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Arbuscular mycorrhizal propagule densities respond rapidly to ponderosa pine restoration treatments

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Summary

1. Mycorrhizae form a critical link between above-ground plants and the soil system by influencing plant nutrition, nutrient cycling and soil structure. Understanding how mycorrhizae respond to disturbances may lead to important advances in interpreting above-ground plant recovery.

2. The inoculum potential for arbuscular mycorrhizae (AM) and ectomycorrhizal (EM) fungi was investigated in thinned-only, thinned and prescribed burned (both restoration treatments) and unthinned and unburned control stands in northern Arizona ponderosa pine forests. The relationships between mycorrhizal fungal propagule densities and plant community and soil properties were quantified.

3. The relative amount of infective propagules of AM fungi was significantly higher in samples collected from both restoration treatments than their paired controls (unthinned and unburned stands). In contrast, the same restoration treatments had no significant effect on the relative amount of infective propagules of EM fungi.

4. The relative amount of infective propagules of AM fungi was significantly positively correlated with graminoid cover and herbaceous understorey species richness and negatively correlated with overstorey tree canopy cover and litter cover.

5. *Synthesis and applications*. These results indicate that population densities of AM fungi can rapidly increase following restoration treatments in northern Arizona ponderosa pine forests. This has important implications for restoring the herbaceous understorey of these forests because most understorey plants depend on AM associations for normal growth. These results also can be applied to other ecosystems that are in a state of restoration or where the role of fire is just beginning to be understood.

Key-words: disturbance, ecological restoration, ecosystem function, ectomycorrhizae, succession.

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Introduction

Prior to Euro-American settlement in the 1880s, ponderosa pine *Pinus ponderosa* Dougl. ex Laws. forests in the American south-west were characterized by herbaceous understoreys dominated by grasses intermixed with large trees (Cooper 1960). Low-intensity grass-fuelled fires every 2–20 years played a major role in determining the structure, composition and stability of these ecosystems (Fulé, Covington & Moore 1997). Euro-American settlers drastically changed these forest ecosystems through heavy livestock grazing, intensive logging of old-growth trees and fire suppression. Today these forests are often characterized by a large number of small trees with closed canopies, deep deposits of dead organic material on soil surfaces, and little herbaceous understorey. Efforts are currently underway to reverse structural and functional ecosystem changes caused by historical land management practices. The aim is to restore ecosystem diversity by returning ponderosa pine forests to a more open savanna-like structure and reintegrating the natural disturbance regime of fire (Covington & Moore 1994). Tree thinning and prescribed burning are two major components of this restoration effort.

A major objective for ponderosa pine forest restoration is to increase herbaceous understorey diversity

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and production to levels that emulate reference conditions (Covington *et al*. 1997). Mycorrhizae are a critical link between above-ground plants and the soil system, playing an important role in plant nutrition, nutrient cycling and the development of soil structure (Allen 1991; Smith & Read 1997). Many tree species are highly dependent on ectomycorrhizal (EM) fungi (Mikola 1970) and most herbaceous plants are associated with arbuscular mycorrhizal (AM) fungi (Smith, Charvat & Jacobson 1998). Consequently, numerous researchers have suggested a relationship between the recovery time of disturbed ecosystems and the abundance of infective propagules of mycorrhizal fungi (Reeves *et al*. 1979; Allen & Allen 1980; Bentivenga & Hetrick 1991; Noyd, Pfleger & Russelle 1995; Gange, Lindsay & Ellis 1999). In addition, Perry *et al*. (1989) refer to the strong link between plants and the soil biota as the 'bootstrapping hypothesis'. This hypothesis suggests that through close mutual interactions some ecosystems are able literally to pull themselves up by their own 'bootstraps', thus creating conditions to allow the system to persist over time (Perry *et al*. 1989). These authors showed that mycorrhizae helped facilitate succession following natural wildfires, where different species of plants formed mutualistic relationships with the same fungi throughout succession in forests in the north-western USA. Other studies suggest that the role of mycorrhizae in facilitating succession may be dependent on the nutrient status of soil (Allen & Allen 1990). Because ponderosa pines are strongly EM and many of the native understorey grasses and forbs are strongly AM, we predicted that densities of AM fungal propagules should increase and EM fungal propagules should decrease in response to restoration thinning and prescribed burning in ponderosa pine forests. The specific objectives of this study were to: (i) quantify the effect of restoration thinning, and thinning and prescribed burning, on AM and EM fungal propagule densities; and (ii) assess the relationships between mycorrhizal fungal propagule densities and plant community and soil properties.

