	Light	0.16 a	0.17 a	0.86 a	0.43 a
	Moderate	0.07 a	0.24 a	0.74 a	0.12 b
	High	0.00 b	0.15 a	1.00 a	0.66 a
AM Fungi					
	Light	0.13 a	0.83 a	0.00 b	0.00 a
	Moderate	0.02 a	0.78 a	0.70 a	0.00 a
	High	0.00 a	0.00 b	0.00 b	0.00 a

Microbial biovolumes, C and N mineralization activity, and glomalin

In summer of 2005, three years after the fire, we were able to determine if recovery was also evident for microbial and fungal biovolumes and C and N mineralization activities. We also measured glomalin levels as proxy for recovery of AM fungal activity in soil after the fire. Bacterial, total fungal, and active fungal biovolumes were determined, but no significant differences were found among treatments 3 years after the Hayman fire (*P=0.151*, *0.252*, and *0.124*, respectively). Significant differences where found for bacterial and fungal C and N mineralization activities, however (Tables 3.4 and 3.5, respectively). Fungal and bacterial respiration was highest in the soil affected by the moderate severity fire and lowest in the soil affected by light severity and high severity fire. Fungal and bacterial nitrogen mineralization rates were highest in the soil affected by light and moderate severity fire and lowest in the nonburned soil and in soil affected by high severity fire. Fungal respiration and nitrogen mineralization activity were slightly greater than those of bacteria, regardless of fire severity.

As shown in Fig 3.6., immediately after the fire (summer 2002) the glomalin level in soil was significantly lower in the high severity burned soil compared to the nonburned and light and moderate severity burned soils. In 2005, 3 years after the fire, the levels were similar to 2002 for the nonburned soil and the soil in the light severity area, but glomalin concentrations in the high severity burned soil had doubled by 2005, so that its value was similar to that in the nonburned soil.

Table 3.4. Carbon mineralization rates of fungi and bacteria as determined by substrate induced respiration during summer 2005. Mean comparisons are among treatments (within columns) and mean values followed by different letters are significantly different at 0.10 level.

Burn Severity	Total	Fungal	Bacterial
	------------$\mu g\ CO_2\ g^{-1}$ soil hr^{-1}-------		
Nonburned	7.62 a	7.04 b	4.85 b
Light	0.85 b	0.93 c	0.66 c
Moderate	10.3 a	10.4 a	7.69 a
High	0.56 b	0.77 c	0.63 c

Table 3.5. Nitrogen mineralization rates for fungi and bacteria as determined by substrate induced respiration during summer 2005. Mean comparisons are among treatments (within columns) and mean values followed by different letters are significantly different at 0.10 level.

Burn Severity	Total	Fungal	Bacterial
	------------kg N g^{-1} soil hr^{-1}----------		
Nonburned	2.07 b	0.31 b	0.11 b
Light	11.3 a	12.7 a	11.6 a
Moderate	10.8 a	11.2 a	10.8 a
High	2.68 b	3.72 b	2.56 b

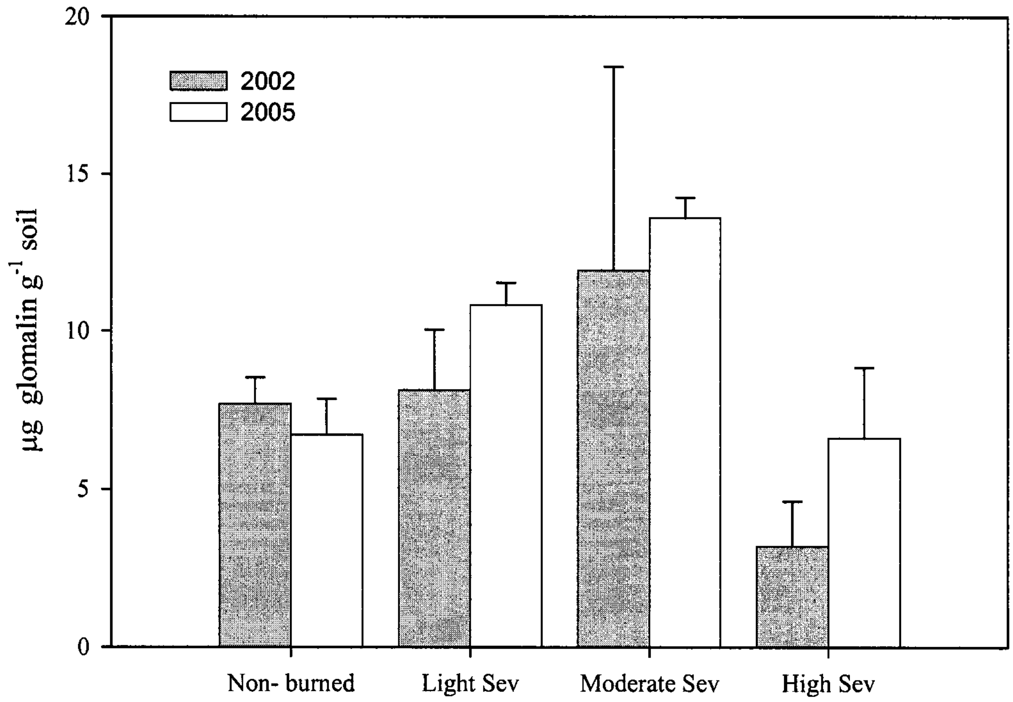
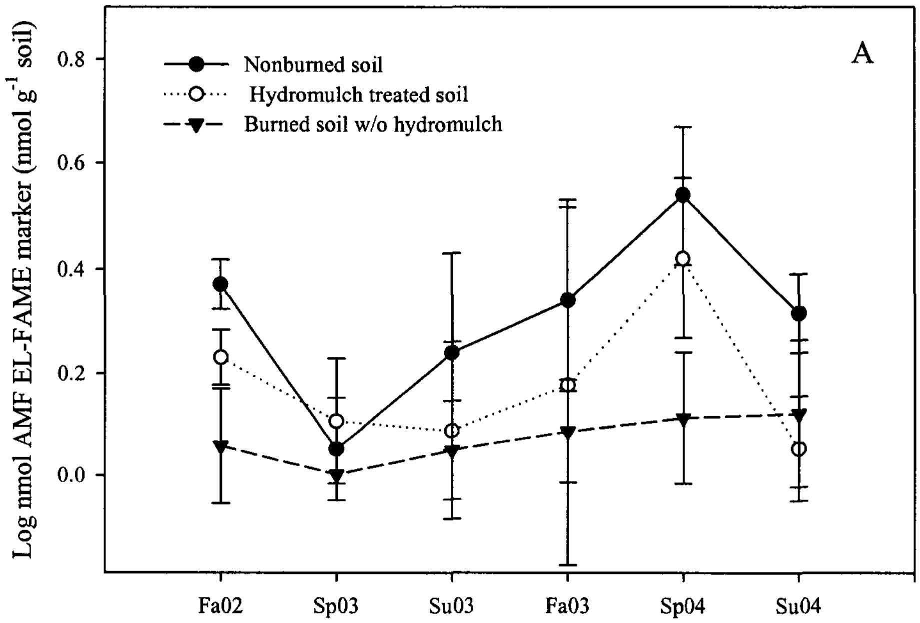


Figure 3.6. Glomalin concentrations from burned and nonburned MEF soil in summer of 2002 and summer 2005. Standard deviation bars (± 2) are shown.

Effects of aerial hydromulching on fungal recovery

Repeated measures ANOVA on microbial EL-FAME data from the hydromulch study revealed a significant treatment effect (RM ANOVA $P= 0.009$). Burned soil treated with hydromulch had significantly greater amounts of the AM fungal marker than burned soils without the treatment (Fig. 3.7a) (RM AOV $P<0.0001$), and the amounts of the marker and its seasonal trends were generally similar and significant in the nonburned and hydromulch-treated burned soil (RM AOV $P<0.0001$). Although the amounts of the AM fungal marker were significantly lower in the nontreated burned soil, its trend was to increase in concentration over time. Total fungal markers followed a similar trend, with greater amounts on fungal EL-FAME markers in burned soil treated with hydromulch than in nontreated burned soil (Fig. 3.7b $P<0.0001$), but there was no significant effect of hydromulch on the recovery of bacterial markers nor were there time effects or aspect effects ($P=0.548$) (data not shown). Gravimetric water contents were generally greater every season in the hydromulch treated soil compared to the nonburned and nontreated burned soil (nonburned soil 7-9 %, hydromulch treated soil 9-14%; nontreated burned soil 7-10%). In the fall of 2002 and 2003, extractable P levels were significantly greater in the hydromulch treated soil compared to the nonburned and the burned soils ($P=0.0006$; data not shown). However, there is no correlation between P content of soil and the AMF biomarker ($P=0.32$).



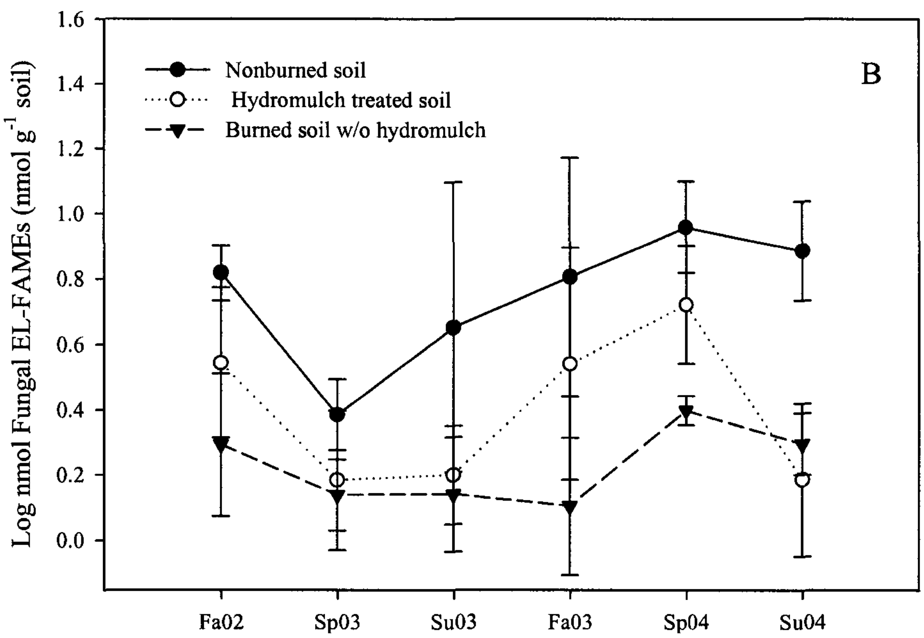


Figure 3.7. **(A)** Repeated measures analysis of AMF marker in hydromulch treated soil. There was a significant treatment effect (*P=0.009*). **(B)** Repeated measures analysis for total fungal markers in soil. There was a significant treatment effect (*P<0.0001*). Standard deviation bars (± 2) are shown.

Discussion

It is well accepted that soil microorganisms contribute greatly to elemental cycling, and disturbances which alter microbial community structure and stability may have repercussions on these ecological processes. The aim of this study was to describe the changes that occurred to soil and soil microbial communities immediately after a fire and to describe how the community recovered after a fire disturbance. We found that the changes in soil chemical properties after fire in the ponderosa pine ecosystem were generally similar to those reported previously in the literature for fire-affected soil. Three years after the fire, soil pH was still elevated (above 6.0) in the moderate severity burn, although in the high severity burn the pH had fallen to 5.8, a value closer to the pH of the nonburned soil. Nutrient availability in soil changed depending on fire severity. Concentrations of extractable elements (P, K, Zn, and Mn) were greater immediately after the fire in the moderate and high severity burns. These findings agree with results from other studies which found that soil available nutrients increase immediately following a fire event (Certini, 2005; Fyles et al., 1991; Pietikainen and Fritze, 1993; Prieto-Fernandez et al., 1998; Prieto-Fernandez et al., 2004). Moreover, three years after the fire, soil burned at moderate severity remained elevated in total C and extractable P and Zn, indicating that fire-induced changes in soil fertility may persist for several years at least, depending on the nutrient. However, as evidenced by the different soil textures reported on Tables 3.1 and 3.2, spatial variability in the samples collected may also account for the nutrient differences observed.

As a quantitative measure of recovery for the microbial community, we calculated indices of resistance and resilience of different microbial groups using EL-FAME data (Table 3.3). The negative value obtained for bacterial resilience in summer 2004 was due to an initial decrease in the amount of bacterial markers in 2002 presumably due to heat sterilization, followed by an increase in bacterial EL-FAMEs concentrations relative to nonburned soil. This increase in bacterial markers over time, relative to nonburned soil, may be due to the increase in total C and nutrients in burned soil, which resulted in an increase in bacterial biomass and thus the markers. Bacterial populations showed high resistance to the moderate severity burn and this might be related to the fact that moderate severity fires are common in the ponderosa pine ecosystem. It has been suggested before that microbial communities previously exposed to fire can adapt to subsequent fire events (Choromanska and DeLuca, 2001). At MEF, the fire regime includes low to moderate severity fires, and the plant community, including ponderosa pine, has physiological adaptations that enable them successfully to inhabit this ecosystem (Romme et al., 2003; Whelan, 1995). It might be that the bacterial community was more resistant and also resilient to moderate severity fire because they too have adapted to the immediate changes produced by a fire of this severity. Although we have no evidence to support a mechanism for adaptation to this fire severity, and although bacteria may experience thousands of generations between fire events in this ecosystem, our observations lend support for a new hypothesis that the bacterial community has attained a degree of tolerance to moderate severity fires.

Molecular tools corroborated the findings of EL-FAME analyses. It is important to note that the observed bands represent only the dominant bacterial species in soil (Nakatsu et al., 2000). Species richness in moderately burned soils was higher than richness in the nonburned soils. These findings parallel with those concluded using EL-FAME data, in which bacterial markers increased in the moderate severity burned soils every summer after the fire compared to the nonburned soil. Nevertheless, the DGGE analysis showed an increase in bacterial species richness in the lightly burned soil as well, which was not seen using the EL-FAME data. In the near future we plan to sequence these bands to assess the composition of the bacterial community structure more in depth.

Light severity burn appeared to have as negative an impact as the high severity burn on several microbial variables, including bacterial, fungal, and AM fungal EL-FAME markers, and on fungal and bacterial respiration. Water content, pH, N, C, and amounts of extractable elements in the soil in the light severity burn were similar to those in the nonburned soil throughout the study, but the EL-FAME markers and fungal and bacterial activity in nonburned soil was higher than in the light severity burn. Therefore, it does not appear that soil fertility and pH are responsible for the detrimental effect of a light severity burn on soil microorganisms. Instead, our results might be due to changes in other variables not measured in this study, such as changes in carbon substrate quality (i.e., greater recalcitrance) due to incomplete organic matter oxidation.

A major finding of this study was that microbial community recovery in fire-affected soil may also be a function of seasonality. For example, the increase in bacterial markers every summer in the moderate severity burn soil may be due to an increase in nutrients because the amounts of total C and total N followed a similar pattern every summer (data not shown). It has been shown before that immediately after a fire, levels of labile C, N, and P increase in soils which in turn increases microbial activity (Fontaine et al., 2004; Fyles et al., 1991). The initial input of nutrients immediately after the fire may have caused a priming effect (an acceleration of mineralization of the organic matter due to an enrichment of other labile substrates) for the bacterial communities in the moderate severity soil that was further enhanced during the summer seasons, presumably by interactions with plants at their peak of the growing season.

Compared to bacteria, fungi appeared to be more affected by fire (regardless of severity) at least for two years following the fire event. The concept of greater fungal sensitivity to fire effects has been previously described by Ahlgren and Ahlgren (1965), Dunn et al. (1985), Vazquez et al. (1993), Theodorou and Bowen (1982), and Fritze and Pietikainen (1993). An increase in soil pH, a decrease in moisture content of soil, and/or an increase in bacterial biomass (increased competition) may be possible reasons why fungi were initially more affected than bacteria by fire effects. However, the exact mechanism is not known and it may depend also on other components of the fire regime, such as fire intensity or season of fire.

AM fungi were not resistant or resilient to fire of any severity and two years after the fire this important fungal group does not appear to have recovered to pre-disturbance levels. The lower amounts of the AM fungal marker may be related to the increase in plant available nutrients in the burned soils. With an increase in extractable phosphate in the burned soil, the plant community present at the disturbed sites may not have the need for symbiotic interactions with AM fungi in order to obtain nutrients (Cornwell et al., 2001; Raznikiewicz et al., 1994). In that regard, some preliminary vegetation recovery data (Table 3.6) suggest that the dominant plant species in the burned soil are not mycorrhizal (*Chenopodium album*- lamb's quarters) or only form mycorrhizal associations in nondisturbed soil (*Linaria vulgaris*- yellow toadflax), and this could also explain the reduced amounts of AMF marker found in the fire affected soil (Fontenla et al., 1999; Harris and Clapperton, 1997).

In regards to the AM fungi, which are an important group of microorganisms for ecosystem function (Zak et al., 2003), we can infer based on our data that AM fungal-mediated nutrient cycling is negatively affected by fire. It is not clear if the marker shows low resilience because of the presence of non-mycorrhizal plants or because P is not limiting or maybe is due to both. It is clear that fire has a negative impact on mycorrhizal associations and this may relate to the shift observed in the above ground community. The extent and duration of these negative impacts of fire on AM fungi are currently under examination; however, studies have linked a decrease in mycorrhizal fungal recovery with tree regeneration problems (Borchers and Perry, 1990). To further elucidate the changes due to fire severity for this important group of fungi, we measured the changes in glomalin immediately after the fire and 3 years later. This protein, secreted by AM fungi, is thought to be an important factor for soil aggregation and stabilization as well as for carbon storage (Wright and Upadhaya, 1996). Our results suggest that light and moderate severity fires can increase glomalin levels in soil. As we previously discussed, moderate severity fire appeared to increase the amount of AM fungal marker in soil and their resilience (at least until summer of 2004), and these changes parallel changes in glomalin production as well. Alternatively, light and moderate severity fires may have altered soil glomalin such that it became easier to extract it from soil. Also in relation to AM fungi, treatment of burned soil with hydromulch immediately after the fire proved to be effective in aiding the recovery of AM fungal biomarker.

Hydromulching is an effective treatment for moisture retention in soil (Robichaud et al., 2004), and because there were no significant differences among soils in pH and total C and N at any season (data not shown), increased water availability and the presence of root systems from the *Triticale* plants were presumably the factors influencing fungal recovery the most in the hydromulch covered soil. Interestingly, pH was higher (pH>6) in hydromulch treated soil after summer 2003. This increase in pH may explain why fungal markers were never greater in concentration in the hydromulch treated soil compared to the nonburned soils as fungi prefer more acidic environments (Penalva and Herbert, 2002) Nonetheless, if we compare the hydromulch treated soil with the burned soil without treatment, hydromulch helped ameliorate the negative fire effects on fungal markers.

Table 3.6. Dominant plant species in nonburned and burned soil at MEF in summer 2004.

	Plant Species	Common name	Family	Life Form	Origin/longevity	Mycorrhizal Association
Nonburned soil	*Artemisia frigida Willd.*	Prairie sagewort	Asteraceae	Forb	Native Perennial	Mycotroph
	Festuca arizonica Vasey	Arizona fescue	Poaceae	Grass	Native Perennial	Mycotroph
Burned soil	*Linaria vulgaris Miller*	Yellow toadflax	Scrophulariaceae	Forb	Introduced, Invasive Perennial	Facultative Mycothroph
	**Chenopodium album L.*	lamb's quarters	Chenopodiaceae	Forb	Native, Invasive Annual	Non-Mycotroph

**Chenopodium album* was present in high frequency (100% of burned plots) and less frequently in the nonburned plots (Ann Lezberg, Forest Service, Personal communication).

The main objective of this study was to asses the changes in microbial community structure after the Hayman fire, but in 2005 we also measured microbial activity as C and N mineralization for fungi and bacteria using substrate induced respiration to asses the recovery in microbial function three years after the fire. Our data suggest that regardless of fire severity, fungi were more active than bacteria in this forest soil because fungal biovolumes were greater than bacterial biovolumes (data not shown). Although no data on mineralization activities were available immediately after the fire, three years after the Hayman fire, respiration and N mineralization rates were greater for both fungi and bacteria in the moderate severity treatment compared to the rest of the treatments. This parallels with the increase in extractable nutrients, total C, and soil water content in the moderate severity treatment by summer of 2005.

Using two culture independent methods (EL-FAMEs and PCR/DGGE) we have been able to track the changes of the soil microbial community structure caused by fire. These culture independent methods (EL-FAMEs and PCR/DGGE) proved more useful than culture-based methods because we were able to monitor microorganisms at the community level, rather than at the level of culturable populations. Also, by using this approach we were able to monitor the recovery of the important but non-culturable AM fungal group. Moreover, the EL-FAME method was particularly useful as it allowed for the analysis of over 150 soil samples in a rapid manner during the course of this study.

It is important to note that this study was conducted on a very small spatial scale in a ponderosa pine forest (i.e. replicate fire severities locations were meters and not km apart), thus it is possible that differences observed for soil chemical data as well as for microbial community responses were due to inherent differences in soil physicochemical or microbiological properties previous to the Hayman fire and not to fire severity alone. Since there is no pre-fire soil data available, as is common in studies regarding unexpected natural disturbaces such as fire, we were unable to characterize the conditions in soil previous to the fire.

In summary, we have shown that changes in soil chemistry and the recovery of microbial communities in soil affected by fire varied depending on the fire severity and also on the season sampled at least in a small spatial scale. Our results suggest that bacteria and fungi in this soil may be more tolerant to the moderate severity burn changes and that the number of dominant bacterial species in soil increased with light and moderate severity fire, a fact that may be related to an increase in soil nutrients and also the frequent occurrence of moderate severity fires in ponderosa pine ecosystem. We have also shown that AM fungi are negatively impacted by fire and their biomarker absence in soil may be related to changes in soil nutrient levels and/or plant community composition. However, hydromulch appeared to facilitate the recovery of AM fungi, perhaps due to the presence of triticale roots as well as increased available water in soil. Resilience indices showed that the microbial communities in soil may structurally recover from a moderate to high severity fire disturbance within 2 years of the disturbance. This does not imply that microbial function has recovered at a similar rate nor that the above ground community will recover at the same rate or return to the same composition as the nonburned sites. Although microbial C and N mineralization activities had recovered in the moderate severity burned soils 3 years after the fire, activity levels in light and high severity burned soils were still well below those in the nonburned soil. More studies are necessary to determine the long term effects of fire on soil microbial communities.

The results of this study are suggestive due to the small extent of the burned area sampled, however the trends implied by the data obtained encourages and excites new research to test hypotheses such as that light severity fire maybe detrimental to soil microorganisms and that bacteria can have adapted to moderate severity fire in this ecosystem. Also, the long term changes in microbial function along with long term changes in vegetation of this forest should be addressed to better understand changes to the overall forest ecosystem.

References

Acea, M.J., and T. Carballas. 1996. Changes in physiological groups of microorganisms in soil following wildfire. FEMS Microbiology Ecology 20:33-39.

Ahlgren, I.F., and C.E. Ahlgren. 1965. Effects of prescribed burning on soil-microorganisms in a Minnesota Jack pine forest. Ecology 46:304-310.

Anderson, J.P.E., and K.H. Domsch. 1975. Measurement of bacterial and fungal contributions to respiration of selected agricultural and forest soils. Canadian Journal of Microbiology 21:314-322.

Barbarick, K.A., and S.M. Workman. 1987. NH_4-HCO_3-DTPA and DTPA extractions of sludge-amended soils. Journal of Environmental Quality 16:125-130.

Bloem, J., M. Veninga, and J. Shepherd. 1995. Fully-automatic determination of soil bacterium numbers, cell volumes, and frequencies of dividing cells by confocal laser-scanning microscopy and image-analysis. Applied and Environmental Microbiology 61:926-936.

Borchers, J.G., and D.A. Perry, (eds.) 1990. Effects of prescribed fire on soil microorganisms., pp. 1-143-162. Oregon State University Press, Corvallis, Or.

Bossio, D.A., and K.M. Scow. 1998. Impacts of carbon and flooding on soil microbial communities: Phospholipid fatty acid profiles and substrate utilization patterns. Microbial Ecology 35:265-278.

Certini, G. 2005. Effects of fire on properties of forest soils: a review. Oecologia 143:1-10.

Choromanska, U., and T.H. DeLuca. 2001. Prescribed fire alters the impact of wildfire on soil biochemical properties in a ponderosa pine forest. Soil Science Society of America Journal 65:232-238.

