Forest Fire: examining the effects of recent fire on soil nutrients and microbes, and above and below ground vegetation

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Study Overview

General Introduction: Fire ecology and the effects of historical suppression

Wildfire is the most dramatic, common, large scale disturbance in Northern Rockies ecosystems, and has coevolved with the landscape. Fire's effects on forest ecology, hydrology, soils, and nutrients are complex, spatially heterogeneous, and time-scale dependent. The suppression of fire by active management in the 19th and 20th centuries, and the current return to natural fire regimes, has further complicated the fire-landscape dynamic and created a large gap between wildfire management and wildfire understanding.

Wildfire is an integral component of forest ecosystems. Intermittent burns are natural disturbances that occur at varying intervals and severities, creating mosaics of even-aged stands and altering many components of the environment. Fire disturbance controls: plant community succession and competition; native species genetics and ecophysiology; soils, nutrients, and erosion; and pest behavior (Brown and Smith, 2000).

The effects of wildfire on a forested landscape are dependent on timescale (Shakesby and Doerr, 2006). Loss of vegetation (high burn intensity), increased risk of erosion, soil hydrophobicity, or loss of organic material (high burn severity), and change in wildlife habitat are instantly observed changes. With increasing time after wildfire, vegetation will likely return, soils will stabilize and regain water holding characteristics, and the ecology of the flora and fauna will progress towards pre-burn conditions.

Wildfire behaves differently in different ecosystems, the effects of which must be evaluated accordingly. Ecological communities will have distinct fire regimes (Brown and Smith, 2000). Fire regimes are the combination of fire factors (frequency, severity, spatial pattern, and vegetation type) that dominate in an area. Brown and Smith (2000) categorized forest fire regimes into four types: understory fires (short return-interval), stand replacing fires (crown fires), mixed severity fires (long return-interval crown fires), and non-fire regimes (no natural fires). Fire severity is determined from charring depth and remaining vegetation, both of which contribute to revegetation (Brown and Smith, 2000). Fire severity is a qualitative description of immediate fire effects on vegetation and soil litter. High-severity fires can be lethal to many trees within the entire stand, and tend to effectively influence forest structure (Rosemary and Veblen, 2006).

Management practices have strong effects on burn severity, in turn affecting the coevolved wildfire-landscape system (Thompson *et al.,* 2007). Since the devastating fires of 1910, the United States government has actively suppressed wildfires. Naturally occurring fire regimes may have been altered across the western United States because of this doctrine. These anthropogenic factors include: reduced fire frequency, increased fuel accumulation, increased human-related ignition, changes in the rate of spread, changes in the incidence of wildfire, loss of fire-resilience in ecosystems, and reduction of fine fuel biomass (Dellasala *et al.,* 2004). For example, wildfire suppression has increased overall tree density and fuel loading in certain ecosystems, promoting large and intense fires rather than the frequent low-intensity fires characteristic of historical regimes (Freeman *et al.,* 2007).

In our study area, the Northern Rocky Mountains, two vegetation zones dominate. The subalpine forests are generally dense and have a long fire interval of around 200-400 years (Brown and Smith, 2000). The wildfires that affect these areas are generally drastic, stand-replacing fires. Due to the long fire interval of subalpine forests, wildfire suppression may not have altered the structure and function of the ecosystem. In contrast, lodgepole pine/Douglas fir communities have a fire return interval ranging from 50-100 years. This study evaluated fire severity effects on soil characteristics, soil biota, and above and below ground vegetation composition in a lodgepole pine-subalpine fir transition zone.

Study Objectives

The two overarching objectives of the 2008 Capstone field research were:

1. To develop specific objectives for four subject areas in relation to wildland fire, burn severity, time since burn, and landscape heterogeneity: soils, microbiology, vegetation & landscape management.
2. To improve our understanding of the importance of interdisciplinary teams when conducting research.

The outcome of objective one was group research on the effects of fire on: (1) soil parameters, (2) soil microbe communities, (3) above and below ground vegetative composition, and (4) how vegetative composition varies with landscape parameters. These four research foci were considered in relation to fire severity which, for the purposes of this research, was used as a qualitative estimate of the immediate effect of fire on the vegetation, litter, and soils.

Study Area

The study site, the Wicked Fire Research Area, is in the Mill Creek drainage south of Livingston, Montana.

Figure 1. *Location of the Wicked Fire Research Area.*

Parts of this drainage basin were burned in both 2006 and 2007. Burn severities were characterized in three polygons ranging from high to low for both the burn seasons. The burn areas were determined by examining burn severity maps created by the Gallatin National Forest Service.

Site Details

- Transition zones between subalpine fir & Douglas fir dominated habitats
- Loam/sandy loam, Typic Haplustep
- Fire affected all aspects & slopes but 3 groups concentrated on northerly aspects & 0-30 degree slopes; the 4th group studied a wider range of aspects/slopes

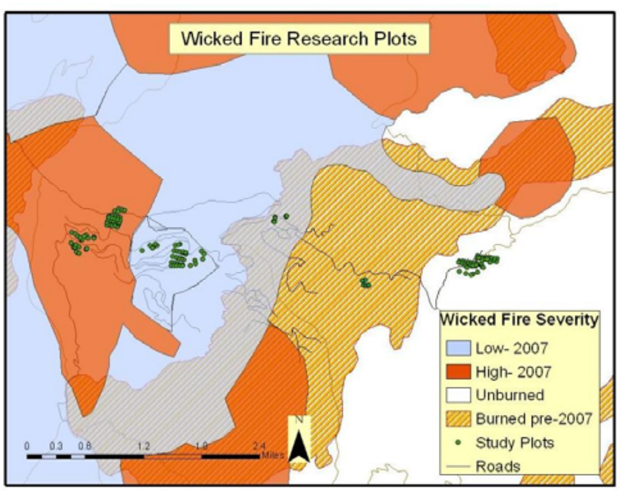

Figure 2. *Detail of Wicked Fire burn area and research plot locations.*

Field Sampling

Research plots were established across the range of fire severities and year of burn as shown in the map above and the table below.

Group	Control plots	Double burn	High 2006	Low 2007	High 2007
Soils	★			★	★
Microbiology	★	★			★
Vegetation	★		★	★	★
Geospatial	★		★	★	★

Table 1. *Research plot stratifications by burn severity and study group.*

Each group conducted a preliminary assessment of the Wicked Fire Research Area and then formulated hypotheses to be tested. Experimental design and procedures were then established to address these hypotheses. In consultation with their faculty advisors, the students were responsible for determining which experiments, measurements, and analytical and statistical procedures were appropriate for their work. Field research was conducted in late-August, 2008. The students then analyzed their data and prepared a written summary of their findings, which are provided below.

Soils Group

Effects of Wildfire on Soil

Fire can adversely affect the chemical, physical, and mineralogical properties of soil (Certini, 2005). Nutrients, water holding capacity, organic carbon, aggregate stability, and hydrophobicity are just a few soil properties that can be significantly altered with fire exposure, compromising the health of the entire ecosystem (Certini, 2005). The severity of a wildfire can play an important role in how severely soil properties can be altered (Certini, 2005). Low to moderate fire severity will not result in irreversible ecosystem changes, but may increase hydrophobicity (Certini, 2005). Severe fires can generally remove organic carbon, deteriorate aggregate stability, decrease nutrient availability, and compromise the microbial health of the soil (Certini, 2005).

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Available nutrients in the soil are also affected greatly by fire severity; nutrient loss occurs with an increase in fire severity due to an increase in volatilization (Certini, 2005). Fire severity and the effects it has on nitrogen in conifer forested areas have been studied greatly in the past. (Duran, 2008). Wildfire stimulates the nitrogen cycle; the heat releases the organic nitrogen through combustion of the organic matter in the soil. This increases the nitrification rates in the soil by increasing the amount of charcoal in the fire. The charcoal absorbs the phenols, which are toxins to the nitrifiers. A study conducted by Duran *et al.,* 2008, stated that available nitrogen levels increase greatly between one to five years after fire in the burned treatment sites. Phosphorus (P) is also affected by wildfire severity; P would have an initial spike after the fire, but there would be a large decrease in P because of the lack of organic P in the soil. The initial increase in available phosphorus is caused by the combustion of organic phosphorus (Kutiel, 2007). As the organic content of the soil is rebuilt along with the microbial community the phosphorus will slowly increase over time.

Soil water content is greatly affected by loss of soil organic carbon, loss of water, increased hydrophobicity, and loss of soil biota. Soil erosion due to the fire severity and the loss of vegetation can cause water runoff and a decrease in water infiltration of water to the soil. Combustion of organic matter increases with an increase in temperature. This combustion causes a reduction, or complete removal of the organic material on the soil surface, and in the upper soil horizons (Certini, 2005). Organic matter exposed to fire can be slightly distilled, charred, or completely oxidized, depending on the fire severity. Organic matter is one of the most important and well known aggregate stabilizing agents in soils (Garcia-Orenes *et al.,* 2001).

The goal of this experiment was to observe the effects of wildfire severity on soil properties. We studied soil texture in unburned, low, and high burn severities. Similar slopes were chosen to select for homogenous soils. We measured soil organic carbon, total and available nitrogen (NO_3^-), and available phosphorus (PO_4^{2-}) in the soils of high burn severity, low burn severity, and control sites, measured the soil volumetric water content, and determined the aggregate stability of the sampled soils. This experiment is aimed to provide more insight to wildfire ecosystems, and how fire severity affects nutrient composition and water holding capacity of a homogeneous soil type.

Materials and Methods

Site Description

The data collected was in a subalpine/Douglas fir transition zone located in Mill Creek that had burned a year prior to data collection. The three sites consisted of areas that had a low burn severity, high burn severity, and a control site that had not been burned or experienced other anthropogenic disturbances such as logging or mining. Each burn severity was at an elevation between 2000-2300 m and a slope of 0-30 percent. The parent material consisted of limestone in the control and high severity, while the low severity contained some granitics. The soil had a texture of loamy sand and loamy in all three areas with a soil classification of Typic Haplustep and a family of loamy-skeletal, mixed, superactive, frigid. The sites have a climate with warm summers and cold winters. During the winter months high amounts of precipitation is common, while during the summer the area tends to dry out.

General Methods

At each site a core sample was removed, placed in plastic bags and brought back to the lab for analysis. The soil from the core samples was used to determine the different soil properties except water content. Fifteen samples were taken from each burn site, high severity, low severity, and control. The core was 5 cm wide and 20 cm deep. All samples were placed into coolers to transport from field to lab, and then placed in refrigeration at 40 degrees Celsius.

Total Nitrogen and Carbon:

In total, forty-five soil samples were collected from the three stratifications. All samples were placed in the oven to dry at 45 degrees C for one week, and the temperature was increased to 50 degrees C for the last two days. The samples were then sieved through 2.0 mm sieve, and then milled to a finer material. Each sample was weighed out to 0.2 grams and placed into the Leco machine where total carbon and nitrogen were given in percent by weight values. Instructions for the Leco Furnace can be found in the online manual.

Extractable Nitrogen

Extractable nitrogen (NO_3^-) is highly soluble in water. This characteristic makes extraction with water possible for most soils of the North Central Region (Gray, C. 1983). Four randomly selected samples were sent to Agvise laboratories in North Dakota to measure extractable nitrogen through the Cadmium Reduction Method. This method uses copperized cadmium to reduce NO_3^- to NO_2^- . Once reduced, the NO_2^- concentration is determined using a modified Griess- Ilosvay method. This method is based on the principle that NO_2^- reacts with aromatic amines. These salts couple with aromatic agents to form colored compounds or dyes. The color intensity is then determined with a spectrophotometer. The range of detection for soil extracts using this method has been reported from 0.2 to 15.0 ppm NO_3^- (Dorich, R.A., 1984).

Olsen P Method

Four samples were randomly selected and sent to AGVISE laboratories in North Dakota. Available P was measured using the Olsen-P method where one gram of soil is extracted with a sodium bicarbonate solution. P in the extract is measured using a flow injection analyzer. The amount of P extracted will vary with temperature and shaking speed. The detection limits of the Sodium Carbonate (Olson) Method are approximately 2.0 mg kg^{-1} (air dried soilbasis) and vary plus or minus 12 percent. Refer to “Estimation of available phosphorus in soils by extraction with sodium bicarbonate” written by Olsen, S. R. *et al.* (1954), for complete methods and procedures.

Water Content

Time Domain Refectrometry (TDR) was used to measure water content within the first 18 cm of soil at each stratification. To obtain sufficient data for analysis three readings were recorded at each sample point. Sample points were all taken from North facing slopes at elevations ranging from 4100-4600 m. Sample points were based on topography determined through the use of ArcGIS (Geographic Information System).

The TDR probe is made of two metal rods attached to a sensor. The metal rods are inserted vertically into the ground at each sample point. A wave/signal is sent from the sensor through the metal rods. Once the signal reaches the end of the metal rod it is sent back to the sensor where a computer program analyses the data and gives a measurement of the soil water content. Standard calibration was done prior to data collection. Rocks within the soil can cause problems with the data, so the shorter 18 cm probes were used. In order for there to be an accurate reflection of water content, the soil needs to contain a measurable amount of water in the soil (Bittelli, 2008).

Aggregate Stability

Aggregate stability measures the soils ability to resist change from externally imposed destructive forces (Burras, 2001). Fifteen samples from each site were used to determine the aggregate stability.

In the laboratory, aggregate stability was determined using an assembly of stable aggregate apparatus, which consisted of sieves, an agitation rack, and a water chamber. All methods were followed from a paper by Burras *et al.,* 2001. The sieves were made of stovepipe 15cm high, and 10cm in diameter with metal screen (0.5 mm opening) connected to the bottom with a muffler clamp. The sieves were labeled according to burn severity.

The stability apparatus was arranged by placing the sieves on the agitation rack in the water chamber. Distilled water was added to the height equal to 20 mm above the base of the sieves. Air bubbles were removed with a syringe. Three hundred grams of soil from each burn severity and the control site were left to air dry overnight. The samples were then gently crushed through a 2mm sieve, and onto a 1mm sieve. Three grams of aggregates between 1.0 and 2.0 mm in size were used as the original weight of the sample (W_{or}). The samples were carefully poured into their appropriate sieve, so that aggregates were evenly distributed across the screen. The sieves were then placed on the agitation rack, and left to soak overnight.

Samples were agitated by raising and lowering the agitation rack 20 times in 40 seconds. On the upward stroke, the sieves were drained, but no air was allowed in the sieves from below. The sieves were removed from the water chamber and oven dried for 2 hours at 105 degrees Celsius. The sieves were then removed from the oven and weighed (W_c). The weight represented the weight of the sieve, aggregates, and sand particles between 1.0 and 0.5mm. The water chamber was then refilled with dispersing solution (37.5g sodium hexametaphosphate/1.0L water), and the agitation rack with sieves were lowered in the chamber. Sieves were agitated until soft before being removed. The aggregates were then rinsed with deionized water until only the sand particles (>0.5mm diameter) were left in the sieve. The sieves were placed back in the oven for 1 hour at 105 degrees Celsius.

Samples were removed from the oven and weighed (W_s). This is the weight of the sieve and sand with diameters between 0.5 and 1.0mm. The purpose of this step was to account for any sand grains between 0.5 and 1.0mm, which would otherwise be counted as aggregates. The soil was then discarded, and the empty sieves were weighed (W_e).

<u>Calculations</u>*:*

Eliminate weight of sieve: $W_c - W_e = W_{as}$ (weight of aggregates and sand)

Find weight of sand: $W_s - W_e = W_{sd}$ (weight of sand)

Fine stable aggregate %: $(W_{as} – W_{sd} / W_{or} – W_{sd})$ x 100%

Texture and Soil Classification

Soil particles, or soil separates were categorized as sand, silt, and clay. Sand particles are 2.0-0.05mm in diameter, silt particles are 0.05-0.002 mm in diameter, and clay particles are <0.002mm. The proportion of sand, silt, and clay determined soil texture. Soil texture was measured using the hydrometer method. In this method the soil particles are dispersed with sodium metaphosphate and then agitated. After dispersion, the amount of each particle size is determined by a hydrometer, by measuring the amount of particles in suspension. The principal of Stokes Law, which states that particles of different sizes will fall out of suspension at different rates over time, is used to determine the amount of each particle size present in a soil (AGVISE Laboratories).

The soils were classified by digging soil pits within each site at random locations. The soils characteristics were obtained by hand texturizing, the use of a pH kit, and color classification by Munsell color chart. The characteristics were observed and a soil taxonomy book was used to classify the soils from each soil pit.

*Statistical method*s

The ANOVA (SPSS 16.0) 1-way analysis program was used to interpret data. Levene's test was used to check for heterogeneity of variance between groups; if the assumption of equal variance across the groups was proved, then Levene's test was passed. Tukey post-hoc tests were used for multiple comparisons among data using significance level of 0.05.

