DISSERTATION

Altered Fire Regime Impacts on the Soil Biogeochemistry and Microbial Community Structure of Mixed Conifer and Ponderosa Pine Forests

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY SARAH HAMMAN ENTITLED 'ALTERED FIRE REGIME IMPACTS ON THE SOIL BIOGEOCHEMISTRY AND MICROBIAL COMMUNITY STRUCTURE OF MIXED CONIFER AND PONDEROSA PINE FORESTS' BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

ALTERED FIRE REGIME IMPACTS ON THE SOIL BIOGEOCHEMISTRY AND MICROBIAL COMMUNITY STRUCTURE OF MIXED CONIFER AND PONDEROSA PINE FORESTS

The practice of fire suppression and intensified grazing of dry western forests in the early 1900s has altered many aspects of forest structure and composition by increasing shade-tolerant species and creating a more continuous vertical and horizontal fuel structure. This has led to increased fire severity and extent throughout many western forests. Projections from climate change models suggest that fire severity and extent in many of the forests of the western United States will continue to rise. To mitigate this risk, managers are conducting prescribed burns when fuels are wetter and weather conditions are more suitable in areas with high fuel loads. The effects of these atypical fires on ecosystem structure and function are largely unknown.

This dissertation assesses the impact of altered fire regime (season, severity) on the soil biogeochemistry and microbial community of two different forest types. To assess effects of altered fire season on soil systems, I studied sites subjected to early season prescribed fire, late season prescribed fire, and no fire (control) in a mixed-conifer forest in Sequoia National Park, CA. Results show that late season fires did have larger, more persistent impacts on the soil system than early season fires, relative to the control

sites, altering several soil abiotic variables and decreasing biotic activity for 2-3 years post-burn. While these within-year treatment effects were significant for many soil environmental and biogeochemical parameters, the effect of year was significant for all variables tested. This suggests that certain climatological variables, such as precipitation, should be considered when analyzing treatment effects over several years.

Post-fire nitrogen availability can be very important for re-colonizing organisms, both above and belowground. Inorganic nitrogen is very difficult to measure, however, due to the myriad of processes impacting net mineralization rates and methodological artifacts. To tease apart some of the problems associated with two different techniques and to assess their sensitivity to important environmental variables, I evaluated fire treatment effects on net nitrogen mineralization rates as measured by the soil core incubation method and the ion exchange membrane (IEM) method. The two methods were not significantly correlated. The core incubation method showed no change in net mineralization rate and a two-fold increase in net nitrification rate with fire while the IEM technique showed nearly a 3-fold increase in net mineralization rate and a 4-fold increase in nitrification rate with fire. The IEM method was also more sensitive to soil environmental variables than the core incubation method.

To evaluate how varying severity fire impacts soil microbial communities, I established sites in dry ponderosa pine forests subjected to low severity fire, high severity fire, and no fire (control) in Pike National Forest, CO. The different fire severities did have different effects on the soil environment (pH, temperature, moisture). Soil microbial communities from low and high severity sites were not distinct, however, they were both significantly different from the unburned sites, according to canonical correspondence

analysis of fatty acid (EL-FAME) biomarkers. Overall microbial biomass and richness were not significantly different among treatments, suggesting a quick recovery of a structurally distinct microbial community in both the low severity and high severity burn sites.

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CHAPTER 1

INTRODUCTION

All aspects of fire regime (frequency, extent, seasonality, intensity, and severity) depend on forest structure, species composition, local weather, and regional climate (Schoennagel et al. 2004; Whitlock 2004). The practice of fire suppression and intensified grazing of dry western forests in the early 1900s has altered many aspects of forest structure and composition by increasing shade-tolerant species and creating a more continuous vertical and horizontal fuel structure (Covington and Sackett 1992). This has led to an increase in wildfire severity and extent throughout several forested regions (Pierce et al. 2004): in the last decade, the number of acres burned in the western U.S. has increased, but the number of fires has actually decreased, reflecting an increase in the spatial extent of fires (Figure 1.). This trend is predicted to continue with future climate change (Grissino-Mayer and Swetnam 2000; Dale et al. 2001), increasing the risk of wildfires and the costs involved in fighting them (Dale et al. 2001).

In order to mitigate the wildfire risk and the cost of fighting fire, land management agencies throughout the western U.S. have begun applying prescribed fire as a fuel reduction tool. Ideally, fire-hazard reduction goals in a management plan are coupled with goals of restoring ecosystem structure and function. Implementing this type of 'process restoration' (Stephenson 1999), however, requires knowledge and understanding of the short- and long-term impacts of the management techniques on key ecological processes (nitrogen dynamics, water movement/uptake, decomposition rates, seed dispersal). For many systems, general fire effects on forest stand structure are well studied, but fire impacts on the spatial and temporal aspects of soil nutrient dynamics, microbial community structure, and plant-soil interactions are not as well understood (Kaye and Hart 1998; Hart et al. 2005; Yeager et al. 2005). In addition, altered fire regimes of the future may influence forest structure and functioning in new ways, setting both the above- and belowground communities on a trajectory of recovery different from the natural history of the site (Acea and Carballas 1996). Altered plant and soil community structure (species composition, abundance, and demographics) could have further implications on nutrient cycling and ecosystem stability (Perry et al. 1990; van der Heijden et al. 1998; Balser and Firestone 2005). Understanding the role of altered fire regimes on ecosystem structure and functioning is vital to developing sound adaptive management policies under a changing climate.

The main objectives of my dissertation were to assess the relative impacts of different fire seasons and different fire severities on soil environmental, biogeochemical, and microbiological parameters. To assess effect of fire season, I sampled sites that experienced early season fire, late season fire, or no fire (control) in the Giant Forest region of Sequoia National Park, CA (Figure 2). Sequoia National Park has one of the oldest, most extensive prescribed fire programs in the country and an active fire ecology research program (Keeley and Stephenson 2000). Fire history and fire effects on vegetation dynamics of the region have been well studied, but research on how fire management affects soil systems is largely lacking. Chapter 2 specifically addresses recovery of soil carbon and nitrogen pools and fluxes in early and late season burn sites both one and two years post-fire. Chapter 3 presents data on the soil environmental conditions and nutrient pools impacting microbial enzyme activity in early and late season burn sites two and three years post-fire. Chapter 4 addresses a methodological experiment I conducted to determine the compatibility of two different methods used to estimate net nitrogen mineralization rates.

Based on the initial effects of early and late season burns on the forest soils in Sequoia, I became interested in studying how altered fire regime effects on soil biogeochemistry might translate into changes in microbial community structure. In order to address these questions, I established sites in unburned, low, and high severity burn sites throughout the Hayman burn area in the Pike National Forest, CO (Figure 3). Chapter 5 describes and quantifies the effects of varying severity wildfire on the soil environment and the subsequent changes in the microbial community structure. In Chapter 6, I summarize my findings and present the main conclusions from my dissertation.

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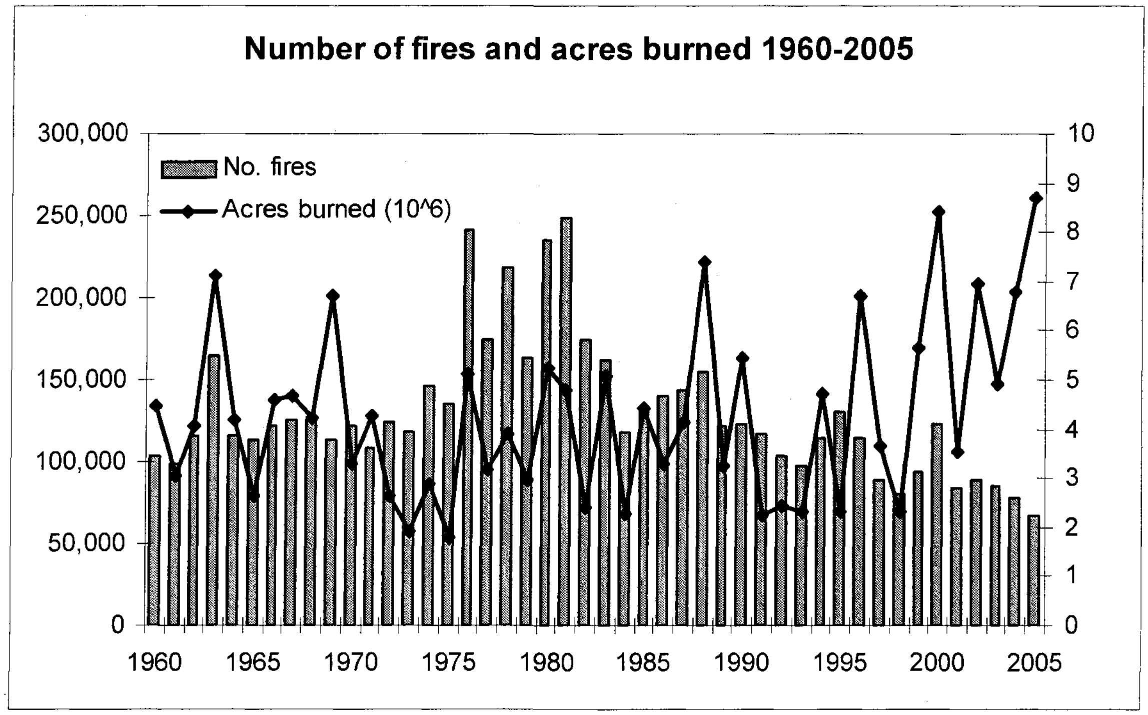

Figure 1. Fire extent has increased over the past decade, with fewer fires burning more acres throughout the western United States. National Interagency Fire Center, Wildland Fire Statistics: http://www.nifc.gov/stats/fires_acres.html

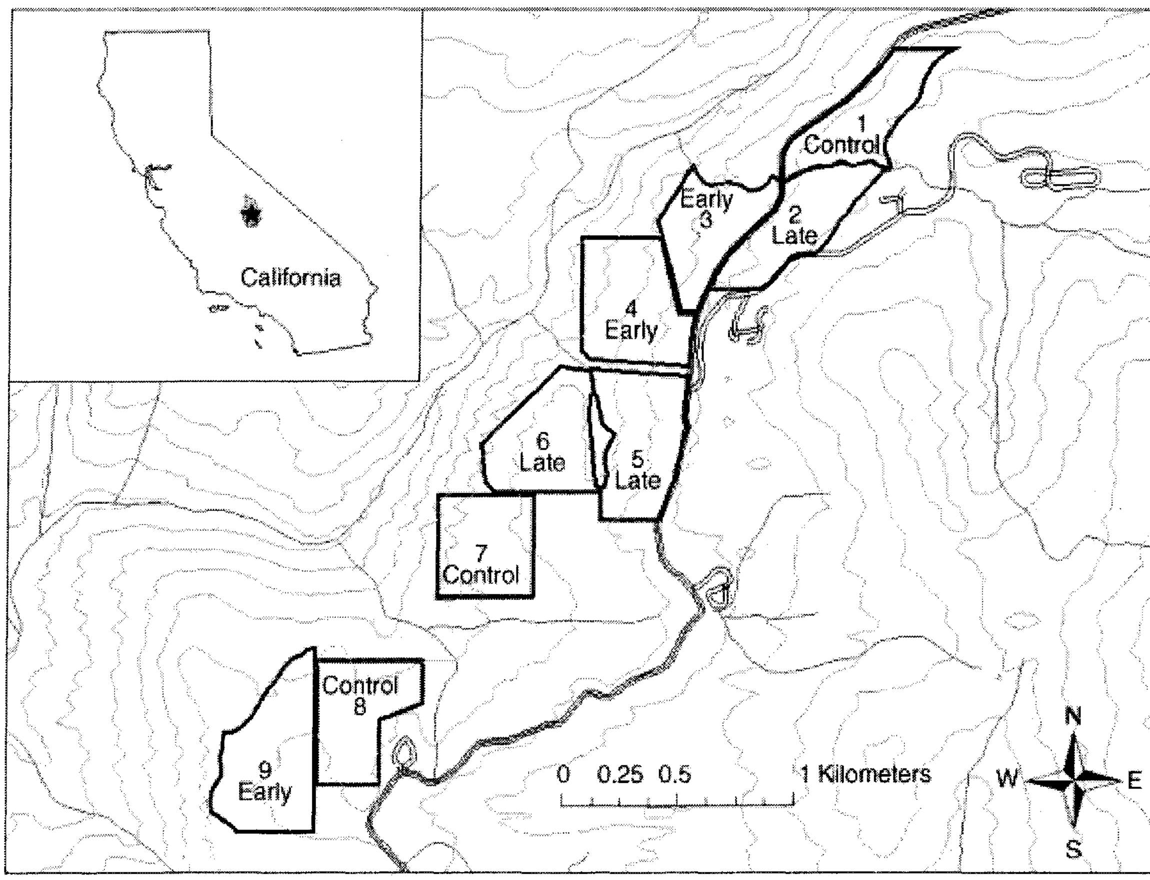

Figure 2. Study sites for project in Sequoia National Park, CA, showing the replicated burn treatments: early season, late season, and control, situated along Generals Hwy.

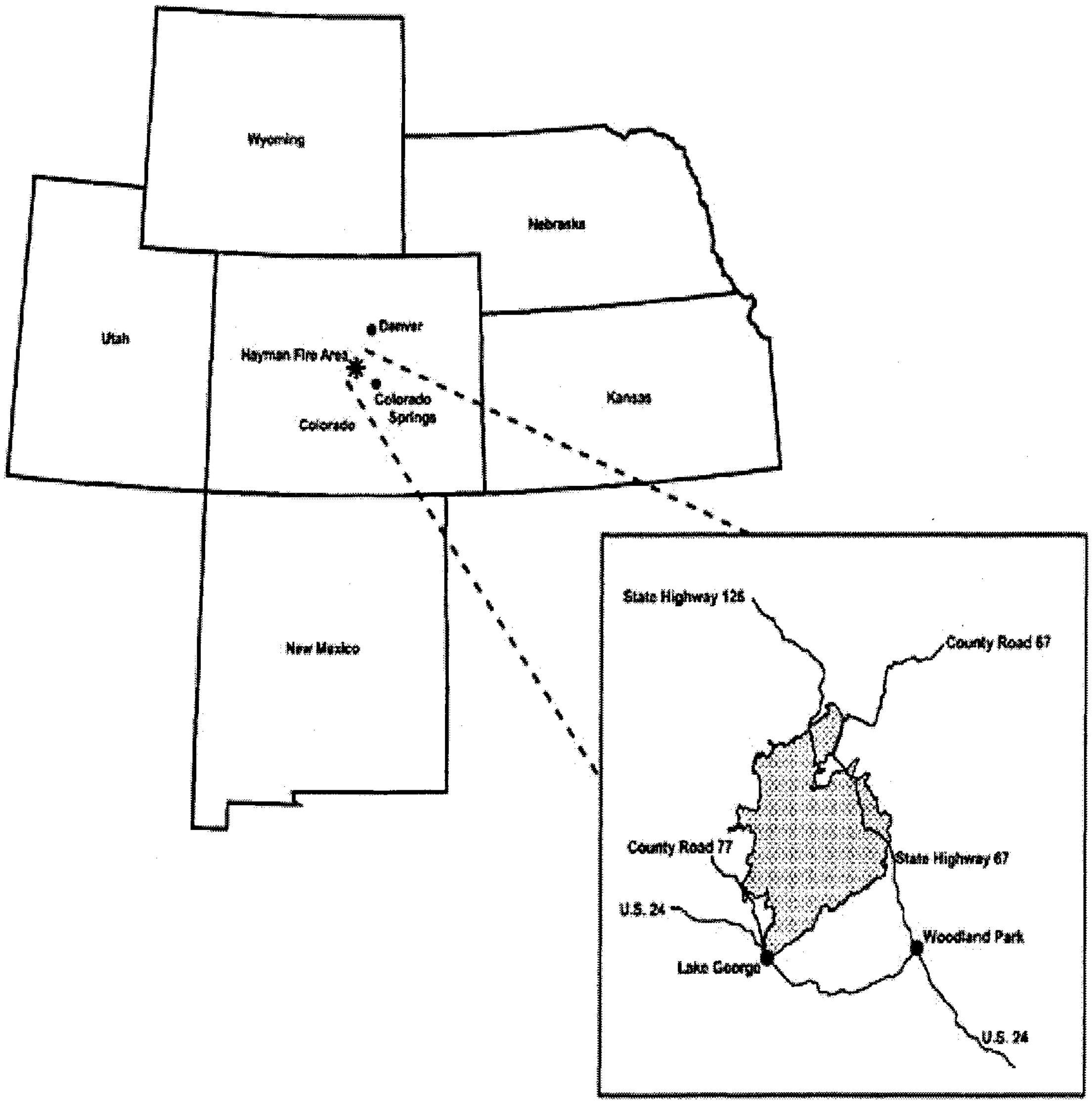

Figure 3. The location of the Hayman burn site in central CO used in this project. Hashed area represents the area of the 55,000 ha burn.

