

**Project Title:** Effect of mechanical mastication followed by prescribed fire on mycorrhizas and hypogeous fungi in mixed hardwood chaparral

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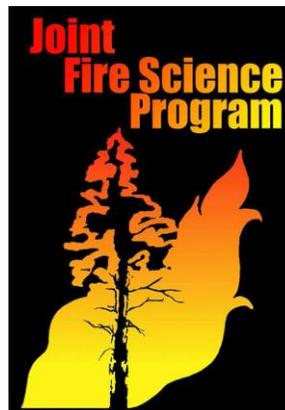
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## **ABSTRACT**

The purpose of this study was to examine the effects of mechanical mastication followed by prescribed fire on mycorrhizal fungi and hypogeous sporocarps. A dense fire-prone mixed hardwood-conifer chaparral comprises a significant component of vegetation at Whiskeytown National Recreation Area. Prescribed burns, as fuel reduction treatments, are limited by air quality restrictions and narrow climatic conditions appropriate for burnings. Brush mastication is a fast and inexpensive tool commonly used by land managers to reduce ladder fuels. However, a dense layer of chipped debris remains, which, when burned, heats the soil more than ladder fuels. The upper layers of mineral soil contain roots of woody plants that form mycorrhizas, symbiotic associations of fungi and roots. The effects of the thick layer of slash created from brush mastication on mycorrhizas are unknown. Changes may occur in the community of mycorrhizal fungi, and in the fungal sporocarps produced belowground. Treatments included mechanical mastication, mechanical mastication followed by prescribed fire, prescribed fire only, and untreated controls.

For mycorrhizas, soil samples were taken at the canopy dripline of ectomycorrhizal trees. Mycorrhizas were described by morphology and by DNA sequences of the ITS region. Hypogeous fungi were sampled by scuffling. Mycorrhizal communities were highly similar in all treatments with no particular changes due to mastication and burning. However, hypogeous sporocarps were greatly reduced by burning, either of standing chaparral or masticated vegetation.

We conclude that prescribed burning of mechanically masticated slash does not harm mycorrhizal communities, but does inhibit fruiting of hypogeous fungi.

## **II. Background and purpose**

The need to reduce fire risk within the urban interface while minimizing adverse impacts to the landscape is an important challenge for land managers, especially in fire-prone areas of the western United States. With the implementation of the National Fire Plan, efforts to reduce hazardous fuels through prescribed fire and mechanical thinning have increased. Because of narrow prescription windows, air quality restrictions, and availability of resources, many management agencies have implemented a multi-faceted fuels management program that includes mechanical treatment of shrubs and small trees.

Mechanical mastication is attractive as a fire surrogate to reduce fuels because it is cost effective and efficient. Potential adverse effects of mechanical reduction and removal of brush are a concern to resource managers because the ecological consequences are unknown. The combination of ground-disturbing activities, reduced canopy cover, and increased fuels at ground level may alter the physical and biological properties of soils.

In contrast to prescribed fire that more closely parallels natural ecosystem processes, mechanical mastication does not mimic natural events. It deposits a layer of woody debris that is relatively resistant to fungal decomposition and may act as mulch that moderates soil temperatures or

decreases aeration. When burned, the masticated debris layer increases burn severity, heating the soil more than a ladder fire and causing greater alterations in ecosystem responses (Busse et al 2006, 2009).

Upper layers of soil are active sites of fine root growth including mycorrhizal hyphae that extend into soil interstices. Mycorrhizal fungi are important components of below-ground biodiversity because they associate with woody roots as normal components of soil biota and are the primary source for transfer of carbon to underground ecosystems. Communities of mycorrhizal fungi are complex; it is common to find over 40 species of fungi associated with the roots of a single tree species at one site. Some fungi form mycorrhizas with both oaks and pines creating the potential for a mycorrhizal network linking diverse species of trees. A mycorrhizal network may sustain fungi for inoculation of seedlings or it may actually transfer nutrients (carbon, nitrogen) among plants.

Among the mycorrhizal fungi associated with oaks and pines are hypogeous fungi (“truffles”) that fruit belowground in the upper few centimeters of mineral soil. These hypogeous fungi, including well known genera such as *Tuber*, develop spores underground; the sporocarps (fruiting bodies) do not open to disperse the spores. Instead, small mammals eat the sporocarps and defecate the spores. Thus hypogeous fungi provide food for small mammals that, in turn, disperse mycorrhizal inoculums for seedlings. Truffle production is highly seasonal. In oak woodlands, truffles develop in spring; in conifer forests, truffles develop in spring and late fall.

