

AN ABSTRACT OF THE THESIS OF

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Title: Effects of Wildfire Burn Severity on Soil Microbial Communities and Invasive Plant Species in the Cascade Range of Oregon.

Abstract approved:

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Fire suppression in the last several decades has resulted in unprecedented accumulations of organic matter on the landscape, leading to an increase in large, intense wildfires. This study investigated the soil microbial community (using phospholipid fatty acid analysis) across recently burned forests on the eastern slope of the Cascade Range in Oregon to examine the effects this belowground community has on the growth of native and non-native plant species in severely burned “red” soil and in less severely burned “black” soil. Long duration, smoldering conditions creating red soils drastically altered both soil nutrients and microbial community structure. Changes in soil properties and biota affected plant growth in a controlled growth chamber, as well as vegetative colonization on red soil plots in natural field conditions. Differential growth was observed between native and non-native plant species when grown in soil from the two burn severities. Native plant growth did not differ between black and red soil, while non-native plants showed reduced growth in

red soil. Although it previously had been reported that fire increased the likelihood of invasion by non-native plant species in a burn area, these results do not support the notion that red soil conditions are more susceptible than moderately burned soil to non-native, invasive plant species colonization. While many factors in addition to mycorrhizal colonization and burn severity influence plant growth, such as soil nutrient availability, our results suggest that a variety of strategies allow plants to grow in disturbed environments. Continued monitoring of microbial communities and re-vegetation in red soil sites could further our understanding of the length of post-fire recovery time of severely burned red soil.

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Effects of Wildfire Burn Severity on Soil Microbial Communities
and Invasive Plant Species in the Cascade Range of Oregon.

by
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I understand my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Cassie Lenae Hebel, Author

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EFFECTS OF WILDFIRE BURN SEVERITY ON SOIL MICROBIAL COMMUNITIES AND INVASIVE PLANT SPECIES IN THE CASCADE RANGE OF OREGON.

1.1 Introduction

Fire disturbance plays an important role in maintaining ecosystems by removing dead and built-up vegetation that hinders new growth, by quickly releasing nutrients bound in litter, thus enriching the soil. Yet, the accumulation of fuels after years of fire suppression in the western United States has led to high severity, stand-replacing wildfire events. The component of fire severity that results in the greatest belowground damage to ecosystems, and hence recovery, is duration of the fire (Neary *et al.*, 1999), and the post-fire impacts on soil biotic and abiotic factors directly affect belowground and aboveground systems. Limited research resources have been directed towards belowground studies that improve our understanding of the sustainability of belowground systems in the presence of natural or anthropogenic disturbances, such as fire (Neary *et al.*, 1999). In addition, the short- and long-term effects of fire on belowground microorganisms, and the resulting effect on ecosystem sustainability, are uncertain.

This study investigated the soil microbial community across recently burned forests on the eastern slope of the Cascade Range in Oregon. The objective was to examine the effects this underground community has on the growth of native and non-native plant species in severely and less severely burned soil. Information from this research will provide forest managers with knowledge about the potential impacts of soil burn severity on microbial communities, and their post-fire effects on native and non-native plant colonization.

1.2 Fire severity and the effects on soil microbes

Fire severity is a qualitative measure that refers to the overall effect of fire on an ecosystem. It relates to the effect of fire on soil and site resources that control ecosystem sustainability (Neary *et al.*, 1999). 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 (OM) content, and time since last burned (Neary *et al.*, 1999). The greatest damage to belowground ecosystems results from the duration of the fire on the soil surface, or smoldering combustion and subsequent heat transfer belowground (DeBano *et al.*, 1998; Neary *et al.*, 1999). Smoldering and glowing combustion temperatures of ground fires can reach 400 to 760°C, but instantaneous temperatures in excess of 1500°C can often occur (Neary *et al.*, 1999). Although the slow movement of this type of fire may affect a small area, it also can have profound soil impacts. Through this process, small areas of soil associated with the complete burning of large, downed woody debris or stumps in direct contact with the soil are oxidized to a reddish color.

These severely burned, reddened sites commonly are found as long, narrow, linear strips ranging from two to over ten feet long and 2 to 14 in wide created as downed logs were consumed by fire; or also can be found as deep, irregular patches sometimes over 20 in in diameter, where stumps and root wads were consumed by fire (Shank, 2004). Smoldering conditions which create red soils are capable of volatilizing soil nutrients to the extent that nutrient availability and plant growth could be reduced in the short or long term (Neary *et al.*, 1999). In addition, severe soil burning not only causes mortality of most soil organisms and plant roots, but also may cause an

alteration of physical properties, such as changes in nutrient cycling patterns (Neary *et al.*, 1999), for a variable period of time after the fire.

Fire has been shown to have negative impacts on soil organisms and has been hypothesized to totally or partially sterilize the soil immediately after a fire (Ahlgren, 1974). This hypothesis has since been confirmed with a large body of research showing evidence of reductions in microbial populations. For example, Pattinson *et al.* (1999) found that fire significantly reduced the levels of bacteria, actinomycetes, and mycorrhizal fungal propagules in the top few centimeters of the soil. Smith *et al.* (2004, 2005) found that overly severe prescribed fires significantly reduced mycorrhizas compared to less severe fire or non-burned treatments.

Alluding to the occurrence of severely burned red soil, Klopatek *et al.* (1988) suggested that high fuel loads in forested ecosystems generate more intense and prolonged burning and incur significant losses of arbuscular mycorrhizal (AM) fungi, and hence impact soil microbial communities as a whole. Time to full recovery of soil biota after fire is uncertain. Some fungi such as fire loving, phoenicoid fungi (Carpenter and Trappe, 1985), emerge only after heat stimulation. Phoenicoid fungi form thick-walled chlamydospores that persist in the mineral soil and survive fire (Baar *et al.*, 1999). Due to limited research on the occurrence of red soil after wildfire, information on slash pile burning effects on soil chemistry, microbial communities, and plant re-colonization inside the slash scar is frequently used as a surrogate for speculating about the role of wildfire induced red soils in a forest.

A common inquiry has been the length of time post-fire that severe soil heating will affect the microbial community structure. Microorganisms have varying

responses following fire (Neary *et al.*, 2005). As a result, the microbial community structure and length of time required before the forest floor microbial community reaches pre-burn levels is unclear. Several researchers have indicated that the decline of soil microbial populations due to fire is rapid and usually transitory, with levels increasing to pre-fire levels within 1-4 yrs after wildfire (Vazquez *et al.*, 1993; Acea and Carballas, 1996; Prieto-Fernandez *et al.*, 1998). Others researchers have recorded a more lengthy recovery time, possibly even up to 12 years (Fritze *et al.*, 1993; Mabuhay *et al.*, 2006a).

1.3 Fire effects on plant populations

Wildfire has been documented to facilitate the introduction of disturbance-dependent (Vitousek *et al.*, 1996) and generalist (Parks *et al.*, 2005) plant species, but the role of fire severity on the introduction and establishment of non-native plants remains unclear (Parks *et al.*, 2005). For example, a recent study of succession after wildfire in a *Pinus ponderosa* Laws. forest in central Montana (Cooper and Jean, 2001), found that high-severity fires result in increased numbers of non-native species. Invasive plants have been documented to have high survival rates, regardless of burn severity; this observation might be due to the fact that most severe fires typically damage roots only 4 in below the soil, while many “weedy” plant species roots penetrate far below this depth (Goodwin and Sheley, 2001).

1.4 Impacts of soil microbes on plant communities

Overwhelming evidence has indicated belowground biotic interactions play a significant role in plant-plant interactions (Allen, 1991; van der Heijden *et al.*, 1998b; Hartnett and Wilson, 2002; Bever, 2003; Hart *et al.*, 2005). These interactions can be

direct or indirect, and range from positive (mycorrhizal fungi) to negative (soil pathogens) (Jones and Smith, 2004; Rillig, 2004). Thus, difficulty arises when determining the role of soil organisms in plant community dynamics. Added complexity is due to the largely undescribed diversity of soil organisms and the technical problems associated with their measurement and manipulation (Bever, 2003).

Over 80% of all terrestrial plant species live in association with arbuscular mycorrhizal (AM) fungi (Smith and Read, 1997). Hyphae of the AM fungi penetrate into plant cortical cells and form branching arbuscules, increasing surface area for nutrient exchange within the plant cell (Peterson *et al.*, 2004). Plants have been documented to respond differentially to colonization by AM fungal mutualists (Sanders and Fitter, 1992; Adjoud *et al.*, 1996; Smith and Read, 1997; Jones and Smith, 2004) or have a differing degree of “mycorrhizal dependency” (van der Heijden *et al.*, 1998a). Likewise, AM fungi also have variable responses to association with individual plant species. For example, AM fungi have the ability to associate with a broad range of plant hosts, but in multiple studies AM fungi have been shown to elicit host-specific growth responses (Bever *et al.*, 1996; Eom *et al.*, 2000; Bever, 2002). Bever (2002) hypothesized that the dynamics within the AM fungal community contributes to the maintenance and diversity within plant communities. Recently, this hypothesis has been demonstrated by several studies (Klironomos, 2002; Stampe and Daehler, 2003; Rillig, 2004).

1.5 Soil biota and exotic plant invasion

The role of soil microbial communities in the invasion process recently has received increased attention from ecologists (Marler *et al.*, 1999; Callaway *et al.*, 2001; Klironomos, 2002; Callaway *et al.*, 2004; Klein *et al.*, 2006; Meiman *et al.*, 2006; Siguenza *et al.*, 2006), yet a fundamental understanding of these interactions remains unclear. Although the specific mechanisms of how soil fungi regulate interactions among plant species is largely unknown, recent research suggests that soil fungal communities determine patterns of plant abundance and invasion success both through negative and positive feedbacks (Klironomos, 2002; Callaway *et al.*, 2004). Callaway *et al.* (2004) proposed a highly species-specific mechanism that allows invasive plants to compete with their neighbors by carbon (C) parasitism via AM fungi. Similarly, in a recent study Carey *et al.* (2004) demonstrated that the interactions between plants and their AM fungal symbionts provide a substantial competitive advantage to the invasive forb *Centaurea maculosa* Lam., by a mediated transfer of C from a native bunchgrass, *Festuca idahoensis* Elmer. Other studies have reported similar findings on the effects of AM fungi and *C. maculosa* (Marler *et al.*, 1999).

Another hypothesized mechanism of invasion suggests that some exotic invaders have escaped control by local soil pathogens and may benefit from soil microbes in invaded regions (Klironomos, 2002). For example, Callaway *et al.* (2004) found that sterilization of soils collected from under populations of *C. maculosa* in its native European range caused an average 166% increase in the total biomass of this forb. In contrast, sterilization of soils collected from under populations of *C.*

maculosa in their non-native northwestern USA range increased their growth only by an average of 24%.

Other non-native invasive plants, such as *Bromus tectorum* L. (Goodwin, 1992), benefit from being facultative for AM fungi (Richardson *et al.*, 2000). Following a major disturbance, their flexibility in response to mycorrhizas (Goodwin, 1992) enables them to establish as non-mycorrhizal plants when inoculum is low and persist by out-competing native mycorrhizal plant species as inoculum levels increase (Richardson *et al.*, 2000). With many recent studies showing the alteration in soil microbial community composition in association with the invasion of non-native plant species (Bever *et al.*, 1997; Belnap and Phillips, 2001; Kourtev *et al.*, 2002; Levine *et al.*, 2003; Zak *et al.*, 2003; Wardle *et al.*, 2004; Wolfe and Klironomos, 2005; Hawkes *et al.*, 2006), as well as changes in soil properties (Ehrenfeld, 2003), it is evident that exotic invasions can have profound and unpredictable effects on ecosystem processes and soil microbial communities.