Methods and materials

EXPERIMENTAL DESIGN

This research was conducted at two sites: the Fort Valley Experimental Forest and adjacent areas near Flagstaff, Arizona (hereafter referred to as Fort Valley), and the Mt Trumbull Resource Conservation Area north-west of Grand Canyon National Park, Arizona (hereafter referred to as Mt Trumbull), USA. Two experimental blocks were established during the summer of 1998 at Fort Valley and four experimental blocks were established during the summer of 1999 at Mt Trumbull. Treatment stands, each *c.* 16 ha, were randomly assigned within each block. Treatments at Fort Valley were (i) no thinning or burning (control) and (ii) thinning to a low level of replacement trees based upon pre-settlement tree densities. Thinning treatments at Fort Valley received prescribed burning treatments following the sampling for this study during the summer of 2001. Treatments at Mt Trumbull were randomly assigned the same two treatments with the addition of prescribed burning to thinning. Specific details of the thinning treatments at Fort Valley are outlined in Fulé *et al*. (2001a) and specific details of the thinning treatments at Mt Trumbull are outlined in Fulé *et al*. (2001b). Twenty systematically located permanent plots were located in each stand at Fort Valley and Mt Trumbull. At each of these permanent plots we sampled herbaceous understorey and overstorey tree composition and abundance, fuel loads and tree canopy cover.

SOIL FIELD SAMPLING

At Fort Valley, soil samples were randomly taken from 10 of the 20 systematically placed plots along a 50-m transect within each of the four stands (2 blocks × 2 treatments) in late May 1999, 6 months after thinning, and again in May 2000, 18 months after thinning. At Mt Trumbull soil samples were randomly taken from 10 of the 20 systematically placed plots along a 50-m transect within each of the eight stands (4 blocks × 2 treatments) in late June 2000, 5 months after thinning and 3 months after burning. Two samples 20 m along each transect (one for the AM bioassay and one for the EM bioassay) were taken and placed immediately into 4 × 20-cm diameter deep Conetainers (Stuewe and Sons Inc., Corvallis, Oregon USA) for bait-plant bioassays (Brundrett & Abbott 1994). Soils were collected to a depth of 15 cm using a hand trowel. We took samples to this depth because AM fungal propagule densities are generally highest in the surface 15 cm (Smith & Walker 1981). A third sample was taken at the same location for soil chemical analyses. This sample was frozen until it was analysed.

VEGETATION FIELD SAMPLING

At Fort Valley, we recorded pre-treatment data for the herbaceous understorey, overstorey tree composition and abundance, fuel loads and tree canopy cover in 1998. Post-thinning treatment data were collected in 1999 and 2000. These data were not significantly different between the 2 years; therefore, only post-treatment data from 2000 is presented because it is further along the restoration trajectory. At Mt Trumbull, we recorded pre-treatment data for the herbaceous understorey, overstorey tree composition and abundance, fuel loads and tree canopy cover in 1999. Post-treatment data was collected in 2000.

We surveyed the herbaceous and shrub understorey community along the same 50-m transects where soil samples were collected for AM and EM bioassays and soil chemical analyses. Every 30 cm along the 50-m transect, for a total of 166 points, substrate (plant, litter, soil, wood, rock) was recorded. If the substrate was a plant, the species was identified and its height was

recorded. We determined plant cover by dividing the number of plant hits by 166 points for each transect. Overstorey tree data were collected in 400-m^2 circular plots centred on each plot centre. Tree species and diameter at breast height (d.b.h., 1·37 m) were recorded for all live and dead trees greater than 1·37 m in height.

In addition, at the same 30-cm intervals along the 50-m transect, tree canopy cover and fire severity in areas treated with prescribed burning were recorded. We determined tree canopy cover by recording the presence or absence of a tree canopy from a vertical projection. Fire severity was determined based upon descriptions in the Western Regional Fire Monitoring Handbook (National Park Service 1992). Areas with charred litter, duff (the fermentation and humus layers of the forest floor) and wood were recorded as lightly burned areas, areas with litter mostly consumed, duff deeply burned and wood recognizable were recorded as moderately burned areas, and areas with litter and duff consumed leaving white ash and soil often reddish were recorded as severely burned. We used a 15-m planar transect located in a random direction from the plot centre to measure forest floor litter and duff depths. Every 1·5 m, litter and duff were recorded following guidelines outlined in Brown (1974).