Cornwell, W.K., B.L. Bedford, and C.T. Chapin. 2001. Occurrence of arbuscular mycorrhizal fungi in a phosphorus-poor wetland and mycorrhizal response to phosphorus fertilization. American Journal of Botany 88:1824-1829.

Dunn, P.H., S.C. Barro, and M. Poth. 1985. Soil-moisture affects survival of microorganisms in heated chaparral soil. Soil Biology & Biochemistry 17:143-148.

Fontaine, S., G. Bardoux, D. Benest, and B. Verdier. 2004. Mechanisms of the priming effect in a savannah soil amended with cellulose. Soil Biology & Biochemistry 68:125-131.

Fontenla, S., I. Garcia-Romera, and J.A. Ocampo. 1999. Negative influence of non-host plants on the colonization of *Pisum sativum* by the arbuscular mycorrhizal fungus *Glomus mosseae.* Soil Biology & Biochemistry 31:1591-1597.

Fritze, H., T. Pennanen, and J. Pietikainen. 1993. Recovery of soil microbial biomass and activity from prescribed burning. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere 23:1286-1290.

Fyles, J.W., I.H. Fyles, W.J. Beese, and M.C. Feller. 1991. Forest floor characteristics and soil nitrogen availability on slash-burned sites in coastal British-Columbia. Canadian Journal of Forest Research 21:1516-1522.

Gee, G.W. and W. Bauder. 1986. Particle size analysis. In D.L. Sparks (ed). Methods of soil analysis part 1. Physical and mineralogical methods. SSSA, Madison WI, pp. 404-409.

Harris, P., and M.J. Clapperton. 1997. An exploratory study on the influence of vesicular-arbuscular mycorrhizal fungi on the success of weed biological control with insects. Biocontrol Science and Technology 7.

Hungerford, R.D., Harrington, M.G., Frandsen, W.H., Ryan, K.C.,and Niehoff, G.J. 1991. Influence of fire on factors that affect site productivity. USDA Forest Service General Technical Report No. INT-280.

Johnson, C.K., M.F. Vigil, K.G. Doxtader, and W.E. Beard. 1996. Measuring bacterial and fungal substrate-induced respiration in dry soils. Soil Biology & Biochemistry 28:427-432.

Klein, D.A., and M.W. Paschke. 2000. A soil microbial community structural-functional index: the microscopy-based total/active/active fungal/bacterial (TA/AFB) biovolumes ratio. Applied Soil Ecology 14:257-268.

Lodge, D.J., and E.R. Ingham. 1991. A comparison of agar film techniques for estimating fungal biovolumes inlitter and soil. Agriculture Ecosystems & Environment 34:131-144.

Moore, R. 1992. Soil survey of Pike National Forest, eastern part, Colorado, parts of Douglas, El Paso, Jefferson, and Teller counties, pp. 106, *In* C. USDA

Forest Serv. and Soil Conservation Serv.; in cooperation with the Colo. Agric. Exp. Stn Denver, (ed.).

Mulvaney, R. 1996. Nitrogen - Inorganic forms, p. 1123-1184 Sparks DL (ed) Methods of Soil Analysis, Part 3. Chemical Methods. SSSA, Madison, WI.

Nakatsu, C.H., V. Torsvik, and L. Ovreas. 2000. Soil community analysis using DGGE of 16S rDNA polymerase chain reaction products. Soil Science Society of America Journal 64:1382-1388.

Nelson, D., and L. Sommers. 1996. Total carbon, organic carbon, and organic matter. , p. 975-977, *In* D. Sparks, ed. Methods of Soil Analysis, Part 3. Chemical Methods. . SSSA, Madison, WI.

Orwin, K.H., and D.A. Wardle. 2004. New indices for quantifying the resistance and resilience of soil biota to exogenous disturbances. Soil Biology & Biochemistry 36:1907-1912.

Penalva, M.A., and N. Herbert. 2002. Regulation of gene expression by ambient pH in filamentous fungi and yeasts. Microbiology and Molecular biology reviews. 66:426-446.

Pietikainen, J., and H. Fritze. 1993. Microbial biomass and activity in the humus layer following burning - short-term effects of two different fires. Canadian Journal of Forest Research 23:1275-1285.

Prieto-Fernandez, A., M.J. Acea, and T. Carballas. 1998. Soil microbial and extractable C and N after wildfire. Biology and Fertility of Soils 27:132-142.

Prieto-Fernandez, A., M. Carballas, and T. Carballas. 2004. Inorganic and organic N pools in soils burned or heated: immediate alterations and evolution after forest wildfires. Geoderma 121:291-306.

Raznikiewicz, H., K. Carlgren, and A. Martensson. 1994. Impact of phosphorus fertilization and liming on the presence of Arbuscular Mycorrhizal spores in a swedish long-term field experiment. Swedish Journal of Agricultural Research 24:157-164.

Robichaud, P., L. MacDonald, J. Freeouf, D. Neary, D. Martin, and L. Ashmun. 2004. Postfire rehabilitation of the Hayman fire. USDA Forest Service Gen. Tech. Rep. RMRS-GTR-114. 2003.

Romme, W., T.T. Veblen, M.R. Kaufmann, R. Sherriff, and C.M. Regan. 2003. Ecological effects of the Hayman fire. USDA Forest Service Gen. Tech. Rep. RMRS-GTR-114.

Schutter, M.E., and R.P. Dick. 2000. Comparison of fatty acid methyl ester (FAME) methods for characterizing microbial communities. Soil Science Society of America Journal 64:1659-1668.

Sullivan, T.S., M.E. Stromberger, M.W. Paschke, and J.A. Ippolito. 2006. Long-term impacts of infrequent biosolids applications on chemical and microbial properties of a semi-arid rangeland soil. Biology and Fertility of Soils 42:258-266.

Theodorou, C., and G.D. Bowen. 1982. Effects of a bushfire on the microbiology of a South-Australian low open (Dry Sclerophyll) forest soil. Australian Forest Research 12:317-327.

Thomas, G.W. 1996. Soil pH and soil acidity, p. 475-490, *In* S. DL, ed. Methods of Soil Analysis, Part 3. Chemical Methods. SSSA, Madison, WI.

Vazquez, F.J., M.J. Acea, and T. Carballas. 1993. Soil microbial-populations after wildfire. FEMS Microbiology Ecology 13:93-103.

Weinbauer, M.G., C. Beckmann, and M.G. Hofle. 1998. Utility of green fluorescent nucleic acid dyes and aluminum oxide membrane filters for rapid epifluorescence enumeration of soil and sediment bacteria. Applied and Environmental Microbiology 64:5000-5003.

Whelan, R.J. 1995. The ecology of fire Cambridge University Press, Cambridge ; New York.

Widden, P., and D. Parkinson. 1975. Effects of a forest fire on soil microfungi. Soil Biology & Biochemistry 7:125-138.

Wright, S.F., and A. Upadhyaya. 1996. Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. Soil Science 161:575-586.

Zak, D. R., W.E Holmes, D.C. White, A.D. Peacock et. al. 2003. Plant diversity, soil microbial communities, and ecosystem function: Are there any links? Ecology 84: 2042-2050.

CHAPTER 4

AMELIORATION OF LONG TERM SOIL SCARIFICATION IMPACTS ON SOIL MICROORGANISMS BY A WILDFIRE

Abstract

Nutrient availability is an important constraint on sustainable forest productivity, so it is crucial to understand the long-term effects of management practices, including soil scarification, on microbial drivers of forest soil nutrient dynamics and storage potential. In addition, because forests are subject to wildfires, it would be useful to understand if the effects of wildfire on forest soil ecosystems are attenuated by management practices. We studied the individual and combined effects of soil scarification and subsequent burning on the microbial community structure and function of a ponderosa pine forest soil in the central Rocky Mountains. Experimental plots (4 m^2) were scarified in 1981, and in 2002, some of the plots were burned during a mixed-severity wildfire. In 2005, soil samples (0-10 cm depth) were collected and assayed for soil chemical properties, bacterial and fungal biovolumes, bacterial and fungal C and N mineralization as determined by the substrate induced respiration-inhibition method, and microbial community structure based on ester-linked fatty acid methyl esters (EL-FAMEs). Both disturbance types increased the soil pH from 5.4 to ~ 6 and reduced fungal

biovolumes relative to bacteria. Scarification alone reduced levels of soil C and organic matter (OM) and biovolumes of both fungi and bacteria. In addition, the bacterial community of scarified-only soil was dominated by Gram-positive EL-FAME markers, presumably due to the relatively oligotrophic nature of the soil. Burning alone increased the P and N nutrient content of soil, reduced fungal but not bacterial biovolumes, and increased bacterial N mineralization rates. The high-severity fire also shifted the bacterial community to one dominated by Gram-negative bacteria which is consistent with an increase in nutrient content in soil. Subsequent to scarification treatment, a high-severity fire event caused soil C and OM levels to increase to levels observed in nondisturbed soil and positively impacted the bacterial component of the microbial community, particularly Gram-negative bacteria. Thus, it appears that soil burning due to a high-severity fire event can accelerate the recovery of soil bacteria in scarified soil, presumably due to increased C and OM availability.

Introduction

Soil scarification is any technique that prepares a site to improve accessible seedbed and thus promotes growth of disseminated seed (Manitoba Conservation, 2001). It may involve tilling, disk trenching, or mounding of inverted humus (Johansson, 1994) but it always entails the shallow removal or mixing of the top organic layer of a soil with mineral soil disturbance depending on the method used. This is a common management practice used in silviculture in the United States, Canada, and Europe that has been found to significantly improve seed establishment in Scot pine and aspen ecosystems (Karlsson and Orlander, 2000; Orlander, 1996) as well as for ponderosa pine ecosystem in the Colorado Rocky Mountains (Shepperd, 2006).

Scarification allows for successful seed establishment because once the mineral soil is exposed, seedling roots can penetrate faster than if they had to pass through a thick litter layer. However, it also has been shown that removal or incorporation of the organic horizon from the soil surface by scarification can have long-term impacts on soil carbon dynamics, nutrient cycling, and nutrient storage (Covington, 1981; Johansson, 1994; Rauni, 1992; Vitousek and Matson, 1985). Nutrient availability is an important constraint of sustainable productivity after silvicultural practices or after disturbance. Therefore it is important to understand the long term effects of management practices, such as scarification, on factors which control nutrient cycling and storage, including soil microbial community structure and function.

Microbial biomass functions as a small but dynamic reservoir of available plant nutrients. As soil bacteria or fungi decompose organic matter, nutrients (C, N, and P) are immobilized as microbial biomass components which prevents their loss from soil. As soil microorganisms senesce or are preyed upon, nutrients are released in their mineral form (mineralized) back into the soil solution where they can be readily taken up by plants. The immobilization-mineralization dynamics that retain and provide available nutrients to plants are highly controlled by microbial biomass and activity. Thus alterations to microbial dynamics may negatively affect soil nutrient retention and the capacity of soils to provide available nutrients to plants.

Changes in microbial community structure in response to soil disturbances may also affect nutrient dynamics as well as other processes in soil. While bacteria perform important soil functions, soil fungi 1) degrade recalcitrant materials such as cellulose and lignin, 2) sequester nutrients in recalcitrant cellular materials such as chitin, 3) physically stabilize soil aggregates by hyphal entanglement of soil particles, and 4) are important mutualists of plant roots. Fungi are also sensitive to many types of soil disturbance, including losses of soil OM or soil heating (Wan et al., 2001; Widden and Parkinson, 1975) so disturbance-driven alterations in the ratio of fungi-to-bacteria may affect soil nutrient dynamics as well as other soil processes.

In 1981 a long term study was established to study ponderosa pine regeneration in the Colorado Front Range with the objective, among others, of comparing the effects of 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 ponderosa pine seedlings. After 21 years of monitoring, some of these original plots were burned by the Hayman wildfire of 2002, a devastating mixed-severity fire which burned more than 130,000 acres of Colorado's Pike National Forest. Prior to the fire, the previously scarified plots were not visibly different from unscarified plots, although some above ground effects of scarification such as increased grass cover and reduced sedge cover were documented in 1996 (Anna Schoettle, personal communication). Based on inspection on the sampling day, the thickness of the litter layer that had accumulated at this point in scarified plots was approximately 2 cm compared to a litter layer thickness greater than 2.5 cm in the nonscarified plots.

The objective of this 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. First, we hypothesized that the 1981 soil scarification treatment would have long term impacts on soil microbial and chemical properties. Specifically, mixing of the litter layer would reduce soil C and 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. Secondly, we hypothesized that burning of a previously scarified soil would ameliorate the effects of scarification on soil microbial communities in part because of the generally positive effects of fire on soil nutrient availability. In addition, we wanted to determine if the effects of a recent wildfire on soil microbial and chemical properties would be muted in previously scarified soil compared to nonscarified soil presumably due to reduced fuel levels and decreased thermal input into scarified soil.

Methods

Site description, experimental design, and sampling

Our study site is located within a larger experiment 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, Colorado. The mean annual temperature of the forest is 5° C, the mean annual precipitation is 40 cm, and its mean elevation is 2400 m (Massman and Frank, 2004). The study area is occupied by an overstory of mature ponderosa pine (+150 y 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 seedtree and shelterwood cutting methods, along with scarification and non-scarification of the forest floor, on ponderosa pine seedling establishment and growth. The study design and results are presented in detail by Shepperd et al. (2006), but in brief, the design of the experiment was a split plot with replications in 7 randomized blocks. Whole plot treatments (91 × 91 m) consisted of seedtree and shelterwood cutting methods, and the split plot treatments (31.8 × 31.8 m) were scarification and non-scarification site preparations. Within the split plots were 16 4-m^2 natural regeneration microplots or a grid of 25 planted trees. The shelterwood cut in 1981 left 50 mature seedbearing trees per hectare, and the seedtree cut left 12 trees per hectare. Scarified plots were rototilled to completely incorporate all understory vegetation into soil with a small rubber-tired tractor that could maneuver between plots.

In June 2002, the Hayman fire burned more than 130,000 acres of Colorado's Pike National Forest,. 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, burning nearly 60,000 acres in one day alone (Graham, 2003). Within two weeks of the fire, burn severity was assessed in the 16 natural regeneration microplots of each split plot by estimating percent cover of scorch to vegetation, litter and duff. In the summer of 2005, we located 4 scarified and 4 nonscarified split plots under shelterwood treatments from the seven blocks of the study. Within each scarified and nonscarifed split plot, we used the 2002 burn assessments to choose 1 microplot that was burned at high severity by the Hayman fire and 1 microplot that was burned at low severity giving four treatment combinations to be examined: nondisturbed (nonscarified and nonburned control soil), scarified but nonburned soil, nonscarified but burned soil, and scarified and burned soil. For the purposes of this study, high severity burning consumed organic layers down to the mineral soils in at least 80% of the microplots, while low severity microplots, referred to as nonburned soil, had unscorched duff, litter, and vegetation in at least 80% of the microplot and no burning of the mineral soil.

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. 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 elements. 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 metals, followed by determination of constituent concentrations on an inductively coupled plasma-atomic emission spectrophotometer (Thermo Jarrell Ash Corp., Franklin, MA).

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 grams 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). After addition of 20 μg nonadecanoic acid (19:0) as an internal standard, samples were analyzed by gas chromatography (GC) analysis with an Agilent 6890 gas chromatograph (Agilent Technologies, Inc., Palo Alto, CA) by the University of Delaware. The GC capillary column was an Ultra 2 Agilent #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 (FID) was achieved at a temperature of 250°C using a carrier gas of hydrogen 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; all functions of the GC were under the control of the computer and this method. 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 according to Sullivan et al. (2006).

Biovolumes of bacteria and fungi in soil were determined by direct microscopy techniques. 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). 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 × resolution, 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 160 cells and 48 hyphal fragments (Klein and Paschke, 2000).

Microbial 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; 15mg 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. After equilibration for 16 hrs 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. After this, samples were incubated at 25 °C for 4 hours at which time the CO_2 evolved was measured by gas chromatography (model GC-8A; Shimadzu Scientific Instruments, 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.

Statistical analyses

Statistical analyses were performed using the SAS Statistical Package version 9.1 (SAS Institute Inc, Cary, NC). N mineralization data and EL-FAME data were log transformed prior to analysis to satisfy the assumption of normality. Analysis of variance tests were performed on univariate data (microbial biovolume, mineralized C and N, individual EL-FAMEs, and soil chemical data); mean comparisons were performed using a Fisher-protected LSD (α=0.10). Principal components analysis (PCA) was performed using the correlation matrix on mol % relativized EL-FAME data.

Results

<u>Soil chemistry</u>

Results from the soil chemical analyses are shown in Table 4.1. Soil pH was significantly higher in disturbed soil, regardless of the disturbance. In nondisturbed soil the pH was 5.4, whereas the pH was ~ 6.0 in soils that were scarified, burned, or both scarified and burned. For other soil properties, the disturbance effect was dependent on disturbance type. Regardless of whether the soil was later burned, scarification resulted in reduced levels of total soil C, OM, and extractable Fe, although Fe values were not significantly different from those of burned-only plots. Scarification also resulted in increased soil water content as well as elevated concentrations of extractable Cu in both burned and unburned plots. The high-severity fire, on the other hand, affected extractable soil P concentrations; regardless of whether plots had been scarified, soil P concentrations were greater in plots burned by the high-severity fire. No significant changes were observed in total soil N and extractable NH_4-N and NO_3-N levels in response to scarification or burning, although the general trends in burned plots followed that of P, where values were elevated in response to soil burning.

<u>Microbial Biovolumes</u>

Scarification significantly decreased soil bacterial biovolumes by approximately 50% (Fig. 4.1a), whereas there was no effect of burning on bacterial biovolumes three years after the high-severity fire (*P=0.675*). Total and active

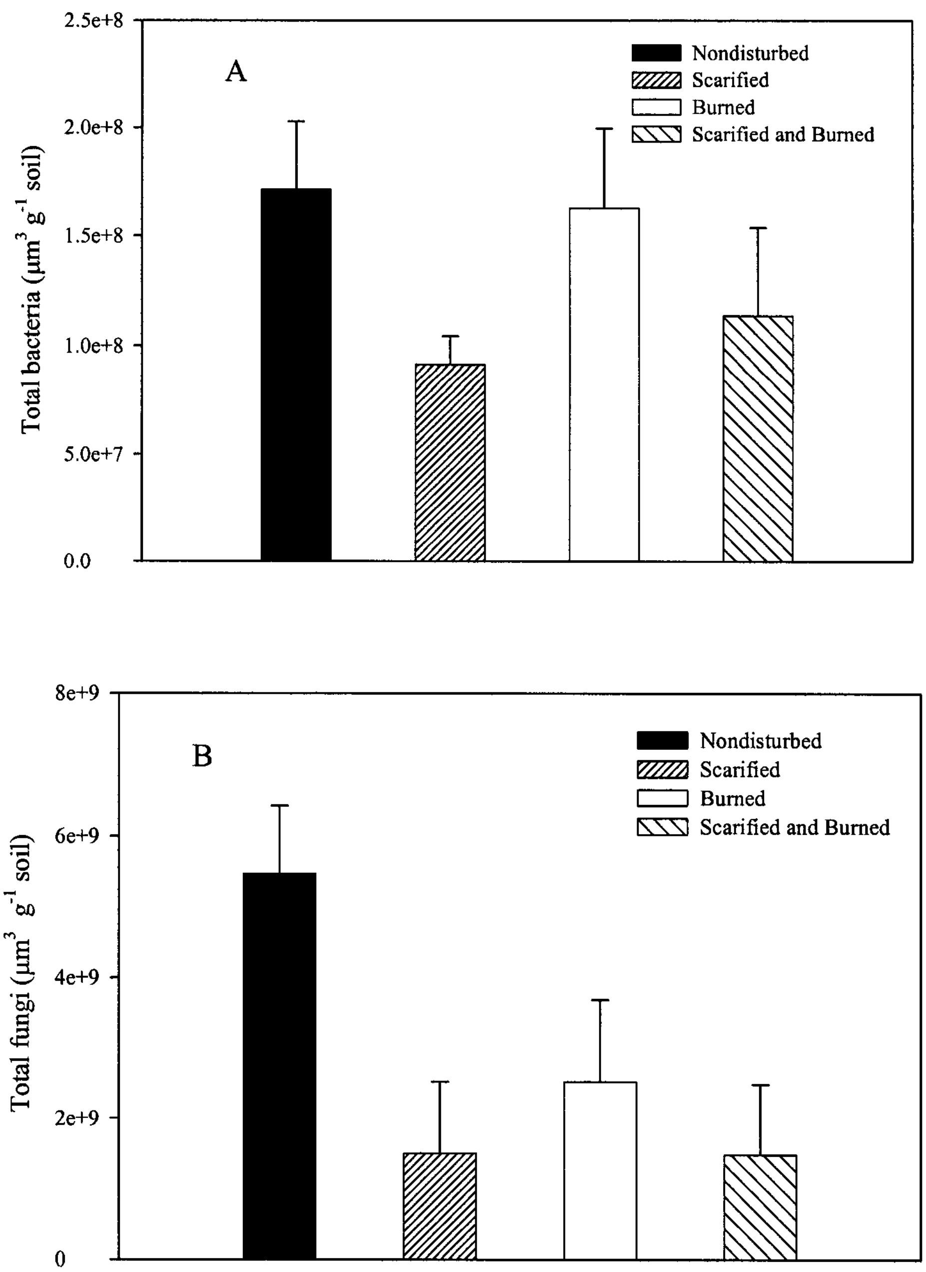
Table 4.1. Soil chemical properties of a forest soil subjected to scarification in 1981 and/or a high-severity fire in 2002. Soil samples (0-10 cm depth) were collected and analyzed in summer 2005. Within columns, mean values followed by different letters are significantly different (α=0.1 level).

Treatment	pH	Water	C	N	OM	NH_4-N	NO_3-N	P	Fe	Cu
		g kg^{-1}				mg kg^{-1}				
Nondisturbed	5.4 b	0.087 b	44 a	2 a	72 a	1.0 a	1.7 a	3.5 b	109 a	0.4 b
Scarified	6.1 a	0.10 a	24 b	1 a	48 b	0.4 a	2.4 a	5.9 ab	56.2 b	0.8 a
Burned	6.0 a	0.94 b	45 a	2 a	69 a	3.8 a	4.4 a	11.2 a	80.2 b	0.4 b
Scarified and Burned	5.8 a	0.12 a	51 a	3 a	67 a	3.8 a	4.8 a	9.6 a	89.9 a	0.9 a

fungal biovolumes were negatively affected by both types of disturbances, either alone or combined; fungal biovolumes were reduced approximately 50% compared to the nondisturbed treatment. As shown in Figs. 4.1b and 4.1c, the negative impact of soil heating on fungi was still visible three years after the fire, although within scarified plots, burning of the soil did not significantly reduce fungal biovolumes any more than did scarification alone. Overall, the ratio of fungal to bacterial biovolumes was lower in disturbed soil than in nondisturbed soil, regardless of disturbance type (Table 4.2). Fungal-to-bacterial biovolume ratios fell from 32:1 in nondisturbed plots to the lowest value of 13:1 in soil that experienced both types of disturbances. In addition, ratio of active-to-total fungal hyphae was significantly lower in burned soil compared to control and scarified-only soil.

