

SHORT- AND LONG-TERM INFLUENCE OF STAND DENSITY ON SOIL
MICROBIAL COMMUNITIES IN PONDEROSA PINE FORESTS

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ABSTRACT

SHORT- AND LONG-TERM INFLUENCE OF STAND DENSITY ON SOIL MICROBIAL COMMUNITIES IN PONDEROSA PINE FORESTS

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Soil microbial communities process plant detritus and returns nutrients needed for plant growth. Increased knowledge of this intimate linkage between plant and soil microbial communities will provide a better understanding of ecosystem response to changing abiotic and biotic conditions. This dissertation consists of three studies to determine soil microbial community responses to reductions in ponderosa pine stand densities and prescribed fire. Chapter 2 relates forest floor and mineral soil (0-5 cm) microbial communities to stand densities across a productivity gradient over a large geographic area, in stands with levels of growing stock that have been maintained over forty years. Chapter 3 investigates the short-term responses of the soil microbial community and soil processes to three wildfire mitigation treatments in northern Arizona. Chapter 4 utilizes the northern Arizona site from Chapter 2 to delve into the interactions among plants, heterotrophic soil microorganisms, and arbuscular mycorrhizal fungi to stand density reductions, and the reintroduction of a low-intensity prescribed fire. Overall, these studies demonstrate a resistance to change in the soil microbial community following stand reductions and low-intensity prescribed fire in ponderosa pine forests. However, spatial attributes of reference conditions of Southwestern ponderosa pine communities, such as uneven tree distributions with large openings, appears to provide a greater potential for increasing native bunchgrasses than simply reducing the stand densities.

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PREFACE

This thesis is divided into five chapters. The first chapter represents a general overview of the research. Both methods and literature reviews for this dissertation have been included into the body of the text and as such there is no formal “methods” or “literature review” section. Chapter 2, 3 and 4 are written in manuscript format of the intended journal submission. Each journal requires method descriptions of the study to be repeatable; therefore, each chapter contains detailed descriptions of the methodologies used. Chapter 2, 3 and 4 are written for submission to the journals *Ecological Applications*, *Forest Science*, and *New Phytologist*, respectively. The manuscript style format of these chapters resulted in some redundancy in the text. An abstract is also included for each chapter. The plural pronoun “we” in these chapters refers to the collaborating authors of each manuscript. The last chapter briefly summarizes the findings of this research and relates these finding to management implications and future research needs.

CHAPTER 1

INTRODUCTION

Most ecological theory is based on information gained from the study of aboveground terrestrial communities and aquatic ecosystems (Standing et al., 2005). The study of above- and below-ground subsystems has primarily been carried out independent of each other, therefore the relationships between these two subsystems within an ecosystem are not well understood. The flow of energy by way of photosynthetically fixed carbon (C) cascades its way through the plant and into soil where it is utilized for growth and metabolic functions of the below-ground community. The below-ground community in turn provides plants with available nutrients in large part derived from the processing of plant detritus. This strong feedback loop of energy and nutrient flows between above- and below-ground components of ecosystems is a major driver of ecosystem community composition and function (Wardle et al., 2004).

Great importance has been placed on C budgets in light of the relatively rapid rise of greenhouse gases. Soil C estimates within forests of the United States exceed 50% of the total C stored in these forests (Heath et al., 2003). This large soil C pool is a function of microbially mediated processes that produce a relatively stable C pool within soils. Yet, we know little about delivery of plant C to the soil subsystem of forested lands and the effect on the soil microbial community.

Reducing present stand densities to decrease the potential risk of high-severity wildfires in western United States forests is currently a primary objective of land management agencies (Agee and Skinner 2005, Noss et al. 2006). Additionally, increasing the resistance of these forests to future wildfires, insect and disease outbreaks,

and climate change would be advantageous to long-term sustainability of these communities (Allen 2002). Tree densities increased and herbaceous and shrub communities diminished in ponderosa pine- (*Pinus ponderosa* P. & C. Lawson) dominated forests of the western United States forests over the last century. These changes also altered plant detrital inputs to the soil have decreased rates of soil microbial processes and possibly altered the soil microbial community (Covington and Sackett 1984, Kaye et al. 2005). Investigation into changes in the soil microbial community has been hampered by the difficulties in determining soil microbial community composition. With the advent of better analytical tools, the relationship between above-ground plant communities and below-ground microbial communities are now being investigated.

Ponderosa pine-dominated forests are one of the most extensive forest types in the United States, ranging from western Canada to northern Mexico. It is also one of the most fire-prone dry forest types. Restoration and fuel reduction efforts are ongoing by way of mechanical thinning and prescribed fire to mitigate the potential for high-severity wildfires.

The studies in this dissertation examine the short- and long-term impacts of stand reductions and prescribed fire on soil microbial communities of ponderosa pine-dominated forests at the stand level. In Chapter 2, I investigated the forest floor and soil microbial community of ponderosa pine-dominated stands at four sites. These four sites (California, Oregon, and Arizona) are geographically distributed to cover the major provenances of ponderosa pine. They are part of a long-term level of growing stock study initiated in the early 1960s to develop relationships between stand density and growth of ponderosa pine. Stand density has the potential to alter abiotic and biotic conditions;

therefore it also has the potential to alter the soil microbial community and processes associated with this community. For Chapter 3 soil microbial community and activity was measured at the Southwestern Plateau site within the Fire and Fire Surrogate (FFS) network following wildfire mitigation treatments (thinning alone, burning alone, thinning and burning, control) that simulated operational scale treatments. The short-term impacts to soil total C and nitrogen (N) concentration, net N mineralization, net nitrification, potential enzyme activity, and soil microbial community structure were measured at six months, one year, and two years following treatments. Operational scale wildfire mitigation treatments are often quite different than treatments applied to experimental restoration studies. Information gained from this and the network scale FFS will be used to adjust wildfire mitigation treatments to mitigate negative impacts created by implementing these treatments. In Chapter 4, I utilized a bioassay to determine the influence of stand density on the arbuscular mycorrhizal symbiosis with two grasses species native to ponderosa pine forests of the Southwest. This study utilized plots from the Arizona site of the level of growing stock study used in Chapter 2. Reestablishment of reference conditions that include native bunchgrasses in ponderosa pine forests of the Southwest is a major goal of restoration efforts.

Emulating reference conditions in ponderosa pine forests is thought to create ecosystem communities with the greatest resistance to perturbations (Fulé et al., 1997). Determining changes to soil microbial communities following wildfire mitigation and restoration efforts will assist in linking below-ground ecosystem processes, such as decomposition and nutrient mineralization, to specific microbial community structures. Awareness of the soil microbial community and its responses to mechanical thinning and

prescribed fire will allow better prescriptions for these treatments to either mitigate negative impacts or take advantage of positive attributes.

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CHAPTER 2

RESISTANCE OF THE SOIL MICROBIAL COMMUNITY TO LONG-TERM CHANGES IN FOREST STAND DENSITY

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Abstract. Ponderosa pine (*Pinus ponderosa* P. & C. Lawson) dominated forests of the western United States are a current priority of wildfire mitigation programs being implemented by public land management agencies. One management alternative for fuel reduction is mechanical thinning to reduce stand densities. The sustainability of these forests is strongly linked to belowground communities in a mutually dependent association. We investigated the long-term influence of stand density along a productivity gradient on total soil microbial biomass, microbial community structure, and fungal-to-bacterial biomass ratios in four ponderosa pine stands from central Oregon to northern Arizona. Stand densities at the study sites have been maintained since the 1960s when the United States Forest Service initiated a study to determine how densities of growing stock affected growth parameters of ponderosa pine. Total carbon and nitrogen contents of the organic horizon and mineral soil were related to stand density. Site productivity and sampling period, but not stand density, significantly influenced microbial biomass, structure, and fungal-to-bacterial biomass ratios. Soil microbial communities in our study were resistant to long-term changes in stand density in ponderosa pine-dominated forests.

Key words: *fuel reduction; microbial community structure; Pinus ponderosa; stand density; site productivity; thinning.*

INTRODUCTION

Reducing current stand densities to decrease the risk of intense stand-replacing wildfires in fire-prone forests of the western United States is a goal of many land management agencies (Agee and Skinner 2005, Noss et al. 2006). Mechanical thinning is an often used treatment to decrease fuel continuity and reduce the occurrence of stand-replacing wildfires (Pollet and Omi 2002). Additionally, reducing stand densities may increase resistance to future wildfires, insect and disease outbreaks, and climate change, providing long-term sustainability of these communities (Allen 2002, Agee and Skinner 2005). While there has been considerable research on ecological responses to restoration and wildfire mitigation practices, our understanding of long-term responses of the soil microbial community structure and dynamics following thinning is limited (Hart et al. 2005, Hart et al. 2006).

Community structure is controlled by environmental conditions, availability of limiting resources, and biotic interactions (Odum 1969, Chapin et al. 1986, Milchunas and Lauenroth 1995, Hooper and Vitousek 1998, Suding et al. 2008). Availability of most nutrients is regulated by soil microbial decomposition and mineralization processes (Meetenmyer 1978, McClaugherty et al. 1985, Hart et al. 1992, Wardle 2004, Fierer et al. 2005). Since Euro-American settlement, decreased rates of decomposition and mineralization in the western United States have been inferred due to altered plant litter, roots, and root exudation inputs to the soil (Covington and Sackett 1984, Kaye and Hart 1998a,b, MacKenzie et al. 2004, Boyle et al. 2005, Kaye et al. 2005). These changes to inputs are due to dramatic increases in tree densities of ponderosa pine (*Pinus ponderosa* P. & C. Lawson) dominated forests and subsequent decline of herbaceous and shrub

communities (Cooper 1960, Covington and Sackett 1984, Covington and Moore 1994). Quality of litter fall, root turnover, and root exudation is a function of plant community composition, which affects the biomass and structure of soil microbial communities (Bardgett et al. 1998, Wardle 1999).

Litter quantity and chemical composition interact with soil moisture, temperature, and mineralogy to control rates of decomposition and mineralization (Paul and Clark 1996, Poranzinska et al. 2003, Bardgett 2005). Bacteria are favored in fertile soils with fast-growing plants that have high quality leaf litter (Pennanen et al. 1999, Leckie et al. 2004, Högberg et al. 2007, Boyle et al. 2008), while fungi dominate on infertile soils with plants that produce lower quality leaf litter and secondary compounds such as lignin and phenolics (Coleman et al. 1983, Bardgett 2005). Fungal-to-bacterial biomass ratios (F:B) reflect this relationship generally, decreasing as site fertility increases (Bardgett et al. 1996, Myers et al. 2001, Siira-Pietikäinen et al. 2001, Grayston and Prescott 2005, Högberg et al. 2007).

Temperature and moisture also regulate soil microbial communities (Atlas and Bartha 1998, Wardle 2004, Schimel et al. 2007, Gordon et al. 2008). Temperature extremes limit the composition of the microbial community (Atlas and Bartha 1996), while microbial decomposition and mineralization processes are sensitive to changes in temperature (Fierer et al. 2005). Reductions in canopy cover decrease the interception of precipitation increasing available soil moisture (Feeney et al. 1998, Stone et al. 1999, Simonin et al. 2007), while greater light penetration increases soil temperatures (Zogg et al. 1997). For example, Zou et al. (2008) found that low-density (250 trees ha⁻¹) ponderosa pine stands consistently had higher soil water content in the total soil profile

than high-density ($2710 \text{ trees ha}^{-1}$) stands over a three year period, yet in surface horizons this increase is not always apparent (Boyle et al. 2005, Simonin et al. 2007). Moisture deficits in soil surface horizons, common in ponderosa pine forests (Oliver and Ryker 1990), can induce shifts in microbial communities to favor fungi and gram-positive bacteria over gram-negative bacteria (Schimel et al. 2007).

Ponderosa pine-dominated communities in the western United States are often limited by nitrogen (N) availability (Klemmedson et al. 1990, Monleon and Cromack 1995, DeLuca and Zouhar 2000, Hungate et al. 2007). Nitrogen limitation in these stands is considered a product of accumulating organic matter with high carbon to nitrogen mass (C:N) ratios (Covington and Sackett 1992). Thinning has resulted in decreased litter fall (Klemmedson et al. 1990, Grady and Hart 2006) with increased (Kaye and Hart 1998b), decreased (Grady and Hart 2006), or no change in net N mineralization (DeLuca and Zouhar 2000, Boyle et al. 2005). The variability of net N mineralization to thinning in these forests has been attributed primarily to the response of the understory vegetation (lower C:N ratios) and temperature changes (Hart et al. 2005, Kaye et al. 2005, Grady and Hart 2006).

In the 1960s, the United States Forest Service initiated a study to determine growth relationships of residual stands thinned to designated growing stock levels (GSL) in ponderosa pine stands across a range of site productivities. This study covered five physiographic regions where two varieties of ponderosa pine (*Pinus ponderosa* P. & C Lawson var. *ponderosa* and *Pinus ponderosa* P. & C Lawson var. *scopulorum*) occur. Stand densities at these study sites have been and continue to be maintained at designated levels of basal area. Continued maintenance at these sites provided an opportunity to

investigate the long-term influence of stand density, site productivity, and their interaction on the soil microbial community. We expected increases in bacterial biomass with stand reductions due to increased available moisture and lower C:N ratios of litter inputs in both the organic horizon (O horizon) and surface mineral horizon (0-5 cm). We also expected less microbial biomass and structure differences due to stand density treatments in the mineral soil than the O horizon due to greater buffering of temperature and moisture extremes in the mineral soil. We believe that larger inputs of plant litter, root, and root exudates at the high productivity sites will produce greater total microbial biomass. Fungal-to-bacterial biomass (F:B) ratios were expected to range from lowest at the low-density, high productivity plots to highest at the high-density low productivity plots.

MATERIALS and METHODS

Study sites and treatment

This study utilized four of the original five GSL sites. These sites are located in the Sierra Nevada of California, the Cascade Range of Oregon, the Blue Mountains of eastern Oregon, and the Colorado Plateau in northern Arizona (Myers 1967). The original GSL study plots (0.10-0.33 ha) were thinned to six designated stand densities in the Arizona and Oregon sites (7, 14, 19, 23, 28, 35 m² ha⁻¹ basal area) and five stand densities in the California site (9, 16, 23, 30 and 37 m² ha⁻¹). Stand densities retained after thinning were specified as a series of GSLs defined by the relationship between basal area and average stand diameter when average stand diameter is 25.4 cm or greater (Myers 1967, Oliver and Edminster 1988). Each site had three replicate plots for each

thinning treatment (Oliver 2005). We selected three GSL treatments, which we will refer to as low, medium and high stand densities for each site (Arizona and Oregon: 7, 14, 28 m² ha⁻¹; California: 9, 16, 30 m² ha⁻¹). We did not select the highest stand density from the original study due to insect and snow damage that occurred at the California site (W.W. Oliver, Pacific Southwest Research Station, Forest Service, United States Department of Agriculture, personal communication). Slash material was lopped and scattered at each site for the initial thinning, which tended to suppress understory growth (Oliver 2005). After initial treatment, measurement of tree growth was conducted every five years and thinned every ten as needed to maintain the designated stand densities. Productivity is reported as the total merchantable volume of tree growth measured every 5 years, and then averaged over the 25 years following initial stand reduction treatments.

The Elliot Ranch study site is located in the Tahoe National Forest 18 km NE of Foresthill, California. This site is at an elevation of 1,212 m on a gentle south-facing slope on the west side of the northern Sierra Nevada (Oliver 1979, 1997). The area is characterized by broad tabular ridges and hillsides with slopes of 2 to 50 percent (Oliver 1979, 1997). The Elliot Ranch site is a plantation of ponderosa pine in a mixed conifer/deerbrush (*Ceanothus integerrimus* Hook. & Arn.) community, growing on soils derived from tuff breccia and classified as fine-loamy, parasesquic, mesic Xeric Haplohumults and fine-loamy, mixed, superactive, mesic Ultic Haploxeralfs (Oliver 1979, 1997).

The Lookout Mountain site is in the Pringle Fall Experimental Forest and part of the Deschutes National Forest in central Oregon (Cochran and Barrett 1999). This site is east of the Cascade Range crest within a naturally regenerated ponderosa pine/velvet

ceanothus (*Ceanothus velutinus* Douglas ex. Hook) community. The elevation of the plots is 1515 m and predominantly on south-facing slopes. The loam soils are derived from dacite tephra parent material resulting from the Mt. Mazama volcanic eruption, and are classified as Xeric Vitricyands (Cochran and Barrett 1999).

The Crawford Creek study site is in the Blue Mountains of northeastern Oregon on the Malheur National Forest (Cochran and Barrett 1995). The plots are located approximately 32 km northeast of Prairie City, Oregon. The site elevation is 1334 m, with slopes ranging from 6 to 29 percent covering all aspects. This ponderosa pine/Idaho fescue (*Festuca idahoensis* Elmer) community is on a soil complex of loamy-skeletal, mixed, superactive, frigid Lithic Haploxerolls and loamy-skeletal, isotic, frigid Vitrandic Haploxeralfs (Hersh McNeil, Malheur National Forest, U.S. Dept. of Agriculture, personal communication). These soils are derived from a mix of volcanic ash and colluvium over colluvium and residuum from basalt, andesite, rhyolitic tuff, and andesitic tuff-breccia.

The Taylor Woods study site, a subdivision of the Fort Valley Experimental Forest, is approximately 14.5 km northwest of Flagstaff, Arizona, at an elevation of 2,270 m (Ronco et al. 1985). All plots are on a gentle (4%), southwest facing slope. The plant community type is ponderosa pine/Arizona fescue (*Festuca arizonica* Vasey) growing on soils derived from basalt flows and classified as fine, smectitic Typic Argiborolls (Ronco et al. 1985).

Experimental Design

Sampling was performed on the three stand densities listed above. Each of the three stand density levels has three replicate plots at each site. Within each plot, three

transects were randomly assigned with three randomly assigned points per transect. No point was sampled closer than three meters of the plot boundary. The O horizon and mineral soil were collected separately (see below) within each plot. These samples were then combined by transect to provide three subsamples of O horizon and mineral soil per plot. Samples were taken in August 2003 for all sites. August at Taylor Woods in northern Arizona coincides with the rainy season, while Elliot Ranch in eastern California was in a normal dry period with high temperatures during the August sampling. Both the Lookout Mt. and Crawford Creek sites were also in a dry period in August, but not as intense as Elliot Ranch. For these reasons, we utilized samples taken in June 2003 from Taylor Woods, the dry part of the growing season in northern Arizona, to compare to the August 2003 samples from the other sites. The large changes in microbial biomass between dry (26.5 nmol PLFA g O horizon⁻¹) and wet (102.4 nmol PLFA g O horizon⁻¹) parts of the growing season at Taylor Woods motivated us to expand the sampling to include both the dry and wet periods for each site. Wet season samples from the California and Oregon sites were then collected in June 2005, coinciding with their growing season wet period. Because these samples were not collected in the same year, we did not statistically compare dry versus wet period samples for California and Oregon sites.

Sampling and laboratory methods

The O horizon total mass (< 6 mm dia.) was measured on combined samples collected within a 0.01 m² litter frame, placed in polyethylene bags, and transported back to the laboratory (2-7 days). All samples were stored in insulated containers with ice to reduce microbial activity during transport. Samples were weighed, and a subsample was

oven dried (70 °C) and weighed again to express total mass on an oven dry basis.

Analysis of the O horizon total C, total N, and total phosphorus (P) concentrations was done on uniformly mixed subsamples that had been air-dried. Dried subsamples were ground (< 0.149 mm dia.), then analyzed for total C and N concentrations on a commercially available elemental analyzer (Flash EA 1112, CE Elantech, Lakewood, New Jersey, USA). Total P concentrations were determined on a flow injection analysis instrumentation (Lachat method 13-115-01-1-B) by the phosphomolybdate method (Murphy and Riley 1962) modified for analysis, following a $\text{CuSO}_4\text{--H}_2\text{SO}_4$ modified Kjeldahl digestion procedure (Parkinson and Allen 1975). Immediately upon return to the laboratory, subsamples (~ 10 g) of uniformly mixed material were placed into paper bags, frozen overnight, and then freeze-dried prior to extraction for analysis of phospholipids fatty acids (PLFA).

Mineral soil beneath the sampled litter was collected with a 5 cm x 5 cm core attached to a slide hammer (AMS, Inc., American Falls, Idaho, USA). Soil samples were placed in polyethylene bags and transported back to the laboratory on ice (3-7 days). Soil samples were weighed immediately upon arrival at the laboratory, then sieved (< 2 mm). The > 2 mm fraction was weighed, submerged in water within a partially filled graduated cylinder to determine the volume of the coarse fragments. The < 2 mm fraction was weighed, then a subsample taken, oven dried (70° C), then reweighed for determination of water content. The volume of the > 2 mm fraction was subtracted from the volume of the original soil core. The mass of the < 2 mm was determined by subtracting the soil water content to provide a total oven dried soil mass for the remaining volume of the soil core to determine bulk density (Mg m^{-3}) of the < 2 mm fraction. Soil samples used for

nutrient pools and pH were air dried prior to analysis. Total C, N, and P concentrations were determined using the same methods described above for O horizon. Additionally, soil pH was determined by immersing a glass electrode into a 1:5 (w/v) soil/0.01 M CaCl_2 solution (Hendershot et al. 1993) connected to a Orion 550A pH meter (Thermo Fisher Scientific, Inc., Waltham, Massachusetts, USA). Mineral soil PLFA subsamples were prepared the same as O horizon subsamples. Calculation of total C, N, and P on a mass per area basis (kg ha^{-1}) was obtained by multiplying concentrations by mass of the dry soil for each site.

The amount and distribution of PLFAs in freeze-dried O horizon and mineral soil subsamples were used to assess microbial biomass and community structure. Analysis of PLFA patterns has been shown to be a powerful approach to describe the structure of the soil microbial communities and to detect changes due to altered ecological conditions (Bååth et al. 1992, Frostegard et al. 1993*a,b*, Zelles 1999, Hassett and Zak 2005, Ramsey et al. 2006). Phospholipid fatty acids are essential components of every living cell with high biological specificity (Zelles 1999). These compounds quickly degrade when cells lyse and are not found in storage products (Tunlid et al. 1985), and therefore are advantageous for determining the active microbial community (White et al. 1979). Individual PLFA can be used as specific biomarkers to identify different microbial groups (e.g., fungi, gram-positive bacteria, gram-negative bacteria, and actinobacter), and summed to provide an index of total microbial biomass.