CHAPTER 2

RECOVERY OF SOIL NUTRIENT POOLS AND FLUXES AFTER TWO DIFFERENT FIRES IN A SIERRA NEVADA MIXED CONIFER FOREST

Introduction

Historically, fire has been an important driver of forest structure and composition for most forest ecosystems throughout the western United States (Covington et al. 1994). The common practice of fire suppression during the first half of the 20th century has led to an increase in aboveground biomass and hazardous fuel levels (Covington and Sackett 1984; Tilman et al. 2000; Schoennagel et al. 2004), leading to a shift in fire regime for many different forest types (Kilgore and Taylor 1979; Swetnam and Baisan 1996; Smith et al. 2004; Kaye et al. 2005). For the past few decades, land management agencies have employed prescribed burning to mediate these fuels and to restore ecosystem structure and function to pre-Euro-American settlement conditions. This can be difficult to do in systems that are far removed from their natural range of variability.

One example of this type of system is the mid-elevation forest of the southern Sierra Nevada mountains of California. Historically, these forests experienced frequent

(5-25 years), low severity, late season fires that maintained open stand structures and low fuel loads (Kilgore and Taylor 1979; Miller and Urban 2000; Keeley and Stephenson 2000). Today, maintaining a fire regime that incorporates low severity, late season burning is extremely difficult from both an environmental and management perspective. The currently high fuel loads combined with low fuel moisture and high air temperatures in the fall increase the risk of fire escaping to adjacent areas, and stable atmospheric patterns typical of fall tend to restrict smoke dispersion, potentially impacting air quality in the neighboring Central Valley (Knapp et al. 2005). This region is plagued by poor air quality so activities that contribute to the air pollution are highly controversial. Conducting prescribed burns earlier in the growing season when fuel moisture is higher and atmospheric conditions are more suitable is a potential solution to this problem, but the short and long-term effects of spring burning on ecosystem properties and processes, such as biogeochemical cycling, are unknown.

Fire affects carbon cycling in forest soils directly by oxidizing many of the available compounds and indirectly by changing environmental constraints on microbial activity. There are often short-term fire-induced changes in the nitrogen budget, as well. Soil inorganic nitrogen (NH_4^+ and NO_3^-) pools typically increase from heat-induced NH_4^+ release from clay complexes (during fire), deposition of organic nitrogen in ash and enhanced mineralization rates (immediately following fire) (Grogan et al. 2000), and nitrogen-fixation (by colonizing plants species post-fire) (Wan et al. 2001). This pulse of available nitrogen has been shown to lead to short-term enhanced site productivity in some systems (Wan et al. 2001).

Season of fire may differentially impact the magnitude and direction of these biogeochemical effects through differences in fire intensity and differences in post-fire weather. Fire intensity of early season fires tends to be lower than late season fires due to relatively high fuel moisture and low air temperatures. The temperatures reached during fire impact the chemical changes that dictate post-fire soil pH, moisture, C and N concentrations, and microbe and root survival (Neary et al. 1999). Therefore, different fire seasons may impact several components of belowground structure and function differently. Post-fire weather can also influence soil physicochemical conditions and nutrient concentrations. In the Sierra Nevadas, the wet season runs between October and April, and the dry season typically lasts throughout the summer months, with only one or two rain events (Kilgore and Taylor 1979). Rain events immediately after a burn often lead to a loss of the nutrient-rich ash layer through erosion and overland flow (Huffman et al. 2001). Consequently, late season burns, through higher intensity and seasonal timing, may be subject to greater post-fire nutrient loss.

My objectives were to determine the short-term effects (1 to 3 years) of early and late season prescribed burning on 1) soil environmental conditions (pH, moisture), 2) soil carbon and nitrogen pools and 3) soil carbon and nitrogen fluxes in a mixed conifer, mid-elevation Sierran forest.

Methods

Study Sites

I conducted this study within the watershed of the Marble Fork of the Kaweah River in the Giant Forest area of Sequoia National Park, California, located in the southern Sierra Nevada Mountains. The Mediterranean climate comprises cool, wet winters and warm, dry summers. Mean annual precipitation is approximately 114 cm, most of it falling as snow. The soils of this region have formed in residuum, colluvium and morainal material that have weathered from granitic rocks. They are classified coarse-loamy, frigid Pachic Xerumbrepts (Huntington and Akeson 1987). The dominant plant species in this mixed conifer, old growth forest are white fir (*Abies concolor* [Gordon & Glend.] Lindley), incense cedar (*Calocedrus decurrens*), sugar pine (*Pinus lambertiana* Douglas), Jeffrey pine (*Pinus jeffreyi* Grev. & Balf.), ponderosa pine (*Pinus ponderosa*), mountain dogwood (*Cornus nuttallii* Audubon), and California black oak (*Quercus kelloggii*). The sites I studied are all on west-northwest facing slopes of approximately 15-25 degrees at elevations ranging from 1900 to 2150 m. The pre-settlement fire return interval has been estimated at 20-40 years; however prior to the recent prescribed burns for this study, the sites had not burned since 1879 (Caprio and Knapp, unpublished data).

Sampling Design and Field Measurements

These sites are part of the National Fire and Fire Surrogate Study, a program developed to evaluate the effects of fuel treatments on ecosystem structure and function (Weatherspoon and McIver 2000). Three replicate early season burn, late season burn, and unburned control sites were established in a completely randomized design, each averaging 15 ha in size. The prescribed fires were conducted in October 2001 (late season) and June 2002 (early season) (see Table 1 for fire characteristics). In each site there were ten modified Whittaker subplots (50m x 20m) for vegetation sampling. In order to avoid disturbing the vegetation, I sampled soils 1m outside the subplots at each corner (4 sub-samples per subplot). I took soil cores to assess nitrogen availability, soil moisture and pH in late season and control plots in June 2002, early season and control plots in June 2003 and in all plots in June 2004. For nitrogen mineralization rates, I used the soil core incubation method, a modified version of the buried bag method (Eno 1960). I collected samples by removing litter and duff layers and driving an aluminum core (15 cm deep x 5 cm diameter) into the mineral soil (Raison 1987). I collected one sample for laboratory analysis (initial) and took another core adjacent to the first, placed it back into the soil with a perforated cap on the bottom of the core (to contain soil but allow for gas and water exchange), and replaced litter and duff layers. I returned 28 days later to collect the second soil core (final). At the time of removal, I immediately placed samples on ice and transferred them to the lab for same-day processing and KCl extraction. I calculated net nitrogen mineralization rate as the change in the total inorganic nitrogen concentration over the incubation time ((final – initial) 28 $days^{-1}$).

I collected and composited 6 mineral soil samples (0-15cm) from each subplot for total C and N analysis. I measured soil respiration at opposing corners of the subplots during the same sampling period (2 sub-samples per subplot) using a PP-Systems EGM-1 Soil Respirometer.

Laboratory Analyses

I sieved soil samples within twelve hours of collection through a 2mm mesh and took 10g sub-samples for moisture and pH. I determined soil moisture using the gravimetric method and soil pH using a 1:1 ratio of soil and de-ionized (DI) water. I dried and ground 10g sub-samples of soil before measuring total soil C and N using a LECO CHN1000 combustion gas analyzer (LECO Corporation, St. Joseph, MI, USA). I extracted 10g subsamples in 50ml 2M KCl, filtered the solutions and analyzed the extracts for NH_4^+ and NO_3^- colorimetrically using an Alpchem Autoanalyzer (College Station, TX).

Statistical Analyses

I tested for the effects of burn season on soil environmental and biogeochemical variables using one-way ANOVA with treatment (early season burn, late season burn, and control) as the independent variable (n=3). Significant differences between treatment means were determined using Tukey's highly significant difference (HSD) (alpha=0.05). I determined the significance of fire treatment, sample year, and the interaction of the two on soil environmental and biogeochemical parameters using a mixed model with treatment and year as fixed effects and site and plot as random variables. I ran all analyses using the Statistical Analysis System (SAS Institute, 1997).

Results

Fire effects on soil environmental variables

There was no change in soil moisture one year after the early season burn, relative to the control, but there was a 22% decrease in soil moisture one year after the late season burn, relative to the control. This difference in the late season burn from the control was still evident, although less pronounced, two years post-fire (Figure 1a). Similarly, soil pH was not significantly different from the control after the early season burns, but there was a large increase in soil pH one year post-fire in the late season burn sites, relative to the control (Figure 1b). By 2004, however, soil pH levels in the late season sites returned to unburned levels.

Fire effects on soil carbon and nitrogen pools

Early season burning did not significantly alter total soil carbon levels, relative to the control; however, one year post-fire soil carbon levels were 17% lower in the late season burn sites (Figure 2a.). This effect continued into 2004 but was not as great. There was a significant effect of year on this variable (Table 1), with lower soil C levels in both 2003 and 2004. I found no significant effect of either early season fire treatment or late season fire treatment on total soil nitrogen levels but I did see a significant year effect (Figure 2b, Table 1).

Fire effects on soil carbon and nitrogen turnover rates

Soil respiration rates were significantly lower than the control one year post-fire in both early season (28% lower) and late season (35% lower) burn sites. This trend continued into 2004 (Figure 3a.). There was also a significant year effect (Table 1), reflecting an overall increase in soil respiration rate in the control sites from year 2002 to 2004. There was a dramatic shift in nitrogen dynamics in the control sites from net mineralization in 2002 to net immobilization in 2003 and then back to net mineralization in 2004 (Figure 3b.), reflecting a significant year effect on this flux (Table 1). Early season fires significantly increased net nitrogen mineralization rates relative to the control one year post-fire but late season fires had no effect on the net nitrogen mineralization rates 8 months post-fire. By 2004 there was no significant difference in net nitrogen mineralization rates between the fire treatments and the control.

Discussion

Late season burns had a much more dramatic effect on soils than early season burns. One year post-fire, there were no significant changes in soil moisture, soil pH, soil C or soil N in the early season sites, relative to the control plots. This suggests that the temperatures reached during these burns were not hot enough to alter the physical and chemical properties of the litter and soil.

The short-term decrease in soil moisture levels in the late season burn sites were likely due to the vaporization of hydrophobic hydrocarbons in the litter during fire, and the subsequent condensation of these compounds on soil aggregates deeper in the soil profile, creating hydrophobic patches in soil (Neary et al. 1999). This phenomenon has been shown to be a primary cause of post-fire increases in runoff and erosion in moderate-high severity burn sites (Huffman et al. 2001). The severity, extent, and duration of post-fire water repellency in soils is dependent on soil texture, fire intensity, antecedent soil water content, and fuel type (Robichaud 2000), so any major difference in these components could lead to large differences in soil hydrophobicity and, hence, infiltration rates. Huffman et al. (2001) found that fire-induced hydrophobicity persisted for at least 22 months in soils experiencing a range of fires in lodgepole and ponderosa pine forests.

Fire intensity also likely played a large role in altering soil pH levels. Higher fire temperatures in the late season burns presumably caused greater rates of combustion of undissociated organic acids in the litter and soil, removing them from the system. Also, the leaching of basic cations from the ash into the soil complex and the associated consumption of hydrogen ions in the formation of water could have led to higher pH levels in the late season burn sites (Neary et al. 1999). Changes in soil pH have been shown to impact soil microbial community structure (Fierer and Jackson 2006). By selecting against certain functional groups that favor lower pH levels (namely, fungi), disturbances such as fire may have short- and long-term impacts on C turnover rates in the soil (Bailey et al. 2002).

There was a decrease in soil C with late season burning, relative to the controls, which persisted throughout the study. This likely reflects the higher severity of these burns. The persistence of this change three years post-fire suggests that there was slow recovery of the above and belowground biota in these sites. Studies are currently being conducted to evaluate the potential impact of early vs. late season burning on aboveground vegetation at these sites. The effect of year on soil carbon stocks was significant ($p<0.0001$), suggesting some climatological or site influence on this parameter. Total annual precipitation at these sites in 2002 (209cm) was twice as high as in 2003 (106cm) and nearly 2.5 times as high as in 2004 (83cm) (National Climate Data Center, http://cdo.ncdc.noaa.gov). Because most of the precipitation falls as snow in this forest system, the vegetation is able to capitalize on the flush of spring runoff. The drop in precipitation in 2003 may have limited both above and belowground productivity, leading to lower levels of total soil carbon stocks.

The drop in total soil nitrogen in 2003 and 2004 followed the same trend, likely for the same reasons. However, within each year, there was no effect of treatment on total soil nitrogen. Fire effects on total soil N pools has been controversial in the literature; studies have reported an increase (Covington and Sackett 1992; Kovacic et al. 1986; Schoch and Binkley 1986), a decrease (Bell and Binkley 1989; Raison et al. 1985), and no change (Knoepp and Swank 1995) in total soil nitrogen pools after fire. This inconsistency in results is likely due to the complex nature of factors influencing nitrogen pools (soil moisture, leaching, soil erosion, plant uptake, microbial immobilization), the spatial heterogeneity of the nitrogen loss during fire, and the soil depth measured. The effects of fire are greatly diminished below 2-5cm (Neary et al. 1999), so if soils are sampled below this depth and mixed, as they were in this study, the effect of the burn is often diluted by the unaffected deeper soil horizons (Gillon and Rap 1989; Wan et al. 2001).