LABORATORY ANALYSIS

Bait-plant bioassays are designed to detect all types of viable mycorrhizal fungal propagules including spores, fragments of mycorrhizal roots and extraradical hyphae. This method quantifies total mycorrhizal fungi more accurately than direct counts of sporocarps, spores or colonized root lengths (Brundrett & Abbott 1994; Johnson, O'Dell & Bledsoe 1999). We used a corn *Zea mays* L. bioassay to determine the relative amount of infective propagules of AM fungi and a ponderosa pine bioassay to determine the relative amount of infective propagules of EM fungi. Corn is mycotrophic with many species of AM fungi and grows rapidly and uniformly; these advantages outweigh the disadvantage of not using a native host-plant (Johnson, O'Dell & Bledsoe 1999). Plants were placed in a greenhouse and watered every 3 days until they were harvested at 6 weeks. Because pine seedlings grow more slowly than corn seedlings, pine seedlings were harvested at 12 weeks to determine inoculum potential. Roots were carefully washed free from the soil and weighed. Following mycorrhizal analysis, roots were oven-dried at 70 °C for 24 h and then reweighed. Shoot lengths and dry root and shoot weights were also measured. Corn roots were prepared for AM analysis by cutting roots into 2·5-cm segments, taking a random subsample of the cut roots of a known mass, clearing roots in 5% potassium hydroxide, and then staining with trypan blue in lactoglycerin (Koske & Gemma 1990). The gridline intersect method using a dissecting microscope was used to measure the proportion of root length containing AM fungal structures: arbuscules, vesicles, coils, internal mycorrhizal hyphae and external mycorrhizal hyphae (Giovannetti & Mosse 1980). We measured pine roots for fungal propagule density through direct examination using a dissecting microscope to quantify the proportion of root tips colonized with EM fungi (Gehring & Whitham 1991). Root tips were classified either as a living or dead EM tip or a living or dead non-mycorrhizal tip. Different morphological types and colours were also recorded.

SOIL ANALYSIS

Soil samples from each transect were analysed for pH, total N, total P and organic C at the Bilby Research Soil Analysis Laboratory, Flagstaff, Arizona, USA. Soil pH was determined in a 1 : 1 slurry by pH meter. Total N and P were measured using a Kjeldahl digestion of the soil material followed with the analysis of N and P by automated colorimetry using a Technicon autoanalyser (Pulse Instrumentation, Saskatoon, SK, Canada) (Parkinson & Allen 1975). Organic C was determined by loss on ignition. Samples were heated in crucibles for 24 h in a muffle furnace at 425 °C and organic matter was estimated from net weight loss.

STATISTICAL ANALYSIS

Multivariate analysis of variance (MANOVA) repeated measures was used to determine the effects of thinning-only on mycorrhizal propagule densities for data collected in 1999 and 2000, with time and treatment as the two main variables in the analysis. We used analysis of variance (ANOVA) for a randomized block design in SPSS version 8 (SAS Institute 1997) to determine the effect of thinning-only and thinning and prescribed burning on vegetation, soil properties and the proportion of AM fungal structures for the 2000 data. ANOVA was also used to determine the effect of thinning and prescribed burning on mycorrhizal propagule densities. Significance for analysis of variance tests was accepted at alpha = 0·05. The Shapiro–Wilks test was used to test data for normality and Leven's test was used to test for homogeneity of the variance (Milliken & Johnson 1984). Root infection data were angular transformed prior to analysis. Herbaceous plant abundance, graminoid cover and soil properties were square-root transformed to improve normality and homoscedasticity assumptions (Zar 1984). Multiple regression was used to determine the relationship between infectivity and plant and community properties. Simple correlation analysis was used to determine the relationship between root infectivity and bait plant characteristics (length and weight).

Results

STAND CHARACTERISTICS

Pre-treatment there were no significant differences between control and treatment stand data for vegetation

Table 1. Vegetation and site characteristics for the Fort Valley control and thinned units for pre-treatment 1998 data and post-treatment 2000 data. Data are expressed as means (n = 2) ± SEM. Herbaceous cover data were square-root transformed prior to analysis

	Pre-treatment 1998		Post-treatment 2000	
Variable	Control stand	Treatment stand	Control stand	Treatment stand
Trees ha^{-1}	1415·1 ± 392	1162 ± 141·4	1426·2 ± 330a	182·3 ± 34b
Tree canopy cover (%)	62·37 ± 5·5	57·61 ± 3·8	61·03 ± 4·41a	34 ± 2·95b
Herbaceous cover (%)	9·67 ± 1·92	9·23 ± 1·2	9·06 ± 1·76	11·22 ± 1·4
Simpson's diversity index	2·92 ± 0·44	2·89 ± 0·38	2·88 ± 0·62	3·61 ± 0·46
Litter load (kg ha^{-1})	14211 ± 1508	14133 ± 1980	14363 ± 1508	15625 ± 2598
Duff load (kg ha^{-1})	25127 ± 2006	25338 ± 3266	25836 ± 3103	26379 ± 3266

Values indexed by a different letter are significantly different at the $P \leq 0{\cdot}05$ level between paired control and treatment units for the same sampling year.

