


# Mycorrhizal propagule densities and soil properties across varying burn severities, three years post-fire, in warm/dry mixed conifer forest in the 416 Fire, southwest Colorado, USA

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**Introduction**

Montane forests of the arid southwestern United States, dominated by ponderosa pine and warm/dry mixed conifer, historically experienced frequent, low severity fire, which regulated forest composition and function. Since the introduction of grazing, fire suppression, and logging with Euro-American settlement of the western United States in the late 1800s, fire frequency, behavior, and intensity have been altered and continue to change with a warming climate (Battaglia et al., 2018). Historically, warm/dry mixed conifer forests were dominated by mixed stands of large, old growth ponderosa pine (*Pinus ponderosa)* and Douglas-fir (*Psuedotsoga menzeisii)* (Cooper, 1960), as well as stable stands of quaking aspen (*Populus tremuloides*) (Fulé et al., 2009), with a robust and diverse understory (Korb et al. 2007). Historic fire regimes consisted of frequent, low-to-moderate intensity surface fires concentrated in the understory with occasional crown fires (Grissino-Mayer et al., 2003; Romme et al., 2009; Swetnam et al., 1996). This disturbance regime allowed for an open and diverse stand structure and canopy with spatial heterogeneity and ample sunlight for shade-intolerant ponderosa pine seedlings (Moore et al., 2004). Anthropogenic processes involved in settlement of the southwestern United States have led to landscape scale change in modern warm/dry mixed conifer forests creating more homogenous stands as well as a thicker, more shade tolerant understory and an accumulation of fuels (Evans et al. 2011; Fulé et al., 1997; White and Vankat, 1993). Due to a recent shift in modern forest management practices, including thinning and prescribed burns following a long, largely fire-free period across the southwest, the ecological effects of wildland forest fires on forest ecosystems, especially soil health, is an area of great interest (Odion et al., 2014). Understanding how fire interacts with forest soils is a key indicator of the potential benefits of re-instating a more "natural" fire regime through future forest management. Further knowledge of fire/soil interactions will be an important tool in understanding the effects of a more fire-prone, climate-changed future.

While the aboveground effects of forest fire have been studied at length, the effects of fire on the rhizosphere have received much less attention (Dove & Hart, 2017; Neary et al., 1999). Understanding how fire affects the soil chemical characteristics, in terms of nutrient availability, within forest soils can help to explain aboveground vegetation responses following forest fire. Soil chemical and biological properties can be greatly affected by soil temperature changes associated with wildland fire. Concentrations of chemical nutrients and organic matter (measured as concentration of carbon) are directly and indirectly related to various chemical processes imperative to healthy forest ecosystems including decomposition, nutrient cycling, and base saturation, and have shown varying responses to differing levels of burn severity (Certini, 2005; Knoepp et al., 2005). Organic matter (OM), carbon (C), nitrogen (N), phosphorus (P), sulfur (S), cations like potassium (K) and ammonium (NH4), and pH are commonly affected by fire and are therefore key indicators of soil responses to heating. Generally, fire decreases the total amount of chemical elements within soil by allowing for nutrient loss through direct gaseous volatilization of chemicals, particulates lost in smoke, and nutrients remaining in ash subsequently lost to erosion and leaching (DeBell & Ralston 1970; Raison et al., 1985 in Knoepp et al., 2005). These fire-induced mechanisms of nutrient loss are potentially detrimental to plant life when concentrations of limiting nutrients like nitrogen and phosphorus, necessary for plant growth, are lowered by soil heating when their relatively low threshold temperatures of 200° C and 774° C , respectively, are achieved (Raison et al., 1985 in Knoepp et al., 2005). Although concentrations of chemical nutrients may decrease post-fire, it is important to note that the change in form of different elements caused by fire can, in many cases, make them more available for uptake by plants and other biological organisms, resulting in short-term increased site productivity (Baird et al., 1999).

The organic soil horizon, where plant roots exist, is typically consumed when soil temperatures exceed 300° C (Neary et al.,1999). Wildland fire consumes aboveground potential organic matter including logs, leaves, and litter as well as belowground OM within the organic matrix, increasing with fire severity. As with certain nutrients, fire can also make OM more readily available to plants by facilitating instantaneous decomposition of organic materials and greatly increasing the rate at which nutrients are released for uptake by trees and plants (St. John & Rundel, 1976).

Among changes in the soil associated with wildland fire are those that occur in the soil's fungal communities including mycorrhizae. Mycorrhiza is a, "mutualistic relationship between plant and fungus localized in a root or root-like structure in which energy moves primarily from plant to fungus and inorganic resources move from fungus to plant" (Allen, 1991). Mycorrhizae act as a bridge between plants and soil and are a key fixture of any healthy forest ecosystem. Mycorrhizae play a critical role in many soil processes such as mediating nutrient and water uptake, protecting roots from environmental extremes and maintaining soil structure (Amaranthus, 1998).

There are two main types of mycorrhizal interactions, endomycorrhizal (arbuscular mycorrhizae, AM), which penetrates into root cells and ectomycorrhizae (EM), which exist outside plant roots and modify root color, shape, and function. At least 95% of plant families are known to form mycorrhizal relationships (Trappe, 1977). The Pinaceae family, a dominant species within warm/dry mixed conifer forests to whom both ponderosa pine and Douglas-fir belong, is known to associate with EM (Harley & Smith, 1983). The majority of herbaceous plants that dominate the understory in warm/dry mixed conifer forests associate with AM (Smith et al., 1998). Both AM and EM mycorrhizae can be measured in propagules, an average count of colony forming spores (Haskins & Gehring, 2004). The presence of mycorrhizae is thought to increase resilience within forests after fire disturbance by facilitating post-fire successional growth (Dove & Hart, 2017; Korb et al., 2004). The response of AM and EM mycorrhizae to changes in the environment can be assessed by measuring changes in the number of mycorrhizal propagules. Researchers have suggested a relationship between disturbance recovery time and abundance of infective propagules of mycorrhizal fungi within disturbed ecosystems (Allen & Allen, 1980 in Korb et al., 2003).

