Applied Soil Ecology 93 (2015) 1–10

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Soil microbial community resilience with tree thinning in a 40-year-old experimental ponderosa pine forest

Steven T. Overby [a,b,*], Suzanne M. Owen [a,b], Stephen C. Hart [b,c], Daniel G. Neary [a,1], Nancy C. Johnson [d]

[a] Rocky Mountain Research Station, United States Forest Service, Flagstaff, AZ 86001, USA
[b] School of Forestry, Northern Arizona University, Flagstaff, AZ 86011, USA
[c] School of Natural Sciences and Sierra Nevada Research Institute, University of California, Merced, CA 95344, USA
[d] School of Earth Sciences and Environmental Sustainability, Northern Arizona University, Flagstaff, AZ 86011, USA

ARTICLE INFO

Article history:
Received 18 September 2014
Received in revised form 20 March 2015
Accepted 29 March 2015
Available online 9 April 2015

Keywords:
Arbuscular mycorrhizal fungi
Phospholipid fatty acids
Soil bioassay
Festuca arizonica
Muhlenbergia wrightii
Fort Valley Experimental Forest

ABSTRACT

Establishment of native grasses is a primary objective of restoration in *Pinus ponderosa* var. *scopulorum* (P. & C. Lawson) forests in the southwestern United States. Interactions among native grasses and soil microorganisms generate feedbacks that influence the achievement of this objective. We examined soil chemical properties and communities of plants and soil microorganisms in clear-cuts and *P. ponderosa* stands thinned and maintained at low and medium tree densities for over 40 years along with high density (unthinned) stands. Phospholipid fatty acids (PLFA) in soils were analyzed to examine arbuscular mycorrhizal (AM) fungi and microbial communities in the three thinning treatments and the unthinned stands with and without a recent broadcast burn. Additionally, two native bunchgrasses, *Festuca arizonica* and *Muhlenbergia wrightii* were grown in containers filled with intact soil cores collected from each field plot to more thoroughly compare the abundance of AM fungi and microbial communities across different stand densities and burn treatments. Tree thinning decreased litter cover and increased the abundance and diversity and altered community composition of both herbaceous vegetation and AM fungi. In the mineral soil layer, the pH, total carbon, nitrogen, phosphorus and PLFA profiles did not differ significantly among the four stand density or burn treatments. Mycorrhizal colonization of the container grown grasses did not significantly differ with tree density or burn treatments; however, *F. arizonica* roots had a strong trend for decreased colonization when grown in soil from high density (unthinned) tree cover. Soil from the containers with *F. arizonica* had a greater abundance of AM fungal spores. Furthermore, bacterial community composition varied with grass species. Concentration of biomarkers for bacteria were higher in soil that supported *F. arizonica* compared to soil in which *M. wrightii* was grown. Our results indicate that the creation of clear-cut openings in forests may increase the abundance and richness of AM fungal propagules and soil bacterial communities were surprisingly resilient to tree thinning and low-intensity fire treatments. These results suggest managing forests to create clear-cut openings generate conditions that favor understory native grasses and AM fungi that are linked to soil bacterial communities.

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1. Introduction

Restoration of overly-dense ponderosa pine (*Pinus ponderosa*) forests to more fire-resilient grass-dominated savannas similar to pre-Euro-American conditions is a primary management goal in the southwestern United States. The overly-dense stand structure of the largest contiguous stand of ponderosa pine in the United States, exacerbated by almost a century of fire suppression, poses a threat to both the ecosystem and human populations in close proximity due to large, often catastrophic wildfires. Reducing tree densities and creating canopy openings can lessen the threat of stand-replacing fires and encourage the establishment of native understory vegetation (Graham et al., 1999; Laughlin et al., 2010). Plant community structure and native plant succession are strongly influenced by communities of soil organisms and plant symbionts such as mycorrhizal fungi (reviewed in Kulmatiski et al.,

* Corresponding author at: Rocky Mountain Research Station, United States Forest Service Flagstaff, AZ 86001, USA. Tel.: +1 928 556 2184; fax: +1 928 556 2130.
E-mail address: soverby@fs.fed (S.T. Overby).
[1] Former Scientist in Charge, Fort Valley Experimental Forest.

http://dx.doi.org/10.1016/j.apsoil.2015.03.012
0929-1393/Published by Elsevier B.V.

2008; Pringle et al., 2009). Thinning of overstory trees can influence both understory plants and soil microbial communities (Jones et al., 2003; Owen et al., 2009; Pickles et al., 2010).

Structural changes following thinning in ponderosa pine forests have been shown to alter soil microbial communities that in turn influence plant diversity and composition (Kaye et al., 2005; Laughlin et al., 2010; Pringle et al., 2009; Schnitzer et al., 2011). Reducing densities of ectomycorrhizal (EM) trees helps arbuscular mycorrhizal (AM) grasses thrive by opening the canopy to provide greater light penetration, reducing competition for resources, and increasing microbial populations that are beneficial to herbaceous plants (Korb et al., 2003; Laughlin et al., 2008). Microbial populations influence ecosystem processes that, in turn, affect plant populations, creating feedbacks between aboveground and belowground communities (Bardgett, 2005; Hart et al., 2005). These feedbacks between plants and soil organisms are important determinants of plant community structure (Klironomos, 2003). Host plants benefit when mycorrhizas acquire nutrients that heterotrophic soil microorganisms mobilize from complex substrates in the soil (Aert, 2002; Talbot et al., 2008; Hodge and Fitter, 2010). Mycorrhizas are typically mutually beneficial, but their influence varies with species of plants, fungi and environmental conditions (Johnson and Graham, 2013). Depending on the manner in which woody debris is treated following tree thinning, AM fungi have been shown to either be more abundant with tree thinning (Korb et al., 2003), or have reduced propagule abundance and richness (Korb et al., 2004; Owen et al., 2009) compared to untreated areas with high tree density.

