Short-term effects of seasonal prescribed burning on the ectomycorrhizal fungal community and fine root biomass in ponderosa pine stands in the Blue Mountains of Oregon

J.E. Smith, D. McKay, C.G. Niwa, W.G. Thies, G. Brenner, and J.W. Spatafora

Abstract: The effects of seasonal prescribed fire on the belowground ectomycorrhizal community and live fine root biomass were investigated before, 1 year after, and 2 years after prescribed underburning. Ectomycorrhizas were sampled from four replications of three treatments (fall underburning, spring underburning, and a nonburned control) in a randomized complete block design. Samples were separated in two subsamples representing the upper 5 cm and lower 5 cm of a soil core. Molecular tools were used to distinguish 140 restriction fragment length polymorphism (RFLP) species of fungi directly from the ectomycorrhizas. Prior to underburning, the number of RFLP species and amount of live root biomass were similar among treatment units and between upper and lower core samples. Fall underburning largely removed live root biomass to a depth of 10 cm and significantly reduced ectomycorrhizal species richness compared with spring underburning and the nonburned control for at least 2 years. RFLP species richness and live root biomass following spring underburning were generally similar to the nonburned treatment. The successful reintroduction of fire to the ecosystem to retain high species diversity of ectomycorrhizal fungi and achieve the desired future condition of large-tree ponderosa pine retention with low fuel loads may require more than underburning in a single season.

Résumé : Les effets du brûlage dirigé saisonnier sur la communauté ectomycorhizienne dans le sol et la biomasse de racines fines vivantes ont été étudiés avant, 1 an après et 2 ans après un brûlage dirigé superficiel. Les ectomycorhizes ont été échantillonnées dans les quatre répétitions de trois traitements (brûlage automnal, brûlage printanier et témoin non brûlé) établis selon un dispositif en blocs aléatoires complets. Les échantillons ont été divisés en deux sous-échantillons représentant les parties supérieure (5 cm) et inférieure (5 cm) d'une carotte de sol. Des outils moléculaires ont été utilisés pour distinguer 140 espèces de champignons sur la base des RFLP directement à partir des ectomycorhizes. Avant le brûlage, le nombre d'espèces et la biomasse de racines vivantes étaient similaires entre toutes les parcelles expérimentales et entre les parties supérieure et inférieure des carottes de sol. Le brûlage automnal a pratiquement éliminé la biomasse de racines vivantes jusqu'à une profondeur de 10 cm et significativement réduit la richesse en espèces ectomycorhiziennes comparativement au brûlage printanier et au témoin non brûlé pendant au moins 2 ans. La richesse en espèces basées sur les RFLP et la biomasse de racines vivantes à la suite du brûlage printanier étaient généralement semblables au traitement non brûlé. La réintroduction réussie du feu dans l'écosystème dans le but de conserver une grande diversité d'espèces de champignons ectomycorhiziens et de recréer les conditions futures souhaitées pour conserver des pins ponderosa de forte dimension avec une faible quantité de combustibles pourrait exiger plus qu'un brûlage superficiel au cours d'une seule saison.

[Traduit par la Rédaction]

Introduction

In the Pacific northwestern region of North America, fire has historically occurred in all major forest types (Franklin and Dyrness 1984), occurring with greater frequency in the drier inland forests of ponderosa pine (*Pinus ponderosa* Dougl. ex Laws) (Agee 1993; Covington and Moore 1994; Heyerdahl et al. 2001). The frequent average fire return interval of approximately 10 years (Robbins and Wolf 1994), attributed to lightning strikes and the historical use of fire by Native Americans, maintained open stands of large fire-resistant ponderosa pine (Biswell 1989; Arno and Allison-Bunnell 2002). A series of uncontrollable wildfires between 1889 and 1910 prompted the fire suppression policy of the

Received 4 March 2004. Accepted 16 July 2004. Published on the NRC Research Press Web site at http://cjfr.nrc.ca on 21 December 2004.

J.E. Smith,[1] D. McKay, C.G. Niwa, and W.G. Thies. US Department of Agriculture, Forest Service, Pacific Northwest Research Station, Forestry Sciences Laboratory, 3200 Jefferson Way, Corvallis, OR 97331, USA.
G. Brenner. Pacific Analytics, P.O. Box 219, Albany, OR 97321, USA.
J.W. Spatafora. Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331, USA.

[1]Corresponding author (e-mail: jsmith01@fs.fed.us).

 doi: 10.1139/X04-124

United States Forest Service (Biswell 1989; Arno and Allison-Bunnell 2002). Fire suppression for nearly a century, livestock grazing, insect infestations, climate change, and logging of the largest trees have led to ponderosa pine stands with increased densities of small trees and unusually high fuel loads (Langston 1995; Bachelet et al. 2003).

Prescribed underburning is a major component of the restoration effort underway in low and mid-elevation ponderosa pine forests in the Blue Mountains of Oregon and Washington to reduce fuels and reverse changes in plant species composition (Langston 1995; Scott 2002). Native plants in these forests can withstand frequent low-intensity fire. Fungi associated with ponderosa pine are likely also adapted to low-intensity fire. However, knowledge of their belowground structure and composition and response to prescribed fire is limited. This knowledge is important because ponderosa pine forms an obligate association with the hyphae of ectomycorrhizal (EM) fungi for efficient nutrient uptake, resistance to drought stress, and protection against some root pathogens (Smith and Read 1997; Read 1998). Most EM fungi are able to colonize multiple host genera, but a few are highly specific to a single host genus such as *Pinus* (Molina et al. 1992; Massicotte et al. 1994, 1999; Horton and Bruns 1998). Arbuscular mycorrhizas may initially colonize species in the Pinaceae (Cázares and Trappe 1993; Cázares and Smith 1996; Horton et al. 1998) and provide nutrients to establishing seedlings (Smith et al. 1998).

Fire, whether prescribed or natural, influences EM community dynamics and succession in coniferous forests to varying degrees depending on intensity and the length of time since fire (Visser 1995; Jonsson et al. 1999; Dahlberg et al. 2001; Horton and Bruns 2001). Fire behavior and effects on soil are influenced by fuelbed structure including duff (decaying organic material) and down wood (Sandberg et al. 2001). Fires of low intensity that leave the organic soil horizons (functionally equivalent to duff) relatively undamaged do not appear to substantially alter EM community composition (Jonsson et al. 1999), whereas fires of high intensity that remove the organic layer and detrimentally burn the mineral soil significantly affect EM community composition (Visser 1995; Baar et al. 1999; Grogan et al. 2000; Dahlberg et al. 2001). Aboveground ecosystem recovery after fire is directly linked to the survival of EM fungi (Perry et al. 1989) that reside mostly in the surface layers of mineral soil and organic matter (Harvey et al. 1986; Swezy and Agee 1991).

Historically, wildfires occurred during the summer and fall months, when fuels are dry and lightning strikes are abundant (Agee 1993). Currently, most prescribed fires are conducted in the spring because there is a long period when fuel and weather conditions permit burning with minimal risk of uncontrolled fires (Scott 2002). Spring burns have higher fuel moisture levels and, therefore, differ substantially from fall burns. The combination of actively growing roots, moist soils with their ability to readily conduct heat, and the low soil temperatures to which roots are adapted after winter may amplify the heat effects of spring underburning (Swezy and Agee 1991).

This study is the first to investigate the response of the ponderosa pine EM community to seasonal underburning. In this study, the EM community is defined as the frequency and abundance of EM fungi colonizing the roots of ponderosa pine. Our objectives were to (*i*) quantify the effect of underburning in fall or spring on the EM fungal community and live fine root biomass; (*ii*) assess the importance of duff depth as a measure of treatment impact; and (*iii*) determine whether the number of EM species and the biomass of live fine roots differ by depth in mineral soil in response to seasonal underburning.

Materials and methods

Description of study site

Research was conducted on the Emigrant Creek Ranger District of the Malheur National Forest, about 30 km north of Burns, Oregon (43.5°N, 118.5°W). Stands within the study area are at 1600–1700 m elevation, with slope gradients from 7% to 23% and soils within the Chernozemic order. Stands contain ponderosa pine as the only EM host species in the Pinaceae; mountain-mahogany (*Cercocarpus ledifolius* Nutt.) also forms ectomorrhizas and occurs on rocky outcrops. The selected stands encompassed a range of plant association groups for the ponderosa pine zone (Franklin and Dyrness 1984), with mountain big sage (*Artemisia tridentata* Nutt.), creeping Oregon-grape (*Berberis repens* Lindl.), and elk sedge (*Carex geyeri* Boott) in the understory. Stands are composed mostly of second-growth ponderosa pine overstory with occasional large residual trees left from logging during the first half of the 20th century. The oldest ponderosa pines on the site are 100–200 years old. Stands contained 200–300 trees·ha^{-1}; tree diameters at breast height (DBH) ranged from 10 to 75 cm, and basal area ranged from 9 to 23 m^2·ha^{-1}.