Specific AM fungi may be essential for the establishment of some plant species; therefore plant diversity within a highly disturbed site may depend not solely on the presence of mycorrhizal fungi, but also on the functional and taxonomic diversity of these fungi (van der Heijden *et al.*, 1998b). It is also possible that plant succession is paralleled by succession of their associated fungal communities (Bever *et al.*, 2001).

1.6 PLFA analysis

Phospholipids are components of cell membranes found only in living cells. Specific groups of soil microbes have signature fatty acids in their biomass that can be

extracted from the soil and used to detect the presence and abundance of these groups (Wolfe and Klironomos, 2005). Phospholipid fatty acid (PLFA) analysis can detect changes in broad groups of soil organisms, such as bacterial and fungal groups, but cannot resolve changes in community composition at the species level. PLFA analysis is a technique commonly used to indicate changes in microbial communities (Zelles, 1999), is visualized by multivariate statistical methods, and has been used to describe changes in microbial community structure in plant invasion studies (Kourtev *et al.*, 2002; Kourtev *et al.*, 2003). Although a commonly used method, few studies have used PLFA analysis to evaluate soil microbial community changes after fire (Pietikainen *et al.*, 2000). To my knowledge, no studies have yet been published on the soil microbial community in severely burned red soils.

1.7 Objectives

Several questions were assessed, comparing severely burned red soil with moderately burned black soil, in this study: 1a) Are there differences in soil microbial community composition between burn severities? 1b) Are there differences in soil chemistry between burn severities? 2a) Are there growth differences between native and non-native plant species with respect to the soil burn severity in which they were grown in? 2b) Are there differences arbuscular mycorrhizal fungi colonization between native and non-native plant species with respect to the soil burn severity in which they were grown in?

EFFECTS OF BURN SEVERITY ON SOIL MICROBIAL COMMUNITIES AND INVASIVE PLANT SPECIES AFTER WILDFIRE IN THE CASCADE RANGE OF OREGON.

2.1 Introduction

With the increase of stand replacing wild fires in the last decade, forest researchers have become interested in the effects of fire severity on forest ecosystem resources, including soil organic matter, soil nutrients, and soil biota (Turner *et al.*, 1997; Cromack *et al.*, 2000; Feller, 2000; Hart *et al.*, 2005). Small areas of soil associated with the complete burning of large downed woody debris or stumps in direct contact with the soil can be found scattered throughout most burned areas. These extensively burned soils have a distinctive color change where the top layer of mineral soil changes to various shades of red due to excessive heating and oxidation of the soil matrix.

Little information exists about the effects of red soil created by a severe wildfire, on the re-establishment of soil microbial and plant communities. It has been demonstrated that the abundance of soil fungi is greatly influenced by the severity of a disturbance (Korb *et al.*, 2004). Fungi have a direct influence on plant community structure and productivity (van der Heijden *et al.*, 1998a). Post-fire landscapes have been shown to facilitate the establishment of invasive, non-native plant species (Vitousek *et al.*, 1996; Korb *et al.*, 2004), whose growing numbers have concerned land managers for nearly a century (Harrod and Reichard, 2001). In particular, land managers have become increasingly concerned with the invasion of non-native plant species in the mountain ecoregions of northwest USA (Parks *et al.*, 2005), especially after high severity surface fire, which has the potential to create red soil conditions.

While the impacts of exotic plant invasions (e.g. competition and loss of biodiversity) on ecosystem structure are evident, little has been documented regarding the alteration of belowground ecosystem processes as a function of the introduction of non-native plant species (Ehrenfeld and Scott, 2001). Finally, the high numbers of fire-killed trees after a severe wildfire is a concern for forest managers because large amounts of dead wood left after fire may increase the potential for additional red soil in the event of a reburn (Poff, 1989; Shank, 2004).

This study investigated the soil microbial community across recently burned forests on the eastern slope of the Cascade Range in Oregon to determine the effects the belowground community has on the growth of native and non-native plant species in severely and less severely burned soil. Specifically, this study addressed the differences in: 1a) soil microbial community composition between burn severities. 1b) soil chemistry between burn severities. 2a) growth of native and non-native plant species with respect to the soil burn severity in which they were grown. 2b) colonization of arbuscular mycorrhizal fungi in native and non-native plant species with respect to the soil burn severity in which they were grown.

2.2 Methods

2.2.1 Study area

This study was conducted within the Booth and Bear Butte (B&B) Fire Complex on the eastern slope of the Cascade Range of Oregon in the Deschutes National Forest. The B&B Fire burned 36,733 ha in the summer of 2003. The area is characterized by an overstory of *Pinus ponderosa*, *Pseudotsuga menziesii* (Mirb.) Franco., *Abies concolor* (Gord. & Glend.) Lindl. Ex Hildebr., *Pinus monticola* Dougl.

ex D. Don, *Abies grandis* (Dougl. ex D. Don) Lindl. and *Larix occidentalis* Nutt., and an understory of *Ceanothus velutinus* Dougl., *Rosa gymnocarpa* Nutt., *Symphoricarpos albus* (L.) Blake, *Mahonia nervosa* (Pursh) Nutt., *Rubus ursinus* Cham.& Schlecht and *Vaccinium parvifolium* Sm. Soils are Aquic Vitrixerands and Alfic Vitrixerands. Elevations range from 1000-1300 m. Average temperatures range from -1 °C in the winter months to 20 °C in the summer months. Average annual precipitation ranges from 50 to 150 cm. Most of the precipitation, about 70 percent, falls during November through April. During the driest months (July, August, and September) the average monthly precipitation is less than 3 cm.

2.2.2 Plot descriptions

One year post-fire, in August 2004, ten site blocks were randomly selected from 30 previously established areas within the fire perimeter. Within each of the ten site blocks, two paired burn severity treatments were present: severely burned “red” soil and adjacent moderately burned “black” soil, for a total of 20 soils (Figure 2.1). A suitable plot contained enough red and black soil to allow for the removal of at least 6.5 kg of soil of each severity from the top 5 cm of soil.

Soil was collected from each paired burn severity treatment plot within each of the ten site blocks and placed into plastic bags for transport back to the lab and stored at 4 °C until microcosm use. A subset of each soil sample was sieved (< 2 mm) and sent to the OSU Central Analytical Laboratory for chemistry analysis. Twenty additional grams of soil were sieved (< 2 mm) and immediately freeze-dried and stored at -20 °C for PLFA analysis.

2.2.3 Soil chemistry

Soil samples were analyzed for: pH (Thomas, 1996); cation exchange capacity (CEC) (cmol_c/kg) (Sumner and Miller, 1996); total phosphorus (P) (ppm) (Kuo, 1996); available nitrate N (NO₃⁻ - N) (ppm); initial extractable mineral N (NH₄⁺-N) (ppm) (Hart *et al.*, 1994); anaerobic incubation N (ppm); net mineralizable N (incubated N minus NH₄-N) (ppm) (Bundy and Meisinger, 1994); total N (%) (Bremner, 1996); and total C (%) (Nelson and Sommers, 1996) using a LECO CNS 2000 Analyzer (LECO Corp., St. Joseph, MO).

2.2.4 Growth room study

Three native plant species with early successional behavior occurring on the study sites were selected: *Ceanothus velutinus*, *Epilobium angustifolium* (L.), and *Elymus elymoides* (Raf.) Swezey. In addition, three non-native plant species that are of concern in the study area were also selected: *Bromus tectorum*, *Brachypodium sylvaticum* (Huds.) Beauv., and *Centaurea maculosa*. Seeds were collected from the areas of occurrence in the region by either myself, or others.

C. velutinus seeds were scarified in 1 L of water at 95 °C, brought to room temperature, soaked in the water for 24 hrs, transferred to 8% water agar plates and stored in the dark at 4 °C for 90 days, then placed under 40 watt gro-lux[®] wide spectrum fluorescent bulbs until germination. Seeds from all other species were surface sterilized with a 0.2% concentration of the DuPont manufactured fungicide Benlate[®], rinsed with distilled water and germinated on 8% sterile water agar plates. Seedlings of each species were removed from their seedcoats and planted separately into 4 inch plastic pots filled with unsieved soil samples (10 site blocks x 2 burn severities x 6 species = 120 pots).

Seedlings were grown in a growth room under 40 watt gro-lux[®] wide spectrum fluorescent bulbs with a 14 hr light/10 hr dark photocycle and watered when necessary for 10 weeks. At harvest, 20 g of soil from each pot was sieved to 2 mm and immediately freeze-dried and stored at -20 °C until PLFA analysis.

2.2.5 Shoot biomass and root colonization by AM fungi

Plant shoots were oven-dried at 37 °C for 12-14 days and weighed to measure within species differences in plant biomass between burn severities. Entire root systems were assessed for colonization of AM fungi and therefore were not dried and weighed. Roots were cleared and stained using a modified Phillips and Hayman (1970) procedure to quantify AM fungal colonization. Roots were washed free of soil, put in 10% KOH at room temperature for 48 hours, rinsed thoroughly with water, neutralized in 1% HCl for 30 minutes and rinsed thoroughly with water. Roots were stained in 0.05% trypan blue in lactoglycerol overnight, again rinsed thoroughly with water, and stored in lactoglycerol until quantification (Phillips and Hayman, 1970).

Quantification of AM colonization was accomplished by direct estimation of the percentage of root length colonized by fungi (Biermann and Linderman, 1981), the most widely used method of determining AM fungal colonization (Hart and Reader, 2002). Cleared and stained roots were placed in grid-lined petri dishes and inspected at 10 - 40 x magnification with stereo-microscopy. More detailed observations were made by mounting root segments in lactoglycerol on microscope slides and examining at 100 – 400 x magnification with compound microscopy. Total length of each root and total length of root colonization were estimated and expressed as a percentage of colonization by AM fungi (Rajapakse and Miller Jr., 1992).

2.2.6 *Vegetation survey*

A survey of vegetation on the two paired burn severity treatment plots was conducted on all ten site blocks two years post-fire in July, 2005. Percent cover of low herbaceous species was assessed according to a modified Quadrat-Charting Method (Mueller-Dombois and Ellenberg, 1974), using a frame (1 m²), drawn to scale on a sheet of graph paper. This was done by subdividing the square meter into 100 decimeters² and by numbering the coordinates of the quadrature frame accordingly from 1 to 10. The frame was set on top of each black and red soil paired treatment plots, percent vegetation cover was recorded, individual plants identified to genus, and constancy (# plots found in / total plots) was calculated for each species identified.

2.2.7 *PLFA analysis*

A hybrid procedure of PLFA and fatty acid methyl ester (FAME) analysis was used to characterize microbial community composition in the initial fresh and post-harvest soil samples (Fraterrigo *et al.*, 2006). Membrane lipids were extracted from 4 g samples of lyophilized soil with chloroform-methanol, phosphate buffer [potassium phosphate (3.6mL), methanol (8mL), and chloroform (4mL)] in glass tubes (25mL), shaken for 1 hr and centrifuged. Supernatant was then decanted to glass tubes (30mL) and potassium phosphate buffer and chloroform were re-added, and the tubes were vortexed for 30 sec. The phases were allowed to separate overnight at room temperature. The top layer was aspirated off (saving the chloroform phase), and volume was reduced in a RapidVap (Labconco Corporation, Kansas City, Missouri, USA). Then, samples were purified following the procedure for FAMEs as given by Microbial ID, Inc. (Hayward, California, USA). Sodium hydroxide was added for

saponification and the solution heated in a water bath for 30 min, followed by mild alkaline methanolysis (Fraterrigo *et al.*, 2006).