Because fire is a natural event, it is not intrinsically harmful to mycorrhizal fungi. Following fire, the mycorrhizal community may change in species richness or species composition, but it is not eliminated or replaced. However, most studies on fuel reduction methods and fire surrogate treatments have not examined the effects of mechanical mastication on mycorrhizal fungi. The increase in downed wood may alter the interaction between wood-digesting saprotrophic fungi and mycorrhizal fungi leading to changes in the mycorrhizal community. The consequences of burning of masticated slash are uncertain. If roots have formed near the surface, a hot fire that burns slowly and smolders may cause death of mycorrhizas in the upper soil layers. If roots have formed in deeper soil layers, the consequences may be less dire.

Fire leads to decreases in production of hypogeous sporocarps. Spring burning may destroy hypogeous fungi at the time of fruiting—resulting in loss of spores for mycorrhizal inoculum and loss of a food source for small mammals. Fall burning before the fruiting season would be predicted to have less effect on hypogeous fungi although it may affect fall-fruiting fungi. A fire that burns a layer of woody chips may heat soil to greater depths for longer periods of time and over a more continuous area. Fungal spores and hyphae are much less resistant to fire than are seeds.

The goal of this project was to evaluate the effect of mechanical mastication and prescribed fire on the ectomycorrhizal community and on hypogeous fungi associated with conifers and hardwoods in a mixed oak shrubland.

We tested the following hypotheses:

1. Mechanical mastication will lead to greater abundance and species richness of ectomycorrhizas and hypogeous fungal sporocarps.
2. Burning without mastication will not reduce the mycorrhizal community, but would decrease hypogeous sporocarps in abundance and species richness.
3. Mechanical mastication followed by burning will decrease the abundance and species richness of both ectomycorrhizas and hypogeous fungal sporocarps.

### **III. Study description and location**

This study was conducted in Whiskeytown National Recreation Area on the southeastern edge of the Klamath Mountains in northern California. The climate is characterized by hot dry summers and cool winters with average annual rainfall 1500 mm. The overstory tree canopy consisted of conifers and hardwoods including knobcone pine, ponderosa pine, black oak, and canyon live oak. The shrub community was dominated by white-leaf manzanita intermixed with toyon, poison oak, buckbrush, and chamise.

To evaluate the effects of fire and fire surrogate treatments on mycorrhizas and hypogeous fungi, we utilized the macroplots designed by Bradley et al. (2006). Macroplots of 0.3 – 1.0 ha were located within 1.2 km of Whiskeytown Lake at elevations of 380 to 530 m. Treatments randomly applied in each macroplot were (1) mechanical mastication only, (2) mechanical mastication followed by prescribed fire, (3) prescribed fire only, and (4) controls. Mechanical mastication treatments were implemented in November 2002 using a low ground pressure, rubber tracked ASV Posi-Track™ with industrial brush-cutter. At least 90 percent of machine operations occurred over surfaces covered with chipped wood to limit soil disturbance and compaction. The treatment thinned shrubs and small trees less than four meters in height, reducing understory density by 60 to 95 percent. No overstory trees were removed.

Spring prescribed fire treatments were conducted in May 2003 with drip torches using a combination of strip and spot ignition patterns. Total woody fuel loads averaged 31.8 Mg ha<sup>-1</sup> at a depth of 5.8 cm (Kane et al. 2009). Burn temperatures averaged hotter in masticated plots at 0.5 meter above ground level, and at the litter surface, but not at the interface of duff and soil (Kane et al 2006, 2009). Mean flame length and flame zone depth were significantly greater in masticated plots than in the nonmasticated plots Bradley et al. 2006).

In spring 2008, we sampled four treatment types (mechanical mastication, mechanical mastication followed by prescribed fire, prescribed fire only, and controls) in five macroplots (A, B, D, E, J), ten samples per treatment. One sample consisted of two combined cores taken at the dripline of a tree (oak or pine). Eight samples were used for mycorrhizas and two for soil assays. Soil samples were collected using a soil corer (2-cm diameter x 30-cm deep).