Changing the plant community from a high density forest to an open canopy forest with an herbaceous community is expected to alter the soil microclimate and chemical properties (DeBano et al., 1998; Waldrop et al., 2003; Grayston and Renneberg, 2006). Following tree thinning, decomposition and N mineralization rates have been shown to increase in the short-term in southwestern *P. ponderosa* (Kaye and Hart, 1998a,b; Grady and Hart, 2006). This increase was assumed to be the result of decreased canopy allowing greater soil insolation to warm the soil surface and increase available soil moisture (Kaye and Hart, 1998a,b). Even with increased soil microbial activity immobilization of N can occur (DeLuca and Zouhar, 2000). If prescribed burning is combined with thinning, lethal temperatures (>100 °C) can negatively affect microbial populations with a disproportionate decrease in fungi especially in the O horizon (DeBano et al., 1998; Hart et al., 2005; Cairney and Bastias, 2007). Additional negative impacts include limiting water infiltration and available soil moisture from the formation of hydrophobic surface conditions and reduced microbial activity (DeBano et al., 1998). Yet increased soil insolation and soil moisture (Hart et al., 2005; Simonin et al., 2007), available N (Covington and Sackett 1992; Kaye and Hart, 1998b; Frey et al., 2004; Kaye et al., 2005), surface soil pH (cation deposition), and the addition of charcoal (Hart et al., 2005) from surface fires has been shown to enhance microbial activity (Pietikäinen and Fritze, 1995; Pietikäinen et al., 2000). To date the majority of studies that have altered ponderosa pine densities for restoration have focused on short-term responses following thinning treatments.

The goals of our study were to examine the long-term (>40 years) influences of varying levels of tree density and a low intensity prescribed fire on understory plant communities, soil chemical properties, microbial biomass, and the abundance and composition of AM fungi. We expected that long-term maintenance of varying stand densities would influence the composition of soil microbial communities. Because AM fungi are obligate symbionts of herbaceous plants including grasses, their abundance and diversity were hypothesized to be lowest in untreated, high-density stands. In clear-cuts we expected abundance and diversity to be the highest due to greater diversity of potential host plants (Korb et al., 2003). Low intensity fire was expected to reduce the amount of litter in the organic horizon, but have only a short-term influence on mineral soil pH, nutrients or microbial community composition as heat penetration should be minimal. We tested four hypotheses: (H_1) long-term *P. ponderosa* stand density reductions should increase the abundance, diversity, and alter the community composition of herbaceous plants, AM fungi, and other soil microorganisms; (H_2) long-term *P. ponderosa* stand density reductions will reduce litter mass and litter C concentration, but increase available N and P; (H_3) low intensity fire would decrease litter mass and possibly increase soil pH, but not influence other plant, microbial or soil variables; and (H_4) different communities of heterotrophic soil organisms would develop from the interaction of varying stand densities and host plants.

2. Materials and methods

2.1. Study sites

In 1962 the United States Forest Service established and has maintained to date an experimental gradient of mechanically-thinned stands of *P. ponderosa* at Taylor Woods, a subdivision of the Fort Valley Experimental Forest. Taylor Woods is approximately 14.5 km northwest of Flagstaff, Arizona, at an elevation of 2266 m (Ronco et al., 1985). Study plots are within a 36.4-ha area on a gentle (4%), southwest-facing slope, in the *P. ponderosa*/Arizona fescue (*Festuca arizonica* Vasey) habitat type. Mean annual air temperature 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. Mean annual snowfall from 1950 to 2006 was 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.

This study examined four tree density treatments: clear-cut (0 trees ha^{-1}), low (145 trees ha^{-1}), medium (471 trees ha^{-1}), and unthinned high density (3200 trees ha^{-1}). Each treatment was replicated three times, with plots ranging in size from 0.30 to 0.50 ha (Ronco et al., 1985; McDowell et al., 2007). The plots were initially thinned to specified stand densities in 1962 and these stand densities maintained by thinning as needed 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, and one half of each plot burned during the fall and winter of 1998–1999 creating a replicate split-plot design. Very low fire intensities were applied to burn understory and surface litter, and no tree mortality occurred within the plots. The high density (unthinned) plots were not burned because it was impossible to perform prescribed burns in a controlled manner.

2.2. Plot vegetation and microbial communities

Plant cover was measured within the different tree density plots to determine if understory plant abundance and diversity would be greater at higher levels of forest thinning. In August, 2003, herbaceous plant canopy cover and frequency

measurements were taken using a line-intercept method. We randomly chose three, 40-m transects that went across both burned and unburned areas in each plot. Percent plant canopy cover was determined by measuring the horizontal linear lengths of each plant along each transect, and the total distance for each species was divided by 40 m for each transect. Total percent plant cover was averaged over each subplot. Total plant, litter, bare ground, life form (forbs, graminoids and shrubs) and individual species cover were estimated in a similar way. Plants were identified to species at the Deaver Herbarium at Northern Arizona University, or at the USDA Rocky Mountain Research Station. Scientific nomenclature follows the PLANTS Database (http://plants.usda.gov).

Arbuscular mycorrhizal fungal spore diversity and soil microbial community based on phospholipid fatty acid (PLFA) profiles were determined from soil cores collected in each of the different tree density plots. Collection techniques and analyses are described below in the Sections 2.4, 2.5 and 3.3.

2.3. Soil and litter sampling and analysis

Samples of the surface organic (O) horizon and mineral soil (0–5 cm) were collected in August 2002, and again in June and August 2003. Within each replicate split-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. The O horizon and mineral soil was collected within a 0.01-m^2 litter frame, placed in polyethylene bags, and transported to the laboratory on ice each day. Mineral soil was collected within the litter frame using 1.9-cm diameter soil probe (Oakfield Apparatus Company, Oakfield, WI, USA).

Litter greater than 6-mm was removed from O horizon samples. 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). These subsamples were dried for 48 h at 70 °C for the O horizon materials and at 105 °C for the mineral soils, 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 C, nitrogen (N), and phosphorus (P) concentrations, while mineral soil was analyzed for pH, and total C and N concentrations.

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, NJ, 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).