Table 2. Vegetation and site characteristics for the Mt Trumbull control and thinned/prescribed burned units for pre-treatment 1999 data and post-treatment 2000 data. Data are expressed as means (n = 4) ± SEM. Herbaceous cover data were square root-transformed prior to analysis

	Pre-treatment 1998		Post-treatment 2000	
Variable	Control stand	Treatment stand	Control stand	Treatment stand
Trees ha^{-1}	2128·47 ± 700·9	1866·67 ± 786·1	2193·1 ± 355a	336·3 ± 119b
Tree canopy cover (%)	58·96 ± 5·8	53·15 ± 6·98	61·51 ± 5·73a	33·49 ± 7·1b
Herbaceous cover (%)	6·54 ± 1·32	6·21 ± 1·52	6·13 ± 1·94	10·61 ± 5·26
Simpson's diversity index	2·26 ± 0·32	2·31 ± 0·26	2·43 ± 0·24	2·33 ± 0·43
Litter load (kg ha^{-1})	13873 ± 1204	13655 ± 1866	13500 ± 199a	6169 ± 2393b
Duff load (kg ha^{-1})	22985 ± 2877	23034 ± 3146	23239 ± 218a	10100 ± 1760b

Values indexed by a different letter are significantly different at the $P \leq 0{\cdot}05$ level between paired control and treatment units for the same sampling year.

and site characteristics at the Fort Valley study site (1998) or at the Mt Trumbull study site (1999) (Tables 1 and 2). Following thinning treatments, there were significantly less trees per hectare and tree canopy cover in the thinned-only and thinned and prescribed burned units than their respective controls (Tables 1 and 2). At the Fort Valley study site, there were approximately 182 trees ha^{-1} in the thinned-only units and 1426 trees ha^{-1} in the paired controls. At the Mt Trumbull study site, there were approximately 336 trees ha^{-1} in thinned and prescribed burn units compared with 2193 trees ha^{-1} in the paired controls. Although the average herbaceous cover tended to be higher in treated units than in control units, the differences were not significant (Table 1). The herbaceous plant communities in the experimental units at Fort Valley were dominated by graminoids followed by non-legume forbs, legumes and woody shrubs. Similar abundance patterns were present at Mt Trumbull except there were more legumes than non-legume forbs. Sedges *Carex* spp. dominated the herbaceous cover for both study sites along with C_3 (a plant that produces a three-carbon molecule during photosynthesis) grasses, including squirreltail *Elymus elymoides* (Rafinesque) Swezey and muttongrass *Poa fendleriana* (Steudel) Vasey. Mountain muhley *Muhlenbergia montana* (Nuttall) Hitchcock was the only abundant C_4 (a plant that produces a four-carbon molecule during photosynthesis) grass in the experimental units at Fort Valley and it was consistently less common than the abundant C_3 grasses. There were no abundant C_4 grasses in the experimental units at Mt Trumbull.

Table 3. Mean substrate burn severity for Mt Trumbull thinned/prescribed burned units. Lightly burned areas had charred litter and duff, moderately burned areas had litter and duff mostly consumed, and severely burned areas have litter and duff consumed leaving white ash. n = 4 ± SEM, 10 replicates in each block

Severity level	Mean (%)
Not applicable (rock)	4·6 ± 3·18
Not burned	16·4 ± 7·77
Lightly burned	19·4 ± 7·66
Moderately burned	37·8 ± 11·15
Severely burned	21·0 ± 5·35

Approximately 21% of the area remained unburned following prescribed burning at Mt Trumbull (Table 3). Of the area that burned, approximately 20% was lightly burned, 38% moderately burned and 21% severely burned. There were no significant differences between soil properties in the treated and control units (Table 4). However, at both sites organic C and total N levels appeared to be higher in both treated units in comparison with their paired controls, and total P was slightly higher in the thinned and prescribed burned units at Mt Trumbull than their controls,