Microbial profiles were identified by analyzing the methyl-ester derivatives from the phospholipid extractions on a gas chromatograph (Hewlett-Packard 6890) equipped with a flame ionization detector and split/splitless inlet and a 25 m × 0.2 mm inside diameter × 0.33 μm film thickness Ultra 2 (5%-phenyl, 95% methyl) capillary column (Agilent) using H₂ as the carrier gas, N₂ as the make-up gas, and air to support the flame. Gas chromatograph conditions were set by the MIDI Sherlock program (MIDI, Inc., Newark, Delaware, USA). Peaks were identified with bacterial fatty acid standards and Sherlock peak identification software (MIDI, Inc., Newark, Delaware, USA). Fatty acids were quantified by comparisons of peak areas from the sample compared to peak areas of two internal standards, 9:0 (nonanoic methyl ester) and 19:0 (nonadecanoic methyl ester), of known concentration (Fraterrigo *et al.*, 2006).

Fatty acids are designated by the total number of C atoms: number of double bonds, followed by the position of the double bond from the methyl end (ω) of the molecule. The prefixes “i” and “a” indicate iso- and anteiso- branching, respectively. Cyclopropane fatty acids are designated by the prefix “cy” and “Me” indicates methyl branching. Total PLFA concentration (nmol PLFA/g C) was used as an index of the total viable microbial biomass. The sum of indicator fatty acids (expressed as mol %) associated with gram-positive (G+) and gram-negative (G-) bacteria, actinomycetes, ECM fungi, AM fungi and protozoa were used as broad taxonomic microbial groupings (Table 2.1). Fungal: bacteria PLFA ratios also were used as a biomass

index to indicate of the changes in the ratio of fungal to bacterial biomass as a function of burn severity (Baath and Anderson, 2003).

2.3 Data analysis

2.3.1 Analysis of shoot growth, AM colonization, % cover and soil chemistry data

Differences in plant shoot growth and percent root colonization by AM fungi between plants grown in soil from the two paired burn severity treatment plots within individual species from each site block were tested with Analysis of Variance (ANOVA). An ArcSine Square Root transformation of the percent root colonization data was necessary to meet model assumptions. No other transformations of the data were necessary. All ANOVA tests were performed on two response variables: soil severity type (red or black) and plot. Differences between burn severity for soil chemistry and percent vegetation cover were tested with paired t-tests. Both ANOVA and t-tests were performed using S-Plus statistical software version 7.0 (Mathsoft, 1988-1999). Linear correlations of plant shoot biomass and soil chemistry were analyzed and the appropriate multiple linear regression model was selected using S-Plus statistical software version 7.0 (Mathsoft, 1988-1999).

2.3.2 Analysis of PLFA data

All statistical analyses of PLFA data were performed using the PC-ORD version 5.0 software package (McCune and Mefford, 1999). PLFA data were relativized by sample unit totals to represent relative abundance (mol % of total PLFA) for each of the 36 identified PLFAs. Mol %, rather than absolute abundance, is most commonly reported and allows for comparisons of community structure

without the effects of differences in microbial biomass or uncertain extraction efficiencies among samples.

Two matrices were used in the original fresh soil analysis: the species matrix (20 sample units x 36 PLFAs) and a second matrix containing explanatory variables (20 sample units x 17 variables) (Table 2.2). Two matrices also were used in the post-harvest soil analysis: the species matrix (114 sample units x 36 PLFAs) and a second matrix containing explanatory variables (114 sample units x 13 variables) (Table 2.3).

Explanatory variables used in matrices include: two categorical variables representing sites and binary indicators for red and black burn severity; quantitative variables representing ten microbial groups: fungi, bacteria, gram-positive bacteria, gram-negative bacteria, actinomycetes, protozoa, AM fungi, total PLFA abundance, fungi to bacteria ratio, and a miscellaneous group for the remaining fatty acids not associated with any particular group. Five additional quantitative chemical variables of the soil: CEC, NO₃-N, NH₄-N, %C, and %N, were used only in the fresh soil environmental matrix.

Microbial community structure was examined using non-metric multidimensional scaling (NMS) with the Sørensen distance measure (Kruskal, 1964; Mather, 1976). NMS is preferred over other ordination methods for community data sets such as principal component analysis because it does not assume normal distribution of the data and ranked distances relieve the “zero-truncation” problem often found in community data sets (McCune and Mefford, 1999). The goal of NMS is to search iteratively for the best sample unit configuration in a reduced ordination

space that minimizes the “stress” or difference with their positions in original species space (McCune and Mefford, 1999).

NMS analyses were run using the “slow and thorough” setting in the autopilot mode with a random starting configuration seeded by the computer’s clock. Forty runs with real data were carried out and Monte Carlo simulations were conducted using 50 randomized runs and a stability criterion of 0.0001. The number of dimensions chosen in the model was assessed by comparing the NMS runs with the real data to Monte Carlo simulations with random numbers. The proportion of variation represented by each axis was assessed by calculating the coefficient of determination (r^2) between distances in the ordination space and Sørensen distances in the original distance matrix. The relationships between plots in ordination space and their corresponding environmental variables were assessed by overlaying the variables as a joint plot.

Blocked multi-response permutation procedure (MRBP) (Mielke, 1991) with Euclidean distance tested the hypothesis of no difference in microbial community structure between burn severities across the 10 site blocks. Average distance function commensuration was incorporated in the MRBP to equalize the contribution of each variable to the distance function, in a sense relativizing the data. Median alignment also was incorporated to focus the analysis on within-block differences among treatments.

Results from the MRBP were compared to a permutation-based multivariate analysis of variance (perMANOVA) with randomized complete block design using the Sørensen distance measure to test the null hypothesis of no difference between the

burn severities. This method is an alternative to MRBP, allowing the use of the non-Euclidean distance measures, such as Sørensen distance, in multifactor designs (Anderson, 2001).

Linear correlations of microbial abundance, measured by total PLFA, and soil chemistry variables were analyzed and the appropriate multiple linear regression model was selected using S-Plus statistical software version 7.0 (S-plus).

2.4 Results

2.4.1 Soil chemistry

All chemical analyses, with the exception of $\text{NH}_4\text{-N}$, showed significant differences between the two burn severities (Table 2.4). Soil pH was highest in red soil, while soil P, $\text{NO}_3\text{-N}$, anaerobic incubation N, net mineralizable N, CEC, total C and N were all highest in black soil.

2.4.2 Shoot growth and AM colonization results

After approximately ten weeks of growth, the average shoot biomass for each of the three non-native plant species was significantly greater when grown in black soil (Table 2.5). However, in contrast, shoot biomass of the three native plant species did not significantly differ between burn severities. Linear correlations were found between shoot biomass and soil nutrients for the non-native plant species. Eighty-four percent of the observed variance ($p < 0.001$, $n = 20$) in the shoot biomass of the invasive grass, *B. sylvaticum*, was explained by linear regression of soil NH_4 ($p < 0.01$) and C ($p < 0.01$) after accounting for multicollinearity between soil chemical measurements. Eighty-three percent of the observed variation ($p < 0.001$, $n = 20$) in *B. tectorum* seedlings shoot biomass was explained by linear regression of soil C

($p < 0.001$) and NH_4 ($p < 0.01$) after accounting for multicollinearity between soil chemical measurements. With a similarly high correlation, eighty-eight percent of the observed variance ($p < 0.001$, $n = 20$) in the shoot biomass of *C. maculosa* seedlings was explained by the linear regression of soil P ($p < 0.01$), C ($p < 0.001$) and NH_4 ($p < 0.01$) after accounting for multicollinearity between soil chemical measurements. In contrast, native plant species shoot biomass did not show linear correlations with soil nutrients, except *C. velutinus*, which showed correlations ($R^2 = 0.84$, $p < 0.001$, $n = 20$) with C ($p < 0.001$) and P ($p < 0.05$) after accounting for multicollinearity between soil chemical measurements.

Percent root colonization by AM fungi for each plant species was highly variable, but typically was greatest in plants grown in black soil (Table 2.6). The greatest difference was found in the non-native species *C. maculosa*, with an average of 47.2% colonization in plants grown in black soil, compared to an average of 1.2% colonization in plants grown in red soil ($p < 0.01$). *C. velutinus* showed no indication of colonization by AM fungi, but did form dense root hairs not found in other plant species in this study.

2.4.3 Vegetation survey

The percent vegetative cover 2 yrs post-fire was significantly greatest on black soil plots ($p < 0.01$) (Table 2.7). The average cover on red soil plots was 24.7%, while the black soil plots contained twice the cover with an average of 51%. Sixteen plant species were identified on the 20 paired 1 m² permanent plots. Of those species, only three (*C. velutinus*, *E. angustifolium* and *R. ursinus*) had constancy > 0.4 (Table 2.8).

2.4.4 PLFA analysis

NMS of PLFA signatures of soil microbial communities from freshly collected red and black soil indicated that a two-dimensional solution best represented the data in reduced ordination space with final stress of 6.3 (Monte Carlo = 14.3, $p < 0.01$). After 87 iterations, the final configuration was deemed stable (final instability < 0.001).

The ordination showed significant separation between the microbial community with respect to burn severity (Fig. 2.2) (MRBP: $p < 0.01$; $A = 0.07$) (PerMANOVA: $p = 0.01$, $F = 5.3$). No separation was apparent between soil collection plots (MRBP: $p = 0.23$, $A = 0.02$) (PerMANOVA: $p = 0.33$, $F = 1.1$). Axis 1 corresponds to burn severity and showed a strong positive correlation with total PLFA ($r = 0.695$), actinomycetes ($r = 0.845$), AM ($r = 0.67$) and protozoa ($r = 0.553$), and had a strong negative correlation with total bacteria ($r = -0.854$) and G- bacteria (-0.866). Axis 2 had weak correlations with all variables.

NMS of microbial communities PLFA signatures' from red and black soil harvested from pots after 10 wks of growth of the six plant species indicate a two-dimensional solution best represented the data in reduced ordination space with final stress of 11.3. After 152 iterations, the final configuration was deemed stable (final instability < 0.001).

Ordinations of post-harvest data displayed trends similar to those of the freshly collected soil, with significant separation between the microbial community with respect to burn severity (MRPP: $p < 0.01$; $A = 0.11$) (Fig. 2.3). Unlike the ordinations of microbial community structure from freshly collected soil, there was significant separation between the microbial communities with respect to plot (MRPP: $p < 0.01$,

A = 0.09) (Fig. 2.4), as well as separation between the particular plant species (MRPP: $p = 0.04$; A = 0.01) (Fig. 2.5).

Linear correlations were found between microbial biomass (measured by total PLFA) and several corresponding soil nutrients. Eighty-three percent of the observed variance ($p < 0.001$, $n = 20$) in microbial biomass was explained by linear regression of soil %C ($p < 0.0001$) after accounting for multicollinearity between soil chemical measurements (Fig 2.6).

2.5 Discussion

2.5.1 Soil microbial community through PLFA analysis

Soil burn severity was the most important determinant of microbial communities tested in our study. Results from PLFA analyses showed that the soil microbial community structure of severely burned red soil differed from that of the surrounding moderately burned black soil both in freshly collected soil and in soil collected after ten wks of plant growth. Total microbial abundance in the freshly collected soil was 60% less in the red soil compared to black soil (Table 2.9). Microbial biomass (total PLFA) was highly correlated with % C in the soil (Fig. 2.6), after accounting for multicollinearity within soil nutrients. Red soil contained significantly lower amount of C than black soil, likely contributing to the microbial community differences between red and black soil. These results are consistent with previous findings indicating that substantial changes in soil temperature during heating can result in volatilization of C and soil nutrients, mortality of soil microbes and shifts in species composition of survivors (Baath *et al.*, 1995; Pietikainen *et al.*, 2000).