Results

Total Carbon

Total soil carbon was measured using the Leco method, which showed differences in total carbon between treatments. The mean values in percent mass were found to be 7.09% in the control site, 2.66% in the low burn severity, and 2.42% in the high burn severity. Levene's test was used to confirm the heterogeneity of variance between groups. Because the assumption that the variance of the data was equal throughout was passed, the Tukey post hoc method was selected for analysis of the individual treatments. It was found that total carbon differed between treatments ($F_{2,42}$=4.41, P=0.02). Tukey post hoc test for multiple comparisons showed a relatively significant statistical difference between control and low (P=0.042), control and high (P=0.041), but no significant statistical difference between high and low severities (P=0.99), where α=0.05 (Figure 3). Through these results it was concluded that the effect of fire severity on total carbon was not determined, or considered statistically significant.

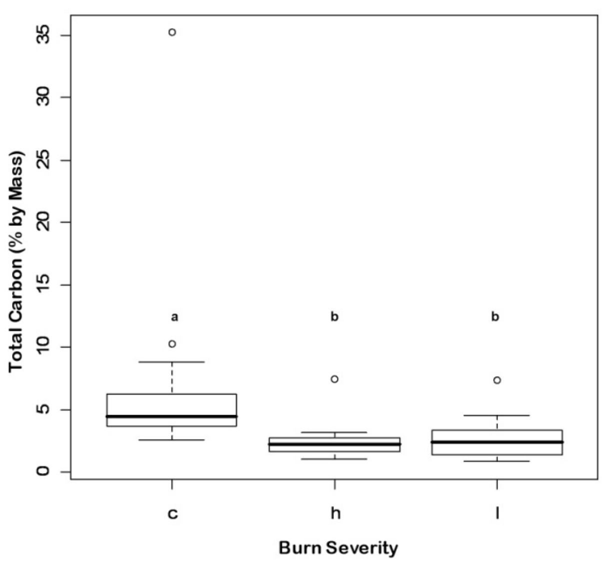


Figure 3. *Shows total carbon in percent by mass for the control, low burn severity and high burn severity sites for the Wicked Creek 2007 fire. Median values are the solid lines, 50% of the data are inside the box and 95% of the data lye within the bars and outliers are denoted by o. Letters denote significant differences based on Tukey post hoc comparisons, α=0.05.*

Total Nitrogen

Total soil nitrogen content was found to be significantly higher in the unburned control site when compared to the low and high burn severity plots (Figure 4). Mean values in percent mass were discovered to be 0.28% in the control site, 0.11% in the low burn severity site, and 0.10% in the high burn severity sites. Levene's test was used to confirm the heterogeneity of variance between treatments. Total nitrogen differed between treatments ($F_{2,42}$= 8.0, P=0.001). Variance was determined to be equal, therefore the Tukey post hoc test for multiple comparisons using α=0.05 was once again used to compare statistical differences. Significant differences were found between the control site and the low burn severity site (P=0.042), the control and high burn severity site (P=0.031), and no significant statistical difference was found between the low burn severity and the high burn severity treatments (P=0.098).

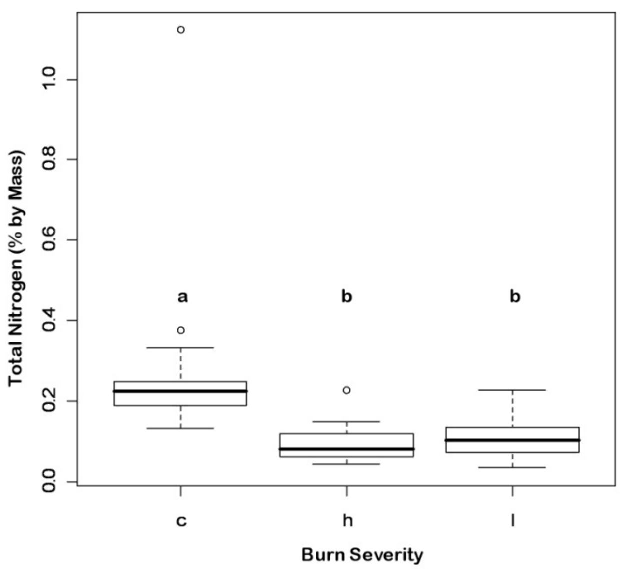


Figure 4. *Shows total nitrogen in percent mass for the control, low burn severity and high burn severity sites for the Wicked Creek 2007 fire. Median values are depicted by the solid lines, 50% of the data are inside the box and 95% of the data lye within the bars and outliers are denoted by o. Letters denote significant differences based on Tukey post hoc comparisons, α=0.05.*

Available Nitrogen

Available soil nitrogen (NO_3^-), assessed using the cadmium reduction method, varied from a mean of 0.5 parts per million (ppm) in the control site to 1.4 and 1 ppm in the low and high burn severities respectively (Figure 5). Analysis of the heterogeneity of variance (Levene's test) showed there was variance between data but was of low significance (P=0.033). Available N did not differ between treatments using α=0.05($F_{2,9}$=0.771, P=0.585). Tukey post hoc test for multiple comparisons was run but found no significant statistical differences between the control site and low and high burn severities (P=0.823), and no significant differences between the low and high burn severities (P=0.98). Results were determined to show that burn severity had no effect on available nitrogen levels.

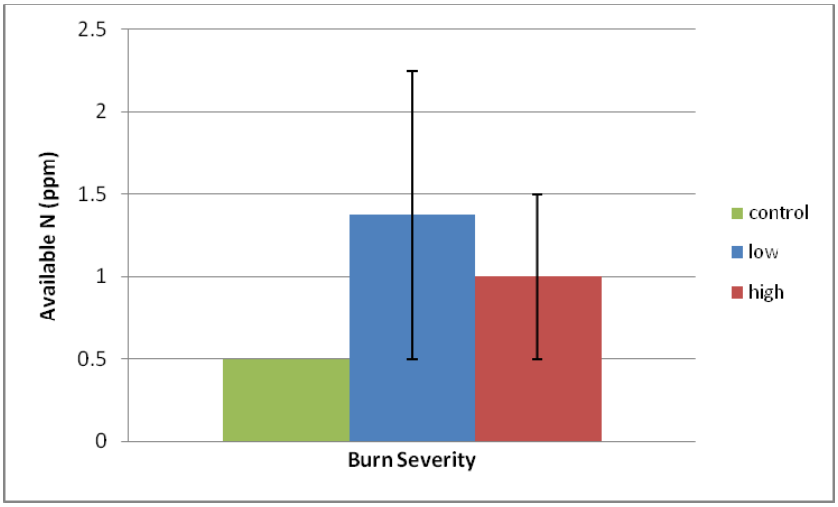


Figure 5. *Available nitrogen (NO_3^-) in parts per million and standard error bars in the control, low severity, and high severity sites from the Wicked Creek 2007 fire. The control site does not have an error bar because all of the samples from that treatment had the same value.*

Available Phosphorus

Available soil phosphorus (PO_4^{2-}) was determined to vary from a mean of 54 parts per million (ppm) in the control site, 62.5 ppm in the low burn severity site, and 54 ppm in the high burn severity site (Figure 6). Levene's test was used to confirm the heterogeneity of variance between sites. Available P did not differ significantly between treatments using $\alpha=0.05$ ($F_{2,9}=3.8$, $P=0.062$). Tukey post hoc test for multiple comparisons showed a relatively significant statistical difference between high and low severities ($P=0.065$). There was no significant difference between control and high and control and low burn severities. Results determined fire severity had an effect on available phosphorus levels, however there was no difference between the control site and either burn severity site.

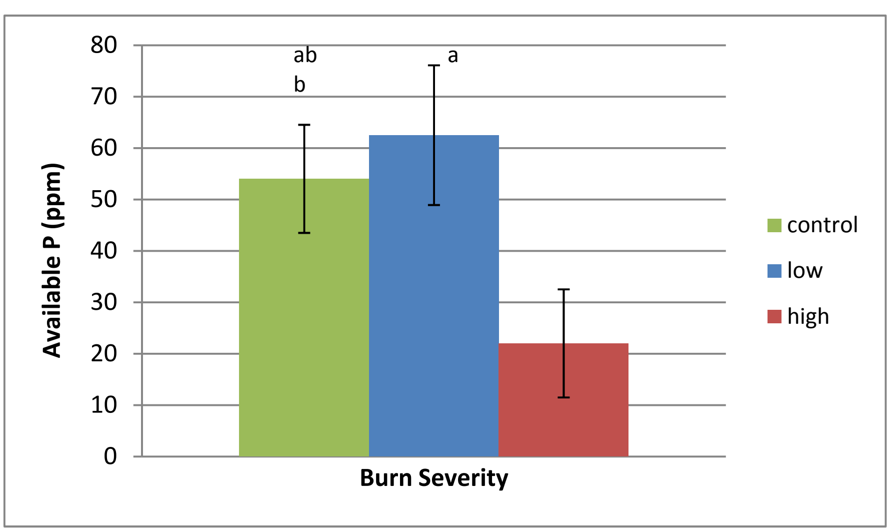


Figure 6. *Available phosphorus (PO_4^{2-}) in*

parts per million and standard error bars as determined by the Olsen Phosphorus Test for the control, low burn severity, and high burn severity sites from the Wicked Creek 2007 fire. Letters denote significant differences based on Tukey post hoc comparisons, α=0.05.

TDR Water Content

Volumetric soil water content, when measured with TDR in August of 2008, showed no differences in water content for the control, the low burn severity, and the high burn severity sites. Water content mean values varied from 0.046 in the control site to 0.067 and 0.085 in the low and high burn severiy sites respectively. Due to high variability and closeness of data values, no statistical analysis was performed (Figure 7).

Aggregate Stability

Aggregate stability refers to the ability of soil aggregates to resist disturbance, usually by water. Tests were conducted to determine the amount of stable aggregates in the soils for each treatment. The control site soil was found to have an aggregate stability of 83.5%, the low burn severity 85.3%, and the high burn severity 83.2%. Because of the similarity in aggregate stability values, statistical analysis were not performed. It was concluded that burn severity had no effect on soil aggregate stability.

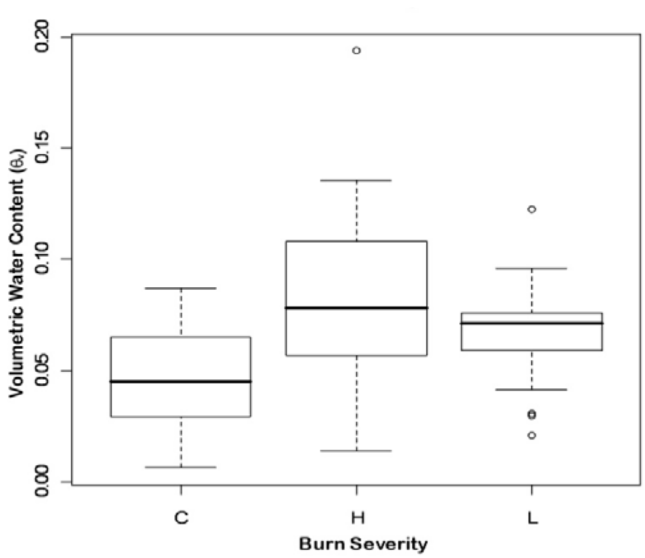


Figure 7. *Soil water content in volumetric water content by time domain reflectometry in the control, low burn severity, and high burn severity sites from the Wicked Creek 2007 fire. Median values are depicted by the solid lines, 50% of the data are inside the box and 95% of*

the data lye within the bars and outliers are denoted by o.

Soil Texture

Soil texture analysis by a hydrometer showed similar size class distribution for soils in the control, the low burn severity, and the high burn severity treatments. The control site soil was found to be a loam with 49% sand, 34% silt, and 17% clay; the low burn severity site soil was determined to be a sandy loam with 59% sand, 36% silt, and 5 % clay; and the high burn severity site soil was also found to be a loam with 43% sand, 36% silt, and 21% clay (Figure 8). Because this data was used only as a stratification method, no statistical analysis was performed.

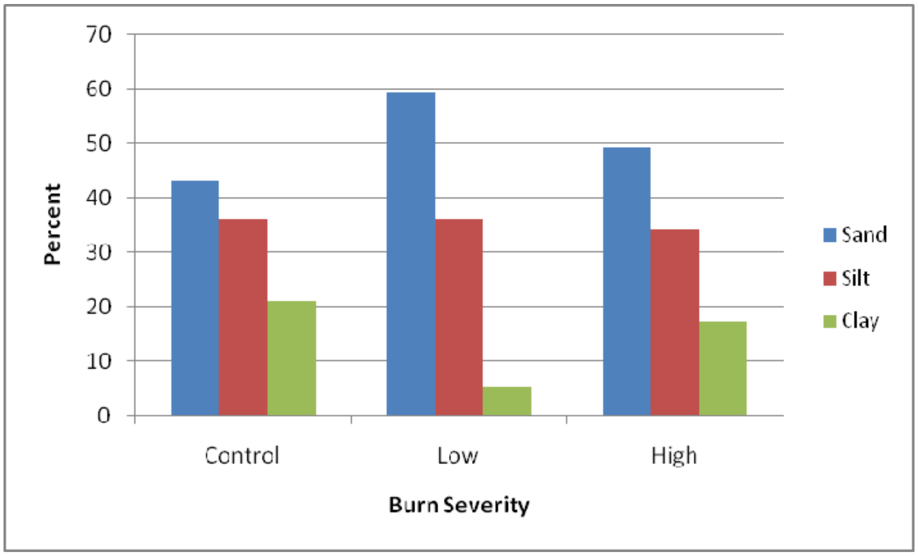


Figure 8. *Soil texture by size class distribution in percent sand, silt and clay for the control, low burn severity, and high burn severity sites from the Wicked Creek 2007 fire.*

Discussion

Carbon Content

Prior to data analysis, it was hypothesized that with an increase in burn severity, the amount of soil total carbon will decrease. As soil temperature increases, so does organic matter combustion, resulting in a reduction or removal of organic carbon in the soil (Certini, 2005). A study by Gonzalez-Perez (2004), found carbon losses higher than 50% in the top 10cm of soil under pine forests.

It is concluded that there is a decrease in total soil carbon following a forest fire when comparing burned to unburned sites, but severity has little effect on total carbon content. It was found that one year after the Wicked Creek fire, there was a 68.8% decrease in average carbon content (18 cm depth) from the high burn to control site. There was a 62.5% decrease in total carbon from the low burn to control site. It was expected that there would be small reduction in C and N capitals following less severe wildfire, where ignition temperature of soil organic matter may not be reached (Barid*, 19*99). There was little difference found between the total amount of carbon between the low and high severity burns. Data was concluded to be insignificant (P=0.99)

The similarity in carbon content from the low burn to high burn site could be due to differences in fire intensity (temperature and duration), slope, and fire type (what was dominantly burned). Increased fire intensity would likely result in increased organic matter combustion. Slope would have an effect on post burn erosion patterns, resulting in possible carbon losses. These variables should be quantified in order to better explain what factors, other than fire severity, affect soil carbon content.

Total Nitrogen

Prior to evaluating total soil nitrogen content, it was hypothesized that total nitrogen would decrease with an increase in fire severity. In a study conducted by Murphy *et al.* (2006), significant decreases in total soil nitrogen were observed in the post burn site compared to the pre burn site, in the Lake Tahoe basin in Nevada. Soil total nitrogen (organic-N) and total carbon are expected to behave similarly after a wildfire because of the effects of wildfire on organic matter, and plant biomass.

After analyzing our data, a significant difference in nitrogen was observed when comparing the control site to low and high burn sites. There was no notable difference in total nitrogen between the high and low burn severity. A decrease in total nitrogen from the control site to the high burn was found to be 79.8%. There was a 78.6% decrease in total nitrogen from the control site to the low burn.

An explanation as to why there was no significant difference in total nitrogen for burn severity can be explained the same way that carbon variance was described, where ignition temperature of the soil organic matter may not be reached (Baird, 1999). If the high severity burn did not have high ignition temperatures, total nitrogen content would not have been drastically affected by severity, yielding a similar loss to the low severity treatment. Factors such as fire intensity, slope, and fire type could also explain any differences seen in total nitrogen concentrations.

Available Nitrogen

Prior to evaluating the soil available nitrogen (NO_3^-), it was hypothesized that available nitrogen would increase with an increase in fire severity. A study conducted by Duran et.al, stated that available nitrogen levels increase immensely one year after fire in burned treatment sites compared to unburned control sites in a *Pinus canariensis* ecosystem. This increase can be attributed to the decrease in plant biomass, or organic matter resulting in a decrease in NO_3^- uptake. Fire causes losses of total nitrogen from the forest floor due to heat-induced degeneration of soil organic nitrogen (Murphy, 2006).

Available nitrogen was found to have no significant difference between high burn, low burn, and control sites. The variance between sites could be due to the low number of soil samples analyzed for available nitrogen. We only used 3 of the 15 samples from each site due to inadequate soil quantities. "NO_3^- released during combustion or post-fire microbial decomposition is highly vulnerable to leaching and erosion loss in a coniferous forest" (Baird, 1999). This could have led to a decrease in NO_3^- in the low and high burn sites post fire.

Available Phosphorus

Prior to data analysis, it was hypothesized that available phosphorus would decrease with an increase in fire severity. The absence of organic phosphorus in the soil after a fire, coupled with a lack of new organic matter causes a decrease in available phosphorus due to leaching (Duran, 2008).