2.4. Container study of AM fungal populations

The purpose of the container study was to grow two native grasses as a bioassay to access the densities of viable propagules of AM fungi in the intact soil cores. Spores, colonized roots, and hyphal networks all function as AM fungal propagules; consequently, growing host plants in intact soil cores will minimize reduced viability of the AM fungal propagules caused by destruction of hyphal networks during mixing (Brundrett and Abbott, 1994).

During August 2003, we collected three intact soil cores for a container study within each replicate split-plot of our four density treatments. These soil cores were obtained using a soil core (5 × 15 cm) attached to a slide hammer (AMS Inc., American Falls, ID, USA), for a total of 63 soil cores. 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 × 18 cm) typically used for containerized tree seedlings. Microbial steam sterilization of the polypropylene containers was accomplished using an autoclave set at 110.3 kPa pressure to obtain 121 °C boiling temperature for 15 min. The remaining core (called "pre") was used to determine the initial field conditions for PLFA profiles and AM fungal spore composition and for later comparison of these variables following 8-weeks of plant growth in an environmental chamber.

Locally harvested native seeds (Native Plant and Seed, Flagstaff, AZ) of, *Festuca arizonica*, the dominant C3 grass species, and *Muhlenbergia wrightii*, a less common C4 species on our plots, were sown into the freshly collected soil cores. These two species were selected due to their availability at the time of the experiment. Five seeds of each species were planted per container and after seed germination, pots were thinned to two plants per core. Plants were maintained in a growth chamber under light (12 h at 25 °C, ~460 $\mu mol\ m^{-2}\ d^{-1}$ photosynthetic photon flux density) and dark conditions (12 h at 20 °C) for 6 weeks. Plants were watered every other day without supplemental nutrients. After 6 weeks, watering was discontinued and the plants were allowed to senesce for an additional 2 weeks to stimulate sporulation by AM fungi.

At harvest shoots were separated from roots, oven dried (60 °C) for 3 days, and weighed. Soil cores were frozen until roots could be separated. Roots were rinsed and random subsamples of at least 100 cm of fine roots were analyzed for AM fungi colonization (McGonigle et al., 1990). The remaining roots were oven dried (60 °C) for 3 days, and weighed. The root subsamples were cleared in 10% (w/v) KOH and stained with blue Shaeffer ink and vinegar (Vierheilig et al., 1998). Percent mycorrhizal colonization was measured using the grid-line intersect method (McGonigle et al., 1990). The AM fungal spores were extracted from a homogenized soil subsample (25 g) from each soil core using the sucrose centrifugation method (Johnson et al., 1999). Spores were mounted onto slides, examined with a compound microscope (magnification of 100–400×), and identified to morphospecies when possible using Schenck and Perez (1990) and INVAM (http://invam.caf.wvu.edu/) as references. Species names follow Schüßler and Walker (2010).

Table 1
Mean (standard error) diversity of plants and arbuscular mycorrhizal fungal spores by *P. ponderosa* stand density treatments in Taylor Woods, AZ. Mean values with different letters within a row designate significant difference ($\alpha = 0.05$) among treatments using Tukey's HSD mean separation test. No letters in a row designates no significant difference among stand density treatments.

Diversity measures	Stand density treatment			
	Clear-cut	Low	Medium	High
Vegetation				
Cover (%)	21.0a (10.16)	5.2b (1.74)	1.0b (0.34)	0.0b (0.00)
Species richness	9.83a (2.10)	8.00a (0.45)	3.83b (0.54)	1.50b (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)
AM fungal spores				
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)