Table 4. Post-treatment soil characteristics (pH, organic C, total N, total P) for the Fort Valley control and thinned units and the Mt Trumbull control and thinned/prescribed burned units in 2000. Data are expressed as means (n = 2) ± SEM at Fort Valley and (n = 4) ± SEM at Mt Trumbull, 10 replicates in each block. All data were square-root transformed prior to analysis

	Fort Valley		Mt Trumbull	
Variable	CT	T	CTB	TB
pH	5·74 ± 0·11	5·7 ± 0·11	6·4 ± 0·02	6·40 ± 0·07
C (% loss on ignition)	8·81 ± 0·63	10·75 ± 1·19	10·97 ± 1·05	12·03 ± 1·43
Total N (mg N g^{-1})	1·38 ± 0·1	1·61 ± 0·16	2·04 ± 0·17	2·27 ± 0·31
Total P (mg P g^{-1})	0·93 ± 0·05	0·91 ± 0·03	1·20 ± 0·14	1·47 ± 0·20

Treatments were: control for Fort Valley thinned units (CT), Fort Valley thinned units (T), Mt Trumbull control for thinned/prescribed burned units (CTB), and Mt Trumbull thinned/prescribed burned units (TB).

Table 5. The relative amount of mycorrhizal propagules indicated by the percentage of the corn root length colonized with AM and the percentage of EM root tips in ponderosa pine bait-plant bioassays from the Fort Valley thinned-only and paired control units in 1999, 6 months after thinning, and 2000, 18 months after thinning. Data are expressed as means (n = 2) ± SEM

	Control		Treatment	
Mycorrhizae type	1999	2000	1999	2000
AM percentage colonized	21·28 ± 3·1	18·26 ± 4·4	44·6 ± 4·3	39·05 ± 4·6
EM percentage colonized	31·42 ± 2·7	34·77 ± 2·6	33·68 ± 3·1	37·0 ± 2·9

Table 6. F-ratios from MANOVA tests on the effects on treatment from the Fort Valley thinned-only and paired control units and time, 6 months after thinning, and 18 months after thinning for the relative amount of AM propagules and EM root tips. Data were analysed on angular-transformed data. Exact P-values are provided in parentheses

	Source of variation			
	Treatment	Time	Treatment × time	d.f.
AM percentage colonized	15·05 (0·0605)	6·78 (0·1211)	0·0097 (0.9305)	3
EM percentage colonized	1·72 (0·3198)	0·697 (0·4927)	1·01 (0·4199)	3

Table 7. The relative amount of mycorrhizal propagules indicated by the percentage of the corn root length colonized with AM and the percentage of EM root tips in ponderosa pine bait-plant bioassays from the Mt Trumbull thinned and prescribed burned, and paired control units for 2000, 5 months after thinning and 3 months after burning. Differences between the units were determined by ANOVA on angular-transformed data. Data are expressed as means (n = 4) ± SEM. Transformed data are presented in parentheses

Mycorrhizae type	Control	Treatment	P	d.f.
AM (% colonized)	17·75 ± 1·94 (0·3425 ± 0·027)	36·63 ± 5·4 (0·578 ± 0·026)	0·008	7
EM (% colonized)	32·82 ± 2·78 (0·576 ± 0·022)	34·73 ± 2·18 (0·606 ± 0·021)	0·3394	7

although none of these differences was statistically significant.

MYCORRHIZAL INFECTIVITY

Mycorrhizal fungi colonized all bait-plants and colonization levels were influenced by restoration treatments at both sites. At Fort Valley infective propagule densities of AM fungi were significantly higher in the thinned units than their paired controls for both study years (Tables 5 and 6). Infective propagule densities of AM fungi at Mt Trumbull were significantly higher in soils from the thinned and prescribed burned units in comparison with their paired controls (F = 39·13, P = 0·008; Table 7). Corn bioassay plants grown in the Fort Valley thinned-only soils had significantly more vesicles and less hyphae than corn grown in their paired control soils (F = 2·1601, P = 0·05; F = 5·4437, P = 0·001; Fig. 1a), whereas there was no difference between the relative proportion of AM fungal structures in the Mt Trumbull thinned and prescribed burned soils and their controls (Fig. 1b). Unlike the AM fungi, there was no significant difference in the relative amount of infective propagules of EM fungi in samples collected from treated units in comparison with their controls for 1999 and 2000 at Fort Valley

Table 8. Multiple regression analysis results for AM and EM infectivity and plant community characteristics for the six Fort Valley study units and eight Mt Trumbull study units combined. Graminoid cover was square-root transformed prior to analysis

