

# Mechanical mastication and prescribed fire in conifer–hardwood chaparral: differing responses of ectomycorrhizae and truffles

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**Abstract.** Fire-prone hardwood–conifer chaparral comprises a significant component of vegetation in seasonally dry areas where prescribed burns of standing vegetation are limited by air-quality restrictions and narrow climatic opportunities for burning. Mechanical mastication is used by land managers to reduce aerial fuels. When burned, the dry masticated slash layer may result in prolonged soil heating, particularly of the upper soil layers, which contain ectomycorrhizal roots and seasonal truffles (hypogeous fungal sporocarps). The purpose of this study was to examine the effects of mechanical mastication followed by prescribed fire on ectomycorrhizae and truffles. We treated blocks with mechanical mastication only, mechanical mastication followed by prescribed fire, prescribed fire only, and no treatment. Five years after the prescribed burn, soils with ectomycorrhizal roots were sampled at the canopy dripline of *Pinus attenuata* and *Quercus kelloggii* and surveyed for truffles. Ectomycorrhizae and truffles were described by morphology and by DNA sequences of the internal transcribed spacer region. Ectomycorrhizal communities did not differ among treatments. However, burning reduced the abundance and species richness of truffles in both controls and masticated vegetation. We conclude that prescribed burning of mechanically masticated slash does not harm ectomycorrhizal communities, but does inhibit fruiting of truffles.

**Additional keywords:** brush mastication, fuels reduction, hypogeous fungi, oak–chaparral.

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## Introduction

Prescribed fire has been the primary tool used for ecosystem restoration and risk reduction in fire-adapted landscapes. However, human development adjacent to public lands or within private lands has exacerbated the level of risk associated with the application of prescribed fire. The goal of land managers has been to reduce risk of wildfire in the wildland–urban interface while minimising the adverse effects of fuel reduction treatments on ecosystem function. Given a century of fire suppression and highly flammable vegetation in a climate with wet winters and hot and dry summers, fire is inevitable (Noss *et al.* 2006). With the implementation of the National Fire Plan, efforts to reduce hazardous fuels through prescribed fire and mechanical thinning increased, especially where frequent prescribed fire was limited by safety concerns, air quality restrictions and limited resources (USDA Forest Service 2001; Glitzenstein *et al.* 2006; Reiner *et al.* 2009). Many land-management agencies have implemented a multifaceted fuels management program that includes mechanical mastication of brush fuels followed by prescribed fire.

Mechanical mastication is attractive as a method of reducing aerial fuels (Busse *et al.* 2005; Bradley *et al.* 2006; Glitzenstein

*et al.* 2006; Kane *et al.* 2009; Reiner *et al.* 2009) because it is efficient for large-scale use. Potential adverse effects of mechanical reduction of shrubs and small trees are a concern to resource managers because the ecological consequences of mechanical mastication followed by prescribed fire 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 and affect soil fungi (Nearby *et al.* 1999; Boerner *et al.* 2009; Schwilk *et al.* 2009).

Mechanical mastication does not mimic natural events or parallel natural ecosystem processes. It deposits a dense layer of woody debris that is relatively resistant to fungal decomposition and that may act as mulch, moderating soil temperature and moisture, and decreasing aeration. When burned, the masticated debris layer increases burn intensity and severity, heating the soil more than a crown fire and causing alterations in ecosystem responses (Keeley 2009). Mechanical mastication of brush increased woody fuel loading in the 10-h time-lag class (Kane *et al.* 2009), leading to higher soil burn severity (Busse *et al.* 2005).

The upper layers of soil are active sites of fine root growth including ectomycorrhizal roots with hyphae that extend into

soil interstices. Ectomycorrhizal fungi are key components of ecosystems, forming symbioses with woody roots (Smith and Read 2008). In addition to large deep roots, trees produce new fine roots annually. Virtually every fine root tip of oaks and pines is ectomycorrhizal (Valentine *et al.* 2004; Moser *et al.* 2005, 2009). Mycorrhizal fungi are the primary source for transfer of carbon to underground ecosystems. Therefore, they are an important metric for investigation of the response of soil organisms to mechanical mastication.

Communities of ectomycorrhizal fungi are complex; it is common to find over 40 species of fungi associated with the roots of a single tree species in a woodland (Valentine *et al.* 2004; Smith *et al.* 2007; Morris *et al.* 2008). Of these, three to five species often make up the majority of ectomycorrhizae whereas the remaining species are rare. Some fungal species can form ectomycorrhizae with both oaks and pines, creating the potential for a mycorrhizal network linking diverse species of trees and shrubs (Kennedy *et al.* 2003; Southworth *et al.* 2005; Simard 2009; Smith *et al.* 2009). A mycorrhizal network may function to sustain diverse fungi for inoculation of seedlings or it may actually transfer nutrients (carbon, nitrogen) among plants (Simard 2009).