The effects of fire on mycorrhizal interactions include immediate physical and chemical changes in soil properties following fire as well as long term effects on fungal community composition caused by changes in host plants' distribution and abundance (Owen et al., 2019). Other factors that play into how fire interacts with soil include fire intensity, duration, and timing, as well as soil type, depth, and moisture content (DeBano et al., 1998; Klopatek, 1988). Short term heat transfer resulting from forest fire combustion directly affects soil biota, resulting in fungal mortality when soil reaches 80° C in dry conditions and 60 ° C in moist conditions (Dunn et al., 1985). Organisms that survive fire experience altered activity and reproduction due to soil heating (Hart et al., 2005). Numerous studies have been conducted in different coniferous forests in post-fire conditions throughout the western U.S. with different experimental designs, study sites, and fire conditions. While multiple studies observed significantly higher infective propagules of AM fungi in post-fire conditions (Korb et al., 2001; 2003), other studies indicated that high-severity fire conditions, typical of mixed-severity wild fire and slash-pile burn scars, significantly reduced AM and EM density compared to lower severity and unburned areas (Korb et al., 2004; Owen et al., 2019; Mott et al., 2021). Studies that investigated species composition, diversity, and richness, as well as mycorrhizal abundance, found that species richness and diversity of both AM and EM tended to drop post-fire while propagule density showed no significant difference among differing burn treatments and severities (Dove & Hart, 2017; Longo et al., 2013). Understanding

mycorrhizal interactions in a specific ponderosa pine forest following a mixed severity fire can help to explain post-fire patterns in tree regeneration and successional growth.

The purpose of this study was to quantify differences in soil nutrients as well as differences in mycorrhizal propagules within a warm/dry mixed conifer forest across areas of differing levels of burn severity: low, moderate, and high, three years after the initial burn. Specifically, research focused on the presence or absence of both AM and EM. We hypothesized that areas burned at a high severity compared to low and moderate severity, which are typical of historical fire regimes, will have reduced soil nutrients as well as lower mycorrhizal propagule densities of AM and EM. Fungal communities that evolved in fire prone ecosystems like the arid Southwest are likely to regenerate more rapidly and benefit from fire more than those that evolved without fire (Remke et al., 2020). However, modern changes in warm/dry mixed conifer structure and fuel load have allowed for unprecedented fire intensity that may be more deadly for mycorrhizal communities than lower severity historical fire regimes. Understanding how forest soil responds to a specific fire severity, especially one occurring in modern climate conditions and burning modern accumulated fuel loads, will provide invaluable evidence indicating how future ecological interactions may proceed as the forest recovers from disturbance and management practices are implemented.

## Methods

### *Study Area*

The study site is located approximately 21 km north of Durango, Colorado in the southern portion of the San Juan National Forest adjacent to Hermosa Creek within the Hermosa Special Management Area and Hermosa Wilderness (BAER 2019). The study area ranges in elevation from 2277 m to 2470 m on steep slopes that range from 30 to 45 degrees. Average daily air temperatures range from a maximum of 26.7°C in July to a minimum of -12.2°C in January. Average annual precipitation is 58.4 cm, with the greatest amounts occurring in July and August due to summer thunderstorm activity. Precipitation from November to March is dominated by snowfall (Western Regional Climate Center, Tacoma, 1971–2000, www.wrcc.dri.edu). Forest types in the study area vary from ponderosa pine and pine oak forest to warm-dry and cool-moist mixed conifer. Aspen is present in the study area and continuous stands of aspen exists adjacent to plots, however, aspen is only a minor component of overstory trees in plots. The study area has never been logged and has a high proportion of large diameter trees for all species present with many stands having old growth characteristics (Mehl 1992). *Pinus ponderosa* (Ponderosa pine), *Pseudotsuga menzesii* (Douglas-fir), *Abies concolor* (white fir), *Abies lasiocarpa* (Sub-alpine fir), *Pinus flexilis* (Limber pine), *Picea pungens* (Blue spruce), and *Populus tremuloides* (Quaking aspen) are the common tree species. Common sprouting shrubs include *Quercus gambelii* (Gambel oak), *Symphoricarpos oreophilus* (snowberry), *Prunus virginiana* (chokecherry), and *Amalanchier alnifolia* (Utah serviceberry).

In 2008, portions of the study area were burned in a broadcast prescribed fire using aerial ignitions. Ten years later in 2018, the study area was burned by an unplanned, artificial ignition that burned a total of 54,130 acres, known as the "416 Fire" (Figure 1).The 416 Fire burned during an extreme drought year resulting in mixed soil burn severities from overall moderate fire behavior driven primarily by available fuels and topography. Suppression efforts focused on the wildland urban interface and no slurry drops or direct attack measures were taken in the study area (Communication with Incident Section Chief).