The significant drop in soil respiration with both early and late season burning one and two years post-fire can be partially explained by the high rates of tree mortality; approximately one-third of the trees died in the early season sites and one-half died in the late season sites, decreasing the autotrophic contribution to total respiration. There was likely an immediate drop in heterotrophic contribution, as well, with direct mortality of soil organisms during the fire and indirect impacts on heterotrophic activity by altered environmental conditions two years post-fire. Understanding how these different soil components respond to different fire applications will aid us in building belowground carbon budgets for forest systems that experience frequent fires.

The changes I saw in the net nitrogen mineralization rates were not consistent with previous studies showing a short-term increase in nitrogen turnover rates post-fire (Hobbs and Schimel 1984; Schoch and Binkley 1986; Kaye and Hart 1998; DeLuca and Zouhar 2000). Because the late season fires killed more trees (and roots) than the early season burns, these fires may have provided a flush of carbon substrate to the microbial populations. This may have increased microbial immobilization rates in the late season burn sites, temporarily lowering the net mineralization rates to control levels. By the time I sampled these plots again in 2004, the net rates were not significantly different from those in the control sites and the initial increase in the early season burn sites (relative to the controls) had subsided. These results may also be attributed to methodology: I used the soil core incubation method. One artifact of this method is that core placement usually involves the severing of roots. The dead and dying roots inside the soil core provide a flush of organic substrate for the microbial population, potentially leading to increased immobilization rates (Adams et al. 1989; Hook and Burke 1995), and, subsequently, lower net mineralization rates.

Conclusions

The decision to burn in the spring or the fall is influenced by several ecological, climatological, social, and political factors. Because of this, the relative importance of belowground processes as one component may seem trivial. However, soil microclimate and nutrient availability immediately post-fire can impact the rate and trajectory of re-colonizing plant and microbial communities (Hart et al. 2005). Therefore, determining the relative impacts of early season vs. late season burning on soil physicochemical conditions will help us understand the limitations to above- and belowground biotic recovery under different management practices. This study shows that late season burning had more dramatic short-term effects on soil abiotic conditions and mineral soil carbon levels than early season burning. However, the total soil nitrogen pools and fluxes and soil respiration rates were not differentially impacted by the two different burn types. This suggests that, despite the differences in the soil environment, the belowground heterotrophic communities responsible for these fluxes recovered at similar rates in the different burn treatments. Incorporating these data with aboveground and belowground community recovery data will help us understand the plant-soil linkage in disturbed systems and will ideally help researchers and managers incorporate ‘process restoration’ in addition to ‘structure restoration’ into their management plans.

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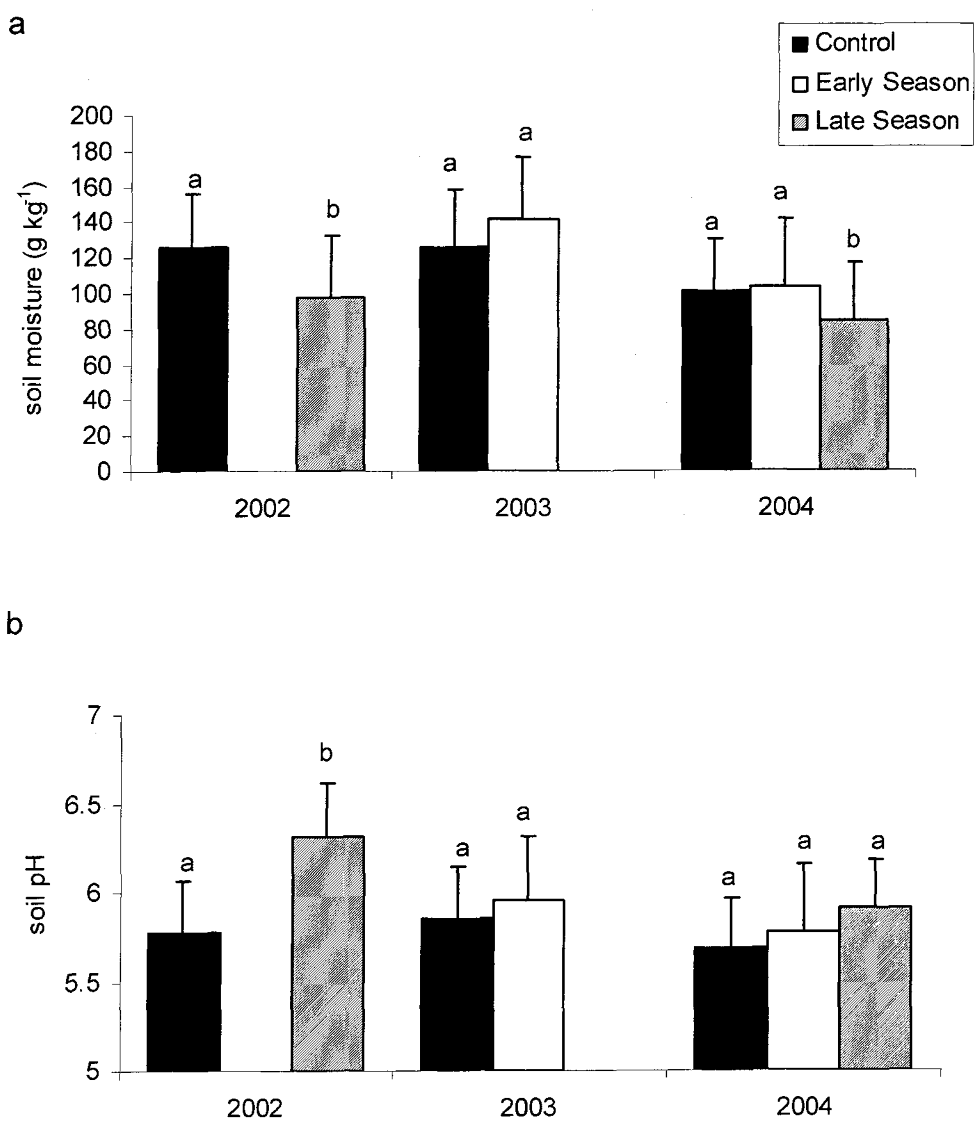
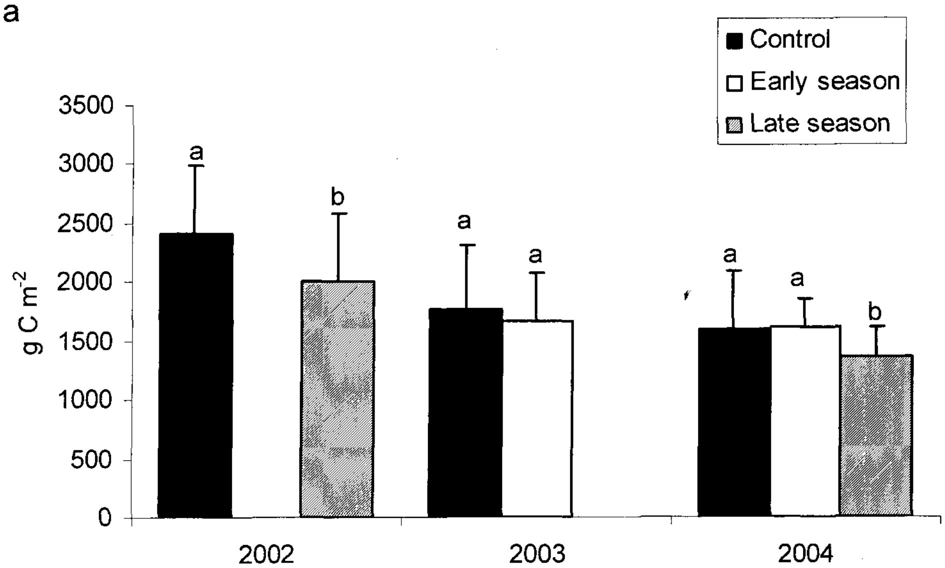


Figure 1. Effects of early and late season fire on soil moisture (a) and pH (b) for each sampling year. Treatment means (+1SD) are presented. Different letters denote a significant treatment difference ($p<0.05$) for the given year.

a



b

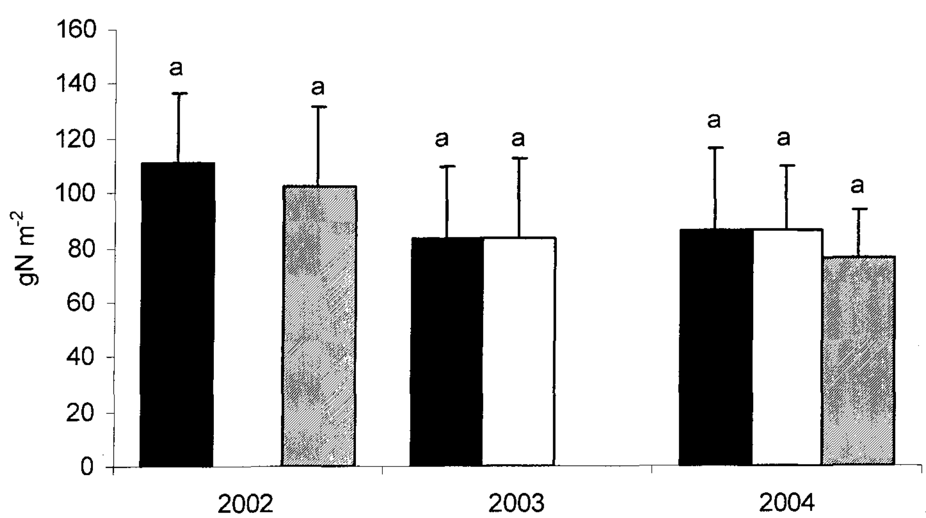


Figure 2. Treatment effects on total soil carbon (a) and total soil nitrogen (b). Treatment means (+1SD) are presented. Within year treatment differences ($p<0.05$) are represented by different letters.

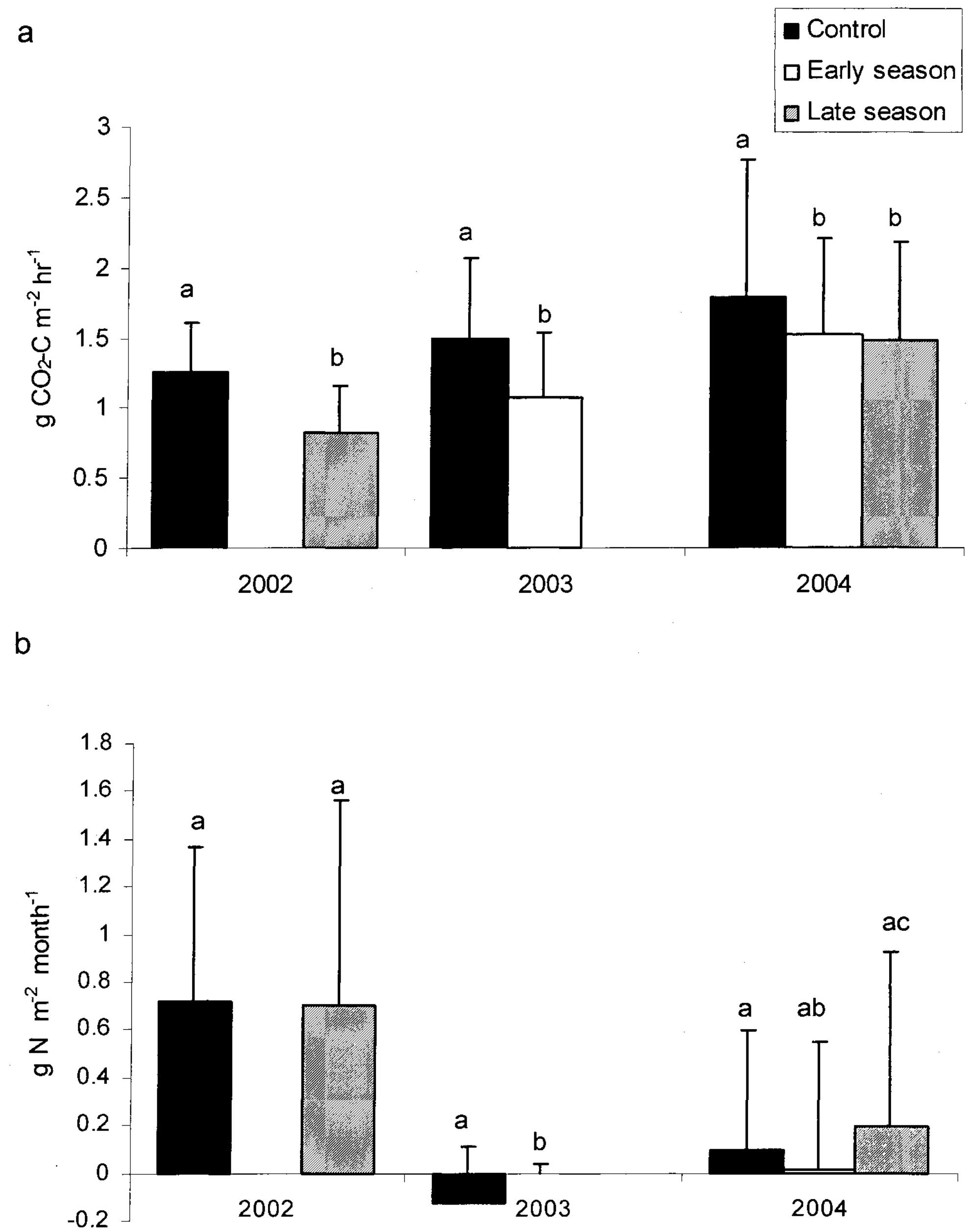


Figure 3. Treatment effects on soil respiration rates (a) and net nitrogen mineralization rates (b). Treatment means (+1SD) are presented. Within year treatment differences ($p<0.05$) are represented by different letters.

Table 1. Characteristics of early and late season prescribed fires conducted in the Giant Forest region of Sequoia National Park, CA. Treatment means are presented. AG=Aboveground

	Scorch height (m)[a]	% area burned[a]	% AG litter and duff consumed[b]	% Tree mortality (2002)[c]	% Tree mortality (2004)[d]
Early Season	11.1	70	66	26	38
Late Season	13.5	88	79	37	56

[a] Data from Knapp et al. (2006)
[b] Data from Knapp et al. (2005)
[c] Results incorporate direct tree mortality from fire (Schwilk - personal communication)
[d] Results incorporate direct tree mortality from fire and post-fire mortality from bark beetle infestation (Schwilk – personal communication)

Table 2. Significance of fire season treatment and year on soil environmental and biogeochemical parameters. P-values are presented for all soil variables. SRR=Soil Respiration Rate.

	% Soil H_2O	Soil pH	Soil C	Soil N	Net Nmin	SRR
Treatment	0.347	0.199	0.254	0.647	0.938	0.009
Year	<0.001	0.004	<0.001	<0.001	0.008	<0.001
Treatment*year	0.478	0.075	0.435	0.955	0.597	0.976

CHAPTER 3

ALTERED SEASON OF FIRE AFFECTS SOIL BIOGEOCHEMSTRY AND MICROBIAL ENZYME ACTIVITY

Introduction

Fire suppression, grazing, and influences of a changing climate have led to an increase in forest biomass, which has created hazardous fuel loads and, consequently, a rise in the extent and intensity of wildfires in certain areas of the western United States (Covington and Sackett 1984; Grissino-Mayer and Swetnam 2000). For the past few decades, land management agencies have employed prescribed burning to mediate some of these effects and to restore ecosystem structure and function (DeLuca and Zouhar 2000). This can be difficult to do without increasing the risk of uncontrollable wildfire in systems that are far removed from their natural range of variability.