Microbial Community Structure

Microbial community structure was assessed by PCA of EL-FAMEs extracted from soil lipids (Fig. 4.2). Principal component 1 (PC 1) separated microbial communities of disturbed soil from microbial communities of the nondisturbed soil. Separation of disturbed soil microbial communities by disturbance type was provided by PC 2, which distinguished between communities of scarified-only versus burned plots. Soil communities to the left of PC 1 (nondisturbed soil) were enriched in fungal markers (18:3ω6c and 18:1ω9c) compared to soil samples on the right of PC 1, which were enriched with Gram-positive and Gram-negative bacterial EL-FAME markers. Based on eigenvector values, communities positioned on the positive side of PC 2 (scarified-only plots)



A
B
Nondisturbed
Scarified
Burned
Scarified and Burned
Total bacteria (µm³ g⁻¹ soil)
Total fungi (µm³ g⁻¹ soil)
2.5e+8
2.0e+8
1.5e+8
1.0e+8
5.0e+7
0.0
8e+9
6e+9
4e+9
2e+9
0

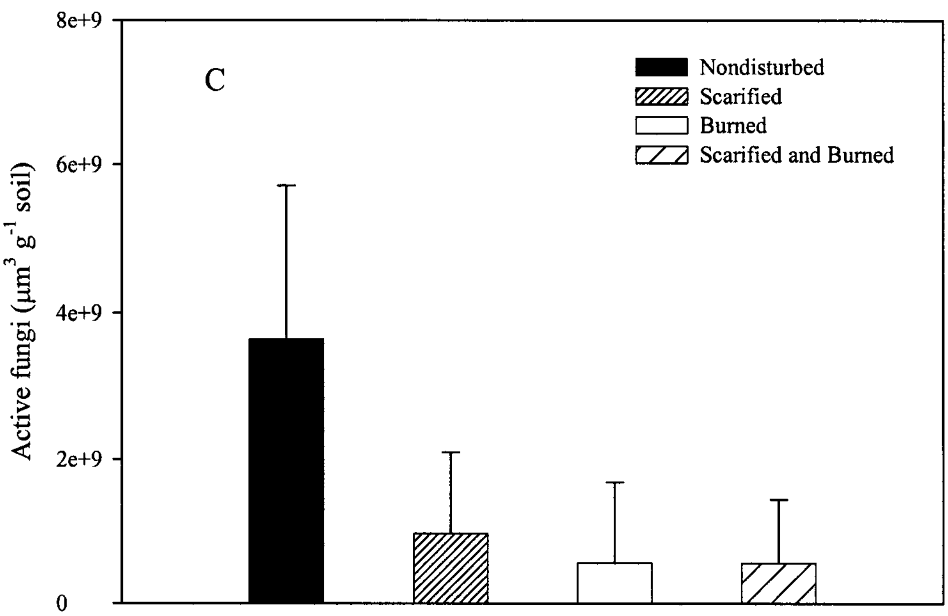


Figure 4.1. Mean biovolumes of total bacteria (A), total fungi (B), and active fungi (C) in forest soil affected by scarification in 1981 and/or high-severity surface fire in 2002. Soil samples (0-10 cm depth) were collected and analyzed in summer 2005. Standard deviation bars (± 2) are shown.

Table 4.2. Ratio of total soil fungal to bacterial biovolumes and active to total fungal biovolumes in a forest soil affected by scarification in 1981 and/or a high-severity fire in 2002. Soil samples were collected and analyzed in summer 2005. Means followed by different letters are significantly different at α=0.10.

Treatment	Fungal:Bacterial Biovolume Ratio	Active fungi:Total fungi Ratio
Nondisturbed	32.0 a	0.67 a
Scarified	15.5 b	0.64 a
Burned	16.4 b	0.22 b
Scarified and burned	13.0 c	0.38 b

contained elevated amounts of Gram-positive EL-FAME markers i15:0, a15:0, i16:0, a16:0, i17:0, a17:0, and the Gram-negative EL-FAME marker 16:1 2 OH. Communities with negative positions on PC 2 (burned only and scarified plus burned plots) were enriched in Gram-negative EL-FAME markers 17:0 and 19:0 cy and the marker for sulfate reducing bacteria, 17:1ω7c.

Bacterial and Fungal C and N mineralization

No significant differences in fungal and bacterial respiration rates were found among the plot treatments (Table 4.3). Total (fungal + bacterial) N mineralization increased two-fold in response to burning but not scarification (Table 4.3). Bacterial N mineralization followed the same trend as total N mineralization, which was ~ two-fold greater in burned plots than in nondisturbed and scarified-only plots. The relative contribution of fungi to N mineralization was measured in this study but no significant differences were found among the plots.

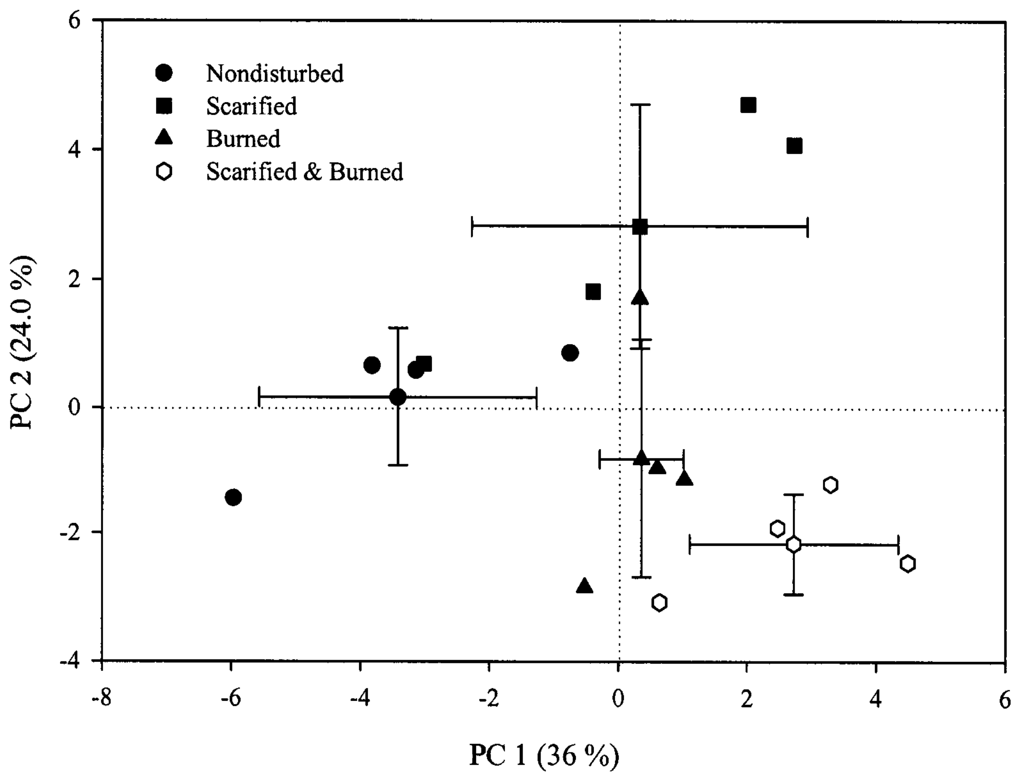


Figure 4.2. Principal components analysis of the microbial community structure based on EL-FAMES profiles in a forest soil that was scarified in 1981 and/or burned in high-severity surface burn in 2002. Soil samples (0-10 cm depth) were collected and analyzed in 2005. Individual samples as well as the average PC scores for each treatment are shown along with standard deviations (± 2). The amount of variability explained by each PC axis is shown in parentheses.

Table 4.3. Amounts of C and N mineralization over a 4-hr period by fungi, bacteria, and both (total) in a forest soil affected by scarification in 1981 and/or a high-severity fire in 2002. Soil samples were collected and assayed in summer 2005. Means followed by the same letter are significantly different at the 0.1 level.

Treatment	**C mineralization**			**N mineralization**		
	Total	**Fungi**	**Bacteria**	**Total**	**Fungi**	**Bacteria**
	------------**$\mu g\ CO_2\ g^{-1}$**-----------			--------------**$mg\ N\ kg^{-1}$**---------		
Nondisturbed	8.03 a	6.23 a	6.36 a	0.29 b	1.94 a	0.51 bc
Scarified	10.2 a	8.51 a	9.38 a	0.74 ab	1.85 a	0.44 c
Burned	7.30 a	5.66 a	6.35 a	1.52 b	2.57 a	1.17 a
Scarified and Burned	7.40 a	5.40 a	5.44 a	2.21 a	2.08 a	0.82 ab

Discussion

Scarification, which was applied to these plots 21 years before the wildfire occurred in 2002, has been known to improve seed establishment and to control invasive plant establishment, (Karlsson and Orlander, 2000; Karlsson et al., 2002) but it also alters organic matter decomposition dynamics and nutrient cycling in soils (Lundmark-Thelin and Johansson, 1997). The effects of scarification on soil organisms are relatively unknown. Ohtonen et al. (1992) reported that scarification had no effects on microbial biovolumes or microbial biomass C and N five years after scarification in a white pine plantation in central Ontario, but our study demonstrates that scarification practices can have long term (> 20 y) impacts on soil properties. These differences may be due to climatic differences between the two sites. Compared to nondisturbed plots, scarification resulted in soils with significantly lower total C and OM contents but greater water with the exception of an elevation in extractable Cu and a reduction in extractable Fe. Differences in time since scarification, climate, and vegetation type could be accountable for the difference between these reports and our observations.

Microbial community structure was still affected 21 years after scarification. Based on biomarker EL-FAMEs, the microbial community of the scarified plots seems to be dominated by Gram-positive bacteria compared to communities on nondisturbed and burned soil. The high-severity fire, in contrast, shifted the bacterial community towards more Gram-negative organisms. Most Gram-positive bacteria are known *k* strategists and are selected for in environments with low nutrients (oligotrophic environments) (Yao et al., 2000), and this is consistent with the fact that scarification led to reduced C supplies in soil. On the other hand, many Gram-negative bacteria are *r*-strategists which favor copiotrophic environments, which also correspond with the observations that fire has increased the available nutrient content of the soil (Andrews and Harris, 1986).

Three years after the Hayman fire, the effects of soil burning were consistent with the effects of fire found by other studies on soil and soil microorganisms (Acea and Carballas, 1996; Acea and Carballas, 1999; Certini, 2005; Hart et al., 2005). We observed an increase in soil pH, greater amounts of extractable P and N, and decreased fungal biovolumes compared to bacterial biovolumes. Also, we observed a significant increase in bacterial N mineralization activity, and although not statistically significant, a slight decrease in fungal C mineralization activity consistent with the idea that fungi were more sensitive to fire than bacteria. While both scarification and soil burning reduced total fungal contents at the time of sampling. Although not statistically significant, the tendency for greater C mineralization activity, as measured by the substrate induced respiration assay, also provides evidence for an apparent C limitation in scarified soil. The reduction in soil C resources may explain the overall reduction in microbial biovolumes in scarified soil, as well as the shift towards more Gram-positive bacteria, which tend to be associated with oligotrophic environments (Yao et al., 2000). Altogether, soil C pools still appear to be negatively impacted by the scarification treatment, despite the litter accumulation over the last 20 years. Reduction of organic matter in soil is associated with an increase in rates of organic matter decomposition. Based on the increased mineralization activity in scarified soil (Table 3), it appears that decomposition is higher in the scarified soil compared to the nondisturbed soil, which might have implications for C sequestration in soil.

Because different scarification techniques have been used in other studies, it is difficult to extrapolate broader impacts regarding the long term effects of scarification on soil nutrient dynamics, and conflicting reports are common. It has been documented that scarification increases the nutrient content of soil in the short term. For example, litter decomposition rates and the release of Ca and K into the mineral soil were faster in scarified soil compared to nonscarified soil (Johansson, 1994), and Orlander et al. (1996) reported an increase in P, Mg, and S in soils that were scarified compared to nonscarified soils. Ring (1996) also reported an increase in soil NO_3-N after scarification. However, our study found little changes to soil nutrients other than C twenty one years after scarification, biovolumes, the short term effects of soil burning may be more detrimental to fungi as evidenced by the ratio of active-to-total fungal hyphae in soil. Whereas scarification did not affect the ratio of active to total fungi, soil burning reduced the proportion of fungal hyphae that is active (cytoplasm-filled).

For soil that experienced both disturbances, some of the scarification effects were ameliorated by the effects of the fire. For example, burning of scarified soil increased the OM content so that its level was similar to that of nondisturbed plots. Burning of scarified soil also altered the microbial community structure; bacterial biovolumes increased slightly compared to biovolumes in scarified-only soil (Fig. 1a), and overall, the microbial community structure became more similar to that of burned-only soil. Thus, a wildfire event can increase the fertility, in terms of OM content, of scarified soil and can alter the microbial community structure of the scarified soil by increasing the relative amounts of bacteria, particularly Gram-negative bacteria. In contrast, we did not observe many differences between burned-only and scarified-burn plots that could be attributed to a scarification-burn interaction. With the exception of extractable Fe content, it did not appear that the scarification treatment altered the impact of the high-severity fire on soil chemical and biological properties. However, it is possible that scarification may have influenced fire impacts in the very short term (< 3 y) or perhaps in the long term (> 3 y).

As hypothesized, soil scarification did result in long term reductions in soil C and OM as well as biovolumes of both fungi and bacteria. However, the impact was more severe on soil fungi than bacteria, with fungal biovolumes being reduced by ~70% compared to the ~50% reduction in bacterial biovolumes. It is not possible to determine if the scarification event itself was directly responsible for the long term loss of microbial biovolumes, but the displacement of the litter layer may have altered subsequent vegetation patterns, litter deposition and decomposition rates, or other soil properties (e.g., soil temperature) which then may negatively impact the soil microbial community in the long term. Second, subsequent burning of scarified plots did ameliorate some of the scarified soil chemical and biological properties, namely soil C and OM levels and the Gram-negative bacterial component of the scarified soil community. Burning did not significantly alter microbial community functions, in terms of C and N mineralization activities, in scarified soil. In addition, the previous scarification treatment did not greatly affect the impacts of a subsequent high-severity fire event on the soil chemical and biological properties measured. However, these conclusions are based on one sampling event in the middle of the growing season (summer of 2005) and thus limited inferences can be drawn. Long term monitoring of these plots is necessary to determine if and when belowground communities in scarified soils are able to recover to sustain soil ecosystem function as required by the aboveground community.

References

Acea, M.J., and T. Carballas. 1996. Changes in physiological groups of microorganisms in soil following wildfire. FEMS Microbiology Ecology 20:33-39.

Acea, M.J., and T. Carballas. 1999. Microbial fluctuations after soil heating and organic amendment. Bioresource Technology 67:65-71.

Anderson, J.P.E., and K.H. Domsch. 1975. Measurement of bacterial and fungal contributions to respiration of selected agricultural and forest soils. Canadian Journal of Microbiology 21:314-322.

Andrews, J.H., and R.F. Harris. 1986. r- and K-selection and microbial ecology, p. 99-147, *In* K. C. Marshall, ed. Advances in Microbial Ecology, Vol. 9. New York, N.Y: Plenum Press, New York.

Bloem, J., M. Veninga, and J. Shepherd. 1995. Fully-automatic determination of soil bacterium numbers, cell volumes, and frequencies of dividing cells by confocal laser-scanning microscopy and image-analysis. Applied and Environmental Microbiology 61:926-936.

Bossio, D.A., and K.M. Scow. 1998. Impacts of carbon and flooding on soil microbial communities: Phospholipid fatty acid profiles and substrate utilization patterns. Microbial Ecology 35:265-278.

Certini, G. 2005. Effects of fire on properties of forest soils: a review. Oecologia 143:1-10.

Covington, W. 1981. Changes in forest floor organic matter and nutrient content following clearcutting in northern hardwoods. Ecology 5:376-380.

Graham, R.T. 2003. Hayman fire case study: Summary. Gen. Tech. Rep., *In* F. S. Department of Agriculture, Rocky Mountain Research Station, (ed.), Vol. RMRS-GTR-115.

Hart, S.C., T.H. DeLuca, G.S. Newman, M.D. MacKenzie, and S.I. Boyle. 2005. Post-fire vegetative dynamics as drivers of microbial community structure and function in forest soils. Forest Ecology and Management 220:166-184.

Johansson, M.B. 1994. The influence of soil scarification on the turn-over rate of slash needles and nutrient release. Scandinavian Journal of Forest Research 9:170-179.

Johnson, C.K., M.F. Vigil, K.G. Doxtader, and W.E. Beard. 1996. Measuring bacterial and fungal substrate-induced respiration in dry soils. Soil Biology & Biochemistry 28:427-432.

Karlsson, C., and G. Orlander. 2000. Soil scarification shortly before a rich seed fall improves seedling establishment in seed tree stands of *Pinus sylvestris*. Scandinavian Journal of Forest Research 15:256-266

Karlsson, M., U. Nilsson, and G. Orlander. 2002. Natural regeneration in clear-cuts: effects of scarification, slash removal and clear-cut age. Scandinavian Journal of Forest Research 17:131-138.

Klein, D.A., and M.W. Paschke. 2000. A soil microbial community structural-functional index: the microscopy-based total/active/active fungal/bacterial (TA/AFB) biovolumes ratio. Applied Soil Ecology 14:257-268.

Lezberg, Ann. Personal communication. January 14, 2006.

Lodge, D.J., and E.R. Ingham. 1991. A comparison of agar film techniques for estimating fungal biovolumes inlitter and soil. Agriculture Ecosystems & Environment 34:131-144.

Lundmark-Thelin, A., and M.B. Johansson. 1997. Influence of mechanical site preparation on decomposition and nutrient dynamics of Norway spruce (*Picea abies* (L.) Karst.) needle litter and slash needles. Forest Ecology and Management 96:101-110.

Manitoba Conservation, Forestry Branch Website. 2001. Scarification for natural regeneration.www.gov.mb.ca/conservation/forestry/forest-renewal/pdfs/scarification2.pdf. Accessed January 2, 2006.

Massman, W.J., and J.M. Frank. 2004. Effect of a controlled burn on the thermophysical properties of a dry soil using a new model of soil heat flow and a new high temperature heat flux sensor. International Journal of Wildland Fire 13:427-442.

Moore, R. 1992. Soil survey of Pike National Forest, eastern part, Colorado, parts of Douglas, El Paso, Jefferson, and Teller counties, pp. 106, *In* C. USDA Forest Serv. and Soil Conservation Serv.; in cooperation with the Colo. Agric. Exp. Stn Denver, (ed.).

Ohtonen, R., A. Munson, and D. Brand. 1992. Soil microbial community response to silvicultural intervention in coniferous plantation ecosystems. Ecological Applications 2:363-375.

Orlander, G., E. Egnell, and A. Albrektson. 1996. Long-term effects of site preparation on growth in Scots pine. Forest Ecology and Management 86:27-37.

Rauni, O., M. Alison, and D. Brand. 1992. Soil microbial communtity response to silvicultural intervention in corniferous plantation ecosystem. Ecological Applications. 2:363-375.

Ring, E. 1996. Effects of previous N fertilization on soil water pH and N concentrations after clear-felling and soil scarification at a *Pinus sylvestris* site. Scandinavian Journal of Forest Research 11:7-16.

Schutter, M.E., and R.P. Dick. 2000. Comparison of fatty acid methyl ester (FAME) methods for characterizing microbial communities. Soil Science Society of America Journal 64:1659-1668.

Shepperd, W., Edminster, C. and S. Mata. 2006. Long term seedfall, establishment, survival, growth of natural and planted Ponderosa pine in the Colorado Front Range. Western Journal of American Forestry 20:0.

Sullivan, T.S., M.E. Stromberger, M.W. Paschke, and J.A. Ippolito. 2006. Long-term impacts of infrequent biosolids applications on chemical and microbial properties of a semi-arid rangeland soil. Biology and Fertility of Soils 42:258-266.

Vitousek, P.M., and P. Matson. 1985. Intensive Harvesting and site preparation decreases soil N availability in young plantations. Southern Journal of Applied Forestry 9:120-125.

Wan, S.Q., D.F. Hui, and Y.Q. Luo. 2001. Fire effects on nitrogen pools and dynamics in terrestrial ecosystems: A meta-analysis. Ecological Applications 11:1349-1365.

Weinbauer, M.G., C. Beckmann, and M.G. Hofle. 1998. Utility of green fluorescent nucleic acid dyes and aluminum oxide membrane filters for rapid epifluorescence enumeration of soil and sediment bacteria. Applied and Environmental Microbiology 64:5000-5003.

Widden, P., and D. Parkinson. 1975. Effects of a forest fire on soil microfungi. Soil Biology & Biochemistry 7:125-138.

Yao, H., Z. He, M.J. Wilson, and C.D. Campbell. 2000. Microbial biomass and community structure in a sequence of soils with increasing fertility and changing land use. Microbial Ecology 40:223-237.

CHAPTER 5

MICROBIAL COMMUNITY STRUCTURE AND ACTIVITY IN A FOREST SOIL SCARRED BY SLASH PILE BURNING

Abstract

In the western United States, thinning for restoration purposes, in combination with traditional harvesting practices, produces large amounts of slash material which are disposed of by burning them in large piles. This results in severe soil scorching which leaves scars behind that are aesthetically unpleasant and may contribute to the establishment and spread of nonnative plants. Moreover, the ability of scarred soils to function normally as a plant growth medium is likely impaired, but for how long is unknown. In this study, we characterized the effects of slash pile burning on soil microbial community structure and function, and we monitored soil microbial recovery over two growing seasons. We established an experimental site at the Manitou Experimental Forest in Colorado in which we where able to obtain real time data on soil temperature, heat flux, and soil moisture contents while a slash pile was burned during spring of 2004. Soil underneath the pile reached temperatures as high as 300 °C in the center of the pile and 175 °C at the edge of the pile. We measured microbial community structure by ester-linked fatty acid methyl ester (EL-FAME) analysis, bacterial and fungal biovolumes by direct microscopy, and bacterial and fungal C and N mineralization activities by

the substrate induced respiration inhibition assay at the end of the burn to correlate changes in soil physical and chemical properties with changes in soil microbiological properties. These sites where monitored seasonally for 15 months after the burn. Our results indicate that slash pile burning increased soil N and P; however, at the conclusion of this study total C was still low in burned soils, pH remained elevated, microbial C and N mineralization activities were still below normal levels, and the AM fungal EL-FAME marker was not detectable in the burned soil. These detrimental changes may have a broad range of implications for ecosystem function and management because of the important roles microorganisms play in providing nutrients to plants and improving soil structure and stabilization.

Introduction

Over 100 years of fire suppression and over grazing by cattle have left profound changes to the some of ponderosa pine forests of Colorado's Rocky Mountains. 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 (Romme et al., 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 currently 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, the geometry of these piles results in a fuel load that when burned, produces an extreme heat pulse into the soil. This results in severe soil scorching which leaves scars behind that are unattractive and may contribute to the establishment and spread of non-native plants (Dickinson and Kirkpatrick, 1987; Korb et al., 2004).

Without restoration efforts, the soil remains charred many years after burning, and vegetation density remains low. This raises the question of how these slash piles are impacting the ecosystem and also whether this management practice meets with restoration objectives. Even though slash pile burning is a means of disposal preferred amongst forest managers, studies considering the short and long term effects of it on below and above ground processes are scarce. Moreover, re-vegetation of scars by native plants may be delayed due to changes in soil chemical and microbiological properties as a result of the slash pile burn. For example Korb et al. (2004) determined that slash pile burning nearly eliminated arbuscular mycorrhizal (AM) fungal propagules and 15 months after the burn, AM fungi were still negatively affected. Also, one year after the burn, they found greater amounts of viable seeds of exotic plants in the perimeters of the slash pile compared to nonburned soil, consistent with an increase in nutrient content after a burn. This study suggested that after a fire, above ground dynamics reflected the changes occurring below ground with particular functional groups of microorganisms.

This research project investigated how slash pile burning can influence forest soil ecosystems by measuring its impact on soil biological properties within the Manitou Experimental Forest. First, we hypothesized that changes to the microbial community at the soil surface will be larger than the changes to the community at lower depths because the insulating capacity of soils. Second, microbial activity will 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, we hypothesized that microbial communities at the edge of the pile will be more active immediately after the fire because of the flush of nutrients associated with soil heating events and the lower thermal input associated to the geometry of the fuel at the edge of the pile compared to the center of the pile. Also, we expected that as soon as the nutrients returned to background levels, the fire effects on soil microorganisms would be similar regardless of location within the pile.

The overall goal of this study was to improve our understanding of soil ecological responses to vegetation manipulation by fire, including the physiological mechanisms for soil regeneration and early stand dynamics, so that we can improve our ability to develop new silvicultural treatments that will sustain and maintain a broader spectrum of ecological conditions.

Methods

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, Colorado. The mean annual temperature of the forest is 5° C, the mean annual precipitation is 40 cm, and its mean elevation is 2400 m (Massman and Frank, 2004). 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 (Shepperd, 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).

The experimental design consisted of two replications each of a nonburned and a slash pile treatment. 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. In August of 2003 four 1.5-m deep pits were dug at locations which would be at the center and at the edge of the yet-to-be-built slash pile as well two areas where no pile was to be built. Trenches were dug from each pit to place the wires connecting the sensors in the pits (thermocouples, transducers, and TDR probes) to the CR23X data loggers (Campbell Scientific, Logan, UT) centrally located 30 m away. Thermocouples (Omega Engineering, Stamford, CT) to measure soil temperature were installed in the pits 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. Heat flux tranducers (East 30 Sensors, Pullman, WA) were installed at depths of 0, 2, 5, 15, and 20 cm, and TDR probes to measure soil moisture were installed at depths of 5 and 20 cm. Specially designed high-temperature TDR probes (Zostrich Geotechnical, Pullman, WA) were used at the slash pile burn site, whereas commercially available TDR probes (Campbell Scientific, Logan, UT) were used at the nonburned site.