Mass spectral analysis identified numerous PLFAs between C14 to C22 in C chain length, of which 16 were used as microbial biomarkers. The 16 compounds are a conservative estimate of known microbial biomarkers (O'Leary and Wilkinson 1988,

Frostegard and Bååth 1996, Zelles 1999). Total microbial biomass was calculated by summing these 16 biomarkers. Gram-negative bacteria biomarkers (cy17:0, cy19:0, 16:1 ω 9, 16:1 ω 7, 18:1 ω 5c, and 18:1 ω 7) and gram-positive bacteria biomarkers (i15:0, a15:0, i16:0, i17:0, a17:0, and 10me16:0) discriminate between guilds. These biomarkers were then summed with the general bacteria biomarkers (C15:0 and C17:0) to estimate total bacterial biomass (O'Leary and Wilkinson 1988, Frostegard and Bååth 1996, Zelles 1999). Two isomers of C18:2n6 (*trans* and *cis*) were used to estimate the fungal group (Frostegard and Bååth 1996). Phospholipid fatty acid analysis provides a coarse level of microbial community structure, but its ability to detect structural changes is quite sensitive. For instance, Ramsey et al. (2006) found PLFA analysis often resolved treatment effects when molecular methods did not.

Two g of freeze-dried ground litter or 5 g of freeze-dried mineral soil was extracted with a single-phase mixture of chloroform, methanol, and phosphate buffer (White et al. 1979) then fractionated into neutral, glyco-, and phospholipids (Frostegard et al. 1991). The phospholipids were then esterified and reconstituted in hexane prior to analysis (Frostegard et al. 1993b). A capillary column was utilized for compound separation. Identification and quantification of the each specific fatty acid methylester (FAME) was established using commercially available standards (Accustandard, Sigma-Aldrich, St. Louis, Missouri, USA) through electron ionization on a quadrapole mass selective detector (Agilent 6890N/5973N gas chromatograph/mass spectrometer, Santa Clara, CA, USA). Quantification (nmol of PLFA g⁻¹ oven-dry material) of samples is based on calibration curves derived from individual FAME standards.

Community-level physiologic profile (CLPP) has been shown to be a useful method to detect functional differences in soil microbial populations, especially when used in conjunction with other methods (Garland and Mills 1991, Classen et al. 2003). The CLPP method cultures microorganisms within microtiter plate wells that contain different sole source C substrates. Some researchers believe that data collected using CLPP method are indicative of the metabolic potential of the microbial community (Garland and Mills 1991), while others assert that CLPP yields information on the functional diversity of the microbial community in question (Kennedy 1994). As used in this study, these culture plates provide a qualitative indicator of a relative responsiveness of bacterial and fungal community to changes in ponderosa pine stand density (Classen et al. 2003).

Bacteria and fungi were extracted from 4 g of ground fresh O horizon and 4 g of sieved fresh mineral soil (< 2 mm), diluted, and then transferred to wells containing a single carbon substrate following procedures outlined in Classen et al. (2003). Bacterial plates were incubated at 25 °C for 48 h, while fungal plates were incubated for 72 h prior to analysis of wells on an EMax absorbance microplate reader (Molecular Devices, Sunnyvale, California, USA) either for color development (Biolog EcoPlate) or turbidity (Biolog SFN2). Each bacterial microtiter plate (Biolog EcoPlate) contained 31 different C sources replicated three times. A tetrazolium dye sensitive to reduction was included with each C substrate. This dye develops a purple color if catabolized that can be measured for absorbance. Fungal CLPP is done on microtiter plates containing 95 individual carbon substrates (Biolog SFN2), but do not include the tetrazolium dye due to its toxicity to some fungi (Dobranic and Zak 1999). Bacterial growth was minimized on the fungal

plates by including antibiotics during inoculation of the microtiter plates (Dobranic and Zak 1999, Classen et al. 2003).

Statistical methods

Statistical analyses of total biomass and nutrient contents for both O horizon and mineral soil (total C, total N, total P, and pH) were performed using two-way analysis of variance (ANOVA), with stand density, productivity (Elliot Ranch > Lookout Mt. > Crawford Creek > Taylor Woods), and the stand density x site productivity interaction as factors. These variables were relatively consistent between dry and wet sampling across stand densities and sites, so we averaged the values from both sample periods to provide better estimates of each variable. Forward stepwise regression analysis was utilized to determine which variables best explained variations in total microbial biomass. Organic horizon total P contents were $\log_{10}$ transformed, while total PLFA values were square root transformed to meet statistical assumptions of normal distribution and homogenous variances. For mineral soil, $\log_{10}$ transformations were performed on total C, N and P contents, while total PLFA values utilized square root transformations. Statistical analyses were performed on a personal computer (SAS for PCs ver. 8.1, Cary, North Carolina, USA).

Microbial community structure (PLFA biomarkers) and CLPPs were analyzed by multi-response permutation procedure (MRPP) using Euclidean distances, a multivariate statistical procedure testing the similarity in multivariate patterns among stand densities or site productivities (Mielke and Berry 2001). Community-level physiological profiles were analyzed only during the dry period sampling conducted in 2003. The CLPP data were normalized for each soil sample prior to statistical analysis by dividing the color or

turbidity development of each well by the total color or turbidity development of the entire plate. This normalization procedure provides a simple method for reducing the influence of differences in initial inoculum densities on CLPP patterns (Classen et al. 2003). Phospholipid fatty acid data were normalized in a similar fashion where the mass of each specific biomarker was expressed relative to the total mass of all biomarkers for a given sample. This allowed us to compare community structure between sites with different total microbial biomasses (McCune and Grace 2002).

Multi-response permutation procedure does not require assumptions of multivariate normality or homogeneity of variances, which are seldom met with ecological community data (McCune and Grace 2002). Simultaneous pairwise comparisons using the Peritz closure method to maintain Type I error rate tested the null hypothesis that all possible pairs are similar (Petrondas and Gabriel 1983). This procedure was performed using Microsoft Excel macros (available from the senior author) following the methodology of Mielke and Berry (2001). All statistical analyses were conducted at the $\alpha=0.05$ significance level.

RESULTS

Organic horizon

There were no significant differences across sites among the three stand densities for total O horizon mass, and C, N and P contents (Table 1). However, total O horizon mass was significantly greater at the high productivity sites, Elliot Ranch and Lookout Mt., than at the low productivity sites, Crawford Creek and Taylor Woods, as was total C contents (Table 2). Total N and P contents were similar across all sites (Table 2). Stand

density by site productivity interactions were not significant for any of the above soil properties.

Multi-response permutation procedure analysis of the O horizon revealed no significant effect of stand density on microbial community structure based on PLFA biomarkers (dry $p = 0.72$, wet $p = 0.92$). Community-level physiological profiles for fungi ($p = 0.062$) and bacteria ($p = 0.32$) also were not different among densities (measured during the dry season only).

Organic horizon microbial biomass, based on the summed PLFA biomarkers, at Taylor Woods increased significantly ($p = 0.014$) from the dry to the wet period during the 2003 growing season (Fig. 1). This pattern was also observed at the other sites, although we did not statistically analyze for differences between dry and wet periods because sampling was done in different years (Fig. 1). Organic horizon microbial biomass was significantly greater at Crawford Creek than the other sites for both wet and dry periods, while microbial biomass at Taylor Woods was significantly lower than the remaining two sites during the wet period (Fig. 1). Forward stepwise regression using abiotic (temperature and precipitation), nutrient (total C, N, P contents, C:N ratios) and productivity factors indicated that the greatest amount of variability in O horizon microbial biomass (39.4%) was explained by site productivity (significant at $\alpha = 0.05$ level).

During the dry period, F:B ratio was greatest at the two highest productivity sites (Fig. 2). However, during the wet period, the F:B ratio at the lowest productivity site was the greatest (Fig. 2). The microbial community structure, evaluated by groups, indicates fungi dominated during both wet and dry sample periods across all sites (Fig. 3). Pairwise

comparisons of microbial community structure by site using MRPP analysis showed significant differences among all sites, with the exception of Elliot Ranch and Lookout Mt. during the dry period, and Lookout Mt. and Crawford Creek during the wet period (Fig. 3).

Mineral soil

Total C, N and P contents of the mineral soil were similar for the three stand densities (Table 1). Soil pH was significantly lower in the high density plots (Table 1). However, total C and N contents were significantly higher at the two high productivity sites, Elliot Ranch and Lookout Mt., compared to the two lower productivity sites, Crawford Creek and Taylor Woods (Table 2). Total P content varied among sites, with Lookout Mt. highest, Taylor Woods intermediate, and Elliot Ranch and Crawford Creek with the lowest values (Table 2). In general across site productivities, pH was similar with the exception of the Lookout Mt., which was significantly lower than the other three sites (Table 2).

Stand density differences did not influence either microbial community structure (dry $p = 0.33$, wet $p = 0.09$) or CLPP (fungi $p = 0.64$, bacteria $p = 0.32$, measured during the dry period only). The same abiotic, nutrient, and productivity factors used in the O horizon forward stepwise regression were also utilized for the mineral soil analysis. Site productivity was the only significant factor related to microbial biomass, explaining almost 54% of the variability (significant at $\alpha = 0.05$ level). Microbial biomass was higher for the wet period compared to dry period for all sites (Fig. 1), yet was not analyzed statistically for the same reasons as for the O horizons. Elliot Ranch had the

highest total microbial biomass during both the dry and wet periods, while Lookout Mt. was intermediate and Taylor Woods the lowest during the wet period (Fig. 1). During the dry period, F:B ratios were significantly higher at Elliot Ranch and Lookout Mt., the high productivity sites, than the two low productivity sites, Crawford Creek and Taylor Woods (Fig. 2). Taylor Woods, the least productive site, had a significantly higher F:B ratio during the wet period than the other three sites (Fig. 2). Community structure was significantly different among all sites during the dry period, while Lookout Mt. and Crawford Creek were similar, but different from the other two sites, during the wet period (Fig. 4).

DISCUSSION

Decreasing stand density by harvesting has been shown to reduce microbial biomass without altering the community structure (Boyle et al. 2005, Hassett and Zak 2005), alter the community structure with little change in microbial biomass (Maassen et al. 2006), result in no change in either microbial biomass or community structure (Siira-Pietikäinen et al. 2001, Hannam et al. 2006), and alter both biomass and structure (Pietikäinen et al. 2007). The microbial biomass and community structure for both O horizon and mineral soil in our study was not responsive to long-term (>35 y) manipulation of stand densities. Microbial communities determined from PLFA biomarkers for both O horizon and mineral soil were resolved primarily by site productivity. We did not detect microbial community differences with stand density manipulation. Community-level physiological profiles mimic this same pattern, showing no difference with stand density, but a strong community association by site productivity.

Reducing stand densities in ponderosa pine forests, without altering the quality of plant inputs to the soil, does not appear to influence the structure of the soil microbial communities, yet there are distinct regional differences among these microbial populations.

Pietikäinen et al. (2007) found significant increases in microbial biomass and F:B ratios of the humus layer with increased stand density in an even-aged Scots pine (*Pinus sylvestris* L.) stands in Finland, where species, soil type, and climatic factors were uniform. This response disappeared once canopy closure suppressed the understory, reducing both microbial biomass and F:B ratio. Microbial community responses to stand density reductions do occur when the herbaceous community responds positively to decreased tree dominance (Hart et al. 2005, Kaye et al. 2005, Hart et al. 2006, Wallenstein et al. 2006). Increased litter quality (lower C:N ratio) inputs from the herbaceous community have resulted in enhanced microbial functions in other ponderosa pine forests (net N mineralization, soil respiration), yet when the herbaceous response is negligible, these processes are relatively unaffected (Hart et al. 2006). According to Oliver (2005), slash residues from the initial thinning treatments left on site suppressed early understory establishment (Oliver 2005), yet in 1999 the Taylor Woods herbaceous community was reported to be ~25% cover at the lowest density with little herbaceous cover at higher densities (McDowell et al. 2007). We observed similar herbaceous establishment at Crawford Creek, and the other sites where shrubs are a dominant cover type of the understory (Elliot Ranch and Lookout Mt.). The O horizon and mineral soil C:N ratios did not differ between stand densities or site productivity. The most influential factor for microbial community change appears to be the result of herbaceous and shrub

responses. Yet, if these understory communities remain suppressed following stand reductions, we expect the microbial community will remain largely unaffected by thinning operations.

Total microbial biomass of the O horizon increased with decreasing site productivity, contrary to our expectations. Crawford Creek, at the low end of the productivity gradient, had the greatest microbial biomass in the O horizon for both dry and wet sampling periods. Also, F:B ratios in the O horizons were lowest at Crawford Creek and Taylor Woods during the dry part of the growing season. Contrary to our findings, Pennanen et al. (1999) found that microbial biomass in the humus layer increased with precipitation and shifted to a greater proportion of bacteria compared to fungi with increased fertility. Other studies have also shown relative increases in bacterial biomass with site fertility (Pennanen et al. 1999, Boyle et al. 2008), while fungi dominate in less fertile sites (Wallenstein et al. 2006). For these studies, site productivity differences were driven by nutrient differences. Our study productivity gradient was primarily influenced by precipitation differences with little soil-based difference in fertility among sites, as documented by the comparable C:N ratios in both the O horizon and the mineral soil. Microbial community structure at the two Oregon sites for both O horizon and mineral soil was most similar during the wet period of the growing season, even though there were large differences in site productivities. While Crawford Creek, in eastern Oregon, and Taylor Woods, in northern Arizona, are most similar with respect to productivity and mean annual precipitation and temperature, the two Oregon sites are geographically the two closest sites. When attempting to predict microbial community response to thinning in ponderosa pine-dominated forests, utilizing results from different

regions may not be applicable, as regional climate appears to be a major factor moderating microbial community responses.

The greatest mineral soil microbial biomass for both sampling periods occurred at the high productivity sites, while the lowest productivity site had the lowest total microbial biomass. In our study, sample periods of greatest soil moisture, based on seasonal patterns of precipitation, generally followed a pattern of increased F:B ratios with decreased productivity. In our case, F:B ratios between sampling periods were primarily due to increased fungal percentage within the community during the wet period, contrary to what we expected. Ponderosa pine-dominated forests occupy relatively dry sites compared to most other forest types, therefore the microbial community in the mineral soil could experience moisture stress even during the wettest part of the growing season. Taylor Woods, our least productive site, consistently had the lowest F:B ratio in the mineral soil, regardless of sampling period. We do not have a good explanation why the Taylor Woods soil microbial community is so different from the other sites. However, differences in growing season precipitation may be important. For instance, Taylor Woods experiences substantial precipitation during the summer growing season while the other sites do not. Again, these results demonstrate that microbial communities respond to thinning of forest stands are regionally dictated.

The GSL study was designed to determine growth yields of ponderosa pine at different stocking levels. The small plot sizes of even-aged, evenly dispersed trees are a limitation on this study, but the wide geographic distribution of the four sites did provide a gradient of productivity driven by climatic conditions over a substantial period of time. Moisture-related differences were illustrated by increases in total microbial biomass from

dry to wet period sampling, especially in the O horizon. Due to dry and wet period sampling in different years except Taylor Woods, we were unable to statistically compare within site microbial community variable differences due to seasonal moisture differences at three remaining sites (Elliot Ranch, Lookout Mt., and Crawford Creek). At Taylor Woods, the total microbial biomass during the wet period did increase in the same year with microbial structural differences in the O horizon, but not the mineral soil. Microbial biomass increased in a similar fashion between dry and wet periods across years at the other sites, but we cannot definitively say if this is a result of moisture differences or just inter-annual variation. As we expected, O horizon F:B ratios responded strongly to sampling period relative to the F:B ratio responses of the mineral soil. Microbial communities in the mineral soil are buffered from climatic extremes, while O horizon microbial communities are comparatively sensitive to climatic variability (Fisher and Binkley 2000, Perry et al. 2008).

Past management policies altered ponderosa pine-dominated ecosystems of the western United States, producing unintended consequences such as stand replacing wildfires (Covington et al., 1997). Mitigation of current wildfire potential in these dry, fire-prone forests will require reductions in stand densities. Sustaining healthy forests into the future needs both functioning aboveground and belowground communities. We found the microbial communities in the O horizon and mineral soil are resistant to long-term stand density reductions in even-aged ponderosa pine-dominated forests when the associated herbaceous and shrub communities are relatively unchanged, yet returning the herbaceous and shrub communities to historical prominence has been a major goal of restoration efforts. Our study further emphasizes the strong linkage between aboveground

and belowground components of the ecosystems, and the importance of regional climate on O horizon and mineral soil microbial communities in seasonally dry forests.

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Figure Legends

Figure 1. Mean total microbial biomass at four ponderosa pine sites (Elliot Ranch, CA; Lookout Mt., OR; Crawford Creek, OR; and Taylor Woods, AZ) of contrasting stand productivity. Microbial biomass determined from phospholipid fatty acid (PLFA) biomarkers measured during dry and wet sampling periods for forest floor (O horizon) and mineral soil (0-5 cm). Means with different letters are significantly different within horizons by sampling period. Vertical lines denote $\pm$ one standard error of the mean. Analysis performed on square root transformed data using two-way ANOVA ($\alpha = 0.05$) and Tukey's HSD mean separation.

Figure 2. Mean fungal-to-bacterial biomass ratios at four ponderosa pine sites (Elliot Ranch, CA; Lookout Mt., OR; Crawford Creek, OR; and Taylor Woods, AZ) of contrasting stand productivity. Microbial biomass determined from phospholipid fatty acid (PLFA) biomarkers measured during dry and wet sampling periods for forest floor (O horizon) and mineral soil (0-5 cm). Means with different letters were significantly different within horizons by sampling period. Vertical lines denote $\pm$ one standard error of the mean. Analysis performed on square root transformed data using two-way ANOVA ($\alpha = 0.05$) and Tukey's HSD mean separation.

Figure 3. Relative abundance of different forest floor (O horizon) microbial guilds (gram-positive bacteria, gram-negative bacteria, actinobacter, fungi) determined from phospholipid fatty acid (PLFA) biomarkers at four ponderosa pine sites (Elliot Ranch,

CA; Lookout Mt., OR; Crawford Creek, OR; and Taylor Woods, AZ) of contrasting stand productivity in the western United States. Multi-response permutation procedures of PLFA biomarkers using Euclidean distances ($\alpha = 0.05$) with simultaneous pairwise comparisons using the Peritz closure method to maintain Type I error rate ($\alpha = 0.05$; Petrondas and Gabriel 1983). All sites were significantly different from each other except Elliot Ranch and Lookout Mt (dry period), and Lookout Mt. and Crawford Creek (wet period).

Figure 4. Proportion of total community based on relativized mineral soil (0-5 cm) microbial guilds (gram-positive bacteria, gram-negative bacteria, actinobacter, fungi) derived from PLFA biomarkers at four ponderosa pine sites of contrasting productivity in the western United States. Multi-response permutation procedures of PLFA biomarkers using Euclidean distances ($\alpha = 0.05$) with simultaneous pairwise comparisons using the Peritz closure method to maintain Type I error rate ($\alpha = 0.05$; Petrondas and Gabriel 1983). All sites significantly different from each other except Lookout Mt. and Crawford Creek (wet period).

Table 1. Mean ($\pm$ standard error) forest floor (O horizon) and mineral soil (0-5 cm) biomass and nutrients in four ponderosa pine stands of varying stand densities in the western United States.

	Stand Density		
	Low	Medium	High
Organic horizon			
Mass (Mg ha^{-1})	13.3 (1.3)	15.7 (1.0)	16.3 (1.3)
Total C (Mg ha^{-1})	5.2 (0.7)	5.9 (0.5)	6.0 (0.5)
Total N (kg ha^{-1})	139 (15.2)	153 (11.6)	161 (14.2)
Total P (kg ha^{-1})	13 (2.2)	13 (0.9)	14.4 (1.3)
Soil (0-5 cm)			
pH [†]	5.11 (0.02)a	5.11 (0.02)a	5.07 (0.02)b
Total C (Mg ha^{-1})	26.2 (2.2)	22.4 (1.4)	24.8 (2.0)
Total N (kg ha^{-1})	1211 (94)	1035 (63)	1146 (78)
Total P (kg ha^{-1})	602 (29)	535 (19)	587 (30)

Notes: [†] Means within rows with different letters differed significantly, means without letters did not differ. Analysis performed using two-way ANOVA ($\alpha = 0.05$) and Tukey's HSD mean separation. Site productivity x stand density interactions were non-significant.

Table 2. Site characteristics, climate, and nutrient pools of four ponderosa pine stands of varying stand densities in the western United States

	Site			
	Elliot Ranch	Lookout Mt.	Crawford Creek	Taylor Woods
Region	Westside Sierra Nevada , CA	Cascade Range, central OR	Blue Mts., eastern OR	Colorado Plateau, northern AZ
Vegetation association	Mixed conifer/ Deerbrush	Ponderosa pine/ Velvet ceanothus	Ponderosa pine/ Idaho Fescue	Ponderosa pine/ Arizona Fescue
Subspecies	Ponderosa	ponderosa	scopulorum	scopulorum
Soil parent material	clay-loam tuff breccia	loam dacitic tephra	loam dacitic tephra	stony clay- loam basalt
Year study initiated	1970	1966	1967	1962
Stand age at initiation of study	45	90	84	83
Mean maximum air temperature (°C)	19.5	14.2	14.2	15.7
Mean minimum air temperature (°C)	3.3	-1.4	-3.4	-3.4
Mean total precipitation. (cm)	132	101	52	56
Mean total snowfall (cm)	65	200	217	209
Stand productivity (m ³ ·ha·yr ⁻¹) [‡]	1.84	1.48	0.96	0.64
Organic horizon				
Total biomass [†] (Mg ha ⁻¹)	18.2 (1.0)a	16.2 (1.3)ab	11.7 (0.8)b	15.5 (1.26)ab
Total C [†] (Mg ha ⁻¹)	6.7 (0.5)c	7.3 (0.5)c	4.9 (0.3)a	5.7 (0.4)b
Total N [†] (kg ha ⁻¹)	164 (12.0)	195 (11.6)	131 (12.8)	157 (15.3)
C:N ratios [†]	41.4 (1.3)	38.0 (1.7)	39.4 (2.2)	37.8 (1.4)
Total P [†] (kg ha ⁻¹)	13 (1.4)	22 (3.7)	18 (2.8)	13 (1.5)
Mineral Soil (0-5 cm)				
pH [†]	5.12 (0.02)a	5.00 (0.03)b	5.15 (0.02)a	5.12 (0.03)a
Total C [†] (Mg ha ⁻¹)	33.06 (1.10)b	30.10 (1.79)b	17.00 (1.02)a	18.81 (0.75)a
Total N [†] (kg ha ⁻¹)	1462 (49.9)b	1332 (71.2)b	816 (49.2)a	895 (38.1)a
C:N ratio [†]	22.8 (0.6)	22.5 (0.3)	20.8 (0.2)	21.1 (0.3)
Total P [†] (kg ha ⁻¹)	652 (16.3)a	670 (26.1)a	513 (19.7)b	451 (8.9)b

Notes: [†] Means averaged across stand densities ($\pm$ standard error of the mean). Means with different letters differed significantly among sites. Analysis performed on log transformed data (all except C:N ratios and pH) using two-way ANOVA ($\alpha = 0.05$) and Tukey's HSD mean separation. [‡] Total merchantable volume for 25 yr following initial thinning. Volumes calculated from measurements repeated every 5 yr. since initiation of study.