One example of a fire-suppressed system is the mid-elevation forest of the southern Sierra Nevada mountains. Historically, most fires in the mixed conifer forests of the Sierra Nevadas burned late in the growing season. Today, maintaining a fire regime that incorporates late season burning is extremely difficult due to limited number of safe prescription days and major air quality concerns (Knapp et al. 2005). The thermal inversion layer that often occurs in late summer and early fall restricts smoke dispersion in an area that is already plagued by poor air quality. Fuel moisture is typically very low after the dry summer months and air temperatures are high. Managers are beginning to conduct prescribed burns earlier in the growing season when these environmental conditions are more suitable, but the effects of spring burning in this forest type on ecosystem properties and processes are largely unknown.

Season of burning largely determines fire intensity and, subsequently, fire severity. Higher fuel moisture levels and lower air temperatures lead to lower intensity fires in the spring while low fuel moisture and dry, hot weather often leads to high intensity fires in the summer and fall. Because the magnitude and direction of many fire effects on soil biogeochemistry depend on fire intensity (Hart et al. 2005), a change in fire season could create large differences in the belowground biotic recovery of sites subjected to these two types of fire. Fire intensity plays a large role in nutrient availability post-burn; higher intensity fires oxidize more of the carbon and nitrogen present in the forest floor (Grogan et al. 2000; Wan et al. 2001) and may have greater impacts on the soil environmental conditions that limit microbial activity. Some of the key soil macronutrients that are important for microbial activity (P, Ca, Mg, K) are also often impacted by high intensity fire (Marion et al. 1991; Romanyà et al. 1994); the temperatures reached during the fire determine the post-fire concentrations of these nutrients.

One way to assess soil quality and microbial activity is through soil enzyme analysis (Boerner et al. 2000). Soil enzymes are sensitive indicators of stress and reflect activities of microorganisms important for C, N, and P cycling (Sinsabaugh et al. 1993; Boerner et al. 2005). Two enzymes, acid phosphatase and phenol oxidase, are commonly used to give a comprehensive picture of the activity of important microbial fractions involved in decomposition and nutrient turnover in forest soils (Sinsabaugh et al. 1993; Boerner et al. 2000). Acid phosphatase is actively excreted by tree roots and microbial cells and passively released by ruptured cells. It is involved in P cycling in soils and is regulated by soil microclimate and organic carbon availability, with optimum activity at pH 5.0-5.5 (Saa et al. 1993). Phenol oxidase is a lignocellulose-degrading enzyme and is primarily limited by substrate and N availability (Boerner et al. 2005). The impact of fire on these enzymes and on the environmental factors that control their activity may help us understand the role of microorganisms in ecosystem recovery from disturbance.

This study has two main objectives: 1) to assess the relative impacts of early season and late season prescribed fire on soil environmental conditions (soil moisture, temperature, pH, total soil C and N, macronutrient content) that can influence microbial activity and 2) to determine the subsequent changes in soil microbial activity of a mixed-conifer forest site in Sequoia National Park, California.

Materials and Methods

Study Site

I conducted this study within the watershed of the Marble Fork of the Kaweah River in the Giant Forest area of Sequoia National Park, California, which is located in the southern Sierra Nevada mountains. The Mediterranean climate involves cool, wet winters and warm, dry summers. Mean annual precipitation is approximately 114cm, most of it falling as snow. The soils of this region formed in residuum, colluvium and morainal material that have weathered from granitic rocks. They are classified as coarse-loamy, frigid Pachic Xerumbrepts (Huntington and Akeson 1987). The dominant plant species in this mixed conifer, old growth forest are white fir (*Abies concolor* [Gordon & Glend.] Lindley), incense cedar (*Calocedrus decurrens*), sugar pine (*Pinus lambertiana* Douglas), Jeffrey pine (*Pinus jeffreyi* Grev. & Balf.), ponderosa pine (*Pinus ponderosa*), mountain dogwood (*Cornus nuttallii* Audubon), and California black oak (*Quercus kelloggii*).

The sites for this study are part of the National Fire and Fire Surrogate Study, a program developed to evaluate the effects of fuel treatments on ecosystem structure and function. They are all on west-northwest facing slopes of approximately 15-25 degrees and at elevations ranging from 1900 to 2150 m. The pre-settlement fire return interval has been estimated at 20-40 years; however prior to the recent prescribed burns for this study, the sites had not burned since 1879 (Caprio and Knapp, unpublished data).

Sampling Design and Field Measurements

Three replicate early season burn, late season burn, and unburned control sites were established in a completely randomized design, each averaging 15 ha in size. The prescribed fires, which were conducted in October 2001 (late season) and June 2002 (early season), removed approximately 88% and 67% of the available surface fuels, respectively (Weatherspoon and McIver 2000). Total tree mortality (from direct fire impacts and indirect post-fire beetle impacts) in 2004 was 38% in the early season and 56% in the late season burn sites (Schwilk, personal communication). In each site there were ten modified Whittaker subplots (50m x 20m) set up for vegetation sampling (Knapp et al. 2005). In order to avoid disturbing the vegetation, I sampled 1m outside the subplots at each corner (4 sub-samples per subplot). I took 15cm soil cores from all plots in June 2004 to assess nitrogen availability, soil moisture and pH. I also collected and composited 6 core samples from around each subplot for soil enzyme and total C and N analysis at this time. All soil samples were immediately placed on ice for transport to the laboratory for further processing. I also measured soil respiration and soil temperature at two opposing corners of the subplots during June 2004 using a PP-Systems EGM-1 Soil Respirometer.

Laboratory Analyses

I sieved fresh soil samples within twelve hours of collection through a 2mm mesh and took 10g sub-samples for moisture and pH. I determined soil moisture using the gravimetric method and soil pH using a 1:1 ratio of soil and de-ionized (DI) water. I dried and ground 5 g subsamples before measuring total soil C and N using a LECO CHN1000 combustion gas analyzer (LECO Corporation, St. Joseph, MI, USA). I extracted 10 g subsamples in 50 ml 1M KCl for macronutrient analysis. Plant-available P was determined using the ascorbic acid method (Greenberg et al. 1992) and soil macronutrients were measured using a ThermoJarrell-Ash Iris Advantage High Resolution Axial Inductively Coupled Plasma Unit. Soil enzyme analysis was conducted at Ohio State University using methods developed by Tabatabai (1982), as modified by Sinsabaugh et al. (1993) and Sinsabaugh and Findlay (1995). Acid phosphatase activities were determined using *p*-nitrophenol (*p*NP)–phosphate, and phenol oxidase activities were measured by oxidation of L-DOPA (L-3,4-dihydroxyphenylalanine) during 1hr incubations (Boerner et al. 2005).

Statistical Analyses

I tested for the effects of burn season on soil environmental, biogeochemical and microbiological variables using one-way ANOVA with treatment (early season burn, late season burn and control) as the independent variable (n=3). Significant differences between treatment means were determined using Tukey's highly significant difference (HSD) with alpha=0.05. I then ran simple regressions to examine the relationship between soil enzyme activity and soil environmental variables. All analyses were run using the Statistical Analysis System (SAS Institute 1997).

Results and Discussion

Seasonal fire effects on soil environmental conditions

Season of burning significantly affected most measured soil environmental variables, likely due to fire intensity. Soil moisture was not significantly different between the unburned control and the early season burn sites, but it was significantly lower than the control ($p=0.04$) in the late season burn sites. The different fire effects of on soil moisture were likely due to the variable effects of fire on water-repellency and, hence, infiltration rates in the soil. Fire-induced hydrophobicity can last anywhere from 3 to 22 months post-fire, depending on the vegetation type, soil texture, and burn severity (Huffman et al. 2001). This water-repellency can lead to lower infiltration rates and high runoff and erosion rates (DeBano 2000; Robichaud, 2000; Ice et al. 2004). In addition to decreased infiltration, the persistence of fire effects on soil moisture in this study was likely influenced by increased evaporation in the burned sites. The opening of the forest canopy and removal of the insulating litter layer allowed for more moisture to be lost to the atmosphere. These changes also allowed more light energy to reach the soil surface, increasing soil temperatures in both the early season ($p<0.01$) and late season ($p<0.01$) burn sites. Soil pH was unaffected by both early and late season fire in 2004, however there was an initial spike in pH in the late season burn sites one year post-fire (see Chapter 2). These data show that the spike in pH was short-lived, returning to pre-burn levels within three years.

In the early season burn sites, there was no significant difference in total soil carbon or nitrogen from the control, but there was a significant drop in carbon ($p=0.01$) in the late season burn sites (Table 1.). This can again be attributed to the higher intensity of the late season fire, with more of the carbon in the top 15cm of soil being oxidized during the burn. Total soil nitrogen in the late season plots was not significantly different than the control three years post-burn, suggesting a relatively quick recovery of this pool. It has also been suggested that the fire-induced changes in the soil nitrogen pool are quite small compared to the size of the entire pool itself. So, although there may be a large loss of nitrogen from the litter during the fire, the majority of the soil pool remains intact (Gillon and Rap 1989).

Three years post-fire, there was no significant change in soil phosphorus with either season of burning, relative to the control. There was an initial drop in phosphorus concentration one year post-burn (data not shown) in the late season burn. These results show that, regardless of season of fire, phosphorus levels returned to pre-fire levels within three years. There was no consistent pattern in soil macronutrient response to fire treatment; soil Mg increased with early season burning but late season burning had no effect on this variable. No other macronutrients were impacted by either early or late season burning. This varied response is largely due to the temperature at which each macronutrient is converted to gaseous form. Phosphorus and potassium vaporize at 1425°F, magnesium at 2025°F and calcium at 2700°F (Raison et al. 1985a and 1985b). Because these elements are only vaporized at very high temperatures, gaseous losses during fire are usually minimal or nonexistent. Instead, these macronutrients generally accumulate in the ash and are then lost through erosion and overland flow (Marion et al. 1991). There was no precipitation during the month immediately following the spring burns so there was minimal initial loss, and in the case of magnesium, slight gain, of macronutrients in these sites that persisted for two years post-burn. Marion et al. (1991) found that low severity fire increased the availability of several macronutrients, but this effect was restricted to the top 5cm of soil. Because the late season burn sites experienced large amounts of precipitation immediately post-burn, a good portion of the nutrient-rich ash and topsoil was likely lost before it was incorporated into the soil complex.

Seasonal fire effects on microbial activity

Decomposition rates are jointly controlled by soil microclimate and by activities of cellulose and lignin-degrading exracelluar enzymes (Sinsabaugh et al. 1992). Inside microbial cells, enzyme production is regulated by moisture, temperature, pH, and nutrient availability. Once enzymes are released into the soil solution, however, their activity is limited by substrate chemistry and availability (Sinsabaugh et al. 1992; Waldrop et al. 2004). So, large changes to either the environmental conditions or the substrate chemistry could significantly alter microbial enzyme activity and, hence, decomposition rates. Acid phosphatase, an enzyme that is often used to estimate overall microbial activity (Boerner et al. 2000), was significantly lower than the unburned control with both early ($p<0.01$) and late season burning ($p<0.01$) (Figure 1). Phenol oxidase, an enzyme responsible for the degradation of lignin (Boerner et al. 2000), showed no significant change in activity with early season fire but had significantly lower activity with late season burning ($p<0.01$) (Figure 1). Because this enzyme targets older litter that is rich in lignin, it is not typically active in early stages of decomposition with fresh litter (Waldrop et al. 2003). The lack of sufficient older substrate in the late season burn was likely limiting phenol oxidase activity in these sites. Boerner et al. (2000) found that low-severity fire had no impact on phenol oxidase activity, suggesting that the fire did not change the composition of the organic matter complex within the soil.

In order to assess how microbial activity varied in relation to soil environmental conditions in fire-affected soils, I ran simple regressions between enzyme activity and all environmental variables. Acid phosphatase activity was positively correlated with soil moisture, total soil C, and total N and negatively correlated with soil pH and temperature (Table 2). These relationships are supported by findings of Deng and Tabatabai (1997), who found that acid phosphatase in agricultural soils was highly correlated with soil organic C, and of Waldrop et al. (2003), who attributed a drop in post-harvest enzyme activity of the forest floor to decreased litter moisture and nutrient content. These results also agree with previous work showing acid phosphatase activity is highest at lower pH levels (5-5.5) (Saa et al. 1993; Acosta-Martínez and Tabatabai 2000). Phenol oxidase, was weakly, although significantly, negatively correlated with soil temperature (Table 2).

These changes in soil environmental conditions and the associated drop in soil enzyme activity reflect the higher severity of the late season burns. The fact that soil enzyme activity had not returned to pre-fire levels within 2-3 years suggests that either overall microbial activity was still lower than unburned sites or that different enzymes were at work in the fire-affected sites. As was the case with phenol oxidase, many enzymes are substrate-specific (Boerner et al. 2005) so if a disturbance decreases substrate availability for the measured enzyme, that measure of microbial activity will decrease. However, if other enzymes become active with a new substrate or altered environmental conditions, this could indicate a shift in microbial community structure. Analyzing a suite of microbial enzymes that target several different types of carbon substrate and that are active under variable environmental conditions would provide a comprehensive estimate of fire effects on microbial activity and a glimpse into the functional changes that occur with disturbances such as fire.

Conclusions

Because a large portion of the forested lands of the American west is vulnerable to high severity fire, it is important to investigate safe and ecologically sustainable management practices to reduce fuel levels. This study shows that different fire treatments can influence the soil abiotic conditions and biotic communities differently. Two years post-fire the early season burn sites had largely returned to pre-burn conditions, but three years post-fire the soils of the late season burn sites were still altered, relative to the control, in many ways. Several soil environmental variables (moisture, temperature, C) were still changed and total enzyme activity was lower in the late season burn sites than in the early season burn sites. The relative sensitivities of the two measured enzymes to environmental conditions may explain some of this difference. Due to the low severity nature of the early season fires, fuel management goals were not met (Knapp et al. 2005). To achieve management goals and provide the benefits of low-moderate severity fires to the soil system, mid-elevation mixed conifer forests of the Sierra Nevada may need to be burned in the fall during the short window when conditions are suitable for prescribed fire treatment. Before implementing this type of management on a large scale, however, it will be necessary to evaluate the relative effects of seasonal fires on aboveground vegetation structure and function.

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Table 1. Seasonal fire effects on key soil environmental variables. Treatment means and standard errors are presented. An asterisk (*) denotes significant difference ($p<0.05$) from the control and a cross (†) denotes significant difference ($p<0.05$) between burn treatments.