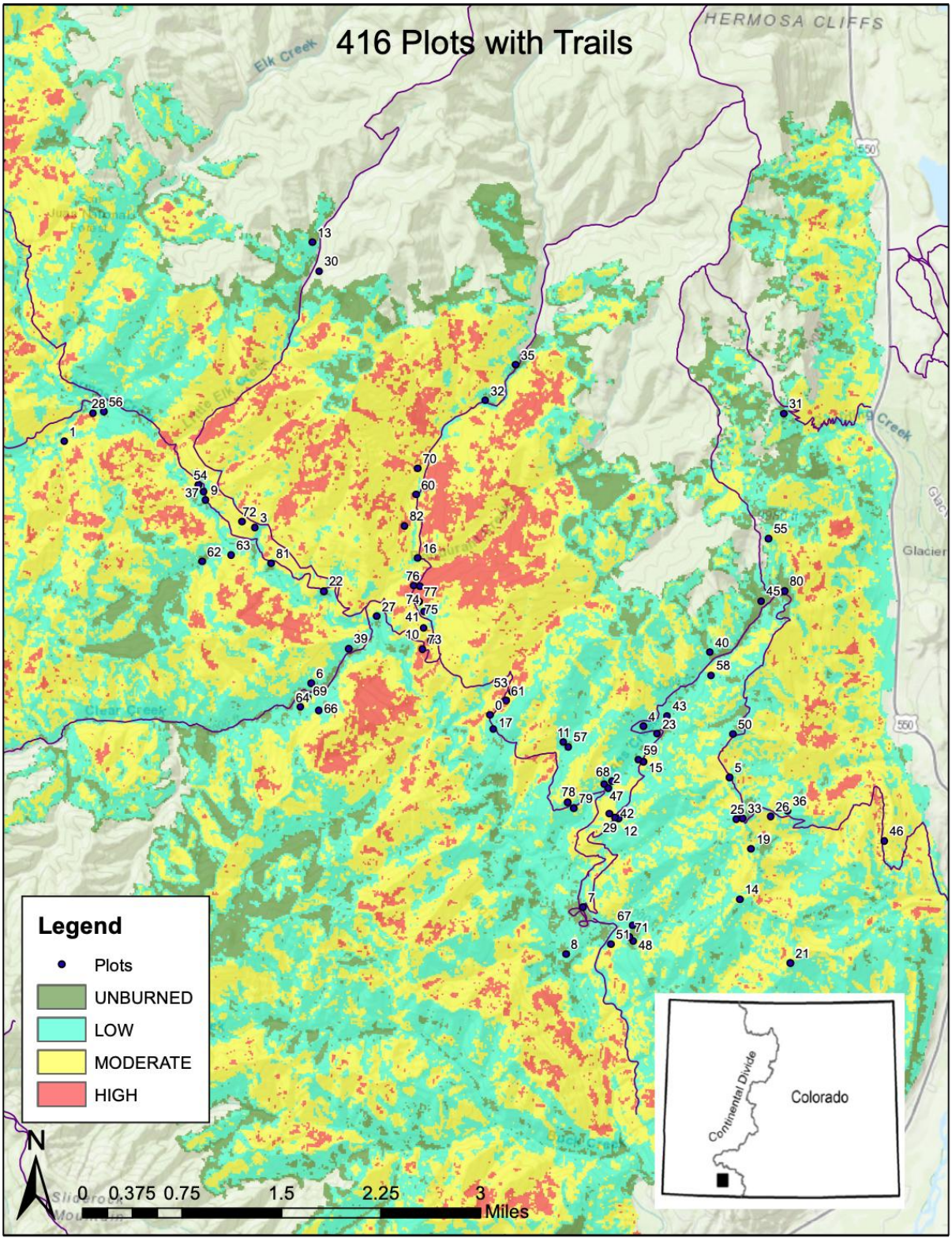


***Figure 1.*** Map of 416 fire with 30 study sites, 10 per burn severity. Sourced from MTBS, USGS and USFS.

*Experimental Design*

Random points were generated using ArcGIS with a minimum spacing distance of 100 meters and within a mile buffer of existing trails to ensure accessibility to sites given the steep slopes of the drainage. These points were stratified across soil burn severities as determined by the Burn Area Emergency Response Team (BAER 2019). Burn severities across the entire fire in percent include: low (54%), moderate (30%), high (3%) and unburned (13%) (BAER 2019) (Figures 1 & 2). Burn severity was derived from satellite imagery comparing pre and post-fire images and were field verified through reconnaissance and adjusted where needed to create a final soil burn severity map (BAER 2019). Once random points were established in GIS, a random number generator was used to select 10 points for each burn severity, not including unburned, to be established as "full" plots that include forest overstory, shrub, tree regeneration, herbaceous understory, and fuel measurements (Table 1).



***Figure 2.*** Low, moderate, and high severity established plots (left to right) in the San Juan National Forest. Photos taken one year post-fire by M. Remke.

***Table 1.*** Environmental Site Characteristics. Mean values (± SEM) for each burn severity are shown describing slope (degrees), aspects (degrees), elevation (m), climatic water deficit (CWD) ($mmH_2O$), and actual evapotranspiration (AET) (mm). There was no significant difference in environmental variables among burn severities.

| Severity | Slope | Aspect | Elevation | CWD | AET |
|---|---|---|---|---|---|
| Low | 22 (2.4) | 183 (34.9) | 2483 (48.5) | 475.19 (16.0) | 572 (4.7) |
| Moderate | 21 (2.7) | 197 (21.7) | 2667 (21.7) | 425.39 (17.5) | 568 (3.0) |
| High | 21 (2.4) | 249 (25.3) | 2432 (22.3) | 491.53 (11.2) | 579 (2.0) |

*Field Methods*

In the summer of 2021, using a small hand trowel, digging to a depth of 15 cm (Vadas et al., 2005), six 4 cm x 20 cm Conetainers (Stuewe and Sons Inc., Corvallis, Oregon USA) of soil were collected from 30 plots, 10 plots per burn severity of low, moderate, and high. Additionally, approximately one liter of soil was collected from each plot, dug from at least four separate locations equidistant across the sample site, and stored in plastic bags in a refrigerator.