Among the ectomycorrhizal fungi associated with oaks and pines, truffles (hypogeous fungal sporocarps), including well-known genera such as *Tuber* in the Ascomycota and *Rhizopogon* in the Basidiomycota, fruit below ground in the upper few centimetres of mineral soil (e.g. Trappe 1979; Luoma *et al.* 1991; Cairney and Chambers 1999; Castellano *et al.* 2004; Frank *et al.* 2006; Trappe *et al.* 2007). Hypogeous sporocarps do not open to disperse the spores; instead, small mammals dig up and eat sporocarps and then defecate spores. Thus, hypogeous fungi provide food for small mammals that disperse ectomycorrhizal inoculum for new fine roots and seedlings (Fogel and Trappe 1978; Johnson 1996; North *et al.* 1997; Frank *et al.* 2006). Truffle production is highly seasonal: in oak woodlands in southern Oregon, truffles develop in spring; in conifer forests, truffles develop in spring and late autumn.

Because fire is a natural event in many plant communities with ectomycorrhizal hosts, it may not be intrinsically harmful to communities of ectomycorrhizal fungi (Cairney and Bastias 2007). Following fire, the ectomycorrhizal 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; Cairney and Bastias 2007). Hotter fires or frequent, repeated fires led to greater reduction in ectomycorrhizal colonisation, especially in the upper 15 cm of soil. Stand-replacing forest fires resulted in decreased mycorrhizal inoculum of vesicular-arbuscular fungi owing to erosion of unvegetated slopes (Amaranthus and Trappe 1993). Post-fire recovery times for mycorrhizal communities varied from 1 to over 40 years – allowing time for host species to regenerate.

Most studies of mechanical mastication and other fuel reduction treatments have not examined the effects on ectomycorrhizal fungi, particularly on individual species or community structure (e.g. Neary *et al.* 1999; Schwilk *et al.* 2009; Boerner *et al.* 2009). The increase in chipped wood after mechanical treatment may alter the interaction between wood-digesting saprotrophic fungi and ectomycorrhizal fungi. Ectomycorrhizal

fungi also respond in diverse ways to the higher soil temperatures resulting from decreased canopy cover. Some effects of mechanical mastication parallel the effects of clear-cutting, with an increase in 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 ectomycorrhizal diversity (Pilz and Perry 1984; Hagerman *et al.* 1999).

The consequences of burning of masticated slash are even less certain. If more roots have formed near the surface owing to the mulch effect, prescribed fire may damage mycorrhizae in the upper soil layers. The maximum temperature and total heat flux to which mycorrhizae are subjected will also vary substantially depending on their depth in the soil and the residence time of fire, especially as affected by smouldering combustion. 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. Smouldering combustion of downed fuels has led to higher soil-surface temperatures for longer periods of time than flaming combustion in which the soils cooled more rapidly (Odion and Davis 2000; Busse *et al.* 2005). Seed germination was poor in areas of prolonged heating, even with propagules 10–20 cm deep. Fungal spores vary in resistance to fire and to heat (Peay *et al.* 2009).

Fire has been shown to decrease production of hypogeous sporocarps. Hypogeous fungi were sparse in the first season following prescribed burning with some recovery in the subsequent post-fire years in an Australian eucalypt forest and in a mixed-conifer forest in California (Meyer *et al.* 2005; Trappe *et al.* 2006).

Soil compression by mastication equipment may decrease root and mycorrhiza growth owing to changes in soil structure. Increased bulk density decreases macropore space, thus limiting exchange of oxygen and carbon dioxide, and reducing the water available for uptake by mycorrhizae. Compaction may lead to formation of a soil crust that reduces infiltration of water, thereby increasing erosion (Kozłowski *et al.* 1991). Forest productivity is correlated with soil porosity; the large pores typical of a healthy forest are fragile and readily compressed by heavy equipment (Poff 1996).

The goal of this project was to evaluate the effect of mechanical mastication followed by prescribed fire on the communities of ectomycorrhizal fungi and hypogeous fungi in a conifer–hardwood shrubland. At Whiskeytown National Recreation Area in California, on the south-eastern edge of the Klamath Mountains, where low-elevation plant communities are characterised by an assortment of oak woodlands, chaparral, mixed conifer and knobcone pine vegetation types, we considered the following two questions. (i) What is the effect of mechanical mastication on the abundance and species richness of ectomycorrhizae and truffles (hypogeous fungal sporocarps)? And (ii) what is the effect of mastication followed by prescribed fire on the ectomycorrhizal community and on truffle production?