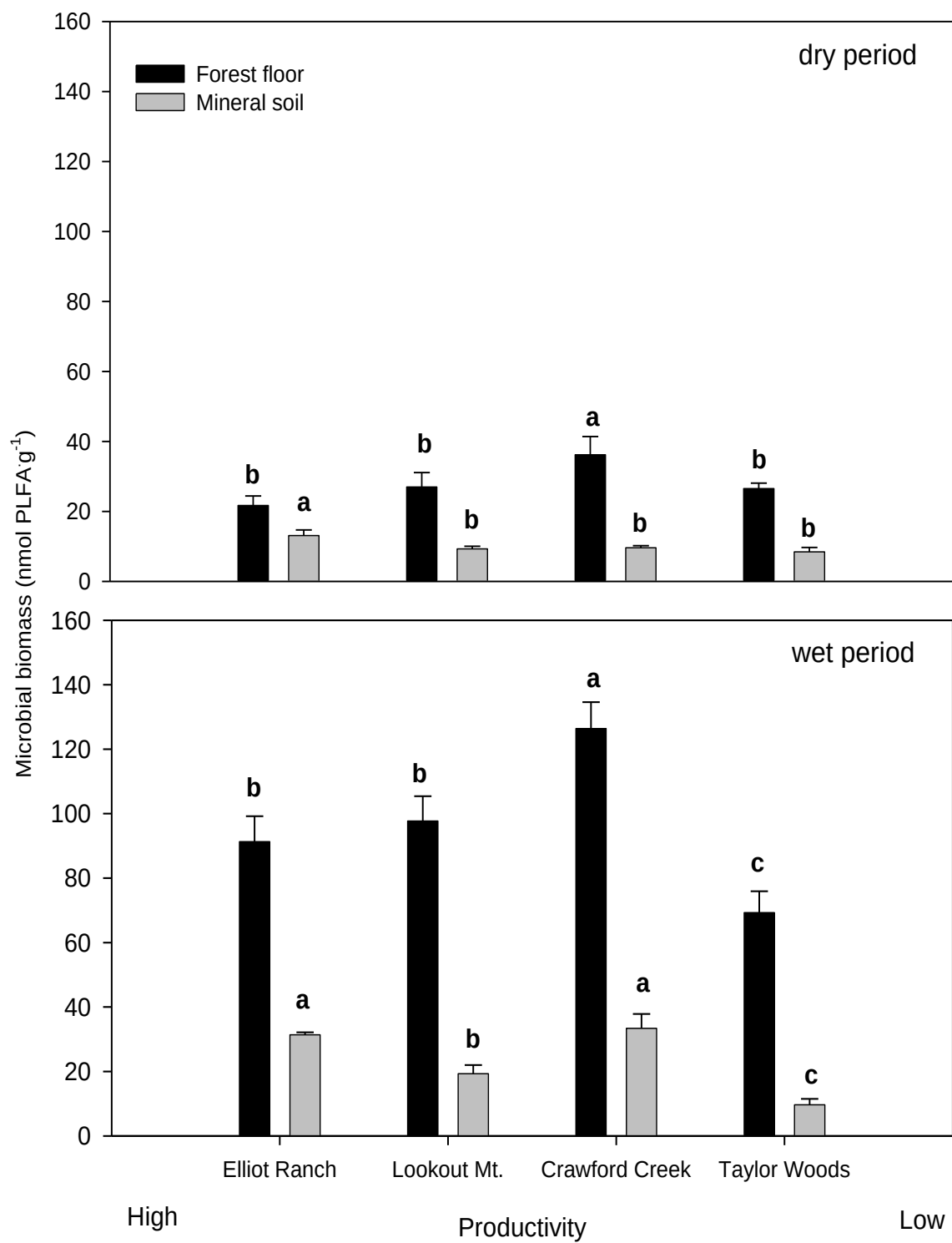


Figure 1

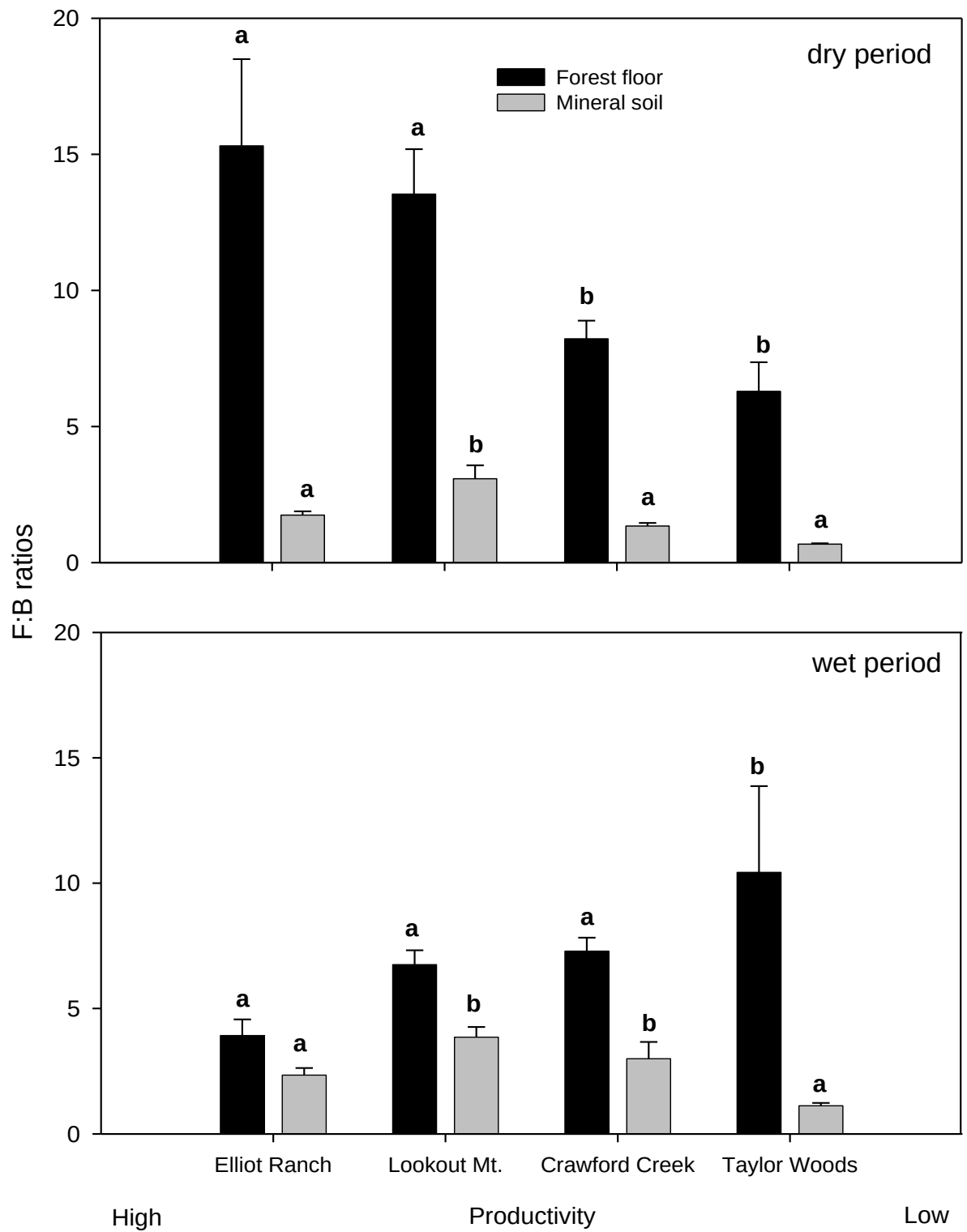


Figure 2

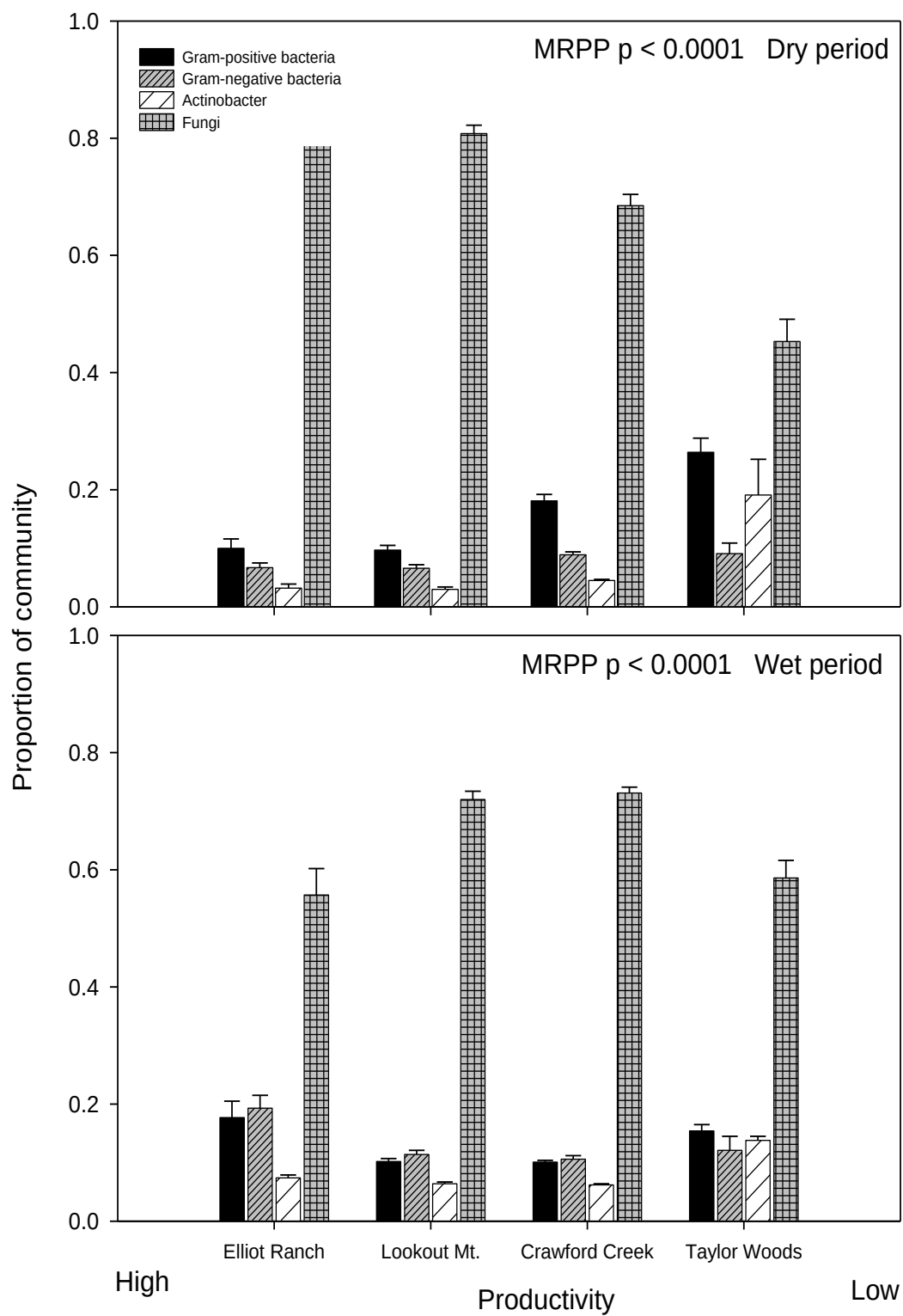


Figure 3

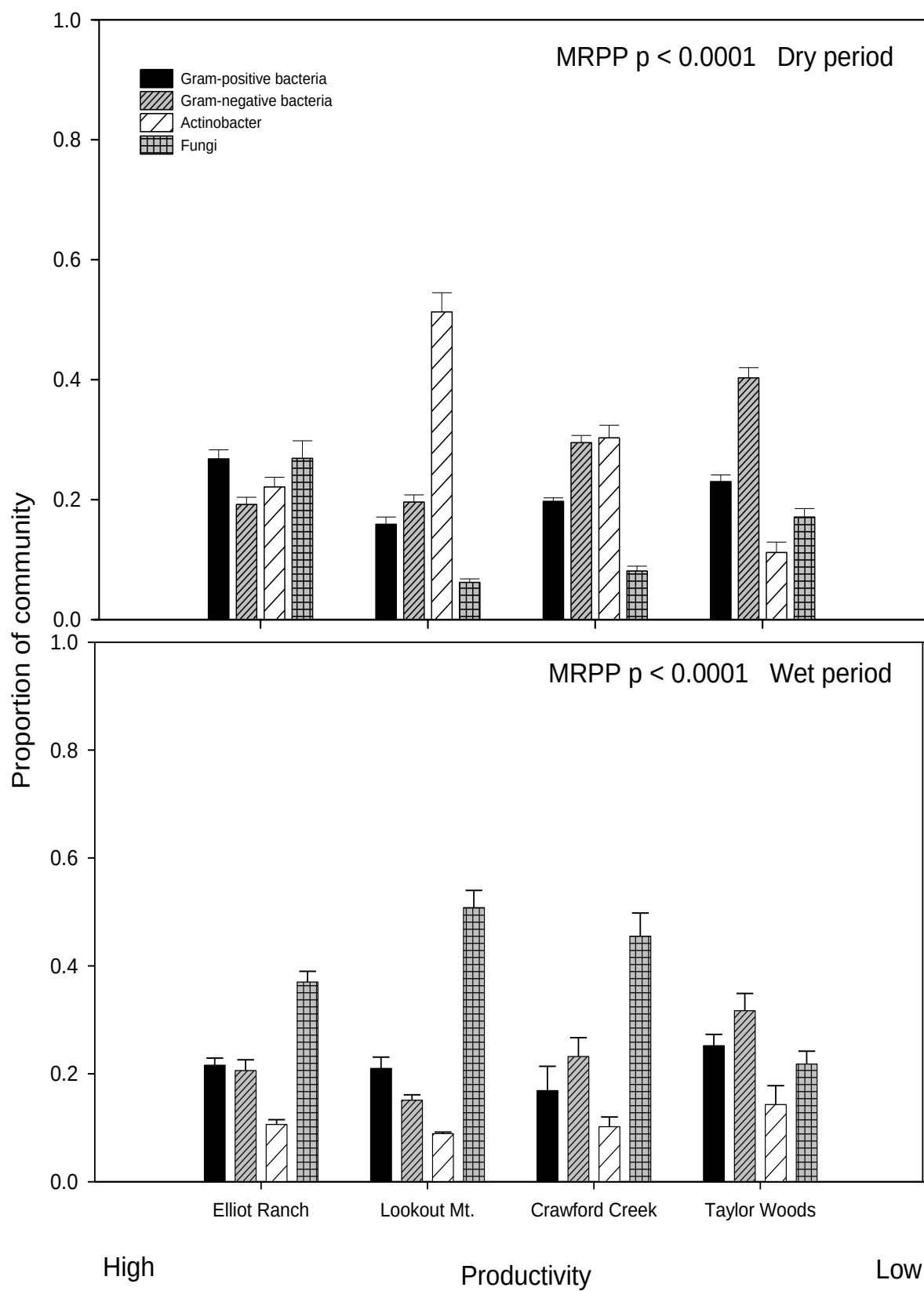


Figure 4

CHAPTER 3
Short-term responses of soil microbial communities to wildfire
mitigation treatments in southwestern ponderosa pine forests

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Abstract

Most western U.S. forests had historically low- and moderate-severity natural fire regimes, but now support high-severity wildfires. The national Fire and Fire Surrogate (FFS) network of 13 sites across the United States was designed to address ecological responses to operational-scale silvicultural treatments and prescribed fire in an effort to provide scientific information on wildfire mitigation to guide management decisions. The southern reaches of the Colorado Plateau in northern Arizona are one of the network sites and the focus of this study. The network experimental design included three replicate units at each site with each unit consisting of mechanical thinning, prescribed fire, and mechanical thinning followed by prescribed fire. The objectives of this study were to determine short-term response to FFS treatments on total soil carbon (C) and nitrogen (N) contents, cation exchange capacity (CEC) and exchangeable cations, pH, net N mineralization and nitrification rates, potential soil enzyme activity, community-level physiological profiles (CLPP), and soil microbial community biomass and structure. Mechanical thinning alone increased total C and N contents of the mineral soil, while prescribed fire had little effect except to lower total N of the forest floor (surface organic horizon). The CEC was significantly increased for burning alone compared to the other fuel treatments due to increased exchangeable Ca. In situ rates of net N mineralization and nitrification in the surface mineral soil (0 – 15 cm) were increased 6 months after thinning and burning treatments, while thinning resulted in net N immobilization. Net N mineralization and nitrification for all treatments returned to rates comparable to the control by the first year post-

treatment. Thinning treatments decreased overall enzyme activity for the period of this study. Community-level physiological profiles and microbial community structure based on assessment of the abundances of phospholipid fatty acids were unchanged in the surface organic horizon and mineral soil (0-5 cm) one year after treatments. Wildfire mitigation treatments had only moderate short-term impacts, yet soil nutrient and microbial populations could potentially be altered with long-term changes to the herbaceous community.

Key Words:, community-level physiological profile, fuel treatments, microbial community structure, nitrification, nitrogen mineralization, soil enzymes.

Introduction

High-severity wildfires in dry, fire-prone forests of the southwestern United States have increased in frequency and size after a century or more of increasing tree density and accumulation of fuels (Cooper, 1960; Covington and Moore, 1994; Mast et al., 1999). Reducing stand densities and fuels in these forests has been shown to reduce fire severity (Wagle and Eakle, 1979; Pollet and Omi, 2002, Strom and Fulé, 2007), but with several alternative fuel reduction strategies available (Boerner et al., 2008) the question becomes: What strategy best serves the long-term sustainability of these fire-adapted forests? Meeting immediate wildfire mitigation targets may be necessary, but restoring the ecological role of fire and increasing the resistance of these communities to future wildfires, insect and disease outbreaks, and climate change are key objectives to attain long-term sustainability (Miller and Urban, 2000; Allen et al., 2002; Agee and Skinner, 2005).

When Euro-Americans settled in the southwestern United States, the ponderosa pine (*Pinus ponderosa* P. & C. Lawson) forests they encountered were more open landscapes than today, with understories dominated by grasses forbs and shrubs surrounding clusters of pine trees (Covington and Moore, 1994; Covington et al., 1997; Fulé et al., 1997). Episodic recruitment of ponderosa pine seedlings in northern Arizona during favorable climatic conditions and inactive fire periods shaped the general forest structure of pre-Euro-American settlement (Brown and Wu, 2005). Pre-settlement trees were often logged, while cohorts established in the 1919 episodic recruitment event (Pearson 1933; Savage et al. 1996,) increased stand densities to the current 727 trees ha⁻¹ on average in Arizona (O'Brian, 2002), with

some stands exceeding 2,000 trees ha⁻¹ (Covington et al., 1997; Kaye et al., 2005; Bakker and Moore, 2007). Frequent fires (2-20 y), herbaceous competition, and drought maintained the pre-Euro-American forest structure of 30-140 trees ha⁻¹ (Cooper, 1960; White, 1985; Swetnam and Baisan, 1996). As tree density increased, herbaceous communities were diminished in many stands, reducing litterfall, root exudation and turnover. Slowed decomposition due to changes in quantity and quality of these organic matter inputs resulted in an accumulating forest floor (surface O horizon) of recalcitrant ponderosa pine litter (Covington and Sackett, 1984).

Fire shapes ecosystems by influencing plant composition, plant growth, soil biota, and nutrient cycling (Hart et al. 2005). In the absence of fire, several researchers have speculated that the soil microbial structure has been altered in ponderosa pine forests of the southwestern United States (Covington and Sackett, 1984; Kaye and Hart, 1998*a,b*; Boyle et al., 2005; Kaye et al., 2005). Soil bacteria and fungi, the primary decomposers, process between 80 and 90% of all plant detritus via production of extracellular enzymes (Bardgett, 2005). Microbial mediated decomposition and mineralization are major drivers of forest productivity (Paul and Clark, 1996). Consequently, understanding the interactions between plant and soil microbial communities is important for maintaining productive forest ecosystems (Wardle et al., 2004; Hart et al., 2005).

The productivity of ponderosa pine forests of the southwestern United States is considered to be limited by nitrogen (N) availability (Hungate et al., 2007). Mechanical thinning alone or in combination with fire can alter N status (Covington

and Sackett, 1986; Kaye and Hart, 1998b; Hassett and Zak, 2005; Grady and Hart, 2006), as well as soil moisture (Boyle et al., 2005; Simonin et al., 2007; Zou et al., 2008) and soil temperatures (Boyle et al., 2005). Following wildfires, the combination of reduced detrital inputs and loss of soil decomposer microorganisms due to lethal temperatures may decrease decomposition and nutrient transformations, resulting in diminished N availability for plants (Paul and Clark, 1996; DeBano et al., 1998). Yet, Covington and Sackett (1984) found no decline in surface organic horizon N immediately following the initiation of a prescribed burning regime in a ponderosa pine stand in northern Arizona. A meta-analysis by Wan et al. (2001) of fire effects on N pools found that total N in fuels, but not N concentration, was reduced following fire due to fuel mass loss. Some of this loss of N in the fuels was transferred to the surface organic horizon replacing some of the combustion losses in the surface organic horizon pool. Soil ammonium (NH_4^+) and nitrate (NO_3^-) also increased following fire, yet total soil N and total soil N concentrations remained unchanged. The magnitude of fire effects varied with vegetation type, fuel type, fire intensity, and fuel consumption amount and percentage.

Our current knowledge of soil microbial community responses to operational wildfire mitigation treatments in ponderosa pine forest of the southwestern United States is insufficient for predicting microbial community and process dynamics in these ecosystems following treatments (Hart et al. 2005). Though soil processes such as N transformations (Kaye and Hart, 1998a), soil CO_2 efflux (Kaye and Hart, 1998b; Grady and Hart, 2006), and potential soil enzyme activities (Boyle et al.,

2005) have been investigated, at issue in these studies is how applicable the experimental designs were for interpreting consequences of operational scale mitigation efforts (Hart et al., 2006). Prescription burning and wildland fire have been promoted to restore forest structure and reduce fuels (Stephens and Moghaddas, 2005), yet both of these options may be inappropriate for the wildland-urban interface (Schwilk et al. 2009). In these situations, mechanical thinning, as a surrogate, attempts to mimic stand-thinning actions of fire (McRae et al. 2001).

