Fire Effects on Forest Soil: Cave Gulch Fire, Helena National Forest

TABLE OF CONTENTS

Acknowledgements……………………………………………..iii

Introduction……………………………………………………...1
 Soil Nutrients……………………………………………1
 Seed bank………………………………………………..2
 Mycorrhizae……………………………………………..3
 Microbes………………………………………………...4

Site Description…………………………………………………6

Material and Methods…………………………………………8
 Site Characterization……………………………………..8
 Soil Nutrients……………………………………………8
 Seed bank…………………………………...………9
 Mycorrhizae……………………………………………10
 Microbes………………………………………………..11

Results…………………………………………………………..15
 Vegetation……………………………………………15
 Soil Nutrients…………………………………………...16
 Seed bank……………………………………………….21
 Mycorrhizae…………………………………………….24
 Microbes………………………………………………..26

Summary……………………………………………………….28

Appendix A…………………………………………………….31
Appendix B…………………………………………………….32
Appendix C…………………………………………………….34

References……………………………………………………...35

ACKNOWLEDGEMENTS

The students of LRES 440C would like to thank the following individuals for their contributions to the course: Sue Farley, Lois Olsen, and Bo Stuart from the Helena National Forest; Don Despain, Clain Jones, and Cliff Montagne for help in the field; Lina Botero-Griffith and the Tim McDermott Lab; Tamara Sperber for help in the lab; Sara Zimmerly the T.A.; and the USDA Higher Education Challenge Grant to Cathy Zabinski, Jon Wraith and Jeff Adams.

INTRODUCTION

Wildfire is an important part of western forest ecosystems. Historically, ponderosa pine (*Pinus contorta*) forest systems have had low intensity fires every few years. These fires typically decreased the fuel load by thinning the understory and creating open forests. Once forest managers began suppressing forest fires, vegetation and debris accumulated in the understory (Dombeck 2001). With increased fuel loads, fire severity increases, resulting in greater impacts to forest ecosystems. In recent years, forest managers have realized the importance of frequent low intensity fires for thinning the understory and decreasing fuel loads. Because this fire is no longer looked at as just having negative effects on the forest, it is no longer suppressed in some cases.

Limited research is available on wildfire impacts on the soil environment. Most fire research relies on laboratory experiments and controlled burns, which rarely mimic the characteristics of wildfires. An understanding of wildfire impacts is needed to effectively manage forest ecosystems, including post-fire management decisions regarding seeding options, erosion control, and other interventions. The objective of this study was to investigate the effects of wildfire burn severity on soil nutrient availability, seed bank density and diversity, mycorrhizal colonization, and soil microbial diversity one year after the Cave Gulch Fire, which burned during the summer of 2000 in the Helena National Forest.

Soil Nutrients

Fire affects soil nutrient cycling through the loss of surface organic matter (OM), additions of ash to the soil, elevated soil temperatures, and loss of vegetation. The

chemical composition of organic matter changes as some elements are released into the atmosphere when the duff layer burns, and other elements such as calcium (Ca^{++}), sodium (Na^{+}), magnesium (Mg^{++}), and ammonium (NH_4^{+}), condense in the top layer of soil (Raison 1979). Because OM correlates to plant productivity (Kimmins 1996), understanding fire effects on OM levels may increase our ability to predict post-fire site productivity.

In combination with soil OM, soil nutrient levels will determine revegetation rates on burned areas. Nitrogen is the single most limiting nutrient in forest systems. Alterations in soil N content vary over time after a fire. Research shows that while there is an increase of available/mineralizable N in the soil immediately after burning, there is a decrease of total N (Andreu et al. 1996; Blank et al. 1998; Brais et al. 2000; Driscoll et al. 1998; Feller 1989; Fernandez et al. 1997; Mroz et al. 1980). Nitrogen may take anywhere from one to 75 years to recover to post-fire levels (Wells et al. 1979; Brais et al. 2000; Driscoll et al. 1999). The amount of available N increases more in boreal forest sites where the cold temperatures tend to immobilize nutrients (Kimmins 1996).

The levels of nutrients and OM greatly determine revegetation rates on burned areas. OM creates macro and micro pore space in the soil that increases water-holding capacity and reduces compaction, which increases the probability of seedling survival. The effect of fire on soil nutrient status is dependent on amount and type of fuels, temperature of the burn, climate, geography, and biota (Hungerford et al. 1990).

Seed Bank

The soil seed bank plays a major role in the recovery of vegetation after a fire. The soil seed bank is the supply of seeds present in a habitat and represents potential for

revegetation of a site. The soil seed bank includes the transient seed bank, seeds that do not live past the second germination season, and the persistent seed bank, those that remain dormant for two or more seasons. Pastures and arable lands tend to have a large number of persistent seeds while coniferous and deciduous forests have few persistent seeds, therefore mainly a transient seed bank (Baskin and Baskin 1998).

The density and species composition of the seed bank is dynamic and changes from year to year as a result of soil disturbance (Jimenez and Armesto 1992). Fire can alter seed bank density and composition in three ways. First, the fire often consumes the seeds present in the duff layer. Once the duff layer is gone, erosion increases and there is no longer this layer to retain the seeds. Secondly, surface organic matter helps trap seeds to build the seed bank (Zabinski et al. 2000). The loss of surface organic matter during a wildfire or subsequent erosion could result in a decrease in seed bank density. Finally, the seeds present in the soil are often heated to a temperature where they are no longer viable. Temperatures lethal to seeds range from 60 to 120°C (Hungerford et al. 1990), depending on seed properties such as thickness of the fruit wall, capsule size, water content, and location in the soil (Baskin and Baskin 1998). Because of these factors, we hypothesized that as fire severity increased, the density and the richness of the seed bank would decrease.

Mycorrhizae

Mycorrhizae are a plant-fungal symbiosis present in most terrestrial plants. Endomycorrhizae, also called arbuscular mycorrhizae (AM), are common in herbaceous plants and some tree species. AM increase plant uptake of phosphorus and other

nutrients, and in exchange for these nutrients, obtain energy in the form of carbon from the plant. Because of this nutrient exchange between plant and fungi, mycorrhizae are considered a critical biotic component of the soil. Although the importance of mycorrhizal fungi is well known, little research exists on the effects of wildfire on mycorrhizae.

Fire effects may include the direct effects of heating on fungal viability and secondary effects associated with erosion of topsoil after burning. Existing research conducted in a laboratory setting shows that heating the top 5 cm of soil to temperatures of 45-70 °C has a deleterious effect on viable propagules (Pattinson et al. 1999). Generally, we would expect that negative effects of fire on AM would increase as fire temperature and duration increase (Hungerford et al. 1990). Erosion may be an important factor in decreasing the number of viable mycorrhizal propagules in soil following fire by removing the top layer of soil where many of the propagules reside (Vilarino and Arines 1991). A study conducted in Oregon found eroded topsoil on a burned slope had a higher infectivity potential than did the eroded slope above (Anaranthus and Trappe 1993).

Microbes

Fire has many effects on soil microbe populations due to the chemical and physical changes associated with high temperatures and ash deposition (Hungerford et al. 1990). There is little data on the effects of wildfire on soil microbes, and most studies were done on prescribed fires (Acea et al. 1996), which tend to have a much lower intensity and severity than wildfires.

Soil microbes are a diverse group of organisms, which include bacteria, actinomycetes, fungi, algae, and protozoa. Soil microorganisms play many essential roles in the overall health of the ecosystem, contributing to nutrient cycling, disease suppression, and decomposition (Pierzynski et al. 2000). Bacteria are the most abundant of all soil microbes (10^{14}-$10^{15}/m^3$) and are crucial for fixing nitrogen and oxidation-reduction reactions (Pierzynski et al. 2000). Without bacteria, life as we know it would not be possible. Besides being essential for soil nutrient cycling and energy flow, soil microbes are very sensitive to environmental change.

Measuring soil microbial diversity and understanding disturbance effects on soil microbes has been limited in the past due to the methods available (Nakatsu et al. 2000). Traditional methods to assess microbial diversity relied on culturing soil samples in the lab, which may measure only 0.3% of soil microorganisms that are able to grow in agar petri plates (Roose-Amsaleg et al. 2001). Modern technology allows soil microbial diversity to be better understood through the use of DNA analysis, which can detect species or groups by providing a “fingerprint” of the community.