Available phosphorus was found to have no significant difference when comparing the control site to the high and low burn severity. However, there was a significant difference in available P between the high and low burn site. The high burn, low burn, and control site had average available phosphorus values of 22ppm, 62.5ppm, and 54ppm respectively. The low available phosphorus in the high burn was expected, however, the spike in available phosphorus in the low burn site was not. One reason for these observed results could be due to the inadequate number of samples tested for available phosphorus (3 samples from each site). In addition, soil pH was not tested, and could have provided insight into our observed results. Phosphorus binds with iron and aluminum at low pHs and binds with calcium at high pHs. Phosphorus availability is highest at a pH of 6.5. Available phosphorus in the low severity site could also be explained by a possible initial increase in phosphorus immediately after fire, or by the presence of organic matter. Phosphorus in the form of organic matter would result in less leaching of available phosphorus through the profile, increasing the P found in the low burn soil. After a fire, the available phosphorus would slowly increase as organic matter is reintroduced into the soil bringing in external sources of nutrients.

Water Content

Prior to evaluating the volumetric soil water content data, it was hypothesized that soil water content would decrease with an increase in fire burn severity. A study by Arnone *et al.* (2004) showed that soil water content was substantially lower in a post fire ecosystem after snowfall (soil water recharge), compared to an adjacent ecosystem unaffected by fire. As a result of this decrease in soil water recharge in the post fire ecosystem, soil water content throughout the entire winter was lower in the post fire site than that of the adjacent control site.

The frequency distribution of soil water content shows that water content increases with an increase in burn severity. However, due to the high level of variability between sites, no significance tests were run, providing inconclusive results for the relationship between water content and fire severity. There are a couple reasons that could have attributed to our observed results. The control site had the highest density of vegetation and cover, and presumably had not been burned for 50 to 75 years, resulting in more plant uptake. Also, when using the TDR in the field, our methods did not include removing the duff layer on the control site floor. This resulted in water content readings in duff material, with only some soil material, likely resulting in lower water content values than readings in soil material, exclusively. Evapotranspiration rates should also be taken into consideration when looking at soil water content data. It is assumed that with a higher plant density, transpiration would increase, thus reducing the soil water content. This could explain why the control site, with a higher vegetation density, would have lower soil water content.

We only did a onetime sampling at the end of August, which could also explain why we see these trends. If we had sampled during a wetter season, the trends may have followed our expected results. Plant density, canopy cover, and other soil physical characteristics may have played a role in our observed results.

Aggregate Stability

Prior to evaluating aggregate stability data, it was hypothesized that percent stable aggregates would decrease with an increase in fire severity. Organic matter is one of the most important and well known aggregate stabilizing agents in soils (Garcia-Orenes *et al.,* 2001). Many studies have found that organic matter decreases with an increase in burn severity, resulting in decreased aggregate stability. Generally, after a severe forest fire, the oxidizable organic content, and the percent stable aggregates decrease due to combustion of cementing organic substances (Garcia-Orenes *et al.,* 2001).

After data analysis, no notable difference in percent aggregate stability was found between the high burn, low burn, and control site. The effects of wildfire on soil aggregate stability have not been studied extensively, making relationships between wildfire and aggregate stability difficult to draw.

When evaluating the degradation level in a soil following wildfire, these soil physical and chemical characteristics would provide more meaning if more samples were taken, and in different seasons. All of these soil characteristics are related, and their relationships are important to understand when trying to characterize their relationship to changes in wildfire severity.

Microbiology Group

Wildfire Impact on the Diversity of Bacterial Ammonia Oxidizing Communities

Introduction

Wildfires are a natural phenomenon and act as essential regulators of natural ecosystems. Wildfires that occur sporadically reduce heavy fuel accumulations (which can ultimately lead to disastrous wildfires), remove dead and built-up vegetation that hinders new growth, and aid in the release of nutrients bound in litter, therefore enriching the soil. However, wildfires can be disastrous and negatively affect plants and microorganisms alike depending on the severity of the fire. Severity is a qualitative measure that refers to the overall effect of fire on an ecosystem. The spectrum of severity that fire produces depends on many interactions, such as burn intensity, fire duration, fuel loading, combustion type, degree of oxidation, vegetation type, slope, topography, soil texture, moisture, organic matter content, and time since last fire occurred (Neary *et al.,* 1999).

Wildfires affect the physical, chemical, and biological properties of the soil due to transfer of heat into the soil (Neary *et al.,* 1999). As a result, the heat transferred into the soil not only kills the microorganisms but also kills or damages plant roots and seeds, destroys soil organic matter, changes soil pH, reduces soil nutrients, and alters soil water holding capacity (Neary *et al.,* 1999). Ahlgren (1974) found that fire has negative impacts on soil organisms and hypothesized that the immediate effect of fire on the soil microbial community depends on the intensity and duration of the fire and can range from complete sterilization to little or no effect. Since then, numerous studies showing evidence of reductions in microbial populations have been conducted that confirms this hypothesis. For example, Pattinson *et al.,* (1999) found that fire significantly reduced the levels of bacteria, actinomycetes, and mycorrhizal fungi in the top few centimeters of the soil. Smith *et al.,* (2004) found that overly severe prescribed fires significantly reduced mycorrhizaes compared to less severe fire or non-burned treatments.

Soil microorganisms represent a significant fraction of living matter in the soil and play an important role in establishing healthy and fertile soils. There are countless microorganisms in the soil and according to Pace (1997) a handful of soil contains billions of microorganisms, so many different species that accurate numbers remain unknown. Soil microorganisms, especially bacteria and fungi, play a major role in breaking down organic material in soil and in the production of humus (Madigan and Martinko, 2006). Many soil bacteria are also involved in nitrogen fixation; a process that benefits plant growth (Madigan and Martinko, 2006). The abundance and diversity of microorganisms is highest in the top 0-10 cm , which receive the largest amounts of potential food sources from plants and animals (Paul, 2007). Even the prevalence of plant roots can have tremendous effects on the abundance of microorganisms; the roots release organic carbon that can be exploited by microorganisms (Paul, 2007).

Soil microorganisms, just like any other organisms, require certain range of temperature, pH, water availability, nutrient availability, and energy sources to survive. With the altered soil conditions due to fire, reductions in the total soil microbial biomass can persist for decades (Prieto-Fernández *et al.,* 1998). However, fast recovery of specific microbial groups has also been observed, and fire has been reported to stimulate microbial numbers and activity shortly after the burn, potentially through the release of readily utilizable carbon and nitrogen substrates (Vazquez *et al.,* 1993, Prieto-Fernández *et al.,* 1998).

There are numerous methods to evaluate the effects of wildfire on soil microbes. In the past the two main options for examining this relationship were the culture- and activity-based methods (Dion *et al.*, 2008). These methods yielded quality data concerning both the microbial activity and population sizes within fire-impacted soils. However, these two methods provided limited data on fire effects in regard to soil microbial community composition and specific functional groups. Recently, more advanced methods using molecular techniques have provided a more thorough evaluation of the effects of wildfire on the composition of microbial communities (Staddon *et al.,* 1996). However, due to the recent development of these DNA- and/or RNA-based techniques there are limited published studies that focus on particular functional groups of soil microbes (Yeager *et al.,* 2005). The sequence of the 16S rRNA gene has been widely used as a tool to estimate relationships among bacteria, but more recently it has also become important as a means to identify an unknown bacterium to the genus or species level.

The aim of this study was to evaluate the effects of wildfires on ammonia oxidizing bacteria (AOB) in forest soils for the Wicked Fire Research Area. The primary objective for this project was to develop a genetic approach to compare the dominance of microbial species between forest soils that have been burned by wildfire once, twice, and unburned (control) using the *amoA* gene (which encodes the active site of ammonia monooxygenase, an enzyme unique to this group of AOB).

H_0: No difference in bacterial communities in burned (high severity), twice burned, and unburned areas will be detected using molecular analysis of the *amoA* gene.

H_1: A change in diversity in the ammonia oxidizing bacterial communities among the burned (high severity), twice burned, and unburned areas will be detected.

Materials and Methods

Environmental sampling

Soil samples were collected from the Mill Creek wildfire area on 27 and 28 August, 2008 within three different sites in the study area corresponding to three different burn severities: high severity (HS), double burned (DB), and a control site that was not burned (C). The global positioning system GPS coordinates for these locations were selected by randomized design and the soil core sites were selected based on the presence of forbs, grasses, and stumps on the soil surface. The samples were collected using a hand-operated, 1-inch diameter soil core sampler. Ash and/or organic matter layers were removed from the top of the soil profile and coring began at the mineral soil surface.

Before each soil sample was collected, the soil core sampler and the scoop used to transfer soil were cleaned and sterilized. Approximately 50mL of soil were collected and stored in sterile, 50mL vials. The soil was not compacted inside of the collection vials. Samples were immediately frozen on dry ice to preserve microbial genetic material. The soil samples were transferred to an -80°C freezer upon returning from the field.

DNA extraction

Due to time constraints, not all of the samples could be used for analysis; instead soil samples from a site with forb cover, control site and double burned site as well as samples from the base of a stump at the double burn site and high severity site were used. Each falcon tube of soil was thawed at room temperature then shaken by hand to homogenize the soil. Based on failed PCR results using DNA extracted using phenol and bead beating, it was determined that the humic acids were not completely removed from the extraction and were inhibiting successful PCR reactions. In order to overcome this inhibition, DNA was extracted from 0.5g of the homogenized soil for each of the samples using MP Bio FastDNA Spin Kit for Soil. The DNA was quantified on 1% agarose gel run at 100V with a high mass ladder.

Polymerase Chain Reaction (PCR)

Amplification of the *amoA* gene was performed in a total reaction volume of 50ul. The PCR reaction mix contained either a pure DNA template from soil extractions or 10-fold dilution of the DNA extract, 2 mM of $MgCl_2$, 200uM of each dNTP, 0.5 uM of each primer amoA-1F-GC and amoA-2R (refer to *Horneck, et al* for primer sequences), 0.4 mg/ml of Bovine serum albumin (Roche Applied Sciences, Indianapolis, Indiana), and 0.5 U of Taq gold polymerase (Promega Corp., Madison, Wisconsin). The thermal profile for amplification of the *amoA* sequence was 5 min at 95 °C; then 40 cycles of 30 s at 94 °C (denaturation), 40 s at 47 °C (annealing), and 40 s at 72 °C (elongation); with a final cycle of 2 min at 72 °C followed by a storage temperature at 4 °C. The PCR products were analyzed on 1% agarose gel electrophoresis run at 100V and stained in ethidium bromide.

Denaturing gradient gel electrophoresis (DGGE)

Approximately 45 µL of each successful PCR product generated from *amoA* primers was mixed with 10 µL of 1% sucrose loading dye and were analyzed on DGGE using the D-gene system (BioRad, Hercules, California). Polyacrylamide gel containing 8% of 37:1 acrylamide-bisacrylamide mixture in 0.5X TAE buffer was made with a 20-80% denaturant gradient (100% denaturant defined as 7 M urea and 40% formamide). The gel was run for 16 hours at 65V in 1X TAE at a constant temperature of 60 °C. The gel was stained for 35 minutes in 1 X TAE containing 1:10,000 dilution of SYBR green before imaging.

Results

Inhibitory effects of humic acids on PCR

The first DNA extraction method did not purify the DNA well enough and humic acids were still present in the samples. The humic acids contain proteins that can interact with the *Taq* polymerase, which then inhibits the enzyme from binding to the DNA strand causing the PCR reaction to fail. To stabilize the *Taq* polymerase, bovine serum albumin was added to the PCR mix, but even this could not overcome the extensive inhibitory effects of humic acids (Tebbe and Vahjen, 1993). To test whether the humic acids in the PCR mix were inhibiting the reaction, a PCR was run with universal 16S bacterial primers (1070F-1392R) with *E. coli* as the positive control. As soon as the *E. coli* was diluted 100:1 with the DNA extraction, the PCR reaction failed indicating the humic acids present in the DNA extraction inhibit the PCR that would otherwise be successful.

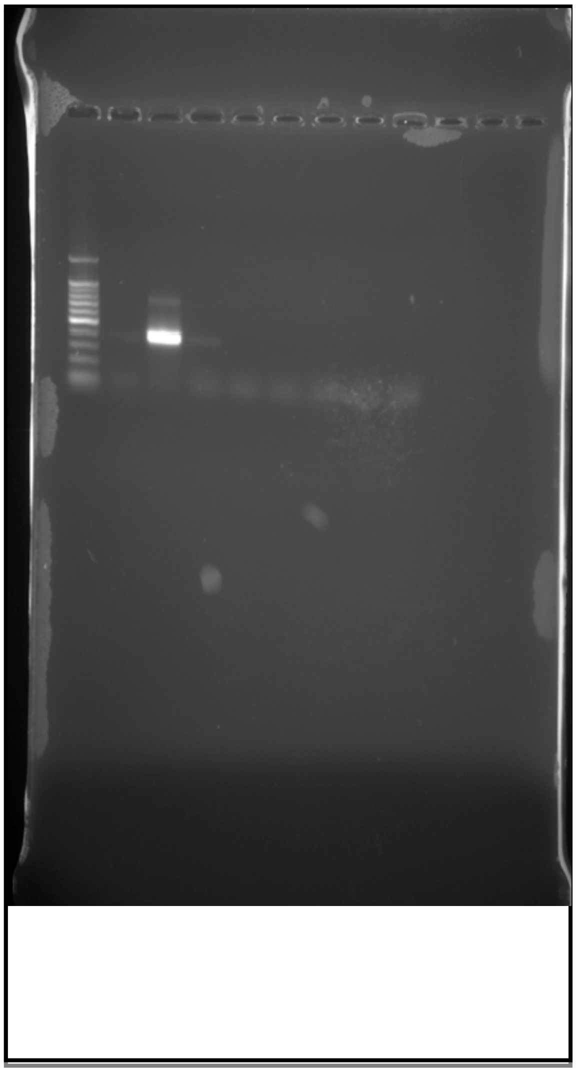


***Figure 9**. Agarose gel with samples from PCR ran using universal bacterial 16S primers. Lane 1: 100bp ladder; lane 2: blank control; lane 3: E. coli only; lanes 4-8: E. coli diluted with DNA extraction.*

DGGE

Environmental soils samples were analyzed using DGGE in order to determine differences among the diversity of ammonia oxidizing bacteria based on genotypic variation. The banding patterns on the DGGE shown in Figure 10 demonstrate that the genotypic diversity differs in each soil site. The banding patterns from the two forb sites (lanes 3-6) do not correlate with one another, therefore it is not possible to determine if the change in AOB diversity is a result of differing burn severity. Each band seen in the DGGE represents a single genotype in the AOB population. By counting the approximate number of bands for each sample, it is evident that the relative species richness is higher in the control (unburned) site than in the other sites. By comparing lanes 5 and 6, it becomes apparent reproducibility of the experiment with replicate, identical samples is low.

According to Dave Ward, the intensity of the DGGE band is associated with the relative abundance of that genotype in the AOB population. By comparing the dominant bands on the DGGE, reproducibility of identical samples is no longer hindering interpretation of the results (Figure 11). Between samples, none of the dominant bands are at the same location meaning that the dominant genotypes in each sample differ based on sequence of the amoA gene.

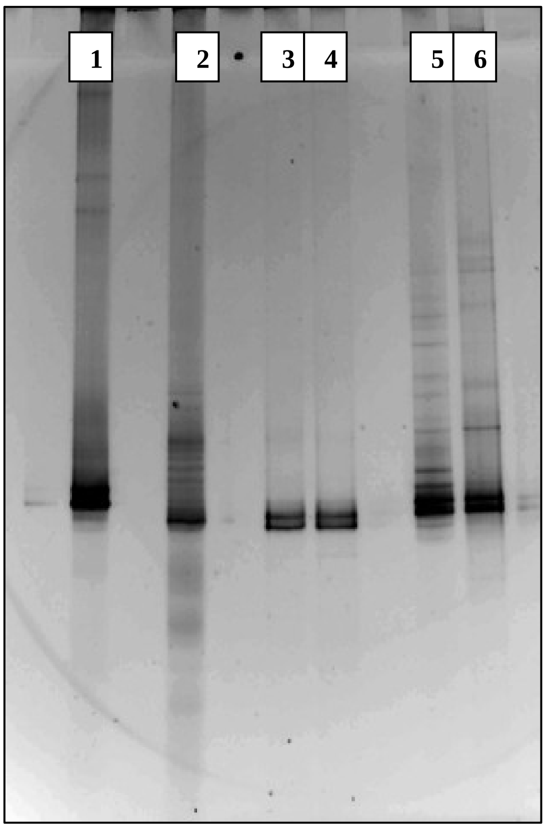


Figure 10. *DGGE with PCR products generated from amoA primers. Lanes: 1. HS_Stump; 2. DB_Stump; 3,4. DB_Forb; 5,6. C_Forb.*

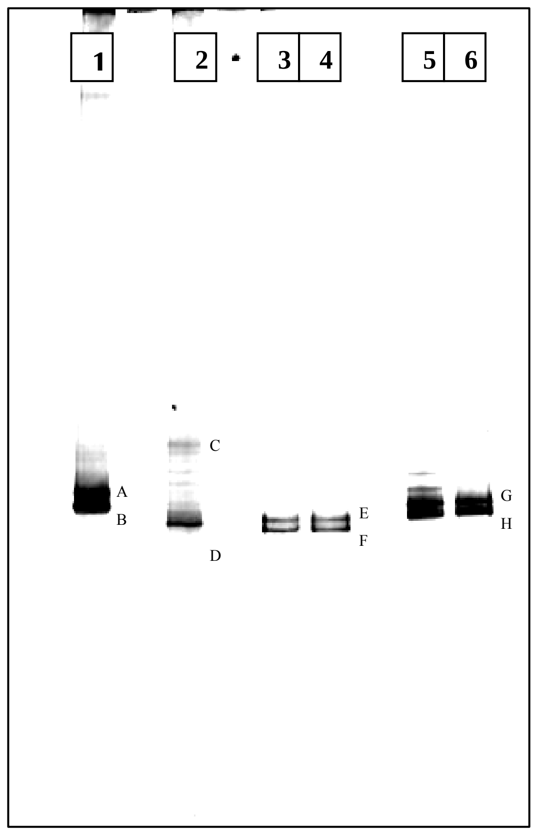


Figure 11. *DGGE with PCR products generated from amoA primers. Contrast has been adjusted to show dominant banding patterns. Lanes: 1.* HS_Stump; 2. DB_Stump; 3,4. DB_Forb; 5,6.