The site, dry in the summer, received a monthly average of 0.12 cm of rain between June and October in 1997 to 1999, and above-average monthly rainfall January to June in 1998 (Oregon Climate Service 1971–2000). Maximum temperatures averaged 27 °C in the summer (June to September 1997 to 1999) and 4 °C in the winter (November to February 1997 to 1999) (Oregon Climate Service 1971–2000).

Fall prescribed underburning occurred in mid-October 1997 and the spring-prescribed underburning in mid-June 1998. Fires were uniformly ignited in a multi-strip ignition pattern with hand-carried torches, designed to maintain a 60-cm flame length. Weather conditions at the time of the burns were similar for the two seasons (temperature: fall, 17–21 °C; spring, 16–21 °C; relative humidity: fall, 26%–35%, spring, 30%–40%; wind speed: fall, 5–6 km·h^{-1}, spring, 3–11 km·h^{-1}). About 55% of the area was burned in both spring and fall underburning treatments, and most trees experienced bark or crown scorch varying from slight to severe (Thies et al. 2001). Basal area, measured four growing seasons after underburning, decreased by about 20% in the fall underburning compared to about 6% in the spring underburning. Seedling regeneration after underburning was sparse, suggesting that roots sampled were mostly from mature trees.

Experimental design and sampling procedures

The study is a randomized complete block design with four replications (blocks) divided into three treatment units. Treatments (fall underburning, spring underburning, and a nonburned control) were randomly assigned to units (approx.

10 ha in size) within blocks. The blocks are designated as Kidd Flat, Trout, Driveway 14, and Driveway 17. The distance between Kidd Flat and Trout is about 3.2 km. These two blocks are 14.5 km west of the two Driveway blocks. The distance between the two Driveway blocks is about 1 km. Treatment unit selection within each block was based on similar stand type, soil type, slope, and aspect.

In July 1997, before the underburning treatments were applied, six soil cores (5 cm diameter × 10 cm depth) were collected on each treatment unit (6 cores × 12 treatment units = 72 soil cores; area sampled: 0.005 $m^2 \cdot ha^{-1}$). One 240-m permanent transect line was established with six permanent plots (approximately 40 m apart) per treatment unit. The ponderosa pine closest to each 40-m mark with DBH ≥ 20 cm was marked with an aluminum tag. One soil core was taken due east of each tree at the tree canopy edge. In July 1998 and 1999, after the underburning treatments were applied (1 and 13 months after spring underburning; 9 and 22 months after fall underburning), three soil cores were collected on each treatment unit (3 cores × 12 treatment units × 2 posttreatment years = 72 cores; area sampled: 0.002 $m^2 \cdot ha^{-1} \cdot year^{-1}$). The three posttreatment soil core locations were randomly selected from the six pretreatment locations and soil cores were collected within 10–20 cm of the pretreatment soil core locations. All the soil cores were separated in two subsamples representing the upper 5 cm and the lower 5 cm of the core. Rocky soils restricted coring to depths deeper than about 10 cm. For the 1997 pretreatment sampling, the six upper core samples from each treatment unit were combined to make a composite upper sample and the six lower core samples from each treatment unit were combined to make a composite lower sample. From the pretreatment sampling, it was determined that composite samples increased the difficulty of sorting ectomycorrhizas into distinct morphological groups. Composite samples were not made for the 1998 and 1999 posttreatment sampling of the soil cores. Duff depth was measured to the nearest 0.5 cm and recorded at each soil core location. The duff layer was removed prior to coring so that a consistent depth was sampled in mineral soil both before and after the prescribed fire treatments. Sporocarps were opportunistically collected on the study site at sample times and from throughout the Blue Mountains from 1997 to 2001 to help determine identities of ectomycorrhizas.

Sorting and processing of ectomycorrhizas

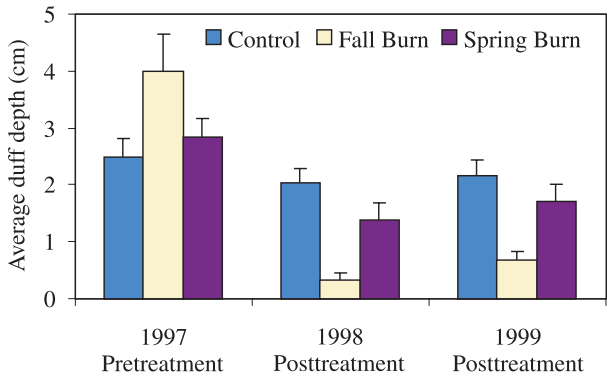
Soil cores were transported to and stored in the laboratory at 4 °C. Soil core samples were soaked in water to loosen debris, then rinsed through a soil sieve with a mesh size of 0.5 mm (No. 35 USA standard testing sieve). Care was taken to rinse all soil from EM root tips. Compound microscopy was used to sort ectomycorrhizas into morphological types (morphotypes) based primarily on color, mantle surface texture, rhizomorph features, and branching pattern (Agerer 1994). Brief descriptions of morphotypes were recorded. Viability assessment of the root tips was based on color and turgidity (Harvey et al. 1976). Those lacking root hairs and well- developed mantles were considered ectendomycorrhizal rather than non-mycorrhizal, and were separated on the basis of color (mostly shades of red-brown) for molecular analysis. Ectomycorrhizas of a single morphotype from a given core sample were placed in individual plastic centrifuge tubes (1.5 mL), quick-frozen in liquid nitrogen, and immediately lyophilized. Morphotype samples were lyophilized within 3 weeks after collection and weighed to the nearest 0.0001 g. In the second posttreatment collection, two root tips from each morphotype within a core sample were placed directly in cetyltrimethyl ammonium bromide (CTAB) buffer and stored at 4 °C. This slight variation in protocol from the previous processing times improved DNA amplification success from the initial two root tips, thereby decreasing the need to extract DNA from additional ectomycorrhizas within the sample. Ectomycorrhizas with slight differences in appearance were separated into several morphotypes and later pooled for data analysis if warranted by identification with molecular techniques. In cases where the two root tips representing a morphotype sample differed, molecular data were obtained for up to 10 additional root tips and the sample biomass apportioned accordingly.

Molecular techniques

DNA extraction, polymerase chain reaction (PCR) amplification, and restriction fragment length polymorphism (RFLP) protocols followed Gardes and Bruns (1993). DNA was extracted individually from at least two root tips from each morphotype within a core sample and also from small pieces of collected sporocarps. Some morphotype groups did not yield PCR product, even after DNA extraction was attempted from up to 10 roots, and were considered not viable. Because these data would potentially overestimate total richness and live fine root biomass, data are presented only for morphotype groups for which molecular data were obtained. Averaging over all sample dates, it was found that more than 80% of the nearly 600 morphotype sample groups yielded PCR product.

Identifications of fungal symbionts were based on PCR amplification of the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA using the fungal specific primer pair ITS-1f/ITS-4 (Gardes and Bruns 1993). The ITS region was characterized by RFLP analysis, which was used to match ectomycorrhizas to one another and to sporocarps collected from the study site and from the Blue Mountains of Oregon. Three restriction enzymes (*Alu*I, *Dpn*II, and *Hin*fI) in single enzyme digests were used to characterize and match fungal ITS-RFLP patterns. Restriction fragments were subsequently separated on agarose gels (3% agarose) and visualized with ethidium bromide under ultraviolet light. Band sizes were measured against a 100-bp DNA ladder using Scanalytics, Inc., Gene Profiler software, which has about a 5%–10% error in size estimation of RFLP bands. All RFLPs were recorded by AlphaImager™, and photos were taken by Sony® Digital Graphic Printer UP-D890. After visual assessment of the RFLP patterns and comparisons of morphotype descriptions and scores of *Dpn*II and *Hin*fI from all photographed images, samples potentially matching were run in adjacent lanes of the same agarose gel with all three restriction enzymes in single enzyme digests. Identical RFLP matches with digests for all three endonucleases determined species-level identification. Restriction fragments resulting from restriction enzyme digest with *Alu*I were used to confirm the matching of samples, but typically were not scored. RFLP types with similar fragment patterns from two enzymes were occasionally split by the third enzyme. There is

Fig. 1. Average duff depth before and after seasonal underburning treatments for ponderosa pine stands. Error bars represent 1 SE at the 95% confidence interval.



a general correspondence between ITS-RFLP types and species (Gardes and Bruns 1996; Kårén et al. 1997; Horton 2002).