	Control	Early Season	Late Season
Soil H2O (g kg^{-1})	101 ± 3.0	104 ± 5.0	84 ± 4.0*†
Soil Temp (°C)	13.2 ± 0.2	14.3 ± 0.2*	15.6 ± 0.3*†
Soil pH	5.7 ± 0.1	5.8 ± 0.1	5.9 ± 0.1
Total soil C (g kg^{-1})	27.0 ± 2.1	26.0 ± 1.5	20.8 ± 1.0*†
Total soil N (g kg^{-1})	1.4 ± 0.1	1.4 ± 0.1	1.1 ± 0.1†
Plant-available P (µg kg^{-1})	141.7 ± 21.5	191.1 ± 27.8	158.2 ± 23.3
soil Ca (mg kg^{-1})	915.8 ± 47.2	1033.5 ± 43.6	983.8 ± 59.3
soil K (mg kg^{-1})	1049.5 ± 119.9	1191 ± 43.6	993 ± 21.9
soil Mg (mg kg^{-1})	86.5 ± 5.1	105.3 ± 5.1*	92.9 ± 6.4

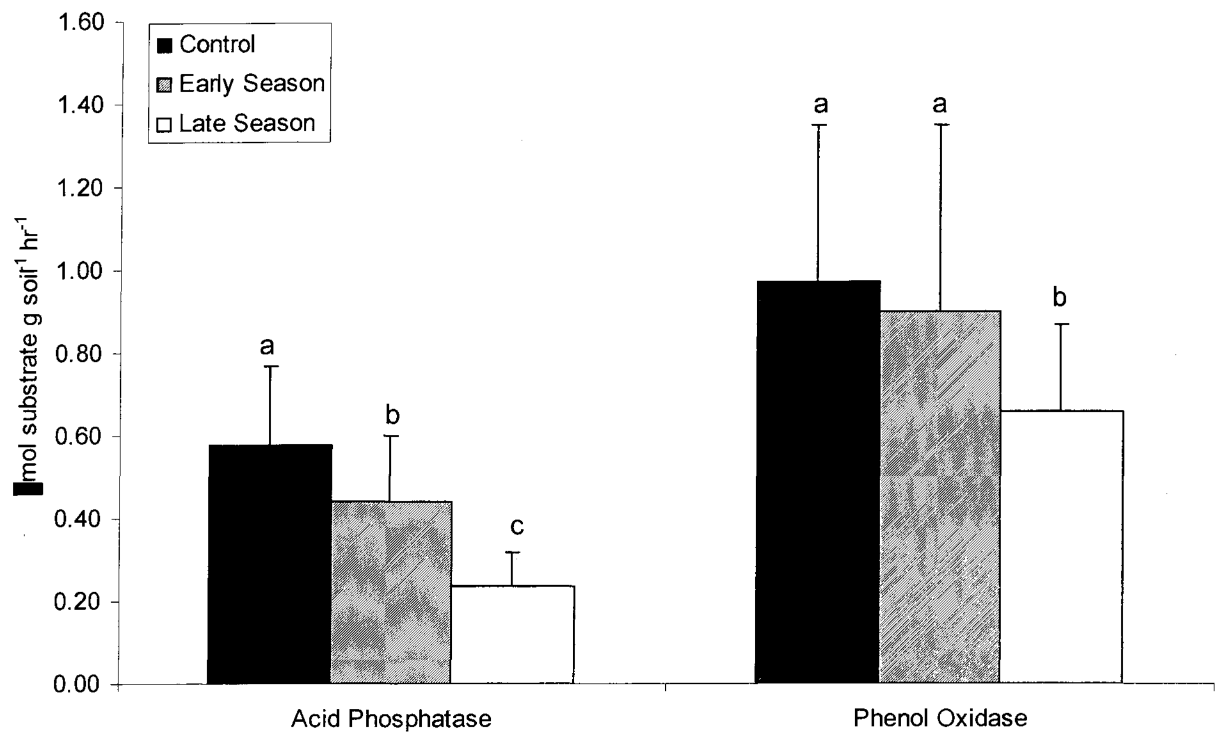

Figure 1. Season of burning impact on soil enzyme activity as measured by the amount of substrate consumed by the enzyme during an hour-long incubation. Treatment means (+1 SD) for each enzyme are displayed with a significant difference of $p<0.05$ represented by different letters.

Table 2. Results of regression analysis between soil enzymes and environmental variables. Correlation coefficients are presented with significance of $p<0.001$ designated by an asterisk (*).

	H_2O (g kg^{-1})	Temp.(°C)	pH	C (g kg^{-1})	N (g kg^{-1})	P (μg kg^{-1})	Ca (mg kg^{-1})	K (mg kg^{-1})	Mg (mg kg^{-1})
Acid Phosphatase	0.44*	-0.55*	-0.44*	0.54*	0.44*	-0.03	-0.15	-0.05	0.04
Phenol Oxidase	0.25	0.35*	0.18	0.02	0.06	-0.05	0.12	0.23	0.20

CHAPTER 4

DETERMINING SOIL NITROGEN MINERALIZATION AND NITRIFICATION RATES – A COMPARISON OF TWO METHODS

Introduction

Primary productivity in most forests is nitrogen-limited (Binkley and Hart 1989). With current and predicted changes in nitrogen deposition rates (Aber et al. 1989), fire regimes (Grissino-Mayer and Swetnam 2000), and land-use activities, the effects of disturbance on nitrogen availability has become a more prominent focus of biogeochemical studies. Despite its importance in ecosystem functioning, however, nitrogen availability remains difficult to assess for several reasons. Temporal and spatial variability, variations in forest stand requirements, differential regulation by microbial mineralization and immobilization, diffusion, mass flow, and plant uptake all affect nitrogen dynamics (Binkley and Hart 1989; Subler et al. 1995; Coté et al. 2000). Considering all of these variables, the chosen methodology and experimental design can greatly affect the results (Raison et al. 1987; Hart and Firestone 1989; Subler et al.1995). Several different methods have been used to assess nitrogen availability, including laboratory and field incubations (Eno 1960; Bell and Binkley 1989; Hook and Burke

1995), bioassays (Lamb 1975), isotopic dilution and enrichment techniques (Vitousek and Matson 1984), and ion exchange resin (IER) techniques (Binkley and Matson 1983; Qian and Schoenau 1995). Each of these methods has different relative sensitivities to environmental parameters, creates different levels of disturbance to the intact soil system, and suffers from inherent artifacts.

The traditional soil core incubation technique involves placing isolated soil columns within capped or uncapped cores in the ground for a designated period of time (Raison et al. 1987). This method, which is a modification of the older buried bag technique (Eno 1960), has the potential to alter moisture and temperature regimes relative to the bulk soil and influence nitrogen cycling via carbon inputs. Because roots are severed by core placement, there is potential for increased immobilization within the core because of the pulse of available carbon from decomposing roots (Adams et al. 1989; Hook and Burke 1995).

Because of its relative simplicity and the minimal soil disturbance, the ion exchange membrane technique (Plant-Root Simulator probe™, Western Ag Innovations, Inc.) is becoming more popular for N mineralization studies. This technique involves placing a 10-cm long cation or anion resin membrane supported by plastic in the mineral soil for a designated period of time. This method assesses ion supply rates by continuously adsorbing charged ionic species over the burial period. Accumulation of ions on exchange resins incubated in the field can provide an estimate of nutrient supply rate in soils. This method has been used extensively in agricultural studies (Huang and Schoenau 1996; Qian and Schoenau 1997) and has recently been applied to natural systems as well (Huang and Schoenau 1997; Johnson et al. 2005).

One ecosystem that provides an interesting and dynamic perspective on nitrogen transformations is a coniferous forest recently touched by fire. Following fire, there are often increased concentrations of soil inorganic nitrogen (NH_4^+ and NO_3^-) from heat-induced NH_4^+ release from clay complexes (during fire), deposition of organic nitrogen in ash (immediately following fire), and nitrogen-fixation (by colonizing plants species post-fire) (Wan et al. 2001). This pulse of available nitrogen has been shown to lead to short-term enhanced site productivity in some systems (Wan et al. 2001). Many studies have evaluated fire effects on nitrogen dynamics (Raison 1979; Mroz et al. 1980; Covington and Sackett 1984; Kovacic et al. 1986; Bell and Binkley 1989; Knoepp and Swank 1995; Baird et al. 1999; DeLuca and Zouhar 2000; Wan et al. 2001), but because of the different methods used to estimate nitrogen availability, overall assessment of fire effects across different ecosystems is difficult (Hart and Firestone 1989). Finding comparable methods to assess nitrogen availability across spatial and temporal scales is key to building cross-site and cross-study conclusions on fire effects on nitrogen availability. Because the biological processes contributing to and the abiotic conditions limiting net nitrogen mineralization rates are layered and complex (Schimel and Bennett 2004), absolute levels of net nitrogen mineralization alone are not very useful. The relative effects of disturbance on net rates may help us better understand the mechanisms leading to altered nitrogen availability and the role disturbance plays in the nitrogen cycling of the soil system. Considering this, the objectives of this study were to: i) test the hypothesis that both the ion exchange membrane method and the core incubation method discriminate between fire treatments equally and ii) examine the relationship of both soil incubation methods with soil environmental variables (soil moisture, pH, cation exchange capacity (CEC), soil C and N).

Materials and Methods

Study Sites

I conducted this study within the watershed of the Marble Fork of the Kaweah River in the Giant Forest area of Sequoia National Park, California, located in the southern Sierra Nevada Mountains. The Mediterranean climate comprises cool, wet winters and warm, dry summers. Mean annual precipitation is approximately 114 cm, most of it falling as snow (Kilgore and Taylor 1979). The soils of this region have formed in residuum, colluvium and morainal material that have weathered from granitic rocks. They are classified coarse-loamy, frigid Pachic Xerumbrepts (Huntington and Akeson 1987). The dominant plant species in this mixed conifer, old growth forest are white fir (*Abies concolor* [Gordon & Glend.] Lindley), incense cedar (*Calocedrus decurrens*), sugar pine (*Pinus lambertiana* Douglas), Jeffrey pine (*Pinus jeffreyi* Grev. & Balf.), ponderosa pine (*Pinus ponderosa*), mountain dogwood (*Cornus nuttallii* Audubon), and California black oak (*Quercus kelloggii*). The sites I studied are all on west-northwest facing slopes of approximately 15-25 degrees and at elevations ranging from 1900 to 2150 m. The pre-settlement fire return interval has been estimated at 20-40 years; however prior to the recent prescribed burns for this study, the sites had not burned since

1879 (Caprio and Knapp, unpublished data). The prescribed fires, which were conducted in October of 2001, removed approximately 88% of the dead and down organic matter (Knapp et al. 2005) and killed approximately 37% of the trees (Schwilk, personal communication).

Sampling Design and Measurements

Three replicate burned and unburned control sites were established in a completely randomized design, each averaging 15 ha in size. In each site there were ten randomly located modified Whittaker subplots (50m x 20m) for vegetation sampling. In order to avoid disturbing the vegetation studies, I sampled 1m outside the subplots on two opposing corners. I collected initial soil cores and placed ion exchange membranes in the ground in June 2002 and collected final soil cores and removed membranes after a 28-day incubation in July 2002.

In Situ Core Incubation

For the soil core incubation, I collected samples by removing litter and duff layers and driving an aluminum core (15 cm deep x 5 cm diameter) into the mineral soil. I collected one sample for laboratory analysis (initial) and took another core adjacent to the first, placed it back into the soil with a perforated cap on the bottom of the core (to contain soil but allow for gas and water exchange), and replaced litter and duff layers. I returned 28 days later to collect the second soil core (final). At the time of removal, I immediately placed samples on ice and transferred them to the lab for same-day processing and KCl extraction. I calculated net nitrogen mineralization rate as the change in the total inorganic nitrogen concentration over the incubation time ((final – initial) 28 days^{-1}) and net nitrification as the change in total nitrate concentration over the incubation time ((final-initial) 28 days^{-1}).

In Situ Ion Exchange Membranes

I placed two ion exchange membranes (one cation, one anion) (Plant-Root Simulator probe™, Western Ag Innovations Inc.) with 10cm^2 resin surface 8-10 cm apart at each sampling point, adjacent to the buried soil cores. As with the soil cores, I removed litter and duff layers before making a slot in the mineral soil for membrane placement. I placed the membranes vertically in the slots and then pressed the soil against the membranes to ensure good contact between the membranes and the soil. They were left in the ground for the same period as the cores (28d). Net nitrogen mineralization rate was calculated as the total inorganic nitrogen adsorbed on the resin membranes over the incubation period; I expressed these on a per cm^2 basis of the probes, rather than on the basis of soil volume. There was no precipitation during the burial time, so the differential effects of saturated soils on each of these methods were not a concern. However, it has been suggested that the soil core incubation method may alter soil moisture conditions over long-term burials (Adams et al. 1989). Because the ion exchange membranes were not contained in a core, they likely experience a more natural range of environmental conditions.

Laboratory Analysis

I sieved soil samples from the cores through a 2mm mesh and took 10g sub-samples for inorganic soil nitrogen (nitrate and ammonium), moisture, and pH analyses. For soil nitrogen analysis, I extracted sub-samples with 50ml 2M KCl-PMA. Extracts were filtered using Whatman no.1 filter paper and analyzed colorimetrically for NH_4-N and NO_3-N on an Alpchem autoanalyzer (College Station, TX). I determined soil moisture using the gravimetric method and soil pH using a 1:1 ratio of soil and deionized (DI) water. Inorganic N values were corrected for a gram dry soil basis, and fine soil bulk density data from the cores were used to express N mineralization on a g N / m^2 basis.

I used bulk soil samples adjacent to the cores for total soil C:N and CEC measurements. I measured total soil C and N using a LECO CHN1000 combustion gas analyzer (LECO Corporation, St. Joseph, MI, USA) and calculated CEC by summing Ca, Mg, K, and Na.

To prepare the ion exchange membranes for analysis, I rinsed them with DI water immediately after removal from the soil and placed them in individual plastic bags for storage at 4°C until analysis. Western Ag Innovations, Inc. conducted the analyses, eluting the membranes with 0.5N HCl and analyzing the eluate for NH_4^+ and NO_3^- using automated colorimetry.

Statistical Analysis

Because absolute rates of net nitrogen mineralization cannot be compared between methods, I looked at treatment response based on the method used. To determine whether the two methods showed similar trends in N availability, I conducted a simple correlation analysis between the data for the two methods, for 1) all net mineralization and 2) net nitrification data. To evaluate whether the two methods showed similar effects of fire on N availability, I used a standard *t* test for each method to individually perform pair-wise (burned vs. unburned) comparisons for net mineralization and net nitrification. To examine the relationship between the incubation methods and soil environmental conditions, I ran simple linear regressions of net mineralization rates for each method and each environmental variable. I used the Statistical Analysis System (SAS Institute, 1997) for all statistical analyses.