In this study, the fungal to bacterial ratio was 35% and 38% greater in red soil compared to black soil in fresh (Table 2.9) and post-harvest soil (Table 2.10), respectively. This result is surprising, as previous research has shown that fire generally results in an increase in bacteria (Ahlgren and Ahlgren, 1960; Vazquez *et al.*, 1993; Pietikainen and Fritze, 1995; Mabuhay *et al.*, 2006a) and a decrease in fungal populations (Baath *et al.*, 1995; Pattinson *et al.*, 1999; Korb *et al.*, 2004). While it is unclear why the fungal to bacterial ratio increased in the severely burned red soil, it is possible that a species of phoenicoid fungi (Carpenter and Trappe, 1985) proliferated in the red soil. Indeed, species of phoenicoid fungi have been identified from the severely burned soil in this study (J. Smith and D. McKay, unpublished data). Our findings are in agreement with a recent study by Mabuhay *et al.* (2006b) who detected only fungi in soil collected 1 wk. post-fire, while bacterial biomass was not detected until 1 mo. post fire. Although PLFA analysis is effective for studying broad groups of microbial organisms (Bossio and Scow, 1998), further study using molecular methods should improve our knowledge of the fungal species inhabiting the soil after fire.

PLFA analysis of post-harvest soils in our study also revealed differences in the microbial community structure with respect to the specific site block where soil was collected (Fig. 2.4). This result is consistent with research indicating temporal and spatial variability in the composition of PLFA fraction and microbial community structure in a wide range of soil types (Steinberger *et al.*, 1999; Zelles, 1999; Fierer *et al.*, 2003) and is directly attributable to the burn severity variability of each plot. Significant differences in microbial communities also were observed between red and

black soils after growth of each plant species (Fig. 2.5). Previous research indicates that microbial communities are influenced by plant associations and in turn, soil microorganisms can differentially influence the growth of individual plant species (Wolfe and Klironomos, 2005; Li *et al.*, 2006).

2.5.2 Shoot growth and AM fungal colonization in native and non-native plants

With the exception of *C. velutinus*, colonization by AM fungi of all species examined in our study was greatest in plants grown in black soil, regardless of whether the plant was native or non-native (Fig. 2.7). Native and non-native plant shoot biomass, however, responded differently to AM fungal colonization. Within individual plant species, shoot biomass of non-native plants grown in red soil was significantly lower than that of the same non-native plant species grown in black soil, while native plant growth did not differ significantly between red and black soil (Fig. 2.8). Similarly, non-native plant species also showed strong linear correlations with soil nutrient availability, while native plants did not.

The non-native grasses, *B. tectorum* and *B. sylvaticum*, had generally low AM fungal colonization (on average $\leq 11\%$), whether grown in red or black soil. Even though mycorrhizal colonization of *B. tectorum* was low in black soil and virtually absent in red soil, this species showed a significant amount of growth in the severely burned red soil compared to the other species in this study. The unexpected growth response of *B. tectorum* in red soil may be explained by its facultative AM status (Goodwin, 1992). This aggressive invasive grass has been shown to establish as non-mycorrhizal plants when mycorrhizal inoculum is low (Goodwin, 1992), such as after a major soil disturbance. Shoot biomass of *B. tectorum* seedlings also was found to be highly correlated with soil nutrient availability. This finding could explain the sharp

growth differences observed between plants grown in the red and black soil, because C was significantly higher in black soil.

B. sylvaticum plants grown in red soil had substantially lower (73%) shoot biomass than those grown in black soil. The absence of literature on the mycorrhizal status of *B. sylvaticum* outside its native range makes it difficult to hypothesize about the growth responses observed, although we did observe low AM fungal colonization rates in *B. sylvaticum*, mostly occurring in plants grown in black soil. Research in this area has indicated that many plant species respond differently to colonization by AM fungi mutualists (Smith and Read, 1997; Jones and Smith, 2004); it is possible that *B. sylvaticum* plants require relatively little AM fungal colonization to benefit from the symbiosis. A more likely explanation of the growth reduction of plants grown in red soil could be linked to the observation that like *B. tectorum*, *B. sylvaticum* seedling biomass was also found to have a linearly correlation with soil nutrient availability, with many soil chemical variables being influential to seedling growth.

In contrast to the non-native grasses, the non-native forb, *C. maculosa*, had relatively high AM fungal colonization (on average 47%) when grown in black soil and, similar to the non-native grasses, had low colonization in roots of plants grown in red soil. Even though shoot growth of this species also was significantly greater in plants grown in black soil, a few individual plants grew well in red soil. Previous studies have indicated that AM fungi both directly (Carey *et al.*, 2004) and indirectly enhance the growth of *C. maculosa* by providing a link for C transfer from native vegetation to the non-native invader (Marler *et al.*, 1999). Although there is ample documentation that this species benefits from AM fungal mutualism, it seems that the

growth response of this species in our study is not reflected in the high colonization rates observed. Due to the variable response of many plant species to different AM fungal species (Smith and Read, 1997; Jones and Smith, 2004), it is possible that the particular AM fungal species residing in these soils may not be as effective in *C. maculosa* as other mutualists. Alternatively, the observed growth differences could be attributable to the linear correlation this species had with soil nutrient availability, with soil many soil chemical variables being beneficial to seedling growth in our study.

Growth responses of the pioneering native species selected for this study were surprisingly similar, regardless of fire severity, AM fungal colonization or plant type (e.g. grass, forb, woody shrub) (Figure 2.7, 2.8). The moderate AM fungal colonization (average 12%, with a range of 0 to 50 %) of *E. angustifolium* roots observed in only a few plants grown in black soil did not confer increased shoot biomass of plants grown in black soil compared to those in red soil. Pioneer species such as *E. angustifolium* are commonly non-mycorrhizal (Allen, 1991). Therefore, the lack of mutualism observed in our *E. angustifolium* plants was not surprising. Unlike *E. angustifolium*, high AM fungal colonization (average 20%) was observed in most *E. elymoides* plants grown in black soil, but again was not reflected in greater shoot biomass. Unlike the three non-native species, both *E. angustifolium* and *E. elymoides* shoot growth had no linear correlation with soil nutrient availability, suggesting an alternate explanation as to why no growth differences were recorded between plants of these two species grown in red and black soils.

The colonization response of the N-fixing woody shrub *C. velutinus* differed compared to the other native species. No AM fungal colonization or nodulation was

observed in the roots of any of these plants. Nodulation has been documented to be an age-related factor in *Ceanothus* plants and mycorrhizal colonization has been noted only on nodulated *Ceanothus* plants (Rose, 1976; Conard *et al.*, 1985). Therefore, the *C. velutinus* plants in our study may have been too young to produce these structures after only 10 wks. of growth. However, dense root hairs were observed on all *C. velutinus* roots, possibly compensating for lack of symbioses. Unlike the other native plants in this study, *C. velutinus* plants did show linear correlations with soil nutrient availability. This finding could explain the suggestive difference in growth response between *C. velutinus* plants grown in red and black soils.

The unexpected differential response of native and non-native plant species to soil nutrient availability could be explained by a theory proposed by Davis *et al.* (2000), based on Grime's triangular model of plant strategies (Grime, 1974). Davis's theory explains how plant communities become more susceptible to invasion with increased amounts of unused resources. The theory describes how disturbances, such as fire, both increase the amount of unused resources of a land area by reducing the rate of resource capture by the resident vegetation, and increasing the resource supply to non-native vegetation, thus heightening the invasibility of that area. Many invasive species tend to be R-selected ruderals which are associated with short life spans and high seed production and have evolved in severely disturbed but potentially productive environments (Grime, 1977). In this study, the invaders chosen were all R-selected ruderals and were observed to have increased growth over the native species rate with increasing nutrient availability and therefore could be validating this theory of invasive plant resource exploitation.

Recent research has shown that the relative growth rate of AM fungi as well as their ability to affect plant growth are highly host-specific and depend on the particular matching of host plant species and fungal species (Adjoud *et al.*, 1996; van der Heijden *et al.*, 1998a; Eom *et al.*, 2000; Bever, 2002; Reynolds *et al.*, 2003; Jones and Smith, 2004). Therefore, AM fungi have the potential to determine plant community structure (van der Heijden *et al.*, 1998a). These specific pairings could be extrapolated to explain the interactions of introduced plants and resident fungi, which may take diverse forms and promote or inhibit plant or fungal growth (Richardson *et al.*, 2000).

The variable but typically lower AM fungal colonization in red soil compared to black soil, is likely attributed to the death of symbiotic microorganisms when the upper organic layers are consumed during high-severity fire (Neary *et al.*, 2005). Hart *et al.* (2005) hypothesizes that fires burning through heavy fuel loads (thus increasing fire severity) are likely to lead to much larger reductions in AM abundance than lower intensity fires. Difficulties arise when one attempts to determine fire's effects on mycorrhizal fungi; since not only is quantifying these soil fungi difficult, but also fire does not uniformly heat the soil. Thus, soil samples from burnt areas are likely to contain highly variable quantities of propagules (Pattinson *et al.*, 1999). However, plants growing in high-severity soils are likely to be less colonized by AM fungi than plants in moderately-burned soils (Korb *et al.*, 2004).

2.5.3 Soil chemistry

Many attributes of soil chemistry differed between red and black soil. Soil pH in red soil was slightly higher compared to black soil. This pH increase in severely burned soil is due to organic acid denaturation at high temperatures (Certini, 2005)

and agrees with previous studies indicating higher pH values inside slash burning scars compared to scar edges and soil outside scars (Korb *et al.*, 2004). Conversely, soil P, CEC, total C and N, NO₃-N, as well as anaerobic incubation N and net mineralizable N were all significantly higher in black soil compared to red soil. It is well established that the total amounts of chemical elements rarely are increased by fire (Neary *et al.*, 2005). However, fire does alter the basic form of many chemical elements, often making them more available for the uptake by plants and other biological organisms (Neary *et al.*, 2005).

The decrement in soil P found in red soil is a result of the conversion of soil organic P to orthophosphate, the sole form of available P in the soil, increasing with burn severity (Cade-Menun *et al.*, 2000). The decrease of CEC in red soil was expected (Badia and Marti, 2003). The decrease in average NO₃-N in red soil could be due to reduced microbial abundance: especially nitrifying bacteria in the severely burned soil. In contrast, NH₄-N was variable from plot to plot and no significant difference was found between red and black soil levels, which could be explained by previous studies that showed the amounts of NH₄-N produced as a result of fire generally increase with the severity and duration of the fire (Korb *et al.*, 2004; Neary *et al.*, 2005). With each plot burning at varying temperatures, and thus severity, it was expected that the NH₄-N concentrations also would be variable. The documented sharp decrease in total soil N in red soil parallels previous research indicating that the total N volatilized during combustion is directly proportional to the amount of organic matter destroyed (Neary *et al.*, 2005).

2.5.4 Vegetation survey

Percent vegetative cover 2 yrs. post-fire was highest in black soil in all plots (Table 2.7). Other researchers have described the decrease in plant biomass with increasing fire severity for up to 2 yrs. post-fire (Feller, 2000). Our observations agree with the hypothesis that red soils will have slower vegetative re-colonization because the high severity burn would have consumed more AM fungal propagules, thus affecting the post-fire community (Rowe, 1983).

Several plant species, such as *C. velutinus*, *E. angustifolium* and *R. ursinus*, were found in relatively high abundance on red soil plots (Table 2.8). Burn severity influences microbial dynamics, soil nutrient availability, and soil aggregation (Whittle *et al.*, 1997); each of these may affect plant initiation and development. High-severity fires can destroy the entire organic horizon and seeds of some plant species (Whittle *et al.*, 1997), but some shrub species, such as *C. velutinus* (Conard *et al.*, 1985), and herbaceous plants such as *E. angustifolium* (Schimmel and Granstrom, 1996; Feller, 2000) are promoted by soil heat treatment (Christensen and Muller, 1975) and have been documented to increase in numbers with burn severity (Schimmel and Granstrom, 1996). Thus, conditions created by high-severity fire or slash burning may improve the establishment of these species (Whittle *et al.*, 1997).