SITE DESCRIPTION

Our study was conducted in the Helena National Forest in the Magpie Gulch drainage. The Cave Gulch fire burned this area during August of 2000. The fire was started on July 23, 2000 by an unknown cause, and burned 29,187 acres of Douglas fir (*Pseudotsuga menziesii*) and lodge pole pine (*Pinus contorta*) forest before it was controlled on September 15, 2000. Our two study sites, Cabin Hill and Upper Grouse Gulch, are in the Magpie Creek drainage (Appendix A). The Upper Grouse Gulch site is at an elevation of 6300 feet above sea level, with a southwest aspect and 20-25% slope, while the Cabin Hill site is 5,300 feet above sea level with a southeast aspect with an approximated 40% slope. These sites were chosen because of adjacent unburned, moderately burned, and severally burned areas so comparisons could be made between severities without differences in site characteristics affecting the results.

Burn severity is defined by the effect of fire on soil and other site characteristics (Baskin and Baskin 1998). The unburned areas at each site had no evidence of ash accumulation, charcoal deposition, or blackened tree trunks (Fig. 1). Moderately burned areas had surviving trees, accumulated ash, some surface organic matter, and blackened trunks

Figure 1. Unburned vegetation at Upper Grouse Gulch.

Figure 2. Moderately burned Douglas fir.

Figure 3. Severely burned site at Upper Grouse Gulch.

lichens still present on some of the upper branches (Fig. 2). Needles were singed, but remained on the trees. Severely burned areas showed high ash and charcoal accumulation, and completely burned trees with no remaining needles (Fig. 3).

MATERIALS AND METHODS

Site Characterization

Vegetation cover, depth of the litter layer, and percent bare ground were measured for each burn severity at both sites. All sampling was done August 30-31, 2001. Three transects were established in each burn severity, placed perpendicular to the slope. Daubenmire frames of one meter by 0.5 meters were randomly located along the transect, with a minimum of 10 plots observed along each transect. Ocular estimations were used to determine percent cover of vegetation, litter, and bare ground. The depth of duff (surface organic matter) was measured at four points within the frame.

Soils

Soils were sampled within each burn plot (unburned, moderate, and severe) at both sites (Cabin Hill and Upper Grouse). Three samples, 5cm deep, including ash, duff, and litter layers, were taken at each sampling site and homogenized in the lab. Soil samples were put directly into a plastic lined paper bag with a sealable top, and stored in a cooler until analysis. In the lab, the soils were dried for 24 hours at 60° C, passed through a 2mm sieve, and sent for analysis at the MDS Harris Laboratories, Nebraska. The samples were analyzed for total nitrogen (N), percent organic matter (% OM), nitrate (NO_3^-), phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), sulfur (S), zinc (Z), pH, soluble salts, sodium (Na), ammonium (NH_4^+), and total cation exchange capacity (CEC).

Soil texture was determined by a hydrometer test (Gee and Bauder 1986). We tested 12 samples using the shaker method. Each sample was tested for percent sand, silt, and clay content.

Seed bank

Soils collected at each site were brought to the greenhouse where germinating seeds present in the soil determined seed bank density and composition. This method allows identification of species when plants are grown to maturity, but only includes those for which germination requirements are met.

Ten randomly located cores were extracted from each burn severity plot at each site for a total of 60 cores. Surface organic matter was kept intact within each core. Cores were 5.6 cm^2 in diameter and 5 cm in depth, including surface organic matter, for a total area of 24.63 cm^2. Pots, 23 cm in diameter, were filled with greenhouse potting soil and labeled. Soil samples were spread on top of the potting soil and placed in the greenhouse to grow for nine weeks. Ten control pots were established that contained only greenhouse potting soil to determine whether seeds were present from another source in the greenhouse. The pots were watered daily for the first week and then reduced to alternate days. Germinants were counted weekly, and seedlings were identified with help from Lois Olsen, Helena National Forest Service Ecologist. Seed bank density was determined by calculating the number of seed present/m^2 in each burn severity. Species composition within each severity was also examined.

Mycorrhizae

We tested fire effects on mycorrhizae by measuring two mycorrhizal parameters: mycorrhizal infectivity potential and field colonization. Mycorrhizal infectivity potential measures the density of viable propagules in the soil. By comparing levels between severities, we can measure the effects that fire severity has on propagules in the soil. Mycorrhizal field colonization measures the proportion of existing root networks that are colonized by mycorrhizal fungi. We hypothesized that as burn severity increases, the negative effects on AM will also increase. Specifically we hypothesized that the number of propagules in the soil will be lowest in severely burned soils, and that AM colonization levels will be lowest in plants colonizing severely burned sites.

Mycorrhizal Infectivity Potential

Cabin Hill and Upper Grouse Gulch soils were collected at three locations within each burn severity. Samples were collected from the top 5 cm of soil and taken to the MSU Plant Growth Center where each sample was placed in a 10 cm pot. Sudan grass seed was spread evenly and in approximately the same quantity over the collected soil. Ten control pots consisting of sterilized sand with the same application of seed were used to reveal any contamination by fungal spores already existing in the greenhouse. Pots were watered daily for the first week, and then on alternate days the following five weeks. After six weeks, the grass was harvested and mycorrhizal colonization levels were measured.

Three to eight cm segments of carefully washed fine roots were cleared of all pigment by soaking roots for 48 hours in 2.5 M KOH solution. Roots were rinsed with water, and soaked in 0.5 M HCl solution for 12 hours, followed by 12 hours in Trypan

Blue. Trypan Blue stains fungal structures only, and allows for identification of mycorrhizal features within the roots. Ninety-six observations were made per plant to determine the proportion of each root system colonized by mycorrhizal hyphae.

Analysis of the data was performed with SPSS statistics software. Comparisons were made between sites and burn severities for the proportion of viable propagules.

Field colonization

Roots of plants grown in each burn severity at Cabin Hill and Upper Grouse Gulch were collected in late August. Species included *Achillea millefolium, Calamagrostis rubescens, Galium boreale, Dracocephalum parviflorum, Geranium bickenellii*, *Aster* sp., as well as an unknown forb, and a *Ranunculaceae* species.

Harvested plants were taken to the laboratory for identification. Roots were washed, cleared, and stained as described above. Mycorrhizal colonization levels were quantified and then statistically analyzed using the SPSS statistical software.

Microbes

From the three burn severities at each site, we randomly collected two 2.5 cm diameter by 10 cm long soil cores, for a total of 12 samples. Samples were extracted with autoclaved (sterilized) PVC pipe, then wrapped in sterile foil, and stored on ice until laboratory analysis. In a laminar flow hood, the samples were homogenized and a subsample of 500 mg was taken to extract total DNA. The extraction was completed using the BIO 101 FastDNA® Spin Kit for Soil (BIO 707, Inc. Carlsbad, CA). This kit is designed to extract PCR-ready genomic DNA in less than 30 minutes. This rapid process

avoids the use of organic solvents, such as phenol and chloroform, which may inhibit subsequent assays.

Using the extracted genomic DNA as a template, the polymerase chain reaction (PCR) was used to amplify the 16S rDNA from all bacterial populations present. Specific primers were used to amplify only bacterial genes (Table 1). The primers used were: bacteria specific forward, B1101F primer to amplify the 1092-1111 region of the 16S rDNA gene according to the *Escherichia coli* numbering system (Amann et al. 1995); and universal reverse primer, U1392RGC amplifying 1392-1406 16S rDNA region and containing a 5' GC clamp to facilitate the separation of PCR products in a DGGE gel (Ferris et al. 1996). The 50 µl PCR reaction consisted of: 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 1 mg ml^{-1} BSA, 0.5 µM each primer, 0.2 mM each dNTP, 5 µl DNA template solution, and 1.5 U Taq DNA polymerase. All components were added to the reaction mixture on ice. PCR was performed in the laboratory using a Gene Amp PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA).

Table 1. Polymerase chain reaction primers specific for the amplification of 16S rRNA genes of bacteria.

Primer	16S rDNA base number targeted*	Primer Sequence	References
B1101F	Bacteria (1092-1111)	5'AAG TCC CGT AAC GAG CGC AA3'	(Amann et al., 1995)
U1392RGC	Universal (1392-1406) **	5'CGC CCG CCG CGC CCC GCG CCC GGC CCG CCG CCC CCG CCC CAC GGG GGG TGT GTA C3'	(Ferris et al., 1996)

* Bases numbered according to the *E. coli* numbering system of the 16S rRNA sequence.
** The GC clamp corresponds to the first 40 bp.

This cycler controlled the temperature of the reaction in three different steps: denaturation, annealing, and extension. The reaction began with an initial 94°C denaturation for 2 minutes, followed by 25 cycles of 94°C for 45 seconds, 55°C annealing for 45 seconds, 72°C extension for 45 seconds, and a final extension at 72°C for 10 minutes, after which it was held at 4°C until removed from the cycler. The presence of PCR products was confirmed using electrophoresis on a 1.5%-agarose gel, which ran for 1 hour at 115 volts, and was then stained with ethidium bromide to make PCR fragments visible on a UV transilluminator (Fig. 4).