After installation, the pit and trench were carefully backfilled and slash piles were build over the instrumented site as well as a second site located approximately 0.48 km south. The slash piles were conical in shape (approximately 6 m x 9 m in diameter) with an approximate fuel loading estimated to be 560 t ha^{-1}. At the noninstrumented site, an area near the slash pile was designated as the second control (nonburned) replicate. On April 26, 2004 the slash piles were ignited and allowed to burn until the fire had consumed the fuel. Measurements of soil temperature, heat flux, and soil moisture content were recorded in the data loggers 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-to-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 elements. 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 metals, followed by determination of constituent concentrations on an inductively coupled plasma-atomic emission spectrophotometer (Thermo Jarrell Ash Corp., Franklin, MA).

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 grams 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 according to Sullivan et al. (2006)

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).

Microbial C and N mineralization activities

Triplicate subsamples (12 g dry weight) of each soil were moistened to 60 % field capacity and inhibitors (4mg g^{-1} soil for streptomycin to inhibit bacteria; 15mg 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 hrs 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. After this, samples were incubated at 25 °C for 4 hours 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.

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 EL-FAME data for each sampling time using the autoregressive covariance structure and time after fire (season), depth, and treatment (pile edge, pile center, or nonburned) as class variables. Analysis of variance tests were performed on univariate data (microbial biovolume, mineralized C and N, individual PCA scores, and soil chemical data); mean comparisons were performed using a Fisher-protected LSD (□=0.10). Principal components analysis (PCA) was performed using the correlation matrix on EL-FAME data that was log transformed and relativized as mol %.

Results

Soil temperature, heat flux, and moisture content during burn

Changes in soil temperature recorded during the burn are shown in Fig. 5.1. 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. Surface soil (0-5 cm) at the edge of the pile experienced temperatures above 100° C for around 10 hours, whereas surface soil at the center of the pile experienced above-100°C temperatures for over 17 hours and over 8 hours at the lower depth (5-15 cm). Peak temperatures in surface soils corresponded to peak fluxes of heat into soil as shown in Fig. 5.2, with large fluxes occurring between 4-14 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.

Moisture levels in the soil at the pile edge (0-5 cm depth) were constant around 0.22 g g^{-1} soil until 18 h after the fire started, and then it dropped to 0.20 g g^{-1} soil (Fig. 5.3). At the lower depth of the pile edge, the water content remained

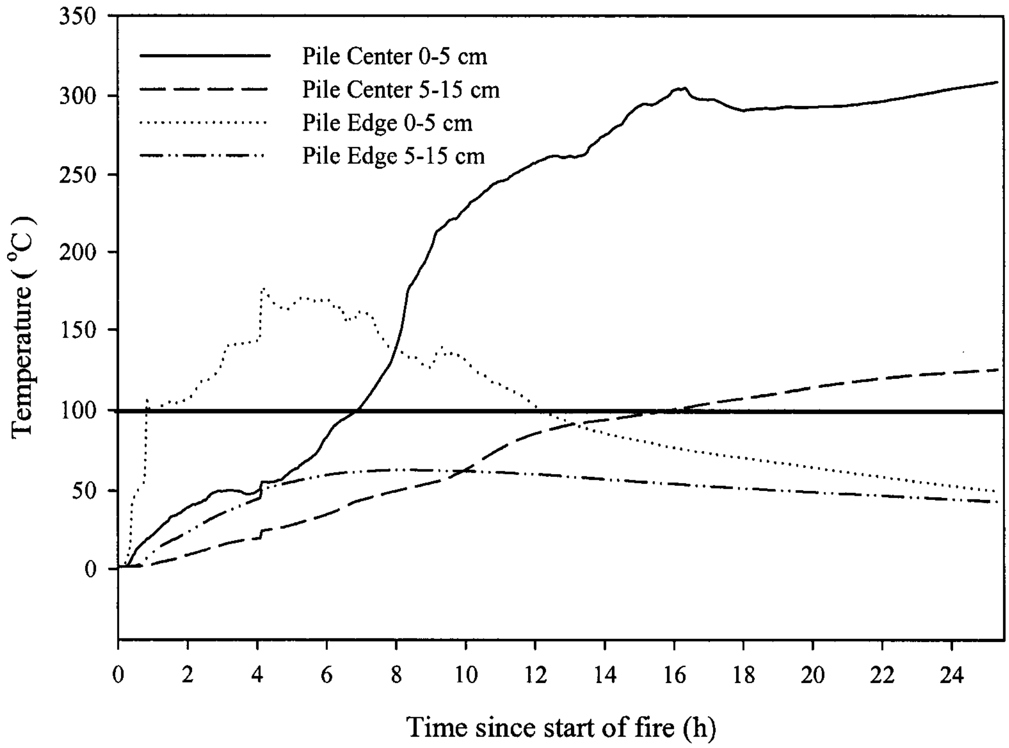


Figure 5.1. Soil temperatures (0-5 and 5-15 cm depths) at the edge and the center of a slash pile as it burned on April 26, 2004. A reference line is set at 100 °C, temperature threshold for bacterial and fungal sterilization in wet soils (Dunn et al., 1979).

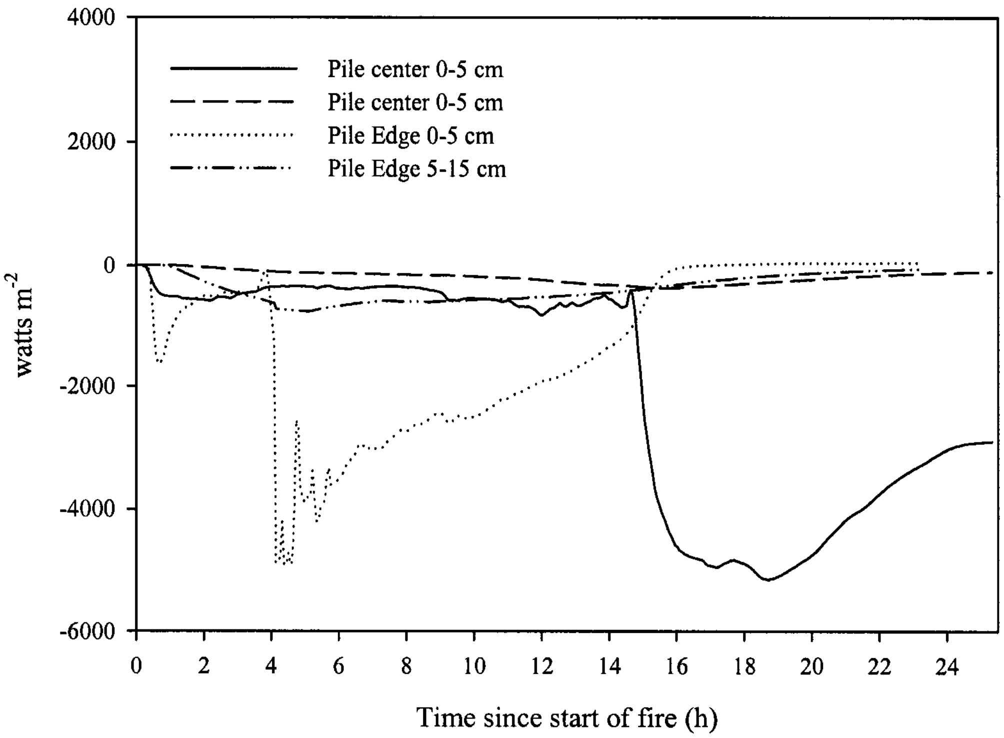


Figure 5.2. Heat flux in soil (0-5 and 5-15 cm depths) at the edge and the center of a slash pile as it burned on April 26, 2004.

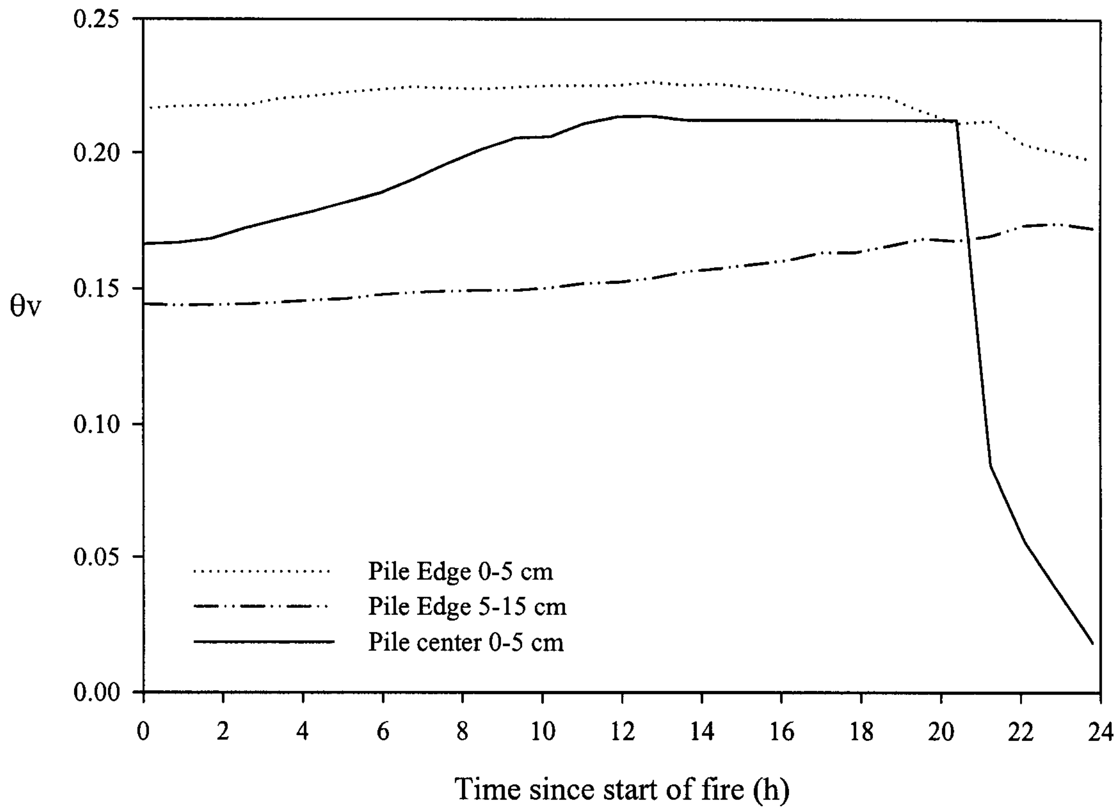


Figure 5.3. Moisture content in soil (0-5 and 5-15 cm depths) at the edge and the center of a slash pile as it burned on April 26, 2004. The moisture level at the lowest depth (5-15 cm) in the center of the pile is not reported here because the sensors were non-functional during the burn.

constant at 0.15 g g^{-1} soil until 18 hours after the fire started and then it slowly increased to 0.16 g g^{-1} soil, 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 g g^{-1} soil during the first 20 h and then suddenly dropped to about 0.01 g g^{-1} soil.

Soil chemical properties

Soil pH in nonburned soil was typically around 5.5, a typical value for soil pH in this ponderosa pine ecosystem. After the slash pile burn, however, the soil pH was between 6.5 and 8 throughout the study (Table 5.1). At the end of this study, summer of 2005, the pH in the burned soil was still above 6.5.

As shown in Table 5.2, total soil C was always higher in the nonburned soil compared to the burned soil at both locations, except immediately (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 nonburned control soil. No differences were seen between soil depths nor was there a time effect on soil C.

Concentrations of inorganic N and extractable P (Tables 5.3 and 5.4, respectively) were significantly greater in the burned soil compared to nonburned soil for the duration of this study. For both inorganic N and extractable P, the changes observed were dependent on fire intensity 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 5.1. pH of burned and nonburned soil determined overtime after a slash pile burn. Mean comparisons are among treatments (among columns) and means with different letters are significantly different at the 0.10 level.

Time (Months)	Nonburned	Pile Edge	Pile Center
0.25	5.76 b	6.35 a	6.38 a
1	5.25 b	6.88 a	6.98 a
3	5.51 b	6.52 a	6.53 a
6	5.40 c	7.20 b	7.65 a
12	5.38 c	7.03 b	7.78 a
15	5.53 b	6.52 a	6.74 a

Table 5.2. Amounts of soil organic C (g kg^{-1} soil) in burned and nonburned soil determined over time after a slash pile burn. Mean comparisons are among treatments (among columns) and means with different letters are significantly different at the 0.10 level.

Time (months)	Nonburned	Pile Edge	Pile Center
	--------------------g C kg^{-1} soil------------------		
0.25	16.6 b	21.1 a	12.2 c
1	24.9 a	15.3 b	13.6 b
3	18.8 ab	21.7 a	14.0 b
6	19.6 a	14.6 a	15.4 a
12	21.7 a	16.1 b	11.4 b
15	30.5 a	16.5 b	17.5 b

Table 5.3. Amounts of inorganic N (NH_4-N and NO_3-N) in burned and nonburned soil overtime after a slash pile burn. Mean comparisons are among treatments (among columns) and means with different letters are significantly different at at the 0.10 level.

Time (months)	Nonburned	Pile Edge	Pile Center
	----------mg N kg^{-1} soil----------		
0.25	6.3 c	37.7 b	61.9 a
1	12.4 b	77.0 a	75.0 a
3	8.0 c	53.7 b	82.0 a
6	4.6 b	55.9 a	56.0 a
12	6.1 b	54.9 a	34.7 a
15	25.9 b	66.4 a	87.4 a

Table 5.4. Extractable P in burned and nonburned soil determined overtime after a slash pile burn. Mean comparisons are among treatments (among columns) and means with different letters are significantly different at the 0.10 level.

Time (months)	Nonburned	Pile Edge	Pile Center
	----------mg P kg^{-1} soil----------		
0.25	2.2 b	7.0 b	38.8 a
1	1.9 b	24.6 a	32.9 a
3	2.2 c	17.7 b	42.5 a
6	24.5 a	24.6 a	56.0 a
12	1.7 b	47.4 a	39.4 a
15	2.9 c	28.1 b	38.4 a

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. 5.4, the immediate effect of slash pile burning was to significantly reduce bacterial biovolumes from 10^8 in the nonburned soil to 10^7 and 10^6 μm^3 g^{-1} soil in the pile edge and pile center, respectively (RM ANOVA $P<0.0001$). There was no significant effect of time nor of depth, and values shown in Fig. 5.4 are averaged across the two soil depths. Fifteen months after the burn, bacterial biovolumes had not recovered to nonburned levels at either location within the pile, but the 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 nonburned soil were generally 100-fold greater than bacterial biovolumes in nonburned soil, but the burning the 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 μm^3 g^{-1} soil initially, and to near zero values 6 months after the fire (Fig. 5.5). Changes to fungal biovolumes depended on depth and seasonality (RM ANOVA $P=0.0014$). At the lower depth, fungal biovolumes were not significantly affected by slash pile burning.

Microbial community structure

Principal components analysis of EL-FAME data collected one month after the burn revealed that microbial community structure was affected significantly by

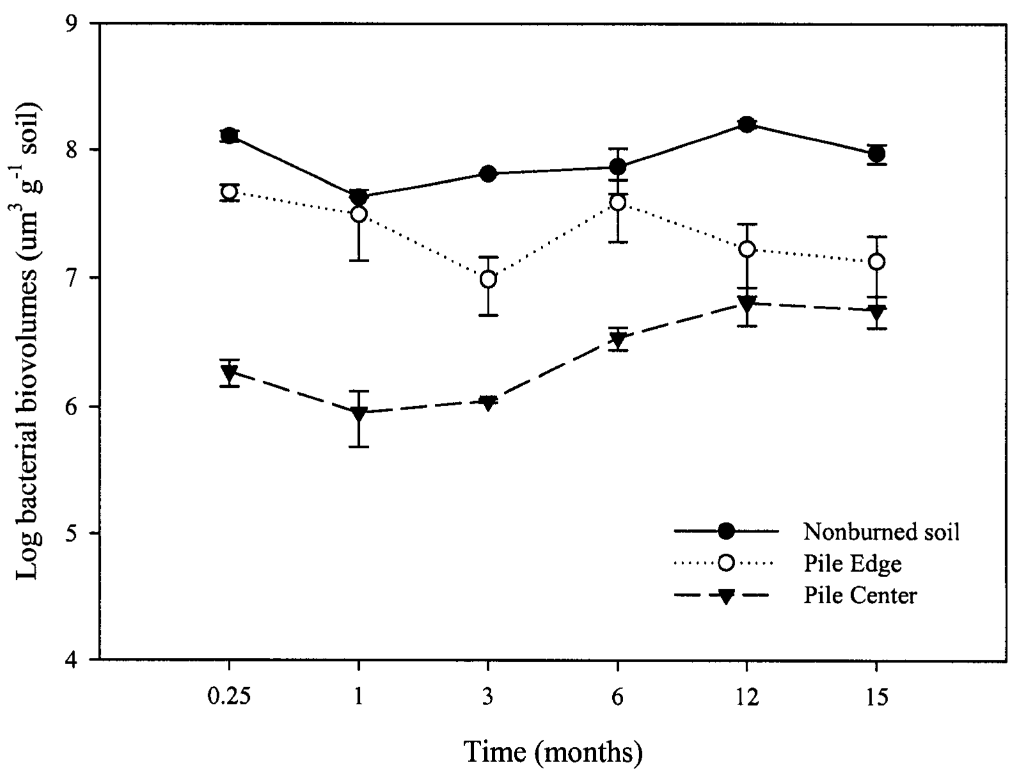


Figure 5.4. Biovolumes of total bacteria (0-15 cm) in soil affected by a slash pile burn. Standard error bars are shown.

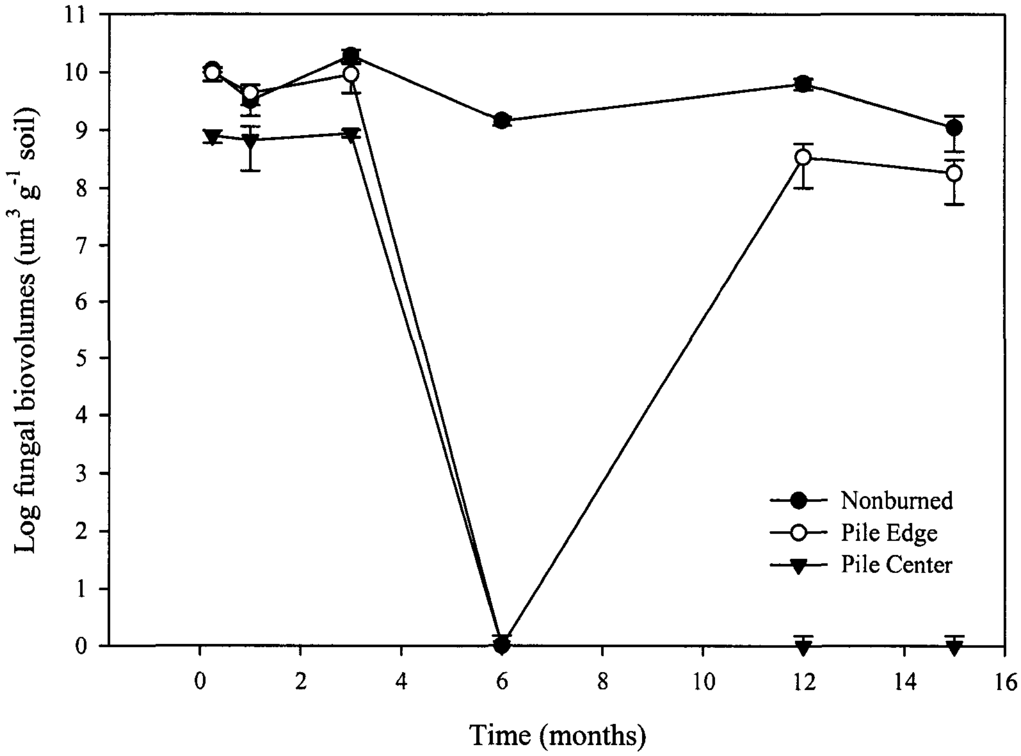


Figure 5.5. Biovolumes of total fungi (0-15 cm) in soil affected by a slash pile burn. Standard error bars are shown.

slash pile burning at both depths sampled. Two principal components (PCs) were extracted that accounted for 71.5 % of the variability in the EL-FAME data set. As shown in Fig. 5.6, microbial communities in the nonburned 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 by PC 1 and PC 2. Communities to the right of PC 1 (pile edge and most of the nonburned soil communities) were enriched with the Gram-negative bacterial markers 16:1ω7, 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 15:0 iso/anteiso, 16:0 iso, and 17:0 iso/anteiso. 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 17:0 iso. Separations along PC 1 and PC 2 were significant (*P=0.08* and *P=0.03*, respectively)

Fifteen months after the burn, principal component analysis showed a distinct separation of microbial communities by treatment (Fig. 5.7). 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 (burned soil) were enriched with the Gram-positive bacterial markers 15:0 iso/anteiso and 17:0 anteiso and Gram-negative markers bacterial 17:0 cy and 19:0 cy. No fungal markers were dominant in the communities on the positive side of PC 1. Microbial communities

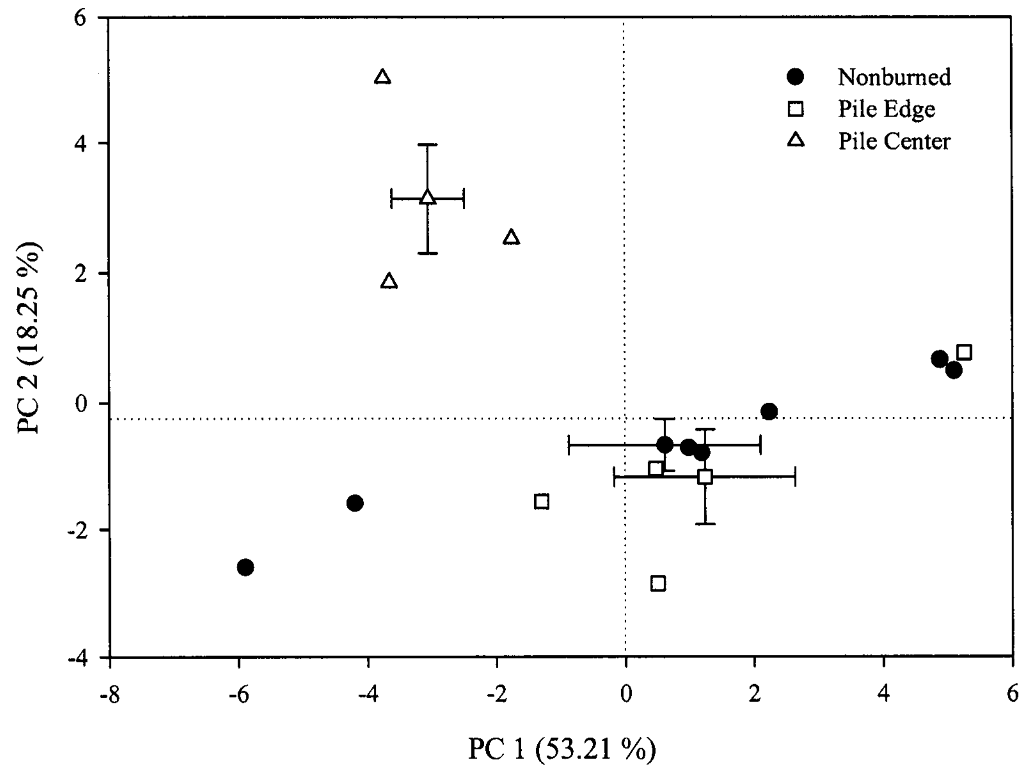


Figure 5.6. Principal components analysis of the microbial community structure in the nonburned and burned soil (0-15 cm) one month after slash pile burning. Individual data points as well as the average PC scores for each treatment are shown along with standard error bars. The amount of variability explained by each PC is shown in parentheses.