No exotic plants were found on our plots 2 yrs. post-fire. This finding could be due to the lack of invasive species before the fire and hence no seed source to allow invasive species to propagate. Although it is possible that non-native species could establish there in the future, it is uncertain what factors may influence exotic plant colonization at a specific location. Many factors besides soil biota can influence the invasion process, including competition, propagule production and dispersal (Levine

et al., 2004). Because individual plant species react differently to soil biota in various ecosystem types, it is important not to generalize about which variables influence non-native plant species invasion. Future research should investigate how individual species respond to these variables in different ecosystem types.

2.6 Conclusions

Fire suppression in the last several decades has resulted in unprecedented organic matter accumulations on the landscape, causing an increase in large, intense wildfires. This increase in fuel load, in addition to the risk of a reburn consuming the dead timber left from previous fires, has the potential to increase severely burned red soil on the landscape after wildfire. Therefore, knowledge of the impact of severe surface burning on soil microbial communities and post-fire plant re-colonization is critical to forest recovery projects.

We found that the long duration of smoldering conditions creating red soils sampled in our study drastically altered both the soil nutrients and microbial community structure in these soils. These changes in soil properties and biota affected plant growth in our controlled growth chamber experiment, as well as vegetation colonization on the red soil plots in natural field conditions. We observed differential growth between native and non-native plant species when grown in soil from the two burn severities. For example, although AM fungal colonization was greatest in both native and non-native plant species grown in black soil, growth of the native plant species did not differ between black and red soil. Non-native plants showed reduced growth in red soil. Although it has been demonstrated that disturbance from fire increases the likelihood of non-native plant species invading a burn area, our results

do not support the notion that red soil conditions are more susceptible to colonization by non-native, invasive plant species after a severe wildfire.

While many factors in addition to mycorrhizal colonization, burn severity, and soil nutrient availability influence plant growth; our results suggest a variety of strategies that allow plants to grow in disturbed environments. Additionally, the effect of soil biota compared with other factors influencing the success of non-native, invasive plant species (e.g. dispersal, propagule production, competition with resident plant species, and interactions with other biota) is unknown. The interactions between fire severity, soil microbial communities, and exotic plant species invasion are complicated and dynamic. We know that microbial communities, especially mycorrhizal fungi, influence plant community structure. These same interactions are, in part, responsible for exotic plant species invasion. Disturbances, such as fire, reduce or change the belowground microbial communities, directly affecting plant community structure aboveground. Even with all the knowledge gathered thus far, we have yet to scratch the surface of these remarkable relationships.

Further experiments, such as the molecular identification of key fungal species contributing to plant success and the extension of similar experiments to the field, are needed to understand the mechanisms behind our results. Continued monitoring of red soil sites could further our understanding of the length of time needed for post-fire recovery in severely burned red soil. Our findings contribute to the expanding body of literature pointing to the importance of plants and soil microbe interactions as determinants of plant community structure and diversity.

2.7 Literature Cited

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Appendix A – Tables

Table 2.1: Indicator PLFAs and the corresponding microbial group for interpretation of PLFA community data.

Indicator PLFAs	Microbial Group
15:0i, 15:0a, 16:0i 17:0i, 17:0a	Gram-positive bacteria
16:1 ω 9, 16:1 ω 7c, 17:1 ω 9, 17:0cy, 18:1w7c, 19:0cy	Gram-negative bacteria
18:0 10Me, 17:0 10Me, 16:0 10Me	Actinomycetes
18:2 ω 6,9	Ectomycorrhizal (ECM) fungi
16:1 ω 5	Arbuscular mycorrhizal (AM) fungi
20:4 ω 6 , 20:2 ω 6	Protozoan

Table 2.2: Summary of variables used in the fresh soil second matrix.

Variable	Description
Groups	Categorical group indicators for sites, numbered 1-10
Severity	Binary indicator for “red” and “black” burn severity
EM	Sum of relative abundance of all fatty acids for ECM fungi (mol %)
AM	Sum of relative abundance of fatty acids for AM fungi (mol %)
Bacteria	Sum of relative abundances of gram-negative and gram-positive bacteria
G+	Sum of relative abundance of all fatty acids for gram-positive bacteria (mol %)
G-	Sum of relative abundance of all fatty acids for gram-negative bacteria (mol %)
Actinomycetes	Sum of relative abundance of all fatty acids for actinomycetes (mol %)
Protozoa	Sum of relative abundance of all fatty acids for protozoa (mol %)
F:B	Ratio of fungi to bacteria based on fatty acids listed above
PLFA	Sum of all fatty acids (nmol PLFA g ⁻¹ C)
MSC	Sum of all misc fatty acids not associated with any particular microbial group
CEC	Cation exchange capacity (cmol _c kg ⁻¹)
NO ₃ -N	Amount of nitrogen comprised of nitrate (ppm)
NH ₄ -N	Amount of nitrogen comprised of ammonium (ppm)
%C	Total carbon (%)
%N	Total nitrogen (%)

Table 2.3: Summary of variables used in the post-harvest soil second matrix.

Variable	Description
Groups	Categorical group indicators for sites, numbered 1-10
Severity	Binary indicator for “red” and “black” burn severity
Species	Categorical indicator for one of six plant species associations
EM	Sum of relative abundance of all fatty acids for ECM fungi (mol %)
AM	Sum of relative abundance of fatty acids for AM fungi (mol %)
Bacteria	Sum of relative abundances of gram-negative and gram-positive bacteria
G+	Sum of relative abundance of all fatty acids for gram-positive bacteria (mol %)
G-	Sum of relative abundance of all fatty acids for gram-negative bacteria (mol %)
Actinomycetes	Sum of relative abundance of all fatty acids for actinomycetes (mol %)
Protozoa	Sum of relative abundance of all fatty acids for protozoa (mol %)
F:B	Ratio of fungi to bacteria based on fatty acids listed above
PLFA	Sum of all fatty acids (nmol PLFA g ⁻¹ C)
Msc	Sum of all miscellaneous fatty acids not associated with any microbial group

Table 2.4: Paired t-test of differences in soil chemistry between two burn severities. Bolded text indicates a significant difference. SE denoted in parentheses (n = 10).

Response Variable	Severity	Average	t_9	p-value
pH	Red	7.9 (0.06)	5.18	< 0.001
	Black	7.3 (0.08)		
P (ppm)	Red	3.5 (0.36)	-3.43	0.007
	Black	12.2 (2.5)		
NO ₃ -N (ppm)	Red	1.4 (0.36)	-2.48	0.035
	Black	4.3 (1.1)		
NH ₄ -N (ppm)	Red	36.7 (10.1)	-1.40	0.193
	Black	45.3 (10.6)		
Incubation-N (ppm)	Red	44.5 (11.3)	-3.48	0.006
	Black	73.6 (12.5)		
Mineralizable-N (ppm)	Red	7.7 (1.6)	-6.66	< 0.001
	Black	28.3 (2.7)		
CEC (cmol _c kg ⁻¹)	Red	9.4 (1.3)	-5.29	< 0.001
	Black	18.3 (2.0)		
% C	Red	1.0 (0.16)	-8.16	< 0.001
	Black	3.5 (0.29)		
% N	Red	0.04 (0.01)	-7.90	< 0.001
	Black	0.13 (0.01)		

Table 2.5: Shoot biomass of each plant species between two paired burn severities. Bolded text indicates a significant p-value. SE found in parentheses (n = 10).

	Species	Severity	Average (kg)	F _{1,9}	p-value
Non-native	CEMA	Red	5.8 (2.2)	11.9	0.007
		Black	39.0 (9.4)		
	BRSY	Red	25.3 (6.7)	19.7	0.001
		Black	93.3 (15.4)		
	BRTE	Red	116.1 (25.0)	5.08	0.051
		Black	173.8 (18.8)		
Native	ELEL	Red	28.8 (6.8)	0.23	0.641
		Black	34.7 (6.3)		
	EPAN	Red	34.4 (13.8)	0.02	0.879
		Black	32.2 (5.5)		
	CEVE	Red	12.5 (3.9)	2.57	0.143
		Black	29.2 (9.1)		

CEMA (*Centaurea maculos*); BRSY (*Brachypodium sylvaticum*); BRTE (*Bromus tectorum*); ELEL (*Elymus elymoides*); EPAN (*Epilobium angustifolium*); CEVE (*Ceanothus velutinus*)

Table 2.6: Percent AM root colonization of each plant species between two paired burn severities. Bold text indicates a significant p-value. SE in parentheses(n = 10).

	Species	Severity	% Colonization	F _{1,9}	p-value
Non-Native	CEMA	Red	1.2 (0.55)	17.1	0.002
		Black	47.0 (10.77)		
	BRSY	Red	1.4 (0.88)	20.6	0.001
		Black	10.9 (2.76)		
	BRTE	Red	1.0 (0.85)	4.41	0.065
		Black	4.6 (1.87)		
Native	ELEL	Red	7.7 (5.57)	4.42	0.065
		Black	19.7 (4.05)		
	EPAN	Red	0.2 (0.13)	5.8	0.039
		Black	11.9 (5.97)		
	CEVE	Red	0.0	----	----
		Black	0.0		

CEMA (*Centaurea maculos*); BRSY (*Brachypodium sylvaticum*); BRTE (*Bromus tectorum*); ELEL (*Elymus elymoides*); EPAN (*Epilobium angustifolium*); CEVE (*Ceanothus velutinus*)

Table 2.7: Total cover of each meter² vegetation survey plots between burn severity.

Plot	Severity	% Cover
Jef-1	Red	28
	Black	78
Jef-5	Red	21
	Black	65
Jef-7	Red	19
	Black	25
Jef-10	Red	35
	Black	85
Jef-12	Red	25
	Black	53
BH-1	Red	18
	Black	35
BH-6	Red	13
	Black	28
1210-3	Red	23
	Black	48
1210-6	Red	27
	Black	43
1280	Red	38
	Black	50

Table 2.8: List of plant species observed in study plots with percent cover and constancy by severity.

Acronym	Species	Severity	% Cover	Constancy
ASTER	unknown asteraceae	Red	3.5	0.2
		Black	0.0	0.0
CEVE	<i>Ceanothus velutinus</i> Dougl.	Red	5.5	0.4
		Black	12.3	0.6
CIVU	<i>Cirsium vulgare</i> (Savi) Tenore	Red	1.0	0.1
		Black	0.0	0.0
CLSI	<i>Claytonia sibirica</i> L	Red	3.0	0.1
		Black	3.0	0.1
COPO	<i>Convolvulus polymorphus</i> Greene	Red	6.0	0.2
		Black	30.0	0.1
EPAN	<i>Epilobium angustifolium</i> L.	Red	12.9	0.8
		Black	19.4	0.7
FRVE	<i>Fragaria vesca</i> L.	Red	0.0	0.0
		Black	15.0	0.1
GRAS	unknown grass	Red	0.0	0.0
		Black	3.5	0.2
PHHA	<i>Phacelia merica</i> Dougl. ex Lehm	Red	8.0	0.1
		Black	1.0	0.1
PINK	unknown <i>Silene</i> spp	Red	3.0	0.1
		Black	3.0	0.1
PSME	<i>Pseudotsuga menziesii</i> (Mirb.) Franco.	Red	0.0	0.0
		Black	1.0	0.1
PTAQ	<i>Pteridium aquilinum</i> (L.) Kuhn.	Red	20.0	0.1
		Black	25.0	0.1
PYPI	<i>Pyrola picta</i> Sm.	Red	0.0	0.0
		Black	2.0	0.1
RUUR	<i>Rubus ursinus</i> Cham.& Schlecht	Red	13.8	0.4
		Black	35.0	0.4
SYAL	<i>Symphoricarpos albus</i> (L.) Blake	Red	0.0	0.0
		Black	7.7	0.3
VIAM	<i>Vicia americana</i> Muhl.	Red	10.0	0.1
		Black	25.0	0.1
Average % cover		Red	5.4	p = 0.001
		Black	11.4	

Table 2.9: Sum of PLFAs for groups of soil microorganisms given as both (Abs) absolute abundance (nmol PLFA g⁻¹ C) and (Rel) relative abundance (mol %) in freshly collected red and black soil at each site block. Groups are defined in Table 2.2.