Discussion

The DGGE results provide evidence of the presence of different AOB genotypes among the various sites, in relation to burn severity and vegetation type. Though the reproducibility of these results is somewhat of a concern, the results did reject the null hypothesis stating that the AOB community composition would not shift following fire disturbance. Even with the reproducibility concerns, the DGGE results for this very limited sampling matrix do show a number of interesting trends in species richness and diversity for varying burn treatments of forest soils.

Due to rapid adaptability of bacterial communities, when a physical or chemical change occurs in an environment, the relative abundances of various populations within the community also change (Baath, *et al.,* 1998). Because of the small micro-niches bacteria inhabit, a disturbance as small as a footstep or the growth of one small plant may be enough to shift the diversity of a community. The shifts in diversity seen in the DGGE exhibit the changes that can easily occur in a particular bacterial population, in this case the AOB. Unfortunately, the sampling matrix tested in this experiment was not broad enough to determine if the shift in diversity among the AOB population was due to burn severity, vegetation type, microclimate, or a myriad of other soil properties. Any of these properties and more would create an environment suitable for different genotypes or species of AOB.

It was thought that many ammonia oxidizing bacterial species would flourish in post-burn soils due to a reduction in soil microbial biomass and a spike in soil ammonia concentration as reported by Smith *et al.,* (2008). However, based on the DGGE results of this study, the control samples showed the greatest species richness, followed by the twice-burned stump sample, then the high severity stump and lastly the twice burned forb samples. The high species richness found in the control site could be due to the fact that there was no major reduction in biomass as there seems to have been in the burned sites. However, the burned sites appear to contain fewer, yet possibly more dominant species of AOB that successfully filled unoccupied microbial niches due to the fire induced sterilization of the soil. Uncertainty arises as to whether the faint banding patterns are accurate indicators of species richness due to the poor reproducibility between replicate samples.

Although PCR reactions typically show some bias towards particular genotypes in environmental samples, past studies suggest that the darkest bands seen in DGGE are the dominant species in the environmental sample (Ward, personal com.). The dominant species in a population are the most abundant, thereby contributing more identical templates for PCR to exponentially amplify, which results in darker bands on DGGE. The dominant genotypes are often the founders of a distinct population, for example the AOB. Since each of the environmental sites had different band positions of the dominant genotype, it appears that the founders for the AOB population in each environment are different from one another at the species level. In order to confirm this theory, sequencing of the dominant bands would need to be performed to compare the genotypes phylogenetically and determine how evolutionarily different they are. The distance of separation between two bands on DGGE is not related to how genetically different the sequences of the *amoA* gene may be, so in order to decipher if two bands (genotypes) are closely related or very distantly related, phylogenetic trees would need to be constructed and analyzed.

The study of soil microbiology, specifically ammonia oxidizing bacteria, is a relatively new area of study with a limited amount of published research. Soil microbes are an integral part of environmental science and there is much yet to be discovered regarding the response of microbial communities to both natural and human disturbances. With further research, the relationships between specific soil disturbances and microbial response can be used for practical applications including post-disturbance management or site remediation. The results from this study, though preliminary, represent positive progress in understanding the response of ammonia oxidizing bacteria in post-wildfire forest soils.

Vegetation Group

Evaluation of Understory Vegetation Effects of the Wicked Hicks Fire

Fire influences on the reestablishment of vegetation

Fire tends to decrease total plant cover and species richness by affecting seedbank viability and underground structures (Wang and Kembell, 2005). Vegetative cover is one of the key factors protecting forest ecosystems from soil erosion and land degradation following a fire (Wittenberg *et al.,* 2007). Preliminary regeneration of forest vegetation after a fire depends on initial vegetation and environmental factors such as climate and terrain. For instance, rapid regeneration generally occurs within the first two years after a fire with slower growth on south-facing slopes (Wittenberg *et al.,* 2007).

The climax successional stage is usually prevented by fire in western forests, and is purely theoretical, but still useful for conceptualizing forest regrowth (Smith and Arno, 1999). A sequence of community compositions typical of a stand replacing fire may be: grasses and forbs; shrubs; saplings and shrubs; pole-size trees; mature forest; and a 'climax' old-growth forest. This is obviously complicated by the pattern of fire severity and the different physiological responses of species to fire. Ecological succession of severely burned areas is generally dominated by early seral species. A study conducted in Yellowstone National Park suggests that areas with severe surface burns had a greater abundance of pioneer plant species, higher cover, and higher density when compared to areas with less severe burns (Turner *et al.,* 1997).

Survival of vegetative generations can influence slope stability, water quality, and therefore repopulation by other plants and wildlife. Following high severity fires, seed banks and propagules are keys to the successful succession of the site. Studies have shown that a majority of plant repopulation following fire is dependent on re-sprouting by survivors within the first three years following fire, not on recruitment from surrounding unburned areas (Stickney, 1990; Turner *et al.,* 1997). Perez-Fernandez *et al.* (2006) suggest seed germination peaks during the growing season following a fire. Fire can stimulate some forbs and woody shrubs to germinate through the cracking of the seedcoat or exposure to chemicals leached from ash (Brown and Smith, 2000). A Northern Rockies study also found areas of more severe fires result in less on-site regeneration from asexual or sexual reproduction because of damage to their sources from the fire (Stickney, 1990). This coincides with the findings from Turner *et al.,* (1997) that suggests that areas of high burn severity will have diminished plant richness when compared to less severely burned areas.

Depending on the depth and intensity to which soil is burned, seeds in the soil seed bank may be destroyed. Typically, lethal temperatures do not penetrate below three cm from the burn boundary, which selects for species deeper than this level in the seed bank (Wang and Kembell, 2005). A study found that in northern conifer communities, such as the area we studied, many grass and annual forb seeds were transient in the litter layer and did not survive burning (Brown and Smith, 2000). Perennial forb species may persist in mineral soils for many years and the most persistent were often shade intolerant, early successional species.

Many metrics have been developed to elucidate differences in plant community composition. Three metrics that were used in this study to look at the response to fire were: species richness, Simpson's diversity index, and the Shannon-Wiener diversity index. Species richness is simply the number of species in a defined area. The two diversity indices take both richness and evenness of the plant community into account. Simpson's index is better suited for small sample sizes, whereas the Shannon-Wiener diversity index takes rare species into account. Both indices were used to see if either one showed significant differences between treatments (Krebs, 1989). This study's objectives were to: (1) determine the differences in plant species composition above and below ground; and (2) determine the effects of fire severity on both above and below ground species richness and diversity.

Methods

Site description

The Wicked/Hicks Fire Complex is located in the Mill Creek and Main Boulder drainages near the Absaroka-Beartooth Wilderness boundary of the Gallatin National Forest. Recent fires burned an extensive area in 2006 and 2007, but were not spatially continuous in regards to slope and aspect. Dominant vegetation types affected by the 2007 fire included: Engelmann spruce/subalpine fir (35%), lodgepole pine and Douglas fir (50%), whitebark pine (10%), and grassland (5%) (Gallatin National Forest, 2007). Soils at the site were medium textured with many rock fragments. Dominant parent materials were alluvium and colluvium over residuum derived from consolidated Tertiary volcanics. Soils were moderately productive and had low to moderate erosivity (Gallatin National Forest, 2007).

Stratification

Random points were generated in a Geographic Information System using burned area polygons stratified by fire severity and chronosequence. These data points were re-checked in the field to ensure desired sampling stratification and habitat consistency. Sampling was limited to one range of aspects 315° – 45° to eliminate confounding effects of aspect on vegetation responses. There were four total stratifications: a control not recently burned; low and high severity areas that burned in 2007; and high severity areas that burned in 2006, no low severity exists for 2006 (Figure 12). Six points were sampled within each stratification.

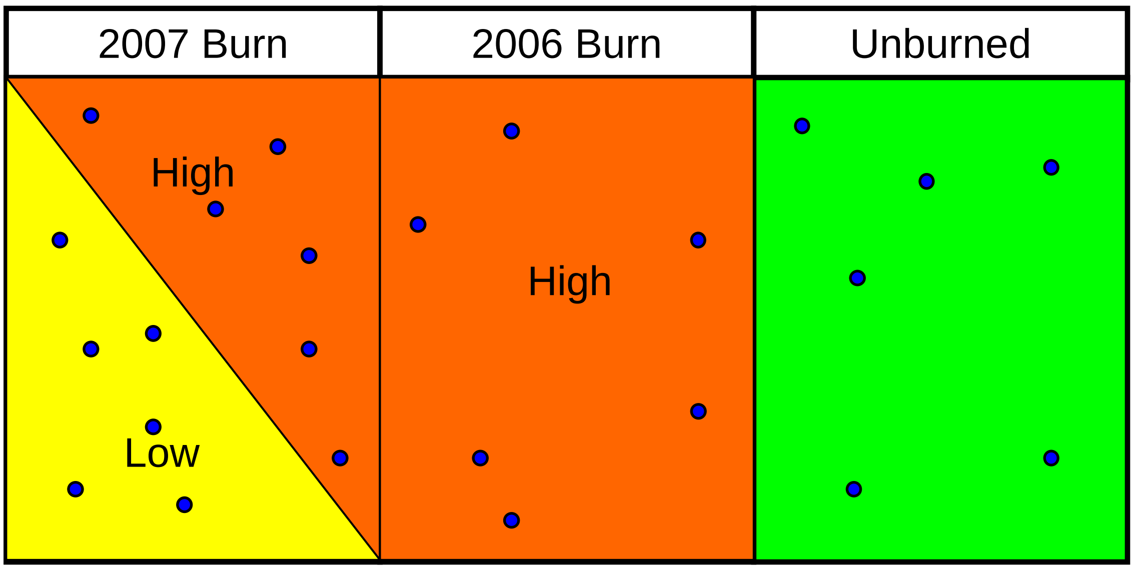


Figure 12: *Sampling stratification design for the Mill Creek Wicked Hicks Fire. Circles represent randomly placed sampling points.*

Determining above ground species composition

At each sampling point a one-square meter frame was used to count understory vascular plant species less than 2 meters in height. The percent cover and number of individuals of each species were counted in each quadrat. Individuals exhibiting a bunch growth habit were counted as bunches, not individual stems.

Determining below ground species composition

At each sampling point soil cores were taken within the quadrat, after above ground sampling, for analysis of seed-bank composition. Four soil cores (about 8 cm in diameter and 8 cm deep) were taken using a tulip bulb corer. The duff layer was sampled separately in the control, and not collected within the other stratifications because it was not present. The soil samples were taken to a greenhouse and mixed with the Montana State University loam/Sunshine peat moss soil mixture to aide with moisture retention. The soil was then placed in trays, positioned under an overhead irrigation system, and monitored daily. After seven weeks, seedlings were removed and recorded. Examples of each species were potted for the purpose of identification once they matured.

Analysis of results

Field data were collated and entered into Excel 2002 (Microsoft®) for analysis. Richness, evenness, diversity, seedling density, and similarity indices of above and below ground composition were calculated and compared between stratifications. Diversity was calculated using Simpson's diversity index ($D = \sum_{i=1}^{s} p_i^2$) and the Shannon-Weiner diversity index ($D = -\sum_{i=1}^{s} p_i \ln p_i$). Normality tests, analysis of variance, non-parametric tests, and regression techniques using Minitab 15 (© 2007 Minitab Inc.) were applied as appropriate to examine the response variables listed above with relation to fire severity and chronosequence. Comparisons were made between the above and below ground samples based on species richness and evenness. Seed-bank density, below ground richness, and below ground evenness were compared between the stratifications.

Analysis was performed using two-sample t-tests and ANOVA with Tukey's comparison test when assumptions of normality and equal variance were met. Normal-probability plots and Levene's equal variance test acted as decision making tools in rejecting ANOVA assumptions of normality. The Mann-Whitney U-test was used for non-parametric two-sample tests, and the Kruskal-Wallis test was used as a non-parametric substitute for ANOVA. The Kruskal-Wallis test is powerful with data from many distributions, but is not robust against outliers. Leverage values greater than 3p/n (where n is observations and p is predictors) identified outliers.

Statistical significance was determined using $\alpha \leq 0.05$ (95% confidence level) for all results. Reported p-values are from two-sample t-test or ANOVA unless identified as non-parametric. Variance p-values are from Levene's test.

Results

Over 2900 individual plants of more than 50 species were counted over two days in late August, 2008.

Above Ground Richness

Species richness in the 2007 low fire stratification was significantly higher (R_j= 14, $p \leq 0.001$) than other areas. All other stratifications were not significantly different (Figure 13).

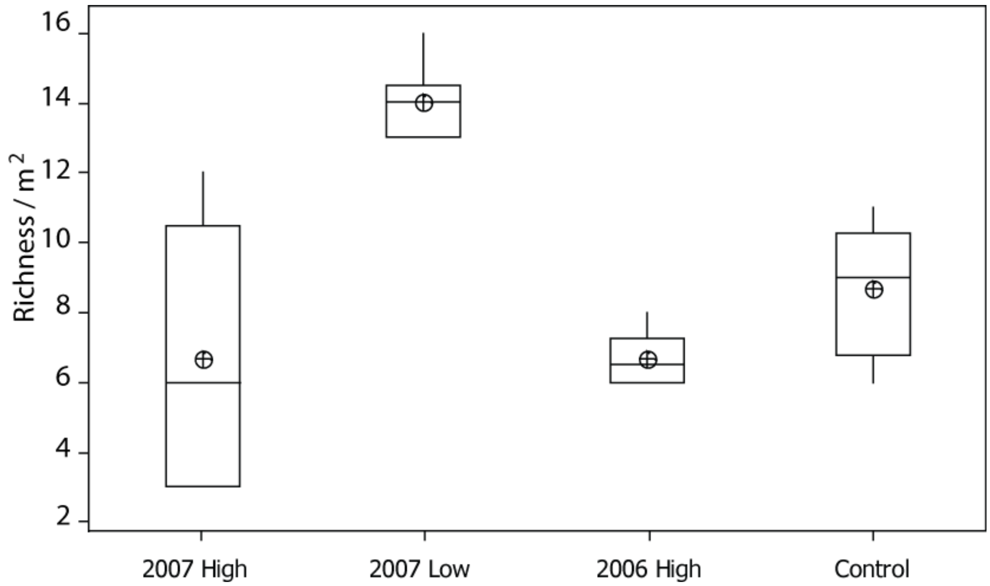


Figure 13: *Above ground species richness in each sampling stratification. Box represents interquartile range, horizontal line is the median, target is mean, and whiskers are upper and lower distribution.*

The high severity burn area of the 2007 fire had significantly higher variance in richness than other stratifications (p=0.005). Slopes greater than or equal to 18 degrees were classified as steep, and a 2-sample t-test was performed for explanation of the high variance. The difference was not significant (p = 0.064), though a trend of increased richness on gentle slopes was evident (Figure 14).

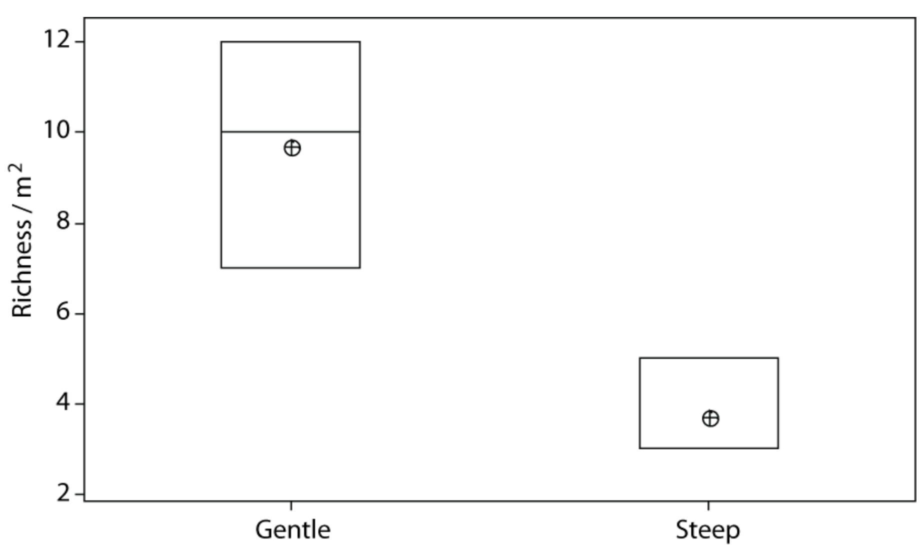


Figure 14: *Above ground species richness of 2007 high severity burn area in regards to slope; steep slopes were ≥ 18°. Box represents interquartile range, horizontal line is the median, target is mean, and whiskers are upper and lower distribution.*

Above Ground Diversity

Simpson's diversity index was significantly ($p = 0.030$) higher in the 2007 low severity area (D_j=0.830) compared to the high severity 2007 area (D_j=0.648). All other stratifications were not significantly different, and were not significantly different than 2007 low severity (Figure 15). Simpson's diversity did not appear to be normally distributed, yet the same significant results were obtained using non-parametric methods.