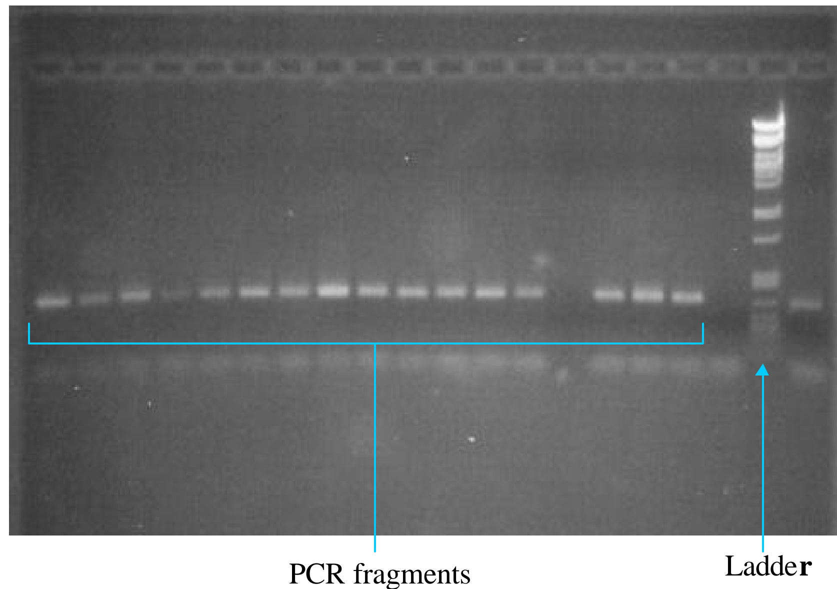


Figure 4. Each band represents the presence of PCR fragments. The ladder is a number of different fragments with known size, used to verify the size of PCR fragments. This gel was used to determine that the PCR amplification was successful, and that the correct sized fragments were amplified.

Denaturing gradient gel electrophoresis (DGGE) was completed using the BioRad Dcode™ electrophoresis system (BioRad, Hercules, CA) to determine apparent bacterial diversity in the PCR products. The gel (16 x 16 cm, 0.1 cm thick) was polymerized with

8% polyacrylamide (37.5:1 acrylamide:bis acrylamide) in 1X TAE buffer (40 mM Tris base, 20 mM acetic acid, 0.5 mM EDTA, pH 8.0) containing a linear denaturant concentration ranging from 16% formamide, 2.8 M urea at the top of the gel to 28% formamide, 4.9 M urea at the bottom. Electrophoresis was conducted overnight at 60°C, at 60 volts, and then gels were stained for 30 minutes in SYBC Green I (Molecular Probes, Eugene, OR), illuminated on a UV transilluminator and photographed.

DGGE is an electrophoretic method to qualitatively identify DNA fragments having different genomic sequences. This technique is sensitive to single base changes in a segment of DNA. In a denaturing gradient acrylamide gel, double-stranded DNA is subjected to an increasing denaturant environment and will melt in discrete segments called “melting domains”. The melting point of these domains is sequence specific. When the fragment completely denatures as it moves through the gel, its migration becomes a function of the sequence. This forms bands in the gel that are visible under UV light, with each band potentially representing a specific microorganism population. The number and location of these bands in the gel will determine the apparent diversity of the bacterial community in the soils sampled.

RESULTS

Vegetation

Vegetation cover varied between sites. At Cabin Hill, there were no differences in vegetation cover between burn severities. Upper Grouse Gulch showed a decrease in cover as fire severity increased (Fig. 5). No differences in vegetation cover between severely burned and unburned plots may be the result of rapid colonization by fire obligate species at severely burned sites. Individual plants growing at that site were large and covered most of the area within frames. Percent bare ground increased with burn severity at both sites (Fig. 6). Percent litter cover was site dependent. Litter cover decreased as the burn

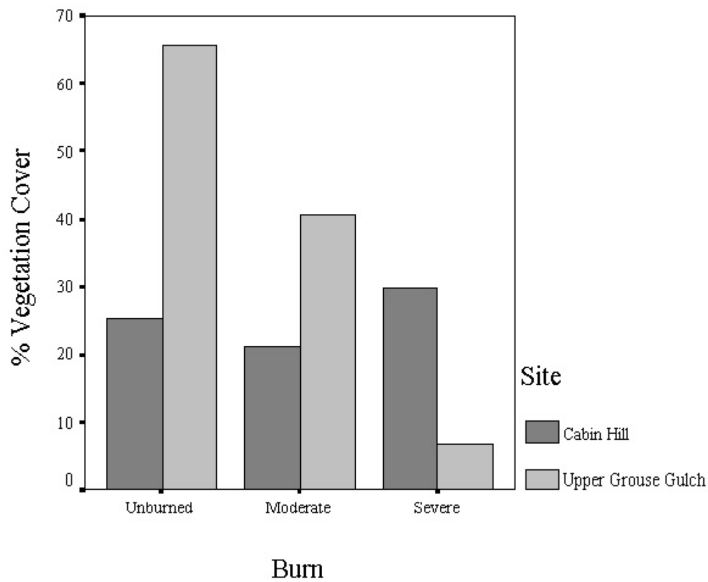


Figure 5. Percent vegetation cover at each site and burn severity.

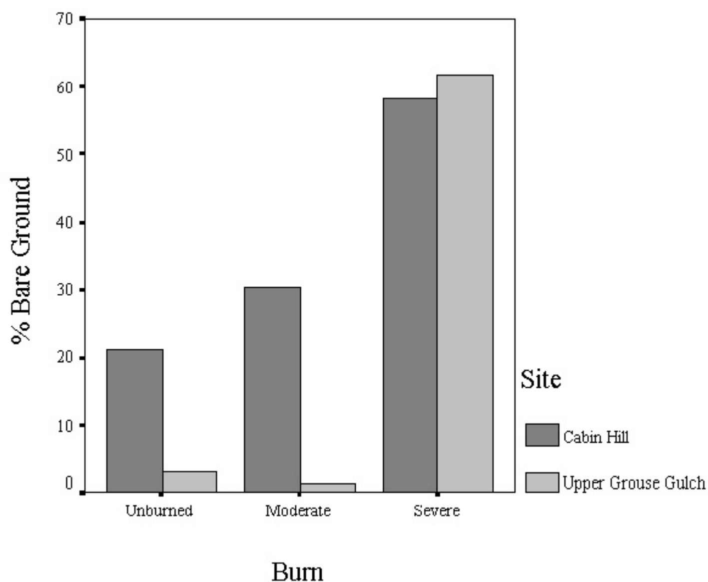


Figure 6. Percent bare ground at each site and burn severity.

severity increased at Cabin Hill. Upper Grouse Gulch showed no difference in the litter cover when comparing unburned and severely burned plots, but the moderate burn had higher litter cover than either unburned or severe burn. This could be due to organic material falling to the ground after the fire. Duff thickness decreased with increasing burn severity (Fig. 7), supporting our assessment of severity types.

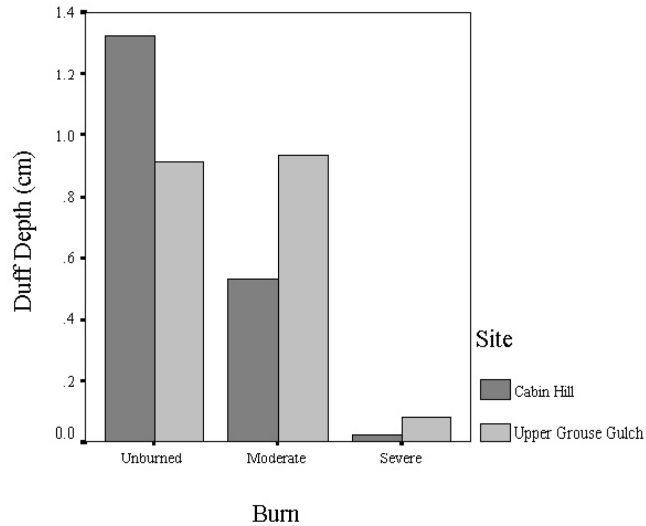


Figure 7. Depth of duff (cm) at each site and burn severity.

Soil Nutrients

Our study focused on pH, OM, total N, NH_3^-, NH_4^+, and P. Potassium, Mg, Ca, S, Z, Na, soluble salts, and CEC were also tested (Appendix C). Significant differences between burn severities were seen in phosphorus, pH, ammonium, and sulfur (Table 2). Organic matter and the other nutrients showed no significant changes.