Soil samples were dispersed in water, allowed to settle, and decanted to remove floating dead organic matter. The sediment was suspended and washed over a sieve (0.6 mm openings) to remove silt. The remaining roots and sand were swirled to suspend organic matter which was trapped on a sieve. Roots were examined under a dissecting microscope at 6.3 to 50 X and the

ectomycorrhizas picked out, rinsed and sorted by morphology. Roots showing any of a suite of characters indicating ectomycorrhizal infection (tip shape, branching pattern, mantle color and pattern, hyphal structure) were described by macroscopic and microscopic characteristics. Digital pictures of macroscopic and microscopic root tip characters were taken with a Spot RT camera. Counts of mycorrhizal tips were estimated by orders of magnitude. Representative specimens were stored in buffer for subsequent DNA extraction.

Two soil cores per tree from two trees were pooled from each of four treatment types in five macroplots. Samples were analyzed at Western Agricultural Laboratories, Stockton, CA, using standard analytic procedures ([www.naptprogram.org](http://www.naptprogram.org)).

To collect hypogeous fungi, we raked away leaf litter and duff with garden cultivators and loosened the upper 10 cm of soil, then looked closely to distinguish sporocarps of hypogeous fungi. Sites were visited in spring. Tissue samples for sequencing were stored in buffer.

For DNA identification of mycorrhizas and sporocarps, DNA was extracted in CTAB with chloroform and amplified in polymerase chain reactions (PCR) with fungal-specific primers. PCR products were purified, prepared with BigDye Terminator Ready Reaction Mix, and sequenced in an ABI 310 Genetic Analyzer in the Biotechnology Center at Southern Oregon University. ITS sequences were compared to other fungal ITS sequences in GenBank ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)) with BLAST and to sporocarps collected at the same site. DNA sequences from mycorrhizas and sporocarps have been deposited in GenBank.

For statistical comparisons, fungal communities and soil variables on all macroplots and treatments were analyzed using Non-metric Multidimensional Scaling (NMS) with PC-ORD Version 5 (McCune and Mefford 1999, McCune and Grace 2002). We analyzed a matrix of 73 mycorrhiza species and four treatment variables from 20 plots (4 treatments x 5 replicates) using the Sørensen distance measure. The hypothesis of no difference on the four treatments among mycorrhizal assemblages and among soil variables were tested by Multi-Response Permutation procedures (MRPP) using Sørensen distance (McCune and Mefford 1999, McCune and Grace 2002). One-way analysis of variance (ANOVA) tests using MiniTab version 15 were used to test for differences in species richness between treatments and to test for differences in soil composition.

#### **IV. Key findings**

Species richness and community composition of ectomycorrhizal fungi were not altered significantly by any of the treatments. However, the abundance and species richness of hypogeous sporocarps decreased by 90% when plots were burned, either with or without mechanical mastication.

##### **A. Mycorrhizas**

Mechanical mastication did not lead to greater abundance and species richness of ectomycorrhizas; neither burning following mechanical mastication nor burning without mastication decreased the abundance and species richness of ectomycorrhizas.

From 629 mycorrhizal root tips of 251 tentative morphotypes, 141 sequences were obtained for 70 species. An additional three unknown morphotypes were included for a total of 73 mycorrhizal species. Species richness of mycorrhizal fungi varied from 23 to 35 species per treatment plot; differences among treatments were not significant. Species richness did not vary by macroplot. Species occurring in >50 % of sample plots include *Cenococcum*, species in *Inocybe* and *Lactarius* and in the Sebacinaceae, as well as resupinate fungi in the Thelephoraceae and Tomentellaceae. Ordination of the entire mycorrhizal dataset showed similarity of all treatment groups. Group differences as tested by MRPP were not significant.

## **B. Hypogeous fungi (“truffles”)**

Mechanical mastication led to greater abundance and species richness of hypogeous fungal sporocarps (fruiting bodies); actual numbers were higher for species richness and for sporocarps on brush masticated plots than on nonmasticated controls. Burning following mechanical mastication and burning of non-masticated plots decreased the abundance and species richness of hypogeous fruiting bodies by 90 %. The fruiting of hypogeous fungi is much more impacted by fire than the ectomycorrhizal community. Fewer hypogeous sporocarps result in reduced food for small mammals and reduced mycorrhizal inoculum for seedlings.