The Joint Fire Science Program (United States Departments of Interior and Agriculture) funded a network of study sites for the long-term evaluation of possible changes to ecological components and processes in fire-adapted forests following operational wildfire mitigation treatments. The Fire and Fire Surrogate (FFS) network consists of 13 sites across the United States; of these, eight were in ponderosa pine-dominated ecosystems. The FFS network study was intended to address the impact of mechanical thinning alone or in combination with fire, and fire alone on forest conditions (Edminster et al., 2000; McIver et al., 2001). Much of the current fuel reduction program (Healthy Forests Restoration Act of 2003, U.S. Public Law 108-148) is targeted at the wildland-urban interface where prescribed burning is often difficult due to aesthetics, air quality, and structural protection (Berry and Hesseln, 2004, Schwilk et al., 2009). The FFS study attempts to compare alternative treatments, such as thinning alone, as a surrogate for stand reductions from wildfire. Under current fuel conditions, even prescribed fire may not produce ecological responses similar to historic wildfire. Stand structural changes that mimic historic conditions also may not mimic the ecological outcomes

associated with historical wildfire, but these structural changes may alter current wildfire behavior to allow wildland fire use in the future (Schwilk et al., 2009). The FFS program hopes to address the needed scientific information to assist managers on appropriate fuel reduction treatments for their site specific situations.

In this study, we evaluated soil response to the three FFS wildfire mitigation treatments. These treatments include mechanical thinning alone, prescribed burning alone, and a combination of mechanical thinning followed by prescribed burning. The experimental design of the FFS study allowed enough flexibility so each of the 13 sites could stand on its own as a research project, yet still provide the necessary core data to meet the larger study objectives (Edminster et al., 2000; McIver et al., 2001). We focus on the Southwestern Plateau site in northern Arizona to evaluate the potential structural and functional changes in soil microbial communities following FFS treatments in ponderosa pine forests of the southwestern United States. The objectives of this study were to determine the effects of FFS treatments on: 1) soil microbial community biomass and structure; and 2) microbial activity (potential enzyme activity, net N mineralization and nitrification, community-level physiological profile). We expected fire to decrease fungal biomass in both the soil organic horizon and mineral soil due to direct heat mortality (Dunn et al, 1985). Mechanical thinning with prescribed fire is expected to increase microbial biomass and activity to a greater degree than either thinning alone or prescribed fire alone. This result is due to greater decomposition and mineralization of residual slash additions with lower carbon to nitrogen (C:N) mass ratios following low-intensity prescribed fire. We expected prescribed burns would result in short-term increased

net N mineralization and nitrification due to lower C:N ratios of organic matter (Wan et al., 2001). We also anticipated changes in microbial structure due to accompanying changes in litter quality, quantity, and microclimate, and differential mortality of soil microbes from fire.

Methods

Study Site, Experimental Design, and Soil Sampling

The Southwestern Plateau FFS study site is located in northern Arizona, with two replicate blocks (Rudd Tank, 35° 14.0'05.9" 111° 44.0'58.4", and Powerline, 35° 12.0' 33.9" 111° 45.0'32.2") on the Coconino National Forest west of Flagstaff, Arizona, and the third block (KA Hill, 35° 12.0' 33.9" 111° 45.0'32.2") on the Kaibab National Forest southeast of Williams, Arizona. Elevations range from 2217 to 2264 m, with overstory dominated by ponderosa pine. There are small populations of alligator juniper (*Juniperus deppeana* Steud.) and some Gambel oak (*Quercus gambelii* Nutt.) trees are present (Faiella and Bailey, 2007). For a greater discussion of vegetation structure and fuels of the FFS study sites, the readers are referred to Schwilk et al. (2009). Currently, these areas are used for recreation and domestic livestock grazing, and have been commercially harvested in the past.

Soils at Powerline and Rudd Tank areas are dominated by Typic Eutroboralfs, while KA Hill area is primarily composed of Lithic Eutroboralfs. These soils are either fine, smectitic or clayey-skeletal, smectitic with 35 to 60% clay in the fine earth fraction (< 2 mm) of the soil. Annual precipitation ranges from 546 mm at Powerline and Rudd Tank to 549 mm at KA Hill with a bimodal distribution.

Nearly half of the precipitation falls as snow (~210 cm depth) and the other half as late summer rains. Temperature averages 7.5 °C, with maximum of 15.7 °C and minimum -3.4 °C (<http://www.wrcc.dri.edu/summary/climsmaz.html>). Little precipitation occurs from May to early July, resulting in low soil moisture content until the onset of summer rains in mid-July.

The experimental design was a randomized complete block with three blocks and four treatments (burn-only (BO), thin-only (TH), thin and burn (TB), untreated (UT)). Treatment blocks were selected in areas identified by National Forest district silviculture staff. Treatment unit boundaries followed existing stand and natural landscape features and contained a core 10 ha sampling area. Treatment units varied in size from 14-16 ha, with at least a 30 m buffer between adjacent units. Permanent boundaries and sampling point centers (see below) were established prior to treatments. Mechanical harvesting began in late 2002 and was completed the spring of 2003. Prescribed fire treatments followed in the fall of 2003 (Faiella and Bailey, 2007).

Thin-only and TB treatment units at each replicate block were harvested to reduce stem density to 116 trees ha⁻¹ with a residual overstory of 12-14 m² ha⁻¹, and created an uneven-aged patchy forest structure (Faiella and Bailey, 2007). Residual slash was piled at the edge of, but outside both the TH and TB treatment units. Slash from TH and TB was burned at the same time as the BO units were treated. Commercial contracts provided for the thinning operations and National Forest personnel performed the prescription burns. These treatments were completed in a

manner comparable to normal fuel reduction operations conducted by the land management agencies.

A grid of thirty-six 20 m x 50 m plots with permanent point centers at 50 m intervals in each 10 ha core treatment was established for vegetation sampling. Due to destructive sampling for soils, ten corresponding soil sampling plots (20 m x 50 m) were established adjacent to odd numbered vegetation plots up to 20 (i.e., 1, 3, 5, ... 19). Surface organic horizon (soil organic horizon) and mineral soil (0-5 cm) were collected at randomly selected points within each soil plot of each unit pre-treatment (fall 2001) and three post-treatment periods (Spring 2004, Fall 2004, Fall 2005). Soil organic horizon and mineral soil (0-5 cm) were collected within a litter frame of known area (0.01 m²), placed in polyethylene bags, and transported to the laboratory on ice. Mineral soil (0-5 cm) was collected under the removed O horizon using a 2-cm diameter soil probe (Oakfield Apparatus Company, Oakfield, WI, USA).

The O horizon samples had all material greater than 6-mm diameter removed, while mineral soil samples were sieved (< 2 mm) immediately upon arrival at the laboratory. All samples were weighed, and then a subsample (~20 g) was taken from both the O horizon and mineral soil (0-5 cm). Each subsample was placed in a drying oven for 48 h (O horizon 70 °C, mineral soil 105 °C), then reweighed to determine water content. Additional subsamples (~10 g) were taken from the O horizon sample for microbial community analysis, and subsamples from the mineral soil (0-5 cm) were also taken for

microbial (~10 g), enzyme (~5 g), and community-level physiological profile (CLPP; ~5 g) analyses. Samples for enzyme assays and CLPP analyses were stored no longer than 12 h at 4 °C prior to analysis. The remaining portion of each sample was then air-dried. For each sample period, soil organic horizon was analyzed for total C and N concentrations, while mineral soil (0-5 cm) was analyzed for pH, total C and N concentrations, potential enzyme activities, and CLPPs. Mineral soil exchangeable cations were measured on pre-treatment and 1-y post-treatment mineral soil (0-5 cm) only. Microbial community analyses were conducted only on 1-y post-treatment O horizon and mineral soil (0-5 cm). For these analyses, three replicate subsamples for each treatment unit were composited from three plots samples (1, 3, 5), (7, 9, 11), and (13, 15, 17) for both O horizon and mineral soil. These subsamples were frozen for 24 h, then freeze-dried (-50 °C, 70×10^{-3} Mbar for 24 h, Edwards Modulyo, Crawley, UK) prior to extraction for phospholipid fatty acids (PLFA). The extraction process occurred within 48 h of returning to the laboratory.

An associated study investigator (S. Haase, Pacific Southwest Research Station, USFS, USDA, 2007, personal communication) collected surficial O horizon (forest floor) for fuel determinations by destructive sampling from thirty-six m² subplots within each treatment unit pre-treatment (summer 2001), between mechanical thinning and burning on the TB units (summer 2003), and 1-y post-treatment (summer 2004). These O horizon mass values

were used with our determinations of O horizon total C and N concentrations to calculate total C and N contents on an areal basis.

Total Carbon and Nitrogen

Air-dried, well-mixed O horizon samples and mineral soil (0-5 cm) were ground until the entire sample passed through a #100 sieve (< 0.149 mm).

Subsamples (20-50 mg) of these materials were then analyzed for total C and N concentrations on a commercially available elemental analyzer (Flash EA 1112, CE Elantech, Lakewood, New Jersey, USA).

Exchangeable cations, and pH

Cation exchange capacity (CEC) is an indicator of soil nutrient holding and buffering capacity. Exchangeable cations (Ca, Mg, K, Na, Fe, Al) and effective CEC of the mineral soil (0-5 cm) were measured using the method described by Hendershot et al. (1993), with elemental concentrations measured with a flame atomic adsorption spectrophotometer (Perkin-Elmer AAnalyst 100, Waltham, Massachusetts, USA). This method was used because it measures cation exchange at the pH of the sampled soil. Exchangeable cations were extracted using 30 ml of 0.1 M BaCl₂ from a 1 g subsample of sieved (< 2 mm), air-dried soil. Calculated CECs were summations of the individual extractable cation concentrations. Both Fe and Al were measured on a subset of pre-treatment samples, but values were below detection limits; therefore, Fe and Al analyses were not performed on post-treatment samples and not included in CEC estimates. Soil pH was determined by immersing a glass electrode into a 1:5 (w/v) soil-to-0.01 M CaCl₂ solution

(Hendershot et al. 1993) connected to a Orion 550A pH meter (Thermo Fisher Scientific, Inc., Waltham, Massachusetts, USA).

Net Nitrogen Transformations

The *in situ* covered-core method (Hart et al., 1994) was used for assessment of net N mineralization and nitrification rates. Intact soil cores for net N transformation measurements were also taken for each sample period within 1-m of the 0.01m² litter frame sample. Two adjacent intact mineral soil cores (0-15 cm) were sampled using a 5 cm x 15 cm corer attached to a slide hammer (AMS, Inc., American Falls, Idaho, USA) containing a 5 cm x 15 cm thin-walled polycarbonate inner sleeves. One core was returned to the original hole left after sampling. The O horizon, if any, was replaced on top of the soil core. The second core was transported to the laboratory on ice, immediately sieved (< 4 mm), then mixed and subsampled (15 g). Gravimetric water content was also measured for these initial cores in the same manner as described above.

Extraction of ammonium (NH₄⁺) and nitrate (NO₃⁻) using 2 M KCl (50 ml) were performed immediately following sieving (Hart et al., 1994). Ammonium and NO₃⁻ concentrations were determined colorimetrically from the KCl extracts using a Flow Injection Analyzer (Lachat Instruments, Inc. 2001 and 2000, respectively). Analytical values were corrected for soil moisture content and reported on an oven-dry weight basis. After 28 days, the *in situ* incubated cores were removed and processed in the same manner as the initial core. Initial cores were taken pre-treatment, 1- and 2-y, with *in situ*

cores incubated for 28 days after these pre- and post-treatment periods.

Annual sampling was done at the end of the rainy season when average maximum temperatures are 28 °C, average minimum temperatures are 5 °C, and average monthly precipitation is 4.9 cm

(<http://www.wrcc.dri.edu/summary/climsmaz.html>). The six month cores were incubated when average maximum temperatures are 15 °C, average minimum temperatures are 3 °C, and average monthly precipitation is 3.3 cm (<http://www.wrcc.dri.edu/summary/climsmaz.html>). Net N mineralization over the incubation period was determined by subtracting the initial inorganic N pools ($\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N}$) from the final post-incubation N pools. Net nitrification was calculated similarly using only the $\text{NO}_3^-\text{-N}$ pool. Mean soil bulk density values (Mg m^{-3}) of the < 4 mm fraction from all intact cores for each plot and each sample period were averaged. These average bulk density values were used to calculate net N transformation rates on an areal basis.

Enzyme assays

To estimate if wildfire mitigation treatments affected microbial activity and function, the potential activities of eight ecologically relevant enzymes were assayed: β -1,4-glucosidase, α -1,4-glucosidase, β -galactosidase, β -xylosidase, cellobiohydrolase, *N*-acetyl-glucosaminidase (NAGase), alkaline phosphatase, and sulfatase. These eight enzymes were measured using the MUB-linked substrates (Boyle et al., 2005). The first five enzymes support decomposition of carbohydrates and polysaccharides into energy sources readily accessible by soil organisms (Eivazi and Tabatabai, 1988; Sinsabaugh, 1994; Eivazi and Bayan, 1996; Boerner et

al., 2000). *N*-acetyl-glucosaminidase contributes to the mineralization of N from chitin (Olander and Vitousek, 2000), phosphatase releases inorganic P by breaking ester linkages (Eivazi and Tabatabai, 1977), and sulfatase breaks ester linkages releasing inorganic forms of sulfur (Ganeshamurthy and Nielsen, 1990; Eivazi and Bayan, 1996).

Methods for enzyme assays followed those outlined by Boyle et al. (2005). Field-moist soil (1 g) was first suspended in 100 mL of 5 mM bicarbonate buffer solution (pH 8.2), then an aliquot (100 μ L) of this soil solution was added with 100 μ L of an enzyme substrate solution to a single microtiter plate well. All eight enzyme substrates followed this procedure six times with quenching standards included on each plate (Sinsabaugh et al., 1991). Plates were immediately read using a Fluoromax fluorometer (Jobin Yvon-Spex, Edison, NJ, USA) with an attached MicroMax Microwell plate reader (excitation of 360 nm, emission 450 nm). Plate incubation was 1 h at 27 °C before the final fluorometric reading.

Community-level Physiological Profiles

The CLPP method cultures microorganisms within microtiter plate wells that contain different C substrates. Some researchers believe that data collected from C substrate utilization plates (Biolog, Inc, Hayward, California, USA) are indicative of the metabolic potential of the microbial community (Garland and Mills, 1991), and others assert that CLPPs yield information on the functional diversity of the microbial community in question (Kennedy, 1994). As used in this study, these culture plates provide a qualitative indicator of bacterial and fungal community changes following wildfire mitigation treatments.

Microorganisms were obtained from 4 g of subsampled mineral soil centrifuged in an inoculating solution, diluted, and then transferred to wells containing a single C substrate following procedures outlined in Classen et al. (2003). Bacterial plates were incubated at 25 °C for 72 h, while fungal plates were incubated for 96 h prior to analysis of wells on an EMax absorbance microplate reader (Molecular Devices, Sunnyvale, California, USA) either for color development (Biolog EcoPlate) or turbidity (Biolog SFN2). Bacterial microtiter plates (Biolog EcoPlate) contain 31 different C substrates, which are replicated three times on each plate. A tetrazolium dye sensitive to reduction is included with each C substrate. This dye develops a purple color if catabolized. Fungal CLPP is done on microtiter plates containing 95 individual C substrates, but do not include the tetrazolium dye due to toxicity to some fungi (Dobranic and Zak, 1999). Bacteria are reduced on the fungal plates with antibiotics during inoculation of the microtiter plates following the procedure outlined by Dobranic and Zak (1999).

Microbial Community Structure

Surface organic horizon and mineral soil PLFAs were assessed for microbial community biomass and structure. Analysis of PLFA patterns has been shown to be a powerful approach to describe the structure of the soil microbial communities and to detect changes due to altered ecological conditions (Bååth et al., 1992; Frostegard et al., 1993a,b; Zelles, 1999; Hasset and Zak, 2005; Ramsey et al., 2006). Individual PLFAs can be used as specific biomarkers to identify different microbial groups and as an index for total microbial biomass. Ramsey et al. (2006)

found PLFA analysis often resolved treatment effects when molecular methods were unable to detect differences.

Mass spectral analysis identified numerous compounds between C14 to C22 in C chain length, of which 16 are used as microbial biomarkers. The 16 compounds are a conservative estimate of known microbial biomarkers (O’Leary and Wilkinson, 1988; Frostegard and Bååth, 1996; Zelles, 1999). Total microbial biomass was calculated by summing these 16 biomarkers. Gram-negative bacterial biomarkers (cy17:0, cy19:0, 16:1 ω 9, 16:1 ω 7, 18:1 ω 5c, and 18:1 ω 7) and gram-positive bacterial biomarkers (i15:0, a15:0, i16:0, i17:0, a17:0, and 10me16:0) discriminate between groups. These biomarkers are then summed with general bacterial biomarker (C15:0 and C17:0) to estimate total bacterial biomass (O’Leary and Wilkinson, 1988; Frostegard and Bååth, 1996; Zelles, 1999). Two isomers of C18:2n6 (*trans* and *cis*) were used to estimate the fungal group (Frostegard and Bååth, 1996).

Five g of freeze-dried mineral soil or 2 g of freeze-dried ground litter was extracted with a single-phase mixture of chloroform, methanol, and phosphate buffer (White et al., 1979) then fractionated into neutral, glyco-, and phospholipids (Frostegard et al., 1991). The phospholipids are then esterified and reconstituted in hexane prior to analysis (Frostegard et al., 1993b). A capillary column was utilized for compound separation. Identification and quantification of each specific fatty acid methylester (FAME) was established using commercially available standards (Accustandard, Sigma-Aldrich, St. Louis, Missouri, USA) through electron ionization on a quadrapole mass selective detector (Agilent 6890N/5973N gas

chromatograph/mass spectrometer, Santa Clara, CA, USA). Quantification ($\mu\text{mol PLFA kg}^{-1}$ oven-dry material) of samples was based on calibration curves derived from individual FAME standards.

Statistical methods

Statistical analysis of total C and N contents, soil water content, pH, and microbial activity (net N mineralization, net nitrification, and individual potential enzyme activity) was performed on plot means using a generalized linear mixed model (GLIMIXX, SAS for PCs ver. 8.1, Cary, North Carolina, USA) to determine differences based on treatment, sampling date, and treatment x sampling date interactions. Within the GLIMIXX model, we designated site as a random effect with sampling date as our repeated measure. Transformation of data to stabilize variance, obtain a linear relationship, and normalize distributed responses to meet assumptions needed for classical statistical frameworks often fix one assumption, but may be inappropriate for another. Generalized linear mixed models start by utilizing an appropriate model for the observed data instead of manipulating the data to fit the model. This allowed us to test for differences in wildfire mitigation treatments on response variables adjusted for any pre-treatment site differences correlated to the response variable. A recent review of generalized linear mixed models argues for the use of these models in ecology and evolution studies to allow greater generalizations of conclusions by incorporating random effects into traditional blocked design experiments (Bolker et al., 2008).

Community-level physiologic profiles, PLFA biomarkers, and the suite of enzymes for each sample were analyzed using multi-response permutation

procedure (MRPP), a multivariate statistical procedure outlined in Mielke and Berry (2001). Data from CLPPs were normalized for each plate by dividing color or turbidity development of each well by the total color or turbidity development of the entire plate. This normalization procedure provides a simple method for reducing the influence of differences in initial inoculum densities (Classen et al., 2003). Phospholipid fatty acid data were normalized in a similar fashion where the mass of each specific biomarker was expressed relative to the total mass of all biomarkers for a given sample. Phospholipid fatty acid biomarkers, grouped by fungi, gram-positive bacteria, and gram-negative bacteria, were also statistically analyzed using GLIMIXX procedure. Multi-response permutation procedure does not require assumptions of multivariate normality or homogeneity of variances, which are seldom met with ecological community data (McCune and Grace, 2002). Simultaneous pairwise comparisons, using the Peritz closure method to maintain Type I error rate and *a priori* alpha level ($\alpha = 0.05$), tested the null hypothesis that all possible pairs are similar (Petronidas and Gabriel, 1983). This procedure was performed using Microsoft Excel macros (available from senior author) following the methodology of Mielke and Berry (2001).

Results

Total soil C and N

Surface O horizon mass was not significantly different by site for pre-treatment samples. However, for the 1-y post-treatment samples, the mass of the O horizon was greater in the UT treatment, while the BO was significantly less than the other treatments (Table 1). Additional data, accessed from the FFS meta-analysis

database and personal communication (S. Haase, Pacific Southwest Research Station, USFS, USDA) showed TB O horizon mass initially gained 23% following harvesting, then lost 36% due to the prescribed fire. Total C contents in the O horizon were similar among treatments for the two years following treatment, yet total N contents were significantly greater for the TH compared to the BO treatment (Table 1).

Mineral soil (0-5 cm) total C and N contents were not significantly different pre-treatment. However, there were significant differences among treatments during the first post-treatment sampling for total N contents. Thin-only soil total N contents were significantly greater than the TB treatment, but not UT or BO treatments. While not significantly different, total C contents exhibited the same pattern as total N content, with the highest values in the TH treatments and lowest values for TB treatments (Table 1).

Exchangeable cations and pH

Pre-treatment samples for exchangeable cations and CEC in the mineral soil (0-5 cm) were statistically similar for all sites. Analysis of the 1-y post-treatment samples showed the BO treatment had significantly greater levels of exchangeable Ca than UT and TH treatments, and significantly lower exchangeable Na than the TB treatment. Cation exchange capacity was also greatest in the BO treatment, and significantly different than either the UT or TH treatments. Soil pH was similar statistically across all treatments (Table 1) for each sampling period.

Net Nitrogen Transformations

Both net N mineralization and nitrification rates were similar among the pre-treatment and 1- and 2-y post-sampling periods (Fig. 1). Only the six-month post-treatment sampling demonstrated any statistically significant differences in net N transformation rates. Net N mineralization rates were significantly higher for the TB treatment than the TH treatment, the latter which showed net N immobilization at this first post-treatment sampling (Fig. 1). Net nitrification rates were not significant different among treatments, except for the six-month samples. Thin and burn treatments had significantly higher rates of net nitrification than the TH and UT treatments (Fig. 1).

Enzyme assays

The GLIMIXX analysis of potential enzyme activity with sampling periods as a repeated measure resulted in significant differences for all individual enzymes, except β -1,4-glucosidase, α -1,4-glucosidase, and xylose (Fig 2.). Two enzymes (β -galactosidase, cellobiohydrolase) showed similar patterns where TH and TB treatments had significantly reduced enzyme activity (Fig. 2). For *N*-acetylglucosaminidase activity, the BO treatment was significantly higher than the TH treatment, but not different than the UT or TB treatments (Fig. 2). Alkaline phosphatase activity was significantly lower in the TB treatment compared to the UT and BO treatments, but was not different than the TH treatment. Sulfatase activity was significantly lower in the TH compared to all other treatments, while in the TB treatment sulfatase activity was also significantly lower than either UT or BO treatment (Fig. 2). Multi-response permutation analysis of enzyme activity as a

group was not significantly different for pretreatment by site ($p = 0.9478$) or post-treatment burning ($p = 0.2913$). Multi-response permutation analysis of enzyme activity grouped by thinning x sample period resulted in significant differences between harvesting (TH, TB) treatments and non-harvested treatments (UT and BO) over the post-treatment period ($p < 0.0001$). There was no difference between TH and TB, or between UT and BO treatments for any sample date. The differences for thinning treatments (TH, TB) compared to unthinned treatments (UT, BO) are illustrated in a nonmetric multidimensional ordination plot (Fig. 3).