Soil texture was similar between each site suggesting texture is not responsible for differences in nutrients. Cabin Hill is a silt loam soil while soils at Upper Grouse are clay loam.

Table 2. Average values of soil characteristics at Cabin Hill and Upper Grouse Gulch. Letters indicate significant differences between burn severities at a site.

	% Total N	**% OM**	**Nitrate** (ppm)	**P** (ppm)	**pH**	$\mathbf{NH_4}$ (ppm)
CH Unburned	.24	5.73	1.67b	10.2a	7.67	3.84a
CH Moderate	.164	3.4	14.2a	39.2b	7.87	15.76b
CH Severe	.177	3.96	13.67a	29.1b	7.56	30.05c
UG Unburned	.29	7.53	1.67	15.22A	6.8A	6.19A
UG Moderate	.317	6.26	1.33	39.89B	7.4B	21.23B
UG Severe	.327	6.99	2.0	64.89C	7.39B	36.37C

Burn severity did not significantly alter pH at the Cabin Hill site (Table 2). However, pH increased in burned soils at the Upper Grouse site. While increases in soil pH of up to three units have been documented immediately after a burn (Raison 1979), there is little literature on soil pH one year post-fire. At this point, plants were recolonizing the area. The associated OM will eventually lower the pH to pre-fire levels. When soil is heated, microbial activity in the soil increases (Pierzynski et al. 2000). The metabolic activity of microbes alters organic elements into a plant available form (N to ammonium) (Pierzynski et al. 2000). As microbial activity increases, pH of the soil also increases.

Organic matter varied more between sites than burn treatments, with 3-12% OM remaining after the fire at each site (Fig. 8). Cabin Hill had less OM than Upper Grouse

in the unburned plots (5.7% vs. 7.5%). Cabin Hill and Upper Grouse burned plots had a 3-8% and 4-12% OM. The slopes are steep and the soils are shallow at both sites, and

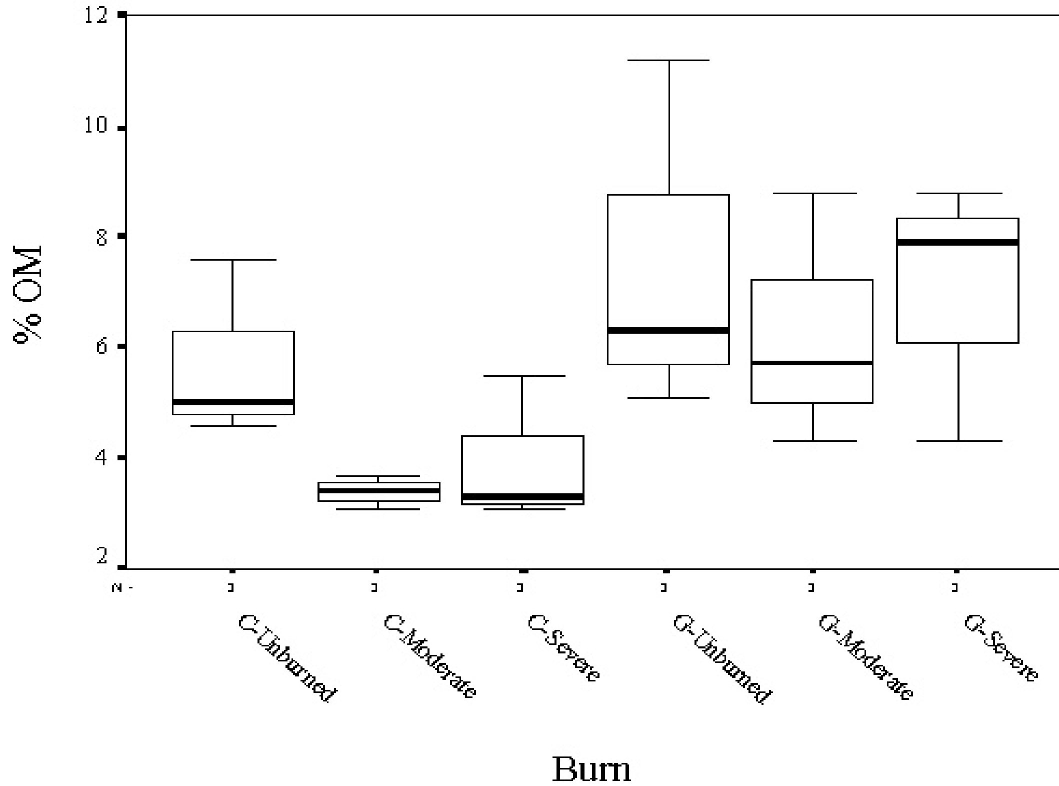


Figure 8. Percent organic matter at Cabin Hill (C) and Upper Grouse Gulch (G) in each burn severity.

OM levels are less than 10%, regardless of disturbance. Organic matter is vital for providing the microclimate needed to produce a healthy and viable plant community.

Total N increased with increasing burn severity at the Upper Grouse site (Table 2), similar to the results of Chromanska and Deluca (2000). This may be from subsequent OM inputs after the fire, including burned debris. Because of the variance between fires and burn types, it is impossible to say what the direct affect on N will be in each study. Nitrate levels decreased in each burn severity but were not significant.

Ammonium increased significantly at each site with increasing burn severity (Fig. 9). Ammonium is often concentrated in the upper soil layers after a fire.

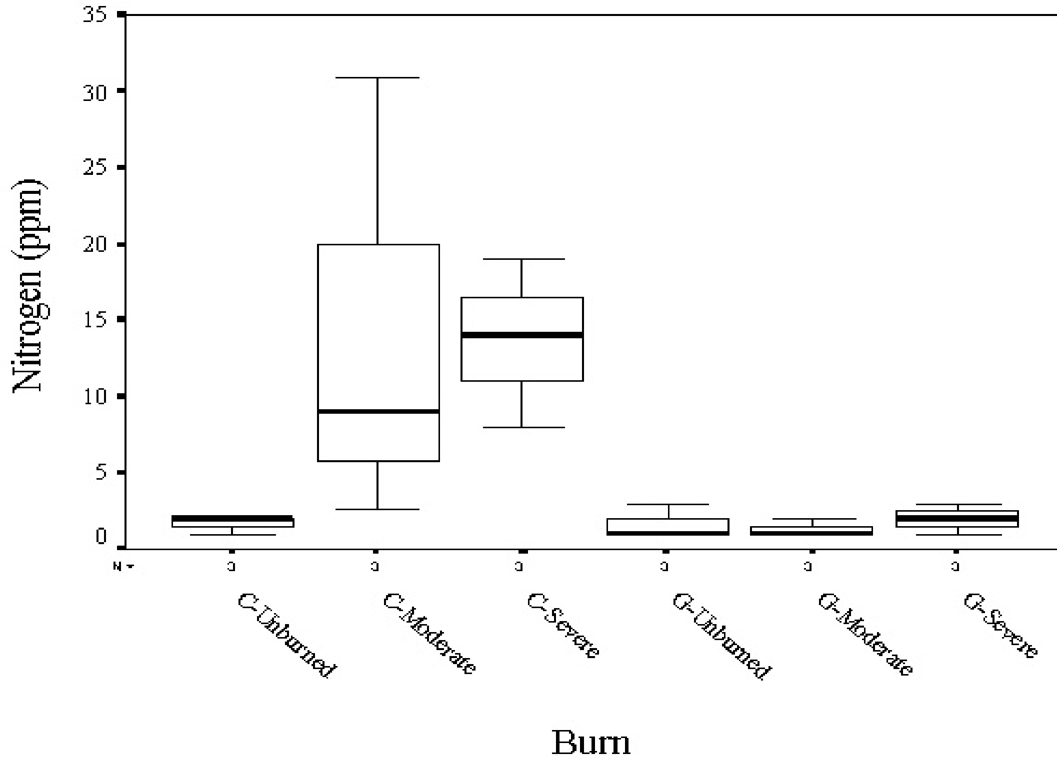


Figure 9. Nitrogen levels (ppm) at Cabin Hill (C) and Upper Grouse Gulch (G) in each burn severity.

Phosphorus is the only element that showed significant differences between both site and fire severity (Fig. 10). Phosphorus increased with increasing burn severity, especially at the Upper Grouse site. Phosphorus typically shows an increase after a fire. Since P is the third most limiting nutrient, these increases are beneficial for new plant establishment in a burned area.