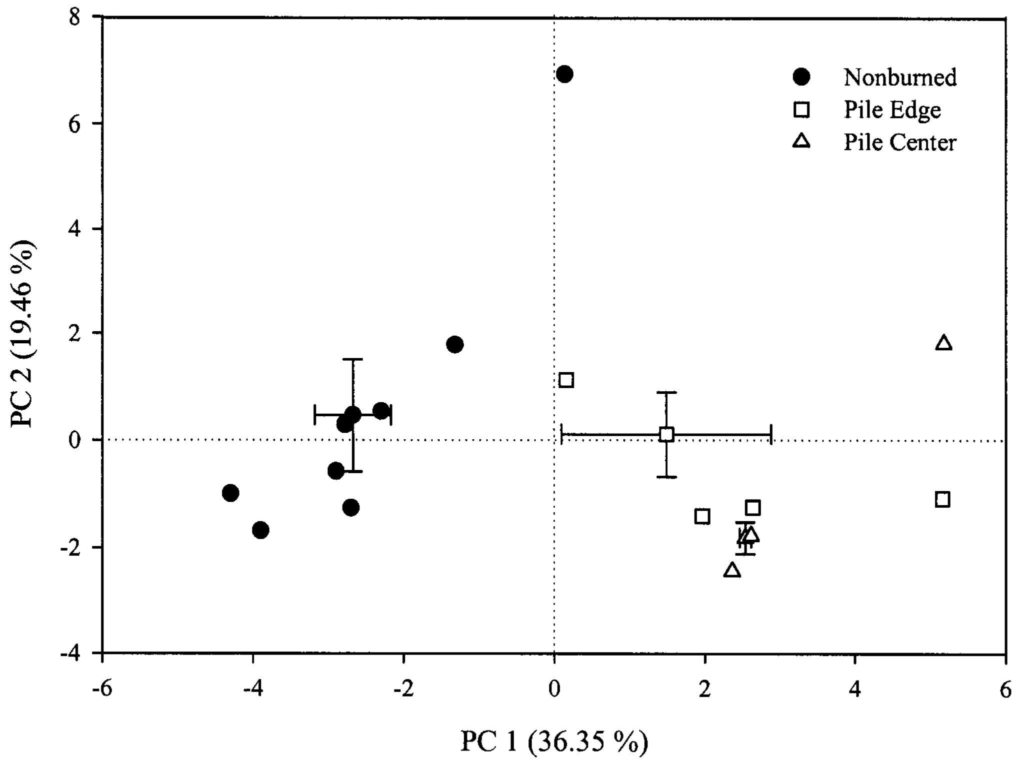


Figure 5.7. Principal components analysis of the microbial community structure in the nonburned and burned soil (0-15 cm) fifteen months after slash pile burning. Individual data points as well as the average PC scores for each treatment are shown along with standard error bars. The amount of variability explained by each PC is shown in parentheses.

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*).

Microbial C and N mineralization activities

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 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. 5.8, bacterial respiration was low in the nonburned site during the first six months after the burn, an apparent seasonality effect that was also evident in burned soil (RM AOV *P=0.07*). Nonetheless, bacterial respiration increased in nonburned soil in 2005 but not in burned 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 shown by the nonsignificant time by treatment interaction (RM AOV *P=0.89*).

Fungal respiration was not influenced by time or depth RM AOV *P=0.59* and *0.81* respectively), but it was negatively influenced by fire. As shown in Fig. 5.9, fungal respiration was reduced by slash pile burning immediately after the fire; 15 months after the fire fungal respiration was still low. (RM AOV *P=0.006*). Regardless of pile location or depth, fungal respiration was lower in burned soil compared to nonburned soil for the duration of this study.

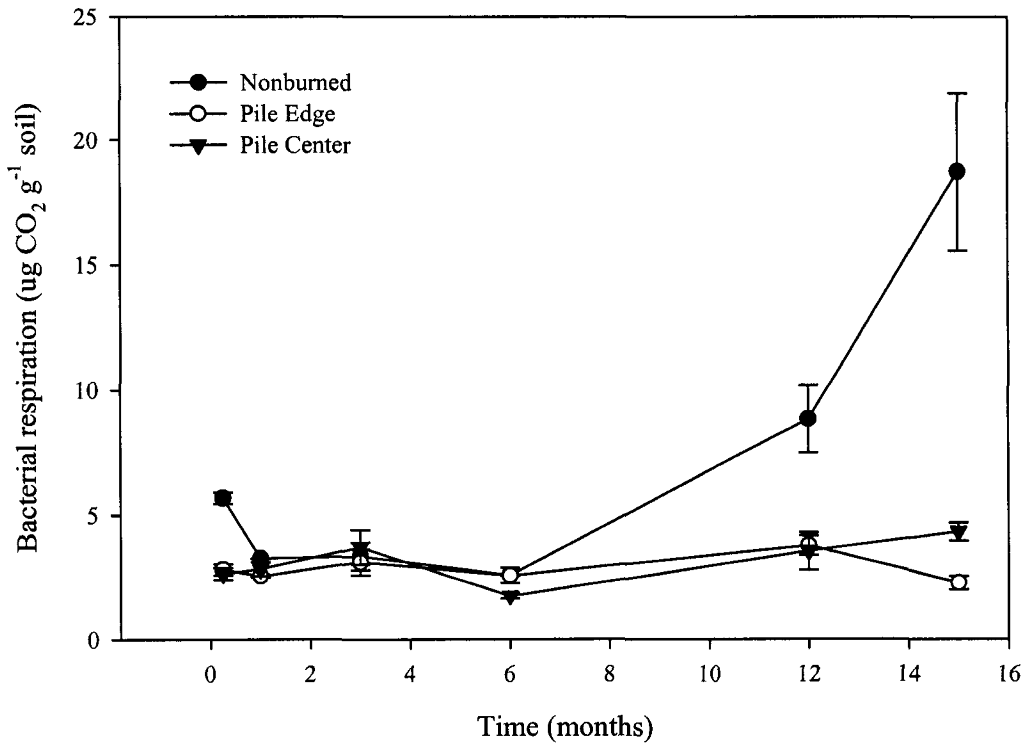


Figure 5.8. Bacterial respiration in burned and nonburned soil (0-15 cm) as determined by a 4-h substrate induced respiration assay. Separations of nonburned and burned soils at the 12 and 15 sampling times are significantly different (*RM AOV P=0.07*). Standard error bars are shown.

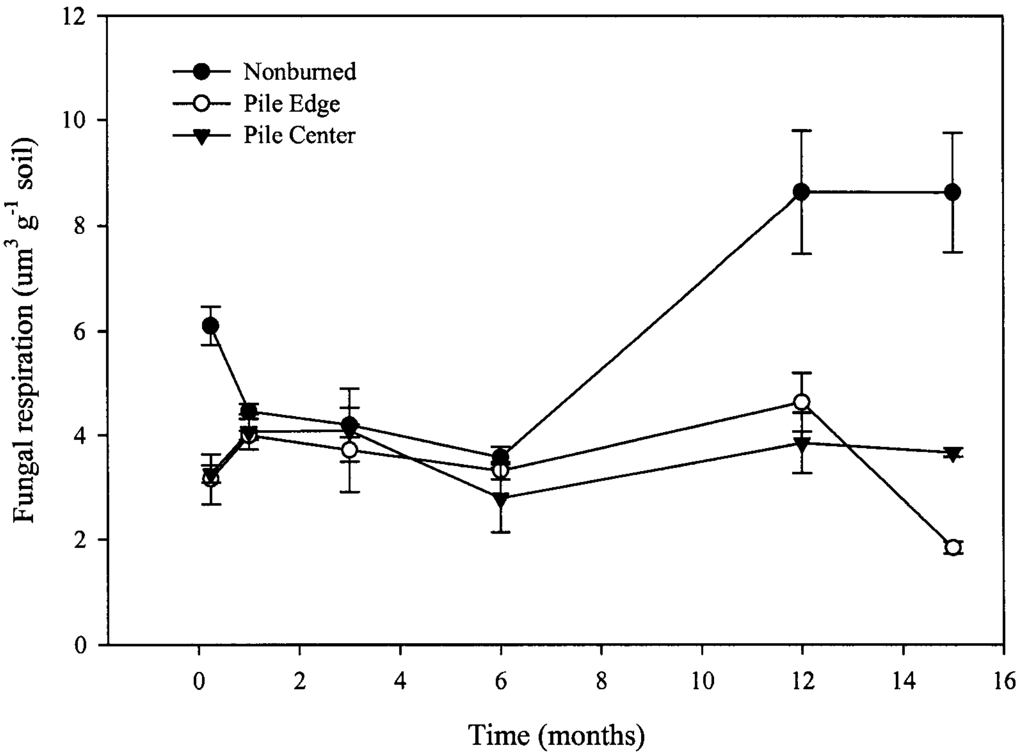


Figure 5.9. Fungal respiration in burned and nonburned soil (0-15 cm) as determined by a 4-h substrate induced respiration assay. Respiration rates between nonburned and burned soils were significantly different throughout the study (RM ANOVA $P=0.006$). Standard error bars are shown.

As shown in Fig. 5.10, net nitrogen mineralization (combined for bacteria and fungi) occurred in nonburned soils at very low rates. In burned soils, 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, net N immobilization occurred in burned soils 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 burned soil compared to nonburned soil. Overall, 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 $P=0.0845$).

Microbial resistance and resilience to slash pile burning

With respiration data as measured by the substrate induced respiration method, we calculated microbial resistance and resilience to this fire disturbance using indices described by Orwin and Wardle (2004). Resistance, defined as the amount of change initially caused by the burn, and resilience, defined as the speed with which the fire affected soil returns to its pre-disturbance levels, was determined for bacteria and fungi. For resistance calculations, we used the nonburned samples from 1 week after the burn as the control (C_0) and the burned samples at this time were used as perturbed soil (P_0) to calculate resistance of microbial activity to the fire. Samples from one week after the burn were also set to be the end of the disturbance for the resiliency calculations. Table 5.5 shows the resistance and resilience indices calculated every sampling time after the burn. For

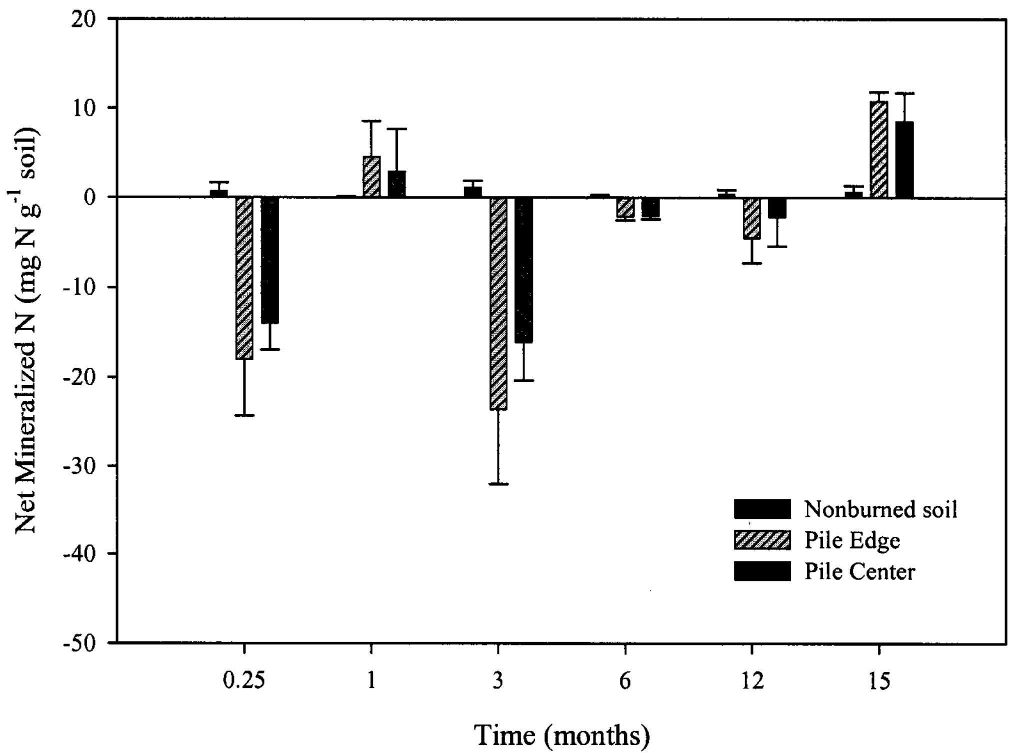


Figure 5.10. Nitrogen mineralization in burned and nonburned soil (0-15 cm) as determined by a 4-h substrate induced respiration. Mineralization rates between nonburned and burned soils were significantly different throughout the study (RM ANOVA $P= 0.0004$). Standard error bars are shown.

the resistance index, a value of 1 means no change due to disturbance and a value of less than 1 implies proportionally more change. A value of 1 for resilience index means full recovery and a value of less than 1 means slower rates of recovery.

Bacterial and fungal activity was equally resistant to the fire disturbance (Table 5.5). However, 15 months after the burn, bacterial activity proved to be less resilient to fire effects than fungal activity (*P=0.025*). Nonetheless, resilience indices were very low for both fungi and bacteria (0.11 and -0.07, respectively), far from the value of 1.00 required for complete recovery of microbial activity in these soils.

Table 5.5. Indices of resistance (0.25 months post-fire) and resilience (all other months) for bacterial and fungal respiration in response to slash pile burning.

Time (months)	Fungi	Bacteria
0.25	0.36 a	0.32 a
1	-0.11 b	0.01 a
3	-0.10 a	-0.10 a
6	0.03 b	0.12 a
12	-0.09 a	-0.14 b
15	0.11 a	-0.07 b

Discussion

Slash pile burning is a very effective slash disposal method; however, this method can degrade the overall quality of the soil leaving it sterile and unproductive in the long term. Our data suggests that slash pile burning may change soil characteristics resulting in a wide range of implications for ecosystem function and management. For example, 15 months after the fire, soil pH was still significantly higher in burned soil compared to nonburned soil. Also, fire had effectively fertilized the soil as the amounts of inorganic N and extractable P were significantly greater compared to nonburned soil. According to our data, nonburned soils at MEF are low on available N and P and have a slight acidic pH (5.4); therefore, microbial communities here are used to oligotrophic and acidic soil, conditions that are not present in the scarred soils at this point. While N and P levels were relatively high in burned soils, burning resulted in a reduction of soil total C that remained 15 months after the burn, 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 (nonburned). 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 soil carbon corresponds to the low microbial and plant biomass of scarred soils. The increase in soil nutrients observed in the soil after the burn parallel the findings observed in previous studies (Ahlgren and Ahlgren, 1960; Borchers and Perry, 1990; Certini, 2005; Choromanska and DeLuca, 2002; Fyles et al., 1991).

Slash pile burning affected soil microbial biomass and community structure. There was an overall reduction in microbial biovolumes in burned soil which has been reported previously (Acea and Carballas, 1996; Fritze et al., 1993; Hart et al., 2005; Pietikainen and Fritze, 1993; Theodorou and Bowen, 1982; Vazquez et al., 1993; Villar et al., 2004). Bacterial and fungal biovolumes were always greater in the nonburned soil compared to the burned soil. Also, except for the six months after the burn sample, microbial biovolumes were greater in soil at the edge of slash pile compared to the center of the pile. While burning reduced total fungal and bacterial biovolumes, the short term effects of soil burning may be more detrimental to fungi as evidenced by the ratio of fungi to bacterial (data not show). Fifteen months after the burn, the fungal-to-bacterial biovolume ratio of these soils decreased about 10 orders of magnitude in the burned soil relative to the nonburned soil, which means that there were more bacteria relative to fungi in burned soils compared to nonburned soil.

These findings are supported by the results of the EL-FAME data. Initially (one month after the fire), the nonburned soil and the soil at the edge of the pile were enriched with Gram-negative bacteria, fungi, and AM fungi in particular. The presence of fungi and of Gram-negative bacteria correlates to the fact that the soil at the edge of the pile had a higher nutrient content which may have negated the initial effects of soil heating. In contrast, the soil at the center of the pile was enriched with Gram-positive bacterial markers. This is expected as some Gram-positive bacteria can form spores and therefore can better withstand heat and desiccation (Coyne, 1999), so if they survived the sterilizing effect of heat as spores, the spores presumably germinated in response to the flush of soil nutrients, thus explaining the apparent increase in the EL-FAME markers.

Fifteen months after the burn, the microbial communities from soil under the edge of the pile clustered with soil communities from under the center of the pile and away from nonburned soil communities. This suggests that changes in soil physicochemical properties and/or absence of vegetation, and not the initial heat pulse received according to the location within the pile, affected microbial community structure in the longer term. Fifteen months after the burn, microbial communities of burned soil were still enriched with Gram-positive and Gram-negative bacterial markers, but no fungal markers. This is in direct contrast to microbial communities of nonburned soil, which were enriched with the fungal markers 18:2ω6c, 18:3ω6c and the AM fungal marker 16:1ω5c. These changes suggest that the fungi are a microbial group most affected by fire as no fungal markers where detected in the burned soils at the end of the study. The concept of greater fungal sensitivity to fire effects has been previously described by Ahlgren and Ahlgren (1965), Dunn et al. (1985), Vazquez et al. (1993), Theodorou and Bowen (1982), and Fritze and Pietikainen (1993).

Microbial activity was also negatively influenced by slash pile burning. Even under the optimal conditions (high water and C availability) created by the substrate induced respiration method, microbial respiration was low in burned soils compared to nonburned soil throughout this study. In regards to this, at the time this study ended, microbial activity had not recovered yet from the fire disturbance. According to resistance and resilience indices calculated for bacterial and fungal respiration, microbial activity was equally resistant to the fire disturbance, fifteen months after the burn, however, bacteria seemed to be less resilient to the disturbance than fungi. Nevertheless bacterial and fungal resilience indices were far from the value of 1.00 required for complete recovery to the microbial activity levels in the nonburned soils. This is in contrast to the reports mentioned above which suggest that fungi are always more affected by fire than bacteria. Our data show that whereas fungal biovolumes were more sensitive to slash pile burning than bacteria, fungal and bacterial activities were equally affected by fire.

Nitrogen mineralization dynamics were affected by burning regardless of location within the pile; and while inorganic N was high in burned soils, net N immobilization occurred regularly in the soils underneath the pile. The reasons for this are not clear, but it is possible that microorganisms in the burned soil are under stress and used to being under N limitation, so they resort to N immobilization when N is abundant.

The lethal temperature for bacteria has been described to be about 110 °C in wet soil, while the corresponding limit for fungi is 100 °C (Dunn et al., 1985). Soil underneath the slash pile experienced temperatures above 100 °C from 8-17 hours depending on the location of the pile, while the soil moisture content was above 14 % during most of the burn. Therefore, there is evidence that partial if not total soil sterilization occurred under the pile at the surface (0-5 cm) and therefore the initial reduction in microbial biovolumes and activity is probably due the sterilizing effect of heat. Four months after the burn, soil temperatures under the pile had returned to background levels (data not shown), thus longer-term changes to microbial community structure and activity, and to fungi in particular, were probably due to long lasting effects of soil pH, lower C content, greater nutrient availability, or other soil physicochemical properties which we were not able to measure. For example, the quality of C remaining in the charred soil may explain why microbial communities in burned soil may differ from communities in nonburned soil long after a fire (Knicker et al., 2005). It has been shown that charcoal (black carbon), which is a recalcitrant form of C due to its aryl content, is found at greater concentrations in soils that were under slash and burning treatments (Rumpel et al., 2006), and this type of carbon can have implications in nutrient cycling due to its absorptive capacity and its refractory nature (Zackrisson et al., 1996).

Overall, some of our hypotheses were supported by our data. For example, there were initial, differential effects of slash pile burning on the microbial response depending on the location under the pile (edge or center) which disappear with time. However, contrary to what we hypothesized, microbial communities at the edge of the pile were not more active immediately after the burn, but microbial activity was low regardless of the location within the pile. Fifteen months after the fire there where no differences in microbial community structure between edge and center soils under the slash pile. But contrary to what we hypothesized, at the time this study concluded soil nutrient levels had not returned to background levels and there were no depth effects in most variables measured except on fungal biovolumes.

In this paper we have presented the effects of slash pile burning on soil chemical and biological properties which are important to soil quality overall. Although burned soils were elevated in plant available N and P, they were also low in C and higher in pH. Fifteen months after the burn, the scarred soils lacked vegetation, microbial biovolumes and activity were below normal levels for this ecosystem, and AM fungi, an important plant symbiont, were still negatively impacted. These detrimental changes to soil microorganisms can have a broad range of implications for ecosystem functions and management because of the important roles of microorganisms in providing nutrients to plants and improving soil structure and stabilization. The long term changes in microbial function along with long term changes in vegetation cover and soil C quantity and presumably quality should be addressed in the future to better understand forest ecosystem changes overall caused by this common management practice.

References

Acea, M.J., and T. Carballas. 1996. Changes in physiological groups of microorganisms in soil following wildfire. FEMS Microbiology Ecology 20:33-39.

Ahlgren, I.F., and C.E. Ahlgren. 1960. Ecological effects of forest fires. Botanical Review 26:483-533.

Ahlgren, I.F., and C.E. Ahlgren. 1965. Effects of prescribed burning on soil-microorganisms in a minnesota Jack pine forest. Ecology 46:304-310.

Anderson, J.P.E., and K.H. Domsch. 1975. Measurement of bacterial and fungal contributions to respiration of selected agricultural and forest soils. Canadian Journal of Microbiology 21:314-322.

Bloem, J., M. Veninga, and J. Shepherd. 1995. Fully-automatic determination of soil bacterium numbers, cell volumes, and frequencies of dividing cells by confocal laser-scanning microscopy and image-analysis. Applied and Environmental Microbiology 61:926-936.

Borchers, J.G., and D.A. Perry, (eds.) 1990. Effects of prescribed fire on soil microorganisms., pp. 1-143-162. Oregon State University Press, Corvallis, Or.

Bossio, D.A., and K.M. Scow. 1998. Impacts of carbon and flooding on soil microbial communities: Phospholipid fatty acid profiles and substrate utilization patterns. Microbial Ecology 35:265-278.

Certini, G. 2005. Effects of fire on properties of forest soils: a review. Oecologia 143:1-10.

Choromanska, U., and T.H. DeLuca. 2002. Microbial activity and nitrogen mineralization in forest mineral soils following heating: evaluation of post-fire effects. Soil Biology & Biochemistry 34:263-271.

Coyne, M.S. 1999. Soil microbiology : an exploratory approach. Delmar, Albany, N.Y.

Dahms, C.W., and B.W. Geils. 1997. An assessment of forest ecosystem health in the Southwest. General Technical Report. , *In* UFSRMF and RESS. Region., (ed.), Vol. RM-GTR-295.

Dickinson, K.J.M., and J.B. Kirkpatrick. 1987. The short-term effects of clearfelling and slash-burning on the richness, diversity and relative abundance of higher-plant species in two types of eucalypt forest on dolerite in Tasmania. Australian Journal of Botany 35:601-616.

Dunn, P.H., L.F. Debano, and G.E. Eberlein. 1979. Effects of burning on chaparral soils 2. Soil microbes and Nitrogen mineralization. Soil Science Society of America Journal 43:509-514.

Dunn, P.H., S.C. Barro, and M. Poth. 1985. Soil-moisture affects survival of microorganisms in heated chaparral soil. Soil Biology & Biochemistry 17:143-148.

Fritze, H., T. Pennanen, and J. Pietikainen. 1993. Recovery of soil microbial biomass and activity from prescribed burning. Canadian Journal of Forest Research 23:1286-1290.

Fyles, J.W., I.H. Fyles, W.J. Beese, and M.C. Feller. 1991. Forest floor characteristics and soil nitrogen availability on slash-burned sites in coastal British-Columbia. Canadian Journal of Forest Research 21:1516-1522.

Hart, S.C., T.H. DeLuca, G.S. Newman, M.D. MacKenzie, and S.I. Boyle. 2005. Post-fire vegetative dynamics as drivers of microbial community structure and function in forest soils. Forest Ecology and Management 220:166-184.

Johnson, C.K., M.F. Vigil, K.G. Doxtader, and W.E. Beard. 1996. Measuring bacterial and fungal substrate-induced respiration in dry soils. Soil Biology & Biochemistry 28:427-432.

Klein, D.A., and M.W. Paschke. 2000. A soil microbial community structural-functional index: the microscopy-based total/active/active fungal/bacterial (TA/AFB) biovolumes ratio. Applied Soil Ecology 14:257-268.

Knicker, H., F.J. Gonzalez-Vila, O. Polvillo, J.A. Gonzalez, and G. Almendros. 2005. Fire-induced transformation of C- and N-forms in different organic soil fractions from a Dystric Cambisol under a Mediterranean pine forest (*Pinus pinaster*). Soil Biology & Biochemistry 37:701-718.