Dependent variable and predictors	Coefficient	*t*	*P*	r^2	d.f.	*F*	*P*
Multiple regressions							
AM infectivity				0·853	4,13	13·12	0·0009
Constant	7·17	0·38	0·715				
Graminoid cover	15·53	3·05	0·014				
Tree canopy cover	–0·34	–2·5	0·034				
EM infectivity				0·029	3,10	0·101	0·9573
Constant	30·89	3·26	0·008				
Trees ha^{-1}	0·0004	0·18	0·861				
Tree canopy cover	–0·054	–0·46	0·655				
Litter cover	0·0804	0·52	0·617				

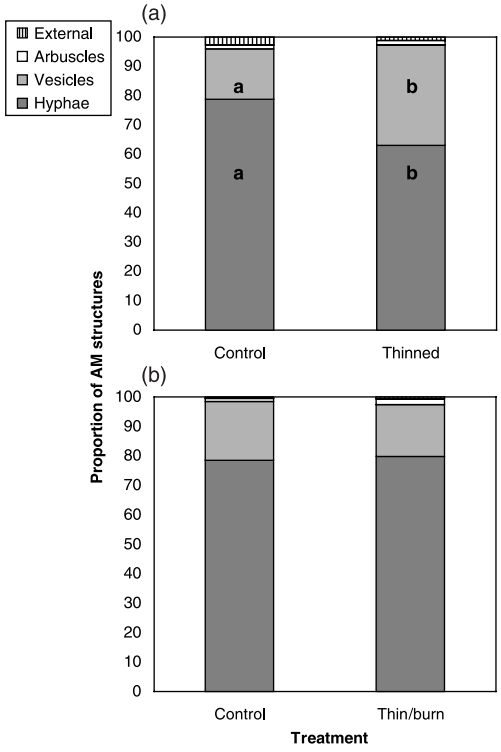

Fig. 1. The proportion of different AM fungal structures within colonized corn roots grown in soils collected from (a) the Fort Valley thinned-only and paired control units and (b) the Mt Trumbull thinned and prescribed burned and pair control units. Values indexed by different letters are significantly different at the $P = 0·05$ level between treatments.

(Tables 5 and 6) or at Mt Trumbull in 2000 ($F = 1·077$, $P = 0·3394$; Table 7). Sparsely branched bifurcated tips were the dominant EM morphotype for all stands.

AM colonization of the corn bait plants was not correlated with corn shoot weight or corn root weight ($r^2 = -0·037$ and $r = 0·031$, $P = 0·05$). EM colonization of the pine bait plants was not correlated with pine shoot weight ($r^2 = 0·11$, $P = 0·74$) but was positively correlated with pine root weight ($r^2 = 0·28$, $P = 0·04$). For all 14 study units combined, about 85% of the variance of AM infectivity was explained by graminoid cover (square-root cover) and tree canopy cover ($F = 13·12$, $P = 0·0009$; Table 8). In contrast, for all study units combined, trees per hectare, tree canopy cover or litter cover did not explain the variance of EM infectivity ($F = 0·101$, $P = 0·9573$; Table 8).

Discussion

During the first 2 years, restoration treatments had no significant effect on the overall cover of herbaceous AM plants, even though plant cover did increase in the restoration treatments. AM abundance was highly correlated with grass cover and therefore areas with higher grass cover (restoration treatments) also had higher propagule densities of AM fungi. Contrary to our expectations, restoration treatments did not change EM propagule densities, illustrating that even though their host-plant density was significantly reduced, EM fungi are able to maintain viable propagules for at least 2 years following thinning and 1 year following thinning and prescribed burning. Other studies have shown variable affects of tree thinning on EM fungal densities, depending on the disturbance intensity. For example, clear-cutting a Douglas-fir *Pseudotsuga menziesii* (Mirb.) Franco forest in western Montana significantly reduced the number of EM roots and affected adjacent uncut stands for at least 7·6 m into the stand (Harvey, Jurgensen & Larsen 1980). In contrast, pre-disturbance population densities of EM fungi were maintained for 2 years after clear-cutting an aspen *Populus tremuloides* Michaux stand (Visser, Maynard & Danielson 1998). Prescribed fire also has varied effects on EM fungi, ranging from dramatic reductions in propagule densities and diversity after intense wildfires (Visser 1995) to no significant changes (Baar *et al*. 1999). A study of a low intensity wildfire showed little change in EM fungal composition or densities (Jonsson *et al*. 1999). This study suggested that if the organic layer, where EM mycelia are concentrated, remains largely undamaged, then fire would have little or no impact on EM fungi. This explanation may account for the lack of changes in EM fungal propagule densities to restoration thinning and thinning and prescribed burning in this study, or there may have been a shift in the source of inoculum or species composition that we were unable to detect. Some studies have detected spores and

sclerotia as the dominant inoculum-types following burning and there is a shift at a high taxonomic level from basidiomycetes to ascomycetes post-fire (Baar *et al.* 1999; Grogan, Baar & Bruns 2000).