Few morphotype RFLP patterns matched those of sporocarps in our database. Therefore, taxonomic identifications were attempted for the majority of RFLP types by obtaining sequences from one or two different DNA regions: (*i*) both spacers of the ITS region of the nuclear ribosomal repeat and the intercalated 5.8S rRNA gene using primer pair ITS-1f/ITS-4 for amplification; and (*ii*) an approximately 400-bp fragment of the mitochondrial large subunit rDNA using primer pair ML-5/ML-6 for amplification (Bruns et al. 1998). Two sequences were typically obtained for RFLP types with samples from multiple core locations. Such confirmation of RFLP species provided a high level of confidence in the ability of the molecular tools to determine actual species. PCR products were cleaned using the Qiagen® QIAquick PCR Purification Kit™ or Qbiogene Geneclean®. Gel electrophoresis was used to verify recovery after purification of 5 μL of purified PCR product; DNA concentrations were estimated by comparison of band intensity to standards, and adjusted to 50 ng before the samples were sent to the Center for Gene Research and Biotechnology at Oregon State University for sequencing on an ABI 377 automated sequencer. The resulting sequences were edited in SeqEd (PE Biosystems) and aligned manually using the PAUP 3.1 (Swofford 1993) and PAUP* (Swofford 1999) and a color font. A sequence similarity search of the National Center for Biotechnology Information (NCBI) database, GenBank, was conducted using Basic Local Alignment Search Tool (BLAST) 2.0 algorithm.

Analysis

All computations were carried out with S-Plus version 2000 software (MathSoft 1988–1999).

Ectomycorrhizal species richness, community patterns, and response to underburning

A Poisson log-linear regression, suitable for modeling count data (McCullagh and Nelder 1991), was used to analyze count data of RFLP species tallied by treatment unit and by core sample depth. Poisson analysis of deviance (ANODEV) tables were derived from the Poisson log-linear models of changes in the number of RFLP species explained by block, treatment (fall underburning, spring underburning, control), and depth (upper, lower) (Hastie and Pregibon 1992). A χ^2 test was used to test for the effects of factors and their interactions, using the deviance attributed to the terms and their degrees of freedom (Hastie and Pregibon 1992).

Ectomycorrhizal RFLP species similarity in space and time

The Sorenson index (SI), designed to equal 1 in cases of complete similarity and 0 if the cases are dissimilar and have no species in common, was used to measure â diversity (Magurran 1988) for RFLP species between all pairs of treatment combinations: (*i*) years, (*ii*) blocks, (*iii*) treatment units, and (*iv*) year.

Ectomycorrhizal root biomass

EM root biomass for each treatment unit was summed across core samples, averaged across blocks for each treatment (fall underburning, spring underburning, control) and depth (upper, lower), and analyzed in an analysis of variance (ANOVA) for each collection year.

Duff depth

To assess whether the depth of duff differed among treatments, we averaged duff depth for each core within treatment units and across blocks for every treatment and analyzed in an ANOVA for each collection year.

Results

Duff depth

Prior to underburning, duff depth was slightly greater in the fall underburning treatment units compared to the control and spring underburning treatment units ($F_{[2,30]} = 3.12$, $p = 0.059$) (Fig. 1). Underburning significantly reduced duff depth on the fall underburning treatment units compared to the control and spring underburning treatment units in both post treatment years (1998: $F_{[2,30]} = 20.12$, $p = 0.0001$; 1999: $F_{[2,30]} = 10.82$, $p = 0.0003$) (Fig. 1).

Ectomycorrhizal species richness, community patterns, and response to underburning

One hundred and forty RFLP species were distinguished on the roots. Of these, 10 matched sporocarps based on RFLP patterns or sequences and an additional 59 were identified to family or genus and 11 to order or class (Table 1). The majority of the identified RFLP species belonged to the Cortinariaceae (30 species), Thelephoraceae (13 species), and Boletaceae (7 species) (Table 1). One hundred and fifteen RFLP species (82%) were found in lower core samples, 88 (63%) in upper core samples and 61 (43%) in both soil core depths. All recurring RFLP species detected in four or more cores (22 species in total) were found in both soil core depths. The cumulative number of RFLP species continued to increase with each sample time, although 40% fewer new RFLP species were found in the second posttreatment year compared to the first. During the course of the study, most (56%) RFLP species were detected in only one treatment unit and 21% were detected in three or more treatment units. The annual occurrence of the 18 frequent RFLP species

Table 1. Annual occurrence within treatment units (n = 12) of ectomycorrhizal restriction fragment length polymorphism (RFLP) species on ponderosa pine identified by polymerase chain reaction (PCR)–RFLP or by nucleotide sequencing.

Taxonomic identification	1997 (Pre)	1998 (Post)	1999 (Post)	Restriction enzyme	
				*Dpn*II	*Hin*fI
Basidiomycetous fungi					
Amanita sp. (rflp 182)†	0	0	1	417/247	355/300
Amphinema sp. (rflp 95)†	0	1	0	375/203	301/268
Basidiomycete (rflp 52)†	1	0	0	396/172	344/311
Basidiomycete (rflp 56)†	1	0	0	406/195	334/273
Basidiomycete (rflp 89)†	0	2	1	355/135	317
Basidiomycete (rflp 115)†	0	2	0	415/280	410/314
Basidiomycete (rflp 154)†	0	4	3	244/215/175	374/310
Basidiomycete (rflp 174)†	2	0	3	399/326	420/193/116
Basidiomycete (rflp 206)†	0	0	1	390/190	315/160/150
Basidiomycete (rflp 224)†	1	0	0	397/232	464/379
Cortinarioid (rflp 44)†	8	1	0	392/208	352/236
Cortinarioid (rflp 178)†	0	0	1	397/160/99	400/330
Cortinarius sp. (rflp 113) #7070‡	0	2	0	409/192	328
Cortinarius sp. (rflp 117) #7204‡	0	3	3	396/194	314
Cortinarius sp. (rflp 40)†	2	0	0	311/173/128	368/168
Cortinarius sp. (rflp 42)†	7	0	0	320	387/182
Cortinarius sp. (rflp 81)†	0	1	0	256/146	395/326
Cortinarius sp. (rflp130)†	0	1	0	394/162/95	386/314
Cortinarius sp. (rflp139)†	0	2	1	394/191	312/292
Dermocybe sp. (rflp 119)†	0	1	0	426/172/112	383/312
Hebeloma sp. (rflp 128)†	0	2	1	432/273	394/333
Hebeloma sp. (rflp 207)†	0	0	1	Not scored	475/400/340
Hygrophoroid (rflp 177)†	0	0	2	400/198	313
Inocybe sororia (rflp 121) #7079‡	0	1	0	411/245	400/326
Inocybe sp. (rflp 17) #7083‡	4	3	0	400/230	379/319
Inocybe sp. (rflp 1)†	2	0	0	245/214/176	388/320
Inocybe sp. (rflp 117)†	0	1	0	419/251	403/332
Inocybe sp. (rflp 148)†	0	1	1	400/280	415/330
Inocybe sp. (rflp 151)†	0	0	1	210/169	346/307
Inocybe sp. (rflp 18)†	3	0	0	413/239	375/316
Inocybe sp. (rflp 194)†	0	0	1	393/260	315/189
Inocybe sp. (rflp 21)†	6	0	0	422/278	420/321
Inocybe sp. (rflp 22)†	1	0	0	445/303	445/348
Inocybe sp. (rflp 24)†	2	0	0	212/173	345/305
Inocybe sp. (rflp 55)†	2	1	3	400/260	320/195
Inocybe sp. (rflp 58)†	1	0	0	402/228	370/311
Inocybe sp. (rflp 61)†	4	1	0	403/229	418/312
Inocyboid (rflp 125)†	0	2	0	413/265	306/286/210
Inocyboid (rflp 134)†	0	1	0	423/267	321/193
Inocyboid (rflp 14)†	2	0	0	383/249/185	291/222/169
Inocyboid (rflp 16)†	2	0	0	397/266	477/371
Inocyboid (rflp 97)†	0	1	0	385/254	309/221/173
Lactarius deliciosus (rflp 84) #7010†‡	2	1	0	273/197/149/119	382/296/88
Piloderma sp. (rflp 75)†	0	1	0	220/168	345/300
Piloderma sp. (rflp 93)†	0	2	0	376/194	284/251
Piloderma sp. (rflp 116)†	0	6	4	374/210	341/214/78
Pseudotomentella sp. (rflp 187)†	0	0	1	451/232	371
Ramaria sp. (rflp 19)†	0	0	1	323/211	333/237/100
Rhizopogon sp. (rflp 158) #7008†‡	0	0	1	255/235/182	447/255/134
Rhizopogon salebrosus (rflp 78) #7018‡	7	1	4	246/230/156	243/230/128/107
Suilloid (rflp 15)†	2	0	1	397/255	401/316
Suilloid (rflp 77)†	0	2	0	244/162	232/127
Suilloid (rflp 118)†	0	2	0	242/169	249/247/166/130
Suilloid (rflp 177)†	0	1	0	402/195	310

Table 1 (*continued*).