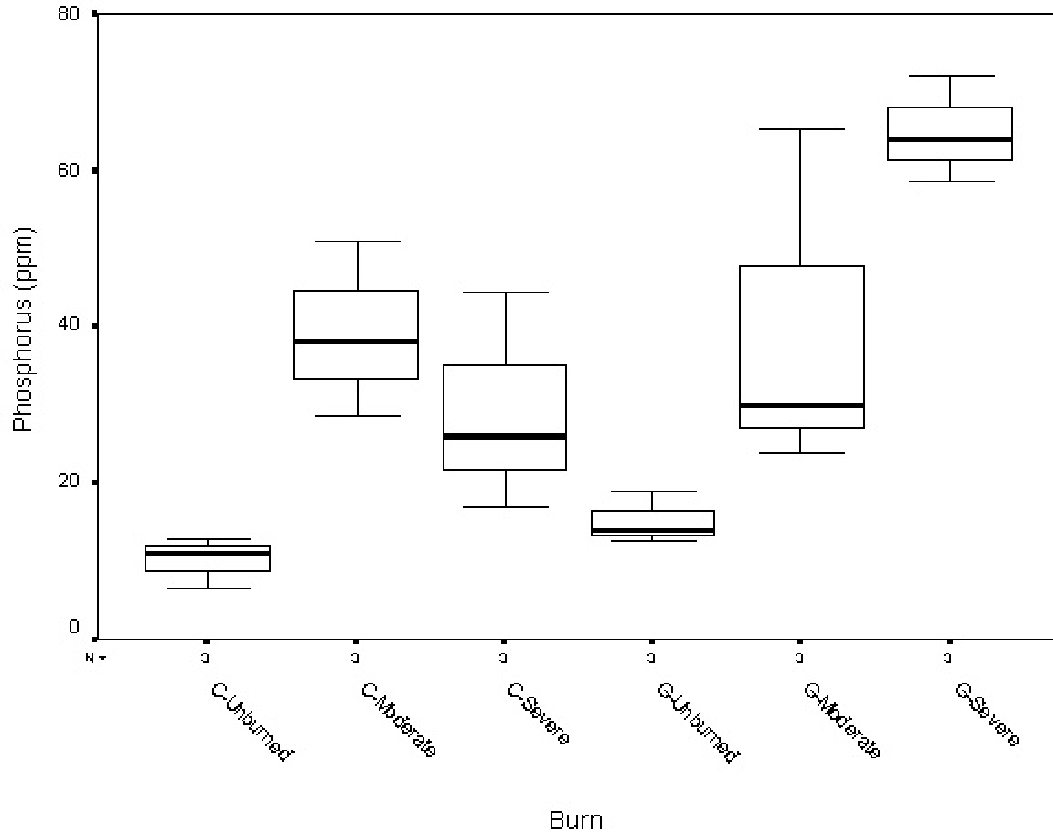


Figure 10. Phosphorus levels (ppm) at Cabin Hill (C) and Upper Grouse Gulch (G) in each burn severity.

Calcium, Mg, K, and Na concentrations increased slightly, but not significantly with increasing fire severity (Appendix C). Available plant levels of Ca, Mg, K, and Na were unaffected by fire.

Fire effects on soil nutrients are site specific. In the Helena National Forest, one year after the fire, the soil contained ample levels of organic matter, an acceptable range of pH and concentrations of all nutrients needed for plant growth and survival.

The question that remains to be answered is why, if the soil was not the problem, weren't more plants established in the burn areas one year after the fire? There is a wide

range of limiting factors in an ecosystem. In this scenario, the Helena National Forest has been exposed to drought conditions for the last several years. It is possible that soil water limited plant growth. Vegetative cover reduction drastically changes the microclimate at the soil surface. Increased direct sunlight, increased evaporation, greater fluctuation in the soil temperature, and increased danger from erosion result. All of these characteristics make plant establishment very difficult.

Post-fire management can be an extremely controversial issue. Fire is a natural form of disturbance, and forest soils and vegetation have evolved to recover after fires. A regression to earlier stages by fire may be beneficial, often improving range condition and habitat and species diversity. Monitoring is the most important way to follow the effects of a fire. Samples can be taken to see if there is a deficit in the soil or if erosion is imminent and needs to be prevented. Each area has to be managed specifically for its needs and uses.

Seed bank

The seed bank results are reported as a seed bank density in seeds/m^2. The density of the seed bank differed between the two sites, which were expected because of differences in aspect, slope, and available water. More importantly, differences between the burn severities were found. At Cabin Hill, there were 528 seeds/m^2 in the

Table 3. Number of germinants in each burn severity in seed/m^2.

Burn Severity	Cabin Hill	Upper Grouse Gulch
Unburned	528	1057
Moderate	81	258
Severe	0	203

unburned plots while the moderate burn had a density of 81 seeds/m^2 (Table 3). The severe burn had no viable seeds. The seed bank at the Upper Grouse Gulch site contained 1,057 seeds/m^2 in the unburned, 258 seeds/m^2 in the moderate burn, and 203 seeds/m^2 in the severe burn.

The number of species decreased in severely burned plots. Combining data from both sites, seven species were present in unburned samples, six in moderately burned samples, and only one in severely burned (Table 4). Bicknell's geranium (*Geranium bicknellii*) was present in the severe burn samples, but was not in either moderately or unburned samples. Bicknell's geranium (*Geranium bicknellii*) requires a disturbance or heating of the soil to germinate (Kershaw et al. 1998).

Table 4. Species that germinated in each severity, combining both sites.

Unburned	Moderate	Severe
Unknown Forb-A	Unknown Forb-A	*Geranium bicknellii*
Unknown Forb-E	Unknown Forb-E	
Achillea millefolium	Unknown Forb-C	
Legume	Unknown Forb-D	
Poa pratenis	*Calamogrostis rubescens*	
Festuca scabrella	Unknown Grass	
Unknown Grass		

Results were as expected, with a decreasing seed bank density with increasing burn severity. The two sites differed significantly in the number of germinants. The Upper Grouse site had more germinants than Cabin Hill. This could be due to different

site conditions including slope and aspect. The species, *G. Bicknellii*, was only found in one out of 20 pots containing soils collected in severe burns. The seed bank present is very patchy.

It is possible that adjacent unburned areas, which could have contributed seeds by natural distribution processes during the year following the fire, could influence the seed bank. If the study was done in the middle of a large severely burned area, the seed bank density may have been lower. Another possible factor influencing our results is time constraints; the samples were not given a cold treatment to simulate winter conditions prior to germination. Providing a cold treatment may have increased the germination rate. The samples were also not grown to maturity, and all species were not identified.

Management implications cannot be effectively derived from this seed bank study alone. This study should be supplemented by studies immediately following a wildfire and in continuing years to track seed bank recovery and to document the role of the seed bank in revegetation of disturbances. The study should look at the effects of the adjacent unburned area, and have a larger sampling size with more sites.

Mycorrhizae

Mycorrhizae Infectivity Potential (MIP)

Our measure of mycorrhizal infectivity potential indicates viable AM fungal propagules are present in the soil. Overall colonization levels of bait plants grown in the greenhouse indicate no difference in propagule density between the two sites, averaged across burn severities (Fig. 11). However, burn severity, averaged across sites, did affect propagule density (Fig. 12). An increase in burn severity causes a significant decrease in infectivity potential.

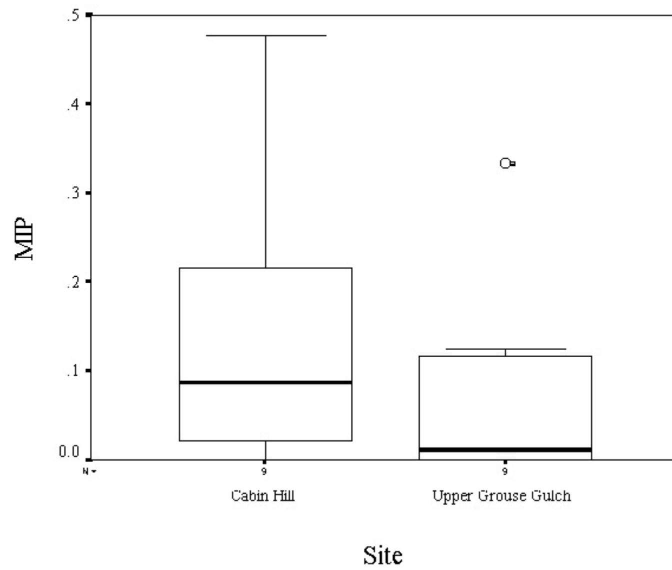


Figure 11. Mycorrhizal infectivity potential at each site.