## Methods

### Study site

We conducted this study in Whiskeytown National Recreation Area on the south-eastern edge of the Klamath Mountains in

northern California (40.64°N, 122.59°W to 40.65°N, 122.60°W). The climate is characterised by seasonal drought, with hot dry summers and cool winters with moderate rainfall (average annual rainfall 1500 mm). Soils are classified as Alfisols (Mallory *et al.* 1973).

The vegetation is chaparral woodland, also called mixed oak–pine shrubland. The overstorey tree canopy included knobcone pine (*Pinus attenuata* Lemmon), ponderosa pine (*P. ponderosa* Laws.), black oak (*Quercus kelloggii* Newb.) and canyon live oak (*Q. chrysolepis* Liebm.). The shrub community was dominated by white-leaf manzanita (*Arctostaphylos viscida* Parry) intermixed with toyon (*Heteromeles arbutifolia* (Lindley) Roemer), poison oak (*Toxicodendron diversilobum* [Torrey & A. Gray] E. Greene), buckbrush (*Ceanothus* spp.) and chamise (*Adenostoma fasciculatum* Hook. & Arn.).

#### Experimental design and prescribed burns

We utilised the macroplots designed by Bradley *et al.* (2006) and analysed by Kane *et al.* (2006, 2009). None of the macroplots were in true forest or in exclusive shrubland. All treatment blocks included at least 10 trees along with dense shrubs, both as understorey and without covering tree canopies. Five replicate macroplots of 0.3–1.0 ha were located within 1.2 km of Whiskeytown Lake at elevations of 380 to 530 m. Treatments were applied randomly to two replicates in each macroplot.

Mechanical mastication treatments were implemented in November 2002 using a low ground-pressure (20.7 kPa) rubber-tracked ASV Posi-Track mounted with an FE slashbuster (Terex Corp., Westport, CT). At least 90% of machine operations occurred over surfaces covered with chipped wood to limit soil disturbance and compaction. The mastication treatment thinned shrubs and small trees less than 4 m in height. No overstorey trees were removed. As determined by plot-based sampling, total woody fuel loads averaged 31.8 Mg ha<sup>-1</sup>, with an average fuelbed depth of 5.8 cm (Kane *et al.* 2006, 2009).

Fires were conducted in May 2003 with drip torches using a combination of strip and spot ignition patterns. Pyrometers constructed of brass tags with OMEGALAQ heat-sensitive paint (Omega Engineering, Stamford, CT) were positioned in three strata: (i) attached to metal posts 0.5 m above the litter surface; (ii) on top of the litter; and (iii) under the litter on top of the soil. Pyrometer sets were placed within 1-m<sup>2</sup> fire-behaviour plots, two to four per treatment block. Mortality, including crown scorch, of *Pinus attenuata* and *Quercus kelloggii* was estimated by frequency classes (0, 1–5, 6–25, 26–50, 51–75, 76–95, 96–100%).

#### Soil sampling and ectomycorrhizal processing

Because ectomycorrhizal fungi are attached to host tree roots, we raked away leaf litter and loosened mineral soil to survey for truffles. Valentine *et al.* (2004) found the greatest species diversity of mycorrhizal fungi at the dripline of *Quercus garryana* in southern Oregon. In spring 2008, we sampled four treatment types: (i) mechanical mastication only; (ii) mechanical mastication followed by prescribed fire; (iii) prescribed fire only; and (iv) untreated controls, in five replicate macroplots, with 10 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 ectomycorrhizae and two for soil assays.

Soil samples were collected using a soil corer (2-cm diameter × 30 cm deep). Samples in plastic bags were carried to the laboratory on ice and kept refrigerated. Soil samples were stirred 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 and the ectomycorrhizae picked out, rinsed and sorted by morphology. Roots showing any of a suite of characters indicating ectomycorrhizal infection (tip shape, branching pattern, mantle colour and pattern, hyphal structure) were described by macroscopic and microscopic characteristics (Agerer 1991; Goodman *et al.* 2002; Valentine *et al.* 2004). Digital images of macroscopic and microscopic root-tip characters were taken with a Spot RT camera (Diagnostic Instruments, Sterling Heights, MI). Counts of ectomycorrhizal tips were estimated by orders of magnitude (1–10, 11–100, >100). Representative specimens were placed in buffer (0.1-M Tris, 0.3-M NaCl, 0.04-M EDTA) and stored at 4°C for subsequent DNA extraction.