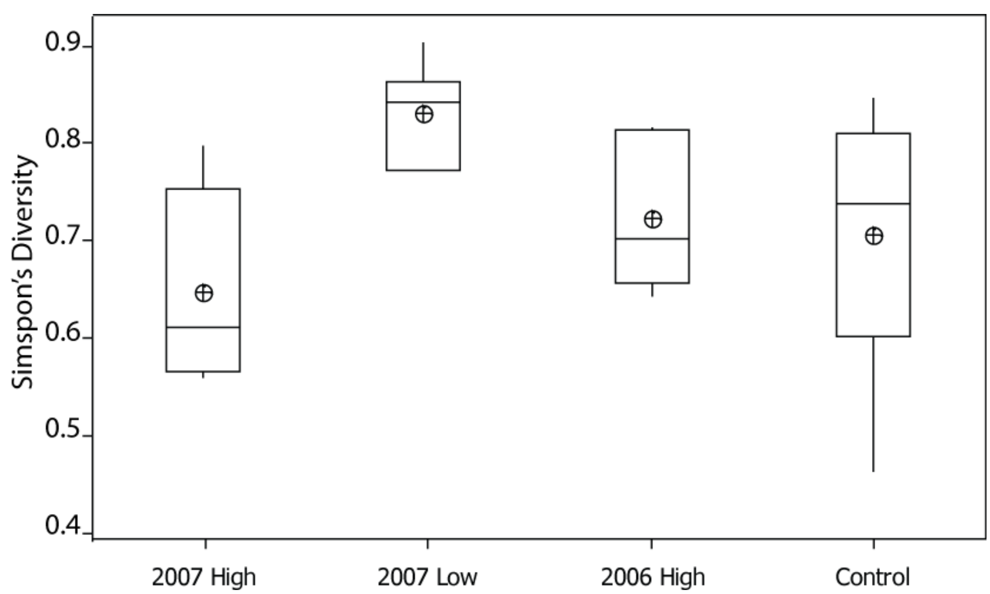


Figure 15: *Above ground Simpson's diversity index for each sampling stratification. Box represents interquartile range, horizontal line is the median, target is mean, and whiskers are upper and lower distribution.*

Distributions and variances were more normal and equal using the Shannon-Weiner diversity index. Results were similar to those found using Simpson's diversity index. The 2007 low severity area was significantly higher (D_j =2.17, p = 0.001) than all other burn-areas (Figure 16). Relationships between all other stratifications were not significantly different.

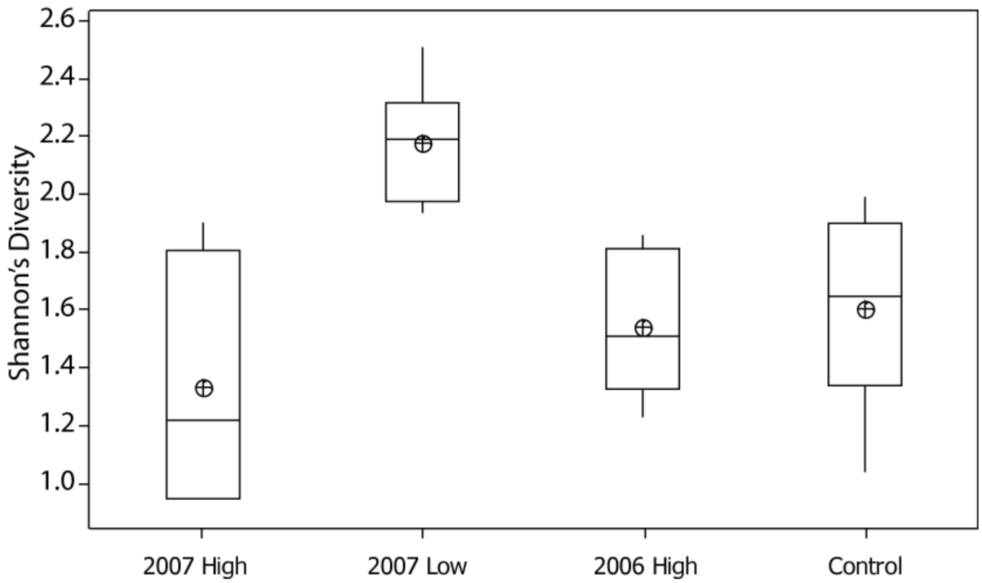


Figure 16: *Above ground Shannon-Weiner diversity index for each sampling stratification. Box represents interquartile range, horizontal line is the median, target is mean, and whiskers are upper and lower distribution.*

Above Ground Relative Abundance

Habit

There were significant differences in relative abundances of habit functional groups between burn-areas when assessed using non-parametric methods (Figures 17-18). Shrubs were significantly more abundant in the 2007 low severity area compared to the 2007 high severity area (p = 0.020). Shrubs were less abundant than forbs in the 2006 high severity burn and control area (both p = 0.001) compared to all other stratifications. Shrubs were also less abundant than graminoids in the 2007 low severity burn (p = 0.001), and more abundant than tree seedlings and saplings in all areas (p = 0.006). Variance of shrub density was significantly different between the 2007 low and 2007 high areas (p ≤ 0.001).

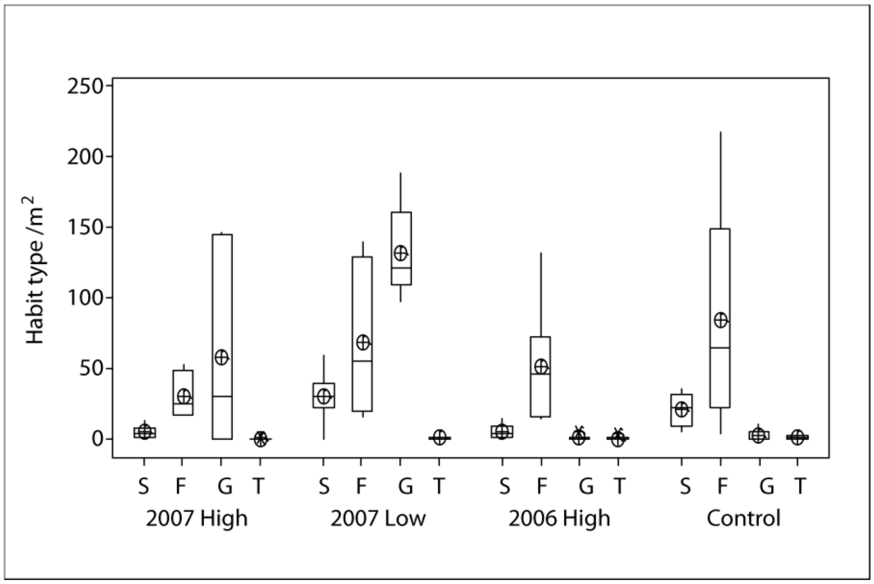


Figure 17: *Boxplot of above ground habit type densities grouped by burn-severity. S= Shrub, F= Forb, G= Graminoid, and T= Tree Seedlings. Box represents interquartile range, horizontal line is the median, target is mean, and whiskers are upper and lower distribution.*

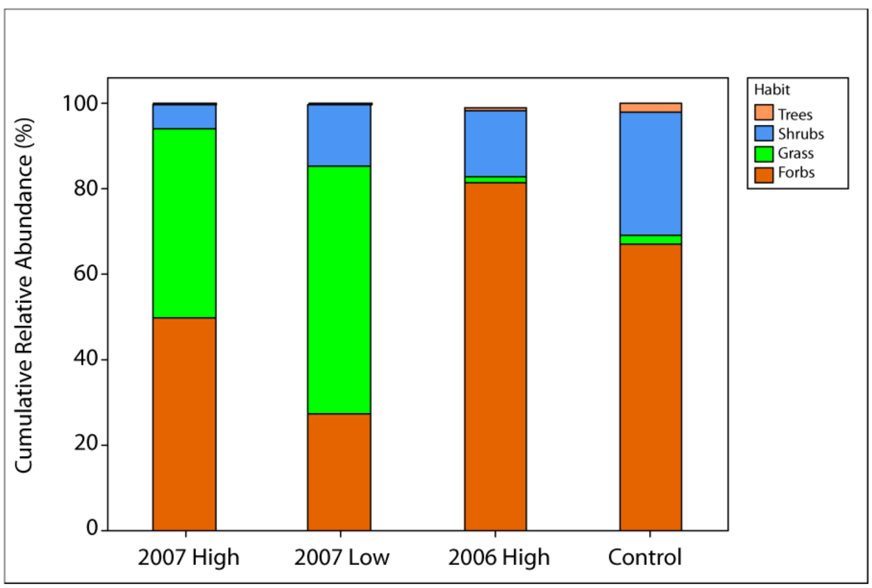


Figure 18: *Cumulative percent relative abundances of functional groups for each sampling stratification.*

Abundance of forbs was not significantly higher in any area and had similar variances between burn-areas. However, forbs were significantly more abundant than trees in all areas (weakest in 2007 high, $p = 0.006$), and all other functional groups in the 2006 high severity area ($p \leq 0.001$).

Graminoids were more abundant than tree seedlings throughout the 2007 burn-areas ($p = 0.005$ in 2007 high), and significantly more abundant than shrubs in the 2007 low severity area ($p \leq 0.001$). The 2007 burns both had more graminoids than either the control or 2006 burn area ($p = 0.005$), as well as greater magnitude variance ($p = 0.001$). Graminoid density was tested in regard to its relation with slope, and low slopes (<18°) had significantly ($p = 0.009$) higher graminoid density, most notably in the 2007 high severity area.

Tree seedlings were equally non-abundant between stratifications, and notably less abundant than any other group except for graminoids in the 2006 burn and control areas.

Duration

Annuals and biennials were not present in the control, and thus significantly higher in all other treatments ($p = 0.014$ from Kruskal-Wallis test). Small numbers of observations in all treatments except some 2007 high plots resulted in significantly different variances ($p = 0.001$), but apparently no real trend for abundance between areas (Figure 19).

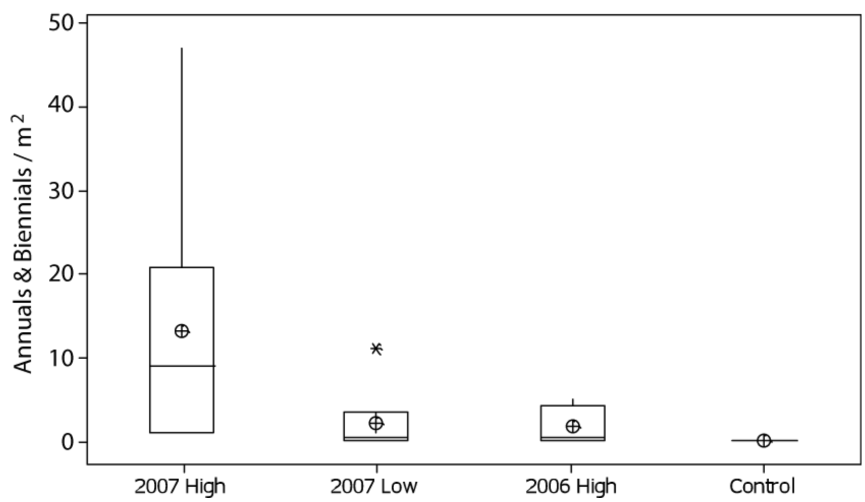


Figure 19: *Annual and biennial combined densities for all sampling stratifications. Box represents interquartile range, horizontal line is the median, target is mean, and whiskers are upper and lower distribution; * represents outlier.*

Nitrogen fixers

No nitrogen fixing plants were observed in the control (except species taller than our sampling limit), which was significantly less ($p = 0.010$ from Kruskal-Wallis) than the mean of 8.83/m^2 observed in the 2007 low severity area (Figure 20).

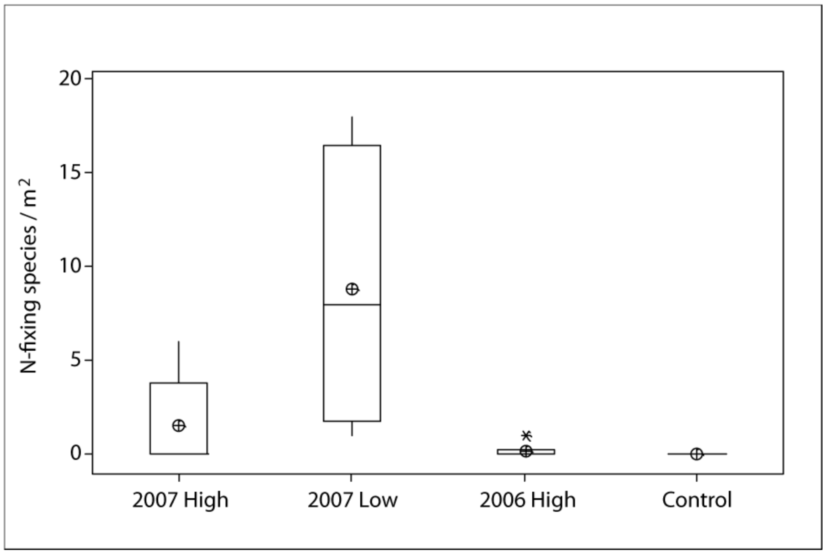


Figure 20: *Nitrogen fixing plant densities for each sampling stratification. Box represents interquartile range, horizontal line is the median, target is mean, and whiskers are upper and lower distribution.*

Non-native species

Two non-native species were observed and recorded in the 2007 low severity burn area. Only *Phleum pratense* and *Taraxacum officinale* were observed in 2006 low stratification, in 2 of 6 plots. No conclusion could be made about the importance or significance of these non-native species due to the small number of observations.

Below ground richness

Belowground seedbank richness was significantly lower in the 2006 high severity burn area than the control ($p = 0.046$ from Kruskal-Wallis). Below ground richness was also significantly lower than the above ground richness in the 2007 low ($p = 0.004$), 2006 high ($p = 0.004$), and control ($p = 0.027$), making it overall significantly different than the above ground ($p = 0.000$ from Mann-Whitney pairs) (Figure 21).

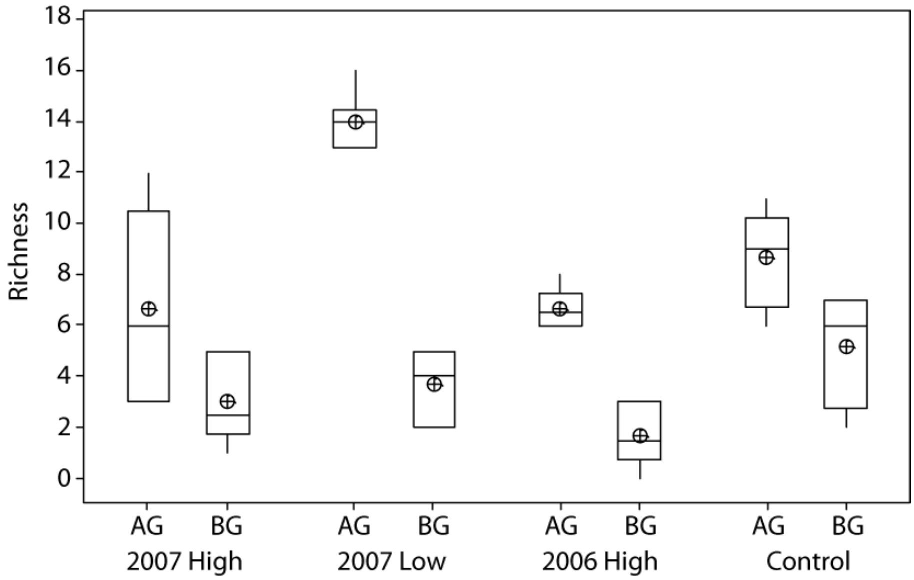


Figure 21: *Below and above ground species richness for all sampling stratifications. AG= Above Ground, BG= Below Ground. Box represents interquartile range, horizontal line is the median, target is mean, and whiskers are upper and lower distribution.*

Below Ground Diversity

Belowground seedbank diversity was significantly lower in the 2006 high plot than in the control plot ($p = 0.043$), and significantly lower than above ground diversity in both 2006 ($p = 0.028$) and 2007 low ($p = 0.010$) severity samples (Figure 22). Pair-wise comparison through all plots showed lower in diversity below ground ($p = 0.0021$).