*Laboratory Methods*

Thirty soil samples were kept cool and brought in plastic bags to the CSU Soil Lab in Fort Collins, Colorado. Soil samples were tested for pH, soil electrical conductivity, organic matter, nitrate, phosphorous, potassium, and ammonium. 90 plant bioassays of corn were planted in 4 cm X 20 cm Conetainers™ using corn (*Zea maiz*) obtained from Johnny's Seed catalog (Stuewe and Sons Inc., Corvallis, Oregon USA). One Conetainer per plot as well as two backups for each plot were planted with multiple seeds per Conetainer, and thinned accordingly. 90 Conetainers were

planted with Ponderosa Pine, obtained from Western Native Seed, with one per plot as well as backups. The same methods of planting and thinning were used. The plants were grown in Durango, CO in the Fort Lewis College Greenhouse. Bioassays were watered every 2 days after initial planting with adjustments made to maintain soil moisture and accommodate varying water requirements of different species. After approximately 6 weeks, the roots and stalks of the corn were measured and weighed. The roots were washed of dirt, and cut into 2.5 cm sections, then placed in small, covered plastic trays. They were boiled for 3-5 minutes in a 1 Molar solution of Potassium Hydroxide. After being rinsed with distilled water they were boiled in a 5% ink and vinegar solution for three minutes and rinsed again. Next, stained roots sat in acidified water for 5-20 minutes to let remaining stain leach out of roots. Roots were stored in 50% ethanol/water solution between harvesting and staining. Using a compound microscope, the percentage of root length colonized by vesicular-arbuscular (VA) mycorrhizae using the magnified intersections method (McGonigle, et al. 1990). AM Mycorrhizae was classified according to structure - hyphae or vesicle (Figure 3). Pine roots were harvested after 12 weeks and directly examined using a dissecting microscope in order to quantify the proportion of root tips colonized with EM Mycorrhizae (Gehring & Whitham 1991). Root tips were classified as living or dead EM tips (Figure 4).

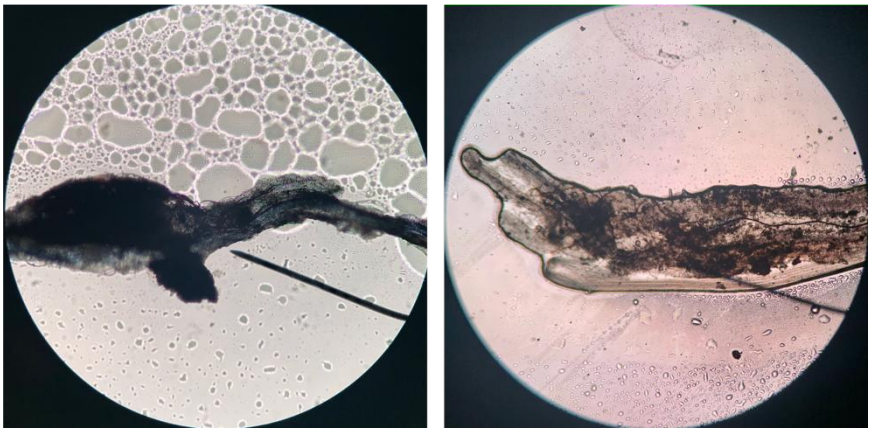

***Figure 3.*** EM Growth under magnification.



***Figure 4.*** Corn roots under 200X magnification, exhibiting stained AM structures: arbuscule (left), vesicles and hyphae (right).

**Statistical Analysis**

SPSS version 16 software was used for all analyses. Non-parametric Kruskal Wallis tests were used to quantify differences between soil conditions in the three treatments (low, moderate, high burn severity) followed by a post-hoc Bonferroni test. Pearson correlation tests were run between variables of interest (e.g., forest canopy cover) and the variable(s) arbuscular mycorrhizae and ectomycorrhizae. Pearson correlation tests were run between the variables of burn treatment, percent colonization of root, and soil nutrients and soil properties. Graphs of all the data were produced using Microsoft Excel.

**Results**

*Soil Chemical Properties*.

Soil chemical properties and nutrients showed no significant differences between burn severities (Table 2). The soil parameters tested showed very little variability. High severity plots showed the highest mean pH (6.95), followed by moderate severity (6.79), and low severity (6.42) ($P = 0.95$). Organic matter also showed little variability among burn severities ($P = 0.521$). Soil nutrient testing showed no significant difference among burn severity in any of the tested chemical nutrients including nitrate nitrogen ($P = 0.890$), phosphorous ($P = 0.384$), potassium ($P = 0.320$), and ammonium ($P = 0.384$).

***Table 2.*** Mean (± SEM) soil chemical properties by burn severity include pH, soil electrical conductivity (EC), organic matter (OM), nitrate nitrogen ($NO_3$-N), phosphorus (P), potassium (K), and ammonium ($NH_4$-N). There were no significant differences in chemical properties among differing burn severities ($P>0.05$).

| Severity | pH | EC (mmhos/cm) | OM (%) | $NO_3$-N (ppm) | P (ppm) | K (ppm) | $NH_4$-N (ppm) |
|---|---|---|---|---|---|---|---|
| **Low** | 6.42 (0.19) | 0.29 (0.06) | 6.68 (0.91) | 30.74 (17.56) | 19.60 (4.29) | 186.86 (26.6) | 3.89 (1.81) |
| **Moderate** | 6.79 (0.19) | 0.21 (0.03) | 5.69 (0.55) | 16.45 (7.01) | 12.93 (1.42) | 135.34 (16.3) | 3.63 (0.77) |
| **High** | 6.95 (0.11) | 0.29 (0.50) | 6.36 (0.60) | 12.54 (4.25) | 15.68 (2.36) | 168.13 (15.1) | 5.09 (1.14) |

*AM Root Colonization – Corn Bioassays.*

Arbuscular mycorrhizal fungi colonized roots of each plant grown in soil from all three burn severities. Errors in laboratory methods caused high severity roots to be stained too dark, making root structures imperceptible and the high severity data unusable. Arbuscular root colonization varied very little among burn severities (Figure 5). Roots grown in soil from low severity plots showed a mean percent colonization (amount of AM "hits"/ total root "hits") of 32.5% and moderate severity roots showed a slightly higher mean of 36.8 % ($P=0.165$). Structural composition of AM colonization also showed little variability among burn severities with no significant difference (Figure 6). Both severities had the highest relative abundance of internal hyphae structures with 45.8% of total AM colonization in low and 47.5% in moderate ($P = 0.481$), followed by vesicle structures with 41.6% and 40.0% of total AM colonization, respectively ($P = 0.529$). Both severities showed the lowest relative abundance of arbuscule structures with 12.6% and 13.1%, respectively ($P = 0.684$).