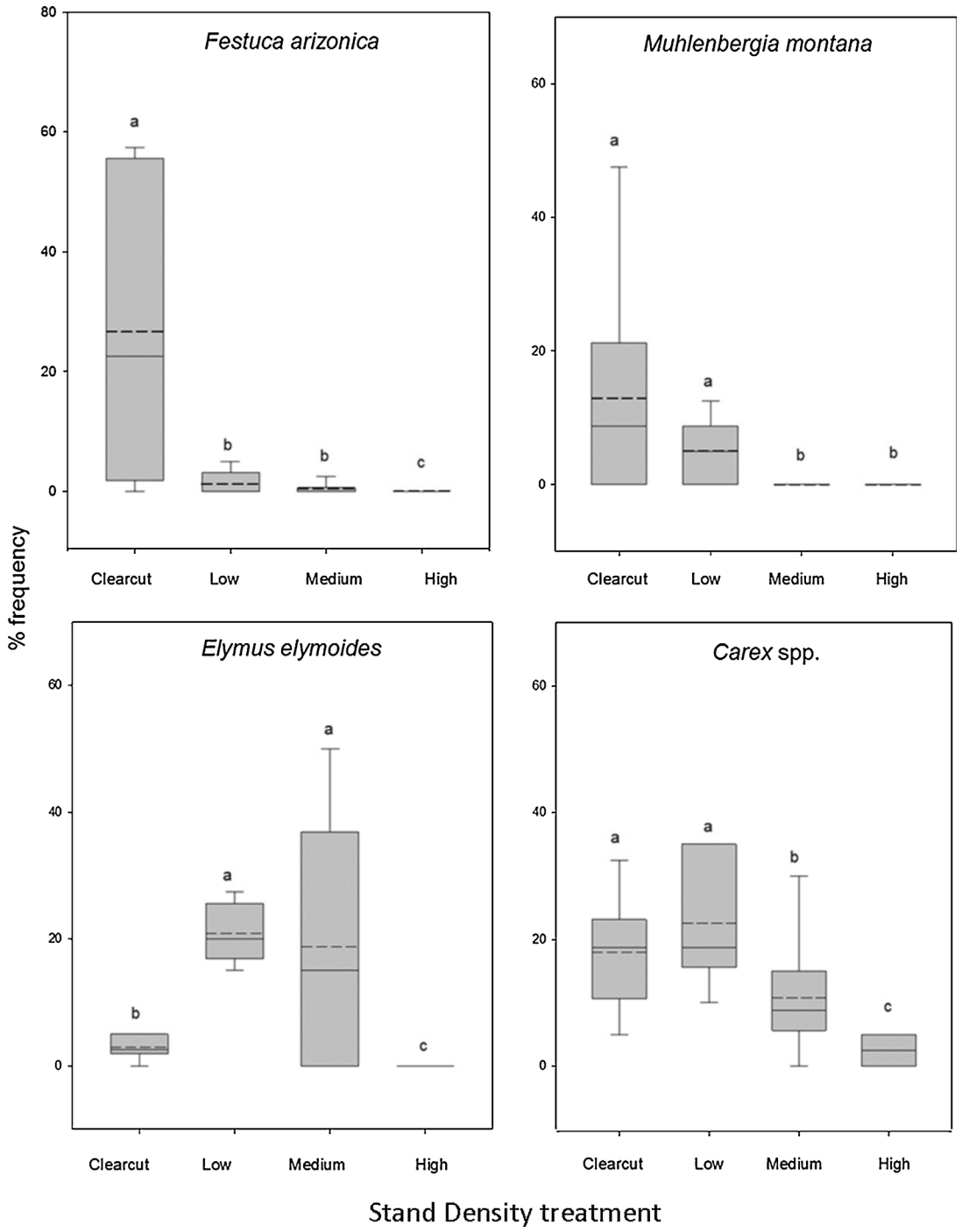

Fig. 1. Mean percent cover (and standard error) of *Festuca arizonica*,*Muhlenbergia montana*, *Elymus elymoides*, and *Carex* spp. by *P. ponderosa* stand density treatments in Taylor Woods, AZ.

2.5. Container study of heterotrophic soil microorganisms

The phospholipid fatty acids (PLFAs) and neutral lipid fatty acids (NLFAs) in O horizon and mineral soils were analyzed prior to planting the two grasses for comparison of microbial communities among stand densities. Final samples from our container study were analyzed for comparison of microbial communities between host plants for each stand density. After collection, these samples 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 PLFA and NLFA. The extraction process occurred within 48 h of returning to the laboratory. Five gram 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), followed by fractionation into neutral, glyco-, and phospholipids (Frostegard et al., 1991). The extraction and analysis method we utilized is described in Schweitzer et al. (2008). Quantification (μmol PLFA kg^{-1} or NLFA kg^{-1} oven-dry material) of samples was based on calibration curves derived from individual fatty-acid methyl esters (FAME) standards.

In addition to estimating total microbial biomass, a conservative approach is to utilize individual PLFAs as biomarkers for fungi and bacteria (Frostegard et al., 2011). Compounds between C14 and C18 in C chain length were used as microbial biomarkers and

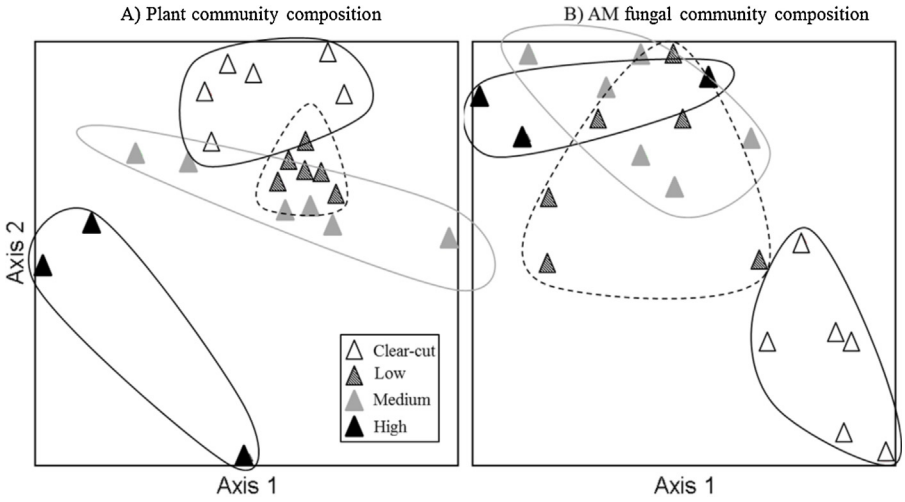

Fig. 2. Nonmetric multidimensional scaling ordination showing species composition for A) Plant and B) AM fungal spore communities by *P. ponderosa* stand density treatments in Taylor Woods, AZ. Different colored triangles represent the entire species composition for each plot in the different stand densities: white = clear-cuts, striped = low, gray = medium, and black = high density.

identified using mass spectrometry. We used biomarkers i15:0, a15:0, i16:0, 10me16:0, i17:0, 17:0, cy17:0, cy19:0, 16:1w9c, 16:1w7, 18:1w5c, and18:1w7 (Hassett and Zak, 2005; Leckie, 2005; Kaye et al., 2005; Zelles, 1999) to represent bacteria; with fungi represented by 18:2w6, 9 biomarkers (Frostegard and Bååth, 1996; Hassett and Zak, 2005; Kaye et al., 2005). The NLFA 16:1ω5 biomarker is found in hyphae and storage structures of AM fungi, such as spores and vesicles (Olsson, 1999). We performed separate extractions for NLFAs and PLFAs using this same methodology except for the fraction collected. Quantifying root biomass within soil cores allowed us to estimate 16:1w5 NLFA per gram oven-dry root.

2.6. Statistical methods

Measures of diversity were calculated for the vegetation and AM fungal spore communities using PC-ORD software (McCune and Mefford, 1999). Regression analysis was performed to assess how herbaceous and litter cover, plant frequency, and AM fungal spore abundance varied with tree densities. Because no significant differences were found between the 'burn' and 'no burn' treatments in any of the treatments, each replicate split-plot burn and no burn result was combined to analyze plant, AM fungal spore, and microbial (PLFA biomarkers) community composition among stand density treatments using MRPP in PC-ORD. 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). The "*A*" statistic is a descriptor of within-group similarity compared to random expectation. An *A* value greater than 0.1 is a strong indicator of a difference among groups (McCune and Grace, 2002). We visualized differences in communities among treatments using NMS ordinations in PC-ORD. The axes have no units, but show how similar or dissimilar each community is in environmental space (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 (Petrondas and Gabriel, 1983). The pairwise comparison procedure was performed using Microsoft Excel macros (available from senior author) following the methodology of Mielke and Berry (2001). If MRPP was significant, we then used the 'Sum-*F*' function (a multivariate permutation test that produces an overall sum and individual *F*-statistic for each species) in PC-ORD on variables to determine the AM fungal species and PLFA biomarkers that are likely driving these community differences (Warton and Hudson 2004).