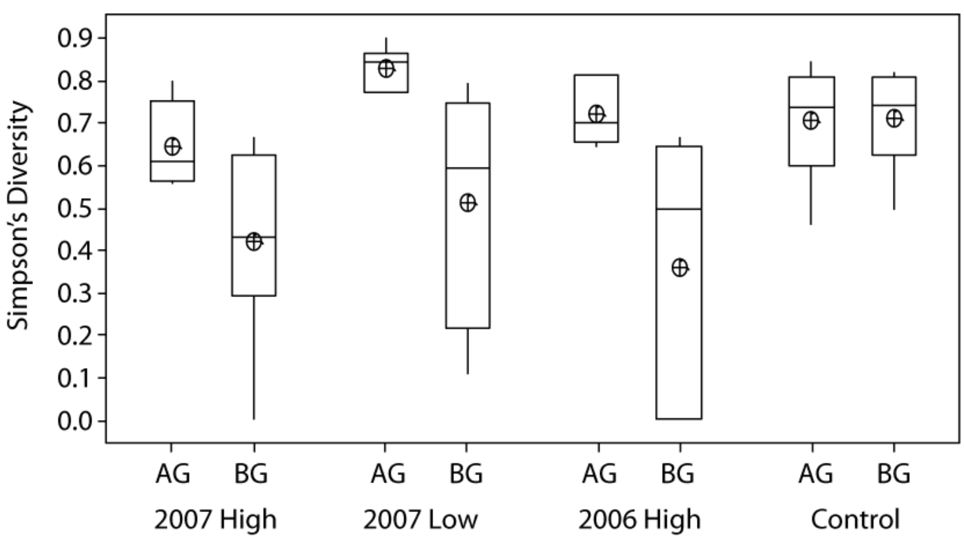


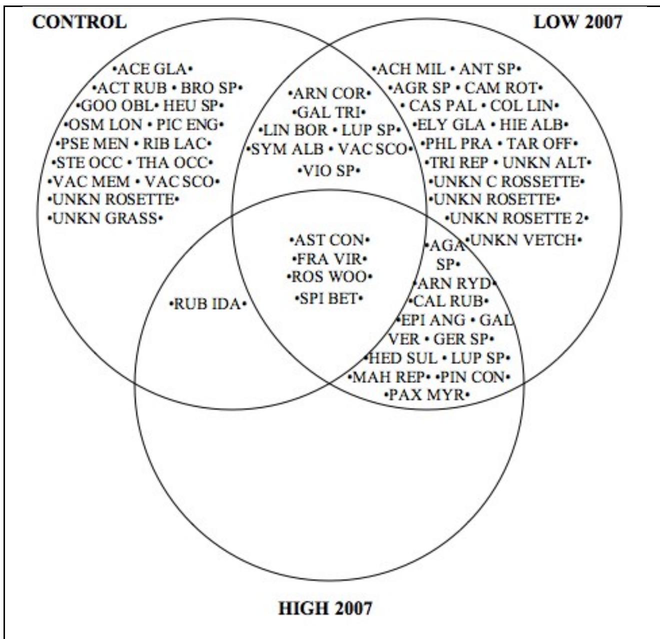
Figure 22: *Simpson's diversity index of above and below ground data for all sampling stratifications. Box represents interquartile range, horizontal line is the median, target is mean, and whiskers are upper and lower distribution.*

Species Richness from Duff Sampling

No statistical difference ($p=0.359$) was shown between soil and duff samples from the control for richness. Mean duff richness was 4.83/m^2, while soil richness was 7.50/m^2.

Species composition

The following Venn diagrams illustrate the species found within each stratification. A complete species list of observed species can be found in Appendix 1. The 2007 high stratification had consistently less species than the other stratifications. *Aster conspicuus*, *Rosa woodsii*, and *Spirea betulifolia* occurred at least once in all above ground stratifications (Figures 25 and 26). While *Aster conspicuus*, *Calamagrostis rubescens*, and *Thlaspi arvense* occurred in all below ground stratifications (Figures 23 and 24).



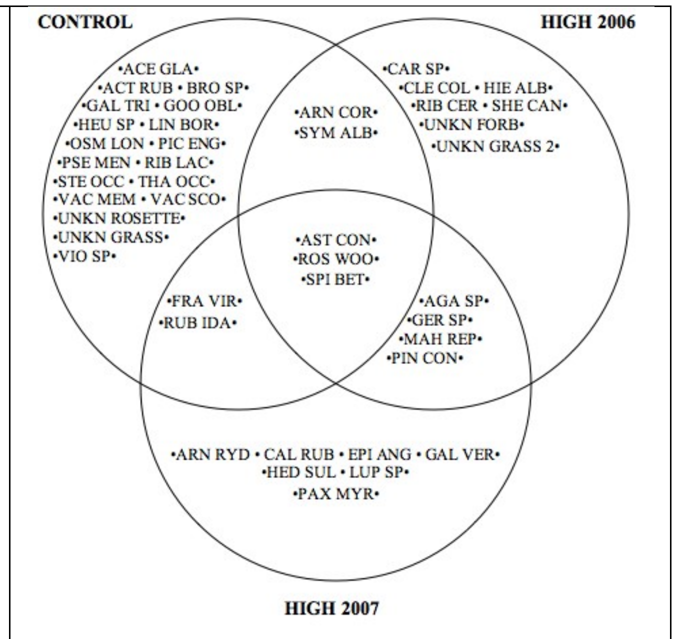


Figure 23: *Species composition of above ground vegetation in varying burn severities (High 2007, Low 2007, and Control).*	***Figure 24:*** *Species composition in above ground vegetation in varying chronosequences (Control, High 2006, and High 2007).*

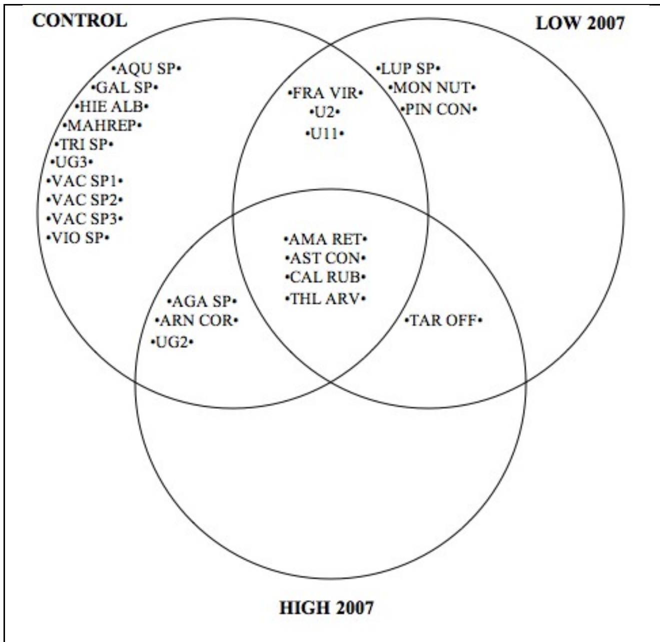


Figure 25: *Species composition from soil seedbank samples in regards to varying burn severities (High 2007, Low 2007, and Control).*

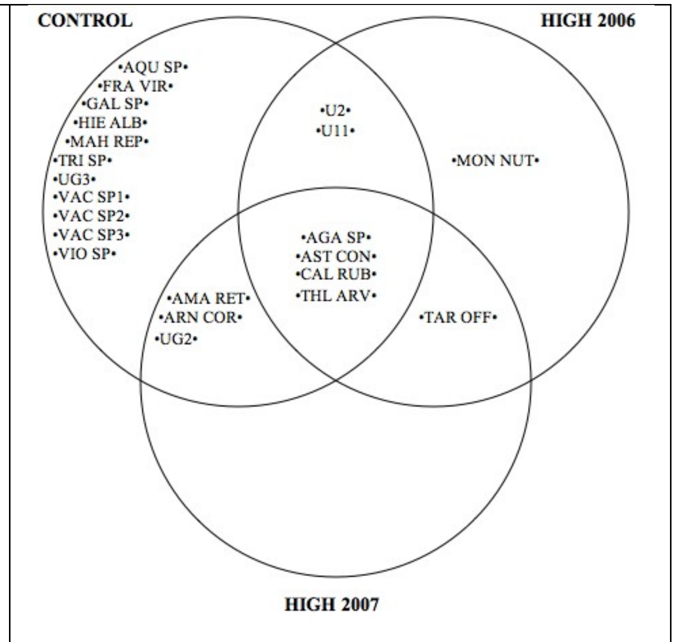


Figure 26: *Species composition from soil seedbank samples in regards to varying chronosequences (Control, High 2006, and High 2007).*

Discussion

Our study showed significant differences in vegetative communities following wildfire; dependent on time since fire and burn severity. Species richness is one way to assess the effects of disturbance on vegetation communities. Our data showed increased species richness in the 2007 low intensity burn area (Figure 15) which was consistent with other studies (Turner *et al.,* 1997; Stickney, 1990). This response was likely due to the thinning of the canopy and the nutrient release from the fire while leaving the seedbank relatively intact. Species richness in both high severity areas was lower than the low intensity burn area which may indicate negative impacts on the seedbank and propagating structures. Shallow slopes had higher species richness than steep slopes which may be a result of increased water retention, accumulation of eroded materials, increasing soil development, and less disturbance leading to increased plant establishment.

Fire causes catastrophic retrogression of the vegetative community. We identified some species unique to the burned areas and absent in the control. These included *Calamagrostis rubescens* (pine grass), *Mahonia repens* (Oregon grape), *Epilobium angustifolium* (fire weed), *Lupinus* sp., *Galium* sp., and *Pinus contorta* (lodgepole pine) According to our observations, the germination of these species is likely enhanced by fire . Nitrogen fixers were also not observed in the control plots (Figure 23), and were most abundant in the 2007 low severity burn-area. This agrees with research conducted in many forest systems (Neary *et al.,* 1999). These results only took into account understory species less than two meters tall and did not account for the presence of alder in the control area which is a known nitrogen fixing species.

Species commonly observed in and unique to the control plots included *Acer glabrum* (rocky mountain maple), *Actaea rubra* (baneberry), *Goodyera oblongifolia* (rattlesnake plantain), *Heuchera sp.* (alum root), *Thalictrum occidentale* (western meadow rue), and *Vaccinium spp.* (huckleberries and whortleberry). These species, as well as *Picea engelmannii* and *Pseudotsuga menziesii* (Engelmann spruce and Douglas fir), may be intolerant to fire.

Seedbank richness from our greenhouse study was not significantly higher than above ground richness, disproving our hypothesis. Seedbank samples showed a significant decrease in richness in the 2006 high severity area compared to the control area (Figure 22). This supports the idea that high severity fires will destroy seeds and propagating structures, but the 2007 burn-area data is inconclusive. More time and a better designed seedbank study are needed to gain further insight into the effects of fire on seedbank viability. The insufficient germination time (7 weeks) and small sample size likely resulted in low overall richness. Though we did apply gibberellic acid (GA) to soil samples, this practice is not common, and our methods were unsubstantiated. We attempted to perform an additional controlled experiment to assess this, but limited time provided inconclusive results.

The soil seedbank composition results showed propagation from underground structures, but only two native perennials occurred in all stratifications of the below ground study; *Aster conspicuus* and *Calamagrostis rubescens* (Figures 25 and 26). A discrepancy in the soil seedbank composition concerned *Thlaspi arvense*., which was not seen in any of the above ground plots, but occurred in every stratification in the greenhouse experiment. It is likely that either: greenhouse conditions were optimal for this species to germinate and field conditions were not; or, the growth medium was contaminated with *T. arvense* seed.

The Mill Creek fires affected vegetative community composition generally as expected, yet correlation and causation have not been conclusively separated. For instance, the community composition differences between stratifications may be a result of differing fire return intervals. Our work does not address this issue, but does provide insight into plant community relationships with fire. It would be interesting to identify the seral association of each species in this study to illustrate a more accurate picture of the successional changes. A continuation of this study for 20-50 years may provide insight into the successional progression of this mixed-conifer forest.

Land Resources Analysis and Management

The effect of fire on plant community diversity and richness across environmental and spatial gradients

Introduction

Wildfire is a dynamic force that plays a multitude of roles within the environment. Depending on one's point of view, it is seen as either a force of destruction or regeneration. Wildfire's destructive side is seen yearly in the late summer and early fall, burning vast swaths of land across the western United States and resulting in the loss of timber resources and homes. At the same time, many plant and animal species depend on fire as a stimulus of growth and a provider of suitable habitat (Perchemlides et al. 2008). The interplay between destruction and regeneration presents a crux to policy makers and forest managers to protect valuable resources while at the same time maintaining healthy forest ecosystems. In order to make the best possible management decisions, it is necessary to understand not only the dynamism of wildfires, but the effect of wildfire policy over the last hundred years.

Modern wildfire policy arose in the late 19th century from a desire to negate the destructive effects of wildfire (Stephens and Lawrence 2005). These initial efforts were successful in suppressing wildfires, but in some areas (not necessarily the greater Yellowstone ecosystem (Romme 1982, Turner and Romme 1997)) led to the build-up of combustible fuels, leading to increasingly intense burns (Stephens and Lawrence 2005, Dombeck et al. 2008). It was not until the late 1960's that controlled burns were acknowledged as a management tool to reduce fuel loads (Stephens and Lawrence 2005). Controlled burns, however, were rarely used and it was only recently that fire policy has shifted away from direct fire suppression to include ecosystem restoration and community assistance as management options (Steelman and Burke 2007, Odion et al. 2004). Despite these recent changes, the amount of burned acreage has continued to increase yearly (Steelman and Burke 2007, Stephen and Lawrence 2005) pointing toward a need for more studies on both the causes and effects of wildfires.

In order to better understand the effects of wildfires, we have chosen to study the influence of burn severity and environmental gradients on the post-fire recovery of plant communities in the Mill Creek drainage in southwestern Montana. Studying the recovery of plant communities is neither simple nor straightforward as a myriad of environmental factors can have a dramatic influence on the regeneration of the community.

Several studies have been performed to examine the succession and diversity of plant communities following disturbance by fire. A study conducted by Turner and colleagues (1997) evaluated the early succession of plant communities following the Yellowstone fires in 1988. Their study sought to quantify early succession by examining several response variables such as species richness, biotic cover and different plant types (trees, herbs, shrubs, or exotic species) as a function of burn severity, size of the burned patch, and distance from unburned area over a four year period from 1990-1993 (Turner et al. 1997). It was found that burn severity and patch size had significant effects on the response of the plant communities, but distance from an unburned area was seldom important in explaining plant densities (Turner et al. 1997). Additionally, it was found that geographic location was the most statistically significant independent variable, implying that the composition post-fire plant communities could be best predicted by pre-fire communities influenced by environmental gradients (Turner et al. 1997).

Another study conducted by Schoennagel and colleagues (2004) sought to examine how the time interval between stand-replacing fires affected the post-fire understory plant communities (consisting of herbs forbs and shrubs) in Yellowstone National Park. The study found that understory communities were dominated by perennials tolerant of a wide variety of fire return intervals, while annuals were more abundant after short intervals between fires (Schoennagel et al. 2004). Furthermore, it was found that total cover and richness in understory plant communities was highest for short time intervals between fires. Similar to the study conducted by Turner and colleagues in 1997, it was found that environmental variability was a significant factor influencing post-fire plant species richness and cover (Schoennagel et al. 2004).

While both studies effectively quantified the post-fire vegetative response and succession, they did not specifically address the influence of environmental gradients upon the recovery of plant communities. Our study of the Mill creek burn area seeks to go one step further by assessing the recovery of plant communities by spatially quantifying plant species richness and diversity as a function of both fire and environmental factors. These factors include burn severity, slope, aspect, elevation, soil type, and distance to unburned area. We hypothesize that burn severity will have a positive effect on plant richness and diversity, with richness diversity increasing with burn severity (Crawford et al. 2001). Slope and aspect will have no significant effect on plant richness or diversity, while the highest plant richness diversities will be found in low elevation areas and in areas with more fertile soil. Distance from burned to unburned areas will also have no significant impact on plant diversity. Previous cover (meadow, forest) will strongly influence vegetative response with meadow areas having higher post-burn diversity than forests. We also intend to spatially model the plant diversity data as a function of these variables and predict that it will be accurate in determining the relative diversity of post-fire plant communities across the landscape.

Methods

Field Sampling

Our region of interest incorporated areas in the Mill Creek drainage that had burned during the 2007 fire season. Within this area, the site was divided into three test sites, high burn severity, low burn severity, and the unburned area (control). The three sampling areas were overlain with a grid created in a geographic information system (GIS) with all data collection points spaced 50 meters apart. A starting point and angle were randomly generated using GIS software in order to help eliminate sampling bias in the data. All grid information was loaded into Trimble geographic positional system (GPS) receivers prior to data collection; the receivers also contained the random starting point and angle for navigation to that point within the pre-defined grid area.

To determine the number of sample points needed, a statistical equation for power tests was used (eq. 1).

$$n = (4[T(1- \alpha/2)]^2 Se^2)/PSD^2 \qquad (1)$$

Where n is the number of samples, α is the p-value (0.05), T is the t-stat for a given α (Since α = 0.05, then T =1.96), S is the standard error, and PSD is the practical significant difference.