#### Soil analyses

For soil physical and chemical properties, soil cores were collected as for ectomycorrhizal roots at the canopy dripline of ectomycorrhizal trees. Two soil cores per tree from two trees were pooled from each of four treatment types in five macroplots ( $n = 20$ ). Samples were analysed at Western Agricultural Laboratories (Stockton, CA) using standard analytic procedures: pH from saturated soil paste; organic matter by loss on ignition at 360°C; phosphorus by the Bray method; potassium, calcium and magnesium by 1.0-M ammonium acetate extraction; and iron and nickel by diethylenetriaminepentaacetic acid (DTPA) extraction and inductively coupled plasma spectrometry (www.naptrprogram.org, accessed 8 August 2011).

#### Collection of hypogeous fungi

In each treatment block, 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 April and May 2007 and 2008. Equivalent sampling effort was applied for each treatment in a macroplot. Hypogeous sporocarps were examined microscopically and hand sections stained with Melzer's reagent. Identifications were based on Trappe *et al.* (2007) and Castellano *et al.* (1989). Tissue samples for sequencing were stored in buffer (as above). Hypogeous sporocarps have been deposited in the Mycological Collection of the Oregon State University Herbarium (herbarium code OSC).

#### Molecular methods

Mycorrhizae and sporocarp tissues were macerated with a micropestle in microcentrifuge tubes. DNA was extracted in 2% cetyltrimethyl ammonium bromide (CTAB) with chloroform. The internal transcribed spacer region (ITS) was amplified in polymerase chain reactions (PCR) with fungal primer ITS1F and universal eukaryote primer TW13 (White *et al.* 1990; Gardes and Bruns 1993; Frank *et al.* 2009). PCR reactions were performed in 20-μL volumes using 0.6 units DNA polymerase

**Table 1. Intensity of prescribed fire**

Frequency of melting temperatures of heat-sensitive paints located on pyrometers in masticated ( $n = 38$ ) and non-masticated ( $n = 32$ ) treatment blocks at three levels: (i) 0.5 m above the litter surface (aerial); (ii) at the litter surface; and (iii) at the soil surface

		Temperatures (°C)						
Treatment	Sensor location	<79	79	107	163	260	399	538
Masticated	Aerial	0.31	0.05	0.18	0.10	0.13	0.25	0
	Litter	0.05	0	0.05	0.10	0.03	0.11	0.66
	Soil	0.97	0.03	0	0	0	0	0
Non-masticated	Aerial	0.75	0	0.13	0.09	0	0	0.03
	Litter	0.41	0.09	0.16	0.09	0.03	0.06	0.16
	Soil	0.97	0	0.03	0	0	0	0

(GoTaq, Promega, Madison, WI) and 4  $\mu$ L of 5 $\times$  colourless buffer (Promega), 200  $\mu$ m of each deoxyribose nucleotide triphosphate (dNTP), 0.3  $\mu$ m of each primer, 2.5 mm of MgCl<sub>2</sub> and 2  $\mu$ L of undiluted DNA template. An initial 3 min at 93°C was followed by 30 cycles of 30 s at 95°C, 2 min at 56°C and 3 min at 72°C, with a final cycle for 10 min at 72°C. PCR products were separated by electrophoresis on 1.5% agarose gels, stained with ethidium bromide (1 mg mL<sup>-1</sup>), and visualised under a Kodak EDAS 290 UV transilluminator (Eastman Kodak, Rochester, NY).

PCR products were purified with QIAquick PCR purification kits (Qiagen, Valencia, CA), prepared with BigDye Terminator Ready Reaction Mix v3.1 (Qiagen) using forward primers ITS1F and ITS1, and reverse primers ITS4 and TW13 and sequenced in an ABI 310 Genetic Analyzer (Applied Biosystems, Foster City, CA) in the Biotechnology Center at Southern Oregon University, Ashland, OR. Sequences were edited with *Chromas 1.45* (McCarthy 1998) and compared with other fungal ITS sequences in GenBank ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov), accessed 19 December 2009) with BLAST and with sporocarps collected at the same site with *ClustalX* (Altschul *et al.* 1990; Thompson *et al.* 1997). DNA sequences from ectomycorrhizae and sporocarps have been deposited in GenBank.