Taxonomic identification	1997 (Pre)	1998 (Post)	1999 (Post)	Restriction enzyme	
				*Dpn*II	*Hin*fI
Suillus pseudobrevipes (rflp 152) #5010‡	1	0	3	233/160/142	223/190/124/75
Thelephoroid (rflp 9)†	2	0	0	274/197/145/111	387/306
Thelephoroid (rflp 10)†	2	0	0	270/192/178	408
Thelephoroid (rflp 13)†	1	0	0	380/233	177/147
Thelephoroid (rflp 28)†	1	0	0	230/152	390/375
Thelephoroid (rflp 30)†	3	1	0	263/234/173	398/320
Thelephoroid (rflp 73)†	0	1	0	213/147	342
Thelephoroid (rflp 94)†	0	1	2	374/222	188/170/150
Thelephoroid (rflp112)†	0	1	0	403/264	410/312/170
Tomentella sp. (rflp 3)†	2	0	0	227/147	347/175
Tomentella sp. (rflp 4)†	1	0	0	226/143	342
Tomentella sp. (rflp 161)†	0	0	1	280/225	350/325
Tomentella sp. (rflp 167)†	0	0	1	360/215	344
Tricholoma sp. (rflp 32)†	1	1	1	265/237/187	398/333
Tricholoma sp. (rflp 83)†	4	5	5	255/227/170	390/321
Ascomycetous fungi					
Ascomycete (rflp 11)†	3	0	0	312/203	328/271
Cenococcum-like (rflp 20)*	1	0	0	390/230	325/198
Cenococcum-like (rflp 34)*	3	0	0	298/193	305/243
Cenococcum (rflp 36)†	1	1	0	298/147	154/122/99
Cenococcum-like (rflp 131)*	0	2	2	427/295/135/113	570/150
Cenococcum-like (rflp 162)*	0	0	2	295/243/185/147	410/261/248
Cenococcum-like (rflp 228)*	0	3	0	240/112	268/160/137
Helotiales (rflp 35)†	2	0	0	310/197	320/260
Otidea sp. (rflp 114) #7001‡	0	2	0	414/339	432/206/123
Pezizales (rflp 221)†	0	0	1	250	190/170/150
Wilcoxina rehmii (rflp 87)†‡	2	3	3	311/211	275/217/138
Unknown fungi					
Unknown (rflp 2)	4	0	0	193/125	266/222
Unknown (rflp 5)	2	0	0	251/186/83	459/262/145
Unknown (rflp 6)	1	0	0	400/302/190	320/260/95
Unknown (rflp 12)	1	0	0	305/196	331/264
Unknown (rflp 23)	4	0	0	190/120	268/225/111
Unknown (rflp 25)	2	0	0	217/173	349/205/114
Unknown (rflp 26)	2	0	0	236/216	345/176
Unknown (rflp 27)	1	0	0	412/255/244	398/372/320
Unknown (rflp 38)	1	0	0	306/202/153	316/251
Unknown (rflp 39)†	2	0	0	313/183	329/113/95
Unknown (rflp 43)	1	0	0	360/249	358/227/118
Unknown (rflp 45)	2	0	0	412/273	433/335
Unknown (rflp 46)	1	0	0	425/250	325
Unknown (rflp 47)	2	0	0	403/193	323/311/270
Unknown (rflp 51)	1	0	0	222/147	407
Unknown (rflp 59)	1	0	0	339/232	362/236
Unknown (rflp 60)	1	0	0	259/202	378/329
Unknown (rflp 64)	1	0	0	422/281	410/324
Unknown (rflp 67)	2	0	0	329/216	230/168
Unknown (rflp 70)	1	0	0	414/239/185	422/320/263/233
Unknown (rflp 72)	1	0	0	442/291	340/209
Unknown (rflp 74)	1	0	0	250/240/190	500/280/160
Unknown (rflp 79)	0	1	0	246/148	471/210/239
Unknown (rflp 82)	0	1	0	256/217/206	378/345
Unknown (rflp 86)	0	2	0	303/249/191/155	413/244
Unknown (rflp 88)	0	1	0	336/203/149	318/206/142/100
Unknown (rflp 90)	0	1	0	359/308/186/160	274/166/108

Table 1 (*concluded*).

Taxonomic identification	1997 (Pre)	1998 (Post)	1999 (Post)	Restriction enzyme	
				*Dpn*II	*Hin*fI
Unknown (rflp 101)	0	1	0	215/188/148/121	449/341
Unknown (rflp 102)†	0	1	0	400/213	315/183/154
Unknown (rflp 103)	0	1	0	395/199	321/297
Unknown (rflp 104)	0	1	1	305/205/195	325/265/225/175
Unknown (rflp 109)	0	1	0	397/322	427/197/107
Unknown (rflp 110)	0	1	0	401/189	318/174/130
Unknown (rflp 124)	0	1	0	413/202	318/155/144
Unknown (rflp 126)	0	1	0	422/281/241	414/314
Unknown (rflp 129)	0	1	0	432/277	418/318
Unknown (rflp 133)	0	1	0	254/164/147/115	232/126/115
Unknown (rflp 141)	0	1	0	368/140/120	200/190/180
Unknown (rflp 142)	0	2	0	400/340	410/205/105/100
Unknown (rflp 144)	0	1	0	350/200/120	450/205/130
Unknown (rflp 145)	0	1	0	200/120/97	280/230
Unknown (rflp 146)	0	2	0	303/160	315/170/100
Unknown (rflp 150)	0	1	0	400/280	315/225/175
Unknown (rflp 156)	0	0	1	245/183/115	173/152/130
Unknown (rflp 160)	0	0	1	265/167/148/108	239/224/113
Unknown (rflp 163)	1	0	1	300/245/196/150	325/258/239/120
Unknown (rflp 165)	0	0	1	334/120	321/300
Unknown (rflp 168)	0	0	1	362/209/118	447/189/129
Unknown (rflp 170)	1	0	1	365/218	180/157/130
Unknown (rflp 181)	0	0	1	381/198	333/297
Unknown (rflp 186)	0	0	1	416/267	379/328
Unknown (rflp 196)	0	0	1	305/270/124	374
Unknown (rflp 197)†	0	0	1	398/180	345/320
Unknown (rflp 202)	0	0	1	300/280/180	315/125/75
Unknown (rflp 209)	0	0	1	Not scored	405/270/250
Unknown (rflp 210)	0	0	1	280/225/180	380/290
Unknown (rflp 216)	0	0	1	310/200	330/270/225/160
Unknown (rflp 220)†	0	0	1	261/224/186	371/310
Unknown (rflp 222)	0	0	1	410/273	394/320
Unknown (rflp 226)	1	0	0	197/156	275/225/110

Note: Numbers in columns for restriction enzymes *Dpn*II and *Hin*fI are the DNA fragment sizes (in base pairs) after the PCR product was cut with that enzyme. Fungi were amplified with ITS1-F and ITS-4. For some unknown fungi, a clean sequence was obtained but not well supported by a match in GenBank. Fungi identified as basidiomycete or ascomycete produced a clean sequence supported by only a partial match to one or more Basidiomycetous or Ascomycetous genera. Voucher collection numbers given are for sporocarps that matched fungi from mycorrhizas.

†RFLP species identified by nucleotide sequencing of partial regions of the mitochondrial DNA and (or) ribosomal DNA.

‡RFLP species identified by RFLP-matching of EM and sporocarp.

*RFLP species tentatively identified based on distinctive morphology.