In contrast to EM fungi, AM fungi responded rapidly to restoration treatments in northern Arizona pine forests. Propagule densities of AM fungi significantly increased by an average of 20% in thinned-only and in thinned and burned units compared with control units. Two main processes control population densities of mycorrhizal fungi following disturbance: immigration of new propagules from nearby areas and survival and spread of residual propagules (Warner, Allen & MacMahon 1987). Rapid colonization of AM fungi has been illustrated in other studies. Gould, Hendrix & Ferriss (1996) found that AM propagule densities increased significantly within 9 months of mining reclamation and increased 10-fold the following spring after seeding. Similarly, Johnson & McGraw (1988) found that taconite tailings that were initially devoid of AM fungi were colonized within weeks of reclamation seeding. It was hypothesized that spores were transferred to the reclaimed tailings by both biotic (animals) and abiotic (wind and water) vectors (Johnson & McGraw 1988).

In the present study, it is likely that AM fungi spread from pre-existing mycorrhizal hyphae in living and dead plant roots. These live and dead root systems and their mutualistic mycorrhizal associations represent the mechanism for the bootstrapping hypothesis (Perry *et al*. 1989). The persistence of these associations under unrestored conditions (dense, closed forest canopies and high fuel loads) and their ability to respond rapidly to tree removal and prescribed fire suggests that AM associations may facilitate succession of this community to its previous dominance of herbaceous AM hosts. A study by Kovacic, St John & Dyer (1984) found that AM hosts and AM fungal abundance were significantly higher in mountain pine beetle *Dendroctonus ponderosae* Hopk.-killed ponderosa pine stands than under live ponderosa pine stands in northern Colorado. While mountain pine beetle outbreaks can be of epidemic proportions, their outcome in reducing live tree stand densities promotes changes in below-ground AM fungal densities and above-ground herbaceous production. Other studies in different ecosystems have also shown changes in AM propagule densities with changes in tree densities. A successional study of AM propagule densities across a grassland to forest chronosequence showed AM inoculum potential increased with increasing grass cover and decreased in later successional sites with EM trees (Johnson *et al.* 1991). Similarly, a study by Benjamin, Anderson & Liberta (1989) illustrated that herbaceous plants had lower AM colonization as tree density and shading increased, possibly because these plants had insufficient photosynthetic capability to support AM infection.

Soil disturbance has been reported to reduce AM propagule densities generally due to the destruction of the hyphal network during the break-up of the soil macrostructure (Reeves *et al*. 1979; Fairchild & Miller 1988). Consequently, one might predict that propagule densities should decrease following tree thinning, due to soil disturbance from mechanized logging equipment. This effect was not observed in our study, which indicates that either soil disturbance was not severe enough to have destroyed AM fungal propagules, that the sites were rapidly colonized by AM fungal spores and residual hyphae, or that the bioassay method may have missed any reduction. Because sunlight and photosynthetic capacity is greater in thinned stands, herbaceous host root densities probably increased with tree removal, which in turn would allow the herbaceous plant community to support more hyphae. Rives *et al*. (1980) also found no reduction in population densities of AM fungi following soil disturbance. It has been suggested that AM propagule densities in seasonal grasslands may be relatively tolerant of disturbance because their AM fungal communities produce high densities of perennating spores (Jasper, Abbott & Robson 1991).