Community-level Physiological Profiles

Community-level physiological profiles based on C substrate utilization showed no significant differences during pre- or any post-treatment sample periods for either bacteria or fungi. Neither total plate activity or normalized C substrate utilization patterns showed any statistical differences among treatments or treatment x sampling date interactions.

Microbial Community Structure

In both the surface organic horizon and surface mineral soil, microbial community structure based on raw data or normalized PLFA biomarkers showed no statistical differences among treatments after 1 y (O horizon $p = 0.2255$, mineral soil $p = 0.7658$). Phospholipid fatty acid biomarkers by groups (i.e., fungi, gram-positive bacteria, and gram-negative bacteria) also did not indicate any treatment differences (Fig. 4, 5).

Discussion

The FFS Network analysis found that operational scale wildfire mitigation treatments produced relatively small immediate effects on soil microbial activity (Boerner et al., 2008), yet the network analysis did not include the more extensive soil microbial community analyses that we employed at the Southwestern Plateau site. Nevertheless, even with this more comprehensive assessment, we also found that soil microbial communities were little affected by wildfire mitigation treatments 1-y post-treatment. Boyle et al. (2005) also found little evidence of thinning impacts on soil microbial communities in a nearby ponderosa pine stand where the residual basal area ($13 \text{ m}^2 \text{ ha}^{-1}$) following thinning was comparable to our thinning treatments, but with a clumpy spatial pattern (Covington et al., 1997). However, Boyle et al. (2005) did find an increase in microbial N with thinning and burning treatments that included repeat burning. Mechanical thinning in other forest types has been shown to reduce microbial biomass without altering the community structure (Hassett and Zak, 2005), change community structure without altering microbial biomass (Maassen et al., 2006; Siira-Pietikäinen et al., 2001; Hannam et al., 2006), and alter both biomass and structure (Pietikäinen et al., 2007).

We did not find statistically significant changes in either the O horizon or the mineral soil microbial communities with either PLFA biomarkers or CLPP in our study 1-y post treatment. Considerable variability within the PLFA and CLPP data was in part due to the spatially variable nature of operational treatments. Both prescription fire and wildfire has been reported to decrease fungal dominance within microbial populations (Hart et al., 2005; Cairney and Bastias, 2007). Fungi

are more susceptible to the heat of fire due to lower mortality threshold temperatures (Dunn et al., 1985). Decreases in fungal populations have been reported in wet sclerophyll forests in eastern Australia between 2- and 4-y prescribed burn intervals (Bastias et al., 2006). The historical influence of fire on microbial populations has been related to frequency, intensity, and season of burning, along with fuel loads and weather conditions at the time of burning (Vasquez et al., 1993, Pietikäinen et al. 2000). One concern with using fire in mitigation treatments is the possible long-term decrease with repeated burning on fungal populations (Cairney and Bastias, 2007). Altering the fuel loads or burning during weather conditions that limit heat transfer to the mineral soil are possible mitigation alternatives to lessen negative impacts to the soil fungi (DeBano et al., 1998).

Extracellular enzyme activity in soils decompose large organic compounds, such as lignin, cellulose, chitin, and proteins, while providing low molecular weight substrates to microorganisms (Marx et al., 2005). Network-scale analysis of the FFS study for pre-treatment and 1-y post-treatment data found no significant differences for phosphatase or chitinase activity, yet phenol oxidase activity was reduced by burning alone compared to controls (Boerner et al., 2008). However, there was reduced phosphatase activity with burning alone in the four western sites of the FFS network, which included our pre-treatment and 1-y enzyme data. With the additional sampling at six months and 2-y post-treatment and the inclusion of additional enzymes, a multivariate analysis of enzyme activity at our site indicated decreased enzyme activity for both treatments that included thinning. The decreased potential enzyme activity was largely due to two enzymes (β -galactosidase,

cellobiohydrolase) that degrade carbohydrate polymers (Sinsabaugh, 1994), NAGase that mineralizes chitin to provide N (Olander and Vitousek, 2000; Waldrop et al., 2003), and sulfatase that hydrolyzes organic esters releasing inorganic S (Eivazi and Tabatabai, 1977; Eivazi and Bayan, 1996).

Our results differed from Boyle et al. (2005), who reported all enzymes except β -1,4-glucosidase and phosphatase increased with thinning compared to the controls. Boyle et al. (2005) also found increased enzyme activity with burning, yet fire did not affect potential enzyme activity in our study. Boyle et al. (2005) measured enzyme activity 8-y after the initial restoration treatments in which forest floor material was removed followed by additions of grass clippings to more closely simulate pre-EuroAmerican settlement conditions prior to burning. These sites were also burned a second time before enzyme activity was measured to mimic a 4-y return fire interval (Covington et al., 1997). Prescribed burning treatments associated with restoration are typically low intensity due to manipulation of the fuel load prior to burning (Covington et al., 1997), while operational scale burn severity may differ considerably based on residue management. Potential enzyme activities, as we measured them in our case, represent the size of the enzyme pools at the time of sampling. This enzyme pool includes both active enzymes and those enzymes stabilized within the soil matrix (Wallenstein and Weintraub, 2008). The additions of fresh residual slash and plant roots following mechanical harvesting provided a pulse of additional C to the forest floor and mineral soil, yet we did not see increased microbial biomass in either the O horizon or mineral soil. One possibility is that rapidly leached labile C from the residual slash degraded the

enzyme pools stabilized in the soil matrix without increasing enzyme production, therefore reducing the overall potential enzyme activity. The decreased potential enzyme activity with thinning may have been limited by temperature, access to the substrate, substrate quality, diffusion limitations *in situ*, or pH (Michel and Matzner, 2003; Wallenstein and Weintraub, 2008).

Nitrogen is often considered the most limiting nutrient for tree growth in natural forests (Vitousek and Howarth, 1991). Net N mineralization and nitrification were not affected by wildfire mitigation treatments in the FFS Network analysis, and there were only minor within site differences (Boerner et al., 2008). Our 1-y post-treatment samples reflect these regional patterns. However, by measuring the six-month post-treatment N transformations, we did observe short-term increases in net N mineralization and nitrification for the thin and burn treatment. Increases in net N mineralization returned to pre-treatment levels for both the 1- and 2-y post-treatment samples. The greatest difference in net N mineralization was between thinning and burning and thinning alone. Increased available N resulting from burning of residual slash and O horizon in the thinning and burning treatment may have decreased the N limitation on decomposition, while residual slash additions from thinning alone increased N limitation resulting in immobilization of N.

Net nitrification rates and available soil NO_3^- pools have increased following prescription burning in southwestern ponderosa pine forests as a result of increases in NH_4^+ availability to nitrifiers from fire-induced oxidation in the O horizon and surface mineral soils (Covington and Sackett, 1986; Klopatek and Klopatek, 1997).

DeLuca and Sala (2006) demonstrated that long-term increases in NO_3^- accumulation occur with frequent fires in ponderosa pine stands. Hypothesized mechanisms for reduced nitrification rates in ponderosa pine-dominated forests with fire suppression include decreased ammonification, polyphenolic complexation of available NH_4^+ , NH_4^+ immobilization due to increased C availability, and accumulation of total phenols (Kaye and Hart, 1998b; DeLuca and Sala, 2006). Fire at our FFS site may have increased available NH_4^+ initially, resulting in short-term increase the nitrification rate, with nitrification rates returning to pretreatment levels within a year. This result is consistent with the meta-analysis findings in Wan et al. (2001), who reported a consistent short-term (7-12 mo) increase in soil NO_3^- pools following fire. The need to measure nitrification over time, with and without repeated burning, will be crucial if we wish to understand how wildfire mitigation treatments affect N dynamics in ponderosa pine forests over the longer term (DeLuca and Sala, 2006). We know fire shaped stand structure and plant composition in these fire-prone forests, yet we do not have a comprehensive understanding of the interaction between post-fire N dynamics and plant community composition in these ecosystems (Hart et al., 2005; DeLuca and Sala, 2006).

A meta-analysis by Johnson and Curtis (2001) concluded that in coniferous stands harvesting method and residue treatment are the major determinants of changes in total mineral soil C and N contents following treatment. Whole tree harvesting decreased total mineral soil C and N, while sawlog harvesting with residue left on site temporarily increased total mineral soil C and N. Boerner et al.

(2008) found increased C:N ratios in the mineral soils across the entire FFS Network for thinning treatments, yet mineral soil C:N ratios at our specific site were unaffected by any treatments. Total C and N contents in the mineral soil following restoration treatments in other ponderosa pine stands in northern Arizona have initially been unaffected (Kaye et al., 2005; Hungate et al., 2007), yet long-term studies have reported increased soil total N (Boyle et al., 2005; Grady and Hart, 2006). The residual slash left at the FFS thinning treatment was 30% greater than the unthinned sites and 82% greater than the thin and burned sites (S. Haase, Pacific Southwest Research Station, USFS, USDA, 2007, personal communication), yet the only significant differences detected were for soil total N between thinning alone, and thin and burn treatments, which did not persist. The differences in slash residue additions may explain changes in the six month sampling, yet these inputs were not enough to result in changes to total C and N contents in the mineral soil after one year. These results support the conclusion that total C and N in the mineral soil is relatively insensitive to wildfire mitigation treatments (Boerner et al., 2009).

At the Southwestern Plateau site, mineral soil (0-5 cm) pH was not affected by the wildfire mitigation treatments at any post-treatment sample period, as was found in the network scale FFS analysis (Boerner et al., 2009). There were two specific sites in the network-scale FFS analysis that showed increases in pH the first year after thinning and burning, yet pH returned to pre-treatment levels by the 2-y sampling (Boerner et al, 2009). The pH increases at these sites were attributed to the higher burn severity that occurred at these sites. Boerner et al. (2009) did not

find significant differences in Ca^{2+} at the network scale, but burning alone did significantly increase Ca^{2+} compared to the unthinned and thin only treatments at our sites. Due to high temperature thresholds, Ca^{2+} is not easily volatilized and often is a major constituent of ash deposited on the soil surface following fire (DeBano et al., 1998). The spatially variable nature of prescribed fire can often confound treatment effects; indeed, fire generally showed small and idiosyncratic effects on soil properties and processes at the network scale in the FFS study (Boerner et al., 2009).

Overall, soil responses to wildfire mitigation treatments were relatively minor and by the second year following treatments most of these differences disappeared. The results from our single FFS site generally support the conclusions from the multisite FFS network meta-analysis on forest soil properties and processes common to all sites (Boerner et al., 2009). Differences between our study and the meta-analysis of the FFS network likely occurred because we measured more immediate effects of the treatments (after 6 months) and included additional response measures (more enzymes assessments and microbial community analyses). Wildfire mitigation efforts attempt to decrease the potential risks of large-scale high-severity wildfires by mechanically thinning trees and use of low-intensity prescribed fire. Increasing the resistance to large high-severity wildfires will also increase resistance to insect outbreaks and regional drought (Allen et al., 2002). Dense ponderosa pine stands are susceptible to infestation by bark beetles, especially under moisture stress related to regional drought (Allen and Breshears, 1998; Kolb et al., 1998; Breshears et al., 2005). Persistent ecological damage to

ponderosa pine forests of the Southwest will continue unless potential risks from large-scale high-intensity wildfire are reduced (Covington et al., 1994). Wildfire mitigation efforts attempt to not only reduce fuels and fuel continuity, but allow low-intensity wildfires to once again shape the vegetation community (Moore et al., 1999, Hart et al., 2005). Our more intensive results from the Southwestern Plateau FFS and the extensive network-wide FFS (Boerner et al., 2009) suggest the mineral soil and associated microbial community is relatively robust in the short-term with respect to wildfire mitigation treatments; nevertheless, given the large spatial scale and potential long-term implications of these planned treatments, continued monitoring of wildfire mitigation treatments with repeated burning is warranted to determine the long-term implications to both plant and soil microbial communities.

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Figure Captions

Figure 1. Net nitrogen mineralization and nitrification rates (0-15 cm) at the Southwestern Plateau Fire and Fire Surrogate study site over three different sampling periods (means and $\pm$ one standard error). Different letters for a given sampling date indicate significant differences ($\alpha = 0.05$) using Tukey-Kramer pairwise comparison following a significant generalized linear mixed model analysis of treatments.

Figure 2. Mean enzyme activity in the mineral soil (0-5 cm) at the Southwestern Plateau Fire and Fire Surrogate study sites over four different sampling periods. Error bars represent plus one standard error. Different letters for a given sampling date indicate significant differences ($\alpha = 0.05$) using Tukey-Kramer pairwise comparison following a significant generalized linear mixed model analysis of treatments.

Abbreviations: β -1,4-glucosidase (blgu), α -1,4-glucosidase (algu), β -galactosidase (galac), β -xylosidase (xylo), cellobiohydrolase (cello), *N*-acetyl-glucosaminidase (nag), alkaline phosphatase (phos), and sulfatase (sulf).

Figure 3. Non-metric multidimensional ordinations results of potential enzyme activities in the mineral soil (0-5 cm) at the Southwestern Plateau Fire and Fire Surrogate study site. Post-treatment sampling periods show mean ordination values with $\pm$ one standard error for both axes. Multi-response permutation procedure analysis showed a significant difference between thinning treatments (thin-only, thin and burn) and treatments with no mechanical harvesting (unthinned, burn-only), while burning and interaction of thinning and burning was not significant.

Figure 4. Forest floor (surface organic horizon) microbial groups at the Southwestern Plateau Fire and Fire Surrogate study sites one-year post-treatment, as assessed using phospholipid fatty acid (PLFA) biomarkers. Bars indicate treatment means and vertical lines are + one standard error. A generalized mixed model analysis showed no significant differences among treatments for any microbial group.

Figure 5. Mineral soil (0-5 cm) microbial groups at the Southwestern Plateau Fire and Fire Surrogate study site one-year post-treatment, as assessed using phospholipid fatty acid (PLFA) biomarkers. Bars indicate treatment means and vertical lines are + one standard error. A generalized mixed model analysis showed no significant differences among treatments for any microbial group.

Table 1. Organic horizon) and mineral soil (0-5 cm) characteristics one year following wildfire mitigation treatments at the Southwestern Plateau Fire and Fire Surrogate study site. Data are means (+ one standard error).

Soil characteristics	Treatment			
	Unthinned	Burn-only	Thin-only	Thin & Burn
Forest floor (O horizon)				
Mass (Mg ha ⁻¹)	20.3 (1.71)b	11.0 (0.95)c	26.3 (1.40)a	14.6 (0.45)c
Total carbon (Mg ha ⁻¹)	7.5 (0.83)	5.8 (0.94)	9.09 (0.86)	5.83 (0.92)
Total nitrogen (kg ha ⁻¹)	200.3 (26.25)ab	101.2 (9.39)c	243.8 (25.78)a	121.1 (4.00)bc
Carbon to nitrogen mass ratio	38.0 (5.81)	27.0 (0.66)	35.3 (2.52)	30.3 (1.44)
Mineral soil (0-5 cm)				
pH	5.3 (0.014)	5.3 (0.006)	5.3 (0.004)	5.3 (0.001)
Total carbon (Mg ha ⁻¹)	33.3 (2.61)a	33.2 (2.45)a	40.3 (4.33)b	31.5 (1.61)a
Total nitrogen (Mg ha ⁻¹)	1480(25)a	1551 (76)a	1740 (27)b	1433 (13)a
Carbon to nitrogen mass ratio	22.5 (0.61)	21.4 (0.88)	23.2 (0.18)	22.0 (0.26)
Exchangeable calcium (cmol kg ⁻¹)	5.80 (0.32)a	7.35 (0.39)b	5.96 (0.35)a	6.43 (0.35)ab
Exchangeable magnesium (cmol kg ⁻¹)	1.70 (0.13)	2.04 (0.13)	1.80 (0.13)	1.95 (0.13)
Exchangeable potassium (cmol kg ⁻¹)	0.22 (0.02)	0.22 (0.01)	0.25 (0.03)	0.23 (0.02)
Exchangeable sodium (cmol kg ⁻¹)	0.157 (0.01)ab	0.132 (0.01)a	0.162 (0.01)b	0.162 (0.01)b
Cation exchange capacity (cmol kg ⁻¹)	7.87 (0.43)a	9.74 (0.48)b	8.16 (0.45)ab	8.78 (0.46)ab

Note: Analysis performed using a generalized mixed model with sampling date as a repeated measure ($\alpha = 0.05$) and Tukey-Kramer pairwise comparisons. Treatment means with different letters differed significantly.