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 × burn interaction as factors. For the soil variables, we used replicate plot values from the three sample periods. The ANOVA for percent AM fungal colonization of two grass species in the container study was performed using log transformed data. Spore numbers and 16:1w5 NLFA per g root by grass species in relation to tree densities was performed using regression analysis. All regression and ANOVA statistical analyses were performed using SAS/STAT® software (SAS Institute Inc., 2011). All statistical analyses were conducted at the $\alpha = 0.05$ significance level.

3. Results

3.1. Vegetation and microbial communities in field plots

In support of our first hypothesis, herbaceous plant cover was higher on clear-cuts than on any other treatment, whereas species richness was higher on both the clear-cut and low density treatments compared to the medium and high density treatments (Table 1). Herbaceous plant cover increased as tree density decreased ($R^2 = 0.75$, $p < 0.001$, $n = 12$). The clear-cut plots had the greatest richness of herbaceous plant species (30 species) compared to low density (18), medium density (11), and high density (2) treatments. Over 90% of the total plant cover in clear-cut and low tree density plots was composed of three strongly mycorrhizal grass species (Fig. 1): *F. arizonica*, *M. montana*, and *Elymus elymoides* (Rowe et al., 2007; Owen et al., 2013). Sedges (*Carex* spp.), which are generally non-mycorrhizal or only facultatively mycotrophic (Muthukumar et al., 2004), were the only herbaceous species in the high density plots (Fig. 1). Plant community composition was significantly different on the high stand density treatment from all other treatments ($A = 0.156$, $p < 0.001$; Fig. 2a). The Sum-*F* for plant species counts cover per treatment was significant ($F = 56.48$, $p = 0.038$), and Wright's deervetch (*Lotus wrightii (A. Gray) Greene)* ($F = 9.2$), *F. arizonica* ($F = 5.8$), *E. elymoides* ($F = 4.6$), and a *Carex* sp. ($F = 3.7$) were the strongest drivers of the observed difference.

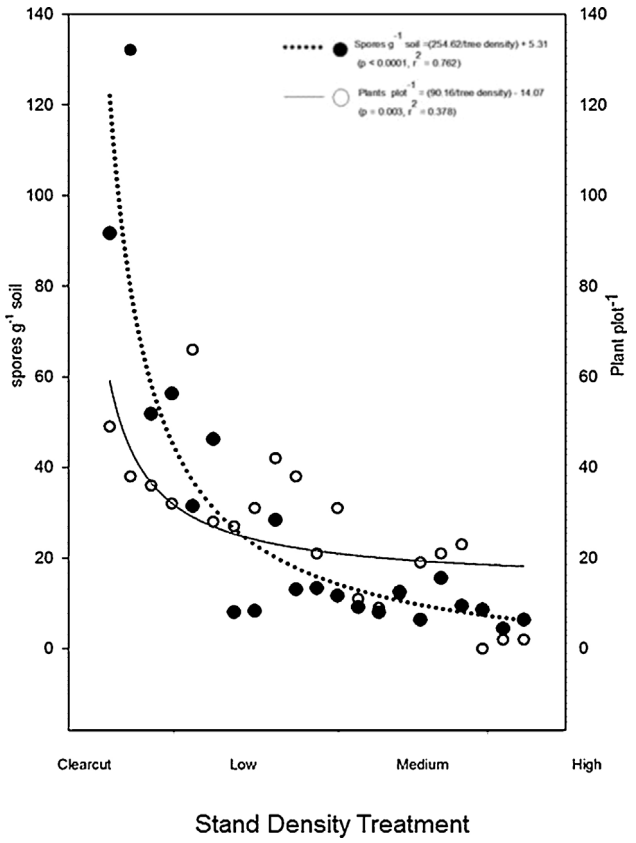

Fig. 3. Field (pre-bioassay) mean AM fungal spore abundance (black circles) and plant cover (white circles), by plot, on four *P. ponderosa* stand density treatments in Taylor Woods, AZ. Regression analyses performed using an inverse power function ($f = y0 + (a \times x^{-1})$, $\alpha = 0.05$).

As plant cover increased, so did AM spore abundance (Fig. 3). The species richness and abundance of AM fungal spores were highest in the clear-cuts compared to the other treatments; however, there were no differences in Shannon's diversity or evenness among treatments (Table 1). Low intensity burning did not affect AM fungal spores or PLFA bioindicators in any of the treatments ($p > 0.05$); therefore, the burn and no burn data were combined for each plot. Clear-cuts contained the greatest richness of AM fungal spore species (23 species) compared to low density (16), medium density (15), and high density (8) treatments. Six unique species were found in the clear-cuts, one in each of the low and medium density treatments, and no unique species were found in the high density treatment (Table 2). The most abundant spore species was *Glomus aggregatum*, which occurred in most plots. The unique species in the clear-cut treatment were *Acaulospora denticulata*, *A. mellea*, *A. undulata*, *Scutellospora calospora* and two morphologically unidentifiable *Glomus* species. The unique species in the low density treatment was a morphologically unidentifiable *Glomus* species, and *Sclerocystis microcarpus* was a species unique to the medium density plots. The clear-cut treatment had a different AM fungal spore community composition compared to all other treatments ($A = 0.165$, $p = 0.001$; Fig 2b). The Sum-*F* for AM fungal spore species counts per treatment was significant ($F = 66.29$, $p = 0.005$), and results indicated that *G. aggregatum* ($F = 12.5$), *Rhizophagus fasciculatus* ($F = 5.67$), *Scutellospora pellucida* ($F = 3.9$), and *A. mellea* ($F = 2.55$) were the most significant species driving the observed difference.