Results and Discussion

Treatment Effect Discrimination

Overall, there was very low correlation of net nitrogen mineralization rates between the two methods (r^2=0.13). This is similar to findings by Binkley (1984), in which short term anaerobic incubations showed no change in N availability with clear-cutting, but ion exchange resin bags showed a 7- to 20-fold increase in N availability. Johnson et al (2005) also found very low agreement between total soil extractable nitrogen and ion exchange membrane nitrogen in tallgrass prairie soils. I found slightly higher correlation, though still a statistically insignificant relationship, between net nitrification rates for the two methods (r^2=0.33). Binkley et al. (1986) found correlation coefficients ranging between 0.87 and 0.92 for nitrate availability determined using IER bags and buried bags. Hart and Firestone (1989) also found that NO_3^--N availability estimates correlated better among the three methods they tested (buried bag, IER-bag and IER-core) than did estimates of NH_4^+ or total inorganic nitrogen availability. This is believed to be primarily due to the mobility of NO_3^- relative to NH_4^+ in the soil and the high sensitivity of the exchange membranes to ion transport.

The two methods did not have similar treatment responses for mean net N mineralization rates. The soil core incubation method showed no significant difference in net N mineralization with burning (p=0.67), but the ion exchange membrane method showed significantly greater rates (nearly 3-fold higher) of net N mineralization with burning (p<0.01) (Figure 1). This difference can likely be attributed to the increased immobilization inside the cores from the pulse of available carbon from the severed roots. Several studies have found that longer incubations often result in decreased net N mineralization rates, due to increased immobilization inside the cores (Adams et al 1989; Binkley and Hart 1989). I also saw a difference in treatment response between the two methods for nitrification, although to a lesser degree. The core method showed nearly a two-fold increase in nitrification rates and the ion exchange membrane method showed over a four-fold increase in nitrification rates with fire (Figure 1). The high nitrification rates with the ion exchange membranes could reflect actual net nitrification (the local microbial process) or, because of the exposure to the bulk soil, the membranes could be absorbing mobile NO_3^- ions from greater distances.

Method Responses to Soil Environment

Nutrient turnover rates are often influenced by several environmental parameters such as soil moisture, CEC, pH, and C:N ratios. Soil moisture content affects physical movement, biological uptake and chemical reactions in the soil. Soil CEC also affects ion concentration and mobility. Soil pH and C:N ratio influence microbial composition and activity which, in turn, affect nutrient turnover rates. All of these variables (percent moisture, CEC, pH, and C:N) were affected by fire in this study (Table 1). Nitrogen mineralization data were weakly correlated with soil pH and C:N ratio for the ion exchange membrane method. Soil pH explained approximately 22% of the variation in N mineralization rates and the C:N ratio explained 21% of the variation. Soil CEC had much less influence at 13% and soil moisture even less at only 3.4%. For the core method, however, none of the environmental variables explained more than 3% of the variation in N mineralization rates. This suggests that the ion exchange membrane method is more sensitive than the core method to environmental parameters that could influence soil nutrient dynamics and plant growth post-fire. This method is constrained, however, by the fact that results cannot be expressed on a soil weight or volume basis. So although this method may be more sensitive to changes in plant-available nitrogen in soil and it is more sensitive to the soil environment, one must use caution when interpreting results.

Conclusions

Both the soil core and the ion exchange membrane method provide a relative index of nitrogen availability, but because the two methods have different artifacts and are sensitive to different environmental conditions, selection of one over the other depends largely on the site and the objectives of the study. Because of the potential for increased immobilization inside the cores, sites with dense aboveground vegetation (and high root density) should be avoided when using the core incubation method. This method is most appropriate when turnover rates are needed on a volume or area basis for short-term incubations (1-3 weeks). The ion exchange membranes are potentially most useful when the investigator has interest in understanding the relative difference in N supply to roots for some treatment effect. The spatial and temporal variability in mineralization, immobilization and ion transport make interpretation of ion exchange membrane results problematic, however. Controlling for environmental variables that can limit microbial activity such as soil pH and C:N ratio would aid in interpreting these data. Conducting incubations with the ion exchange membranes inside and outside soil cores would also help to determine the effects of root severing on immobilization, provide a volume-based estimate of soil contributing to the results, and potentially answer some interesting questions regarding ion delivery to the membranes.

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Table 1. The effect of burn treatment on soil properties one year post-fire and correlation among soil properties and net nitrogen mineralization rates using both the ion exchange membrane (IEM) and core incubation methods.

Soil Property	Control	Burn	P-value	R^2 (IEM)	R^2 (core)
H_2O ($g\ kg^{-1}$)	125.4	99.8	<0.01	0.03	0.03
pH	5.78	6.32	<0.01	0.22	0.002
CEC (meq/100g)	17.75	16.05	0.02	0.13	0.01
C:N ratio	21.92	19.90	<0.01	0.21	0.01

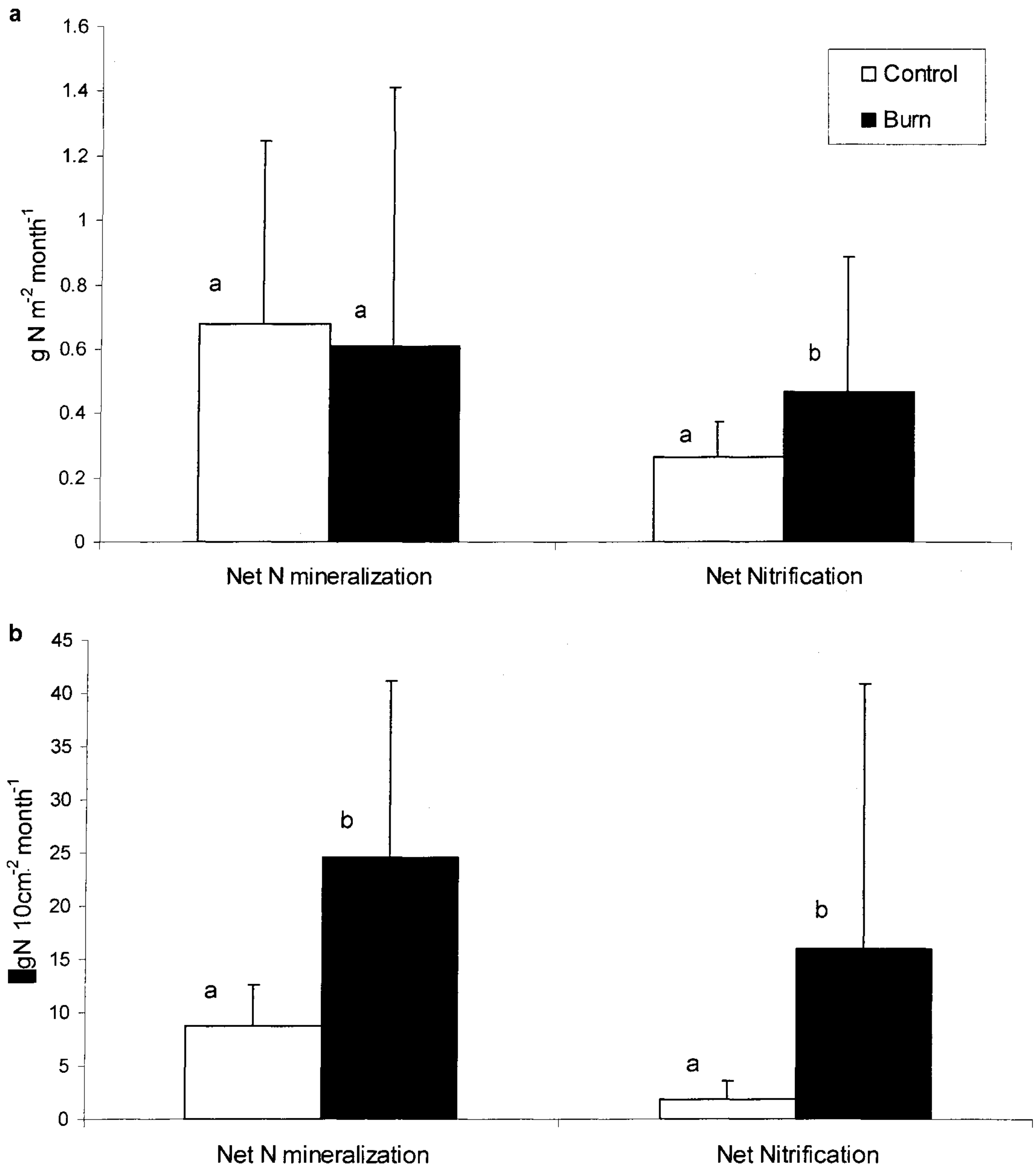

Figure 1. Estimates of net nitrogen mineralization and net nitrification rates according to the core incubation method (a) and ion exchange membrane method, expressed as the total inorganic N adsorbed on the $10cm^2$ of resin surface during the month-long incubation (b). Treatment means (+1 SD) are presented. Different letters represent significant difference ($p<0.01$) between treatments.

CHAPTER 5

RELATIONSHIP BETWEEN MICROBIAL COMMUNITY STRUCTURE AND SOIL ENVIRONMENTAL CONDITIONS IN A RECENTLY BURNED SYSTEM

Introduction

Throughout the last few decades, parts of the western United States have seen a dramatic annual increase in area burned by wildfire (Grissino-Mayer and Swetnam 2000; Conard et al. 2001). This shift in fire regime is largely due to an increase in aboveground biomass and the subsequent hazardous fuel levels that have developed from the early practice of fire exclusion (Neary et al. 1999; Tilman et al. 2000; Schoennagel et al. 2004). Understanding the consequences of these wildland fires on plant and microbial communities is vital to our understanding of the role of fire in ecosystem functioning. Fire affects carbon cycling in forests directly by oxidizing many of the available compounds and indirectly by changing environmental constraints on microbial activity. The primary effects of fire on the carbon dynamics of a forest ecosystem are an immediate loss of carbon upon the combustion of organic matter, changes in above- and belowground primary production, and changes in decomposition and soil respiration rates. Soil respiration, the combined carbon dioxide efflux from root and mycorrhizal respiration and soil organic matter (SOM) decomposition, is controlled by aboveground litter inputs (Raich and Nadelhoffer 1989), soil temperature (Raubuch and Jorgensen 2002; Certini et al. 2003; Raich and Mora 2005) and soil moisture levels (Certini et al. 2003). Root and microbial death, as well as changes in soil environmental conditions, may alter soil respiration, thereby changing the belowground carbon budget for the system.

Fires are often extremely heterogeneous, depending on localized fuel loads, and wind strength and direction, which result in large patches of unburned and moderately burned areas within the boundaries of the fire. The belowground carbon dynamics of these patches (which can reach up to several square kilometers in size) are potentially very different than those of severely burned areas, due to fewer trees killed and a potential shift in the belowground microbial community. These unburned and moderately burned patches may act as a biotic source for the microbial re-colonization of the severely burned areas post-fire (Visser 1995; Smith et al. 2005). Depending on the nutrient status and aboveground vegetation present, the recovery trajectory of soil microbial communities in the moderately burned patches could be very different than in large contiguous areas experiencing high severity fire. Several studies have provided data on total microbial biomass and activity in fire-impacted soils (Fritze et al. 1993; Pietikäinen and Fritze 1993; Dumontet et al. 1996; Villar et al. 2004), but there is very little published information on the composition of these populations (Hart et al. 2005) and consequences of altered community structure. Bisset and Parkinson (1980) noted that the taxonomic shift in soil microbial communities post-fire could lead to a fundamental change in soil energy pathways, and Balser and Firestone (2005) suggested that disturbance-induced changes in the soil microbial community could influence ecosystem scale events such as seedling establishment, trajectory of vegetative recovery, and competitive advantages between plants.

Most soil carbon transformations are governed by fungal and bacterial populations (Bailey et al. 2002). These two groups of organisms are differentially regulated by environmental factors (temperature, moisture, pH, organic carbon source), so as fire changes the soil environment, the relative abundance of fungi and bacteria may change as well. Fungi are more sensitive to disturbance than bacteria and they tend to respond more quickly to changes in organic carbon levels and soil pH (Pietikäinen and Fritze 1995; Mamilov and Dilly 2002). Because of this, the fungi:bacteria ratio may decrease post-fire. The mycorrhizal species present may be differentially altered by fire, as well, impacting re-colonization of the aboveground vegetation (Smith et al. 2005).

Methodologies such as ester-linked fatty acid methyl ester (EL-FAME) analysis provide a microbial abundance and diversity index that can aid in our understanding of these belowground microbial community dynamics, possibly providing an index of soil biotic recovery and restoration progress in disturbance-impacted areas (Schutter and Dick 2000; Mummey et al. 2002). Coordinating microbial community data with key soil biogeochemical and environmental variables may give us some insight into what factors are important for post-fire microbial community recovery.

The three main objectives of this study were 1) to determine the relative effects of varying severity wildfire on belowground carbon dynamics and soil environment conditions of a mid-elevation ponderosa pine (*Pinus ponderos*a Dougl. ex. Laws.) forest, 2) to determine the relative effects of varying severity wildfire on belowground microbial community structure, and 3) to determine if/how changes in the microbial community structure are correlated with fire-induced changes in soil biogeochemical and environmental parameters.

Materials and Methods

Site Description and Field Methods

The Hayman fire burned 55,000 ha of mid-elevation forest in central Colorado over a period of three weeks during the summer of 2002 (Graham 2003). Two large runs burned the bulk of the area on two days of particularly high-severity conditions (low humidity, high winds). Much of the forest was subjected to very high-severity fire, but approximately 48% of the area within the perimeter of the burn experienced either low severity fire or no fire at all (unburned) (Robichaud et al. 2003). During August of 2003, I sampled three separate sites within each of these 'treatments' (high-severity, low-severity, unburned) to assess fire severity effects on soil environmental variables and soil microbial community structure. Severity was determined by percent tree mortality; low-severity sites had <50% tree mortality and high-severity had >75% tree mortality. The sites all had similar slope, aspect, and elevation (approximately 1375m). The dominant vegetation at the sites is ponderosa pine/slimstem muhly (*Muhlenbergia filiformis* Vasey) and the soil parent material is Pikes Peak granite, which weathers to fine gravel and coarse sand. The soils consisted of the Sphinx and Legault series, both characterized by well-drained, highly erosive, coarse, sandy loam soils (Robichaud et al. 2003). Average annual precipitation at these sites is around 40cm with no persistent winter snow pack (Huckaby et al. 2001).

Within each site, I randomly set a 100 meter transect and positioned a sampling point every 20 meters, totaling 5 samples per site. At each sampling point, I measured soil respiration rate and soil temperature with a LI-COR 6400-09 (LICOR Technologies, Lincoln, NE). I set out soil respiration collars at least 24 hours prior to sampling to avoid a disturbance-induced flux of soil CO_2. I then collected and composited two soil samples (0-15cm) from beneath the respiration collar at each sampling point to assess soil moisture, pH, total carbon content, and microbial community structure.

Laboratory Analyses

Samples were returned to the laboratory and sub-sampled for the various analyses. I used 10g sub-samples for soil moisture, pH and total C analyses and stored these soils at 4°C until analysis. I used 4g sub-samples for the microbial analyses and stored these sub-samples at -80°C until analysis. I measured soil moisture using the gravimetric method, and used 1:1 slurry of soil and deionized water to measure soil pH. I air-dried and ground sub-samples before measuring total soil C using a LECO CHN1000 combustion gas analyzer (LECO Corporation, St. Joseph, MI, USA).