Korb, J.E., N.C. Johnson, and W.W. Covington. 2004. Slash pile burning effects on soil biotic and chemical properties and plant establishment: Recommendations for amelioration. Restoration Ecology 12:52-62.

Lodge, D.J., and E.R. Ingham. 1991. A comparison of agar film techniques for estimating fungal biovolumes inlitter and soil. Agriculture Ecosystems & Environment 34:131-144.

Massman, W.J., and J.M. Frank. 2004. Effect of a controlled burn on the thermophysical properties of a dry soil using a new model of soil heat flow and a new high temperature heat flux sensor. International Journal of Wildland Fire 13:427-442.

Moore, R. 1992. Soil survey of Pike National Forest, eastern part, Colorado, parts of Douglas, El Paso, Jefferson, and Teller counties, pp. 106, *In* C. USDA Forest Serv. and Soil Conservation Serv.; in cooperation with the Colo. Agric. Exp. Stn Denver, (ed.).

Orwin, K.H., and D.A. Wardle. 2004. New indices for quantifying the resistance and resilience of soil biota to exogenous disturbances. Soil Biology & Biochemistry 36:1907-1912.

Pietikainen, J., and H. Fritze. 1993. Microbial biomass and activity in the humus layer following burning - short-term effects of 2 different fires. Canadian Journal of Forest Research 23:1275-1285.

Romme, W., T.T. Veblen, M.R. Kaufmann, R. Sherriff, and C.M. Regan. 2003. Ecological effects of the Hayman fire. USDA Forest Service Gen. Tech. Rep. RMRS-GTR-114.

Rumpel, C., M. Alexis, A. Chabbi, V. Chaplot, D.P. Rasse, C. Valentin, and A. Mariotti. 2006. Black carbon contribution to soil organic matter composition in tropical sloping land under slash and bum agriculture. Geoderma 130:35-46.

Schutter, M.E., and R.P. Dick. 2000. Comparison of fatty acid methyl ester (FAME) methods for characterizing microbial communities. Soil Science Society of America Journal 64:1659-1668.

Shepperd, W., Edminster, C. and S. Mata. 2006. Long term seedfall, establishment, survival, growth of natural and planted Ponderosa pine in the Colorado front range. Western Journal of American Forestry 20:0.

Sullivan, T.S., M.E. Stromberger, M.W. Paschke, and J.A. Ippolito. 2006. Long-term impacts of infrequent biosolids applications on chemical and microbial properties of a semi-arid rangeland soil. Biology and Fertility of Soils 42:258-266.

Theodorou, C., and G.D. Bowen. 1982. Effects of a bushfire on the microbiology of a South-Australian low open (Dry Sclerophyll) forest soil. Australian Forest Research 12:317-327.

Vazquez, F.J., M.J. Acea, and T. Carballas. 1993. Soil microbial-populations after wildfire. FEMS Microbiology Ecology 13:93-103.

Villar, M.C., V. Petrikova, M. Diaz-Ravina, and T. Carballas. 2004. Changes in soil microbial biomass and aggregate stability following burning and soil rehabilitation. Geoderma 122:73-82.

Weinbauer, M.G., C. Beckmann, and M.G. Hofle. 1998. Utility of green fluorescent nucleic acid dyes and aluminum oxide membrane filters for rapid epifluorescence enumeration of soil and sediment bacteria. Applied and Environmental Microbiology 64:5000-5003.

Zackrisson, O., M.C. Nilsson, and D.A. Wardle. 1996. Key ecological function of charcoal from wildfire in the boreal forest Oikos 77:10-19.

CHAPTER 6

INCREASES IN SOIL pH AND BACTERIAL ACTIVITY CAN INFLUENCE FUNGAL RECOVERY AFTER A FIRE

Abstract

Soil biological, physical and chemical properties can be altered by the heat released during a fire and the magnitude and duration of these changes are dependent on fire severity and intensity. It has been previously reported that soil fungi recover more slowly than bacteria after a fire event. Thus, in an effort to determine the mechanisms by which fire affects soil fungi compared to bacteria, we conducted two laboratory studies to elucidate which post-fire soil properties affected fungal recovery. In the first study, soil was limed to increase the pH to levels similar to those observed after a fire in a pine forest; and in the second study the effects of reduced bacterial activity (bacteriostasis) were measured after heating the soil at various temperatures to determine fungal responses to heat in the presence and absence of bacteria. Increasing soil pH to levels similar to those reached after a forest fire (from 5.4 to 7.8) significantly decreased the total and active fungal biovolumes in soil after 96 hours of incubation. Therefore, changes in soil pH may be responsible for the observed lag in recovery of fungi after a soil heating event. Fungal biovolumes were positively affected by mild increases in soil temperatures and also by reduced bacterial activity. However, when soil was

heated at 121° C, fungal activity was positively affected by bacterial activity, suggesting that in disturbed soil, fungi and bacteria do not compete but might have commensal or synergistic relationships. Based on these data, I concluded that bacterial competition is not a factor inhibiting initial fungal recovery after a fire.

Introduction

Soil physical and chemical properties can be altered by the heat released during a fire. The magnitude and the duration of soil physicochemical alterations are dependent on components of the fire regime such as fire severity and intensity. In field studies, I assessed the effects of forest fires of different severities and intensities on total and active fungal hyphae, ester-linked fatty acid methyl ester (EL-FAME) fungal biomarkers in soil, and fungal C and N mineralization activities (Jimenez-Esquilin, 2006). All studies resulted in a similar outcome: soil fungi recovered more slowly than bacteria after a fire event; ratios of active-to-total fungi decreased with increasing fire severity and intensity, and in soil burned by a high intensity fire, the ratio of active-to-total fungi was still zero one year after the fire. I also assessed chemical (pH, C, N, and available nutrients) and physical (temperature, moisture content) properties in recovering soils, although the cause of slow fungal recovery could not be directly correlated to one particular soil physicochemical change, presumably because of the interacting effects of such changes.

Other studies regarding fire effects on soil microorganisms have shown similar effects on soil fungi (Ahlgren and Ahlgren, 1965; Dunn et al., 1985; Fritze et al., 1993; Theodorou and Bowen, 1982; Vazquez et al., 1993). Some of these studies suggested that the reasons for the delay in soil fungal recovery may be the increase in soil pH typically associated with a fire event, an initial decrease in soil moisture content, or an increase in bacterial biomass (increased competition); however the exact mechanism by which fungi recovery slowly after a fire are not known.

In an attempt to elucidate why fungi are less resilient to fire disturbances compared to bacteria, I conducted two laboratory studies to determine the mechanisms by which fire may affect soil fungi. I hypothesized that fungal recovery is slow due to 1) increased soil pH because fungi in the nondisturbed system experience a slightly acidic soil pH and 2) increased competition by soil bacteria as bacteria showed more resilience to fire than fungi (Jimenez-Esquilin, 2006). By identifying specific soil properties and/or mechanisms which influence the ability of soil fungi to recover following a fire event, a better understanding of fire effects on soil microbial communities, which I draw on as indicators of forest soil ecosystem recovery, can be gained.

Methods

Sampling and Soil characteristics

The soil used in the following experiments was collected from the Manitou Experimental Forest (MEF) which is located in the montane zone (6,500 to 8,000 feet) of the Colorado Front Range. The over story of this forest is composed of mature ponderosa pine (*Pinus ponderosa*) and Douglas fir (*Pseudotsuga menziesii*)

(+ 150 y), and the soil is a fine-loamy, mixed, frigid, pachic Argiustoll derived from weathered red arkosic sandstone and Pikes Peak Granite. Five kilograms of soil were collected from the top 10 cm of the A horizon aseptically with a trowel, stored in a cooler, and transported to the laboratory were they were analyzed for texture, pH, total C and N, NH_4-N, NO_3-N, and ammonium bicarbonate-diethylenetriaminepentaacetic acid (AB-DTPA)-extractable elements. 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 metals, followed by determination of constituent concentrations on an inductively coupled plasma-atomic emission spectrophotometer (Thermo Jarrell Ash Corp., Franklin, MA). Results of the soil analyses are shown in Table 6.1.

Experiment 1: Effects of increased pH

I measured the pH of 2 analytical replicates of forest soil prior to liming to determine the basal soil pH by the saturated paste method of Thomas (1996). To determine the amount of $CaCO_3$ necessary to raise the soil pH to the levels observed after forest fires (6.5-7.8), I used a shotgun approach according to the methods of Islam et al. (2004). Subsamples of 10 g dry soil were weighed into plastic cups (100 mL capacity) and lime ($CaCO_3$) was added at the following rates:

0, 1.0, 2.5, 3.0, 4.0, 5.0, 7.0, and 10.0 g. After adding the lime, the samples were homogenized by stirring, and the pH was measured at 2 h and again at 48 h to verify that the pH had stabilized. The amount determined to increase the soil pH by 2 units was between 2.5 and 3.0 g of $CaCO_3$. The actual amount used was 2.5 g of $CaCO_3$ per 10 g soil. After determining the liming requirements, I limed a 200-g sample of soil and incubated it for 96 hours to determine fungal biovolumes and fungal respiration as described below.

Table 6.1. Properties of a Manitou Experimental Forest soil (n=3) from summer 2005, including total (C and N) and extractable (inorganic N, P, K, Zn, Fe, Mn, and Cu) nutrients.

Property	Unit	
Texture		Loam
Water content	$g\ g^{-1}$	0.12
pH		5.4
Organic matter	$mg\ cm^{-3}$	71.3
Total C	$mg\ cm^{-3}$	31.1
Total N	$mg\ cm^{-3}$	1.92
Inorganic N (NH_4-N + NO_3-N)	$mg\ cm^{-3}$	0.01
P	$\mu g\ cm^{-3}$	2.35
K	$\mu g\ cm^{-3}$	430
Zn	$\mu g\ cm^{-3}$	3.99
Fe	$\mu g\ cm^{-3}$	73.4
Mn	$\mu g\ cm^{-3}$	12.0
Cu	$\mu g\ cm^{-3}$	1.46

Experiment 2: Effects of soil heating and bacteriostasis

Soil (1.5 kg) was spread on an aluminum tray in a 1-cm thick layer. A laboratory oven was brought up to the required temperature (25, 65 or 121 °C) and the soil was heated for one hour at each temperature. From here on, I refer to the 25° C-treated soil as unheated soil. These temperatures were chosen based on temperature thresholds for bacteria (110 °C) and fungi (100 °C) determined by Dunn et al. (1985). The total energy input into the soil based on these temperatures was 0.02 and 0.04 watts m^{-2}. After heating, soils were cooled to room temperature for one hour and the 1.5 kg samples per temperature treatment were further divided into two 750-g samples in separate cardboard trays. To both trays I added glucose (4 mg g^{-1} soil) and enough water to bring the soil to 60% field capacity; soils then were incubated at 25 °C for 48 hours. This was done to activate the surviving microorganisms as required by the substrate induced respiration assay (Anderson and Domsch, 1975; Johnson et al., 1996). The concentration of glucose added was empirically determined in previous optimization experiments.

After 48 hours, one of the trays was randomly selected and ampicillin (Sigma Aldrich Chemicals) was added at a rate of 15 mg g^{-1} soil to inhibit bacterial growth and mixed well. This rate was previously determined by adding different concentrations of ampicillin (0, 2, 4, 6, 8, 10, 12, 15, 20, 50 mg g^{-1} soil) to unheated soil, incubating soils for 24 hours and spread-plating a serial dilution (10^{-3} to 10^{-7}) of soil slurry onto TSA and Phenylethyl Alcohol Agar media (BBL diagnostics). Plates were incubated at 25 °C for 48 hours and bacterial counts were determined. The concentration that resulted in the lowest bacterial count (4.1

x 10^3 cells g^{-1} soil compared to highest value of 8.4 x 10^8 cells g^{-1} soil present in soil without ampicillin) was 15 mg of ampicillin g^{-1} soil.

Once ampicillin was added and mixed in, the soil from each tray was divided into 3 mason jars (~160 g soil in each jar) for destructive sampling of each jar at each time interval. Jars were incubated at 25 °C for a period of 14 days and four jars (heated + ampicillin, heated without ampicillin, unheated with ampicillin, unheated without ampicillin) were destructively sampled 3, 7, and 14 d later. This was a 3-way factorial design with four, paired temperature levels (25 and 65°C) and (25 and 121 °C), two bacteriostasis levels (with and without ampicillin), and three incubation time levels (3, 7 and 14 d) for a total of 24 samples.

<u>Fungal respiration and fungal biovolumes</u>

Triplicate samples (12 g dry weight) of soil from each treatment of Experiments 1 and 2 were amended with inhibitors (4mg g^{-1} soil for streptomycin to inhibit bacteria or 15mg g^{-1} soil for cyclohexamide to inhibit fungi) as required for the substrate-induced respiration inhibition assay (Anderson and Domsch, 1975; Johnson et al., 1996). Triplicate samples were also incubated in the presence of both inhibitors as well as in the absence of inhibitors to determine total C mineralization. After equilibrating 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. After this, 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).

Biovolumes of fungi in soil were determined in duplicate by direct microscopy techniques. Soil subsamples were serially diluted in filter-sterilized water and quantification of soil fungal hyphae was performed with the coverslip-well slide method of Lodge and Ingham (1991). Fungal slides were observed at 400 × resolution 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. Fungi were counted in a total of 30 fields of view per sample. Images of 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 fungi were determined based on the average diameter of at least 60 hyphal fragments (Klein and Paschke, 2000).

Results

Effects of increased pH

As shown in Fig. 6.1, most of the fungi present in the soil were active (i.e., cytoplasm-filled) regardless of soil pH, but increasing the soil pH to a level similar to that reached after a forest fire significantly decreased the total and active fungal biovolumes in soil from 10^9 to 10^8 um^3 hyphae g^{-1} soil (total hyphae) and from 10^9 to 10^7 um^3 hyphae g^{-1} soil (active hyphae). However, fungal C mineralization activity was not significantly affected by liming the soil (*P=0.234*; data not shown).

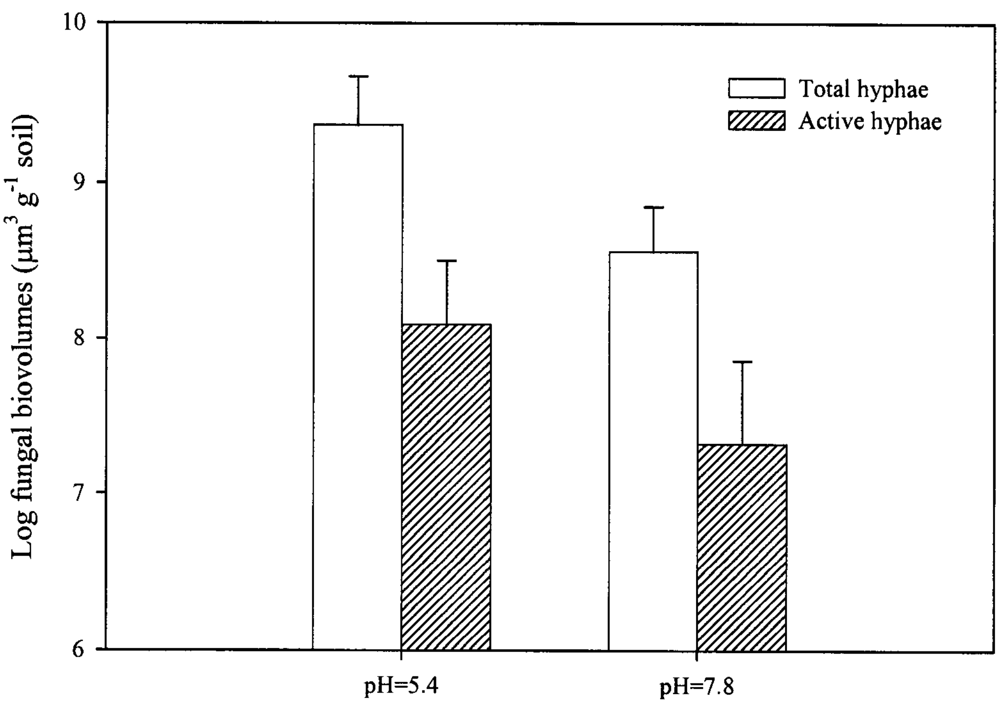


Figure 6.1. Total and active fungal biovolumes determined in nonlimed (pH=5.4) and limed (pH=7.8) MEF soil. Standard deviation bars (± 2) are shown.

Effects of heating and bacteriostasis

Total fungal biovolumes

Total and active fungal counts were measured to determine the effects of heating and bacteriostasis on soil fungal biomass. Repeated measures analysis of variance (RM ANOVA) revealed that the changes in fungal biovolumes depended on temperature, bacteriostasis, and time of incubation as shown by the significant 3-way interaction (RM ANOVA *P=0.0538*). Figure 6.2 shows that similar temporal dynamics were observed in the nonheated soil and the soil heated at 65 °C. Under soil bacteriostasis, fungal biovolumes increased initially and then decreased by day 21. However, under no bacteriostasis, fungal biovolumes decreased from 10^9 to 10^8 um^3 of hyphae g^{-1} of soil in nonheated soil, and from 10^{10} to 10^9 in soil heated at 65 °C. Also, in the 65 °C treatment, fungal biovolumes were slightly greater compared to biovolumes in the nonheated soil throughout the study. In the soil heated at 121 °C, fungal biovolumes decreased with time in both treatments (bacteriostasis and no bacteriostasis), and at day 14 fungal biovolumes were significantly lower in the bacteriostatic soil than in soil which did not receive ampicillin. Trends in active fungal biovolumes were less evident and active fungal biovolumes were not significantly affected by soil temperature or bacteriostasis (data not shown).

Fungal C mineralization

Changes in fungal respiration depended on the combined effects of heating and bacteriostasis as well as the time of incubation as shown by the 3-way interaction (RM ANOVA *P=0.0252*). As shown in Fig. 6.3, fungal respiration

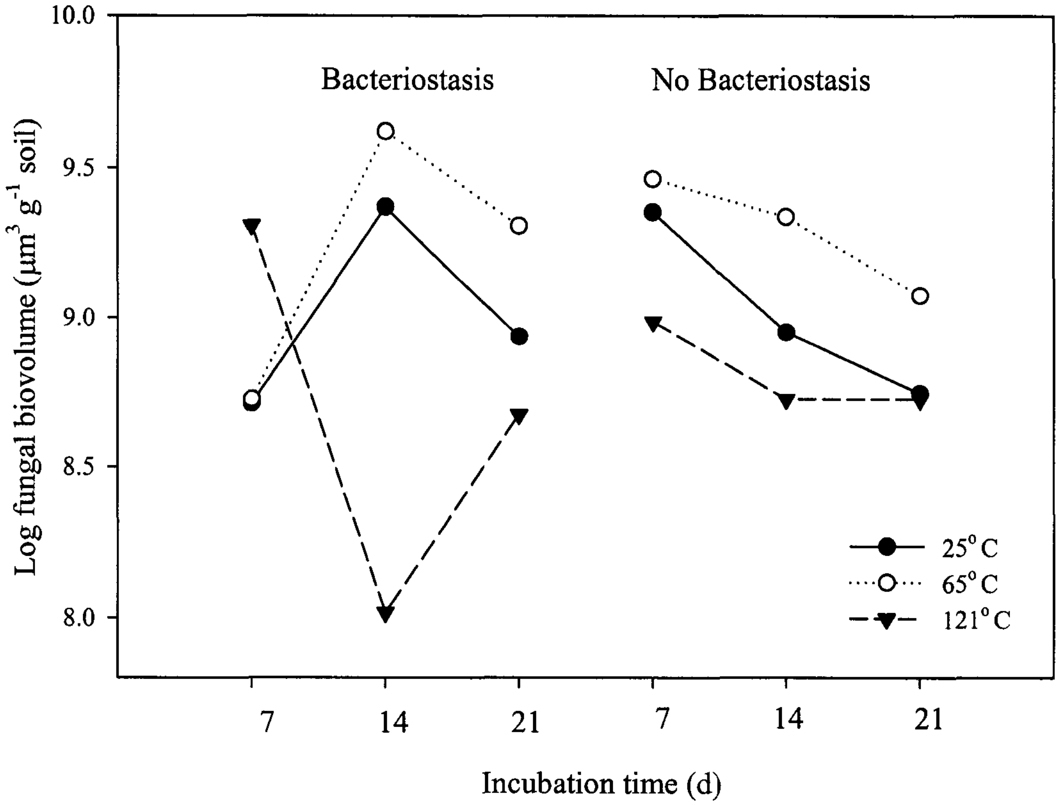
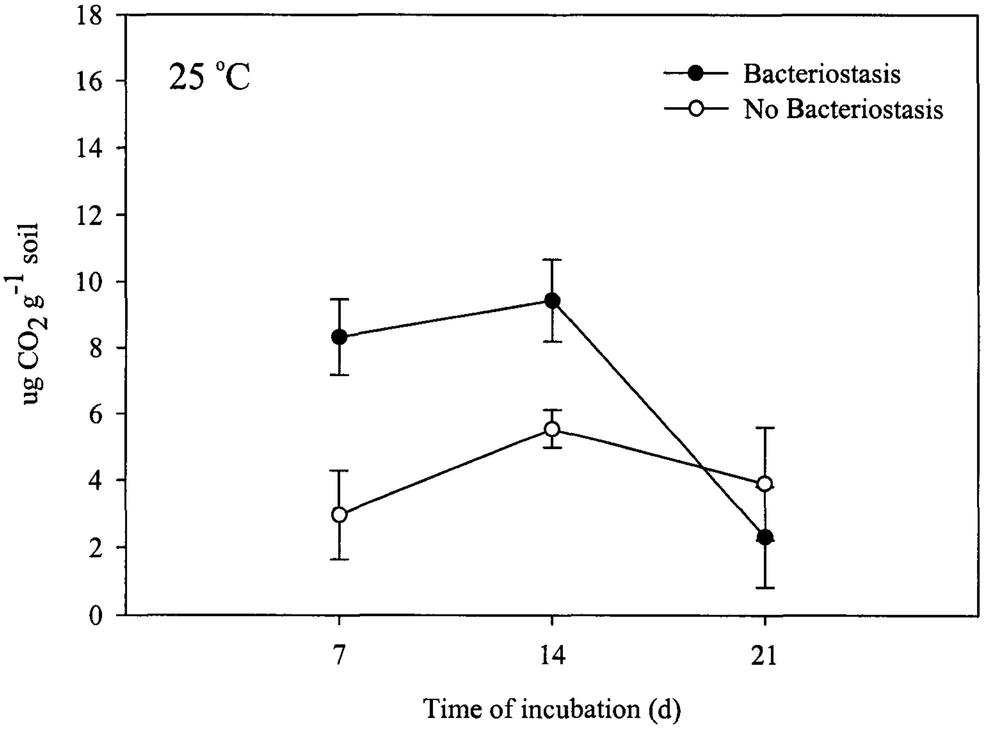
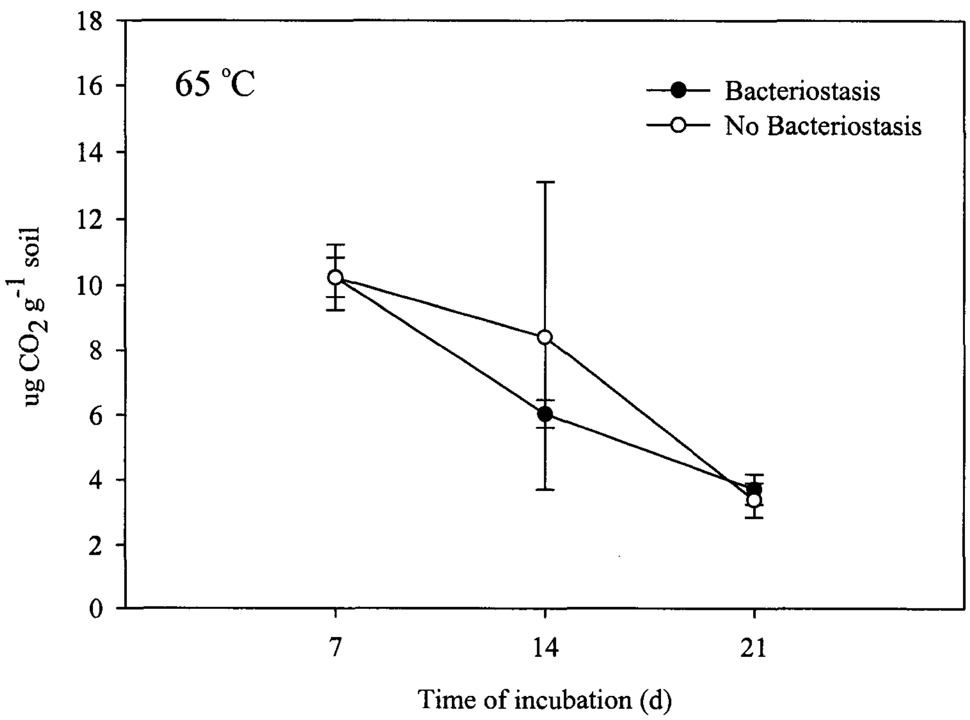


Figure 6.2. Total fungal biovolumes in nonheated (25 °C) and heated (65 or 121°C) soil treated with or without ampicillin to achieve bacteriostasis.