#### Data analysis

Fungal communities and soil variables of all macroplots and treatments were analysed using non-metric multidimensional scaling (NMS) with *PC-ORD Version 5* (McCune and Mefford 1999; McCune and Grace 2002). We analysed a matrix of 86 total fungal species, 73 from ectomycorrhizae and 13 from truffles, with four treatment variables from five replicate macroplots using the Sørensen distance measure. Fungal communities were analysed using: (i) all species; (ii) all species with Helotiales removed because they might be from ericoid hosts; (iii) all species with the outlier, *Cenococcum*, removed; and (iv) all species by taxonomic group or sporocarp habit (epigeous, hypogeous or resupinate). NMS was performed with 50 runs of real data along with 100 runs with randomised data for a Monte Carlo test of significance. The hypothesis of no difference in the four treatments among ectomycorrhizal assemblages and among soil variables was tested by Multi-Response Permutation procedures (MRPP) using Sørensen distance (McCune and Mefford 1999; McCune and Grace 2002).

One-way analysis of variance (ANOVA) using *MiniTab v.15* (Minitab Inc., State College, PA) was used to test for differences

in species richness between treatments. MANOVA was used to test for differences in soil composition (percentage organic matter, P, Mg, Ca, Na, pH, cation-exchange capacity, nitrate and S) between treatments.

## Results

### Environmental variables

Prescribed-burn temperatures at the soil surface were similar for masticated and non-masticated blocks, but aerial and litter surface temperatures were hotter in masticated blocks (Table 1). At soil level in both masticated and non-masticated treatments, 97% of sensors did not reach 79°C. One masticated block pyrometer reached 107°C and one non-masticated block pyrometer 79°C. At the masticated litter surface, 66% of sensors reached 538°C, but without mastication, only 16% of sensors reached 538°C. For masticated litter, the median melting temperature was 538°C, but only 107°C for non-masticated litter. Aerial temperatures reached 399°C above masticated litter, but only 163°C above non-masticated litter.

Most ectomycorrhizal trees survived the prescribed burns. With burning following mastication, 25% of trees had scorched or burned canopies, with no significant difference between pines and oaks. However, with burning of standing chaparral, 39% of oak canopies were scorched or burned and 8% of pines were burned.

Soil nutrient composition did not vary among treatments (Table 2). No differences among soil samples were detected by ANOVA, by ordination with NMS, or by MRPP.

### Mycorrhizae

From 629 ectomycorrhizal root tips sorted into 251 tentative morphotypes, 141 sequences were obtained for 67 taxa (Table 3, Accessory publication, Table A1). An additional three unknown morphotypes were included for a total of 70 ectomycorrhizal species. Eleven sequences came from five species of ectomycorrhizal fungi (Helotiales and *Oidiodendron*) that are specific for ericoid hosts such as manzanita. The remaining 130 sequences representing 62 species were dominated by Basidiomycota, which were chiefly epigeous (mushrooms) and resupinate (crusts) rather than hypogeous (truffles). In contrast, the Ascomycota were nearly all hypogeous.

Numbers of ectomycorrhizal fungal species on burned and unburned blocks were similar, 54 and 56 respectively. Total numbers of species were higher on non-masticated blocks (47)

**Table 2. Soil characteristics**

Mean values with 1 s.d. in parentheses on five replicate macroplots with four treatments: C, control; B, non-masticated and burned; M, masticated only; and MB, masticated and burned ( $n = 20$ ). Differences among treatments were not significant (ANOVA,  $P > 0.1$ ). CEC, cation exchange capacity

Treatment	% organic matter	P (mg L <sup>-1</sup> )	K (mg L <sup>-1</sup> )	Mg (mg L <sup>-1</sup> )	Ca (mg L <sup>-1</sup> )	Na (mg L <sup>-1</sup> )	pH	CEC (mg L <sup>-1</sup> )	NO <sub>3</sub> (mg L <sup>-1</sup> )	S (mg L <sup>-1</sup> )
C	4.1 (1.1)	4.0 (1.9)	113 (39)	282 (88)	1147 (282)	13.8 (4.5)	5.6 (0.1)	9.5 (5.1)	2.4 (0.5)	5.8 (6.9)
B	4.3 (1.0)	4.0 (1.6)	106 (23)	204 (66)	917 (331)	16.8 (7.3)	5.4 (0.2)	9.4 (3.0)	3.4 (3.1)	30.0 (51.6)
M	4.3 (0.8)	4.2 (1.5)	89 (15)	253 (132)	1116 (307)	15.6 (7.8)	5.5 (0.2)	10.5 (2.9)	2.4 (0.5)	16.4 (15.5)
MB	4.4 (1.2)	3.8 (0.8)	100 (25)	291 (103)	1358 (238)	16.6 (2.4)	5.6 (0.2)	12.4 (2.8)	3.2 (2.2)	30.4 (58.5)