Plot	Severity	PLFA	F:B	Bacteria		G +		G -		EMF		AMF		Actino		Proto	
		Abs		Abs	Rel	Abs	Rel	Abs	Rel	Abs	Rel	Abs	Rel	Abs	Rel		
Jef-1	Red	87.2	0.07	46.8	53.7	10.0	11.4	36.9	42.3	3.4	3.9	2.6	3.0	2.6	2.9	0.0	0.0
	Black	311.8	0.09	123.1	39.5	48.0	15.4	75.2	24.1	11.4	3.7	7.2	2.3	16.6	5.3	3.6	1.1
Jef-5	Red	55.9	0.22	28.1	50.3	3.3	6.0	24.8	44.3	6.2	11.0	1.3	2.3	1.7	3.1	1.2	2.1
	Black	437.7	0.06	133.9	30.6	43.2	9.9	90.8	20.7	8.3	1.9	10.5	2.4	26.6	6.1	10.0	2.3
Jef-7	Red	140.1	0.10	63.3	45.2	18.2	13.0	45.1	32.2	6.0	4.3	2.0	1.4	6.0	4.3	1.8	1.3
	Black	231.9	0.09	95.7	41.3	30.1	13.0	65.7	28.3	8.8	3.8	3.8	1.6	11.5	5.0	7.7	3.3
Jef-10	Red	282.1	0.29	78.0	27.7	27.1	9.6	50.9	18.0	22.3	7.9	26.1	9.3	20.9	7.4	30.0	10.6
	Black	377.4	0.07	145.8	38.6	53.4	14.2	92.3	24.5	10.6	2.8	4.9	1.3	16.5	4.4	18.3	4.9
Jef-12	Red	233.3	0.07	94.9	40.6	28.0	12.0	66.8	28.6	6.6	2.8	2.4	1.0	10.8	4.6	6.4	2.7
	Black	259.1	0.08	105.5	40.7	37.0	14.3	68.5	26.5	8.5	3.3	3.4	1.3	11.7	4.5	6.8	2.6
BH-1	Red	84.8	0.07	46.8	55.2	10.0	11.7	36.9	43.5	3.4	4.0	2.6	3.1	2.6	3.0	0.0	0.0
	Black	312.4	0.09	123.1	39.4	48.0	15.4	75.2	24.1	11.4	3.7	7.2	2.3	16.6	5.3	4.2	1.4
BH-6	Red	138.8	0.08	76.8	55.3	25.8	18.6	51.0	36.7	6.4	4.6	2.3	1.7	1.8	1.3	1.1	0.8
	Black	433.8	0.09	162.9	37.5	79.4	18.3	83.5	19.3	14.5	3.3	9.3	2.2	24.9	5.7	15.2	3.5
1210-3	Red	42.6	0.13	21.3	50.0	2.0	4.8	19.3	45.2	2.8	6.5	0.0	0.0	0.0	0.0	0.0	0.0
	Black	205.1	0.05	91.0	44.4	30.9	15.1	60.1	29.3	4.8	2.3	4.7	2.3	10.0	4.9	3.4	1.7
1210-6	Red	141.0	0.05	73.1	51.9	7.9	5.6	65.2	46.2	3.9	2.8	0.0	0.0	5.6	4.0	14.8	10.5
	Black	381.9	0.05	142.3	37.3	76.5	20.0	65.8	17.2	7.5	2.0	8.7	2.3	25.3	6.6	15.2	4.0
1280	Red	155.8	0.10	75.3	48.3	19.2	12.3	56.2	36.0	7.6	4.9	2.1	1.4	3.0	1.9	0.0	0.0
	Black	528.0	0.06	205.5	38.9	85.4	16.2	120	22.7	13.0	2.5	9.7	1.8	33.7	6.4	25.1	4.7

Table 2.10: Sum of PLFAs for groups of soil microorganisms given as both (Abs) absolute abundance (nmol PLFA/g C) and (Rel) relative abundance (mol %) in post-harvest red and black soil for each plant species grown. Microbial group abbreviations are defined in Table 2.3. (SE in parentheses, n = 10)

Species	Severity	PLFA	F:B	Bacteria		G +		G-		EMF		AMF		Actino		Proto		
		Abs		Abs	Rel	Abs	Rel	Abs	Rel	Abs	Rel	Abs	Rel	Abs	Rel	Abs	Rel	
Non-natives	CEMA	Red	97.2 (20.8)	0.13 (0.01)	44.0 (7.1)	49.8 (2.7)	11.3 (3.4)	9.8 (1.5)	32.7 (4.3)	40.0 (3.9)	5.5 (0.9)	6.7 (0.8)	1.9 (0.5)	2.0 (0.4)	3.9 (1.4)	2.8 (0.8)	1.2 (0.6)	0.7 (0.3)
		Black	300.3 (41.1)	0.07 (0.01)	109.0 (13.6)	37.5 (1.7)	39.5 (3.9)	13.9 (0.8)	69.5 (10.1)	23.6 (1.4)	6.9 (0.8)	2.4 (0.3)	18.1 (5.0)	5.7 (1.4)	17.3 (2.8)	5.8 (0.5)	7.7 (1.4)	2.6 (0.5)
	BRSY	Red	65.2 (15.7)	0.15 (0.04)	29.5 (6.5)	48.2 (1.9)	7.3 (2.2)	11.6 (2.0)	22.2 (4.7)	36.6 (2.2)	4.1 (1.0)	6.9 (1.6)	1.6 (0.5)	1.9 (0.5)	2.7 (1.1)	3.1 (0.8)	0.9 (0.5)	0.7 (0.4)
		Black	213.4 (43.3)	0.10 (0.01)	74.6 (16.5)	34.0 (2.3)	32.1 (7.9)	13.9 (1.1)	42.5 (8.8)	20.1 (1.5)	6.9 (1.6)	3.3 (0.3)	11.1 (2.7)	5.4 (1.3)	11.7 (3.3)	4.7 (0.6)	8.7 (2.7)	4.2 (1.3)
	BRTE	Red	97.5 (16.1)	0.13 (0.02)	46.6 (6.3)	50.8 (2.5)	12.2 (2.3)	12.5 (1.3)	34.4 (4.6)	38.3 (2.6)	5.8 (0.6)	6.7 (0.9)	1.9 (0.4)	1.7 (0.3)	3.9 (1.1)	3.4 (0.6)	1.0 (0.4)	0.8 (0.3)
		Black	245.2 (23.6)	0.10 (0.01)	95.4 (8.4)	39.5 (1.0)	40.5 (4.7)	16.1 (0.6)	54.9 (4.2)	23.4 (1.4)	9.3 (1.0)	4.0 (0.4)	5.2 (0.6)	2.2 (0.2)	13.0 (1.7)	5.1 (0.3)	5.9 (1.0)	2.4 (0.4)
Natives	ELEL	Red	115.2 (33.6)	0.18 (0.05)	46.3 (11.2)	43.6 (2.8)	15.9 (6.9)	11.1 (1.5)	30.4 (4.4)	32.5 (3.2)	6.2 (0.9)	7.4 (1.6)	4.9 (3.4)	2.4 (0.8)	7.8 (3.2)	6.0 (1.9)	1.8 (0.9)	1.1 (0.4)
		Black	212.0 (34.1)	0.10 (0.02)	78.7 (11.0)	39.2 (1.9)	32.3 (5.9)	14.9 (1.2)	46.5 (5.5)	24.3 (1.8)	7.7 (1.8)	3.9 (1.1)	6.1 (1.5)	2.6 (0.4)	13.9 (3.2)	5.8 (0.7)	7.6 (1.7)	3.9 (1.2)
	EPAN	Red	125.3 (29.1)	0.20 (0.03)	49.8 (10.7)	42.3 (1.7)	14.8 (4.7)	9.1 (1.9)	35.0 (6.5)	33.3 (3.3)	8.9 (2.3)	8.5 (1.3)	2.1 (0.7)	1.3 (0.3)	6.3 (2.0)	3.8 (0.8)	3.2 (1.1)	1.9 (0.7)
		Black	277.6 (38.5)	0.13 (0.03)	94.5 (12.4)	34.2 (1.4)	36.8 (6.1)	12.4 (1.0)	57.8 (6.5)	21.8 (1.4)	13.4 (3.8)	4.5 (0.8)	6.7 (1.6)	2.2 (0.3)	15.9 (3.0)	5.5 (0.4)	9.6 (1.2)	3.6 (0.2)
	CEVE	Red	112.5 (15.1)	0.11 (0.01)	52.1 (5.1)	48.2 (1.8)	15.9 (1.9)	14.4 (0.8)	36.2 (3.5)	33.8 (1.7)	5.6 (0.8)	5.3 (0.6)	1.9 (0.3)	1.6 (0.2)	4.9 (1.0)	4.1 (0.4)	1.3 (0.7)	0.9 (0.5)
		Black	287.7 (25.6)	0.07 (0.01)	112.8 (8.2)	39.8 (0.9)	51.7 (5.0)	17.8 (0.4)	61.1 (3.8)	22.0 (1.2)	7.8 (0.7)	2.9 (0.3)	7.2 (1.5)	2.4 (0.3)	17.7 (2.2)	6.0 (0.3)	8.2 (1.4)	2.7 (0.3)

Appendix B - Figures

Figure 2.1: B&B Fire Complex perimeter map with ten randomly selected site blocks with paired burn severity treatments.