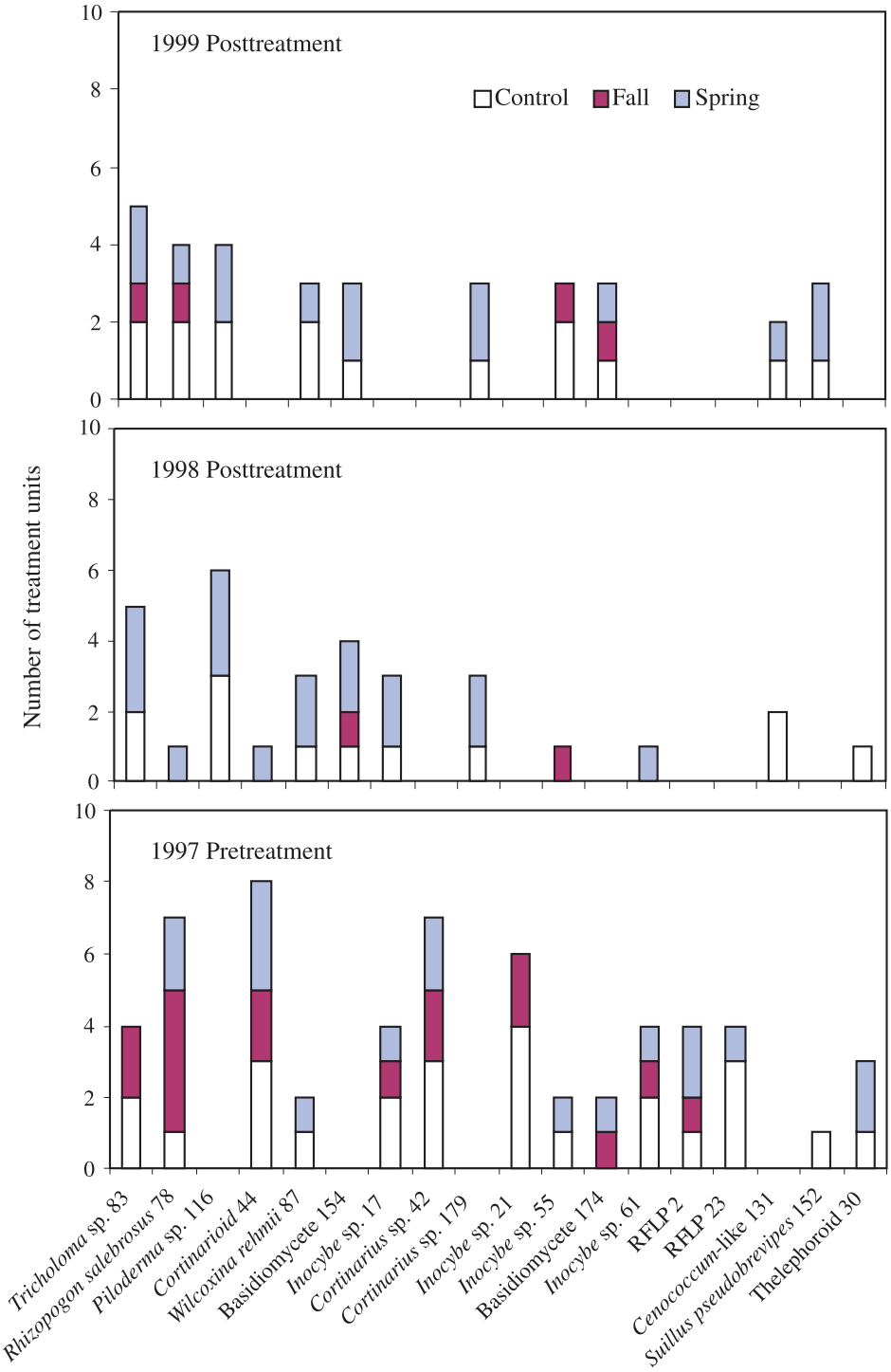
(those detected in four or more treatment units) is presented by treatment in Fig 2. These results indicate a mycorrhizal community consisting of a large number of RFLP species scattered at low frequencies across the site before and after prescribed fires.

Prior to underburning, the number of RFLP species was similar among treatment units ($\chi^2_{(2)} = 2.43$, $p = 0.296$) and between upper and lower core samples ($\chi^2_{(1)} = 2.28$, $p = 0.131$) (Table 2). After underburning, the number of RFLP species differed among treatments in both posttreatment years (1998: $\chi^2_{(2)} = 35.09$, $p = 0.0001$; 1999: $\chi^2_{(2)} = 31.93$, $p = 0.0001$) (Table 2). The spring underburning and control treatment units had about six times more RFLP species than the fall underburning treatment units in both posttreatment years (Table 2). There was no difference in the number of RFLP species between soil sample depths in either the first ($\chi^2_{(1)} = 1.47$, $p = 0.225$) or second ($\chi^2_{(1)} = 0.38$, $p = 0.536$) posttreatment year, nor was there evidence of an interaction between treatment and core sample depth (1998: $\chi^2_{(2)} = 0.50$, $p = 0.780$; 1999: $\chi^2_{(2)} = 2.14$, $p = 0.343$).

Ectomycorrhizal species similarity in space and time

Persistence of RFLP species through time was evident from 20 species recurring within particular blocks for at least 2 years, and three species (*Rhizopogon salebrosus* 78 A.H. Sm., *Tricholoma* sp. 83, and *Wilcoxina rehmii* 87 Yang & Korf) were detected within particular blocks for all 3 years. In addition to these three RFLP species, *Inocybe* sp. 55 was also found all 3 years (Fig. 2), but not within the same block. Ten and 11 RFLP species were common between the pretreatment and posttreatment years one and two, respectively (SI = 0.18); 15 RFLP species were common to the

Fig. 2. Pre- and post-treatment occurrence within treatment units (n = 12) for the 18 most frequent ectomycorrhizal species. Species are listed in descending order of total frequency.



post-treatment years (SI = 0.27). Most (10) of the RFLP species recurring in both post-treatment years occurred in neighboring cores from control and spring underburning treatment units.

RFLP species similarity across the landscape was evident from the relatively high similarity between a pair of distant blocks (Trout and Driveway 17, SI = 0.38), as well as between a pair of nearby blocks (Trout and Kidd Flat, SI = 0.35). The number of RFLP species in common between pairs of blocks ranged from 9 to 24 (SI = 0.23–0.38). Five species (Cortinarioid 44, *Inocybe* sp. 21, *Rhizopogon salebrosus* 78, *Tricholoma* sp. 83, and RFLP 23) were detected in all four blocks. In addition, similarity was evident between treatment units, with all pair combinations sharing at least 1, and as many as 13, RFLP species (SI = 0.06–0.49).

Posttreatment fall underburning comparisons contained few total RFLP species (6 and 5 RFLP species in posttreatment

Table 2. Means and standard errors (SE) for number of restriction fragment length polymorphism (RFLP) species and live root biomass in stands of ponderosa pine before and after seasonal underburning.

		Pretreatment	Posttreatment	
Treatment	Depth (cm)	1997	1998	1999
No. of RFLP species				
Control	0–10	13.00 (2.12)	11.25 (2.50)	9.75 (2.75)
Fall underburning	0–10	9.50 (2.63)	1.75 (0.85)	1.25 (0.63)
Spring underburning	0–10	10.25 (1.11)	10.00 (1.87)	7.75 (2.87)
Upper	0–5	5.83 (0.93)	4.25 (1.16)	3.67 (1.10)
Lower	5–10	7.42 (0.58)	5.33 (1.12)	4.17 (0.89)
Control upper	0–5	NA	6.25 (2.39)	5.75 (1.75)
Control lower	5–10	NA	8.00 (1.47)	7.00 (1.41)
Fall underburning upper	0–5	NA	1.00 (1.00)	0.25 (0.25)
Fall underburning lower	5–10	NA	0.75 (0.48)	1.00 (0.41)
Spring underburning upper	0–5	NA	5.50 (1.66)	5.00 (2.04)
Spring underburning lower	5–10	NA	7.25 (0.85)	4.50 (0.65)
Live root biomass (g)				
Control	0–10	0.15 (0.03)	0.05 (0.01)	0.07 (0.01)
Fall underburning	0–10	0.11 (0.04)	<0.01 (<0.01)	0.01 (0.01)
Spring underburning	0–10	0.11 (0.03)	0.06 (<0.01)	0.05 (0.02)
Upper	0–5	0.06 (0.02)	0.01 (<0.01)	0.02 (0.01)
Lower	5–10	0.06 (0.01)	0.03 (0.01)	0.03 (0.01)
Control upper	0–5	NA	0.01 (<0.01)	0.03 (0.01)
Control lower	5–10	NA	0.06 (<0.01)	0.04 (0.02)
Fall underburning upper	0–5	NA	0.00 (0.00)	0.00 (0.00)
Fall underburning lower	5–10	NA	<0.01 (<0.01)	0.01 (0.01)
Spring underburning upper	0–5	NA	0.01 (<0.01)	0.03 (0.01)
Spring underburning lower	5–10	NA	0.04 (0.01)	0.03 (0.01)

Note: Twice as many samples were collected in the pre-treatment year than in the post-treatment years. NA, not applicable (i.e., no overall significant difference).

years 1 and 2, respectively) and, consequently, shared the least number of RFLP species (0–4 species, SI = 0.00–0.21) with other time and treatment combinations (Fig. 3). Relatively high similarity was seen among pretreatment comparisons (13–17 RFLP species, SI = 0.41–0.49) and among posttreatment spring underburning and control comparisons (5–13 RFLP species, SI = 0.15–0.37) (Fig. 3). Only one species, *Rhizopogon salebrosus* 78, was detected in all treatments both before and after underburning.

Ectomycorrhizal root biomass

Nineteen biomass-dominant RFLP species, those with 2% or more of the total mycorrhizal biomass, accounted for 54% of the total mycorrhizal biomass. Their annual abundance is presented by treatment in Fig. 4. An additional 30 RFLP species each contributed 1% to the total biomass. Similar to frequent species distribution, the EM biomass distribution among years and across the site (Fig. 4) also indicated that the EM community consisted of a few dominant species and a relatively large number of infrequent species. Ten of the biomass-dominant species were also among the most frequent species (Figs. 2 and 4), indicating some correlation between EM biomass and frequency. Three (*Cortinarius* sp. 42, *Inocybe* sp. 21, and RFLP 23) of these 10 species were detected only prior to underburning.