To characterize microbial community structure, I used ester-linked fatty acid methyl ester (EL-FAME) analysis. This technique identifies microbial functional groups (biomarkers) based on the type of fatty acids present. I first extracted lipids from the soil sub-sample in a 1:2:0.8 extractant mixture of chloroform: methanol: phosphate buffer using a modified method of White et al. (1979). I then employed the mild alkaline transesterification method of Schutter and Dick (2000) to extract fatty acids from lipid samples. I added an internal standard (20 μg of 19:0) to each EL-FAME sample before the hexane solvent was completely evaporated off with nitrogen. Samples were analyzed with an Agilent 6890 gas chromatograph (GC) (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}. Peaks were identified using bacterial FAME standards and MIDI peak identification software (Microbial ID, Newark, DE); all functions of the GC were under the control of the computer and this method. To clean the column between samples, the 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. I assigned biomarkers of specific functional groups according to Sullivan et al. (2006).

Fatty acid nomenclature denotes the number of carbons: number of double bonds, followed by the double bond location(s) from the aliphatic (ω) end of the molecule. For example, 18:1 ω6 indicates a fatty acid with 18 carbons and a double bond at carbon 6.

The suffixes *c* and *t* indicate *cis* and *trans* geometric isomers. The prefixes 'a' and 'i' refer to anteiso and iso branching and Me and OH specify methyl groups and hydroxyl groups, respectively.

Statistical Analyses

In order to determine the relative effects of varying severity wildfire on soil biogeochemical and environmental variables, I used a simple ANOVA with a mixed model in the Statistical Analysis Software (SAS) 9.1 package (SAS Institute, Inc., Cary, North Carolina, USA). I tested burn severity, site, and their interaction effects on soil moisture, temperature, pH, and total C. Treatment differences were considered significant at $p<0.05$.

In order to address fire effects on belowground community structure, I used only known EL-FAME fungal and bacterial biomarkers. I used a simple ANOVA with a mixed model in SAS, with burn severity and site as class variables and microbial functional group abundance (nmol EL-FAME g^{-1} dry soil) as the response variable.

Before analyzing the community data, I excluded outliers in the EL-FAME biomarker data set using a Euclidean distance measure in PC-ORD, version 4.20 (MjM Software, Gleneden Beach, Oregon, USA) to identify samples that fell outside of two standard deviations from the mean. I analyzed the soil microbial community EL-FAME and soil environmental data using Canonical Correspondence Analysis (CCA) (Ter Braak 1986). CCA identifies an environmental basis for community ordination by extracting the maximum relationship between community composition and the measured environmental variables. Because fatty acid and environmental scores are obtained simultaneously, relationships between the patterns can be determined from ordination biplots of the data. The ordination axes are constrained to be linear combinations of the environmental variables, maximizing the dispersion of the EL-FAME scores. The resulting ordination diagram displays EL-FAME biomarkers as points and soil environmental variables as vectors. Vectors of greater magnitude and forming smaller angles with an ordination axis are more strongly correlated with that axis and, therefore, more closely related to the pattern of community variation shown in the diagram. Only variables that were highly correlated ($r^2>0.70$) with the ordination axes were included in the CCA biplot. All soil environmental variables were tested for significant contribution to the explanation of the variation in the EL-FAME data with the Monte Carlo permutation test associated with the subroutine in PC-ORD. This tests the null hypothesis that there is no relationship between the two matrices (EL-FAME data and environmental data).

Results and Discussion

Fire effects on soil environmental and biogeochemical variables

The impact of fire on the soil microclimate depended on severity. High-severity fire sites had significantly greater soil moisture levels than both the control and the low-severity fire sites ($p=0.01$) (Table 1). This is likely due to the variable effects of fire on water-repellency and, hence, infiltration rates in the soil. Soils heated between 175°C and 250°C (low to moderate severity fire) have high levels of hydrophobicity caused by the distillation, transfer, and condensation of hydrophobic hydrocarbons from the soil organic matter down through the soil profile (DeBano 2000; Robichaud, 2000; Ice et al. 2004). If temperatures exceed 250-300°C, as in a high intensity fire, the hydrophobic substances undergo combustion and therefore no longer contribute to the hydrophobic nature of the soil (DeBano 2000). This, along with the complete removal of the litter layer, presumably led to greater infiltration rates and, consequently, greater soil moisture levels post-fire. Soil temperature increased significantly with burning ($p<0.01$), with no difference between severities (Table 1). This was due to several factors, including the opening of the forest canopy, the blackening of the soil surface, and the combustion of the insulating litter layer (Ice et al. 2004).

Soil pH was not affected by low-severity fire but was significantly lower in high severity burn sites than the control ($p=0.01$) (Table 1). This is contrary to our current understanding of chemical effects of fire on soil pH levels. However, these soils likely experienced more than just chemical effects; because there was substantial erosion in many of the high severity burn areas within the Hayman burn perimeter (Graham 2003), a majority of the topsoil may have been removed before sampling time, potentially removing a good portion of the fire-affected soil.

Soil respiration rates were significantly lower in both low and high severity fire sites, relative to the unburned sites ($p<0.01$), with no significant difference between the two severities (Table 1). The primary cause of this difference was likely the lack of root respiration in these sites. Because of the high tree mortality rate (nearly 100%) in the high-severity burn sites, I expected soil respiration rates to be even lower due to the lack of autotrophic activity. However, the dead and dying roots may have provided a flush of organic carbon substrate for the surviving microbial populations, stimulating heterotrophic respiration rates. There was no significant difference between treatments in total soil carbon one year post-fire. This suggests that the carbon from the dead and dying roots in the burned sites had already been incorporated into the bulk soil by sampling time, bringing the total soil carbon values close to those found in the unburned sites.

Fire effects on belowground microbial community structure

The fire had variable effects on the abundance of different microbial functional groups. There was no significant change in the bacterial abundance with fire, however there was a significant decrease in the fungal biomarkers with both low and high severity burning (Figure 1). Fungi have been shown to be more sensitive to changes in environmental conditions than bacteria (Pietikäinen and Fritze 1995), possibly explaining the different fire effects. The arbuscular mycorrhizal fungi (AMF) were more abundant in the low-severity burn sites, and there was no significant change in AMF abundance in the high-severity burn sites relative to the unburned (Figure 1). These changes can likely be attributed to the effects of fire on the vegetative associates of these organisms. Because most trees are dependent on ectomycorrhizal (EM) fungi and most herbaceous plants are associated with AMF, any significant shift in the aboveground structure (killing the overstory and allowing more understory vegetation to establish) could correspond with a shift in the belowground mycorrhizal community (Johnson et al. 1992; Hart et al. 2003).

Korb et al. (2003) found that low intensity burning increased infective AMF propagules by 20%, which is consistent with the increase in biomarkers for AMF found in this study. The presence of these mycorrhizal organisms could speed the understory vegetative recovery in both the low and high severity fire sites (Treseder et al. 2005).

There was no significant difference among treatments in microbial biomass (as estimated by total concentration of EL-FAMEs) (Figure 2), nor was there change in microbial community richness with either low or high severity burning (as measured by total number of fatty acids). However, there was a shift in total community structure. CCA ordination of the microbial communities separated burned and unburned sites, but did not separate communities based on fire severity (Figures 3 and 4). Based on these results, there appears to be a rapid (within one year) re-colonization of the microbial community in both the low and high severity sites, but these communities are structurally different from the pre-fire population. This could be due to the altered environmental conditions and the lack of vegetative associates for some mycorrhizal species, as mentioned above.

Identifying the effects of varying severity fire on specific functional groups (N-fixers, ammonifiers, methanogens, specific arbuscular mycorrhizal and ectomycorrhizal fungal groups) through genetic analysis would further increase our understanding of post-fire above- and belowground community recovery. Smithwick et al. (2005) and Yeager et al. (2005) have both looked at changes in N-cycling bacteria post-fire, but they focused their efforts solely on stand-replacing fires. Because so many of the fires that occur throughout the western U.S. involve both low and high severity patches, it will be useful to evaluate the re-colonization of important microorganisms in areas affected by both types of fire.

Relationship between environmental variables and microbial community structure

I identified the soil environmental factors that best explain the pattern of microbial community profiles across fire intensities. Soil characteristics that were correlated with the microbial community ordination axes included soil temperature, soil pH, and soil carbon (Table 2). Utilizing the distribution of the communities along three axes, these three environmental variables explained roughly 23% of the variability in the distribution of the community data. These variables have previously been found to influence community structure. Drenovsky et al. (2004) found differences in the microbial community with changes in soil carbon and moisture levels in California agricultural soils, and Zogg et al. (1997) documented a shift in microbial community composition of hardwood forest soils with increasing temperatures. Steenwerth et al. (2003) also found that differences in soil microbial community composition of grassland and agroecosystems were highly correlated with soil microbial biomass, pH and certain management factors that influence resource (N and water) availability. These studies all stress the importance of abiotic factors in influencing belowground community recovery.

One of the main assumptions of CCA is that the environmental variables chosen are the most important variables that could affect community composition. The variables I chose to measure in this study were those most affected by fire and, therefore, I considered, those likely to influence any shift in microbial community structure. The vectors in the ordination diagrams representing these variables represent environmental gradients, pointing in the direction of maximum change (Figures 3 and 4). Drawing a perpendicular line from the different samples and EL-FAME biomarkers to these vectors gives a coarse approximation of where they fall along the gradients. Using this approach, we see that the fungal biomarker 18:1ω9*t* is correlated with high soil C levels, low soil temperatures and moderate to high pH, while 18:3ω6*c* is correlated with moderate soil C and soil temperatures and high pH levels. The AMF biomarker, 16:1ω5*c*, is found at moderate to high soil temperatures and moderate soil C and pH levels. Most of the bacterial biomarkers fall near the center of the biplots (not labeled), with some unidentified biomarkers appearing at the edges of the ordination diagrams.

Because recovery times for different fractions of the microbial community are likely variable (Smithwick et al. 2005), sampling time is very important when studying ecosystem recovery from disturbance. I sampled these sites 14 months post-fire. Initial fire effects on the soil biogeochemical and environmental parameters and direct impacts on the microbial populations were not likely captured with this sampling schedule. Sampling along a chronosequence of time-since-fire to capture short-term and long-term changes in the belowground community in both low severity and high severity sites would be a valuable approach to tracking the re-colonization rates of different fractions of the microbial community. Tracking these changes along with the shifts in abiotic conditions throughout ecosystem recovery from fire will provide information on limiting factors for biotic recovery in disturbed systems.

Conclusions

The soil biogeochemistry of a site post-fire can greatly influence the trajectory of biotic recovery. The soil microbial community both controls and is controlled by several environmental and biogeochemical parameters that are subject to change with fire. Depending on the fire severity, environmental conditions are significantly altered, potentially influencing the survival and growth of certain fractions of the soil microbial community. My research shows that microbial biomass recovered to pre-fire levels 14 months post-fire in both low and high severity burn sites, but the structure of these communities was different, mainly due to altered fungal densities in the burned sites. While there are certainly many variables influencing microbial community structure, 23% of the variability in the microbial communities can be predicted by temperature, pH and carbon.

Incorporating empirical field data on ecosystem dynamics from small-scale studies such as this into landscape and regional scale models will make it possible for researchers and managers to utilize this information at larger temporal and spatial scales. Because of the spatial and temporal heterogeneity of these effects, however, the information must be applied carefully. Integrating soil community structure data with vegetation patterns will help us understand the role the belowground community plays in the recovery of the aboveground vegetation post-fire (Hart et al. 2005). As the threat of fire in western forests of the U.S. becomes greater each year, recognizing the spatial heterogeneity of fire effects on a landscape becomes important for recovery and rehabilitation efforts.

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Table 1. Varying severity fire effects on soil environmental and biogeochemical variables one year after the Hayman fire. Treatment means and standard error are presented. Asterisk denotes significant difference between treatment and unburned at $p<0.05$.

Soil variable	**Unburned**	**Low Severity**	**High Severity**
Soil moisture (g kg^{-1})	66.0 ± 9.0	54.0 ± 1 0.0	103.0 ± 14.0*
Soil temperature (°C)	13.1 ± 0.2	18.6 ± 0.6*	18.1 ± 0.4*
Soil pH	6.7 ± 0.1	6.8 ± 0.1	6.5 ± 0.1*
Soil respiration (μmol CO_2 m^{-2} s^{-1})	3.3 ± 0.3	1.5 ± 0.2*	1.6 ± 0.3*
Total soil C (g kg^{-1})	11.0 ± 1.9	10.0 ± 1.0	10.7 ± 1.3

Table 2. Correlation coefficients[†] for the soil environmental variables and overall FAME-environment correlations for each of the three ordination axes.

Soil variable	Axis 1	Axis 2	Axis 3
Soil Temperature	-0.015	0.867	-0.438
Soil Moisture	-0.251	0.255	0.111
Soil pH	0.405	-0.312	-0.859
Soil C	-0.942	-0.315	-0.072
Pearson Correlation‡	0.739	0.796	0.741

[†]Intra-set correlation coefficients relate to the rate of change in community composition per unit change in the corresponding environmental variable.
[‡]Correlation between sample scores for an axis derived from the FAME data and sample scores that are linear combinations of the environmental variables.

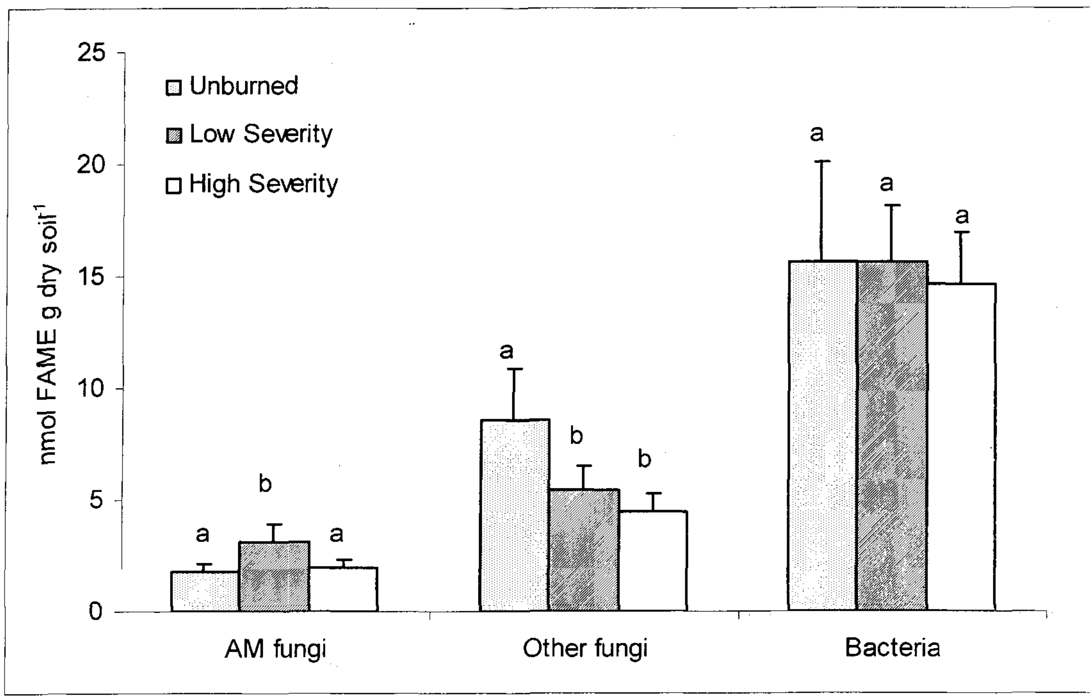

Figure 1. Varying severity fire effects on microbial abundance as determined by EL-FAME analysis. Bars represent one standard error. Different letters denote significant ($p<0.05$) difference between treatments.