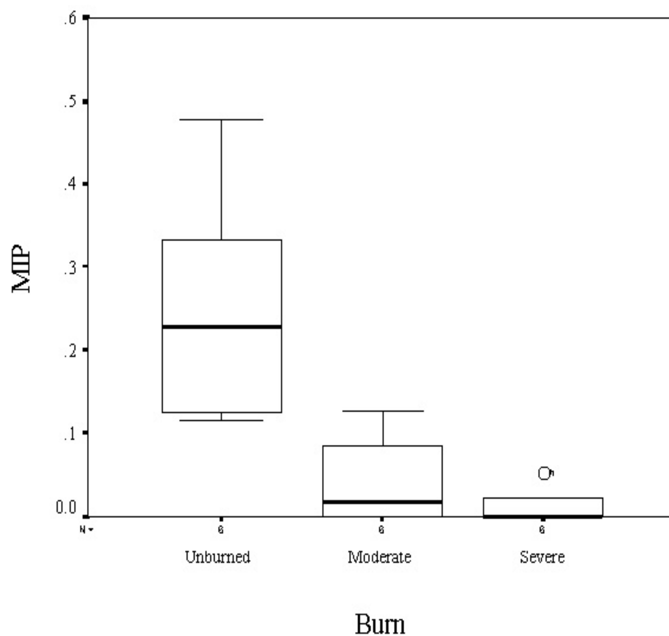


Figure 12. Mycorrhizal infectivity potential at each burn severity.

Field Colonization

The significant decline in viable propagules in the soil supports our hypothesis that burn severity has an effect on mycorrhizae, particularly on severely burned sites. One-year post fire, viable propagules were lower in severely burned soils as compared to unburned soils (Fig. 13). The reduction of mycorrhizal fungi in the soil may slow the growth and establishment of colonizing vegetation. Where viable propagules are present, vegetation would be expected to thrive. This may help to explain the sporadic distribution of the vegetation in severely burned sites. Plants may colonize primarily where mycorrhizal fungi are present. No significant differences were observed between the Cabin Hill and Upper Grouse site (Fig. 14).

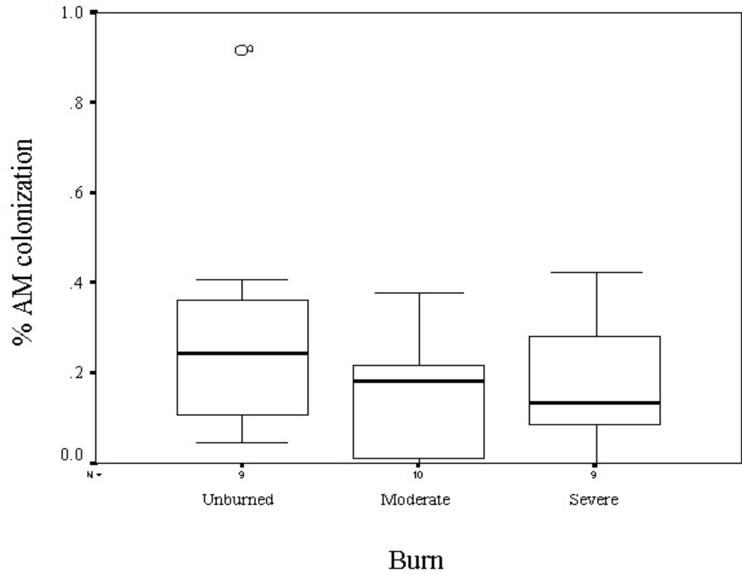


Figure 13. Percent AM colonization at each burn

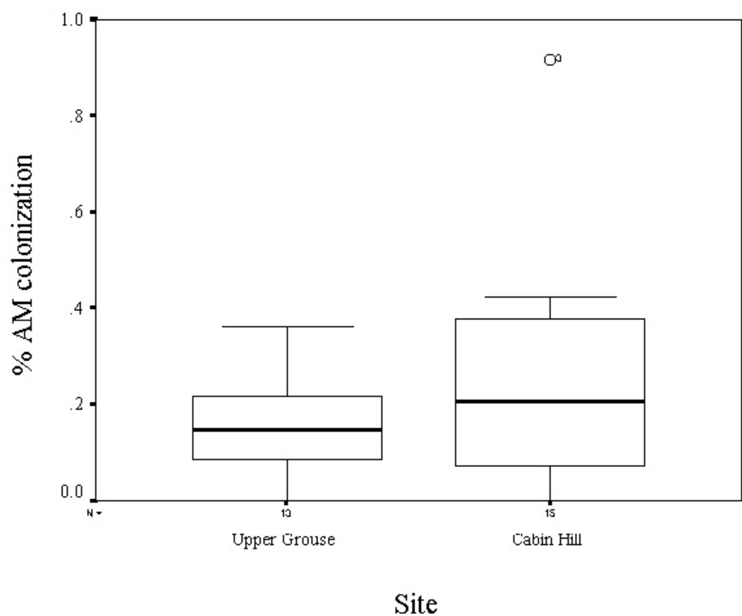


Figure 14. Percent AM colonization at each site.

Microbes

The DGGE profiles indicate that there are hundreds of different bacterial 16S rDNA nucleotide sequences in each of the PCR products (Fig. 15). This indicates high apparent bacterial diversity in these soils. Some of the bands in the sequence appear to be brighter than others. This may suggest that there is more of that particular sequence present than that in the lighter bands. However, this may also mean that the bright bands are a result of a clustering of populations with a similar nucleotide sequence (Nakatsu et al. 2000).

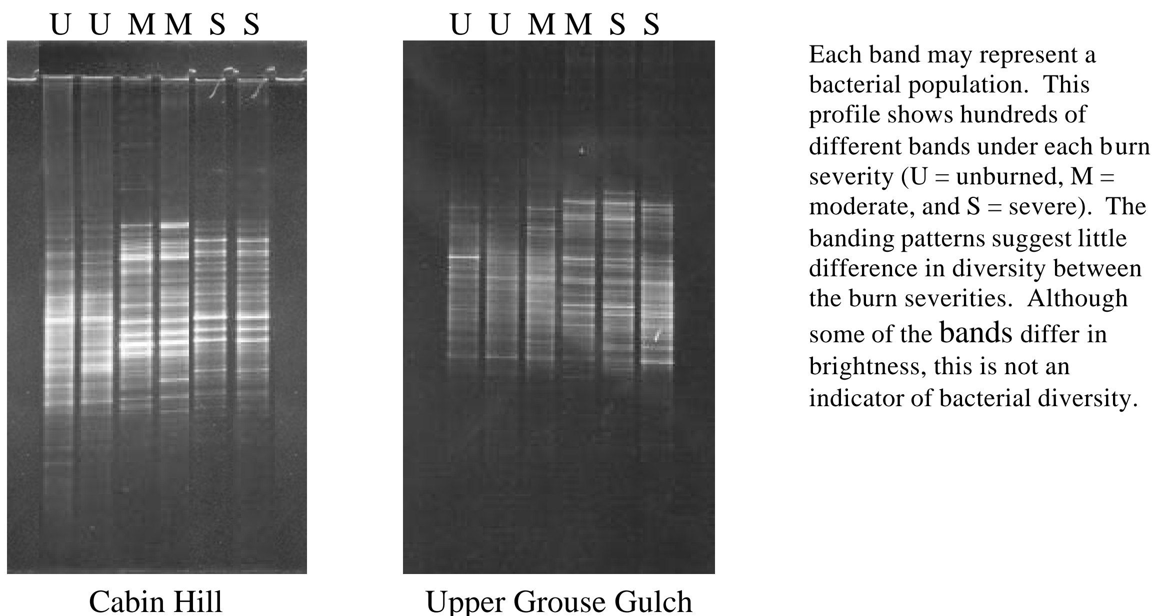


Figure 15. Denaturing Gradient Gel Electrophoresis

The DGGE banding patterns for each sample suggest that there is very little difference in diversity between burn severities. There are numerous explanations for our results. First, we sampled soils to a 10 cm depth, and the sites we sampled may not have been affected 10 cm below the surface by the heat of the fire. Since the 10 cm soil samples were homogenized, the bacterial communities affected may have been diluted.

If this were true our results would be a misrepresentation of the fire's effect on the bacterial population.

The soils were collected one year following the fire. We cannot rule out the possibility that the bacterial community was negatively impacted by burn temperatures, and recolonized or recovered in the time between the fire and our sampling. Without apparent catastrophic damage to the community and because of rapid exponential reproduction abilities, bacterial populations could easily recover.

Alternatively, bacteria could easily recolonize the site, possibly on the hooves of wildlife containing bacteria on their hooves, or by us transporting bacteria on the soles of our boots. The Cabin Hill site had very steep slopes where erosive effects were observed. The severe site was located at the bottom of the slope, which may have been contaminated by soil movement from the unburned and moderately burned sites upslope.