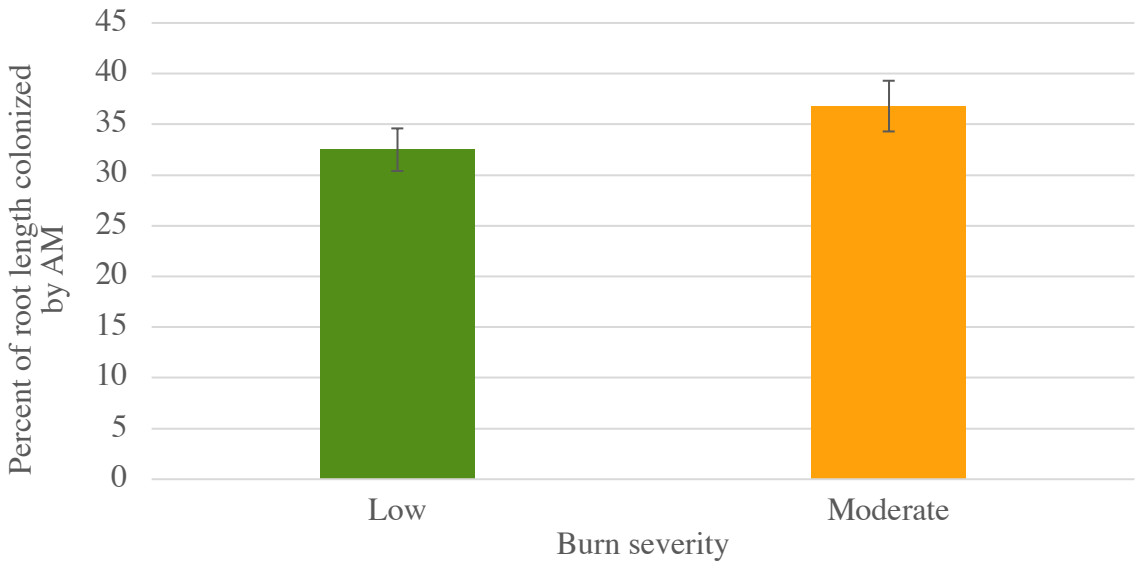


***Figure 5.*** Inoculum potential (mean percent colonization) of AM fungi from corn bioassays, grown in soil from low and moderate burn severities. No statistical difference was detected between burn severities (Mann-Whitney, P>0.05).

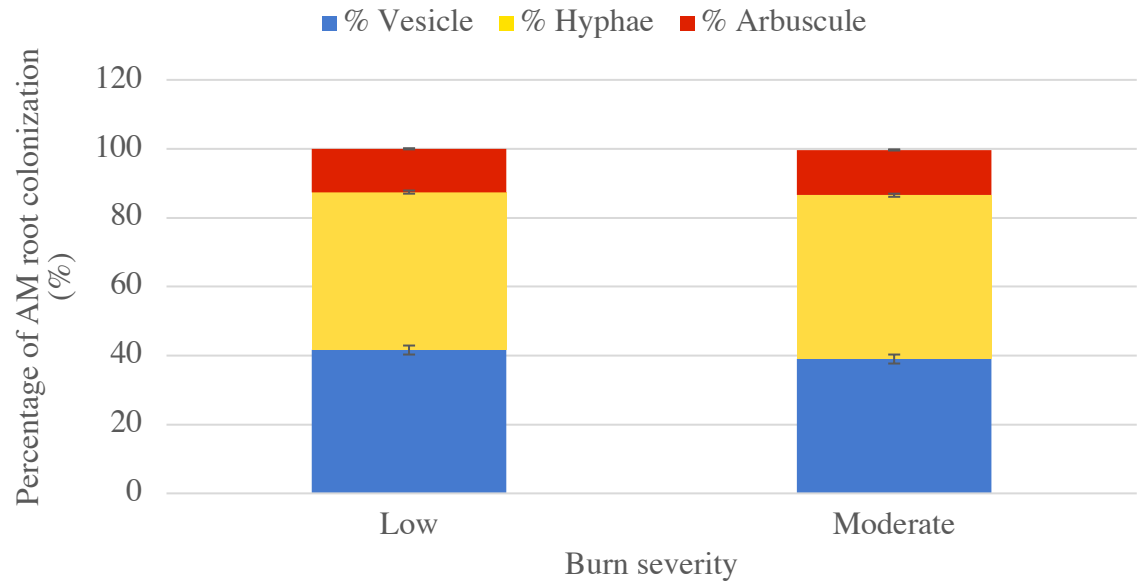


***Figure 6***. Relative percent colonization of three different AM structures: vesicle, hyphae, and arbuscule present within corn roots grown soil from low and moderate severity plots. There was no significant difference in structure between different severities (Mann-Whitney, P>0.05).

EM *Root Colonization – Ponderosa Pine Bioassays.*

Ectomycorrhizal fungi colonized each plant grown in soil collected from every burn severity. Percent root colonization by EM (amount of EM "hits"/ total "hits") of ponderosa pine roots was highest in roots grown in low severity soil (32.72%), followed by high severity (29.44%), and moderate severity (28.07%). No significant difference was found among severities (P = 0.86) (Figure 7).