25 °C
Bacteriostasis
No Bacteriostasis
ug CO2 g-1 soil
Time of incubation (d)


65 °C
Bacteriostasis
No Bacteriostasis
ug CO2 g-1 soil
Time of incubation (d)

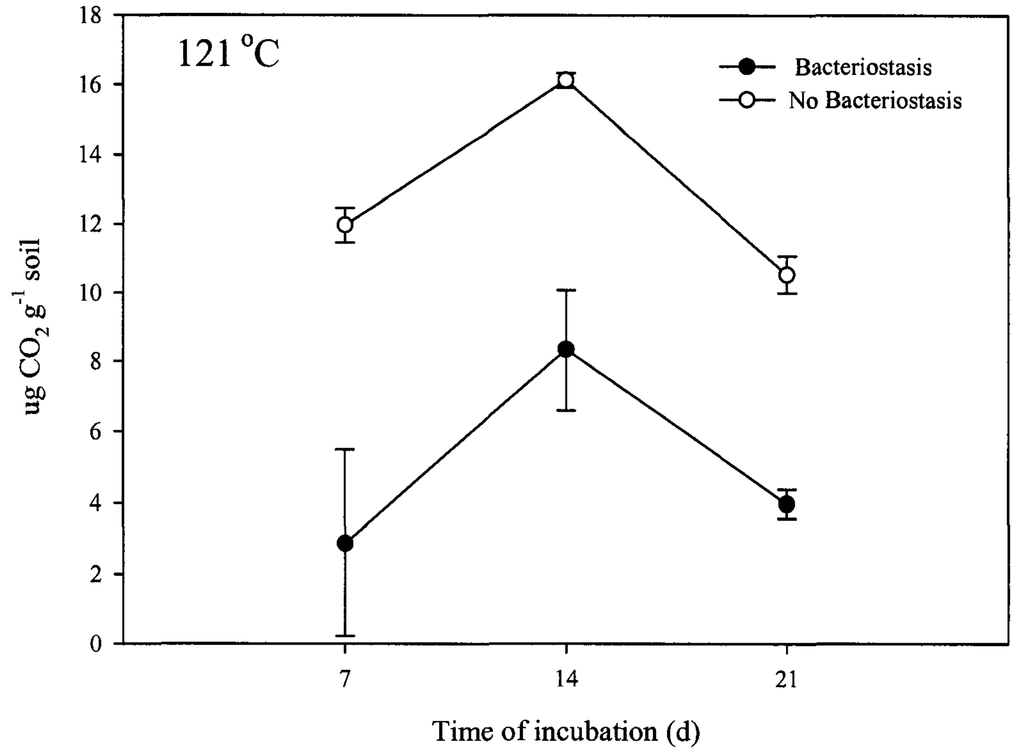


Figure 6.3. Fungal respiration as determined by substrate induced respiration inhibition assay in nonheated (25 °C) and heated (65 or 121 °C) soil treated with or without ampicillin to achieve bacteriostasis. Standard deviations bars (± 2) are shown.

in nonheated soil was significantly greater in the absence of bacteria, but 21 days after incubation the amount of CO_2 evolved decreased to 2-to-4 ug CO_2 g^{-1} soil regardless of bacteriostasis. In soil heated at 65 °C, fungal activity decreased with time from 10 to 4 ug CO_2 g^{-1} regardless of the bacteriostasis treatment. Lastly, fungal activity was highest (> 10 ug CO_2 g^{-1}) in soil heated at 121 °C in the presence of bacterial activity.

Discussion

The overall purpose of this study was to identify specific chemical or biological properties of soil that may influence the ability of soil fungi to recover following a fire event so that we can better comprehend fire effects on the soil microbial community structure and function. This is important because microorganisms, including soil fungi, contribute largely to nutrient cycling and aggregate stabilization in soils. Fungi are particularly adept at stabilizing soils via aggregate formation by physically entangling soil particles with their hyphae as well as cementing soil particles together with excreted polysaccharides and glycoproteins (Six et al., 2004; Wright et al., 2000). Thus recovery of soil fungi after a fire effect can be a good indicator of forest soil ecosystem recovery.

Soil pH increases after a forest fire due to release of basic cations (Mg^{2+}, K^+, Ca^{2+}) from oxidized organic matter or due to the volatilization of soil organic acids, and these changes may last several years after a fire (Certini, 2005). Our findings suggest that increasing soil pH to levels similar to those reached after a forest fire (from 5.4 to 7.8) can significantly decrease the total and active fungal biovolumes in soil after 96 hours of incubation. Moreover, the reduction in the active-to-total hyphae ratio from 0.26 in nonlimed soil to 0.07 (average of 7 replicates) in limed soil implies that fungal hyphae are not able to exploit nutritional resources and grow when soil pH is raised. This may be due to the fact that fungi are usually favored in acidic environments due to reduced competition with pH-sensitive bacteria or because their physiology is not supported by in slightly neutral or basic pH (Penalva and Herbert, 2002). In a related study, Raznikiewics et al. (1994) showed that liming soil from pH 5.1 to 6.3 significantly affected soil fungi, specifically spores of arbuscular mycorrhizal fungi which decreased presumably due to the pH-associated increase in soil P.

Another explanation for the reduced fungal biovolumes can be the increased osmotic stress due to the addition of high amounts of $CaCO_3$. However, in previous studies (Jiménez-Esquilín, 2006) we found that immediately after and one year following a fire event, fungal EL-FAMEs markers were negatively and significantly correlated with changes soil pH as shown in Table 6.2 implying that the amounts of fungal markers decreased with increasing soil pH.

Table 6.2. Pearson correlation coefficients for soil pH and fungal EL-FAMEs markers.

Sampling time	Pearson Correlation Coefficients	*p value*
Summer 2002	-0.59	0.044*
Spring 2003	-0.39	0.021*
Summer 2003	-0.53	0.075*

* significant at 0.1 level

These correlations along with the biovolume changes presented in this study lend support for the hypothesis that increased soil pH may be an important factor influencing fungal recovery after a fire.

In the second experiment, the effects of bacterial competition were measured after heating the soil at various temperatures. Bacterial competition against fungi was reduced in soil by bacteriostasis, which was achieved by adding ampicillin to inhibit production of bacterial cell wall material peptidoglycan. Repeated measures analysis of variance showed that bacterial activity was significantly lower in ampicillin-treated soil compared to nontreated soil for the duration of this study (RM ANOVA $P < 0.0001$; data not shown). The purpose here was to inhibit bacterial growth and function after the soils were heated at different temperatures so that fungal responses to heat in the presence and absence of bacteria could be determined. My results showed that lowering bacterial activity in nonheated (25 °C) or moderately heated (65 °C) soil resulted in greater fungal biovolumes after 14 d of incubation. The suppression of soil fungi in the presence of bacteria or due to nutrient depletion in soil is commonly known as soil fungistasis (de Boer et al., 2003). In this study, soil fungistasis was alleviated when the soil was heated at 65 °C and when bacterial activity was inhibited. Moderate soil heating increased fungal biovolumes compared to nonheated soil presumably due to an increase in nutrient concentrations caused by oxidation of organic matter during soil heating (Certini, 2005). Furthermore, the reduction of bacterial activity would likely have removed competition pressures, thus explaining the positive response of fungal biovolumes to ampicillin in unheated soil and soil heated to 65 °C. However, heating soil to 121 °C resulted in different bacteriostasis effects on soil fungi. While fungal biovolumes in soil heated to this temperature were lower than in nonheated soil and soil heated to 65 °C, fungal biovolumes were even lower when bacterial activity was low. Thus, it appears that fungal biomass was more resilient to the effects of high temperature soil heating in the presence of bacteria.

Responses of fungal C mineralization activity to soil heating and bacteriostasis were somewhat similar to those of fungal biovolumes. Substrate induced respiration assays demonstrated that in the absence of bacterial activity (bacteriostasis) in nonheated soil, fungal respiration was higher for the first two weeks of the study compared to soil with intact bacterial activity, which suggests that fungistasis was relieved by suppression of bacterial function. In soil heated at 65 °C no effects of bacteriostasis were seen and fungal respiration decreased continuously for the duration of the study. However, fungal activity was highest in the soil heated at 121 °C when bacterial activity was present (no bacteriostasis).

My hypothesis regarding early competition by bacteria was rejected by the data collected. This study showed that after the initial sterilizing effect of heat (7 days later), fungal populations in high temperature heated soil may be positively affected by bacterial activity and that the dominant interaction between the two microbial groups is not competition but commensal or perhaps synergistic. As it was suggested by de Boer et al. (2005) bacteria can benefit fungal populations by producing compounds such as vitamins and growth factors that may aid fungal growth. Also, synergistic strategies have evolved between bacteria and fungi to degrade recalcitrant organic substrates like those that may be present in soil after a fire (Knicker et al., 2005). Moreover, positive and significant correlations between fungal and bacterial EL-FAMEs markers were found in soil burned at different fire severities (Jiménez-Esquilín, 2006) as shown in Table 6.3 further supporting the idea of bacterial and fungal synergistic interactions after a fire.

Table 6.3. Pearson correlation coefficients for fungal and bacterial EL-FAMEs markers in burned soil.

Sampling time	**Pearson Correlation Coefficients**	***p value***
Summer 2002	0.99	<0.001*
Spring 2003	0.85	0.0003*
Summer 2003	0.65	0.021*
Fall 2003	0.89	<0.001*
Spring 2004	0.94	<0.001*
Summer 2004	0.54	0.06*
Fall 2004	0.48	0.1
Summer 2005	0.51	0.12

* significant at 0.1 level

These findings contradict the hypothesis suggested by some that early bacterial competition may negatively influence fungal recovery in fire-affected soils (Ahlgren and Ahlgren, 1965; Dunn et al., 1985; Fritze et al., 1993; Theodorou and Bowen, 1982; Vazquez et al., 1993). In light of these findings, I propose that fungal recovery in fire affected soil may be highly influenced by soil pH or other factors not tested in this study (e.g., C substrate quality after the fire), but not so much by initial bacterial competition. Further studies investigating the effect of recalcitrant forms of C in soil fungal recovery are needed.

Also it is important to note that the higher negative impact of fire on fungi compared to bacteria may be explained by their choice of habitat in soil (Ranjard and Richaume, 2001; Six et al. 2005). Fungi tend to be located in the surfaces of soil aggregates, more exposed to soil predators and soil surface disturbances such as temperature increases during a fire, whereas bacteria tend to inhabit inside macro and microaggregates and thus are more structurally protected from the sterilizing effect of heat or from the changes that may occur in soil during a fire.
The data collected in this study motivates further research to better elucidate the interaction among microbial groups in soil and how these interactions reflect the changes observed aboveground in relation to ecosystem recovery after a disturbance.

References

Ahlgren, I.F., and C.E. Ahlgren. 1965. Effects of prescribed burning on soil-microorganisms in a Minnesota Jack Pine forest. Ecology 46:304-310.

Anderson, J.P.E., and K.H. Domsch. 1975. Measurement of bacterial and fungal contributions to respiration of selected agricultural and forest soils. Canadian Journal of Microbiology 21:314-322.

Certini, G. 2005. Effects of fire on properties of forest soils: a review. Oecologia 143:1-10.

de Boer, W., L.B. Folman, R.C. Summerbell, and L. Boddy. 2005. Living in a fungal world: impact of fungi on soil bacterial niche development. Fems Microbiology Reviews 29:795-811.

de Boer, W., P. Verheggen, P. Gunnewiek, G.A. Kowalchuk, and J.A. van Veen. 2003. Microbial community composition affects soil fungistasis. Applied and Environmental Microbiology 69:835-844.

Dunn, P.H., L.F. Debano, and G.E. Eberlein. 1979. Effects of burning on chaparral soils 2. Soil microbes and Nitrogen mineralization. Soil Science Society of America Journal 43:509-514.

Dunn, P.H., S.C. Barro, and M. Poth. 1985. Soil-moisture affects survival of microorganisms in heated Chaparral soil. Soil Biology & Biochemistry 17:143-148.

Fritze, H., T. Pennanen, and J. Pietikainen. 1993. Recovery of soil microbial biomass and activity from prescribed burning. Canadian Journal of Forest Research 23:1286-1290.

Islam, M.A., P.J. Milham, P.M. Dowling, P.C. Jacobs, and D.L. Garden. 2004. Improved procedures for adjusting soil pH. Communications in Soil Science and Plant Analysis 35:25-37.

Jimenez-Esquilin, A.E. 2006. Fire effects in soil microbial community structure and function in a ponderosa pine ecosystem. Dissertation, Colorado State University, Fort Collins.

Johnson, C.K., M.F. Vigil, K.G. Doxtader, and W.E. Beard. 1996. Measuring bacterial and fungal substrate-induced respiration in dry soils. Soil Biology & Biochemistry 28:427-432.

Klein, D.A., and M.W. Paschke. 2000. A soil microbial community structural-functional index: the microscopy-based total/active/active fungal/bacterial (TA/AFB) biovolumes ratio. Applied Soil Ecology 14:257-268.

Knicker, H., F.J. Gonzalez-Vila, O. Polvillo, J.A. Gonzalez, and G. Almendros. 2005. Fire-induced transformation of C- and N-forms in different organic soil fractions from a Dystric Cambisol under a Mediterranean pine forest (*Pinus pinaster*). Soil Biology & Biochemistry 37:701-718.

Lodge, D.J., and E.R. Ingham. 1991. A comparison of agar film techniques for estimating fungal biovolumes in litter and soil. Agriculture Ecosystems & Environment 34:131-144.

Penalva, M.A., and N. Herbert. 2002. Regulation of gene expression by ambient pH in filamentous fungi and yeasts. Microbiology and Molecular biology reviews. 66:426-446.

Ranjard, L. and A. Richaume. 2001. Quantitative and qualitative microscale distribution of bacteria in soil. Research in Microbiology 152: 707-716.

Raznikiewicz, H., K. Carlgren, and A. Martensson. 1994. Impact of phosphorus fertilization and liming on the presence of Arbuscular Mycorrhizal spores in a swedish long-term field experiment. Swedish Journal of Agricultural Research 24:157-164.

Six, J., C. Bossuyt, D. Degryze, and K. Denef. 2004. A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. Soil and Tillage Research 79:7-31.

Theodorou, C., and G.D. Bowen. 1982. Effects of a bushfire on the microbiology of a South-Australian low open (Dry Sclerophyll) forest soil. Australian Forest Research 12:317-327.

Thomas, G.W. 1996. Soil pH and soil acidity, p. 475-490, *In* S. DL, ed. Methods of Soil Analysis, Part 3. Chemical Methods. SSSA, Madison, WI.

Vazquez, F.J., M.J. Acea, and T. Carballas. 1993. Soil microbial-populations after wildfire. FEMS Microbiology Ecology 13:93-103.

Wright, S.F., M.C. Rillig, and K.A. Nichols. 2000. Glomalin: A soil protein important in carbon sequestration. Abstracts of Papers of the American Chemical Society 220:U396-U396.

CHAPTER 7

SUMMARY

The effects of wildfire as well as slash pile burning on soil microbial community structure and function were examined. Generally, burning increased the soil pH and P and N nutrient content of soil; 3 years after the wildfire soil fertility and pH remained high. In regards to the effects of fire severity on microbial responses, my results suggested that fire severity should be taken into account when designing soil restoration programs in fire-affected ecosystems because rates of microbial community recovery, and thus rates of soil recovery, will differ depending on fire severity. I showed that soil bacteria and fungi were more tolerant structurally and functionally to a moderate severity fire than a light or high severity, which may be related to the frequency of occurance of these fires in the ponderosa pine ecosystem. Also, soil microbial community structure appeared to have recovered from the moderate and high severity fire within 2 years of the disturbance and potential microbial C and N mineralization activities had recovered in the moderate severity burn after 3 years, but microbial activity had not recovered in soil burned at light or high severity 3 years after the fire. Microbial community structure changed with fire but often these changes were dependent on fire severity and fire intensity.

Although my data showed that a wildfire may have detrimental effects on microbial community structure and function for at least the first two years and that these changes may be related to the establishment of invasive species above ground, fire can ameliorate the effects of other disturbances common to the ponderosa pine ecosystem such as soil scarification. Scarification reduced levels of soil C and organic matter (OM) and biovolumes of both fungi and bacteria. Also, the bacterial community of scarified soil was dominated by Gram-positive EL-FAME markers, presumably due to the relatively oligotrophic nature of the soil. Nonetheless, according to my results a high severity fire can accelerate the recovery of soil bacteria in scarified soil presumably due to increased C and OM availability after a fire.

Slash pile burning may cause detrimental changes that may have a broad range of implications for ecosystem function and management. Fifteen months after a slash pile burn, soil total C remained low in burned soils, pH remained above 6, microbial C and N mineralization activities were below normal levels, and the AM fungal EL-FAME marker was not detected in the burned soil. Based on temperature data collected after the burn, it seems that longer-term changes to microbial community structure and activity (and those of fungi in particular) were probably due to the long-lasting effects of soil pH, lower organic C, higher nutrient availability, or other soil physicochemical properties which we were not able to measure (e.g., changes to C quality).

In all of my studies, the general trend was for fungi to be more affected by heat and changes in soil due to fire than bacteria. Arbuscular mycorrhizal fungi in particular were greatly negatively impacted by fire. Thus, in trying to determine the mechanisms by which fire affects soil fungi compared to bacteria, I found that increasing soil pH to a level similar to that reached after a forest fire (from 5.4 to 7.8) significantly decreased total and active fungal biovolumes in soil, indicating that changes in soil pH may be responsible for the observed lag in recovery of fungi after a soil heating event. However, bacterial competition was not a factor inhibiting fungal recovery immediately after a high-severity fire.

Even though there were general trends among these studies, in particular regarding soil nutrient levels and lack of fungal resiliency to fire, these findings suggest that the dynamics of soil microbial recovery after a fire are not simple and interactions exist among some of the components of the fire regime (fire severity, fire intensity, season of fire) that will affect the outcome of the soil recovery and therefore of the above ground community. Thus more research is needed with a multidisciplinary and integrated approach in order to design successful soil restoration programs in fire-affected ecosystems. Thus, we should commit to carrying out such studies, which would fill in gaps in our knowledge making it possible to link disturbance, biodiversity, and the biological functioning of soils.

BIBLIOGRAPHY

Acea, M.J., and T. Carballas. 1996. Changes in physiological groups of microorganisms in soil following wildfire. FEMS Microbiology Ecology 20:33-39.

Acea, M.J., and T. Carballas. 1999. Microbial fluctuations after soil heating and organic amendment. Bioresource Technology 67:65-71.

Agnelli, A., J. Ascher, G. Corti, M.T. Ceccherini, P. Nannipieri, and G. Pietramellara. 2004. Distribution of microbial communities in a forest soil profile investigated by microbial biomass, soil respiration and DGGE of total and extracellular DNA. Soil Biology & Biochemistry 36:859-868.

Ahlgren, I.F., and C.E. Ahlgren. 1960. Ecological effects of forest fires. Botanical Review 26:483-533.

Ahlgren, I.F., and C.E. Ahlgren. 1965. Effects of prescribed burning on soil microorganisms in a Minnesota Jack pine forest. Ecology 46:304-310.

Amann, R.I., W. Ludwig, and K.H. Schleifer. 1995. Phylogenetic identification and in-situ detection of individual microbial-cells without cultivation. Microbiological Reviews 59:143-169.

Anderson, J.P., and K.H. Domsch. 1975. Measurement of bacterial and fungal contributions to respiration of selected agricultural and forest soils. Canadian Journal of Microbiology 21:314-322.

Andrews, J.H., and R.F. Harris. 1986. r- and K-selection and microbial ecology, p. 99-147, *In* K. C. Marshall, ed. Advances in Microbial Ecology, Vol. 9. New York, N.Y: Plenum Press, New York.

Baath, E., and T.H. Anderson. 2003. Comparison of soil fungal/bacterial ratios in a pH gradient using physiological and PLFA-based techniques. Soil Biology & Biochemistry 35:955-963.

Barbarick, K.A., and S.M. Workman. 1987. NH_4-HCO_3-DTPA and DTPA extractions of sludge-amended soils. Journal of Environmental Quality 16:125-130.

Beare, M.H., C.L. Neely, D.C. Coleman, and W.L. Hargrove. 1990. A substrate-induced respiration (SIR) method for measurement of fungal and bacterial biomass on plant residues. Soil Biology & Biochemistry 22:585-594.

Beare, M.H., C.L. Neely, D.C. Coleman, and W.L. Hargrove. 1991. Characterization of a substrate-induced respiration method for measuring fungal, bacterial and total microbial biomass on plant residues. Agriculture Ecosystems & Environment 34:65-73.

Bloem, J., M. Veninga, and J. Shepherd. 1995. Fully-automatic determination of soil bacterium numbers, cell volumes, and frequencies of dividing cells by confocal laser-scanning microscopy and image-analysis. Applied and Environmental Microbiology 61:926-936.

Bollen, G.J. 1970. Effect of pasteurization on spore germination of some saprophytic fungi from soil. Acta Botanica Neerlandica 19:117-&.

Borchers, J.G., and D.A. Perry, (eds.) 1990. Effects of prescribed fire on soil microorganisms., pp. 1-143-162. Oregon State University Press, Corvallis, Or.

Bossio, D.A., and K.M. Scow. 1998. Impacts of carbon and flooding on soil microbial communities: Phospholipid fatty acid profiles and substrate utilization patterns. Microbial Ecology 35:265-278.

Boyle, S.I., S.C. Hart, J.P. Kaye, and M.P. Waldrop. 2005. Restoration and canopy type influence soil microflora in a ponderosa pine forest. Soil Science Society of America Journal 69:1627-1638.

Certini, G. 2005. Effects of fire on properties of forest soils: a review. Oecologia 143:1-10.

Choromanska, U., and T.H. DeLuca. 2001. Prescribed fire alters the impact of wildfire on soil biochemical properties in a ponderosa pine forest. Soil Science Society of America Journal 65:232-238.

Choromanska, U., and T.H. DeLuca. 2002. Microbial activity and nitrogen mineralization in forest mineral soils following heating: evaluation of post-fire effects. Soil Biology & Biochemistry 34:263-271.

Corbet, A.S. 1934. Studies on tropical soil microbiology II: The bacterial numbers in the soil of the Malay peninsula. Soil Science 38:407-416.

Cornwell, W.K., B. Bedford, and C.T. Chapin. 2001a. Occurence of arbuscular mycorrhizal fungi in a phosphorus-poor wetland and mycorrhizal response to phosphorus fertilization. American Journal of Botany 88:1824-1829.

Cornwell, W.K., B.L. Bedford, and C.T. Chapin. 2001b. Occurrence of arbuscular mycorrhizal fungi in a phosphorus-poor wetland and mycorrhizal response to phosphorus fertilization. American Journal of Botany 88:1824-1829.

Covington, W. 1981. Changes in forest floor organic matter and nutrient content following clearcutting in northern hardwoods. Ecology 5:376-380.

Coyne, M.S. 1999. Soil microbiology : An exploratory approach. Delmar, Albany, N.Y.

Dahms, C.W., and B.W. Geils. 1997. An assessment of forest ecosystem health in the Southwest. General Technical Report , *In* UFSRMF and RESS Region., (ed.), Vol. RM-GTR-295.

de Boer, W., L.B. Folman, R.C. Summerbell, and L. Boddy. 2005. Living in a fungal world: Impact of fungi on soil bacterial niche development. Fems Microbiology Reviews 29:795-811.

de Boer, W., P. Verheggen, P. Gunnewiek, G.A. Kowalchuk, and J.A. van Veen. 2003. Microbial community composition affects soil fungistasis. Applied and Environmental Microbiology 69:835-844.