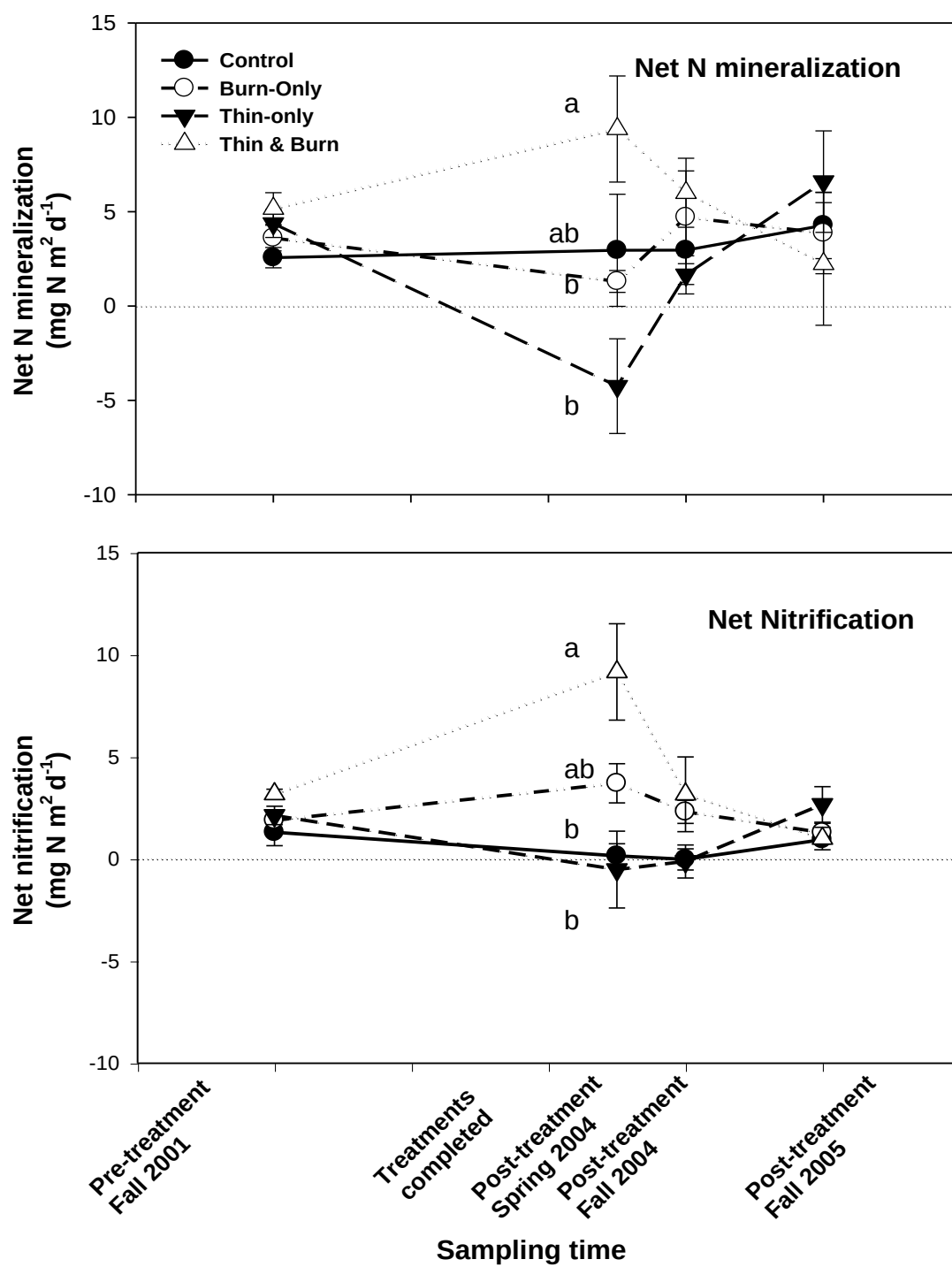


Figure 1

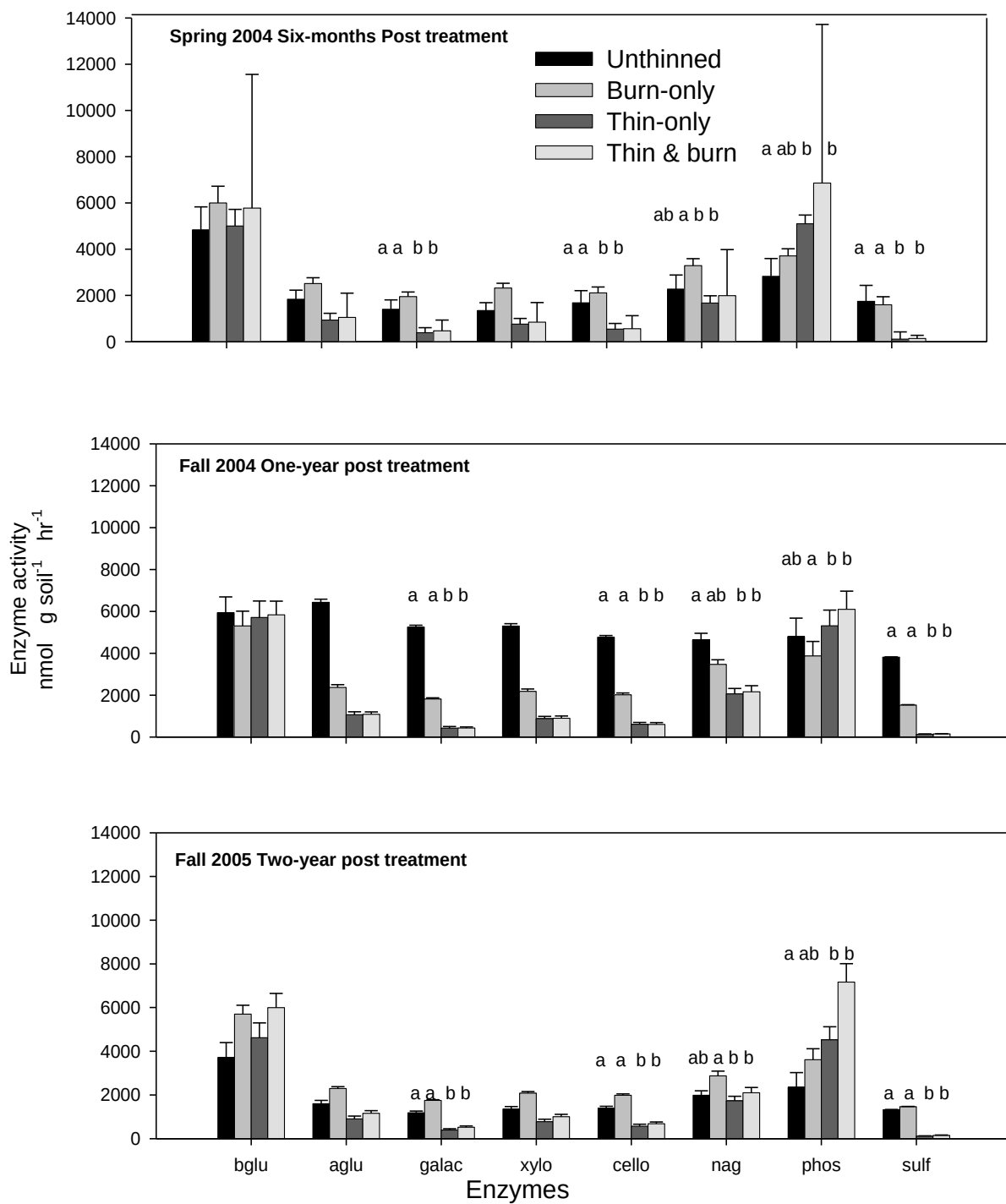


Figure 2

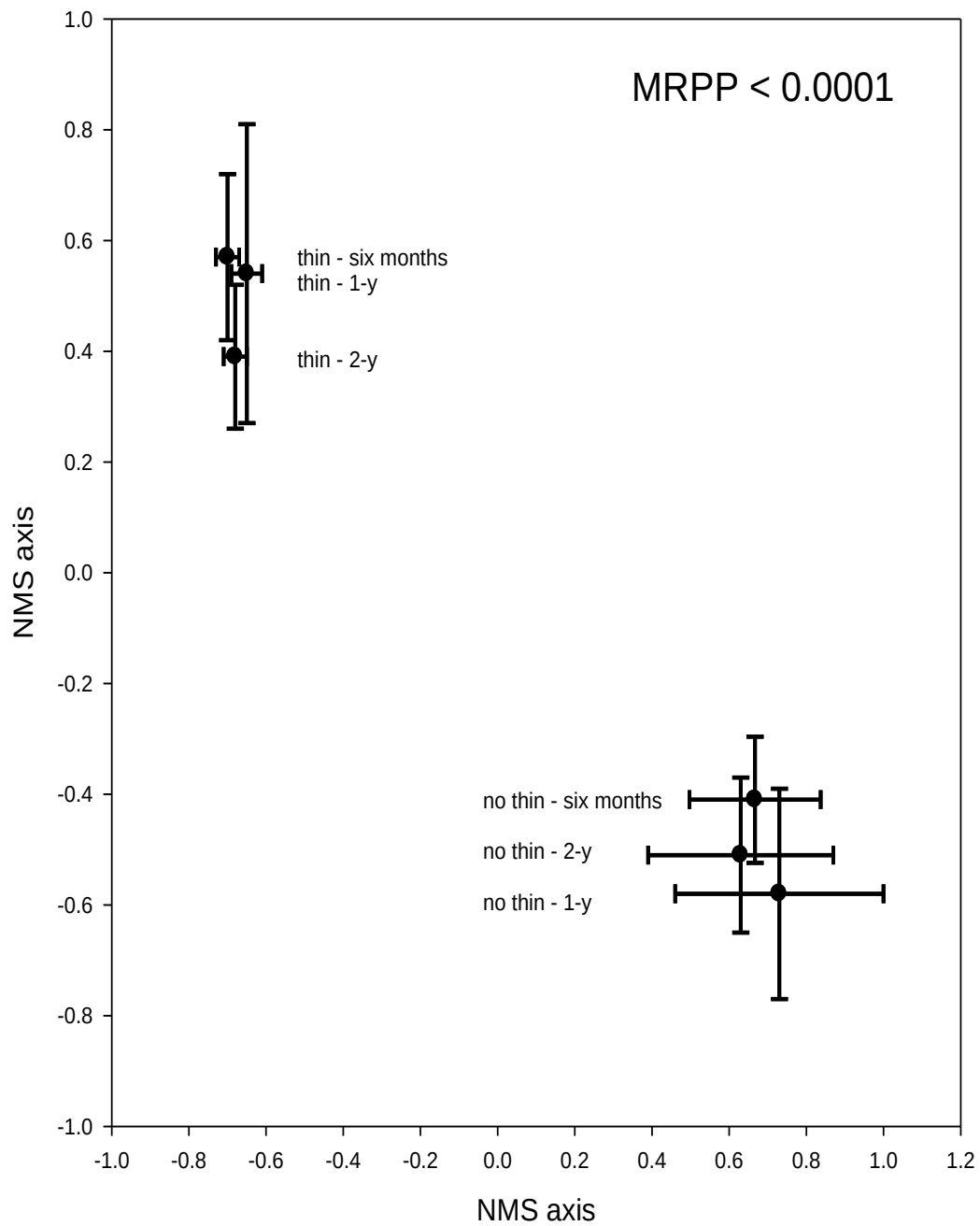


Figure 3

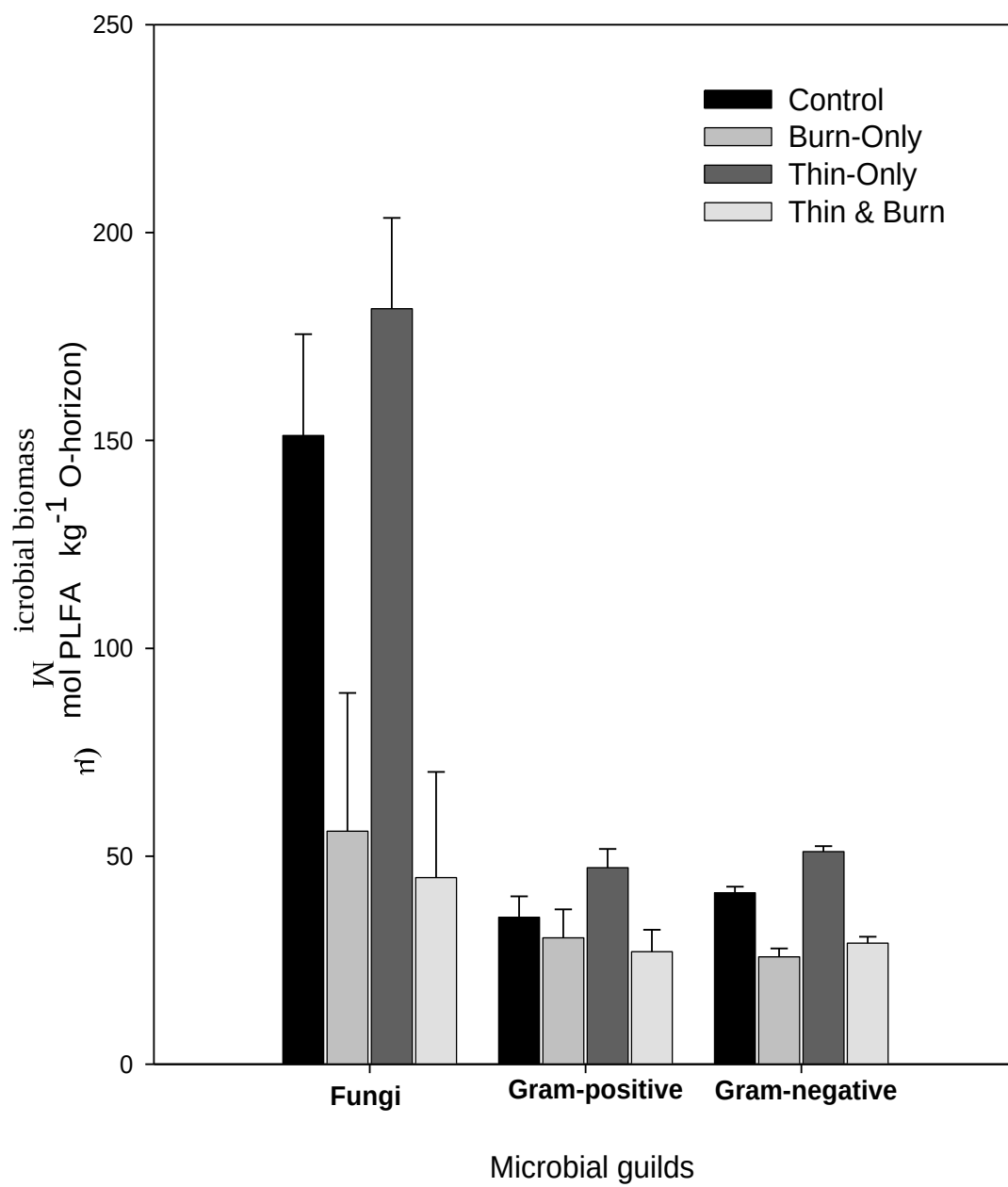


Figure 4

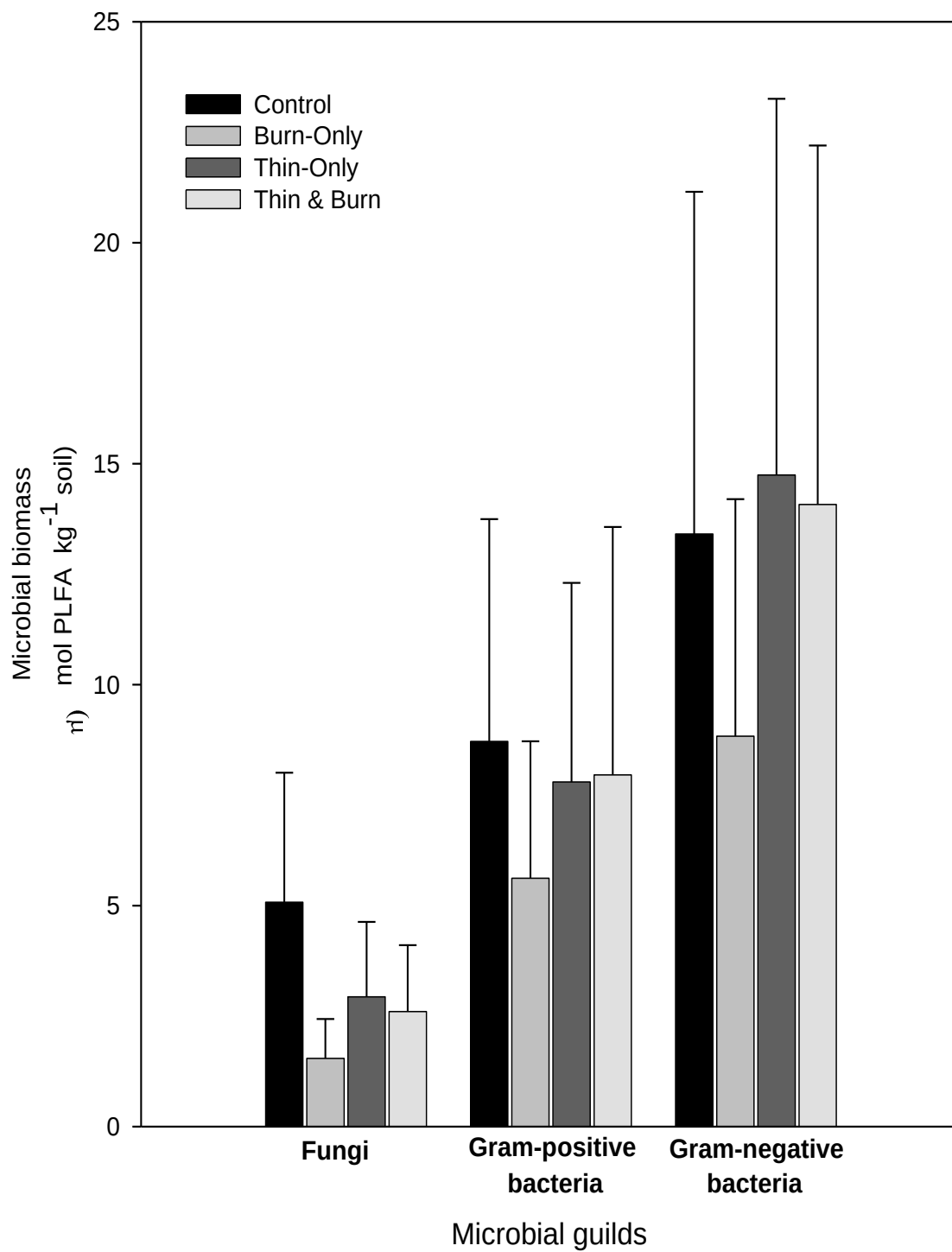


Figure 5

CHAPTER 4
**STAND DENSITY AND FIRE IMPACTS ON NATIVE GRASS-
ARBUSCULAR MYCORRHIZAL-HETEROTROPHIC SOIL MICROBIAL
COMMUNITIES IN A PONDEROSA PINE FOREST**

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Summary

- Linkages between herbaceous vegetation, arbuscular mycorrhizal fungi (AMF), and heterotrophic soil microbial communities were investigated in a ponderosa pine stand in southwestern USA thinned over forty years ago and maintained to different stand densities. Prescribed fire was recently completed on half of each original plot, except for the high density plots for safety reasons.
- Initial arbuscular mycorrhizal fungal (AMF) spores and soil microbial community were measured at four stand densities, with and without fire. The soil microbial community was analyzed using phospholipid fatty acids (PLFA).
- Intact soil cores from four stand densities, burned and unburned, were used in a bioassay of two native bunchgrasses (Arizona fescue (*Festuca arizonica*), and spike muhly (*Muhlenbergia wrightii*)) to determine AMF colonization and spore production. Soil microbial communities from the intact cores were measured following a bioassay to determine the influence of native grasses on microbial community structure.
- Complete removal of overstory canopy had the greatest affect on both herbaceous vegetation and AMF spore numbers in the mineral soil, yet stand density did not affect AMF inoculum potential of the two native grasses used in the bioassay.
- Arizona fescue significantly increased gram-negative and gram-positive bacteria and actinobacter, but not heterotrophic fungi, in the intact mineral soil cores compared to spike muhly.
- Reducing canopy densities and creating canopy gaps in southwestern USA ponderosa pine forests increased the presence of AMF propagules, yet did not increase AMF inoculum potential.

Introduction

When Euro-Americans settled in the southwestern United States, they encountered open landscapes with clusters of ponderosa pine (*Pinus ponderosa* P. & C. Lawson) trees and an understory dominated by grasses, forbs and shrubs (Covington and Moore, 1994; Covington et al., 1997; Fulé et al., 1997). Frequent fires (2-20 y), herbaceous competition, and drought maintained this pre-Euro-American forest structure of 30-140 trees ha⁻¹ (Cooper, 1960; White, 1985; Swetnam and Baisan, 1996). Post-settlement, selective logging of mature trees and active fire suppression, which increased recruitment survival (Pearson 1933; Savage et al. 1996), reduced average stand diameter and increased stand densities. Contemporary forests in Arizona average 727 trees ha⁻¹ (O'Brian, 2002), with some stands exceeding 2,000 trees ha⁻¹ (Covington et al., 1997; Kaye et al., 2005; Bakker and Moore, 2007).

After a century or more of increasing stand density and accumulation of woody fuels in ponderosa pine forests of the southwestern United States, wildfire size and severity has increased (Cooper, 1960; Covington and Moore, 1994; Mast et al., 1999). Diminished herbaceous communities due to increased tree density, domestic livestock grazing, and fire suppression efforts (Covington and Moore, 1994; Covington et al., 1997; Fulé et al., 1997) eliminated fine fuels that were characteristic of pre-Euro-American settlement ponderosa pine forests, but also changed litterfall, root exudation and turnover. Slowed decomposition due to changes in quantity and quality of these inputs has resulted in an accumulating surface organic (O) horizon of recalcitrant ponderosa pine litter (Covington and Sackett, 1984). Mechanical thinning and prescribed fire are management tools used to lessen the severity of wildfires (Wagle and Eakle,

1979; Pollet and Omi, 2002; Strom and Fulé, 2007) and recreate pre-EuroAmerican settlement community structure (Covington et al., 1997).

Bunchgrasses dominated the pre-EuroAmerican settlement understory, yet under heavy grazing pressure these bunchgrasses were drastically reduced (Arnold, 1950). Restoration in southwestern ponderosa pine forests necessitates an increase in understory diversity and production to reflect reference conditions (Covington et al., 1997). Successful restoration will include establishing dominant native bunchgrasses along with reducing stand densities in ponderosa pine forests of the Southwest. Ectomycorrhizal associations are critical for survival and growth of ponderosa pines (Harvey et al., 1986), while arbuscular mycorrhizal fungi (AMF) are important to the native understory vegetation (Kovacic et al., 1984). Korb et al. (2003) found a persistence of AMF propagules in unthinned stands of ponderosa pine in northern Arizona, and a rapid increase in propagule numbers soon after restoration treatments were applied. They speculated this persistence and rapid response following restoration treatments may facilitate the recovery of the herbaceous community to a prominence resembling reference conditions.

Arbuscular mycorrhizal fungi are essential for plant nutrient uptake and productivity (Cromack et al., 1979; Read et al., 1992; Smith and Read, 1997; Jakobsen et al., 2003). Over 60% of all terrestrial plant species form associations with AMF (Trappe, 1987). These AMF associations can alter competition between plants (Hetrick et al., 1989; Allen and Allen, 1990; West, 1996) and influence plant community composition (van der Heijden et al., 1998; Klironomos et al., 2000; van der Heijden et al., 2003). Symbiosis with AMF has been shown to promote fine root development (Smith and

Read, 1997), but also provides root protection from pathogens and moderate metal toxicity while enhancing soil structure (Harley and Smith, 1983; Perry et al. 1989).

The objectives of this study were to determine: 1) AMF inoculum potential and diversity of AMF at different stand densities maintained for over forty years with prescribed fire recently introduced; 2) the influence of two native bunchgrasses, one dominant the other uncommon, on AMF colonization rates, spore production, and extraradical hyphae, and; 3) the influence of two native grass-AMF symbioses on mineral soil microbial community structure. We expected lower colonization rates for the high stand density plots due to reduced propagule densities (Korb et al., 2003), and higher colonization rates for Arizona fescue (*Festuca arizonica* Vasey), a dominant grass in Southwest ponderosa pine forests, than spike muhly (*Muhlenbergia wrightii* Vasey), an uncommon grass. The increased colonization rates on Arizona fescue should also increase extraradical hyphae and spore production compared to spike muhly.

Materials and Methods

Study sites

In the 1960s, the United States Forest Service initiated a study to determine growth relationships of residual stands thinned to designated growing stock levels (GSL) in ponderosa pine (*Pinus ponderosa* var. *scopulorum* P. & C. Lawson) stands across a range of site productivities. Taylor Woods was chosen to represent the low end of this productivity gradient. Taylor Woods, a subdivision of the Fort Valley Experimental Forest, is approximately 14.5 km northwest of Flagstaff, Arizona, at an elevation of 2,266

m (Ronco et al., 1985). Study plots are within a 36.4 hectare area on gentle (4%), southwest facing slope, in the ponderosa pine/Arizona fescue habitat type. Mean annual air temperature, measured at Fort Valley Experimental Forest Headquarters (~3.5 km west), is 6.1 °C; mean daily air temperatures range from -3.9 °C in January to 17.2 °C in July. Mean maximum air temperatures in January and July are 5.6 °C and 27.2 °C, respectively. Mean annual precipitation is 55.9 cm, of which approximately 29% falls in July and August, the wettest months of the year. The summer rainy season is bracketed by spring and fall droughts. Total annual snowfall averages 246 cm (all climate data from <http://www.wrcc.dri.edu/summary/climsmaz.html>).

The soil at Taylor Woods is derived from flow and cinder basalt and is classified as Brolliar stony clay loam, a fine, smectic, frigid Typic Argiboroll (Meurisse, 1971). The A horizon is rather shallow, extending to only 10 cm, but the remainder of the soil profile reaches a depth of 114 to more than 152 cm before bedrock of fractured basalt is encountered.

Mechanical thinning treatments of growing stock levels (GSL) along with a clearcut were initiated in 1962. We utilized the clearcut, two GSL levels (14, 28 m² ha⁻¹ basal area), and unthinned areas for this study (Ronco et al., 1985). Each treatment was replicated three times, with plots ranging in size from 0.30 to 0.50 ha (Ronco et al., 1985). The unthinned plots basal area of 45 m² ha⁻¹ corresponds to a density of 3200 trees ha⁻¹ (McDowell et al., 2007). The plots were thinned in 1962 for the first time, then treated again in 1972, 1982 (Ronco et al. 1985), 1992, and 2003 (C. Edminster personal communication 2003, Rocky Mt. Research Station, U.S. Forest Service, Flagstaff, AZ). During the fall of 1998, the plots were split with half of each plot burned during the fall

and winter of 1998-1999. The untreated high density plots were not burned due to inability to perform prescribed burns in a controlled manner.

Sampling Design

Sampling was performed on two levels of GSL (14, 28 m² ha⁻¹ basal area), hereafter referred to as low (145 trees ha⁻¹) and medium density (471 trees ha⁻¹), along with three replicate plots each for high density (3200 trees ha⁻¹) and clearcut (0 trees ha⁻¹) treatments. The surface organic (O) horizon and mineral soil (0-5 cm) samples were taken August of 2002, and again in June and August of 2003. Within each replicate treatment plot, three soil transects were randomly assigned. Three randomly selected soil samples were collected and composited per transect, providing three samples per split-plot for nutrient and pH analyses (see below). The O horizon and mineral soil was collected within a litter frame of known area (0.01 m²), placed in polyethylene bags, and transported to the laboratory on ice each day. Mineral soil was collected within the litter frame using 2-cm diameter soil probe (Oakfield Apparatus Company, Oakfield, WI, USA).

The O horizon samples had all material greater than 6-mm removed, while mineral soil samples were sieved (< 2 mm) immediately upon arrival at the laboratory. All samples were well mixed, weighed, and a subsample of O horizon and mineral soil removed (~20 g). This subsample was placed in a drying oven for 48 h at 70 °C for the O horizon and 105 °C for the mineral soil, and then reweighed to determine water content to report analytical results on an oven-dry weight basis. The remaining portion of each sample (except that used for microbial analyses, see below) was then air-dried. For each

sample period (August 2002, June 2003, August 2003), the O horizon was analyzed for total carbon (C), nitrogen (N), and phosphorus (P) contents, while mineral soil was analyzed for pH, and total C and N contents. Microbial analyses were also conducted on a subsample (~ 5 g) of mineral soil (0-5 cm) collected in August 2003. After sieving, these subsamples were immediately frozen for 24 h, then freeze-dried (-50°C , 70×10^{-3} Mbar for 24 h, Edwards Modulyo, Crawley, UK) prior to extraction for phospholipid fatty acids (PLFA). The extraction process occurred within 48 h of returning to the laboratory.