**Table 3. Frequency of ectomycorrhizal fungi categorised by genus or family**

For identification of ectomycorrhizal fungi, see Table A1 of the Accessory publication. Number of species per taxon equalled one unless number given in parentheses. Treatments included C, control; B, non-masticated burned; M, masticated unburned; and MB, masticated burned. Frequency refers to number of plots on which each occurs out of five replicates

Ectomycorrhizal fungus	Phylum	Treatment				Total
		C	B	M	MB	
<i>Cenococcum</i>	Ascomycota	5	5	4	5	19
Thelephoraceae (14)	Basidiomycota	4	4	2	3	13
<i>Inocybe</i> (9)	Basidiomycota	3	3	3	3	12
Sebacinaceae (6)	Basidiomycota	4	4	1	3	12
<i>Lactarius</i> (4)	Basidiomycota	3	2	2	4	11
<i>Russula</i> (6)	Basidiomycota	3	3	2	1	9
<i>Laccaria</i>	Basidiomycota	1	1	2	2	6
<i>Cortinarius</i> (2)	Basidiomycota	1	2	1	1	5
<i>Hydnoplicata</i>	Ascomycota	2	0	2	0	4
<i>Rhizopogon</i> (2)	Basidiomycota	1	0	0	3	4
<i>Boletus</i>	Basidiomycota	1	1	0	0	2
<i>Pachyphloeus</i>	Ascomycota	0	1	0	1	2
Pyronemataceae	Ascomycota	1	1	0	0	2
<i>Tylospora</i>	Basidiomycota	1	1	0	0	2
<i>Amanita</i>	Basidiomycota	0	0	1	0	1
Cantharellales	Basidiomycota	0	1	0	0	1
<i>Clavariadelphus</i>	Basidiomycota	0	0	1	0	1
Corticaceae	Basidiomycota	0	0	0	1	1
<i>Craterellus</i>	Basidiomycota	1	0	0	0	1
<i>Genabea</i>	Ascomycota	1	0	0	0	1
<i>Genea</i>	Ascomycota	0	1	0	0	1
<i>Hebeloma</i>	Basidiomycota	1	0	0	0	1
<i>Helvella</i>	Ascomycota	0	0	0	1	1
<i>Leucophleps</i>	Basidiomycota	0	1	0	0	1
Pezizomycotina	Ascomycota	0	0	0	1	1
<i>Tricholoma</i>	Basidiomycota	0	0	1	0	1

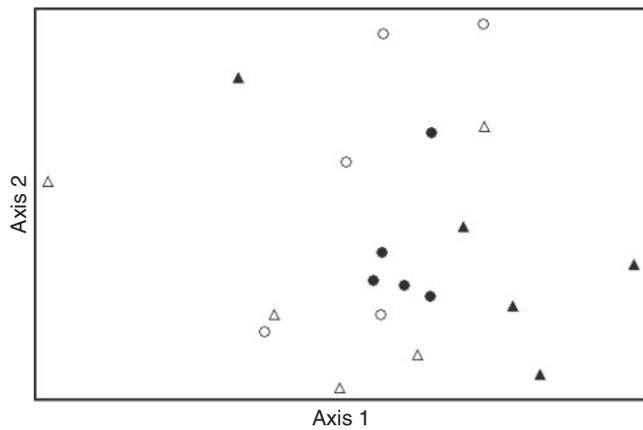
than on masticated blocks (34), but the average number of species per plot did not differ significantly. Twenty-two species occurred only on unburned blocks, 18 species only on burned blocks (see Accessory publication, Table A1). Species richness of ectomycorrhizal fungi ranged from 23 to 35 species per treatment type; differences in species richness among treatments were not significant (ANOVA,  $P = 0.107$ ). Species richness also did not differ by macroplot (ANOVA,  $P = 0.734$ ). Species groupings occurring in  $>50\%$  of sample blocks include *Cenococcum*, *Inocybe*, *Lactarius*, Sebacinaceae and resupinate fungi in the Thelephoraceae (Table 3).

Ordination of the entire ectomycorrhizal community dataset showed similarity among all treatment groups (Fig. 1). Groups

did not differ as tested by MRPP. Similar results showed no differences among treatments of ectomycorrhizal communities with or without Helotiales, the outlier *Cenococcum*, singletons or hypogeous sporocarps.

#### Hypogeous fungi

Truffles were sparse on all blocks. A total of 26 collections in 12 genera were made during spring surveys (Table 4). For nine collections, identification was confirmed by DNA sequences (Table 5). Even with this small dataset, differences in frequency among treatments were significant (Chi-square:  $P = 0.016$ ), with fewer hypogeous fungi on burned blocks whether masticated or not. The largest number of collections was on



**Fig. 1.** NMS ordination plot of mycorrhizal communities with fuel-reduction treatments: triangles, non-masticated; circles, masticated; open symbols, unburned; closed symbols, burned.