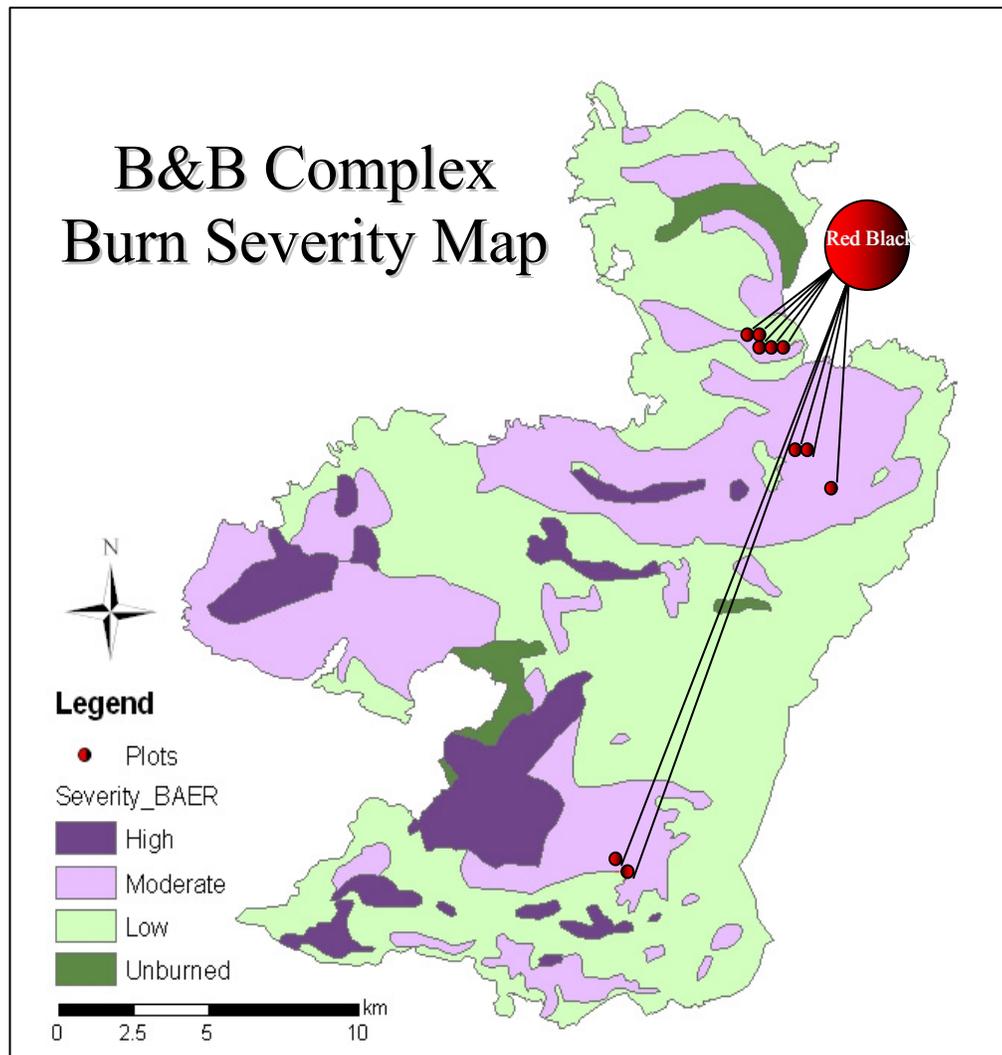


Figure 2.2: NMS of PLFA relative abundance by burn severity. Symbols represent the microbial community of freshly collected soil from each plot separated by burn severity. R^2 axis 1 = 0.84, axis 2 = 0.058. Vectors are based on summed abundances of specific PLFA groups. The length of the vector is proportional to the correlation between that variable and the NMS axis.

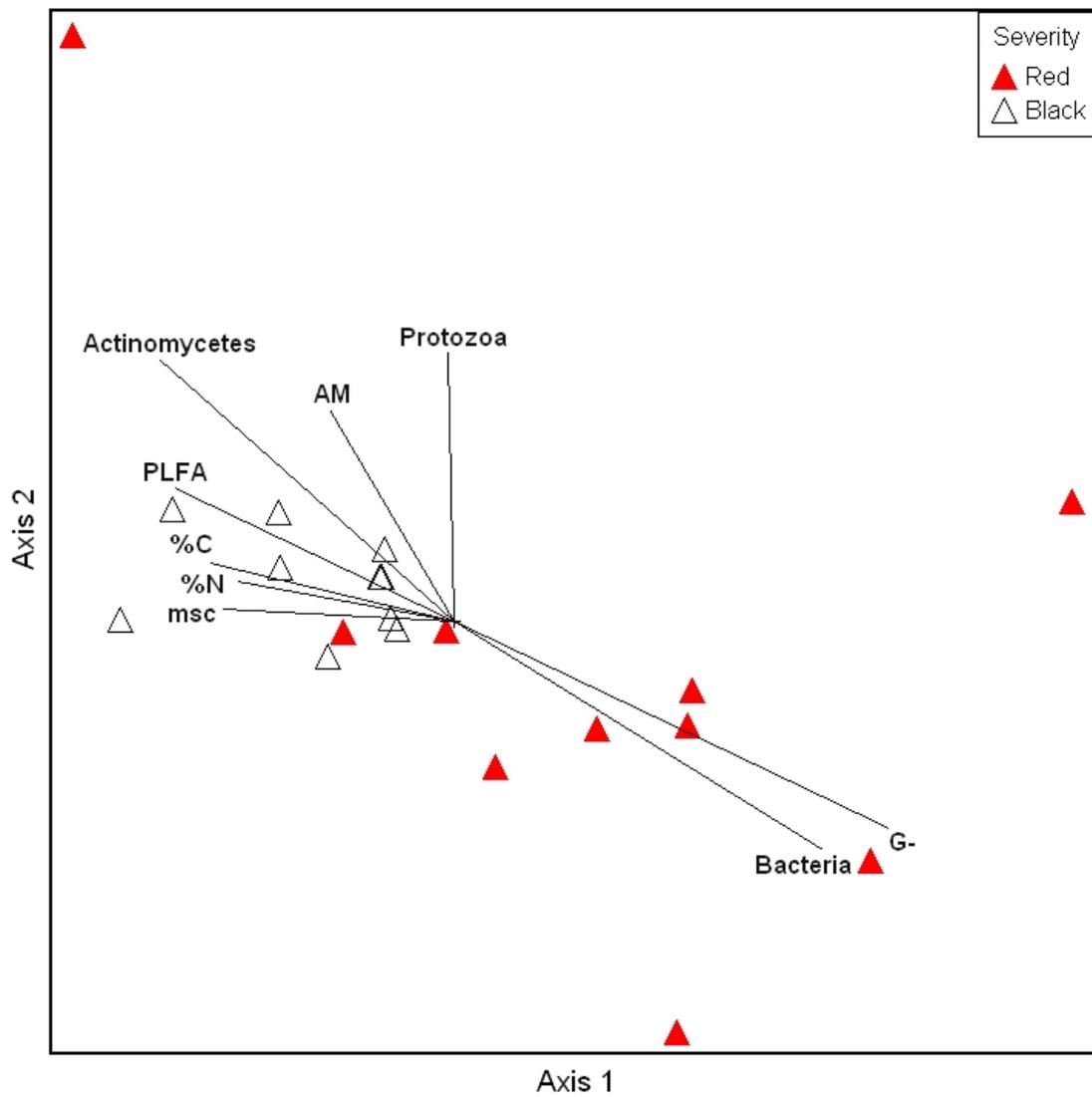


Figure 2.3: NMS of PLFA relative abundance by burn severity. Symbols represent the microbial community from red or black soil harvested from pots after 10 weeks of growth of one of three native and three non-native species in a growth chamber. R^2 axis 1 = 0.624, axis 2 = 0.272. Vectors are based on summed abundances of specific PLFA groups. The length of the vector is proportional to the correlation between that variable and the NMS axis.

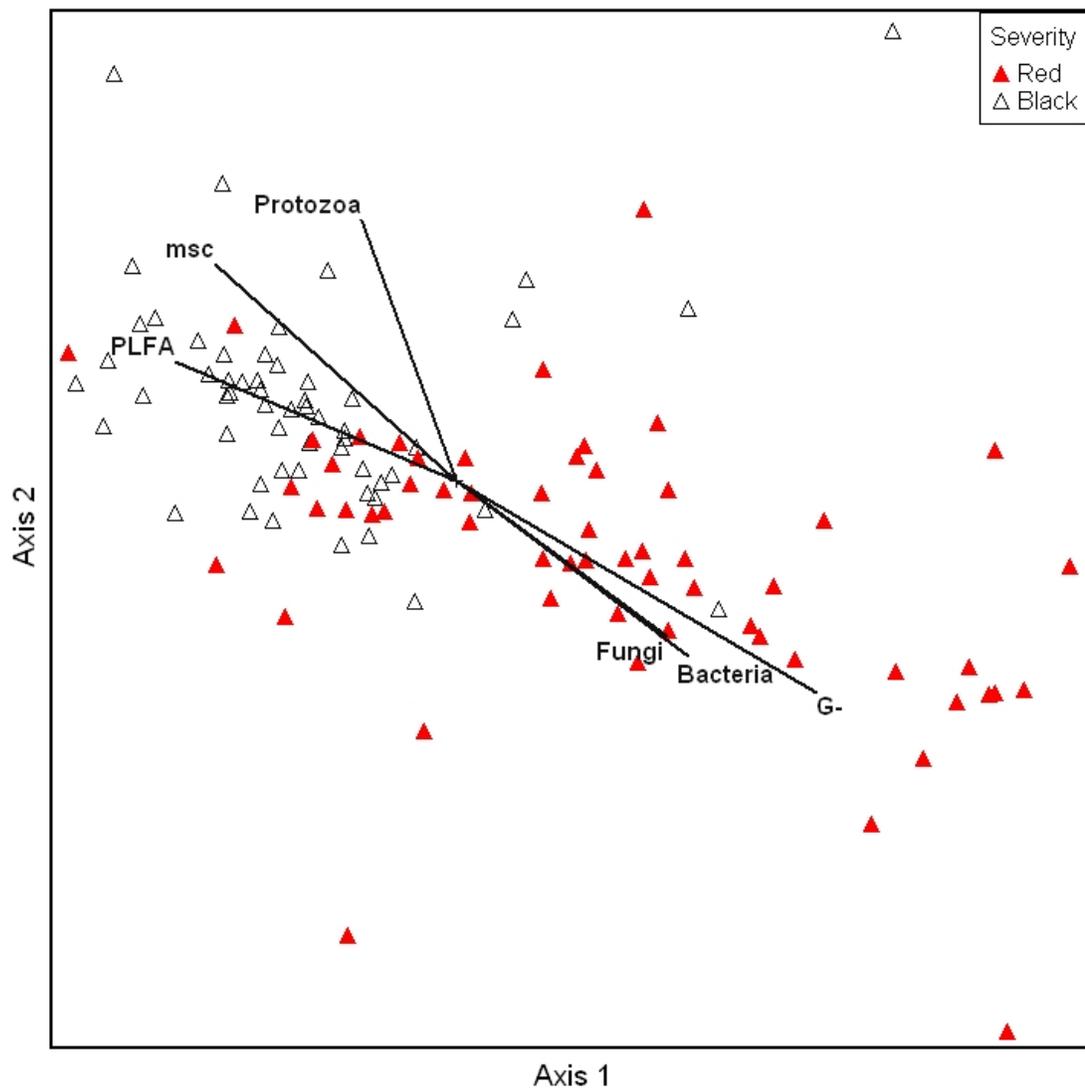


Figure 2.4: NMS of PLFA relative abundance by soil collection plots. Symbols represent the microbial community from 10 paired plots of field collected soil after 10 weeks of growth of three native and three non-native species in a growth chamber.