Prior to underburning, live root biomass was similar among treatment units ($F_{[2,6]} = 3.54$, $p = 0.097$) and between upper and lower core samples ($F_{[1,15]} = 0.01$, $p = 0.945$) (Table 2). After underburning, live root biomass differed among treatments in both posttreatment years (1998: $F_{[2,6]} = 18.03$, $p = 0.003$; 1999: $F_{[2,6]} = 6.30$, $p = 0.033$). The fall underburning treatment units had less live root biomass than the control and spring underburning treatment units in both posttreatment years (Table 2). Averaging across all treatment units, it was found that the live root biomass was less in upper core samples compared to the lower in the first posttreatment year ($F_{[1,15]} = 29.72$, $p = 0.0001$), but there was no evidence that the live root biomass differed between core sample depths after the second posttreatment year ($F_{[1,15]} = 0.82$, $p = 0.381$) (Table 2). There was evidence of an interaction between treatment and core depth for live root biomass in the first posttreatment year ($F_{[2,15]} = 6.64$, $p = 0.009$), but not after the second ($F_{[2,15]} = 0.22$, $p = 0.802$). In the first posttreatment year, the live root biomass was much less in upper core samples from fall underburning treatment units compared with the control and spring underburning treatment units. Most upper core samples from fall underburning treatment units had negligible or no live root biomass in either posttreatment year (Table 2).

Discussion

Fall underburning in dry ponderosa pine stands significantly reduced duff depth, live root biomass, and EM RFLP

Fig. 3. Matrix showing the number of restriction fragment length polymorphism (RFLP) species in common and Sorenson indices for ponderosa pine between time and treatment pairs. Shaded, similarity coefficients from the Sorenson index; unshaded, number of species in common. Pre, pretreatment 1997; Post1, posttreatment 1998; Post2, posttreatment 1999; C, control; F, fall; S, spring.

	Pre: C	**Pre:** F	**Pre:** S	**Post1:** C	**Post1:** F	**Post1:** S	**Post2:** C	**Post2:** F	**Post2:** S
Pre: C	1.00	0.41	0.49	0.16	0.05	0.18	0.14	0.15	0.13
Pre: F	13	1.00	0.43	0.09	0.00	0.17	0.13	0.18	0.15
Pre: S	17	13	1.00	0.08	0.05	0.16	0.15	0.16	0.14
Post1: C	6	3	3	1.00	0.17	0.37	0.29	0.04	0.15
Post1: F	1	0	1	4	1.00	0.05	0.10	0.18	0.06
Post1: S	6	5	5	13	1	1.00	0.30	0.11	0.25
Post2: C	5	4	5	11	2	10	1.00	0.20	0.40
Post2: F	3	3	3	1	1	2	4	1.00	0.13
Post2: S	4	4	4	5	1	7	12	2	1.00

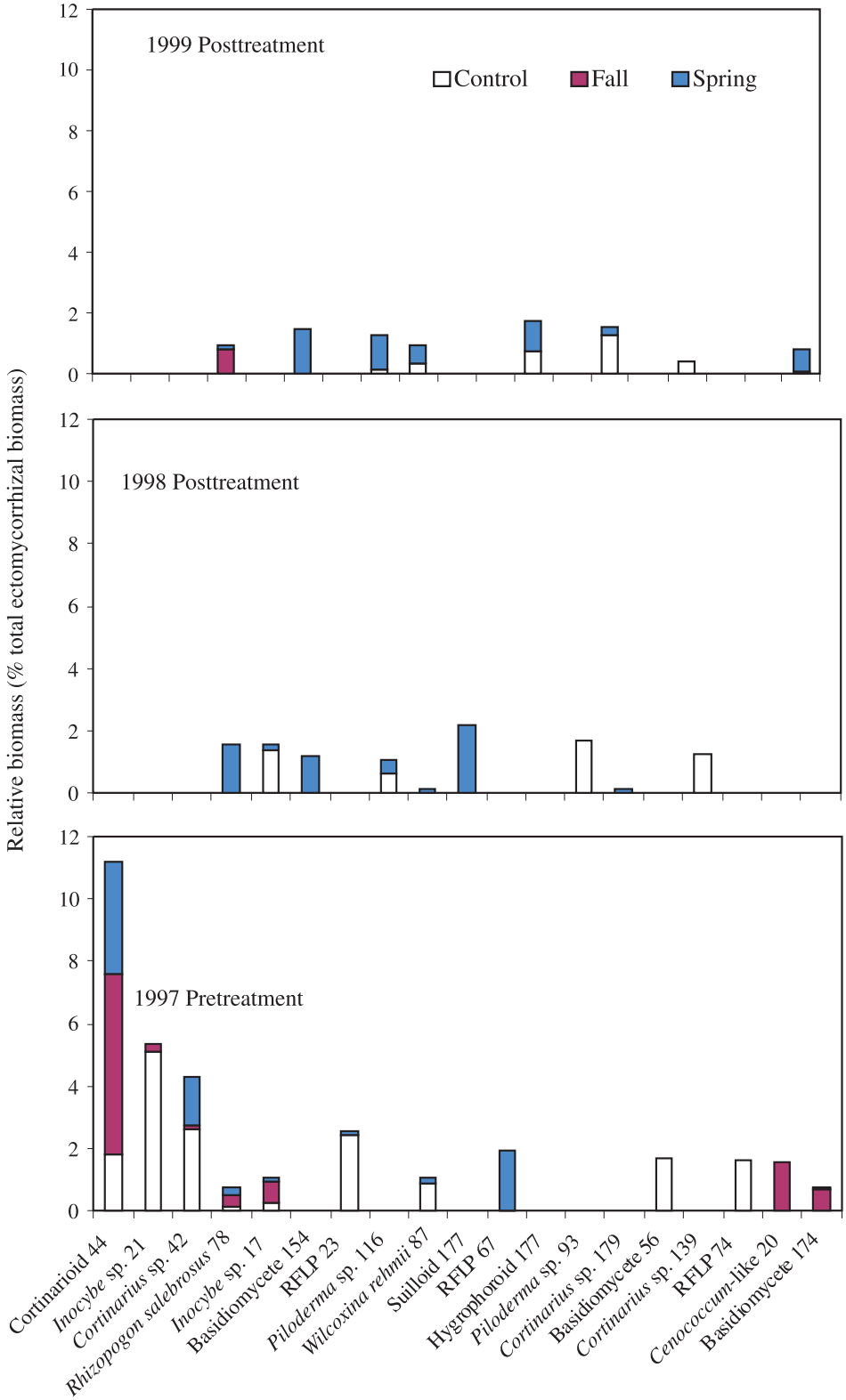
species richness compared with spring underburning, for at least 2 years. Spring underburning response for these variables was generally similar to that of the nonburned treatment. Dahlberg et al. (2001) found that the mortality of ectomycorrhizas increased with burn intensity and tree mortality. On our study site, the probability of mortality of residual mature trees was greater after fall underburning compared with spring (Thies et al. 2001), suggesting that the short-term burn effects detected in our study predict long-term differences in soil and stand recovery.

In this study, the duff was largely removed by fall underburning. Duff reduction to reduce the risk of stand-replacing wildfires, combined with large tree survival, is an integral objective of most prescribed fire treatment programs (Scott 2002). Deep accumulations of duff around mature ponderosa pines place them at risk of root and root-crown cambial injury when burned (Scott 2002). Complete consumption of duff that results in exposure of mineral soil and leads to old-growth pine mortality appears more typical of fall underburning than spring underburning in the Blue Mountains of Oregon (Scott 2002), as well as in a study in Arizona (Kaufman and Covington 2001). In contrast, ponderosa pine mortality was greater in early- compared with late-season burns in studies by Harrington (1987) in southwestern Colorado and by Swezy and Agee (1991) in southwestern Oregon. Burning conditions in the fall vary considerably in the Blue Mountains and some fall underburning is so light as to appear like spring underburning (Scott 2002).

Differences in site and annual weather conditions that influence fire intensity accentuate the difficulty of generalizing outcomes based solely on the season of burning. Above-average precipitation in the months preceding the spring underburning in this study likely increased understory vegetation and fuel moisture and reduced fire intensity relative to the fall underburning and to typical spring underburning in this area. In the study by Harrington (1987), higher ambient temperatures in spring and summer compared with fall likely increased fire intensity in the spring and summer burns. Soil characteristics also mediate responses to fire. For example, in the study by Swezy and Agee (1991), pumice soils, derived from the eruption of Mount Mazama, may conduct heat differently than the Mazama ash soils of the Blue Mountains.