Prescribed fire has variable effects on AM propagule densities, ranging from decreased AM propagule densities (Klopatek, Debano & Klopatek 1988; Dhillion & Anderson 1993), to no change (Anderson & Menges 1997; Rashid *et al*. 1997) to increases in AM propagule densities (Bentivenga & Hetrick 1991). Similar to soil disturbance, the effect of prescribed fire on AM propagules is highly dependent on the disturbance intensity. Because fire intensity can span three orders of magnitude (Pyne, Andrews & Laven 1996), soil is not heated uniformly during prescribed burns and therefore soil samples from burned areas are likely to contain highly variable levels of AM fungi, making the assessment of fire on AM fungi difficult. In our study, similar variance in the inoculum potential of soils from thinned-only and thinned-burned units suggests that these forests were not significantly affected by prescribed fires, which were mostly low-intensity burns of short duration. Approximately 40% of the Mt Trumbull study area was lightly burned or remained unburned following prescribed fire and approximately 38% was only moderately burned. At the Fort Valley study site, a stand that was treated with thinning and a low-intensity burn of short duration showed a 22% increase in AM propagule densities similar to the increases found at Mt Trumbull (J. E. Korb, unpublished data). In contrast, high-intensity burns of long duration in slash pile burning at the Fort Valley study site significantly reduced AM propagule densities by 98% (Korb 2001). Simulated laboratory fires have shown that heating field soil above 80 °C almost completely eliminates AM fungi (Pattinson *et al*. 1999). Understanding the maximum soil temperature and duration that AM fungi can withstand at different soil depths will be crucial to understanding the effects of prescribed fire on AM fungi propagule densities in south-western ponderosa pine forest restoration.

IMPLICATIONS FOR RESTORATION

Some evidence suggests that ponderosa pine understorey communities prior to the mid-1880s were dominated by warm-season (C_4) grasses, which are often strongly mycotrophic (Cooper 1960; Pearson, Mann & Howard 1971; Wilson & Hartnett 1998). However, current ponderosa pine forests are dominated by species that often form no mycorrhizal associations (e.g. *Carex geophila*) or C_3 grasses that are often weakly mycotrophic (Wilson & Hartnett 1998). These forests will probably continue to lack productive understorey communities, unless treatments are implemented that favour herbaceous host-plants and AM fungi by reducing tree canopy cover and litter cover. Noyd, Pfleger & Russelle (1995) demonstrated that strongly mycotrophic C_4 grasses big bluestem *Andropogon gerardii* Vitman and little bluestem *Schizachyrium scoparium* (Michaux) Nash were unable to grow or survive as seedlings in soil where AM fungi were eliminated, but establishment of the C_3 grass Canada wild rye *Elymus canadensis* L. was unaffected by AM fungi availability. Similarly, Hetrick *et al*. (1994) found that C_4 grasses were competitively superior to C_3 grasses when grown in the presence of AM fungi, but C_3 grasses were competitively superior in soils without AM fungi. As a result, one can predict that increased populations of AM fungi in the thinned-only and thinned and prescribed burned restoration stands may assist in the replacement of weakly mycotrophic species by strongly mycotrophic species, thereby increasing C_4 grass cover and leading to the maintenance of mycorrhizal-compatible species (Francis & Read 1994; Johnson 1998).

Enhancing AM propagules does not always result in a favourable community, as shown in a study by Marler, Zabinski & Callaway (1999) where AM fungi enhanced the competitive superiority of spotted knapweed *Centaurea maculosa* Lam. over a native bunchgrass. In addition, our results suggest that restoration thinning may have caused a shift in the species composition of the AM fungal community at the Fort Valley study site, where taxa that form abundant vesicles increased in the thinned treatments, while they did not increase at the Mt Trumbull study site even though the proportion of vesicles in the controls at these two sites were similar. These changes in AM species may directly influence above-ground plant community composition and structure because taxa of AM fungi differ greatly in their effects on plant fitness. Specifically, species of the Gigasporaceae family do not form vesicles and therefore an increase in vesicles could suggest a reduction in AM species of this taxon or an increase in other species that do readily form vesicles. Future studies of AM in ponderosa pine restoration areas will require analysis of the species composition of the fungal communities to determine if a shift in the fungal community is occurring in response to restoration treatments, and whether these shifts impact the plant community composition.

Recent research in a variety of environments has shown that mycorrhizal interactions may be important determinants of plant diversity, ecosystem variability and productivity (van der Heijden *et al*. 1998a,b; Hartnett & Wilson 1999; Klironomos *et al*. 2000). Host-plant species composition influences AM fungal species composition (Johnson, Tilman & Wedin 1992; Bever *et al*. 1996; Eom, Hartnett & Wilson 2000), which provides evidence for current feedback models between soil communities and plant community structure (Bever, Westover & Antonovics 1997). Future studies of ponderosa pine restoration treatment areas are needed to more fully understand responses of AM fungal communities and assess feedback between AM fungal and plant communities. Our results indicate that AM propagule densities respond rapidly to thinning and prescribed burning in south-western ponderosa pine restoration treatments. This has important implications for land managers trying to restore the herbaceous understorey of these forests because most understorey plants depend upon AM associations for normal growth.

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