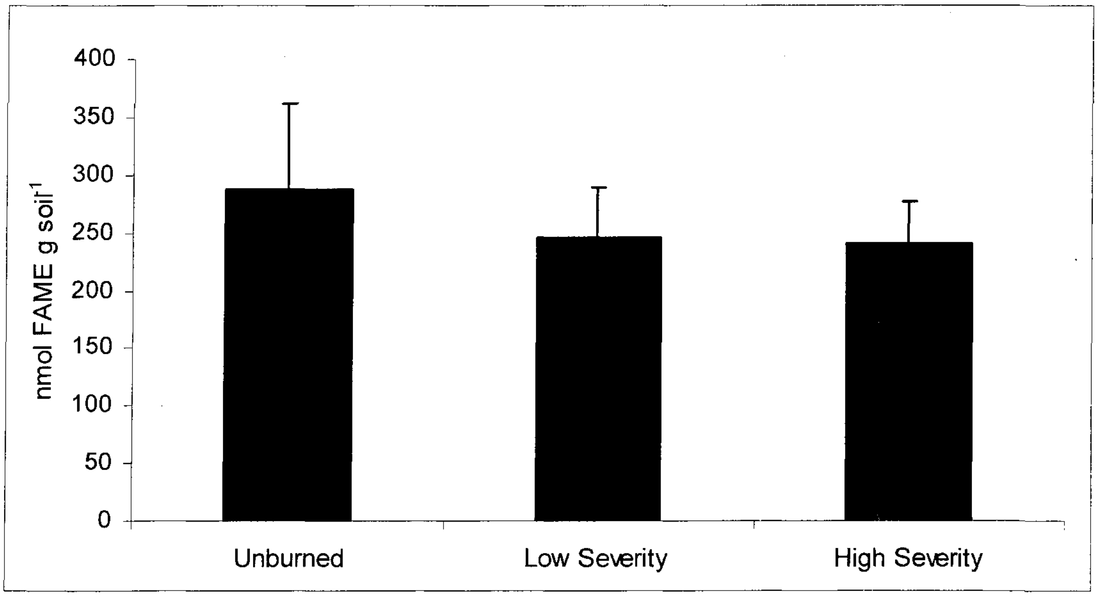

Figure 2. Microbial biomass for each of the burn treatments, as estimated by total concentration of EL-FAMEs. Treatment means and standard errors are presented.

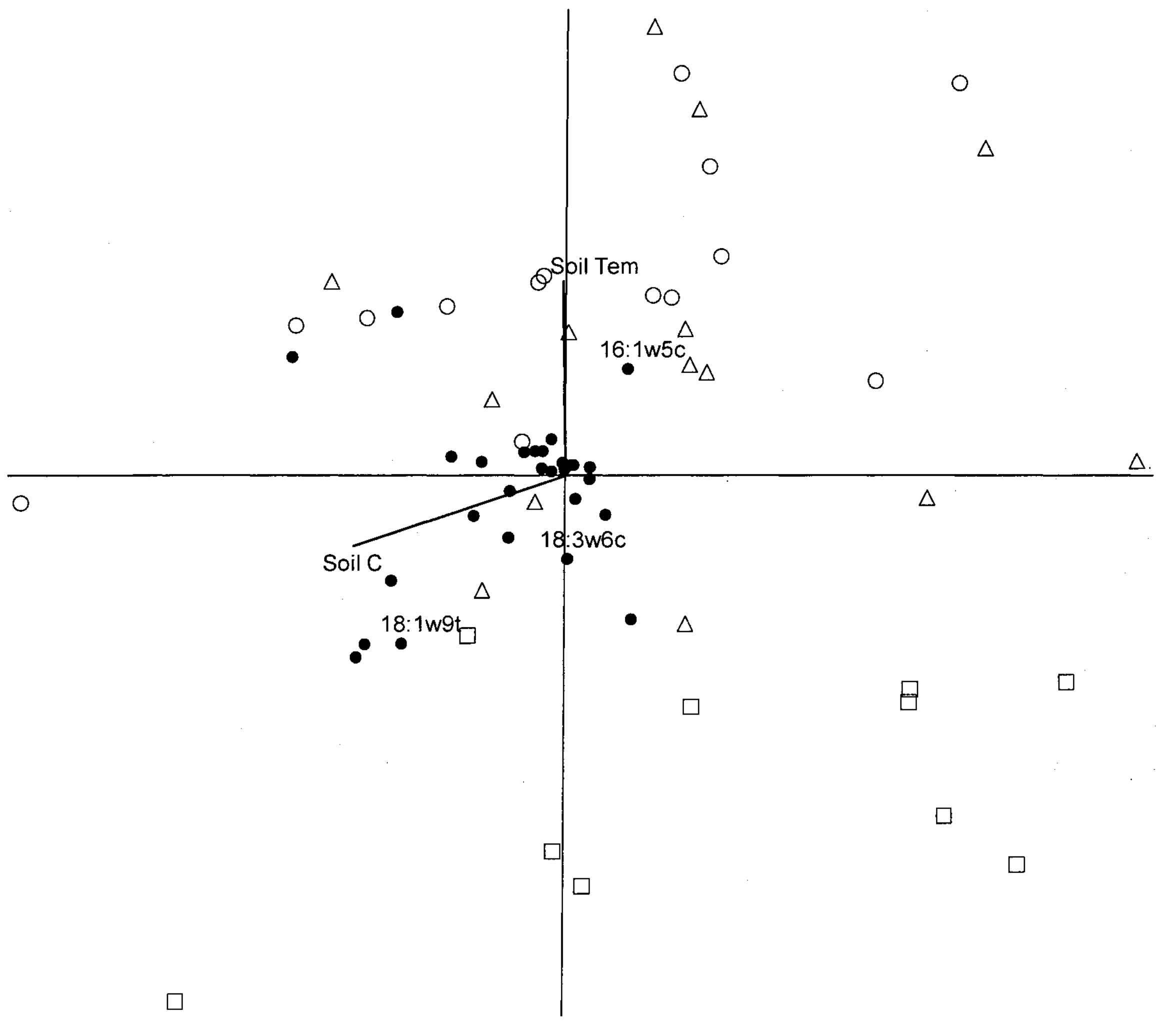

Figure 3. CCA ordination biplot of FAME community profiles for unburned (), low severity (Δ) and high severity (○) sites. Sites that are close together on the ordination diagram are more similar (in terms of their microbial community structure) than sites that are farther apart. Individual FAME biomarkers (•) are plotted and fungal biomarkers that were affected by fire are labeled. Vectors affiliated with each of the axes represent environmental variables. Axes 1 (horizontal) and 2 (vertical) account for 9% and 8% of the variation in the data, respectively.

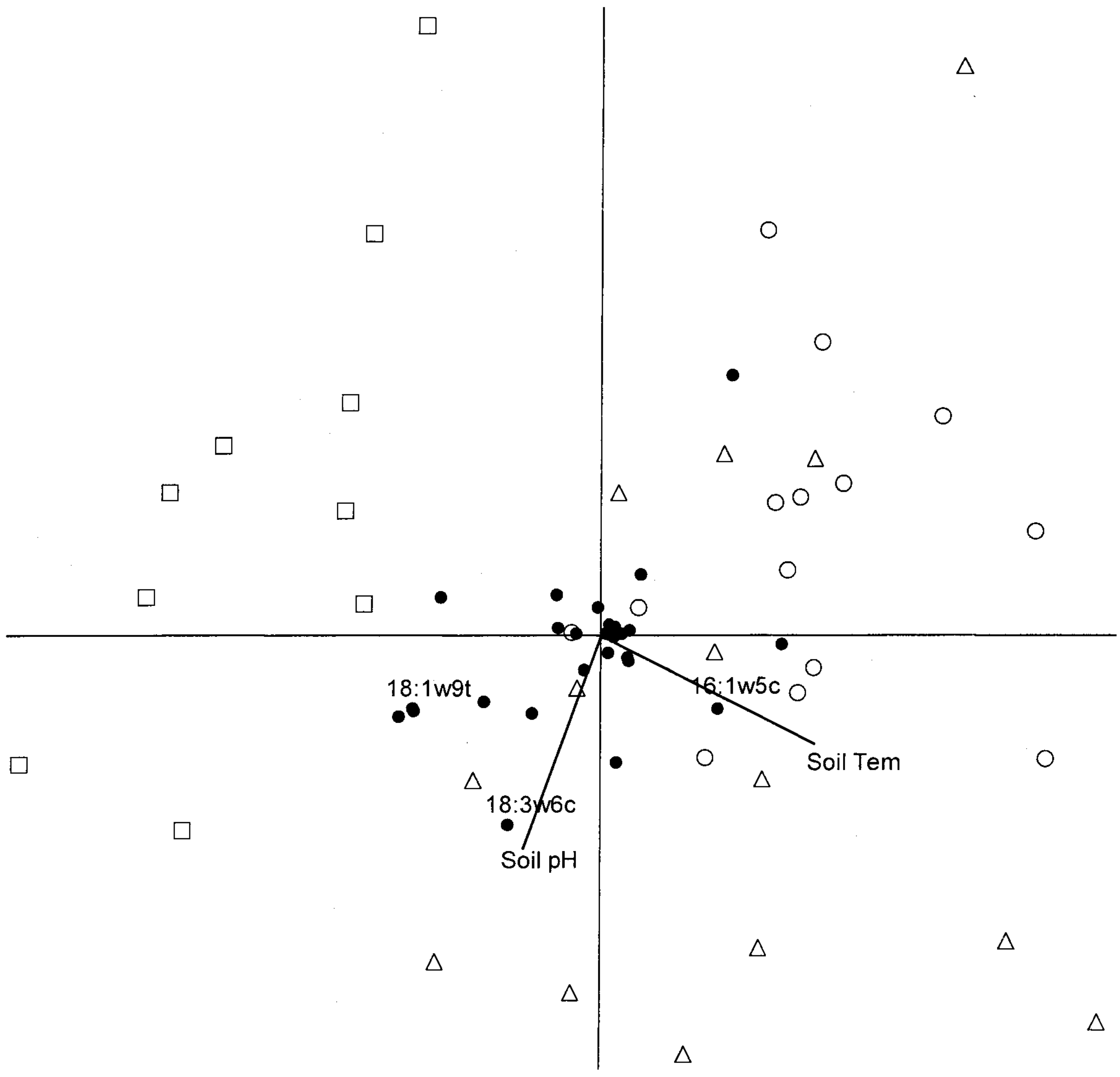

Figure 4. CCA ordination biplot of FAME community profiles for unburned (), low severity (Δ) and high severity (○) sites. Sites that are close together on the ordination diagram are more similar (in terms of their microbial community structure) than sites that are farther apart. Individual FAME biomarkers (•) are plotted and fungal biomarkers that were affected by fire are labeled. Vectors affiliated with each of the axes represent environmental variables. Axes 2 (horizontal) and 3 (vertical) account for 8% and 6% of the variation in the data, respectively.

CHAPTER 6

SUMMARY AND CONCLUSIONS

The primary objectives of my dissertation were to quantify effects of altered fire regimes (different fire seasons and severities) on the soil physiochemical characteristics, carbon and nitrogen dynamics, and microbial communities of two different forests. To address these objectives I first analyzed a suite of soil environmental conditions and biogeochemical pools and fluxes in a mixed conifer forest site that experienced two different seasons of prescribed fire in Sequoia National Park, CA. To address questions surrounding soil microbial community response to varying severity fire, I measured soil environmental variables, soil carbon dynamics, and microbial community structure in ponderosa pine forests subjected to low and high severity wildfire in the Front Range of Colorado.

The results from Chapter 2 showed that season of fire differentially impacted soil environmental variables and C and N pools and fluxes. These initial changes and the persistence of some of the effects in the late season burn until 2004 suggests that the temperatures reached during these burns were hot enough to alter the physical and chemical properties of the litter and soil.

In Chapter 3, I evaluated the effects of early and late season fire on soil nutrients important for microbial activity, and the subsequent impacts of varied fire season on soil enzyme activity. Overall microbial enzyme activity was lower than the controls in both early and late season fire sites but the two enzymes were differentially correlated with the measured soil physiochemical variables. Acid phosphatase was correlated with changes in soil pH, temperature, and soil C levels, while phenol oxidase was only correlated with soil temperature. These results suggest that microbial activity is impacted by a host of environmental factors and that fire-induced changes in these variables can limit microbial activity two to three years post-fire. Ultimately, the results from Chapters 2 and 3 will be imported into a biogeochemical simulation model to evaluate long-term (incorporating several fire cycles) implications of early and late season burning on ecosystem C and N dynamics.

To better understand the impacts of fire on net nitrogen mineralization rate and to evaluate methods used to measure this rate, I conducted a comparative experiment between the traditional soil core incubation method and the newer ion exchange resin membrane method. The results from this chapter (4) showed that the traditional soil core incubation method underestimated net nitrogen mineralization rates, likely due to elevated immobilization rates inside the cores. The ion exchange resin membranes were more sensitive to environmental conditions and are much easier to use, however the artifacts associated with this method make interpretation difficult. Overall, the two methods were not well correlated, indicating the need for caution when comparing N availability estimates derived from these methods.

In Chapter 5, I found that there was quick (within one year) recovery of the microbial communities in both fire severities but these communities were structurally different from those in the unburned sites. There was no single environmental variable responsible for variation in the community ordination; instead I found that temperature, pH and soil C explained 23% of the variability in the community composition. This research shows that these three soil physiochemical variables are important for microbial recovery from fire, considering that they were significantly correlated with both microbial enzyme activity in Sequoia soils and community structure in Colorado soils. However, due to the low amount of variability explained in community ordination data by these variables, it is likely that several other structural and chemical factors such as soil pore space and bulk density, influence post-fire microbial community dynamics.

Altering certain aspects of the fire regime (season, severity) under which a particular forest system has evolved can impact both the structure and biogeochemical function of the soil communities. In the mid-elevation forests of the Sierra Nevada, where late season prescription burning is often difficult to perform safely due to dry fuels and air quality issues, early season burning may provide the benefits of a low severity fire to the soil complex. However, because of the low severity nature of these fires, it may be difficult to reach management goals of fuel reduction with just one burn. Future management plans for this region will need to balance the fuel mitigation goals with ecosystem effects. In Colorado, where the risk of high severity wildfire is a growing concern for land managers, understanding the impacts of fire severity on belowground structure and function will be important for future restoration planning. Developing structural and process-based ‘targets’ for restoration requires knowledge of the organisms present and an understanding of the environmental conditions and biogeochemical processes essential for successful ecosystem recovery.