During August 2003, we also collected three intact soil cores for a bioassay within each replicate split-plot of our four density treatments using a corer (5 x 15 cm) attached to a slide hammer (AMS, Inc., American Falls, Idaho, USA). The sample location within each replicate split-plot was selected randomly. The three soil cores were taken within 25 cm of each other. Two cores were immediately placed into sterilized polypropylene containers (5 x 18 cm) typically used for containerized tree seedlings. The remaining core was used to determine the initial microbial and AMF spore community for later comparison to the final bioassay soil microbial community.

Plot Vegetation and Soil Characteristics

Herbaceous understory measurements were taken using the Daubenmire method in August of 2003 (Daubenmire, 1959). A 30-m transect was randomly selected within each split-plot. Three 20 x 50 cm quadrats were randomly located along each transect. Estimates of ground cover by cover class (Daubenmire, 1959) for each species, and species identification within each quadrat were recorded. Canopy cover was visually

estimated as a vertical projection of a polygon drawn around the extremities of each plant within the quadrat. The projections are summed and recorded in a corresponding cover class. Six cover classes were used and converted to class midpoints for reporting (Daubenmire, 1959). These estimates are used for descriptive purposes of the vegetation at the time of removing the soil cores for the bioassay.

Air-dried mineral soil subsamples were ground (< 0.149 mm dia.), then analyzed for total C and N concentrations on a commercially available elemental analyzer (Flash EA 1112, CE Elantech, Lakewood, New Jersey, USA). Total P contents were determined by the phosphomolybdate method (Murphy and Riley, 1962) modified for analysis on flow injection analysis instrumentation (Lachat method 13-115-01-1-B) using a CuSO_4 - H_2SO_4 modified Kjeldahl procedure (Parkinson and Allen, 1975). Soil pH was determined using a glass electrode immersed in a 1:5 soil/0.01 M CaCl_2 solution (Hendershot et al., 1993).

Bioassay of mycorrhizal community

We conducted a bioassay using two native grasses to access the viable propagule densities of AMF (Brundrett and Abbott, 1994; Abbott et al., 1995) in intact mineral soil cores. Spores, colonized roots, and hyphal networks all function as AMF propagules; consequently, using intact soil cores as the growth medium is important to overcome the problem of reduced viability of the AMF propagules caused by destruction of hyphal networks during mixing (Perry et al., 1989). We used locally harvested native seeds (Native Plant & Seed, Flagstaff, AZ) of Arizona fescue, the dominant grass species, and spike muhly, an uncommon species, to test for differences in AMF responses between

these two native grass species. Sampled soil cores (5 x 15 cm) were planted with five seeds of individual species per container for both grasses. After seed germination, plants were reduced to two plants per core. Plants were grown in a growth chamber under light (12 h at 25 °C) and dark conditions (12 h at 20 °C) for 6 weeks. Plants were watered every other day without supplemental nutrients. After 6 weeks, we discontinued watering for an additional 2 weeks to allow sporulation to occur.

Plants were harvested following the two week dry period and shoots were separated from roots and dried in an oven for 3 days at 60 °C, then weighed for amount of dry biomass. Intact cores were frozen until roots could be separated. Roots were rinsed and small random subsamples (25 g) of fine roots were separated from the main bulk, weighed, and frozen for subsequent analysis of AMF colonization. The remainder of the roots were treated in the same fashion as the shoots to estimate total root biomass and used in our calculations of shoot-to-root biomass ratio. The root sub-samples were cleared in 10% (w/v) KOH and stained by the ink and vinegar method using blue Shaeffer ink (Vierheilig et al., 1998). Percentage mycorrhizal colonization in roots was measured for each core using the grid-line intersect method (McGonigle et al., 1990). From each soil core, a homogenized soil subsample (25 g) was used for AMF spore extraction using the sucrose centrifugation method (Johnson et al. 1999). Spores were mounted onto slides, examined with a compound microscope (magnification of 100-400x), and identified to morphospecies when possible using Schenck and Perez (1990) and INVAM (<http://invam.caf.wvu.edu/>) as references.

Bioassay of heterotrophic soil microorganisms

The O horizon and mineral soil PLFAs were conducted using intact soil cores (0-15 cm) that comprised the initial and final samples from our bioassay. Analysis of PLFA patterns has been shown to be a powerful approach to describe the structure of the soil microbial communities and to detect changes due to altered ecological conditions (Bååth et al., 1992; Frostegard et al., 1993*a,b*; Zelles, 1999; Hasset and Zak, 2005; Ramsey et al., 2006). Individual PLFAs can be used as specific biomarkers to identify different microbial groups and as an index for total microbial biomass. Ramsey et al. (2006) found PLFA analysis often resolved treatment effects when molecular methods were unable to detect differences. The PLFA 16:1 ω 5 biomarker has been correlated with AMF hyphae in colonized roots and is not found in plant roots (Peng et al., 1993; Olsson et al., 1997).

Mass spectral analysis identified numerous compounds between C14 to C22 in C chain length, of which 16 were used as microbial biomarkers. The 16 compounds were a conservative estimate of known microbial biomarkers (O'Leary and Wilkinson, 1988; Frostegard and Bååth, 1996; Zelles, 1999). Total microbial biomass was calculated by summing these 16 biomarkers. Gram-negative bacterial biomarkers (cy17:0, cy19:0, 16:1 ω 9, 16:1 ω 7, 18:1 ω 5c, and 18:1 ω 7) and gram-positive bacterial biomarkers (i15:0, a15:0, i16:0, i17:0, a17:0, and 10me16:0) discriminate between bacterial groups. These biomarkers were then summed with the general bacterial biomarker (C15:0 and C17:0) to estimate total bacterial biomass (O'Leary and Wilkinson, 1988; Frostegard and Bååth, 1996; Zelles, 1999). Two Isomers of C18:2n6 (*trans* and *cis*) are used to estimate the fungal group (Frostegard and Bååth, 1996).

Five g of freeze-dried sieved (< 2 mm) mineral soil or 2 g of freeze-dried ground litter (< 2 mm) was extracted with a single-phase mixture of chloroform, methanol, and phosphate buffer (White et al., 1979) then fractionated into neutral, glyco-, and phospholipids (Frostegard et al., 1991). The phospholipid fraction was then esterified and reconstituted in hexane prior to analysis (Frostegard et al., 1993b). A capillary column was utilized for compound separation. Identification and quantification of the each specific fatty acid methylester (FAME) was established using commercially available standards (Accustandard, Sigma-Aldrich, St. Louis, Missouri, USA) through electron ionization on a quadrupole mass selective detector (Agilent 6890N/5973N gas chromatograph/mass spectrometer, Santa Clara, CA, USA). Quantification (nmol PLFA kg⁻¹ oven-dry material) of samples is based on calibrations curves derived from individual FAME standards.

Statistical methods

Statistical analyses of total C, N, and P concentrations of the mineral soil were performed using two-way analysis of variance (ANOVA), with stand density, burn, and the stand density x burn interaction as factors. For the above soil characteristics, we used replicate plot values from the three sample periods to provide better estimates of each parameter. Additionally, statistical analyses of number of spores per gram soil, microbial groups (gram-negative and gram-positive bacteria, actinobacter, fungi) in the final soil cores and two individual biomarkers that represent AMF extraradical hyphae, and soil fungi (PLFA 16:1 ω 5, PLFA 18:2n6c,t) were performed using two-way ANOVA, with stand density, burn, and the stand density x burn interaction as factors. Spore numbers were log₁₀

transformed to meet assumptions of homogeneity of variance and normal distributions required for ANOVA. Statistical analyses were performed on a personal computer (SAS for PCs ver. 8.1, Cary, North Carolina, USA).

Microbial community (PLFA biomarkers) and spore community structure were analyzed by a multi-response permutation procedure (MRPP), using Euclidean distances to test the similarity of the communities based on stand density, burn treatment, and stand density x burn interactions (Mielke and Berry, 2001). Multi-response permutation procedure does not require assumptions of multivariate normality or homogeneity of variances, which are seldom met with ecological community data (McCune and Grace, 2002). Simultaneous pairwise comparisons using the Peritz closure method to maintain Type I error rate tested the null hypothesis that all possible pairs were similar (Petronidas and Gabriel, 1983). This procedure was performed using Microsoft Excel macros (available from senior author) following the methodology of Mielke and Berry (2001). All statistical analyses were conducted at the $\alpha=0.05$ significance level. Measures of diversity were calculated for both AMF spore community and the vegetation community using PC-ORD (Ver. 4.03, MjM Software Design, Gleneden Beach, Oregon, USA).

Results

Plot vegetation and soil characteristics

Herbaceous cover estimates for clearcut plots were greater than low, medium, or high stand densities (Table 1). Low intensity burning on the split-plot within the four different

density treatments did not affect the measured herbaceous cover, species richness, or diversity (Table 2.). The clearcut contained the greatest number of herbaceous species (30 species) compared to low density (18), medium density (11), and high density (2) treatments (Table 3), and exhibited the highest species richness (# of species per sample area; Table 2).

Total C, N, and P contents of the mineral soil (0-5 cm) were not significantly different among stand density levels or with or without fire; additionally, no interactions between stand density and fire treatments occurred (Table 4).

Bioassay of mycorrhizal community

Initial soil cores from the clearcut plots had the highest species richness and significantly greater numbers of AMF spores compared to low, medium, and high density plots (Fig. 1; Table 5). Measures of AMF spore diversity and evenness were comparable among all stand densities (Table 2). Table 5 lists the species of AMF spores we found in the initial and final soil cores. Inoculum potentials (%) by AMF were similar between grass species among stand densities and between burn treatments. Final soil core AMF spore communities associated with Arizona fescue were significantly different than initial soil cores among stand densities (MRPP, $p < 0.0001$), where clearcut treatment was significantly greater than low and medium density, but not high density. Initial and post-bioassay spike muhly soil cores were similar among all stand densities. There was also significantly greater root and shoot biomass with Arizona fescue compared to spike muhly at all measured stand densities (Table 6), but no significant difference between stand densities within the same grass species. Shoot:root biomass ratio was not significantly different between the two grass species (Table 6). One PLFA biomarker

(16:1 ω 5) that represents extraradical AMF hyphae (Olsson, 1999) was significantly higher for Arizona fescue than spike muhly regardless of stand density or burning treatments (Fig 2).

Bioassay of heterotrophic soil microorganisms

Multi-response permutation procedure analysis of the heterotrophic microbial community from the initial soil cores using PLFA biomarkers indicated similar communities for the four different stand densities burned ($p=0.1007$), and unburned ($p=0.6608$). Bioassay cores had similar heterotrophic microbial communities within grass species across stand densities burned and unburned (Arizona fescue $p=0.2695$, spike muhly $p=0.1007$), yet differed significantly from the initial soil heterotrophic microbial community (Arizona fescue $p<0.0001$, spike muhly $p<0.0001$) and between grass species ($p<0.0001$). We utilized specific PLFA biomarkers for microbial groups (gram-negative and gram-positive bacteria, actinobacter, fungi) to determine which microbial groups within the final soil cores were different (Fig. 3). Arizona fescue had significantly higher gram-negative and gram-positive bacteria and actinobacter populations compared to the spike muhly soil cores (Fig. 3).

Discussion

The greatest concentration of AMF spores and the highest AMF diversity index in the mineral soil was from the openings created by clearcutting at Taylor Woods. While the values were significantly higher in the clearcut openings, the AMF spore density and diversity was similar among the three stand densities with overstory canopy. Understory

vegetation species richness declined by 85% from the clearcut openings to high stand density plots, yet AMF species richness only declined 66%.

Our results did not show an increase AMF inoculum potential with stand density reductions, but other ponderosa pine stands in the Southwest have shown rapid increases in AMF propagules and inoculum potential following restoration treatments when compared to adjacent untreated sites (Korb et al., 2003). Differences in methodologies to estimate AMF inoculation potential between these previous studies and our approach may account in part for the divergent results. For instance, Korb et al. (2003) use corn as the bait-plant in their study due to its known symbiosis with numerous AMF and its rapid uniform growth (Johnson et al., 1999), while we used native grasses in our study. As native communities of both plants and AMF have been shown to be locally adapted (Klironomos, 2003), we believed that using native grasses in our bioassay may provide more realistic inoculum potentials. The creation of openings by clearcutting resulted in significant increases in AMF propagules and AMF species richness, yet AMF inoculum potentials between the clearcut and plots with overstory canopy were not different. This suggests that propagule densities and species richness are not a limiting factor in colonization with native grasses in ponderosa pine forests of northern Arizona.

Fire has been shown to alter composition of AMF communities (Vilariño and Arines, 1991; Bastias et al., 2006), yet AMF spore density was not impacted by the low intensity prescribed burning at our sites. Fire effects on AMF inoculum potential has been shown to be quite variable. Klopatek et al. (1988) reported up to 50% reductions of AMF inoculum potentials when temperatures exceeded 60 °C in a microcosm study of woodland communities of the Southwest, while Haskins and Gehring (2004) reported

AMF inoculum potentials were unaffected by fire in similar woodlands 5-y after burning. The impact of fire on AMF communities is linked to heat intensity and duration (Covington et al., 1997; DeBano et al., 1998). One practice that is deleterious to AMF communities is the utilization of piling and burning as a slash disposal method following thinning. Burning large residual slash piles can create conditions of extreme lethal temperatures eliminating AMF propagules, and creating potential vectors for exotic plant species invasion (Korb et al., 2004).

A field experiment in tallgrass prairie by Harnett and Wilson (1999) found the dominant grass species were more strongly mycotrophic than coexisting plant species, and that AMF colonization enhanced their dominance. The favorable conditions of the growth chamber resulted in greater Arizona fescue biomass, AMF extraradical hyphae, spore production, and rhizosphere bacterial and actinobacter microbial communities. The increase in extraradical hyphae with Arizona fescue may provide greater access to nutrients, improved water relations, and reduce pathogenic infection (Newsham et al., 1995; Smith and Read, 1997), while enhancing the dominance of Arizona fescue in the herbaceous community of ponderosa pine forests of the Southwest.

Plant species are influential in shaping soil heterotrophic microbial communities (Stephan et al., 2000), and have been shown to influence bacterial communities associated with the rhizosphere (Hawkes et al., 2007). The soil heterotrophic microbial community from the bioassay associated with Arizona fescue had greater gram-negative and gram-positive bacteria and actinobacter populations than soil cores with spike muhly, but similar fungal populations. These final soil heterotrophic microbial communities originated from equivalent initial microbial communities with comparable soil

characteristics. The short time period of the bioassay (8 wks), and the rapid change in soil heterotrophic microbial community between grass species, suggests a strong linkage between labile root exudates of recently fixed photosynthate and soil microbial community structure and activity (Högberg and Read, 2006). Root exudation of photosynthate into surrounding soil varies with plant species, and the quality and quantity of this input is a determining factor in the composition of the proximate soil microbial community (Reynolds et al., 2003; Hawkes et al., 2007).

Fire has been shown to reduce bacteria, actinobacter, and fungi in the surface horizon of mineral soils (Deka and Mishra, 1983; Pattison et al., 1999). Temperatures of 100 °C can be fatal to most organisms, yet these lethal temperatures are dependent on fuel characteristics and weather conditions (DeBano et al., 1998). If fires are of low severity, which is typical of prescribed fires, our results suggest little impact on the soil microbial populations.

Reductions in stand densities by mechanical thinning often combined with prescription burning attempts to both mitigate potential risks from large high-severity wildfires and restore sites to reference conditions. The openings created by clearcutting at Taylor Woods responded with significant increases in herbaceous community, yet the reintroduction of a single low-intensity burn event had no effect. Thinning in ponderosa pine stands of the Southwest has resulted in increased establishment of Arizona fescue (Naumburg and DeWald, 1999; Naumburg et al., 2001), while repeated fall burns can potentially decrease native bunchgrasses (Laughlin et al., 2008). Thinning and burning reduces overstory dominance, which can lead to increased herbaceous production in ponderosa pine stands of the Southwest (Moore et al., 2006; Laughlin et al., 2008), yet

successful reestablishment of the native bunchgrass community may depend on the symbiotic relationships among plants-AMF-heterotrophic soil microorganisms.

The creation of openings in the overstory canopy reduced tree dominance (Moore and Deiter, 1992) and increased AMF propagule densities and species richness. The increased AMF propagule density and species richness did not increase the inoculum potential of our native grasses, suggesting inoculation potential may not be a limiting factor for restoring the native grass community in these ecosystems. Root colonization by AMF with increased bacteria and actinobacter in Arizona fescue was coincident with greater shoot and root biomasses, suggesting that the plant-AMF-heterotrophic interaction may enhance the dominance of this grass. Spatial variability created large openings by within ponderosa pine forests of the Southwest appears to provide a greater potential for increasing native bunchgrasses than simply reducing stand densities (Sabo et al., 2009).

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Figure Legends

Figure 1. Pre-bioassay mean arbuscular mycorrhizal fungal spore concentrations determined from intact mineral soil cores (0-15 cm) taken at four treatment density levels (clear-cut = 0 m² ha⁻¹ basal area, low = 14 m² ha⁻¹ basal area, medium = 28 m² ha⁻¹ basal area, unthinned = 45 m² ha⁻¹ basal area) of ponderosa pine at Taylor Woods, AZ. Stand density means with different letters were significantly different. Vertical lines denote $\pm$ one standard error of the mean. Analyses performed using log₁₀ transformed data in a two-way ANOVA ($\alpha = 0.05$) and Tukey's HSD mean separation test.

Figure 2. (a-b) Mean values of arbuscular mycorrhizal fungal extraradical hyphae (PLFA 16:1 ω 5) from an eight week bioassay utilizing intact soil cores taken at the level of growing stock study site, Taylor Woods, AZ. Intact soil cores (0-15 cm) are from growing stocking levels (clear-cut = 0 m² ha⁻¹ basal area, low = 14 m² ha⁻¹ basal area, medium = 28 m² ha⁻¹ basal area, unthinned = 45 m² ha⁻¹ basal area) of ponderosa pine, with and without burning, at Taylor Woods, AZ. Grass species PLFA 16:1 ω 5 values are significantly different from each other for each stand density with and without burning. Vertical lines denote $\pm$ one standard error of the mean. Analyses performed using two-way ANOVA ($\alpha = 0.05$) and Tukey's HSD mean separation.

Figure 3. Soil microbial guild biomass as determined by phospholipid Fatty Acid (PLFA) analysis. PLFAs were pooled across stand densities and burn treatments at Taylor Woods, AZ, because there were no significant differences among stand densities or with burning. The bioassay utilized two native grasses (*Festuca arizonica*, *Muhlenbergia wrightii*)

grown in intact cores from four different stand densities (clear-cut = 0 m² ha⁻¹ basal area, low = 14 m² ha⁻¹ basal area, medium = 28 m² ha⁻¹ basal area, unthinned = 45 m² ha⁻¹ basal area) of ponderosa pine, with and without burning. Means with different letters are significantly different between grass species for each individual microbial guild. Vertical lines denote + one standard error of the mean. Analyses performed using two-way ANOVA ($\alpha = 0.05$) and Tukey's HSD mean separation tests.

Table 1. Mean cover values based on Daubenmire cover classes for growing stock levels (clear-cut = 0 m² ha⁻¹ basal area, low = 14 m² ha⁻¹ basal area, medium = 28 m² ha⁻¹ basal area, unthinned= 45 m² ha⁻¹ basal area) of ponderosa pine sampled in August of 2003 at Taylor Woods, AZ. (Daubenmire 1959).

Stand density	% Litter	% Rock	%Bare soil	% Vegetation
Clear-cut	37.5	2.5	37.5	22.5
Low	85	2.5	2.5	5
Medium	97.5	0	0	1
Unthinned	97.5	0	0	0

Table 2. Mean diversity of vegetation and arbuscular mycorrhizal fungi (AMF) spores (+ one standard error) at Taylor Woods, AZ. Values are for vegetation and arbuscular mycorrhizal fungal spores sampled at growing stock levels (clear-cut = 0 m² ha⁻¹ basal area, low = 14 m² ha⁻¹ basal area, medium = 28 m² ha⁻¹ basal area, unthinned= 45 m² ha⁻¹ basal area) of ponderosa pine.

Diversity measures	Density			
	Clearcut	Low	Medium	High
Vegetation				
species richness	9.83a (2.10)	8.00a (0.45)	3.83b (0.54)	1.5b (0.50)
evenness	0.77 (0.03)	0.85(0.02)	0.76 (0.05)	0.50 (0.50)
Shannon's diversity	1.72a (0.22)	1.77a (0.07)	1.01b (0.16)	0.35b (0.35)
Simpson's index	0.72a (0.05)	0.79a (0.02)	0.54b (0.07)	0.25c (0.25)
AMF Spore				
species richness	11.67a (1.28)	7.33b (0.92)	6.17b (0.70)	4.00b (0.58)
evenness	0.63 (0.03)	0.81 (0.04)	0.83 (0.02)	0.73 (0.18)
Shannon's diversity	1.54 (0.12)	1.56 (0.07)	1.49 (0.10)	1.04 (0.32)
Simpsons index	0.67 (0.03)	0.73 (0.02)	0.71 (0.03)	0.54 (0.18)

Mean values with different letters within a row designate significant difference ($\alpha=0.05$) among stand density using Tukey's HSD mean separation test. Mean values within a row without letters are not significantly different.

Table 3. Presence-absence of herbaceous species at growing stock levels plots (clear-cut = 0 m² ha⁻¹ basal area, low = 14 m² ha⁻¹ basal area, medium = 28 m² ha⁻¹ basal area, unthinned= 45 m² ha⁻¹ basal area) of ponderosa pine at Taylor Woods, AZ.