There is the possibility that the fire did not significantly affect soil bacteria. To better understand our results, further studies need to be conducted. Cloning the individual sequences in the DGGE bands would enable us to identify the apparent communities. Other possibilities include separating the bacterial community using specific primers designed to focus only on specific species, such as nitrite oxidizers, ammonifiers, etc. This could further our knowledge of fire impacts on microbial species. These studies in combination with an assessment of nutrient cycling could help to build an understanding of fire effects on long-term site productivity.

SUMMARY

Fire is a natural disturbance that dictated patterns of ecological succession in the forests of the western United States until the advent of fire management in the last century. As a result of aggressive fire suppression from the early 1900s through the 1970s, western forest ecosystems were reshaped, creating unprecedented dense understories (Dombeck 2001). Some land managers and scientists feel that the dense understory contributes to more intense wildfires (Dombeck 2001).

Western Montana experienced two intense fire seasons during 2000 and 2001. Natural resource managers have a profound need for accurate, relevant information in support of policy and management decisions. Over a semester, the Montana State University Land Resources and Environmental Sciences Department capstone course students, in conjunction with Helena National Forest personnel, investigated fire effects on soil nutrient availability, seed bank and vegetation, mycorrhizal fungi and microbial diversity.

One year following the fire, burning and burn severity did not appear to affect soil nutrient availability and therefore was not a limiting factor in regeneration of plant species. Although vegetation had established at each site, significant bare ground existed in contrast to unburned areas. Below normal rainfall and resulting low soil water availability may offer a better explanation than nutrient status for limited vegetative responses occurring at Cabin Hill and Upper Grouse Gulch. Additionally, we documented a decrease in seed bank density in soils showing signs of severe burn. Lack of propagules in the soil at some sites may slow vegetation establishment.

With decreased seed bank density caused by fire, the establishment of seedlings from viable seeds may be enhanced by the presence of mycorrhizal fungi. Examination of root systems growing in unburned, moderately burned and severely burned soils showed equivalent levels of mycorrhizal colonization, indicating this component of soil biota was extant one year after fire. However, mycorrhizal propagules decreased with increased burn severity. The distribution of mycorrhizal vegetation may indicate the distribution of viable fungi in the soil. Other microorganisms did not show any apparent change in diversity. A better understanding of microbial responses to fire is essential to elucidate fire impacts because of the dependence of nutrient (particularly N) cycling on microbial activity.

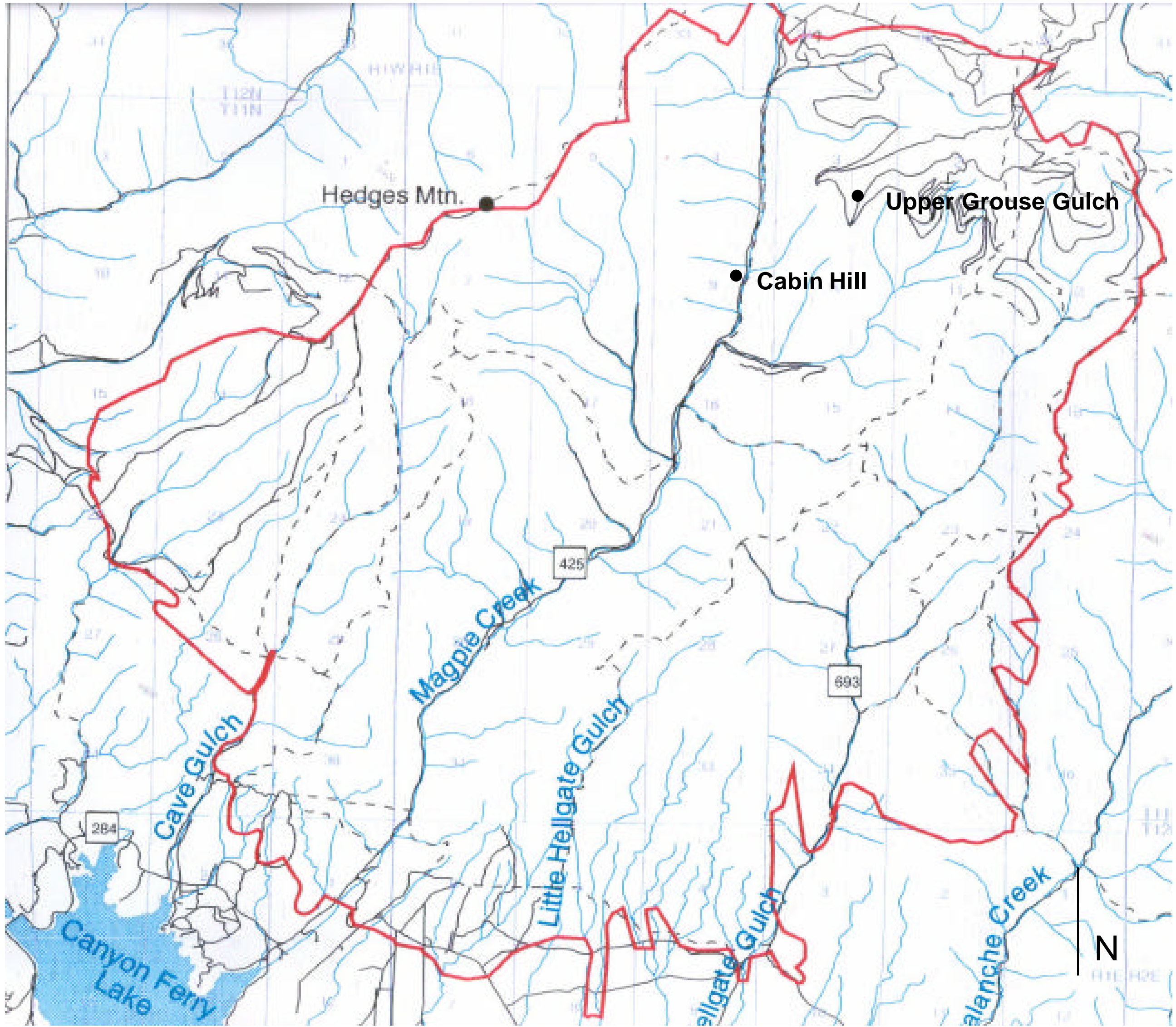
An important aspect of this study was the sampling time frame: one year post-fire. Immediate fire impacts were missed in this study; in combination with long-term effects, these would have provided a more complete picture of fire effects on soils. Site selection also influenced the results. Study sites were selected for proximity of unburned, moderately burned, and severely burned areas while minimizing expected differences in soil heterogeneity and microclimate. This proximity, however, may have increased the likelihood of colonization from adjacent unburned sites.

Fire has turned back the successional clock on the Helena National Forest. Returning to an equivalent level of productivity in the forest ecosystem may take many years. Although fire consumes organic matter and increases soil temperatures for a relatively short duration, these events may have less of an impact on soil characteristics and subsequent site productivity levels than secondary processes at the burn site. Sparse post-fire vegetation, combined with steep mountainous landscapes prevalent in western

forests, potentially contribute to significant soil erosion. Consequently, soil biota and seed bank may be distributed down slope from their original position, leaving intact soils upslope low in organic matter and nutrients essential for plant growth. These post-fire effects may be more important than the actual fire in delaying a return to pre-burn vegetative communities.