In this study, EM biomass was largely removed by fall underburning to the deepest measurement of 10 cm, and particularly in the upper 5 cm of mineral soil. Fire typically reduces EM biomass in the litter (recognizable plant material) and organic soil horizons (Dahlberg 2002), but has little impact on EM fungi if the organic layer remains largely undamaged (Jonsson et al. 1999; Korb et al. 2003). Swezy and Agee (1991) found that live, fine roots of ponderosa pine were concentrated in the duff and upper 10 cm of mineral soil, and that these depths received lethal temperatures (>60 °C) during prescribed underburning. Sims (1976) reported lethal temperatures in a jack pine (*Pinus banksiana* Lamb.) burn in the upper 5 cm of mineral soil. Other studies in pine forests using a similar partition for soil depths sampled show noticeable short- and long-term effects of fire on nutrient availability in the upper 5 cm (Prieto-Fernandez et al. 1993; Monleon et al. 1997). An early summer prescribed underburning in ponderosa pine in California reduced EM biomass eightfold in the litter and organic layers, with little change in the mineral layers (Stendell et al. 1999). The overall decrease in live root biomass in the upper core samples in the first posttreatment year of this study, including in the nonburned controls, may have been influenced in part by weather conditions. However, the near absence of live roots in the fall underburning upper core samples in both posttreatment years, despite a rebound in root biomass in the spring underburning and control upper core samples, suggests that the reduction seen in the fall underburning treatment was the result of fire intensity. A trend in increased live fine root biomass through time is evident in the lower 5 cm of mineral soils in the fall underburning and in the upper 5 cm of mineral soils in the spring underburning (Table 2).

Fig. 4. Pre- and post-treatment biomass of the 19 biomass-dominant species within treatments. In certain cases, relative mycorrhizal biomass for a particular restriction fragment length polymorphism (RFLP) species was so small that it is beyond the resolution of the figure. Species are listed in descending order of total biomass.



The relatively low-intensity sampling of ectomycorrhizas provided clear evidence of differences between seasonal underburning in EM RFLP species richness, but obviously limited detection of EM RFLP species occurring at low frequencies. Nevertheless, it allowed annual and repeated within-year detection of commonly occurring species. A similar low sampling intensity scheme in the study by Jonsson et al. (1999) also yielded detection of common EM RFLP spe-

cies. Individual RFLP species in our study were typically detected too infrequently to enable the impacts of seasonal underburning on particular EM RFLP species to be assessed. For example, several of the most frequent and abundant RFLP species were detected only prior to the application of underburning. *Rhizopogon salebrosus* was the only species detected in all treatments both before and after underburning. In nonburned or low-intensity spring underburning, about 10% of the observed RFLP species recurred either in the same experimental treatment unit (8–12 ha) or within a short distance (10 cm) of the previous year's sample location, evidence of the ability of some EM species to persist through time and to withstand low-intensity disturbance. By contrast, most (80%) of the recurring RFLP species detected in the fall underburning treatment units before burning were not detected in those units after the comparatively high-intensity fall underburning. Even though these data suggest that such recurring species were reduced or eliminated with higher intensity underburning, it is possible that they could have been present on the roots, but missed because of patchy distributions and low sampling intensity. The patchy distribution of ectomycorrhizas is well established (Gardes and Bruns 1996; Dahlberg et al. 1997; Horton and Bruns 2001; Taylor 2002).

Recovery of EM communities after fire is influenced by the extent of survival within burned areas, as well as by the recolonization abilities of the species affected. Three species *(Rhizopogon salebrosus* 78, *Tricholoma* sp. 83, and RFLP 174) were detected before and 2 years after fall underburning. The widespread and uniform presence of *Rhizopogon* species is well documented in other studies both before fire (Taylor and Bruns 1999; Kjøller and Bruns 2003) and after (Horton et al. 1998; Baar et al. 1999; Stendell et al. 1999; Grogan et al. 2000) and suggests that spores rather than mycelium are the primary inoculum source (Horton et al. 1998; Baar et al. 1999). Prescribed and natural burns typically are spatially heterogeneous, leaving refugia of nonburned and low-intensity burned areas within most sites. EM fungi surviving within these areas may facilitate reestablishment of EM propagules. Mah et al. (2001) suggest that differences in EM community structure among clear-cut, clear-cut and burned, and mature sites depended on propagules of fungi capable of surviving burns and moisture stress. Spores and sclerotia of some mycorrhizal species, including species of *Rhizopogon*, *Wilcoxina*, and *Morchella*, persist in forest soils (Danielson 1991; Miller et al. 1993, 1994; Kjøller and Bruns 2003) and have been shown to survive fire (Danielson 1982; Horton et al. 1998; Baar et al. 1999; Grogan et al. 2000).

This study showed high RFLP species diversity of EM fungi in dry ponderosa pine forests where sporadic and low-level production of sporocarps is typical. Studies in *Picea*-, *Pinus*-, and *Pseudotsuga*-dominated forest communities have repeatedly shown that sporocarp production is a poor reflection of the composition of subterranean EM communities (Gardes and Bruns 1996; Dahlberg et al. 1997; Kårén et al. 1997; Jonsson et al. 1999). Over half of all observed RFLP EM species in this study occurred only once, reflecting a pattern of rarity of most species and dominance by a few that is typical of EM fungal communities (Gehring et al. 1998; Baar et al. 1999; Grogan et al. 2000; Smith et al. 2002) and taxonomic groups in general (Magurran and Henderson 2003). Also mirrored in this study was the common pattern of high EM species diversity in forest communities with few or even a single EM host species (Goodman and Trofymow 1998; Kranabetter and Wylie 1998; Byrd et al. 2000; Smith et al. 2002). Vertical stratification among recurring EM RFLP species was not detected in this study, although others have reported that some taxa occur more frequently in the organic layer and some in the mineral soil (Stendell et al. 1999; Grogan et al. 2000; Dickie et al. 2002; Rosling et al. 2003).

EM communities after fire sometimes exhibit a relatively short-lived shift in dominance from basidiomycetous to ascomycetous fungi (Visser 1995; Torres and Honrubia 1997; Baar et al. 1999; Grogan et al. 2000). Such a shift in dominance at this higher taxonomic level was not detected in this study or in the study by Fujimura et al. (2004), conducted on the same fall underburning sites. Most frequent and abundant RFLP species in this study were basidiomycetes; Fujimura et al. (2004) report that only 15% of their RFLP species obtained from 32 soil cores were ascomycetes. *Wilcoxina* was among the ascomycetous fungi detected by Fujimura et al. (2004), although it was not detected in samples from fall underburning units in this study. An increase in the frequency of *Wilcoxina* on seedlings after burns has been reported in several studies (Torres and Honrubia 1997; Baar et al. 1999; Grogan et al. 2000). In contrast, on roots from mature trees in this study, *Wilcoxina* occurred with similar frequency in spring underburning and nonburned controls. These differences among studies in the colonization frequency of *Wilcoxina* suggest that initial post-fire dominance by ascomycetous fungi may be more often observed on the roots of seedlings than on mature trees, and that dominance at the higher taxonomic levels possibly shifts with host seedling age as well as with short time periods since disturbance. Indeed, bishop pine (*Pinus muricata* D. Don) seedlings exhibited rapid colonization and shifts in dominance of several higher taxonomic fungal groups after fire (Horton et al. 1998).

Results of this study show that prescribed fall and spring underburning differentially influenced the community structure and abundance of EM fungi, and would seem to suggest that spring underburning is a better alternative than fall underburning for reducing forest fuel loads, if an objective is to maintain high EM diversity. However, the successful reintroduction of fire into an ecosystem (where decades of wildfire suppression have resulted in heavy fuel accumulations) may not be as simple as selecting a single season to burn. It is important to consider the complexity of the historical condition and the recovery potential of the site (Cromack et al. 2000). Clearly, the goal of reducing fuel loads must be tempered with retaining ponderosa pines established before Euro–American settlement. Complete duff reduction at the time of only one fire entry does not achieve the desired future condition of large-tree pine retention with low fuel loads (Scott 2002). A combination of spring and fall underburning treatments may best return stands to historic conditions (Scott 2002). Duff accumulations around large trees could be reduced through a series of spring-prescribed underburning treatments before prescribed underburning is used on the natural fire return interval and season (Scott 2002). Further examination on the impact of frequent low-

intensity spring burns on fine root survival and the EM community would expand the limited knowledge that currently exists.

Acknowledgements

We gratefully acknowledge the Emigrant Creek Ranger District (previously Burns Ranger District), and especially Roland "Buddy" Crisafi, for help and timely burning of the stands. We thank Kei Fujimura, Yana Valachovic, and Bob Peck for help with field collections; Sara Ashkannejhad, Jonathon Bishop, Mary Bowen, and Renee Rider for lab assistance; and James Trappe and Martin Bidartondo for identification of *Rhizopogon* sporocarps. This study was improved by the instruction and advice of Tom Horton on molecular techniques and access to Paula Brooks' sporocarp collections from the Blue Mountains. The comments of Kermit Cromack, Jr., Tom Horton, Steve Miller, Randy Molina, Patricia Muir, and two anonymous reviewers improved the manuscript. Mention of trade or firm names does not constitute an endorsement by the U.S. Department of Agriculture.