Scientific names	Common names	Density			
		Clear-cut	Low	Med.	High
<i>Achillea millefolium</i> L.	White yarrow	X		X	
<i>Agropyron desertorum</i> (Fisch. ex Link)	Desert wheatgrass	X			
<i>Androsace septentrionalis</i> L.	pygmyflower rockjasmine	X			
<i>Antennaria parvifolia</i> Nutt.	small-leaf pussytoes			X	
<i>Artemisia carruthii</i> Alph.	Carruth's sagewort	X		X	
<i>Artemisia dracunculus</i> L.	False Tarrogon	X			
<i>Bahia dissecta</i> (A. Gray) Britton	ragleaf bahia	X	X	X	
<i>Bouteloua gracilis</i> (Willd. ex Kunth)	Blue Grama	X			
<i>Bromus inermis</i> Leyss.	Smooth Brome	X	X	X	
<i>Carex</i> L.	sedge	X	X	X	X
<i>Cercocarpus ledifolius</i> Nutt.	curl-leaf mountain mahogany			X	
<i>Chenopodium</i> L.	goosefoot	X	X		
<i>Cirsium wheeleri</i> (A. Gray)	Wheeler's Thistle	X	X		
<i>Convolvulus arvensis</i> L.	Field Bind Weed				
<i>Elymus elymoides</i> (Raf.)	squirreldail	X	X	X	
<i>Erigeron divergens</i> Torr. & A. Gray	spreading fleabane	X			
<i>Erigeron formosissimus</i> Greene	beautiful fleabane				
<i>Eriogonum racemosum</i> Nutt.	redroot buckwheat			X	
<i>Euphorbia</i> L.	spurge	X	X		
<i>Festuca arizonica</i> Vasey	Arizona fescue	X	X	X	
<i>Geranium caespitosum</i> James	pineywoods geranium	X	X		
<i>Helianthus annuus</i> L.	common sunflower	X			
<i>Hieracium fendleri</i> Sch. Bip.	yellow hawkweed				
<i>Iris missouriensis</i> Nutt.	Rocky Mountain iris				
<i>Linaria dalmatica</i> (L.) Mill.	Dalmatian toadflax	X			
<i>Lotus wrightii</i> (A. Gray) Greene	Wright's deervetch		X		
<i>Lupinus argenteus</i> Pursh	silvery lupine	X	X		
<i>Lupinus kingii</i> S. Watson	King's lupine	X	X		
<i>Machaeranthera canescens</i> (Pursh)	Hoary Aster				
<i>Mirabilis linearis</i> (Pursh) Heimerl	narrowleaf four o'clock	X			
<i>Muhlenbergia montana</i> (Nutt.) Hitchc.	mountain muhly	X	X		
<i>Packera neomexicana</i> (A. Gray)	New Mexico groundsel			X	
<i>Potentilla plattensis</i> Nutt.	Platte River cinquefoil				
<i>Ribes cereum</i> Douglas	wax currant				
<i>Robinia neomexicana</i> A. Gray	New Mexico locust		X		X
<i>Senecio wootonii</i> Greene	Wooton's ragwort	X	X		
<i>Solidago missouriensis</i> Nutt.	Missouri goldenrod	X			
<i>Thalictrum fendleri</i> Engelm. ex A. Gray	Fendler's meadow-rue	X	X		
<i>Thinopyrum intermedium</i> (Host)	intermediate wheatgrass	X	X		
<i>Tragopogon dubius</i> Scop.	yellow salsify	X			
<i>Verbascum thapsus</i> L.	common mullein	X			
<i>Vicia pulchella</i> Kunth	sweetclover vetch		X		

Table 4. Mean total nutrient concentration values (+ one standard error) for mineral soil (0-5 cm) at Taylor Woods, AZ. Values are partitioned by split- plots of growing stock level (clear-cut = 0 m² ha⁻¹ basal area, low = 14 m² ha⁻¹ basal area, medium = 28 m² ha⁻¹ basal area, unthinned= 45 m² ha⁻¹ basal area) of ponderosa pine by burn treatment.

	Clear cut		Low Density		Medium Density		Unthinned
Mineral soil (0-5 cm)	No Burn	Burn	No Burn	Burn	No Burn	Burn	No Burn
Total C (%)	3.83 (0.59)	4.89 (0.25)	4.0 (0.27)	3.5 (0.40)	3.16 (0.73)	3.49 (0.83)	4.13 (0.41)
Total N (%)	0.21 (0.03)	0.25 (0.01)	0.17 (0.02)	0.16 (0.02)	0.17 (0.04)	0.18 (0.05)	0.17 (0.02)
Total P (%)	0.11 (0.01)	0.11 (0.01)	0.10 (0.004)	0.11 (0.002)	0.11 (0.02)	0.10 (0.01)	0.09 (0.002)

Mean values with different letters designate significant difference ($\alpha=0.05$) for burn effect within stand density using Tukey's HSD mean separation test. Mean values without letters are not significantly different.

Table 5. Presence-absence data for arbuscular mycorrhizal spores sampled in mineral soil cores (0-15 cm) from growing stock levels (clear-cut = 0 m² ha⁻¹ basal area, low = 14 m² ha⁻¹ basal area, medium = 28 m² ha⁻¹ basal area, unthinned = 45 m² ha⁻¹ basal area) of ponderosa pine at Taylor Woods, AZ. Pre-cores are initial field sampled cores analyzed prior to bioassay and post-cores are spores harvested post bioassay.

Arbuscular Mycorrhizal Fungi	Pre-Core	Post-Core <i>Festuca arizonica</i>	Post-Core <i>Muhlenbergia wrightii</i>
<i>Acaulospora denticulata</i>	X	X	X
<i>Acaulospora lacunosa</i>		X	X
<i>Acaulospora laevis</i>	X	X	X
<i>Acaulospora mellea</i>	X	X	X
<i>Acaulospora nicholsonii</i>		X	X
<i>Acaulospora rehmsii</i>		X	X
<i>Acaulospora scobiculata</i>		X	X
<i>Acaulospora trappei</i>	X	X	X
<i>Acaulospora undulata</i>	X	X	X
<i>Gigaspora albida</i>		X	X
<i>Glomus aggregatum</i>	X	X	X
<i>Glomus ambisporum</i>	X	X	X
<i>Glomus cerebriforme</i>	X	X	X
<i>Glomus clarum</i>	X	X	X
<i>Glomus constrictum</i>	X	X	X
<i>Glomus etunicatum</i>	X	X	X
<i>Glomus fasciculatum</i>	X	X	X
<i>Glomus fecundisporum</i>	X	X	X
<i>Glomus heterosporum</i>	X	X	X
<i>Glomus interadices</i>	X	X	X
<i>Glomus invermaynum</i>	X	X	X
<i>Glomus microaggregatum</i>	X	X	X
<i>Glomus microcarpa</i>		X	X
<i>Glomus microcarpum</i>	X	X	X
<i>Glomus mossea</i>	X	X	X
<i>Glomus patchycaulus</i>		X	X
<i>Glomus s rubiformis</i>		X	X
<i>Paraglomus occultum</i>	X	X	X
<i>Scutellospora alborosea</i>		X	X
<i>Scutellospora calospora</i>		X	X
<i>Scutellospora heterogama</i>		X	X
<i>Scutellospora pellucida</i>		X	X

Table 6. Mean dry weight (+ one standard error) values for harvested shoots and roots of two native grass species harvested from soil cores (0-15 cm) collected at graduated stocking levels (clear-cut = 0 m² ha⁻¹ basal area, low = 14 m² ha⁻¹ basal area, medium = 28 m² ha⁻¹ basal area, unthinned= 45 m² ha⁻¹ basal area) of ponderosa pine at Taylor Woods, AZ following an 8 week bioassay.

	Root biomass (g)	Shoot biomass (g)	Shoot:Root ratio
<i>Festuca arizonica</i>	0.35a (0.05)	0.27a (0.01)	0.99 (0.19)
<i>Muhlenbergia wrightii</i>	0.17b (0.03)	0.20b (0.02)	1.51 (0.26)

Mean values with different letters designate significant difference ($\alpha=0.05$) between grass species using Tukey's HSD mean separation test. Mean values without letters are not significantly different.

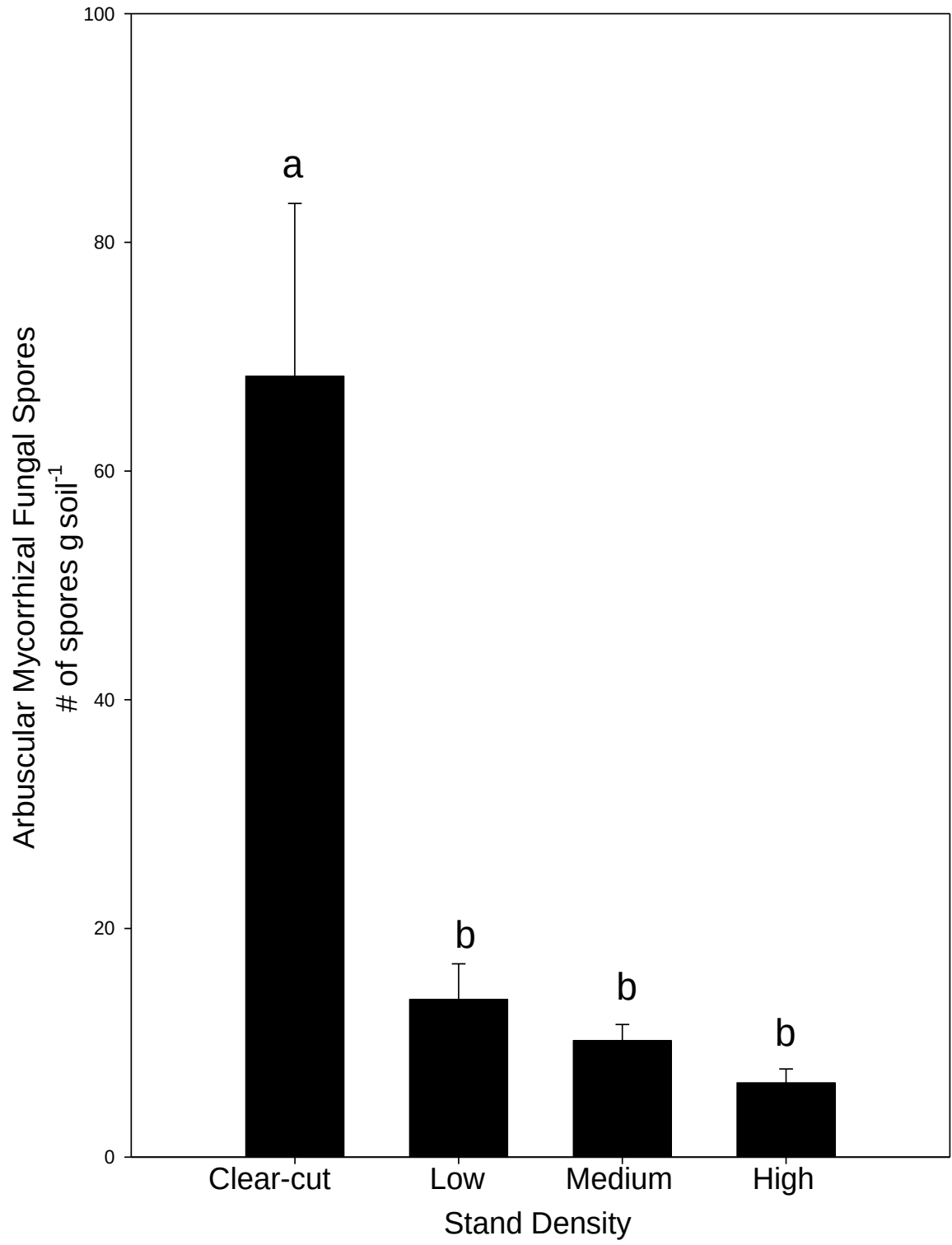


Figure 1

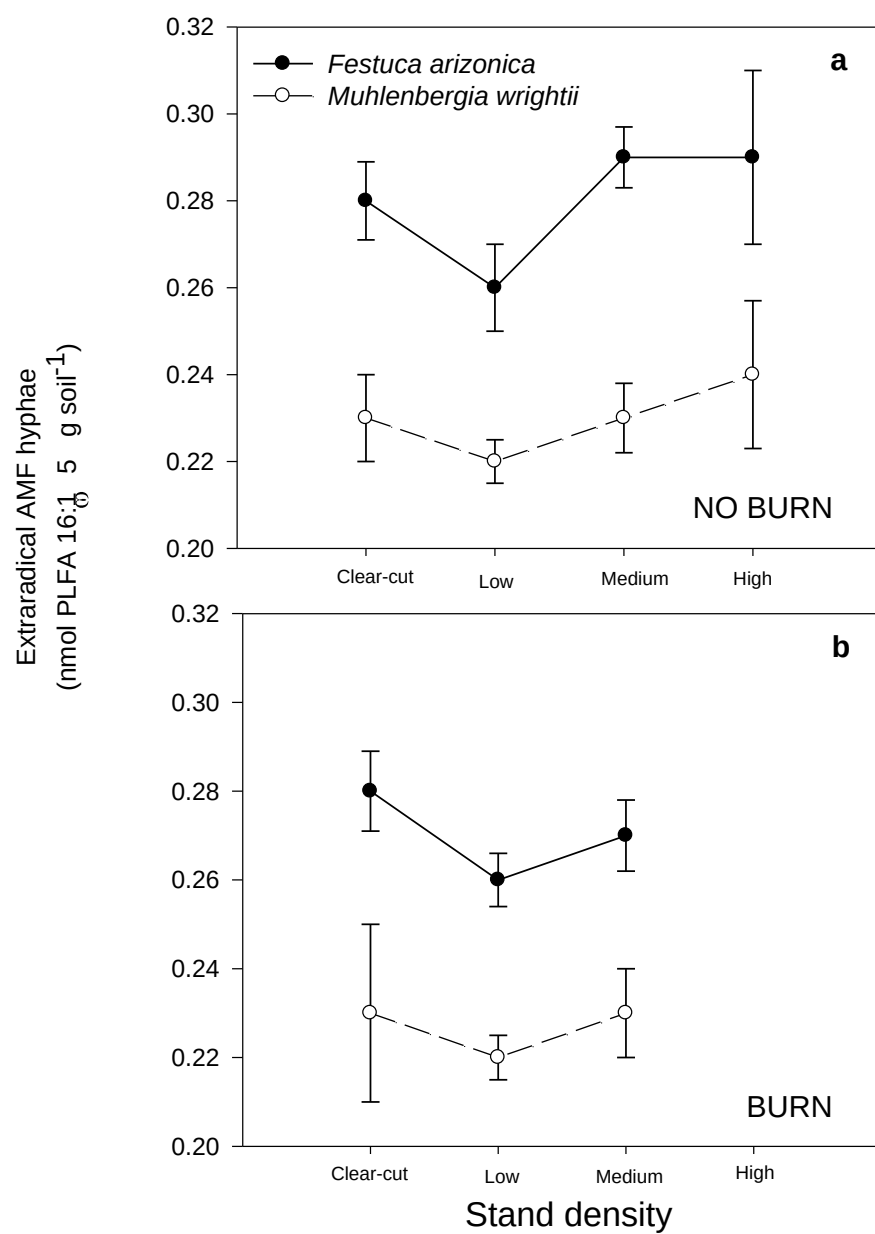


Figure 2

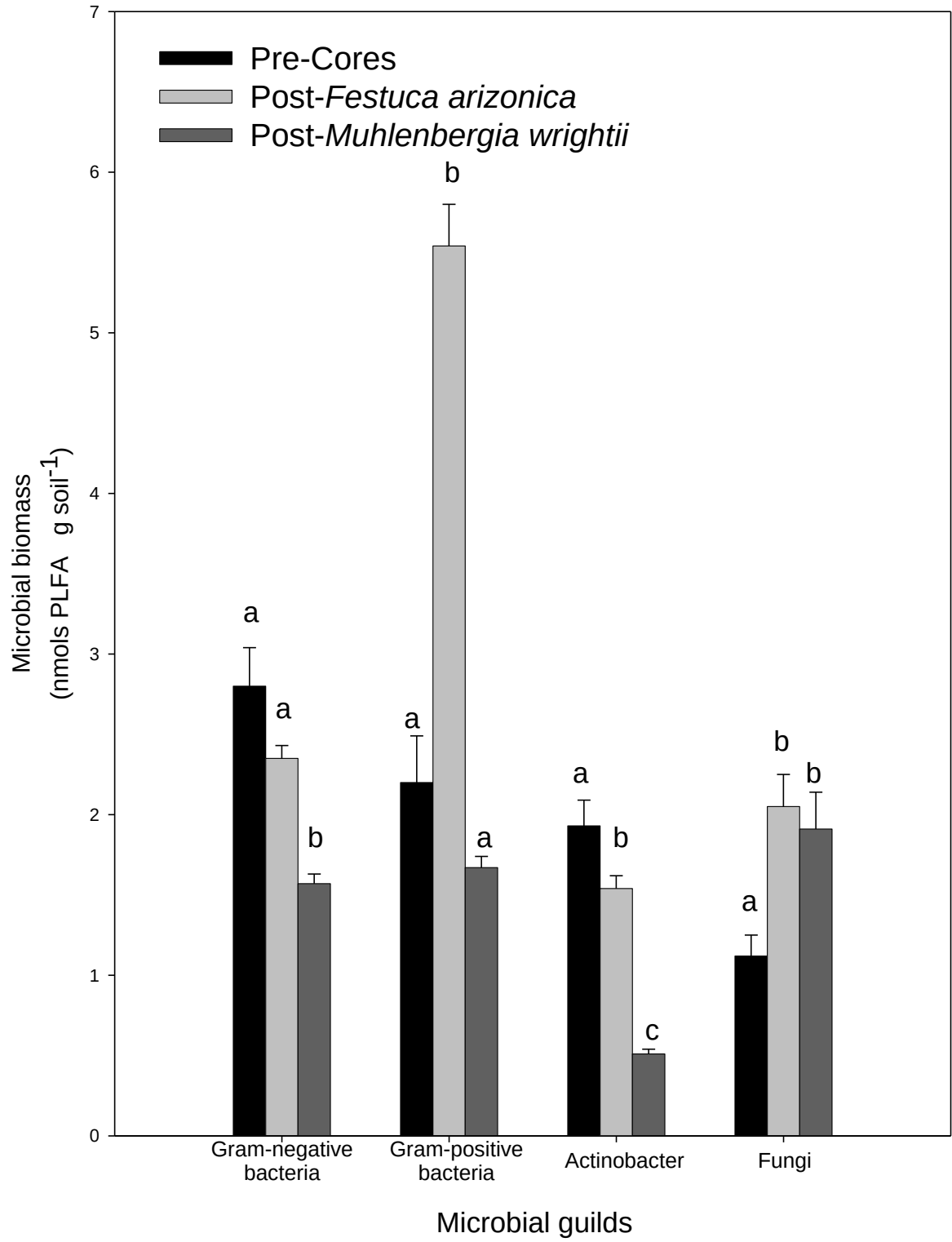


Figure 3

CHAPTER 5

CONCLUSIONS

My dissertation provides useful information to land managers and scientists involved in wildfire mitigation and restoration efforts. I investigated the influence of stand density reductions of ponderosa pine by mechanical thinning to different levels, with and without prescribed fire, on the soil microbial community and processes they catalyze. A century or more of fire suppression, grazing, logging, and climate change has created dense stands of ponderosa pine, and loss of much of the herbaceous and shrub community of these forests (Covington et al., 1997). Decreased soil process rates and possible soil microbial community changes are speculated to have occurred with the increased stand densities in ponderosa pine forests (Hart et al., 2005). As landscape-scale wildfire mitigation and forest restoration efforts move forward, a better understanding of the linkage between reduced stand densities and the soil microbial community is imperative to the long-term success of these efforts.

Recommendations developed from these studies should take into account both the location and the nature of treatment applications used (e.g., how slash residues were treated or the severity of applied prescribed burns). The first objective of this dissertation was to determine the long-term impact of ponderosa pine-dominated stand densities on soil microbial community biomass and structure. I found that at the stand level, soil microbial community structure is robust to changes in overstory structure. This is true for ponderosa pine forests over a large geographic area with contrasting productivities and site conditions. The different site conditions (e.g. climate, soils, geology) and productivities over this large geographic area exerted the greatest influence on the soil

microbial community. These regional differences emphasize the need to develop site-specific protocols for wildfire mitigation and restoration efforts.

The second objective was to quantify short-term impacts of operational-scale wildfire mitigation treatments on soil microbial community and processes. The Fire and Fire Surrogate (FFS) network study, a large national project, attempted to quantify the ecological, economic, and social consequences of alternative fire and fire surrogate treatments in several forests types of the United States (Edminster et al., 2000). The FFS network applied operational-scale wildfire mitigation treatments to three units on two national forests of northern Arizona. The soil microbial community biomass and structure at this Southwestern Plateau FFS site were gain relatively unaffected by wildfire mitigation treatments. I did find increased net N mineralization and nitrification with thinning and burning six months after completion of the treatments, but these increases returned to pre-treatment levels within the first year. I also measured reduced soil enzymatic activity for two years following treatments that included thinning. This reduction in potential enzyme activity following thinning treatments may be the result of increased degradation of existing, soil-stabilized enzymes rather than due to a reduced microbial response (Wallenstein and Weintraub, 2008), as microbial populations did not appear to be significantly affected by thinning. Additional indicators of microbial activity, such as net N mineralization and net nitrification at the Southwestern Plateau burn units were not different than the untreated plots after one year for any of the treatments, including those incorporating prescribed fire. Wan et al. (2001) meta-analysis of fire impacts on forest floor and mineral soil N emphasized fire severity as a major determinant of N dynamics, and we know that low-severity is an attribute of prescribed

fire. Again, the results for this study further the hypothesis that soil microbial communities are robust to treatments that reduce stand densities, but also the low impact of prescribed fire on these microbial communities.

The third objective of my dissertation was to determine what influence long-term stand reductions and prescribed fire had on the capacity of arbuscular mycorrhizal fungi (AMF) to colonize native grasses. I identified AMF spores from intact soil cores used in a bioassay taken from replicate split-plots of four different stand densities, with and without prescribed fire. I also utilized two native grasses, a dominant and uncommon species, to determine plant species-specific effects on AMF inoculum potentials and the heterotrophic microbial community of the rhizosphere. I found that burning had no significant effect on mycorrhizal properties or on the soil microbial community. Furthermore, stand density did not affect AMF inoculum potentials for these two grass species, yet creating openings in the canopy by clearcutting did increase AMF densities and species richness. Finally, I found different soil microbial communities between the two grass species that had been colonized by the local AMF community. The rhizosphere of Arizona fescue (*Festuca arizonica* Vasey), a dominant grass species in ponderosa pine forests of the Southwest, had higher bacteria and actinobacter populations than the rhizosphere of mountain muhly (*Muhlenbergia wrightii* Vasey), an uncommon species in ponderosa pine forests of the Southwest. Arizona fescue, and the associated rhizosphere microbial community, resulted in greater plant shoot and root biomasses and greater extraradical hyphae and AMF spores concentrations than the spike muhly system. This suggests that dominance of Arizona fescue in the herbaceous communities of ponderosa pine forest of the Southwest is enhanced by AMF colonization and increased bacterial

and actinobacter populations within the rhizosphere. In contrast to Korb et al. (2003) who reported a rapid increase in AMF inoculum potentials following restoration treatments, I found no change in AMF inoculation potentials with stand reductions in my study. It is clear from these studies that microbial communities in ponderosa pine forests are robust to both long- and short-term reductions in stand densities and prescribed fire across a wide range in site characteristics. Furthermore, my dissertation suggests that maintaining the couplings between understory species and the microbial communities that they support may be important to the success of restoration efforts.

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