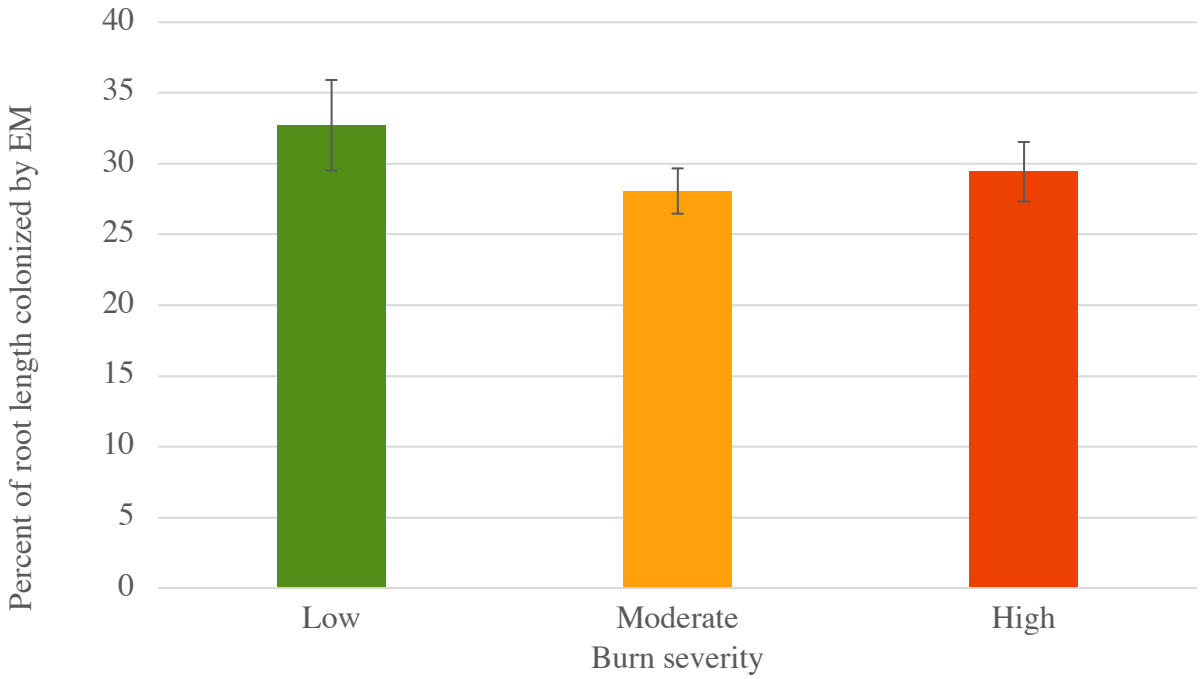


***Figure 7.*** Inoculum potential (mean percent colonization) of EM fungi from ponderosa pine bioassays, grown in soil from different burn severities. No statistical difference was detected between burn severities (Kruskal-Wallis, P>0.05).

**Discussion**

*Soil Chemical Responses to 416 Fire.*

There were no significant differences in soil chemical properties among burn severities three years post-fire, indicating no lasting effects of wildland fire on nutrient levels or soil properties. High severity fire, determined by peak temperatures and duration of fire, and characterized by greater than 75% tree mortality and significant mineral soil exposure (Figure 2), is known to have a greater impact on soil nutrients than lower severities (Certini, 2005; Neary et al., 1999). However, results in this study indicated similar, sometimes even higher levels of soil nutrients including carbon, nitrogen, and phosphorous in high severity plots compared to low and moderate severity. Across burn severities, soil chemical properties assessed three years post-fire are consistent with un-burned conditions observed in past studies conducted in the San Juan National Forest as well as other similar forest types across the Western US, which can provide insight into mixed conifer ecosystems of the San Juan (Barton & Korb, 2015; Remke et al., 2021). A long-term soil productivity study conducted in mixed-conifer forests of the Sierra Nevada, California, USA, found levels of organic matter within (5-12%) and pH within (5.8- 6.2), consistent with 416 soil data three-years post-fire (Busse et al., 2021). Additionally, pre-fire abundance of nitrogen, phosphorous, and various cations recorded in other studies in the Sierra Nevada were also comparable (Chorover et al., 1994; Hamman et al., 2008). A more recent study in conifer forests of Northern Arizona also showed comparable levels of pH, P, N, and C in unburned plots (Korb et al., 2001). It is common after fire, especially in fire-adapted warm/dry mixed conifer forests, for minerals to increase “remarkably but ephemerally” in accordance with differing levels of burn severity and resulting nutrient volatilization (Certini, 2005). Although results failed to show significant, lasting changes in nitrogen and phosphorus three years post-fire,

in-field observations of localized "remarkable" understory plant growth indicates the possibility of short-lived nutrient spikes following the 416 fire (Korb & Post, personal communication). Further research is necessary in the form of long-term soil nutrient monitoring in the 416 burn area to assess "recovery" status of soil nutrition and fully describe the impact of the 416 fire on belowground sustainability (Neary et al., 1999). Additionally, collection of data describing other physical soil properties including soil infiltration and conductivity as well as soil stability would be valuable in understanding how other abiotic environmental factors can affect plant growth and post-fire regeneration by their impact on forest soil conditions (Hart et al., 2005; Hamman et al., 2008).

*Bioassays and Root Colonization of AM/EM Following Fire*.

Each bioassay "bait plant" grown in soil from three different burn severities of the 416 fireshowed fungal colonization to varying amounts. Weights and lengths of roots were not taken into account for corn or ponderosa pine seedlings as many plants were under severe water deficit stress due to human error in watering practices and lack of proper watering systems. Differing burn severity appeared to have no lasting effects on the inoculum potential of AM or EM analyzed from bioassays grown in forest soils, with no significant differences in AM or EM root colonization found among burn severities. Aside from short-term changes in soil microbial communities caused by heat-induced mortality, fire has been known to affect soil fungal communities on a long-term scale by directly altering plant community composition which in turn affects plant/fungal relationships (Hart et al., 2005). EM diversity and abundance are especially sensitive to reductions in host plant distributions (Amaranthus, 1999; Steidinger et al., 2020). Recent studies have found that fire can further alter plant communities by accelerating climate-related range shifts, especially in mountainous areas, of certain trees (in the context of warm/dry mixed conifer forests we have seen type shifts from dominant conifer trees to shrub species like Quercus) which can lead to shifts in ecosystem scale symbioses of specific plant communities and fungal communities (AM and herbaceous understory, EM and conifer trees) (Amaranthus, 1999; Hill & Field, 2021; Remke et al., 2021). An adjacent research project conducted in the same study area of the San Juan National Forest three years post-fire, found no significant difference in live shrub density among differing burn severities (known to associate with AM) as well as only slight differences in live conifer density (known to associate with EM), with high severity plots showing slightly lower density while moderate, low, and unburned plots showed no significant difference (Post et al., 2021). Additionally, no significant differences were found in species of surviving trees among burn severities, indicating that species composition has not shifted three years post-fire. These findings may indicate the absence of a post-fire species shift as well as lessened long-term threats to fungal communities in the soils of the 416 burn area as understory and conifer communities appear to have largely returned to "unburned" conditions three years post-fire (Figures 8 & 9).