**Table 4.** Sporocarps of hypogeous fungi collected from Whiskeytown National Recreation Area, CA, USA

Treatments: C, control; B, non-masticated burned; M, masticated unburned; and MB, masticated burned. Frequency of sporocarps per treatment differed (Chi-square,  $P = 0.016$ )

Taxon	Treatment				Total
	C	B	M	MB	
<i>Balsamia alba</i>		1	1		2
<i>Balsamia magnata</i>	3	1			4
<i>Elaphomyces</i> sp.			2		2
<i>Endogone</i> sp.			1		1
<i>Geopora cooperi</i>			1		1
<i>Gilkeya compacta</i>		1		1	2
<i>Gymnomyces</i> sp.	1		1		2
<i>Hymenogaster</i> sp.			1		1
<i>Hysterangium</i> sp.	1				1
<i>Melanogaster</i> sp.			1		1
<i>Peziza infossa</i>				1	1
<i>Schenella pityophilus</i>		2		2	4
<i>Rhizopogon</i> sp.	1		3		4
Total collections	6	5	11	4	26
Total species	4	4	8	2	13

masticated blocks though there was no significant difference from non-masticated controls.

Based on Sørensen's index of similarity, species differed among pair-wise treatments: control and burned ( $S = 0.3$ ), masticated and masticated burned ( $S = 0.0$ ), control and masticated ( $S = 0.6$ ), burned and masticated burned ( $S = 0.5$ ). Burned blocks, whether of standing brush or masticated brush, were more similar to each other than were burned blocks to their controls. The most similar were the unburned controls and the unburned masticated blocks.

Among truffle species, the overlap between sporocarps and ectomycorrhizae was poor. Only *Rhizopogon* species, collected as sporocarps, were detected on roots; none of the other hypogeous sporocarp taxa were detected on roots. Conversely, five additional hypogeous taxa, *Genabea*, *Genea*, *Hydnoplicata* and

**Table 5.** GenBank accession numbers for hypogeous sporocarps from Whiskeytown National Recreation Area, CA, USA

Taxon collection numbers are in parentheses. Site letters refer to macroplots (A, B, D, E, J) and treatments (NU, non-masticated unburned; NB, non-masticated burned; MU, masticated unburned; and MB, masticated burned)

Taxon	GenBank accession number	Length (base pairs)	Site
<i>Balsamia</i> cf. <i>alba</i> (871)	GU184098	778	JMU
<i>Elaphocordyceps</i> (872)	GU184099	316	JMU
<i>Elaphomyces</i> sp. (999)	GU184100	264	BMU
<i>Geopora</i> cf. <i>cooperi</i> (1000)	GU184101	565	BMB
<i>Gilkeya compacta</i> (870)	GU184102	778	JMB
<i>Gilkeya compacta</i> (1169)	GU184103	931	BNB
<i>Gymnomyces xerophilus</i> (880)	GU184104	590	DNU
<i>Peziza infossa</i> (869)	GU184105	926	JMB
<i>Schenella pityophilus</i> (852)	GU184106	461	EMU

*Pachyphloeus* (Ascomycota) and *Leucophleps* (Basidiomycota), were detected as ectomycorrhizae, but not collected as sporocarps. Ectomycorrhizae of *Genea*, *Pachyphloeus*, *Leucophleps* and *Rhizopogon* were found on six burned blocks, indicating that the species were present, though they did not form sporocarps.

## Discussion

Ectomycorrhizal communities and truffle assemblages responded differently to mechanical mastication and to mastication followed by prescribed fire. Ectomycorrhizal communities were slightly less species-rich overall, but the communities did not differ significantly among any of the treatments. However, truffle assemblages were lower by 50% on blocks treated with prescribed fire, whether masticated or not.

Species richness and community composition of ectomycorrhizal fungi were not altered significantly by any of the treatments. Mechanical mastication did not lead to greater abundance and species richness of ectomycorrhizae, nor did the addition of burning decrease the abundance and species richness of ectomycorrhizae. Burning without mastication did not reduce the ectomycorrhizal community. Apparently, in spite of the alteration of fuels by mechanical mastication and the resulting higher litter-surface fire temperatures, ectomycorrhizal roots were resilient. Relatively cool soil temperatures and insulation by soil at depth may have prevented damage to roots, hyphae or spores. In addition, because overstorey host trees were not masticated, the soil directly under the trees may have experienced less heating. The mastication protocol that preserved ectomycorrhizal host trees during mastication may have coincidentally preserved their root-associated symbionts.