Appendix A

Site Locations



----- Fire Perimeter

Appendix B

Upper Grouse Gulch:

Unburned

Arrowleaf balsamroot	*Balsamorhiza sagittata*
Aster	*Aster sp*
Carrot family	*Apiacea*
Dandelion	*Taraxacum officinale*
Dwarf blueberry	*Vaccinium cespitosum*
Idaho fescue	*Festuca idahoensis*
Lupine	*Lupinus sericeus*
Northern bedstraw	*Galium boreale*
Oregon grape	*Mahonia repens*
Pinegrass	*Calamagrostis rubescens*
Rockcress	*Arabis sp*
Snowberry	*Symphoricarpos albus*
Spiraea	*Spiraea betulifolia*
Strawberry	*Fragaria virginiana*
Yarrow	*Achillea millefolium*

Moderate

Aster	*Aster sp*
Bicknell's geranium	*Geranium bicknellii*
Dragon's tongue	*Dracocephillusalum parviflorum*
Heart arnica	*Arnica cordifolia*
Mint family	*Lamiaceae sp.*
Oregon grape	*Mahonia repens*
Pine grass	*Calamagrostis rubescens*
Showy aster	*Aster conspicuus*
Snowberry	*Symphoricarpos albus*
Spiraea	*Spiraea betulifolia*
Sticky geranium	*Galium boresle*
Violet	*Viola sp*

Severe

Bicknell's geranium	*Geranium bicknellii*
Dragon's tongue	*Dracocephillusalum parviflorum*
Pine grass	*Calamagrostis rubescens*
Showy aster	*Aster conspicuus*
Spiraea	*Spiraea betulifolia*

Cabin Hill:

Unburned

Arrowleaf balsamroot	*Balsamorhiza sagittata*
Aster	*Aster sp*
Bearberry	*Arctostaphylos uva-ursi*

Beardstongue	*Penstemon procerus*
Bluebunch wheatgrass	*Agropyron spicatum*
Carrot Family	Apiaceae sp.
Cut-leafed fleabane	*Erigon compostus*
Dandelion	*Taraxacum officinale*
Dogbane	*Apocynum androsaemifolium*
Onion	*Allium sp.*
Poa sp.	*Poa sp.*
Pussy toes	*Astragalus sp*
Ross's sedge	*Carex rossii*
Snowberry	*Symphoricarpos albus*
Spiraea	*Spiraea betulifolia*
Strawberry	*Fragaria virginiana*
Yarrow	*Achillea millefolium*

Moderate

Aster	*Aster sp*
Bicknell's geranium	*Geranium bicknellii*
Dogbane	*Apocynum androsaemifolium*
False Solomon's seal	*Maianthemum stellatum*
Heart arnica	*Arnica cordifolia*
Onion	*Allium sp.*
Oregon grape	*Mahonia repens*
Pine grass	*Calamagrostis rubescens*
Rose	*Rosa acicularis*
Ross's sedge	*Carex rossii*
Showy aster	*Aster conspicuus*
Snowberry	*Symphoricarpos albus*
Spiraea	*Spiraea betulifolia*
Yarrow	*Achillea millefolium*

Severe

Aster	*Aster sp*
Bicknell's geranium	*Geranium bicknellii*
Dandelion	*Taraxacum officinale*
Dragon's tongue	*Dracocephillusalum parviflorum*
Heart Arnica	*Arnica cordifolia*
Lupine	*Lupinus sericeus*
Milk vetch	*Astrogolus sp*
Mint family	*Lamiaceae sp.*
Onion	Allium sp.
Oregon grape	*Mahonia repens*
Snowberry	*Symphoricarpos albus*
Spiraea	*Spiraea betulifolia*

Appendix C

Nutrient Data

	Calcium (ppm)	**Potassium** (ppm)	**Magnesium** (ppm)	**Sodium** (ppm)	**Sulfur** (ppm)	**Zinc** (ppm)	**CEC** Total	**Soluble Salts** mmhos/cm
CH Unburned	2444.3	303.9	250.3	6.0	3.2	3.9	15.1	.219
CH Moderate	2942.3	408.5	293.1	9.8	35.3b	4.4	18.2	.393
CH Severe	2973.7	245.1	257.4	8.4	18.9a	4.3	17.7	.366
UG Unburned	2677.3	310	216.6	10.9	12.1	5.7	16.0	.274
UG Moderate	3461	329.1	209.3	12.7	40.9a	6.7	19.9	.356
UG Severe	4080.3	257	170.4	11.7	43.8a	8.2	22.5	.399

REFERENCES:

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

Amaranthus, M. P. and J. M. Trappe. 1993. Effects of erosion on ecto- and VA-mycorrhizal inoculum potential of soil following forest fire in southwest Oregon. Plant and Soil, 150:41-49.

Andreu, V., J.L. Rubio, J. Fortenza, and R. Cerni. 1996. Postfire effects on soil properties and nutrient losses. International Journal of Wildland Fire, 6:53-58.

Bakin, C.C. and J.M. Baskin. 1998. Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. Academic Press, San Diego, pp. 133-179.

Bissett, J. and D. Parkinson. 1979. Long-term effects of fire on the composition and activity of the soil microflora of a subalpine, coniferous forest. Canadian Journal of Botany, 58:1704-1721.

Blank, R.R. and D.C. Zamudio. 1998. The influence of wildfire on aqueous-extractable soil solutes in forested and wet meadow ecosystems along the eastern front of the Sierra-Nevada Range, California. International Journal of Wildland Fire, 8:79-85.

Brais, S., P. David, and R.Ouimet. 2000. Impacts of wild fire severity and salvage harvesting on the nutrient balance of jack pine and black spruce boreal stands. Forest Ecology and Management, 137:231-243.

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

Dombeck, M. 2001. How can we reduce the fire danger in the interior west? www.fs.fed.us/land/wdfire.htm. 61:5-13.

Driscoll, K.G., J.M. Arocena, and H.B. Massicotte. 1999. Post-fire soil nitrogen content and vegetation composition in Sub-Boreal spruce forests of British Columbia's central interior, Canada. Forest Ecology and Management, 121:227-237.

Feller, M.C. 1989. Estimation of nutrient loss to the atmosphere from slash burns in British Colombia. In Proceedings 10th Conference on Fire and Meteorology, April 17-21, 1989, Ottawa, Canada, pp. 126-135.

Fernendez, I., A. Cabaneiro and T. Carballas. 1997. Organic matter changes immediately after a wildfire in an Atlantic forest soil and comparison with laboratory soil heating. Soil Biology and Biochemistry. 29:1-11.

Gee, G.W. and J.W. Bauder. 1986. Particle-size analysis. Methods of Soil Analysis.1. Physical and Mineralogical Methods. Agronomy Monograph Methods 9. 2nd ed. American Society of Agronomy-Soil Science Society of America, pp. 383-411.

Hungerford, R.D., M.G. Harrington, W. H. Frandsen, K. C. Ryan, and Gerald J. Niehoff. 1990. Influence of flora on factors that affect site productivity, pp. 32-50.

Jimenez, H. E., and J. J. Armesto. 1992. Importance of the soil seed bank of disturbed sites in Chilean Matorral in early secondary succession. Journal of Vegetation Science, 3:579-586.

Kershaw, L., A. MacKinnon, and J. Pojar. 1998. Plants of the Rocky Mountains. Lone Pine Publishing, Edmonton, Canada.

Kimmins, J.P. 1996. Importance of soil and role of ecosystem disturbance for sustained productivity of cool temperate and boreal forests. Journal of Soil Science Society of America, 60:1643-1654.

Mroz, G.D., M.F. Jurgensen, A.E. Harvey, and M.J. Larsen. 1980. Effects of fire on nitrogen in forest floor horizons. Soil Science Society of America Journal, 44:395-400.

Pattinson, G. S., K. A. Hammill, B. G. Sutton, and P. A. McGee. 1999. Simulated fire reduces the density of arbuscular mycorrhizal fungi at the soil surface. Mycological Research, 103:491-496.

Pietikainen, J. and H. Fritze. 1992. Microbial biomass and activity in the humus layer following burning: short-term effects of two different fires. Canadian Journal of Forest Restoration, 23:1275-1285.

Pierzynski, G.M., J.T. Sims, and G.F. Vance. 2000. Soils and environmental quality, 2nd edition. CRC Press LLC, Boca Raton.

Raison, R.J. 1979. Modification of the soil environment by vegetation fires, with particular reference to nitrogen transformations: a review. Plant and Soil, 51:73-108.

Roose-Amsaleg, C.L., E. Garnier-Sillam, and M. Harry. 2001. Extraction and purification of microbial DNA from soil and sediment samples. Applied Soil Ecology, 18:47-60.

Tugel, A.J., and A.M. Lewandowski, eds. February 2001 – August 2000. Soil Biology Primer [online]. Available: www.statlab.iastate.edu/survey/SQI/soil_biology_primer.htm [accessed on October 18, 2001].

Vazquez, F.J., M.J. Acea, and T. Carballas. 1993. Soil microbial populations after a wildfire. FEMS Microbiology Ecology, 13:93-104.

Vilarino, A. and J. Arines. 1991. Numbers and viability of vesicular-arbuscular fungal propagules in field soil samples after wildfire. Soil Biology and Biochemistry, 23:1083-1087.

Wells, C.G., R.E.Campbell, L.F. Debano, C.E. Lewis, R.L.Fredriksen, E. Carlyle, R.C. Franklin, Froelich, and P.H.Dunn, 1979. Effects of fire on soil: a state-of-knowledge review. United States Department of Agriculture Forest Service General Technical Report WO-7, Washington, DC.

Zabinski, C., T. Wojtowicz, and D. Cole. 2000. The Effects of recreation disturbance on subalpine seed banks in the Rocky Mountains of Montana. Canadian Journal of Botany. 78:577-582.