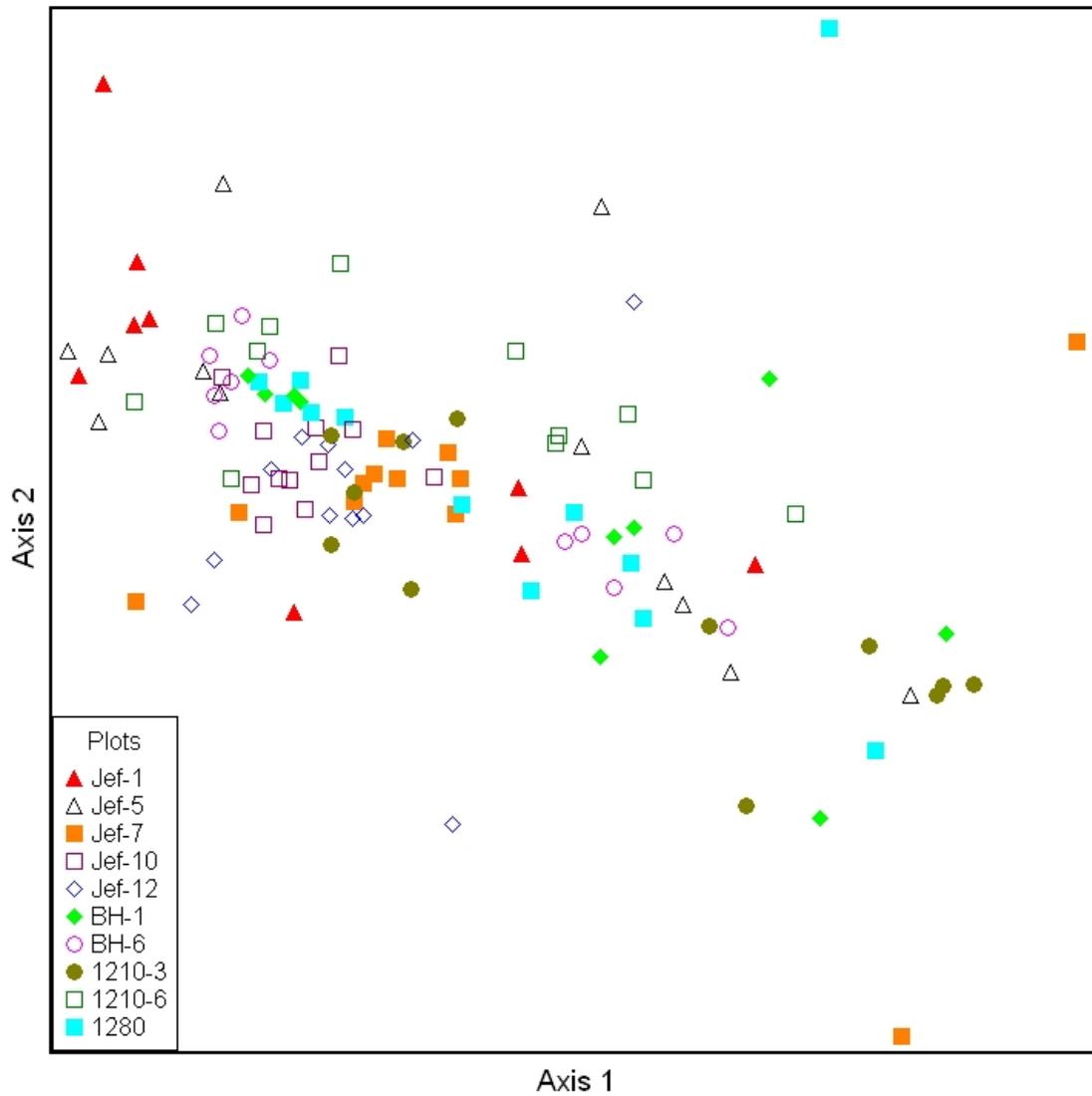


Figure 2.5: NMS of PLFA relative abundance by species association. Symbols represent the microbial community from field collected soil after 10 weeks of growth of three native and three non-native species in a growth chamber.

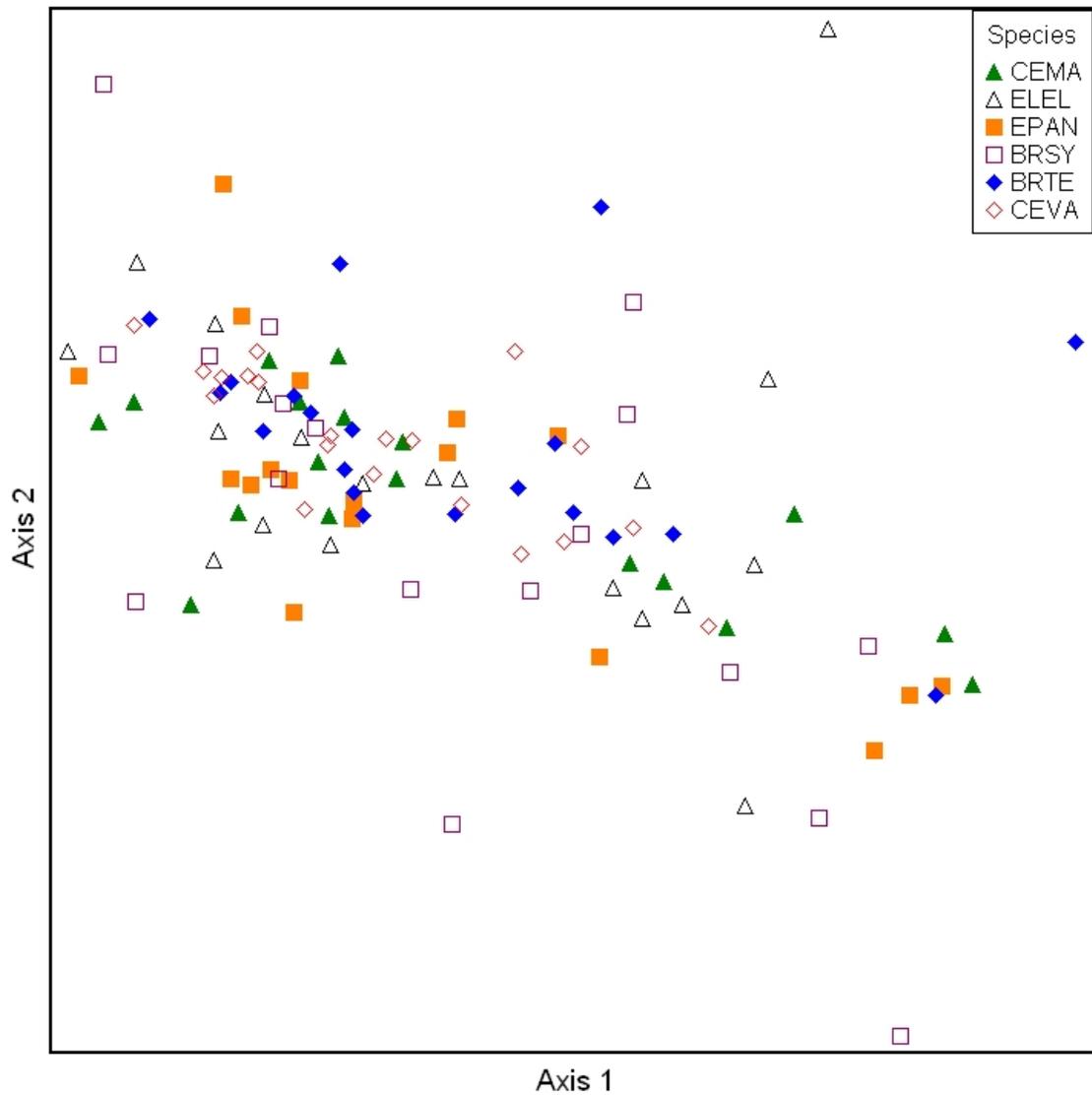


Figure 2.6: The effect of soil C on microbial biomass (total PLFA)

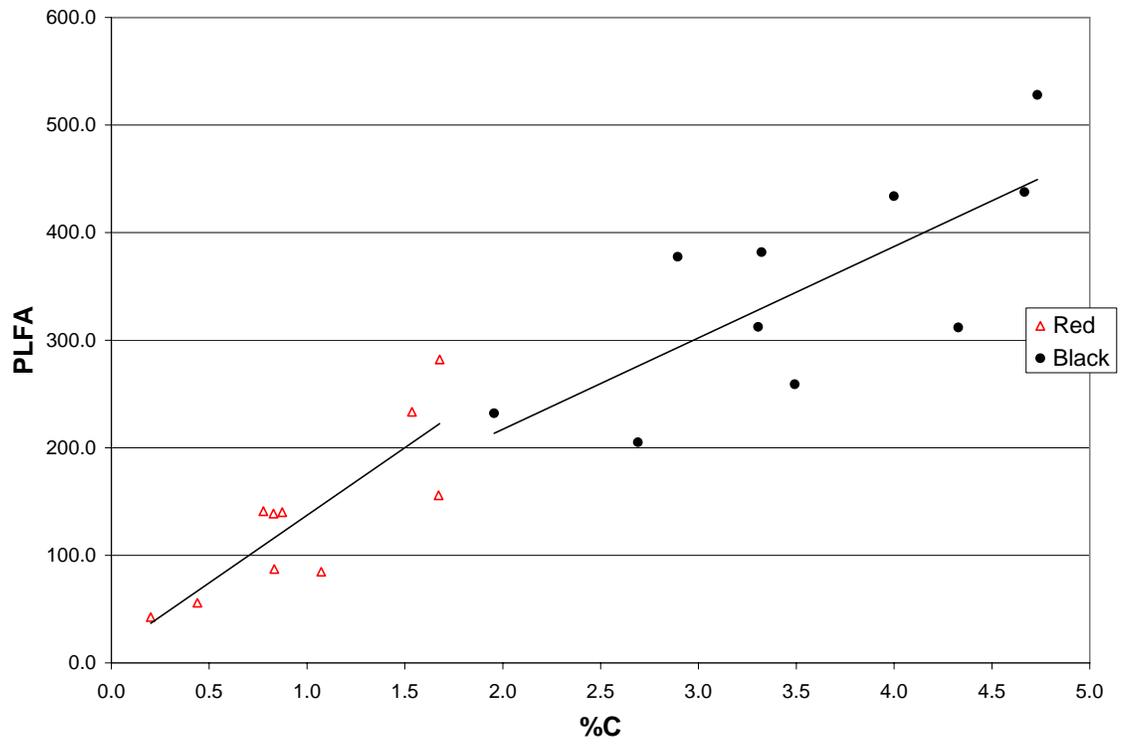


Figure 2.7: AM fungal root colonization of the three native and three non-native plant species grown. Significant differences denoted with an (*).

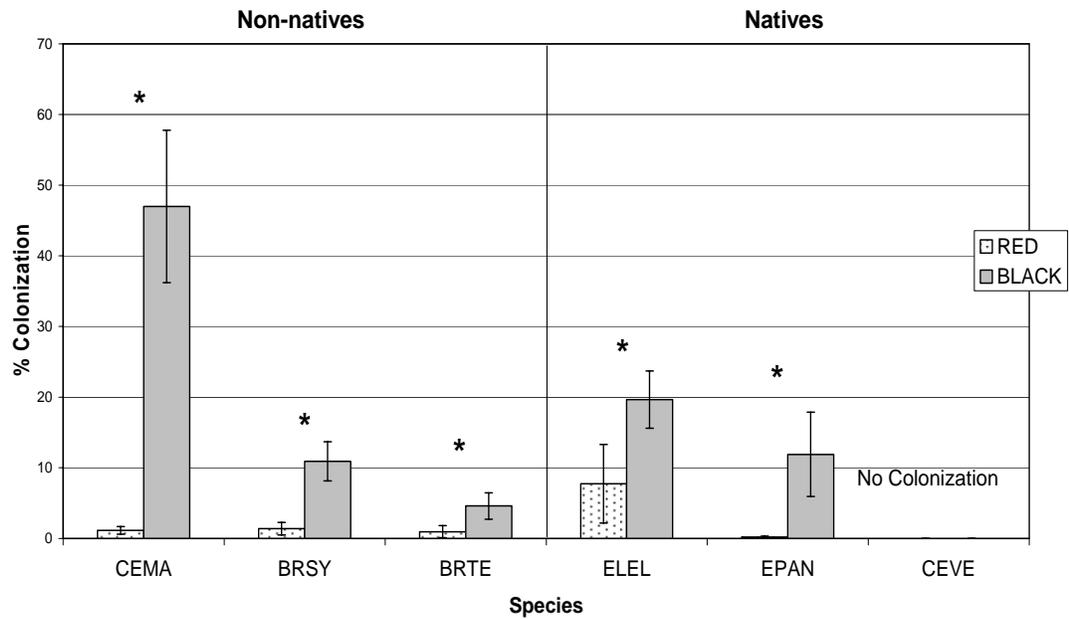


Figure 2.8: Shoot biomass of the three native and three non-native plant species grown. Significant differences denoted with an (*).