Wildfire and prescribed burns may result in an alteration of ectomycorrhizal communities (Baar *et al.* 1999; Hart *et al.* 2005; Cairney and Bastias 2007). In mixed-conifer forests, prescribed burns on either thinned or unthinned stands reduced the number of ectomycorrhizal species to ~60% that of the control (Smith *et al.* 2004, 2005). Hyphae recovered from in-growth bags differed in species composition among control and 2- and 4-year post-burn blocks (Bastias *et al.* 2006). However, ectomycorrhizal species richness and community structure did not differ

following wildfire in either oak or boreal forests (Jonsson *et al.* 1999; de Román and de Miguel 2005). Differences in the response patterns of ectomycorrhizal communities to fire may reflect heterogeneity in fire intensity, fuel properties and the insulating qualities of soils, as well as differential survival of fungal spores (Baar *et al.* 1999; Peay *et al.* 2009).

The vegetation of the Klamath Mountains is highly fire-adapted, with fire return intervals from 1700 to 1850 of 4–7 years, followed by an increase in fire return intervals until 1925 with almost complete suppression of fire (Fry and Stephens 2006). Plant communities including their associated fungal symbionts may have adapted to survive fire, particularly fast-moving crown fires and fire mosaics.

Mechanical mastication resulted in greater abundance and species richness of hypogeous fungal sporocarps, maintaining a suitable habitat for fruiting of hypogeous fungi. More truffle species and more sporocarps were collected on masticated blocks than on control blocks. The presence of a masticated litter layer did not correlate with decreased truffle production.

Although chemical properties of soil have been observed to change following wildfire (Boerner *et al.* 2009), in our study they did not change with mastication followed by burning. We analysed samples at the depth of ectomycorrhizal roots (20 cm) and allowed time to pass (3 years) after treatment to see what changes would persist. This time delay and the depth of soil samples may not have captured subtle changes present at the surface immediately post fire.

Fewer truffles occurred in burned plots of either standing chaparral or masticated chaparral. Changes due to direct consequences of fire, e.g. charred ground surface with its hydrophobic properties and loss of organic matter, may have created conditions not conducive to fruiting. In addition, the indirect effects of vegetation removal may have rendered the soils hotter and drier over several years.

Both wildfire and prescribed fire with or without fuel reduction treatments often reduce truffle production. Wildfire in Australian eucalypt forests reduced hypogeous fruiting to nearly zero (Trappe *et al.* 2006). Prescribed burns in Yosemite National Park resulted in fewer truffle fruiting bodies, but did not decrease species richness or biomass (Meyer *et al.* 2008).

The truffle species found on burned blocks, *Gilkeya compacta* and *Peziza infossa*, are known to occur on unburned sites also (Frank *et al.* 2009). These are not characteristic post-fire fungi, which are saprobic rather than mycorrhizal (Fujimura *et al.* 2005; Claridge *et al.* 2009). The truffle species *Hydnoplicata* sp., *Genabea* sp. and *Pachyphloeus* sp. that occurred as ectomycorrhizae on burned blocks but not as sporocarps might be less able to withstand fire or the fruiting bodies may occur at other times or simply be rare.

The consequences of a decrease in truffles are a loss of food for small mammals and diminished spore dispersal as inoculum for seedlings of ectomycorrhizal trees (oaks, pines, tanoaks), particularly those located away from the root zone of mature trees, and for new fine roots of mature trees (Johnson 1996; North *et al.* 1997; Meyer *et al.* 2005; Frank *et al.* 2006, 2009; Trappe *et al.* 2006). Seedling survival may be limited by the dependability and timing of ectomycorrhizal inoculation. Truffles typically produce sporocarps in the spring. Although hypogeous fungal species make up a small fraction of the total

ectomycorrhizal fungal community, they are particularly well adapted to drought (Castellano *et al.* 2004).

We conclude that neither mastication alone nor mastication followed by prescribed fire result in large effects on ectomycorrhizal fungal communities. Detection of small-scale effects may have been limited by the conditions of this study (spring burns only) and the sampling design (control and effect only). What matters to ectomycorrhizal fungi is the survival of host plants. The host plants in this study survived mastication and prescribed fire. Ectomycorrhizae on plants that were killed also would have perished because they are obligate symbionts. The long-term persistence of ectomycorrhizae in areas where vegetation is killed by treatments would depend on recolonisation of these sites by the same species. Size of treatment areas and intensity of treatments will affect availability of inoculum from the surviving rhizosphere hyphae of tree roots.

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