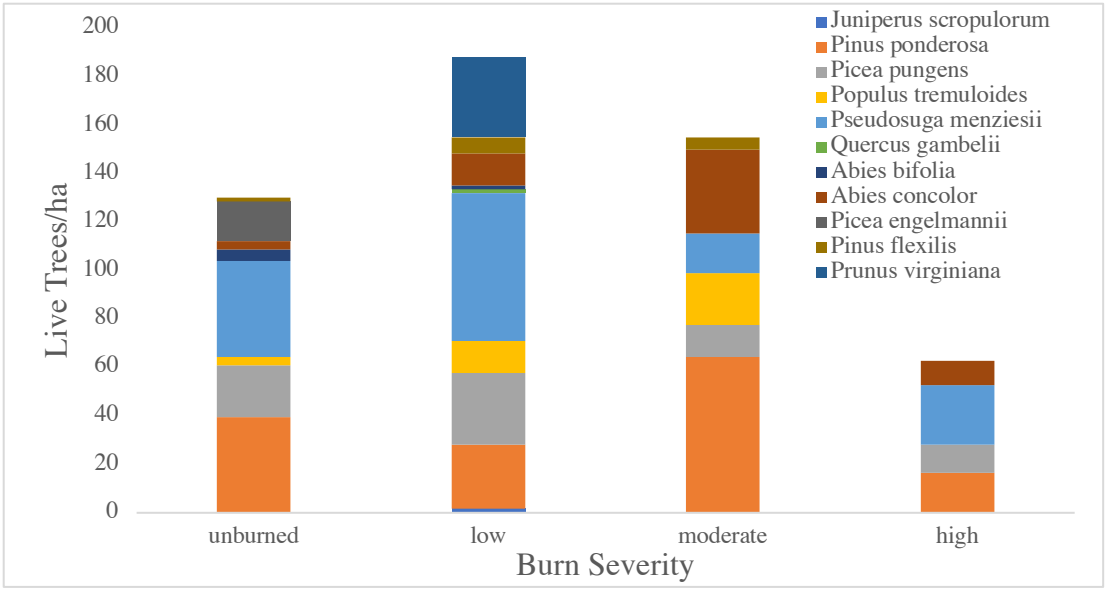


***Figure 8.*** Mean live tree density/hectare (ha) by species in the 416 burn area by burn severity. N = 15/burn severity. High severity was statistically different than unburned, low, and moderate severities in total live tree density/ha (Kruskal-Wallis, $P < 0.05$). There was no significant difference found within individual tree species/ha (Kruskal-Wallis, $P > 0.05$).

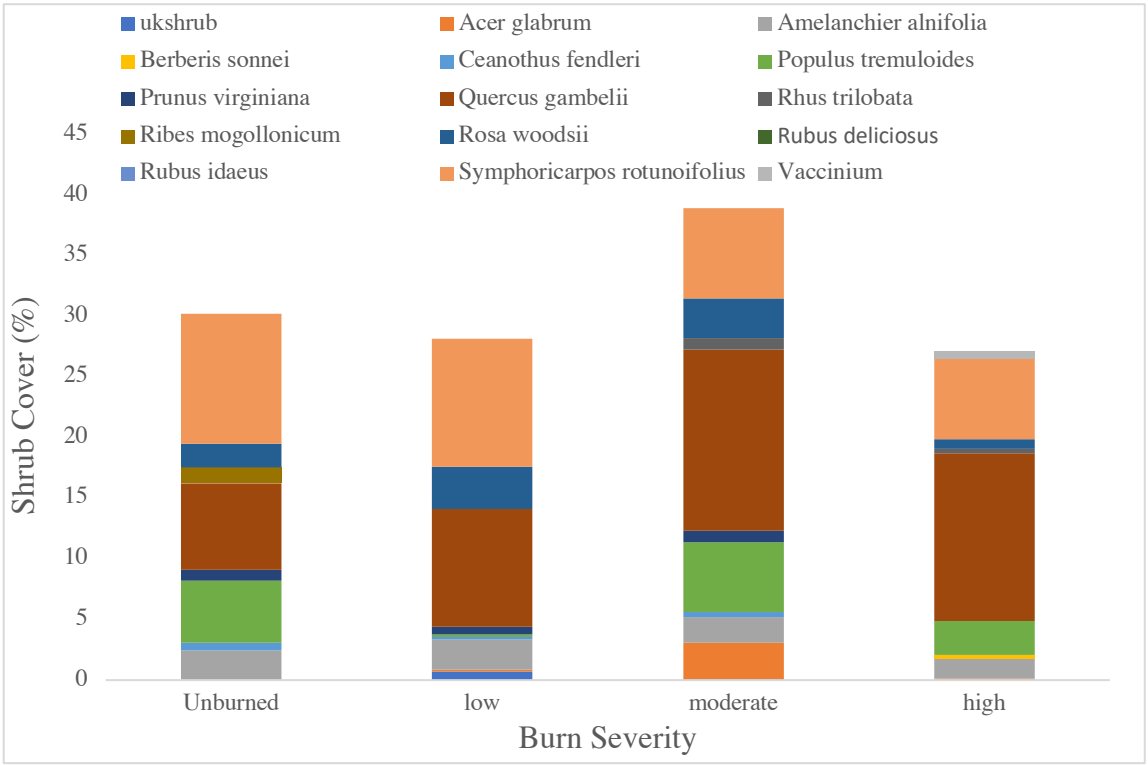


***Figure 9.*** Mean live shrub cover (%) by species ("ukshrub" refers to unknown species) in the 416 burn area by severity. N=15/burn severity. There was no significant difference among burn severities for total shrub cover (Kruskal-Wallis, $P > 0.05$).