Debano, L.F., G.E. Eberlein, and P.H. Dunn. 1979. Effects of burning on Chaparral soils 1. Soil nitrogen. Soil Science Society of America Journal 43:504-509.

Diaz-Ravina, M., A. Prieto, and E. Baath. 1996. Bacterial activity in a forest soil after soil heating and organic amendments measured by the thymidine and leucine incorporation techniques. Soil Biology & Biochemistry 28:419-426.

Diaz-Ravina, M., A. Prieto, M.J. Acea, and T. Carballas. 1992. Fumigation extraction method to estimate microbial biomass in heated soils. Soil Biology & Biochemistry 24:259-264.

Dickinson, K.J.M., and J.B. Kirkpatrick. 1987. The short-term effects of clearfelling and slash-burning on the richness, diversity and relative abundance of higher-plant species in 2 types of eucalypt forest on dolerite in Tasmania. Australian Journal of Botany 35:601-616.

Drijber, R.A., J.W. Doran, A.M. Parkhurst, and D.J. Lyon. 2000. Changes in soil microbial community structure with tillage under long-term wheat-fallow management. Soil Biology & Biochemistry 32:1419-1430.

Driver, J.D., W.E. Holben, and M.C. Rillig. 2005. Characterization of glomalin as a hyphal wall component of arbuscular mycorrhizal fungi. Soil Biology & Biochemistry 37:101-106.

Duineveld, B.M., G.A. Kowalchuk, A. Keijzer, J.D. van Elsas, and J.A. van Veen. 2001. Analysis of bacterial communities in the rhizosphere of chrysanthemum via denaturing gradient gel electrophoresis of PCR-amplified 16S rRNA as well as DNA fragments coding for 16S rRNA. Applied and Environmental Microbiology 67:172-178.

Dunn, P.H., L.F. Debano, and G.E. Eberlein. 1979. Effects of burning on chaparral soils 2. Soil microbes and nitrogen mineralization. Soil Science Society of America Journal 43:509-514.

Dunn, P.H., S.C. Barro, and M. Poth. 1985. Soil-moisture affects survival of microorganisms in heated chaparral soil. Soil Biology & Biochemistry 17:143-148.

Fenn, M.E., M.A. Poth, P.H. Dunn, and S.C. Barro. 1993. Microbial N and biomass, respiration and N-mineralization in soils beneath two chaparral species along a fire-induced age gradient. Soil Biology & Biochemistry 25:457-466.

Ferry, G.W., R.G. Clark, R.E. Montgomery, and R.W. Mutch. 1995. Altered fire regimes within fire-adapted ecosystems. Status and Trends.

Fioretto, A., S. Papa, and A. Pellegrino. 2005. Effects of fire on soil respiration, ATP content and enzyme activities in Mediterranean maquis. Applied Vegetation Science 8:13-20.

Fontaine, S., G. Bardoux, D. Benest, and B. Verdier. 2004. Mechanisms of the priming effect in a savannah soil amended with cellulose. Soil Biology & Biochemistry 68:125-131.

Fontenla, S., I. Garcia-Romera, and J.A. Ocampo. 1999. Negative influence of non-host plants on the colonization of *Pisum sativum* by the arbuscular mycorrhizal fungus *Glomus mosseae*. Soil Biology & Biochemistry 31:1591-1597.

Frandsen, W.H., and K.C. Ryan. 1986. Soil-moisture reduces belowground heat-flux and soil temperatures under a burning fuel pile. Canadian Journal of Forest Research 16:244-248.

Fritze, H., T. Pennanen, and J. Pietikainen. 1993. Recovery of soil microbial biomass and activity from prescribed burning. Canadian Journal of Forest Research 23:1286-1290.

Frostegard, A., E. Baath, and A. Tunlid. 1993. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty-acid analysis. Soil Biology & Biochemistry 25:723-730.

Fyles, J.W., I.H. Fyles, W.J. Beese, and M.C. Feller. 1991. Forest floor characteristics and soil nitrogen availability on slash-burned sites in coastal British-Columbia. Canadian Journal of Forest Research 21:1516-1522.

Gardiner, D.T., R.W. Miller, and R.W. Miller. 2004. Soils in our environment. 10th ed. Pearson/Prentice Hall, Upper Saddle River, N.J.

Gee, G.W. and W. Bauder. 1986. Particle size analysis. In D.L. Sparks (ed). Methods of soil analysis part 1. Physical and mineralogical methods. SSSA, Madison WI, pp. 404-409.

Giovannini, G., S. Lucchesi, and M. Giachetti. 1990. Effects of heating on some chemical-parameters related to soil fertility and plant-growth. Soil Science 149:344-350.

Gonzalez-Perez, J.A., F.J. Gonzalez-Vila, G. Almendros, and H. Knicker. 2004. The effect of fire on soil organic matter - a review. Environment International 30:855-870.

Graham, R.T. 2003. Hayman fire case study: Summary. Gen. Tech. Rep., *In* F. S. Department of Agriculture, Rocky Mountain Research Station, (ed.), Vol. RMRS-GTR-115.

Guerrero, C., J. Mataix-Solera, I. Gomez, F. Garcia-Orenes, and M.M. Jordan. 2005. Microbial recolonization and chemical changes in a soil heated at different temperatures. International Journal of Wildland Fire 14:385-400.

Hardy, C.C., S.G. Conard, J.C. Regelbrugge, and D.R. Teesdale. 1996. Smoke emissions from prescribed burning of southern California chaparral, pp. 1-8 Usda Forest Service Pacific Northwest Research Station Research Paper.

Harris, P., and M.J. Clapperton. 1997. An exploratory study on the influence of vesicular-arbuscular mycorrhizal fungi on the success of weed biological control with insects. Biocontrol Science and Technology 7.

Hart, S.C., T.H. DeLuca, G.S. Newman, M.D. MacKenzie, and S.I. Boyle. 2005. Post-fire vegetative dynamics as drivers of microbial community structure and function in forest soils. Forest Ecology and Management 220:166-184.

Hartnett, D.C., A.F. Potgieter, and G.W.T. Wilson. 2004. Fire effects on mycorrhizal symbiosis and root system architecture in southern African savanna grasses. African Journal of Ecology 42:328-337.

Hastings, R. 1999. Application of denaturing gradient gel electrophoresis to microbial ecology, p. 333, *In* C. Edwards, ed. Methods in Biotechnology 12: Environmental Monitoring of Bacteria. Humana Press, Totowa, N.J.

Hatten, J., D. Zabowski, G. Scherer, and E. Dolan. 2005. A comparison of soil properties after contemporary wildfire and fire suppression. Forest Ecology and Management 220:227-241.

Hedrick, D.B., A. Peacock, J.R. Stephen, S.J. Macnaughton, J. Bruggemann, and D.C. White. 2000. Measuring soil microbial community diversity using polar lipid fatty acid and denaturing gradient gel electrophoresis data. Journal of Microbiological Methods 41:235-248.

Hernandez, T., C. Garcia, and I. Reinhardt. 1997. Short-term effect of wildfire on the chemical, biochemical and microbiological properties of Mediterranean pine forest soils. Biology and Fertility of Soils 25:109-116.

Hillel, D. 1998. Environmental soil physics Academic Press, San Diego, CA.

Huffman, E.L., L.H. MacDonald, and J.D. Stednick. 2001. Strength and persistence of fire-induced soil hydrophobicity under ponderosa and lodgepole pine, Colorado Front Range. Hydrological Processes 15:2877-2892.

Hungerford, R.D., Harrington, M.G., Frandsen, W.H., Ryan, K.C.,and Niehoff, G.J. 1991. Influence of fire on factors that affect site productivity. USDA Forest Service General Technical Report No. INT-280.

Islam, M.A., P.J. Milham, P.M. Dowling, P.C. Jacobs, and D.L. Garden. 2004. Improved procedures for adjusting soil pH. Communications in Soil Science and Plant Analysis 35:25-37.

Jimenez-Esquilin, A.E. 2006. Fire effects in soil microbial community structure and function in a ponderosa pine ecosystem. Dissertation, Colorado State University, Fort Collins.

Johansson, M.B. 1994. The influence of soil scarification on the turn-over rate of slash needles and nutrient release. Scandinavian Journal of Forest Research 9:170-179.

Johnson, C.K., M.F. Vigil, K.G. Doxtader, and W.E. Beard. 1996. Measuring bacterial and fungal substrate-induced respiration in dry soils. Soil Biology & Biochemistry 28:427-432.

Johnson, J. 1916. Preliminary studies on heated soils. Science 43:434-435.

Jones, T. P. & Rowe, N. P. 1999. The earliest known occurrences of charcoal in the fossil record. In: Ninth Annual V. M. Goldschmidt Conference, August 22-27, 1999, Harvard University, Cambridge, MA, USA. LPI Contribution No. 971, Lunar and Planetary Institute, Houston, Texas, USA. pp. 143-144.

Jordan, N.R., J. Zhang, and S. Huerd. 2000. Arbuscular-mycorrhizal fungi: potential roles in weed management. Weed Research 40:397-410.

Karlsson, C., and G. Orlander. 2000. Soil scarification shortly before a rich seed fall improves seedling establishment in seed tree stands of *Pinus sylvestris*. Scandinavian Journal of Forest Research 15:256-266

Karlsson, M., U. Nilsson, and G. Orlander. 2002. Natural regeneration in clear-cuts: effects of scarification, slash removal and clear-cut age. Scandinavian Journal of Forest Research 17:131-138.

Klein, D.A., and M.W. Paschke. 2000. A soil microbial community structural-functional index: the microscopy-based total/active/active fungal/bacterial (TA/AFB) biovolumes ratio. Applied Soil Ecology 14:257-268.

Knicker, H., F.J. Gonzalez-Vila, O. Polvillo, J.A. Gonzalez, and G. Almendros. 2005. Fire-induced transformation of C- and N-forms in different organic soil fractions from a Dystric Cambisol under a Mediterranean pine forest (*Pinus pinaster*). Soil Biology & Biochemistry 37:701-718.

Knorr, M.A., R.E. Boerner, and M.C. Rillig. 2003. Glomalin content of forest soils in relation to fire frequency and landscape position. Mycorrhiza 13:205-210.

Komarek, E.V. 1973. Ancient fires: A quest for ecological understanding. Proceedings 13th Tall Timbers Fire Ecology Conference, Tallahasse, FL. 12: 219-241.

Korb, J.E., N.C. Johnson, and W.W. Covington. 2004. Slash pile burning effects on soil biotic and chemical properties and plant establishment: Recommendations for amelioration. Restoration Ecology 12:52-62.

Kuyumjian, G. 2004. The BAER team: Responding to post-fire threaths. Southwest Hydrology September/October.

Landi, L., L. Badalucco, F. Pomare, and P. Nannipieri. 1993. Effectiveness of antibiotics to distinguish the contributions of fungi and bacteria to net nitrogen mineralization, nitrification and respiration. Soil Biology & Biochemistry 25:1771-1778.

Laughlin, R.J., and R.J. Stevens. 2002. Evidence for fungal dominance of denitrification and codenitrification in a grassland soil. Soil Science Society of America Journal 66:1540-1548.

Lezberg, Ann. Personal communication. January 14, 2006.

Lipson, D.A., C.W. Schadt, and S.K. Schmidt. 2002. Changes in soil microbial community structure and function in an alpine dry meadow following spring snow melt. Microbial Ecology 43:307-314.

Lodge, D.J., and E.R. Ingham. 1991. A comparison of agar film techniques for estimating fungal biovolumes inlitter and soil. Agriculture Ecosystems & Environment 34:131-144.

Lundmark-Thelin, A., and M.B. Johansson. 1997. Influence of mechanical site preparation on decomposition and nutrient dynamics of Norway spruce (*Picea abies* (L.) Karst.) needle litter and slash needles. Forest Ecology and Management 96:101-110.

Mabuhay, J.A., N. Nakagoshi, and T. Horikoshi. 2003. Microbial biomass and abundance after forest fire in pine forests in Japan. Ecological Research 18:431-441.

Manitoba Conservation, Forestry Branch Website. 2001. Scarification for natural regeneration.www.gov.mb.ca/conservation/forestry/forest-renewal/pdfs/scarification2.pdf. Accessed January 2, 2006.

Marschner, P., E. Kandeler, and B. Marschner. 2003. Structure and function of the soil microbial community in a long-term fertilizer experiment. Soil Biology & Biochemistry 35:453-461.

Massman, W.J., and J.M. Frank. 2004. Effect of a controlled burn on the thermophysical properties of a dry soil using a new model of soil heat flow and a new high temperature heat flux sensor. International Journal of Wildland Fire 13:427-442.

Meyer, V.F., E.F. Redente, K.A. Barbarick, R.B. Brobst, M.W. Paschke, and A.L. Miller. 2004. Plant and soil responses to biosolids application following forest fire. Journal of Environmental Quality 33:873-881.

Moore, R. 1992. Soil survey of Pike National Forest, eastern part, Colorado, parts of Douglas, El Paso, Jefferson, and Teller counties, pp. 106, *In* C. USDA Forest Service and Soil Conservation Service in cooperation with the Colorado Agricultural. Experimental Station Denver, (ed.).

Mulvaney, R. 1996. Nitrogen - Inorganic forms, p. 1123-1184 Sparks D.L. (ed) Methods of Soil Analysis, Part 3. Chemical Methods. SSSA, Madison, WI.

Muyzer, G., E.C. Dewaal, and A.G. Uitterlinden. 1993. Profiling of complex microbial-populations by denaturing gradient gel-electrophoresis analysis of polymerase chain reaction-amplified genes-coding for 16s ribosomal-RNA. Applied and Environmental Microbiology 59:695-700.

Nakatsu, C.H., V. Torsvik, and L. Ovreas. 2000. Soil community analysis using DGGE of 16S rDNA polymerase chain reaction products. Soil Science Society of America Journal 64:1382-1388.

Neary, D.G., C.C. Klopatek, L.F. DeBano, and P.F. Ffolliott. 1999. Fire effects on belowground sustainability: a review and synthesis. Forest Ecology and Management 122:51-71.

Neely, C.L., M.H. Beare, W.L. Hargrove, and D.C. Coleman. 1991. Relationships between fungal and bacterial substrate-induced respiration, biomass and plant residue decomposition. Soil Biology & Biochemistry 23:947-954.

Nelson, D., and L. Sommers. 1996. Total carbon, organic carbon, and organic matter. , p. 975-977, *In* D. Sparks, ed. Methods of Soil Analysis, Part 3. Chemical Methods. . SSSA, Madison, WI.

Niemi, R.M., I. Heiskanen, K. Wallenius, and K. Lindstrom. 2001. Extraction and purification of DNA in rhizosphere soil samples for PCR-DGGE analysis of bacterial consortia. Journal of Microbiological Methods 45:155-165.

Ohtonen, R., A. Munson, and D. Brand. 1992. Soil microbial community response to silvicultural intervention in coniferous plantation ecosystems. Ecological Applications 2:363-375.

Olson, F.C. 1950. Quantitative estimates of filamentous algae. Trans American Microscopy Society 69:272-279.

Orlander, G., E. Egnell, and A. Albrektson. 1996. Long-term effects of site preparation on growth in Scots pine. Forest Ecology and Management 86:27-37.

Orwin, K.H., and D.A. Wardle. 2004. New indices for quantifying the resistance and resilience of soil biota to exogenous disturbances. Soil Biology & Biochemistry 36:1907-1912.

Palese, A.M., G. Giovannini, S. Lucchesi, S. Dumontet, and P. Perucci. 2004. Effect of fire on soil C, N and microbial biomass. Agronomie 24:47-53.

Penalva, M.A., and N. Herbert. 2002. Regulation of gene expression by ambient pH in filamentous fungi and yeasts. Microbiology and Molecular biology reviews. 66:426-446.

Pietikainen, J., and H. Fritze. 1993. Microbial biomass and activity in the humus layer following burning - short-term effects of two different fires. Canadian Journal of Forest Research 23:1275-1285.

Pietikäinen, J. 1999. Soil microbes in boreal forest humus after fire. Dissertation, University of Helsinki, Helsinki

Piotrowska-Seget, Z., and A. Mrozik. 2003. Signature lipid biomarker in determining changes in community structure of soil microorganisms. Polish Journal of Environmental Studies 12:669-675.

Piotrowski, J.S., T. Denich, J.N. Klironomos, J.M. Graham, and M.C. Rillig. 2004. The effects of arbuscular mycorrhizas on soil aggregation depend on the interaction between plant and fungal species. New Phytologist. 164:365-373.

Prieto-Fernandez, A., M.J. Acea, and T. Carballas. 1998. Soil microbial and extractable C and N after wildfire. Biology and Fertility of Soils 27:132-142.

Prieto-Fernandez, A., M. Carballas, and T. Carballas. 2004. Inorganic and organic N pools in soils burned or heated: immediate alterations and evolution after forest wildfires. Geoderma 121:291-306.

Prieto-Fernandez, A., M.C. Villar, M. Carballas, and T. Carballas. 1993. Short term effects of a wildfire on the nitrogen status and its mineralization kinetics in an Atlantic forest soil. Soil Biology & Biochemistry 25:1657-1664.

Pritchett, W.L. 1979. Properties and management of forest soils Wiley, New York.

Pritchett, W.L., and R.F. Fisher. 1987. Properties and management of forest soils. 2nd ed. Wiley, New York.

Pyne, S.J., and J.G. Goldammer. 1997. The culture of fire: An introduction to anthropogenic fire history. . In: Clark, J. S., Cachier, H., Goldammer, J. G. & Stocks, B.(eds). Sediment Records of Biomass Burning and Global Change. NATO ASI Series Springer-Verlag, Berlin, Heidelberg. 51:71-114.

Rajapaksha, R., M.A. Tobor-Kaplon, and E. Baath. 2004. Metal toxicity affects fungal and bacterial activities in soil differently. Applied and Environmental Microbiology 70:2966-2973.

Rauni, O., M. Alison, and D. Brand. 1992. Soil microbial communtity response to silvicultural intervention in corniferous plantation ecosystem. Ecological Applications. 2:363-375.

Ranjard, L. and A. Richaume. 2001. Quantitative and qualitative microscale distribution of bacteria in soil. Research in Microbiology 152: 707-716.

Raznikiewicz, H., K. Carlgren, and A. Martensson. 1994. Impact of phosphorus fertilization and liming on the presence of Arbuscular Mycorrhizal spores in a swedish long-term field experiment. Swedish Journal of Agricultural Research 24:157-164.

Rillig, M.C. 2004a. Arbuscular mycorrhizae, glomalin, and soil aggregation. Canadian Journal of Soil Science 84:355-363.

Rillig, M.C. 2004b. Arbuscular mycorrhizae and terrestrial ecosystem processes. Ecology Letters 7:740-754.

Rillig, M.C., and P.D. Steinberg. 2002. Glomalin production by an arbuscular mycorrhizal fungus: a mechanism of habitat modification? Soil Biology & Biochemistry 34:1371-1374.

Ring, E. 1996. Effects of previous N fertilization on soil water pH and N concentrations after clear-felling and soil scarification at a *Pinus sylvestris* site. Scandinavian Journal of Forest Research 11:7-16.

Robichaud, P., L. MacDonald, J. Freeouf, D. Neary, D. Martin, and L. Ashmun. 2004. Postfire Rehabilitation of the Hayman Fire. USDA Forest Service Gen. Tech. Rep. RMRS-GTR-114. 2003.

Robichaud, P.R., and R.D. Hungerford. 2000. Water repellency by laboratory burning of four northern Rocky Mountain forest soils. Journal of Hydrology. 231:207-219.

Rochelle, P.A. 2001. Bacterial community profiling using DGGE or TGGE analysis, p. 177-187 Environmental molecular microbiology : protocols and applications. Horizon Scientific, Wymondham, Norfolk.

Romme, W., T.T. Veblen, M.R. Kaufmann, R. Sherriff, and C.M. Regan. 2003. Ecological effects of the Hayman fire. USDA Forest Service Gen. Tech. Rep. RMRS-GTR-114.

Rosenzweig, W.D., and G. Stotzky. 1979. Influence of environmental-factors on antagonism of fungi by bacteria in soil - clay-minerals and pH. Applied and Environmental Microbiology 38:1120-1126.

Rosenzweig, W.D., and G. Stotzky. 1980. Influence of environmental-factors on antagonism of fungi by bacteria in soil - nutrient levels. Applied and Environmental Microbiology 39:354-360.

Rumpel, C., M. Alexis, A. Chabbi, V. Chaplot, D.P. Rasse, C. Valentin, and A. Mariotti. 2006. Black carbon contribution to soil organic matter composition in tropical sloping land under slash and bum agriculture. Geoderma 130:35-46.

Sawamoto, T., R. Hatano, T. Yajima, K. Takahashi, and A.P. Isaev. 2000. Soil respiration in Siberian Taiga ecosystems with different histories of forest fire. Soil Science and Plant Nutrition 46:31-42.

Schutter, M.E., and R.P. Dick. 2000. Comparison of fatty acid methyl ester (FAME) methods for characterizing microbial communities. Soil Science Society of America Journal 64:1659-1668.

Shepperd, W., Edminster, C. and S. Mata. 2006. Long term seedfall, establishment, survival, growth of natural and planted Ponderosa pine in the Colorado front range. Western Journal of American Forestry 20:0.

Six, J., C. Bossuyt, D. Degryze, and K. Denef. 2004. A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. Soil and Tillage Research 79:7-31.

Smithwick, E.A.H., M.G. Turner, M.C. Mack, and F.S. Chapin. 2005. Postfire soil N cycling in northern conifer forests affected by severe, stand-replacing wildfires. Ecosystems 8:163-181.

Song, H.G., O.S. Kim, J.J. Yoo, S.O. Jeon, S.H. Hong, D.H. Lee, and T.S. Ahn. 2004. Monitoring of soil bacterial community and some inoculated bacteria after prescribed fire in microcosm. Journal of Microbiology 42:285-291.

Sparks, D.L. 2003. Environmental soil chemistry. 2nd ed. Academic Press, San Diego, Calif.

Stendell, E.R., T.R. Horton, and T.D. Bruns. 1999. Early effects of prescribed fire on the structure of the ectomycorrhizal fungus community in a Sierra Nevada ponderosa pine forest. Mycological Research 103:1353-1359.

Sullivan, T.S., M.E. Stromberger, M.W. Paschke, and J.A. Ippolito. 2006. Long-term impacts of infrequent biosolids applications on chemical and microbial properties of a semi-arid rangeland soil. Biology and Fertility of Soils 42:258-266.

Sylvia, D.M. 2005. Principles and applications of soil microbiology. 2nd ed. Pearson Prentice Hall, Upper Saddle River, N.J.

Theodorou, C., and G.D. Bowen. 1982. Effects of a bushfire on the microbiology of a South-Australian low open (Dry Sclerophyll) forest soil. Australian Forest Research 12:317-327.

Thomas, G.W. 1996. Soil pH and soil acidity, p. 475-490, *In* S. DL, ed. Methods of Soil Analysis, Part 3. Chemical Methods. SSSA, Madison, WI.

Treseder, K.K., M.C. Mack, and A. Cross. 2004. Relationships among fires, fungi, and soil dynamics in Alaskan boreal forests. Ecological Applications 14:1826-1838.

United States Department of Agriculture. 2004. National Plant Data Center: The PLANTS Database Version 35 [Online] (verified December 12, 2003).

United States Forest Service. 2002. Hayman fire assistance website. http://www.fs.fed.us/r2/psicc/hayres/.

van der Heijden, M.G.A., J.N. Klironomos, M. Ursic, P. Moutoglis, R. Streitwolf-Engel, T. Boller, A. Wiemken, and I.R. Sanders. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396:69-72.

Vazquez, F.J., M.J. Acea, and T. Carballas. 1993. Soil microbial-populations after wildfire. FEMS Microbiology Ecology 13:93-103.

Vestal, J.R., and D.C. White. 1989. Lipid analysis in microbial ecology - Quantitative approaches to the study of microbial communities. Bioscience 39:535-541.

Villar, M.C., V. Petrikova, M. Diaz-Ravina, and T. Carballas. 2004. Changes in soil microbial biomass and aggregate stability following burning and soil rehabilitation. Geoderma 122:73-82.

Vitousek, P.M., and P. Matson. 1985. Intensive harvesting and site preparation decreases soil N availability in young plantations. Southern Journal of Applied Forestry 9:120-125.

Walker, L.R., and R. Willig. 1999. Ecosystems of disturbed ground, p. xii, 868, *In* L. Walker, ed. Ecosystems of the world ; 16., 1st ed. Elsevier, Amsterdam ; New York.