In contrast to our first hypothesis, PLFA profiles did not differ among stand density treatments. Also, we did not find any differences in individual PLFA marker community composition for unburned treatments among stand density treatments ($p = 0.101$), or for burned treatments among stand density treatments ($p = 0.661$).

3.2. Soil and litter characteristics

In support of our second hypothesis, litter mass and C concentration were lower with reduced stand density, but in contrast to this hypothesis, mineral soil total C, N, and P concentrations were not different among stand density treatments (Table 3). Litter cover ($R^2 = 0.587$, $p < 0.001$, $n = 12$) and mass (Table 3) were lowest on the clear-cut treatment and comparable among the remaining treatments. Total C and N concentrations of the O horizon for the clear-cut and low density treatments were significantly lower than the high density treatment, whereas total P was different between the medium density treatment and the high density treatment (Table 3). In contrast to our third

Table 2
List of arbuscular mycorrhizal spore species in intact mineral soil cores (0–15 cm) for each *P. ponderosa* stand density treatment in Taylor Woods, AZ.

Stand density treatment			
Clear-cut	Low	Medium	High
Acaulospora denticulata			
Acaulospora laevis	*Acaulospora laevis*		
	Ambispora leptoticha	*Ambispora leptoticha*	
Acaulopsora mellea			
Acaulospora undulata			
Archeaospora trappei	*Archeaospora trappei*	*Archeaospora trappei*	*Archeaospora trappei*
Claroideoglomus etunicatum			
Funneliformis constrictum		*Funneliformis constrictum*	
Funneliformis mosseae	*Funneliformis mosseae*	*Funneliformis mosseae*	*Funneliformis mosseae*
Glomus aggregatum	*Glomus aggregatum*	*Glomus aggregatum*	*Glomus aggregatum*
Glomus ambisporum	*Glomus ambisporum*	*Glomus ambisporum*	
Glomus cerebriforme		*Glomus cerebriforme*	
Glomus heterosporum	*Glomus heterosporum*	*Glomus heterosporum*	
Glomus invermaium	*Glomus invermaium*		
Glomus microaggregatum	*Glomus microaggregatum*	*Glomus microaggregatum*	*Glomus microaggregatum*
Glomus microcarpum	*Glomus microcarpum*	*Glomus microcarpum*	*Glomus microcarpum*
Paraglomus occultum	*Paraglomus occultum*	*Paraglomus occulatum*	*Paraglomus occulatum*
Rhizophagus clarus	*Rhizophagus clarus*	*Rhizophagus clarus*	
Rhizophagus fasciculatus	*Rhizophagus fasciculatus*	*Rhizophagus fasciculatus*	*Rhizophagus fasciculatus*
Rhizophagus intraradices	*Rhizophagus intraradices*	*Rhizophagus intraradices*	*Rhizophagus intraradices*
		Sclerocystis microcarpus	
Scutellospora pellucida	*Scutellospora pellucida*		
Scutellospora calospora			
Unknown Glomus sp. *#1*			
Unknown Glomus sp. *#2*			
	Unknown Glomus sp. *#3*		

Table 3
Mean (standard error) total nutrient concentration for the organic horizon and mineral soil (0–5 cm) in burned and unburned plots by stand density treatment. Mean values with different letters designate significant differences ($\alpha = 0.05$) among treatments using Tukey's HSD mean separation test. No letters in a row designates no significant difference among treatments.

Stand density treatment							
	Clear cut		Low		Medium		High
O-horizon	No Burn	Burn	No Burn	Burn	No Burn	Burn	
Litter mass (Mg ha^{-1})	19.6b (4.0)	19.6b (3.1)	37.8a (6.9)	47.1a (4.9)	46.6a (6.4)	49.7a (8.5)	44.8a (9.9)
Total C (%)	30.48b (3.32)	26.76b (3.56)	26.94b (1.82)	32.02b (2.83)	31.52ab (1.99)	33.35ab (2.14)	39.65a (1.17)
Total N (%)	0.85b (0.06)	0.74b (0.07)	0.80b (0.02)	0.88b (0.04)	0.90ab (0.05)	0.96ab (0.07)	1.09a (0.08)
Total P (%)	0.10ab (0.02)	0.10ab (0.02)	0.09ab (0.02)	0.10ab (0.01)	0.13a (0.01)	0.11a (0.01)	0.07b (0.01)
Mineral soil (0–5 cm)							
pH	5.30a (0.1)	5.29a (0.02)	5.10a (0.19)	5.01ab (0.07)	4.92b (0.08)	5.11a (0.06)	5.0ab (0.05)
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 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)

hypothesis, low intensity fire did not influence litter mass or pH; however, as expected, it did not influence total concentrations of C, N, or P in either the O horizon or mineral soil (Table 3). No interactions between stand density and fire treatments occurred. The prescribed fire, as applied in this study, was observed to be of very low intensity and is reflected in the above results.

3.3. Container study of native grasses and soil microbial communities

In contrast to our first hypothesis, mycorrhizal colonization of the two bioassay grasses were similar, and AM spore communities and PLFA/NLFA profiles did not differ when grasses were grown in containers with soil from the clear-cut treatment compared to the high density treatment. Root colonization by AM fungi was similar between grass species ($p = 0.604$) and between burn treatments ($p = 0.917$), but there was a strong trend for higher colonization in plants grown in soils from clear-cut and low density treatments ($p = 0.059$; Fig. 4). There were no significant interactions among stand density by burn treatments ($p = 0.942$), stand density by grass species ($p = 0.233$), or tree density by burn treatment by grass species ($p = 0.670$). The root and shoot biomass of container-grown *F. arizonica* was greater than *M. wrightii* (Table 4), but there was no significant difference among stand density treatments within the same grass species (*F. arizonica*: $p = 0.270$, *M. wrightii*: $p = 0.101$; data not shown). Furthermore, shoot:root biomass ratio was not significantly different between the two grass species (Table 4). The NLFA biomarker for AM fungi (16:1ω5; Olsson, 1999) was significantly higher ($p < 0.0001$) in soils planted with *F. arizonica* compared to *M. wrightii*, regardless of stand density or burning treatments, and was higher when *F. arizonica* was grown in soil from the clear-cut treatment compared to other treatments (Fig. 5).