For determining the standard error and practical standard difference (PSD), plant species data was used from data from Measuring Plant Species Diversity, treatment 0 (Rew, unpublished data). The p-value is the probability that results observed in the study could have occurred by chance. The study used a p-value of 0.05, which means a random sample from identical population would lead to a difference smaller than the observed 95% of the time. Standard deviation was found to be 0.255 from the Shannon Index value of the 13 plots. Therefore, Se = 0.255 /√(13) = 0.071. Our measure of diversity is the Shannon's diversity index and our chosen PSD value was 0.05 (The PSD is a measure of the precision of the data). Therefore, the value of each plots Shannon diversity index will have an uncertainty of ± 0.05. Thus, the number of samples needed is:

$$n = 4[1.96\ (1-0.05/2)]^2 * (0.071)^2 / (0.05)^2 = 29 \text{ samples} * 3 \text{ sites} = 87 \text{ total samples needed.}$$

Initial navigation to each grid site within a burn intensity was done using GPS units. Within each grid area, a 100 m tape and compass were used to navigate to individual sample points. At each sample point a 1/4 m^2 circular quadrat was laid. Within each quadrat the number of plant species and percent coverage for each species were tabulated and recorded by hand. Each plot was photographed for referencing or re-calculating of species if needed. Other data recorded at each sample point included slope (recorded with a clinometer), aspect, and GPS position.

Data Analysis

The plant data collected was analyzed using three indices: species richness (eq. 2), the Shannon index (eq. 3), and the Simpson diversity index (eq. 4).

S = the number of species; also called species richness. (2)

$$H' = -\sum_{i=1}^{S} p_i \ln p_i \quad (3)$$

.

$$D = \frac{\sum n(n-1)}{N(N-1)} \quad (4)$$

Range (m)	*Percentage of Sample Points*
0–0.15	–
0.15–0.30	–
0.30–0.50	0.9
0.5–1	30.8
1–2	36.9
2–5	23.8
>5	7.6

Table 2. *Estimated accuracies of GPS data collected at Mill Creek, MT between 8/27 and 8/28, 2008.*

Species richness is the measure of the number of different species present in an area and thus is simply a summation of each different species (s) (eq. 2). The Shannon index is a measure of the species evenness in a community and is calculated as a function of the relative abundance of each species (p_i). The relative abundance is the number of individuals of a certain species divided by the total number of individuals in the community. While increasing evenness increases the Shannon index, it also increases by adding more unique species and thereby introduces some bias into the index. Simpson's index is also a measure of species diversity and takes into account the number of individuals in a species (n_i) and the total number of individuals in the community (N). It should be noted that percent cover was used to calculate the species diversity indexes.

Each of these indices was then regressed individually (using S+ statistical software package) against slope, aspect, and burn severity as well as GIS slope and GIS aspect as obtained from 30 meter digital elevation model (DEM) of the study area. This was done because the main goal of the study is to develop a predictive model and thus GIS measurements of slope and aspect will be the most likely inputs for land managers, not field measurements. Aspect was analyzed as both a categorical and a continuous variable by either dividing it into five or nine categories or using a cosine transformation to make it a continuous variable. A multiple regression analysis was also conducted for each index against the combinations of slope, GIS slope, aspect, GIS aspect (all categories), and burn severity. The focus of these regressions was to elucidate a relationship between plant richness and diversity and environmental gradients of aspect and slope, which then may be used to create a predictive geospatial model. This model was intended to be used to create a predictive map of post-fire plant community richness and diversity for use by forest and land managers.

Geographical positioning system (GPS) data was post-processed using the CORS, Mammoth, WY base station at 44°58'24.34293"N, 110°41'21.47780"W, 1,824.35 m. 99.2% of the data was able to be corrected. The estimated accuracies and precision of the GPS data are presented in Table 2 and Table 3.

	Positional Dilution of Precision			Horizontal Precision		
	High	*Low*	*Control*	*High*	*Low*	*Control*
Minimum	2.8	2.1	2.2	0.7	0.5	0.8
Maximum	16.6	6	14.7	9.4	5.6	2.2
Mean	4.99	4.27	4.55	2.23	1.5	1.47

Table 3. *Estimated precision of GPS data collected at Mill Creek, MT between 8/27 and 8/28, 2009.*

Results

Across all three of the burn severities, species richness showed large differences in mean values in addition to a large amount of variation (Figure 27). This shows that while the mean values of richness decrease as the burn severity increases, there is enough variation to make the differences observed insignificant (except between the control and high severity areas). However, these results do not indicate that each of the sites supported similar plant communities, as the percent composition of forbs, shrubs, grasses, and trees was different for each of the study sites (Figure 28).

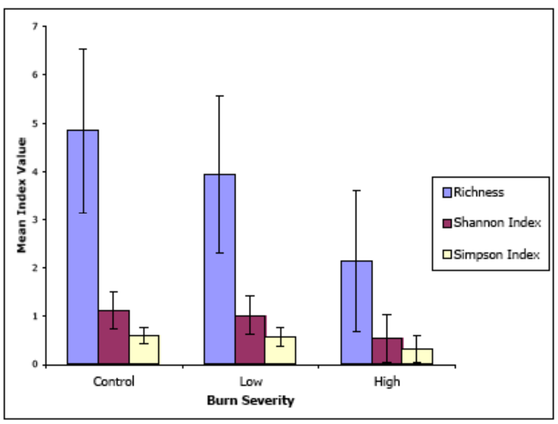


Figure 27. *Graphical representation of average and standard deviation of richness and diversity indexes across all burn severities.*

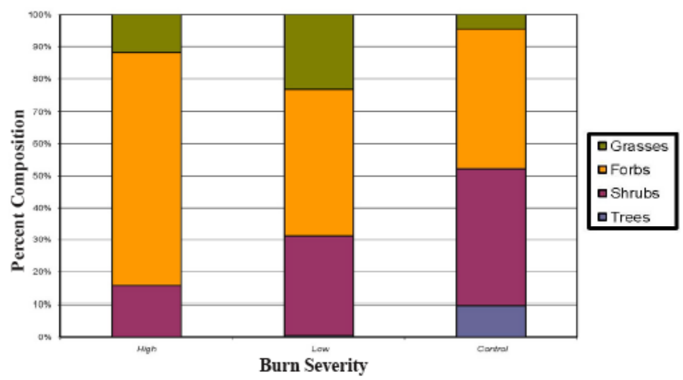


Figure 28. *Percent composition of plant functional groups from collected data for each study site.*

As species richness forms the basis for the calculation of the other diversity indexes, we find that the large variation in the richness values propagates through both the Shannon and Simpson index values. In turn, they too show a large amount of variation across all three study areas (Figure 27). These results show that, in spite of a significant difference between control and high severity sites with richness, there is relatively little difference between all three study areas in terms of species diversity.

Percent composition of functional groups showed some distinctive trends between low, high, and control study areas. The high intensity burn area showed the greatest percent composition of forbs for all three areas, while grasses had the greatest percent composition in the low burn intensity. The percent composition of shrubs increased as burn intensity decreased (Figure 28).

In contrast to species diversity, slope varied widely between each site. Frequency distributions of slope measured in the field show the high severity burn site having the highest frequency of steeper slopes (> 20˚) while the low intensity had the lowest frequency of steep slopes (Figure 29). The unburned control site had a few steeper slopes, but these were the result of a dramatic change in topography in a small section of the study site.

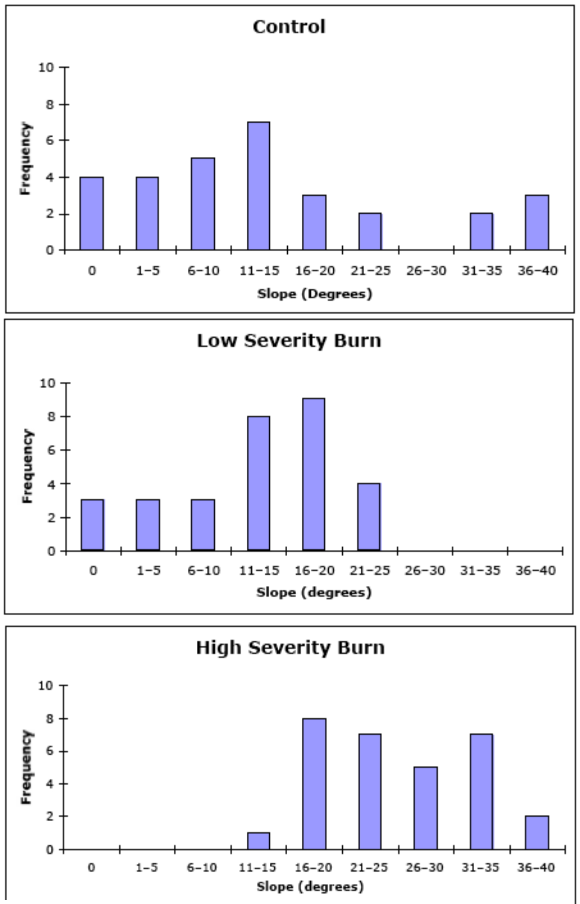


Figure 29. *Histograms showing the frequency distribution of slope between study sites.*

Unlike slope, all of the study sites were dominated by a narrow range of aspects. For all sites the main aspects were northerly, being north, northwest or northeast (or around 1 for the cosine transformation). Some discrepancy exists between the aspect measured in the field and the data obtained by GIS analysis (Figure 30). During analysis by GIS to obtain aspect points, some of the aspect categories present in the field data were eliminated. This is due to the coarseness of the DEM used for the analysis and the short spacing of the actual data points, likely causing an oversampling of the same area to determine the aspect value for different points. The elimination of categories is very evident in the high intensity burn area (Figure 30) as well as the other study sites. A similar phenomenon was observed for GIS slope, but to a lesser degree.

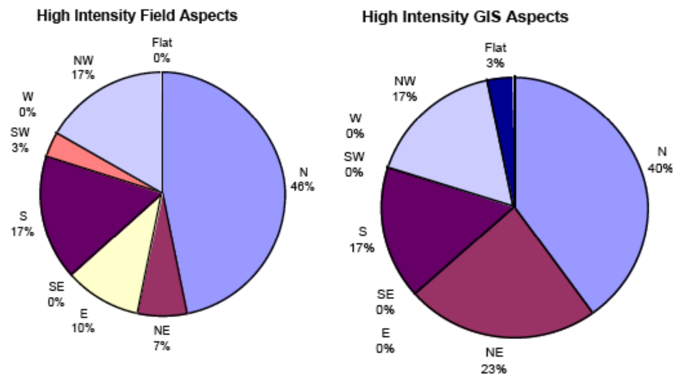


Figure 30. *Percentage of sample points in each aspect category as determined by field measurements and GIS analysis.*

For the individual (one variable) regression analysis burn severity showed the strongest and most significant ($p < 0.05$) correlation with species richness (Table 4). Alone, it explained between 26-34% of the observed variation in the data. The strongest correlation was with species richness, showing that the number of plant species present was best predicted by burn severity. Other variables did show significant correlations with species richness and diversity. Most notable is the GIS aspect with nine categories as it has a lower p-value and an equivalent or higher r-squared value than its field counterpart (Table 4). The other aspect measurements (five categories and cosine aspect) showed either insignificant correlations or significant correlations with low r-squared values (Table 4). GIS slope showed significant correlations with richness and the two diversity indices, but had very small r-squared values. Aside from a significant but weak correlation with richness, field slope was not a significant predictor variable.

The most significant variables of the slope and aspect categories (GIS slope and GIS aspect, nine categories, from the individual regression), in addition to burn severity, were used in the multiple regression analysis against the species richness and diversity indices. Other variables were investigated (such as field slope and field aspect), but the results were highly insignificant and hence are not reported. In all cases for the three variable multiple regression (Table 5), GIS slope and GIS aspect were insignificant. Removing the least significant (greatest p-value) of the two and rerunning the regression did not dramatically increase the significance of the regression and the individual p-value of the GIS variable remained insignificant (Table 5). As with the individual regressions, burn severity remained the most significant variable in predicting plant richness and diversity response. The small gain in r-squared values in the multiple regression analysis as compared to the individual regression clearly indicates that the burn severity, slope, and aspect variables exhibit a negative cooperativity (in other words, the r-squared value from the multiple regression is smaller than that expected from a simple linear combination of the individual r-squared values). Overall, the results of the multiple regression analysis did not yield a high enough r-squared value (≥0.78 needed) to warrant the creation of a model or predictive map.

Index	Burn Severity	Slope		Aspect 5[c]		Aspect 9[c]		Cosine Aspect[c]	
		Field[b]	GIS[b]	Field	GIS	Field	GIS	Field	GIS
Richness									
r^2	0.337	0.0630	0.0939	0.115	0.0828	0.163	0.161	0.0349	0.0310
p-value[a]	1.77×10^{-8}	0.0170	0.00330	0.0146	0.115	0.0193	0.0102	0.0777	0.0967
Shannons									
r^2	0.264	0.0204	0.0855	0.116	0.0981	0.151	0.181	0.0245	0.0289
p-value	1.57×10^{-6}	0.180	0.00517	0.0139	0.0641	0.0302	0.00432	0.140	0.109
Simpsons									
r^2	0.248	0.0248	0.0855	0.0965	0.1105	0.161	0.183	0.0204	0.0301
p-value	4.09×10^{-6}	0.138	0.00516	0.0324	0.0392	0.0211	0.00399	0.180	0.102

[a]p-values >0.05 are considered not significant.
[b]Field indicates values measured in the field, GIS indicates values measured using GIS software.
[c]Aspect was treated as a categorical variable using 5 or 9 categories or as a continuous variable using a cosine transformation.

Table 4. *Results of the individual regression analysis for each variable.*

Index	Overall values		Individual p-values[a]		
	r^2	p-value[a]	Severity	GIS Slope	GIS Aspect 9
Richness					
Three values	0.356	1.22×10^{-5}	1.0×10^{-7}	0.715	0.809
Two values	0.338	8.97×10^{-8}	1.0×10^{-7}	0.710	–
Shannons					
Three values[b]	0.301	2.09×10^{-4}	2.30×10^{-6}	0.901	0.524
Two values[b]	0.301	8.81×10^{-5}	1.90×10^{-6}	–	0.520
Simpsons					
Three values	0.295	2.84×10^{-4}	5.0×10^{-6}	0.907	0.386
Two values	0.295	1.20×10^{-4}	4.3×10^{-6}	–	0.380

[a]p-values >0.05 are considered not significant.
[b]Three values indicates three variables were used in the regression, while two values indicates that two variables were used and that the least significant value from the three value regression was removed.

Table 5: Summary of significant multiple regressions for each species richness and diversity indices

Discussion

Our original objectives were to evaluate a variety of environmental gradients and their effect on post-fire plant recovery with the intention of using the data to create a predictive map. This map would then be used to identify areas of the landscape where the diversity of post-fire plant communities may be negatively impacted or hindered by environmental factors. Unfortunately, many of the factors we sought to evaluate where either confounded (elevation) or unimportant to the study site (previous cover was all forest) and thus limiting our study to examining slope, aspect, and burn intensity as a means of predicting post fire plant diversity.

Examining the data for species richness and diversity revealed some definite trends. Species richness increased (with plenty of variation) from the high severity burn area to the unburned control area (Figure 27). The trend is likely due to the control area having a greater number of individual species compared to the other sites, thereby giving it a higher species richness. Additionally, its composition was a much more diverse mix between shrubs forbs, grasses, and trees (Figure 28) compared to the low and high severity burn sites, which were primarily forbs in the high burn severity and forbs and shrubs in the low burn severity (Figure 28). This is not an unexpected result as certain species may dominate after a fire, depending on burn severity and soil fertility (Schoenagel et al. 2004). The dominance of forbs on the high burn severity sight is somewhat anomalous as it directly contradicts previous findings showing that more severely burned sites having lower cover of forbs (Turner et al. 2003). However, the lower mean values of species richness, Shannon index, and Simpson index in the high burn severity area corroborate the percent composition data, implying the predominance of a few species in this area.

This result alone seems to suggest the operation of other factors than just burn severity influencing the species richness and diversity of post-fire plant communities. However, the results of our regressions (both multiple and single) show that burn severity by far showed the best and most significant correlation with plant species richness and diversity (Table 4 and 5). Of the three response variables, species richness showed the strongest correlation and by far the most significant (several orders of magnitude) for both individual and multiple regressions (Table 4 and 5). This implies that in areas with higher burn severities there is lower species richness and they may be dominated by relatively few species, a trend that may also be seen qualitatively from the percent composition data. This is comparable to results found by Turner and colleagues (1997), in which lower richness of vascular plants was found in areas of higher severity burns and higher richness in areas of lower severity burns.

Slope and aspect did show some significant individual correlations with plant diversity (Table 4) in contrast to previous findings showing their insignificance (Turner et al. 1997). This may be explained by the simple fact that our study had much more slope and aspect data than these earlier studies. The r-squared values obtained for slope and aspect, however, were low and did not translate to increased correlation in multiple regressions when coupled with burn intensity. As such, we were unable to explain enough of the variation in the data (need r-squared of at least 0.78) to create a model.