Sporocarps of hypogeous fungi were sparse on all plots. Identification was confirmed by DNA sequences for nine collections. A total of 25 collections in 12 genera were made during spring surveys. Even with this small dataset, differences were significant with fewer hypogeous fungi on burned plots whether masticated or not. The largest number of collections was on masticated plots though the difference from controls was not significant.

## **C. Soil components**

Mechanical mastication followed by burning did not significantly change soil nutrients at the depth of fine roots and mycorrhizal fungi. We chose deep samples (20cm) and allowed time to pass (5 years) since treatment so that we might observe persistent changes in the soil surrounding mycorrhizal roots. Soil nutrient composition did not vary among treatments as compared by ANOVA or by ordination with NMS.

## **V. Management implications**

What matters to mycorrhizal fungi is the survival of host trees; where there are no pines and oaks, there are no roots for the mycorrhizal fungi to colonize.

### **A. Mechanical mastication without prescribed fire**

**Retain trees greater than 4 m (12 ft) in height in mechanical mastication treatments—**  
Virtually all root tips of oaks and pines are colonized by mycorrhizal fungi, an essential

symbiont for tree survival. Mechanical mastication without prescribed fire did not damage the ectomycorrhizal community of standing trees (oaks and pines).

**Woody debris from mechanical mastication did not reduce the fruiting of hypogeous fungi**—These fungi are mycorrhizal with the trees and form their fruiting bodies under and beyond the canopy edge. Apparently, competition with saprotrophic fungi (wood-rotting fungi) and the change in soil surface properties due to woody debris did not restrict the fruiting process.

## **B. Mechanical mastication followed by prescribed fire**

**Retain larger trees to reduce soil heating above the tree root zone**—Mycorrhizal communities of oaks and pines were not reduced in species richness or altered in species composition five years after prescribed fire following mechanical mastication. Because the mastication treatment avoided trees taller than 4 m (12 ft), the woody debris under trees may have been less than in dense shrubland. Although depth of masticate was not part of the experimental design, the preservation of larger trees may have worked to decrease soil heating under tree canopies above the root zone. Alternatively, soil depth above mycorrhizal roots (10-20 cm/4-8 in) may have insulated the roots. New fine roots grow annually at this depth; they appear to have found their mycorrhizal fungi.

**Prescribed fire on masticated and unmasticated sites nearly obliterated the production of fruiting bodies by hypogeous fungi**—The same amount of fuel, whether masticated or standing, sufficiently altered soil properties so that hypogeous fungal fruiting was reduced by 90%. This is a serious loss of fungi with several consequences. Hypogeous fungi form part of the diet of small mammals, particularly in the spring when seeds are scarce (Frank et al. 2006, Taylor et al. 2009). Rodents disperse and defecate spores which serve as inoculum for establishment of pine and oak seedlings. Some species of hypogeous fungi are threatened and endangered. The loss of fruiting bodies over a period of several years may result in population decreases and extinctions.

## **VI. Relationship to other recent findings and ongoing work on this topic (one to two pages)**

**Effect of fire on hypogeous fungi**—Hypogeous fungi were sparse in the first season following prescribed burning with some recovery in the subsequent postfire years in an Australian Eucalypt forest (Trappe et al. 2006) and in mixed conifers in California (Meyer et al. 2005). Spring burning may destroy hypogeous fungi at the time of fruiting—resulting in loss of spores for mycorrhizal inoculum and loss of a food source for small mammals (Johnson 1996, Frank et al. 2006, Taylor et al. 2009). Fall burning before the fruiting season would be predicted to have less effect on hypogeous fungi although it may affect fall-fruiting fungi. Hypogeous fungi might be less affected by summer wildfires, the natural season for fire. The species found on burned plots, *Balsamia* sp., *Gilkeya compacta*, and *Schenella pityophilus* (formerly *Pyrenogaster*), are not characteristic post-fire fungi which are saprobic rather than mycorrhizal (Fujimura et al. 2005).

**Effect of fire on mycorrhizal communities**--Fire is not intrinsically harmful to mycorrhizal fungi (Cairney and Bastias 2007). Following fire, the mycorrhizal community may change in species richness or species composition, but it is not eliminated or replaced (Visser 1995, Miller et al 1998, Baar et al 1999, Jonsson et al 1999, Stendell et al 1999, Smith et al. 2004). Hotter

fires or frequent repeated fires lead to greater reduction in mycorrhizal colonization, especially in the upper 15 cm of soil. Stand-replacing fire resulted in decreased mycorrhizal inoculum due to erosion of unvegetated slopes (Amaranthus and Trappe 1993). Recovery times to pre-existing mycorrhizal communities varied from one year to over forty years, most likely dependent on fire intensity.