The fourth hypothesis that the interaction between host species and stand density will result in different microbial communities was not supported as the final container study cores included similar biomarker values within grass species across stand density treatments (*F. arizonica*: $p = 0.2695$; *M. wrightii*: $p = 0.1007$). Host species by itself was significant (MRPP; $p < 0.0001$, $A = 0.208$) with concentrations of bacterial biomarkers showing higher *F*-ratio values compared to fungal biomarkers using the Sum-*F* statistical procedure (Table 5).

4. Discussion

An assumption often associated with restoration treatments of *P. ponderosa* forests of the southwestern U.S. is that reductions in stand densities will result in environmental conditions that more closely resemble pre-Euro-American settlement conditions; therefore other biological structures within the ecosystem such as herbaceous and microbial communities will also be restored. The bulk of studies to date relate short-term responses of these biological components to restoration treatments. The southwestern U.S has great climatic variability that often makes short-term responses transient in nature. Taylor Woods offered a unique situation in which the original thinning treatments were performed forty years ago and the gradient of density treatments has been maintained over the forty years since initial treatment.

Our field measurements support the first hypothesis that the abundance and species richness of herbaceous plants and AM fungi

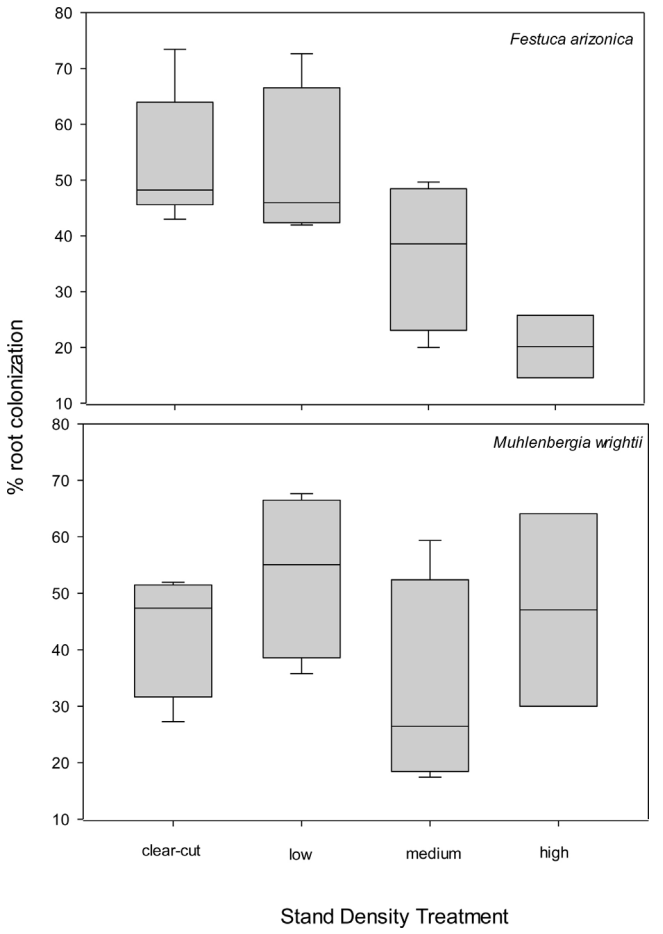

Fig. 4. Arbuscular mycorrhizal fungi root colonization (%) of two grass species (*Festuca arizonica* and *Muhlenbergia wrightii*) grown in environmental chambers. Plants established in containers with intact soil cores on four *P. ponderosa* stand density treatments in Taylor Woods, AZ.

Table 4
Mean dry weight (standard error) of shoots and roots of two native grasses grown for 8 weeks in containers of intact soil cores in environmental chambers. Due to no significant stand density treatment (shoot biomass $p = 0.611$; root biomass $p = 0.096$) or burn (shoot biomass $p = 0.671$; root biomass $p = 0.803$) effect, data were pooled for each species.

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

Table 5
Univariate *F*-ratio values for soil phospholipid fatty acid (PLFA) concentrations using Sum-*F* statistical procedure. The values contrast between *Festuca arizonica* (Fear) and *Muhlenbergia wrightii* (Muwr) grown for 8 weeks in containers with intact soil cores collected at Taylor Woods, AZ.

Phospholipid fatty acid	*F*-ratio	Indicator type
i-15:0	262.5600	Bacteria
a-15:0	9.2021	Bacteria
15:0	47.08100	Bacteria
i-16:0	108.9500	Bacteria
10me16:0	96.9220	Bacteria
16:1w9	8.3051	Bacteria
16:1w5	59.5090	Bacteria
17:0	59.5090	Bacteria
cy17:0	23.4480	Bacteria
C18:2n6t	0.8522	Fungi
C18:2n6c	0.2786	Fungi

are inversely related to tree density. The creation and maintenance of openings in overstory canopies of *P. ponderosa* provide opportunities for establishment of herbaceous plants. Many of the herbaceous plants we found in the clear-cuts are obligatory mycotrophic while the highest density plots had only weakly or non-mycotrophic plant species. Both understory plants and AM fungi community composition differed between the clear-cut treatments and the high density treatments. Host plant species composition can influence AM composition (Eom et al., 2000), and different AM species influence host plant success differently (Klironomos 2003). Specific AM species may be lost with high overstory densities of non-AM host species and this could influence understory plant response (Stampe and Daehler 2003). These results support the hypothesis of feedback mechanisms between the greater herbaceous plant cover and the abundance and richness of AM fungi in the clear-cuts compared to the higher density treatments. In contrast to our first hypothesis, PLFA microbial biomass did not differ among tree densities. Gundale et al. (2005) did not observe changes in the PLFA profile with thinning alone in a *P. ponderosa* stand in Montana, although they did observe differences post thinning and burning combined.