It is important to note that GIS slope and GIS aspect (nine categories) showed stronger and more significant individual correlations with plant diversity and richness than the actual field measurements (Table 4). Again, the best possibility for the incongruence of the GIS and field measurements is that the GIS significantly changes the number of sample points in each category and even eliminates an aspect category all together (Figure 28). This is likely the result of area averaging and oversampling caused by the small distance between sample locations (50 m) compared to the coarseness of the DEM (30 m). Thus, while this result could imply that the area effects of slope and aspect are more significant in affecting plant diversity than local ones, it is most likely an artifact of the methods used to generate the data. Originally, the data points were to spaced at 100 m intervals, which may have alliviated the discrepency between field and GIS aspect. But it was deemed that the time needed to collect all the data would take too long given the rough terrain and short amount of field time.

Overall, the result that burn intensity showed the strongest correlation to plant diversity and richness is unsurprising and has been shown in previous studies (Turner et al. 1997). However, in previous studies done in Yellowstone National Park, slope and aspect have been shown to be not significant and were not as extensively quantified (Turner et al. 1997). Thus, the fact that some of the individual regressions have shown slope and aspect to be significant factors (though with low r-squared values) is an important result in as it indicates that more work is needed to better elucidate the mechanism by which they can affect post-fire plant species richness and diversity.

While this is an important result, there are several limiting factors that could have confounded the results of the study, thereby preventing a fuller understanding of the effect environmental gradients on the recovery post-fire plant recovery. The primary limitation is elevation as all three study sites had widely different elevations, which in turn has a direct effect on local climate and hence plant communities. Another factor is that the high intensity burn site had greater frequency of steeper slopes than the other two sites (Figure 29), which again may affect the establishment of plant communities and the local climate. The predominance of north facing aspects across the study sites will also skew the relationships observed as the aspect directly affects the amount of sunlight available for plant growth in addition to evapotranspiration and soil temperature.

Thus, in order improve results and to better quantify the effect of environmental gradients, it is necessary to choose a study site that has the fewest confounding variables, thereby magnifying any differences arising from the environmental gradients being studied. Selecting such a site is not an easy task and would, ideally, be small enough that confounding variables could be controlled or accounted for. While there are often many problems with scaling-up relationships derived on the small scale, it may be a necessary evil as such relationships can provide insight and focus for larger studies on the effect of environmental gradients on post-fire plant species richness and diversity. Future studies could also examine the effects of local climate (temperature and moisture), soils (only if the area consists of very different soil orders or textures, which is not the case in our study area), and hydrology as any of these factors could display a more synergistic relationship with slope and aspect in accounting for plant diversity and richness.

General group discussions

The Soil Group's Interdisciplinary Relevance

The overall implication of this interdisciplinary study was that wildfire has significant effects on a landscape. As soil temperature increases, so does organic matter combustion, resulting in a reduction or removal of organic carbon in the soil (Certini, 2005), therefore it was predicted that with an increase in burn severity, the amount of soil total carbon would decrease. From our study we concluded that there was a decrease in total soil carbon in burned sites compared to unburned sites, but fire severity had little effect on total carbon content. Soil total nitrogen (organic-N) and total carbon were expected to behave similarly after a wildfire because of the effects of wildfire on organic matter, and plant biomass. With severe wildfire, "Promoted recovery of the vegetation is essential, and is often limited by the scarcity of available C and N which leads to a very slow rate of nutrient turnover" (Fernández, 1997). It was expected that there would be small reduction in total C and N following less severe wildfire, where ignition temperatures of soil organic matter may not be have been reached (Barid, 1999). With small reductions in total C and Nresulting from low severity burns, sufficient nutrient levels for plant reestablishment should be created and maintained. The results of the vegetation group appear to support this statement. They found, "There were significant differences in vegetative communities following wildfire… Species richness in the 2007 low fire stratification was significantly higher than other areas". Similar richness results were observed by the Land Resources and Management group (LRAM) with overall species richness in the control, followed by low, and finally high burn plots. However, it should be noted that they did sampled a wider range of sites than the soils or vegetation group and therefore our results are more comparable with the vegetation group. Plant species diversity indices also followed similar patterns.

Prior to evaluating the soil available nitrogen (NO_3^-), it was hypothesized that available nitrogen would increase with an increase in fire severity. A study conducted by Duran *et al.* (2008) stated that available nitrogen levels increased immensely one year after fire in burned treatment sites compared to unburned control sites in a *Pinus canariensis* ecosystem. Our observed results showed that available nitrogen was highest in the low burn site, and lowest in the control site. However, available nitrogen was found to have no significant difference between high burn, low burn, and control sites. Interestingly, the Vegetation Group stated that, "Nitrogen fixers were not observed in the control plots, and were most abundant in the 2007 low severity burn-area". This agrees with research conducted in many forest systems (Neary *et al.*, 1999) though we are unsure how this relates to our soils data.

The Microbiology Group's Interdisciplinary Relevance

Soil microorganisms have a defined range of temperatures, pH, water availability, nutrient availability, and energy sources in which they can thrive. Changes in soil properties, vegetation, and landscape slope and aspect can significantly change the microbial community composition. The abundance and diversity of microorganisms is highest in the top 0-10 cm of the soil layer, which receives the largest amount of nutrients from plants and animals (Paul, 2007). However, this layer is affected most by disturbances such as wildfires which may result in a shift in the community structure. Using molecular techniques to analyze ammonia oxidizing bacteria (AOB), we examined the effects of the Wicked Hicks fire in the Mill Creek, Montana drainage on the soil microbial community and compared these results to those found by the Soils, Vegetation and LRAM teams.

Soils

Based on the DGGE results , the control soil samples showed the greatest AOB species richness, followed by the twice-burned stump sample, the high severity burned stump and lastly the twice burned forb samples. We expected ammonia oxidizing bacteria would be more prevalent in post-fire soils as more ammonia becomes available in soils after the occurrence of fire (Smith *et al*. 2008).Even though the control soil had the least water content, least nitrogen content and possibly the least ammonia content, it had the highest AOB species richness and abundance. This may be due to higher amount of organic matter and prevalence of plant roots in the unburned soil. The burned sites appear to contain fewer, yet possibly more dominant species of AOB that may have successfully filled unoccupied microbial niches caused by fire induced sterilization of the soil. The decrease in richness and abundance of AOB in the burn sites might be due to an increase in soil heterotrophic bacteria which can out compete AOB for resources (Horz *et al.,* 2004). However, competition by heterotrophic bacteria can not be the overriding factor in determining soil community composition since the control soil has the highest carbon content and is the richest in AOB.

Water content of the soil may be a main factor in AOB soil abundance. A larger increase in soil moisture can depress AOB abundance by decreasing the diffusion of oxygen into the soil (Horz *et al.* 2004).This correlates with the results found by the soils group that the burned treatment soils have higher moisture content than compared to the control treatment soil. Although, macro-nutrients such as nitrogen, carbon and ammonia can have a significant effects on the AOB soil abundance our findings suggest moisture content may be the main determining factor in our treatment site soils.

Vegetation

Wildfires tend to burn heterogeneously across landscapes due to variations in slope, aspect, soil properties, and vegetation. The interplay of these multiple environmental variables can confound the relationship between biologic responses and wildfire severity. The vegetation study examined species richness following wildfire disturbance and determined that variation in slope and aspect played a significant role in observed values. Plant species richness and diversity varied among sample sites without showing a significant trend. Although we did not compare sites of varying slopes and aspects, we suspect these variables play an important role in the response of microbial species richness as well as fire severity.

Land Resources Analysis and Management (LRAM)

The LRAM team hypothesized that higher burn severity would result in increased plant diversity; slope and aspect would have no impact on diversity, but previous cover would influence the post-burn plant diversity. Aspect, slope and previous cover could also be important determinants of post-fire soil microbial community structure. The LRAM team predicted that diversity would be higher at lower elevations due to more fertile soils as suggested by Högberg *et al.* 2006 who found higher total nitrogen in toe slope areas due to N influxes from the surrounding areas. This could substantially impact AOB communities located at lower sections of hill-slopes.

The LRAM team examined the influence of aspect, slope and burn severity on plant diversity. Significant relationships were found between all three factors and plant species richness and diversity. The LRAM team also determined that species richness and diversity was highest in the unburned area, which is consistent with the effects of wildfire on AOB community structure observed by the Microbiology group.. However, both group's results are preliminary, suggesting a need for further research to better quantify the relationships between landscape, vegetation and soil microbial communities in post-wildfire soils.

The Vegetation Group's Interdisciplinary Relevance

The time since a fire and the degree of burn severity were correlated with differences among post-fire vegetation communities. Species richness is one way to assess the effects of disturbance on vegetation communities. Our data show increased species richness in the 2007 low intensity burn area (Figure 4); which was consistent with other studies (Turner et al., 1997; Stickney, 1990). This response was likely due to the thinning of the canopy from the fire which releases nutrients while leaving the seedbank relatively intact. Species richness in both high intensity areas was lower than the low intensity burn area, which suggests a negative impact of fire on the seedbank and propagating structures. However, the LRAM group found the highest richness and diversity in unburned plots, followed by the 2007 low and 2007 high burn severity areas. This discrepancy is likely due to variations in sampling protocol and objectives between the two projects. The LRAM group used a large number of quarter meter diameter circularplots and sampled across a range of slopes and aspects. In contrast, the vegetation group used a $1m^2$ quadrat and sampled relatively few locations all on northerly aspects. The LRAM group's data also suggests that the low severity burn had greater richness and diversity than the high severity burn area when more aspects were analyzed. The control plots had the greatest range of aspects which may explain why the richness and diversity was so high in these plots. Furthermore, the soil group found trends of elevated available nutrient levels (although these results were not statistically significant) particularly in the low severity burn area. These data suggest that a flush of nutrients may be responsible for higher richness and diversity in the 2007 low severity plots.

Fire causes catastrophic retrogression of the vegetative community. Evidence of such retrogression was identified by early seral species found in our fire affected plots and those of the LRAM group. More severely burned areas were brought back to an earlier seral stage than less severely burned areas. For example, the LRAM group found the high severity burn areas dominated by forbs while the low severity burn area had forbs as well as a later seral habit type, shrubs. We found shrubs to be more common in the 2007 low severity burn plots than the 2007 high severity burn plots (Figures 8 and 10), which confirms this claim.

Nitrogen fixers were not observed in our control plots (Figure 12), and were most abundant in the 2007 low severity burn-area. This agrees with research conducted in many forest systems (Neary et al. 1999) and may relate to research performed by the microbiology group in the Mill Creek Fire complex. The microbiology group inferred that the control plots may have had higher richness of ammonia-oxidizing bacteria than all other stratifications. Our data suggests that no nitrogen fixing plant species existed in the control plots, which could explain the need for more ammonia-oxidizing bacteria. However, these results only took into account understory species less than two meters tall and did not account for the presence of alder (*Alnus sp.*) in the control area which is a known nitrogen fixing species.

In general, the Mill Creek fires affected plant community composition as expected, but correlation and causation have not been conclusively separated. Despite extensive communication between other research teams within the same fire complex, we have yet to identify a statistically significant rationale for the plant responses observed in the Mill Creek area. Continuation of this research for several years may clarify the post-fire ecological responses.

The Spatial Vegetation (LRAM) Group's Interdisciplinary Relevance

Interdisciplinary and cooperative studies are essential to comprehensive scientific research. During this interdisciplinary exercise, four groups examined a broad range of abiotic and biotic influences on post-fire ecosystem recovery from the Wicked-Hicks fire complex. Specifically the groups examined the soils, microbiology, vegetation, and geospatial controls of post-fire vegetation within burned and unburned areas of the Mill Creek drainage. The results gained through this research, while unique to each group's focus, allow broad connections to be observed. As the Land Resource Analysis and Management group we looked at the geospatial controls on species richness and diversity in differing burn severities. While our data and results correlated with all the groups, the best relationships were seen with the vegetation group's results.

Perhaps the most interesting finding was the effect of sample frame size and number of samples. In our study, we observed lower richness and diversity than the vegetation group. However, our sample frames were one-quarter of the size used by the vegetation group. Diversity and richness values similar to those found by the vegetation group were obtained when we scaled-up our sample area to equal that used by the vegetation group. Though we found similarities in species richness and diversity, we did not find the same trends as the vegetation group. This difference is likely due to the number of samples collected. Our group collected 30 samples in each of the burn severities while the vegetation group collected 6 samples in each burn severities. The influence of the number of samples can also be seen when comparing our work to Turner and colleagues. They only looked at a few samples and could not find correlations between slopes and aspects; we looked at more samples and were able to find correlations between spatial controls and both species richness and diversity.

In comparing our results with the soils group, few correlations could be drawn. This could be due to the number of samples that they collected and some inconclusiveness in their data. One possible significant finding was that as available phosphorus decreased so did species richness and diversity. This suggests that phosphorus may be a limiting factor to plant growth in post-fire recovery.

The microbiology group found higher microbial diversity in the unburned areas relative to the burned areas. Higher microbial diversity in the control plots corresponds with the plant diversity that we observed. In a healthy, undisturbed soil microbial richness is likely to be higher and the same could be argued for plant richness. Both these trends were observed in the unburned site. The high severity burn had the third highest richness. This agrees with the soils data which show lower available nitrogen in the high severity burn.

Even though our data showed strong agreements with the vegetation group, comparisons between our results and those of other groups was minimal though several interesting trends and correlations were identified. While the relationships may be tenuous, they provide some insight into the broad-scale patterns of post-fire ecosystem recovery. Working with other research groups can help broaden the knowledge gained by individual research. Thus, interdisciplinary groups are vital for large scale research such as forest fire recovery.

Concluding Remarks

We believe that our work has proved some new insight into the effects of wildfire on a landscape and the response in the soil, microbial and vegetative community. We have also learnt a considerable amount about study design, sampling techniques and statistical analysis. Three of our groups stratified their sampling on northerly aspects at similar elevation and pre-fire habitat but even this approach provided considerable variability in our data due to our often low sample size. The fact that the LRAM group sampled different aspects highlighted the benefits of the sample stratification used by the other groups and simultaneously demonstrated that the lack of data clarity provided by many of the previous studies we read may have been in part due to their lack of incorporation of environmental variables into their study design or analysis. The value of interdisciplinary work became more apparent to us as the project continued and in retrospect we would have liked to have all sampled within a small area (~10 m^2) and sampled all of the burn severities.

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Appendix A: Scientific and common names and species codes for observed plant species

Latin Name	Common Name	Code
Acer glabrum	rocky mountain maple	ACE GLA
Achillea millefolium	common yarrow	ACH MIL
Actaea rubra	red baneberry	ACT RUB
Agastache foeniculum	blue giant hyssop	AGA FOE
Agropyron sp.	unknown wheatgrass	AGR SP
Antennaria sp.	unknown pussytoes	ANT SP
Arnica cordifolia	heartleaf arnica	ARN COR
Arnica rydbergii	Rydberg's arnica	ARN RYD
Aster conspicuus	showy aster	AST CON
Bromus sp.	unknown brome	BRO SP
Calamagrostis rubescens	pine grass	CAL RUB
Campanula rotundifolia	harebell	CAM ROT
Carex sp.	unknown sedge	CAR SP
Castilleja pallescens	pale indian paintbrush	CAS PAL
Clematis columbiana	rock clematis	CLE COL
Collomia linearis	narrowleaf collomia	COL LIN
Elymus glaucus	blue wild rye	ELY GLA
Epilobium angustifolium	fire weed	EPI ANG
Fragaria virginiana	Virginia strawberry	FRA VIR
Galium trifidum	threepetal bedstraw	GAL TRI
Galium verum	yellow spring bedstraw	GAL VER
Geranium carolinianum	Carolina geranium	GER CAR
Goodyera oblongifolia	rattlesnake plantain	GOO OBL
Hedysarum sulphurescens	white sweetvetch	HED SUL
Heuchera sp.	alum root	HEU SP
Hieracium albiflorum	white hawkweed	HIE ALB
Linnaea borealis	twinflower	LIN BOR
Lupinus sp.	unknown lupine	LUP SP
Mahonia repens	Oregon grape	MAH REP
Osmorhiza longistylus	sweet cicely	OSM LON
Paxistima myrsinites	mountain lover	PAX MYR
Phleum pratense	timothy	PHL PRA
Picea engelmannii	Engelmann spruce	PIC ENG
Pinus contorta	lodgepole	PIN CON
Pseudotsuga menziesii	Douglas fir	PSE MEN
Ribes cereum	squaw currant	RIB CER
Ribes lacustre	black currant	RIB LAC
Rosa woodsii	Wood's rose	ROS WOO
Rubus idaeus	greyleaf red raspberry	RUB IDA
Shepherdia canadensis	russet buffaloberry	SHE CAN
Spiraea betulifolia	white spirea	SPI BET
Streptopus amplexifolius	twisted stalk	STE AMP
Symphoricarpos albus	snowberry	SYM ALB
Taraxacum officinale	dandelion	TAR OFF
Thalictrum occidentale	western meadow rue	THA OCC
Trifolium repens	white clover	TRI REP
Vaccinium membranaceum	thinleaf huckleberry	VAC MEM
Vaccinium scoparium	grouse whortleberry	VAC SCO
Viola sp.	unknown violet	VIO SP