Average percent colonization of EM ranged from 28-33% while AM percent colonization ranged from 32-37%. Both findings are similar to other studies performed post-fire in warm/dry mixed conifer forests (Barton & Korb, 2015; Korb et al., 2001, 2003; Longo et al., 2014). Results from the 416 fire, three years post-fire are consistent with "unburned" conditions found in past studies conducted in the San Juan National Forest and in similar forest types in Northern Arizona with inoculum potentials of EM in control plots ranging between 30-35% and AM ranging between 25-30% (Barton & Korb, 2015; Korb et al. 2001). Findings are inconsistent with initial hypothesis that higher severity fire would show decreased amount of mycorrhizal propagules and instead indicate fungal recovery in areas that likely experienced fungal mortality during and shortly after the 416 fire due to direct soil heating (Dunn et al., 1985; Pulido-Chaves et al., 2020). All types of soil biotic organisms are capable of full recolonization of burned areas post-fire given that land use does not change (e.g. for agricultural use) and soil stability is not lost (Certini et al., 2021). Soil biota in warm/dry mixed conifer forests have co-adapted alongside native species to a harsh, semi-arid climate with frequent disturbance and their mutual success is intimately linked (Pulido-Chavez et al., 2020; Remke et al., 2020). Given that the 416 fire exhibited similar patterns to fire regimes of the past (mixed severity, fewer high severity burn patches than other severities), fungal communities of the San Juan National Forest that were affected by the 416 fire may have achieved a state of recovery and be readily contributing to vegetative regeneration three years post-fire.

*Future Studies.*

This project encountered many obstacles and roadblocks that provided valuable insight into adjustments and optimizations for future research in this area. Long term studies in the 416 burn area would bring forth necessary information to fully understand soil responses to mixed severity fire in this specific ecosystem. Future projects working in the same study area would benefit greatly from an increase in established plots within high severity (secured through cooperation with local private land-owners in order to gain access to high severity burned areas) as well as data collection from "unburned" plots. This would result in a higher number of sampling replicates, which would allow for more accurate mean measurements as well as decreased error. Additionally, future use of local soil testing avenues would alleviate potential for project-altering human errors (lost soil samples, staffing changes, etc.) and be a much more cost efficient as well as timely method for determining soil nutrition than sending soil across state to another institution's lab. This could involve updating soil testing equipment in the labs at Fort Lewis College, as well as establishing a relationship with local soil labs such as Green Analytical Laboratories in Durango, CO, a company that is well versed in post-fire soil testing and has a larger capacity for more diverse nutrient testing. Utilizing a different lab would also present an opportunity to test more niche soil chemicals with greater volatilization rates like methylmercury.

Movement among researchers of soil fungal communities to unify sampling techniques as well as update techniques for AM root staining could provide beneficial in consolidating research findings and describing general post-fire ecologic responses of mycorrhizal communities. This would include producing further research on in-situ mycorrhizal sampling and improving accessibility of said techniques. Additionally, if future student studies also include the use of bioassays grown in field-collected soils, more time and resources should be allocated to the proper cultivation of these bioassays. Corn and ponderosa pine seedlings have different water and light preferences and should be tended to accordingly. Skilled cultivation of bioassays could provide more data relevant to soil effects on plant growth in terms of germination, growth rates, root/shoot size and weight, etc. While updates have been made to staining practices for AM measurement since the 1990s moving from methods using a majority of toxic chemicals to the utilization of pen

ink and vinegar after clearing roots with KOH, current supply chain shortages may lead to these methods becoming obsolete (Vierheilig et al., 2000). Future innovation of new, safer and more accessible methods of root staining and subsequent objective characterization of root colonization is necessary for the continued development of this field of study.

*Management Implications.*

Although soil chemical properties and mycorrhizal colonization rates observed in this study have shown similarities to unburned conditions, indicating a possible state of full recovery in forest soils three years after the 416 fire, other variables affecting conifer regeneration such as climate water deficit (CWD), and temperature as a driver of drought (with environmental warming amplifying water limitation) should be studied further (Korb et al., 2019; Odion et al., 2015; Williams et al., 2012). Multiple studies conducted in regenerating warm/dry mixed conifer forests have found that actual evapotranspiration rates and climatic water deficit are strong indicators of climatic restraints on seedlings (Dobrowski et al, 2015; Puhlick et al., 2012; Rodman et al., 2020; Tepley et al., 2017). It is important to note as well that although the 416 fire resulted in less high severity fire than has become typical of modern wildland fire (burning modern accumulated fuel loads), much of the existing literature indicates that higher severity fire can be more detrimental to various biotic and abiotic factors of forest ecosystems than low and moderate severity (Korb et al., 2004). It is thus imperative that future management practices seek to limit high severity burns by managing fuel loads through prescribed fire and taking other measures to encourage forest regeneration in an increasingly dryer and warmer climate instead of attempting to restore warm/dry mixed conifer forests to pre Euro-American settlement conditions (Stoddard et al., 2015). Efforts like those of US Forest Service to replant ponderosa pine seedling in areas where live seed trees are no longer present may need to be expanded throughout multiple burn severities as natural regeneration of conifer seedlings remains low throughout the burn area (Post et al., 2021; MSI, personal communication). Findings from this study provide hope for efforts like these to regenerate ponderosa pine and other dominant conifer species as presence of EM has been indicated and its symbiotic associations will no doubt prove beneficial for a regenerating forest.

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