Our results partially supported the second hypothesis that long-term density reductions in *P. ponderosa* stands will reduce litter mass and litter C concentration, but increase available N and P. As predicted, thinning reduced the litter mass and C concentration of the O-horizon. The clear-cut treatments also had lower total N and increased total P concentration by mass in the O-horizon compared to the high density treatments, yet we did not observe nutrient differences in the mineral soil. The total nutrient pools in the surface mineral soil are resistant to long-term changes in quantity and quality of plant inputs (Johnson and Curtis, 2001) and alteration of abiotic conditions (Hart et al., 2006; Simonin et al., 2007). Total nutrient pools in surface mineral soils of *P. ponderosa* of the Southwest reflect both past abiotic and biotic conditions.

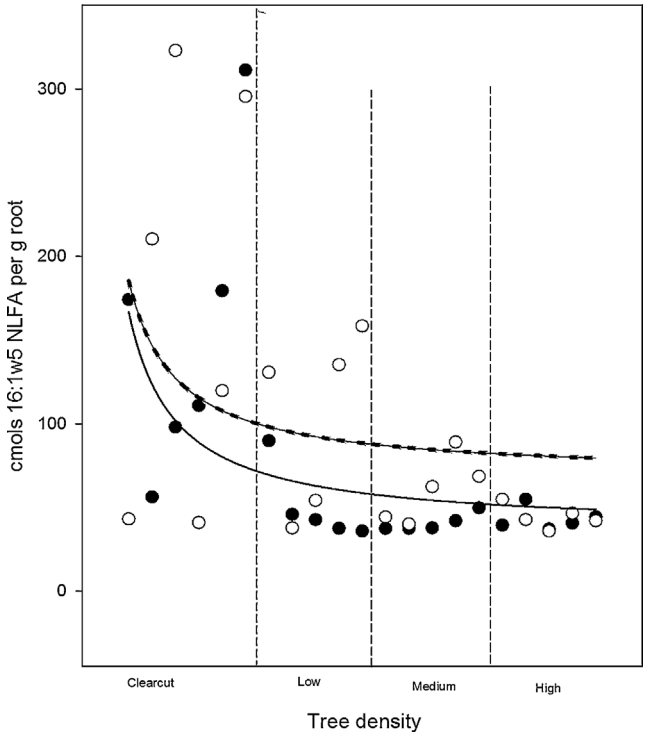

Fig. 5. Plot level mean concentration of 16:1w5 NLFA per g root from containers with *Festuca arizonica* (white circles, $p = 0.032$) and *Muhlenbergia wrightii* (black circles, $p = 0.170$) grown in soil collected in four *P. ponderosa* stand density treatments in Taylor Woods, AZ. Regression analyses performed using an inverse power function ($f = y0 + (a \times x^{-1})$, $\alpha = 0.05$).

In support of our third hypothesis, low intensity fire did not measurably influence the litter mass, soil pH, or plant and microbial communities in our study. High soil temperatures of 100 °C or more and long duration can be fatal to most soil organisms (DeBano et al., 1998). Intense fires have been shown to reduce populations of soil microbes (Deka and Mishra, 1983; Pattison et al., 1999), yet a recent meta-analysis of fire effects on microbial biomass showed prescribed fires did not significantly impact soil microbial populations (Dooley and Treseder, 2012). Low severity fires can decrease AM fungi propagules in surface soils (Klopatek et al., 1988; Pattison et al., 1999), yet other studies have shown that low severity fires have little impact on AM propagules (Korb et al., 2004; Haskins and Gehring 2004) with recovery to pre-fire quantities or greater occurring within one year (Dhillion et al., 1988). As expected, the fire intensity on our plots was likely too low to have an influence; also, any ephemeral effects of burning would not be observed by our study 3–4 years after the fire treatment.

The container study partially supported our fourth hypothesis that different heterotrophic soil organisms develop from the interaction of stand densities and host plant. The soil heterotrophic microbial community associated with *F. arizonica* had greater bacterial populations than soil cores with *M. wrightii*, yet had similar fungal populations. Molecular techniques have shown there is a degree of specificity in the rhizosphere bacterial communities for different plant species, even though this rhizosphere community is derived from a common microbial community (Hawkes et al., 2007). In our study there was a common soil bacterial community, yet there was a difference as AM fungi richness and abundance increased with decreasing canopy cover. The increases in bacterial populations associated with *F. arizonica*, a C3 plant, are coincident with greater plant vigor we measured compared to *M. wrightii*, a C4 plant, but were not different with decreases in canopy density and the associated increased AM fungi abundance and richness. Root exudation of photosynthate into surrounding soil varies with plant species, and the quality and quantity of this input could be a determining factor

in the composition of the proximate soil microbial community (Reynolds et al., 2003; Hawkes et al., 2007).

5. Conclusions

The changes in the vegetation in the clear-cuts we observed were accompanied by increased abundance and diversity of AM fungal species. The understory community is able to recover from past management practices that promoted overly dense forests by thinning treatments that reduce stand density and creating openings within the overstory canopy. Others have found mechanical thinning of *P. ponderosa* trees may lead to increased production of native grasses and other herbaceous plants depending on the reduction in stand density and the spatial arrangement of the thinning (Laughlin et al., 2008; Sabo et al., 2009). Clear cutting eliminates tree competition for soil resources, decreases litter cover, and increases insolation which facilitates herbaceous plant establishment (Baumeister and Callaway 2006; Laughlin et al., 2008). Our study demonstrates that understory plants, and AM fungal and soil microbial communities are resilient to reductions in stand densities maintained over time and low intensity prescribed fire.

Acknowledgements

This work was funded by Joint Fire Sciences Program (99-01-3-13), National Fire Plan, and the National Science Foundation (DEB-0842327) to NCJ. The authors wish to thank Dana Erikson, Lauren Hertz, and Laura Levy for sampling and laboratory assistance.

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