### **Effect of fuel reduction methods and fire surrogate treatments on ecosystem components**

**belowground**--Most studies on fuel reduction methods and fire surrogate treatments have not examined the effects of mechanical mastication on mycorrhizal fungi (e.g., Schwilk et al., 2009, Boerner et al. 2009). Some effects of mechanical mastication parallel the effects of clear cutting with an increase canopy opening and a decrease in mycorrhizal transfer of photosynthetic carbon to the soil. In subalpine fir and Douglas-fir forests, clear-cut logging resulted in reduction in active fine roots and in mycorrhizal diversity (Pilz and Perry 1984, Hagerman et al 1999). Removal of understory vegetation in ponderosa pine forests decreased soil carbon and nitrogen (Busse et al 1996). Hot ground fires cause death of mycorrhizas in the upper soil layers (Smith et al. 2004).

**Mycorrhizal networks**—A diverse mycorrhizal community has the capacity to form mycorrhizal networks in which the mycelium of single fungus associates with multiple trees including those of different species (e.g., oaks and pines) (Simard et al. 2002, Kennedy et al. 2003, Southworth et al. 2005, Simard and Durall 2004, Simard 2009). A mycorrhizal network may function to sustain diverse fungi for inoculation of seedlings or it may actually transfer nutrients (carbon, nitrogen) among plants.

## **VII. Future work needed**

These results point to questions where more information would help in management decisions:

- How does depth of masticated woody debris affect hypogeous fungal fruiting? Are there depths created by mechanical mastication of dense shrubs that would inhibit fruiting?
- What size of unburned tree islands would preserve rodent populations and hypogeous fruiting? This is a question of scale.
- Following mechanical mastication and prescribed fire, what soil treatments might promote recovery of hypogeous fungi? Fire makes an impervious surface—would some form of surface loosening allow water to penetrate better?
- Which rare fungi and rodents are in areas that require fuel reduction?

## VIII. Deliverables Cross-Walk

Proposed	Delivered	Status
Annual reports	Written documents including the rationale for the work, methods, results, discussion and conclusions	Completed
Presentations	(1) Donohue et al. (poster) <i>Effect of brush mastication on belowground mycorrhizal fungi in a mixed hardwood chaparral</i> , 3rd International Fire Ecology and Management Congress, San Diego, CA 2006 (2) Donohue et al. (poster) <i>Effect of brush mastication on mycorrhizas and hypogeous fungi in mixed hardwood chaparral</i> , Ecological Society of America, San Jose, CA 2007 (3) Southworth et al. (public presentation) <i>The hidden life of native plants: mycorrhizas and plant roots</i> , California Native Plant Society, Redding, CA 2008 (4) Southworth et al. (oral presentation) <i>Effect of brush mastication and prescribed fire on mycorrhizas and hypogeous fungi in mixed hardwood chaparral</i> , Botanical Society of American/Mycological Society of America, Snowbird, UT 2009	Completed
Publication	Manuscript submitted to scientific journal	In progress
Webpage	Images, data, analyses, PDF files of papers and talks; at Whiskeytown NRA and at SOU	In progress
Field trips	Visit to project site for universities, agencies, and the general public	In progress
Symposia	Organized at scientific meetings with talks on fire surrogate methods and on guidelines for use and monitoring of brush mastication.	In progress

## X. Additional Reporting (Appendices and other inputs to JFSP)

### A. Input into Findings Database (available from [www.firescience.gov](http://www.firescience.gov))

Table 1. Concensus taxa with GenBank accession numbers, based on BLAST matches, for ectomycorrhizas from Whiskeytown National Recreation Area, CA.

Table 2. Frequency of the more common ectomycorrhizal fungi grouped to genus or family by treatment.

Table 3. Concensus taxa based on BLAST matches and GenBank accession numbers for hypogeous sporocarps from Whiskeytown National Recreation Area, CA.

Table 4. Fruit bodies of hypogeous fungi collected from Whiskeytown National Recreation Area, CA.

B. Digital Photo Library (Photos available on CD)

C. Completed Deliverables (available on CD and entered into citation database at [www.firescience.gov](http://www.firescience.gov))

## IX. Literature cited

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