References

Agee, J.K. 1993. Fire ecology of Pacific Northwest forests. Island Press, Washington, D.C.

Agerer, R. 1994. Characterization of ectomycorrhizae. *In* Techniques for mycorrhizal research *Edited by* J.R. Norris, D. Read, and A.K. Varma. Academic Press, London. pp. 25–73.

Arno, S.F., and Allison-Bunnell, S. 2002. Flames in our forest: disaster or renewal? Island Press, Washington, D.C.

Baar, J., Horton, T. R., Kretzer, A., and Bruns, T.D. 1999. Mycorrhizal colonization of *Pinus muricata* from resistant propagules after a stand-replacing wildfire. New Phytol. **143**: 409–418.

Bachelet, D., Neilson, R.P., Hickler, T., Drapek, R.J., Lenihan, J.M., Sykes, M.T., Smith, B., Sitch, S., and Thonicke, K. 2003. Simulating past and future dynamics of natural ecosystems in the United States. Global Biochem. Cycles, **17**(2): 1–21.

Biswell, H.H. 1989. Prescribed burning in California wildlands vegetation management. University of California Press, Berkeley, Calif.

Bruns, T.D., Szaro, T.M., Gardes, M., Cullings, K.W., Pan, J.J., Taylor, D.L., Horton, T.R., Kretzer, A., Garbelotto, M., and Li, Y. 1998. A sequence database for the identification of ectomycorrhizal basidiomycetes by phylogenetic analysis. Molec. Ecol. **7**: 257–272.

Byrd, K.B., Parker, V.T., Vogler, D.R., and Cullings, K.W. 2000. The influence of clear-cutting on ectomycorrhizal fungus diversity in a lodgepole pine (*Pinus contorta*) stand, Yellowstone National Park, Wyoming, and Gallatin National Forest, Montana. Can. J. Bot. **78**: 149–156.

Cázares, E., and Smith, J.E. 1996. Occurrence of vesicular-arbuscular mycorrhizae in *Pseudotsuga menziesii* and *Tsuga heterophylla* seedlings grown in Oregon Coast Range soils. Mycorrhiza, **6**: 65–67.

Cázares, E., and Trappe, J.M. 1993. Vesicular endophytes in roots of the Pinaceae. Mycorrhiza, **2**: 153–156.

Covington, W.W., and Moore, M.M. 1994. Post-settlement changes in natural fire regimes and forest structure: ecological restoration of old-growth ponderosa pine forests. *In* Assessing forest ecosystem health in the Inland West. *Edited by* R.N. Sampson, D.L. Adams, and M.J. Enzer. The Hayworth Press, New York. pp. 153–181.

Cromack, K., Jr., Landsberg, J.D., Everett, R.L., Zeleny, R., Giardina, C.P., Strand, E.K., Anderson, T.D., Averill, R., and Smyrski, R. 2000. Assessing the impacts of severe fire on forest ecosystem recovery. J. Sustain. For. **11**: 177–228.

Dahlberg, A. 2002. Effects of fire on ectomycorrhizal fungi in Fennoscandian boreal forests. Silva Fenn. **36**(1): 69–80.

Dahlberg A., Jonsson L., and Nylund, J-E. 1997. Species diversity and distribution of biomass above and below ground among ectomycorrhizal fungi in an old Norway spruce forest in south Sweden. Can. J. Bot. **8**: 1323–1335.

Dahlberg, A., Schimmel, J., Taylor, A.F.S., and Johannesson, H. 2001. Post-fire legacy of ectomycorrhizal fungal communities in the Swedish boreal forest in relation to fire severity and logging intensity. Biol. Conserv. **100**: 151–161.

Danielson, R.M. 1982. Taxonomic affinities and criteria for identification of the common ectendomycorrhizal symbionts of pines. Can. J. Bot. **60**: 7–18.

Danielson, R.M. 1991. Temporal changes and effects of amendments on the occurrence of sheathing (ecto-) mycorrhizas of conifers growing in oil sands tailings and coal spoil. Agric. Ecosyst. Environ. **35**: 261–281.

Dickie, I.A., Xu, B., and Koide, R.T. 2002. Vertical niche differentiation of ectomycorrhizal hyphae in soil as shown by T-RFLP analysis. New Phytol. **156**: 527–535.

Franklin, J.F., and Dyrness, C.T. 1984. Forest zones of western Washington and northwestern Oregon. *In* Natural vegetation of Oregon and Washington. Oregon State University Press, Corvallis, Ore. pp. 53–109.

Fujimura, K.E., Smith, J.E., Horton, T.R., Weber, N.S., and Spatafora, J.W. 2004. Pezizalean mycorrhizas on ponderosa pine (*Pinus ponderosa*) after prescribed fires in eastern Oregon, USA. Mycorrhiza. In press.

Gardes, M., and Bruns, T.D. 1993. ITS primers with enhanced specificity of basidiomycetes: application to the identification of mycorrhizae and rusts. Mol. Ecol. **2**: 113–118.

Gardes, M., and Bruns, T.D. 1996. Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: above- and below-ground views. Can. J. Bot. **74**(10): 1572–1583.

Gehring, C.A., Theimer, T.C., Whitham, T.G., and Keim, P. 1998. Ectomycorrhizal fungal community structure of pinyon pines growing in two environmental extremes. Ecology, **79**: 1562–1572.

Goodman, D.M., and Trofymow, J.A. 1998. Comparison of communities of ectomycorrhizal fungi in old-growth and mature stands of Douglas-fir at two sites on southern Vancouver Island. Can. J. For. Res. **28**: 574–581.

Grogan, P., Baar, J., and Bruns, T.D. 2000. Below-ground ectomycorrhizal community structure in a recently burned bishop pine forest. J. Ecol. **88**: 1051–1062.

Harrington, M.G. 1987. Ponderosa pine mortality from spring, summer, and fall crown scorching. West. J. Appl. For. **2**: 14–16.

Harvey, A.E., Larsen, M.J., and Jurgensen, M.F. 1976. Distribution of ectomycorrhizae in a mature Douglas-fir/larch forest soil in western Montana. For. Sci. **22**(4): 393–398.

Harvey, A.E., Jurgensen, M.F., Larsen, M.J., and Schlieter, J.A. 1986. Distribution of active ectomycorrhizal short roots in forest soils of the inland Northwest: effects of site and disturbance. USDA For. Serv. INT-RP-374.

Hastie, T.J., and Pregibon, D. 1992. Generalized linear models. *In* Statistical models in S. *Edited by* J.M. Chambers and T.J. Hastie. Wadsworth and Brooks/Cole Advanced Books and Software, Pacific Grove, Calif. pp. 195–248.

Heyerdahl, E.K., Brubaker, L.B., and Agee, J.K. 2001. Spatial controls of historical fire regimes: a multiscale example from the interior west, USA. Ecology, **82**(3): 660–678.

Horton, T.R. 2002. Molecular approaches to ectomycorrhizal diversity studies: variation in ITS at a local scale. Plant Soil, **244**: 29–39.

Horton, T.R., and Bruns, T.D. 1998. Multiple-host fungi are the most frequent and abundant ectomycorrhizal types in a mixed stand of Douglas-fir (*Pseudotsuga menziesii*) and bishop pine (*Pinus muricata*). New Phytol. **139**: 331–339.

Horton, T.R., and Bruns, T.D. 2001. The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. Mol. Ecol. **10**(8): 1855–1871.

Horton, T.R., Cázares, E., and Bruns, T.D. 1998. Ectomycorrhizal, vesicular-arbuscular and dark septate fungal colonization of bishop pine (*Pinus muricata*) seedlings in the first five months of growth after wildfire. Mycorrhiza, **8**: 11–18.

Jonsson, L., Dahlberg, A., Nilsson, M-C, Zackrisson, O., and Kårén, O. 1999. Ectomycorrhizal fungal communities in late-successional Swedish boreal forests, and their composition following wildfire. Mol. Ecol. **8**: 205–215.

Kårén, O., Högberg, N., Dahlberg, A., Jonsson, L., and Nylund, J.E. 1997. Inter- and intraspecific variation in the ITS region of rDNA of ectomycorrhizal fungi in Fennoscandia as detected by endonucleases analysis. New Phytol. **136**: 313–325.

Kaufmann, G.A., and Covington, W.W. 2001. Effect of prescribed burning on mortality of presettlement ponderosa pines in Grand Canyon National Park. *In* Proceedings: Ponderosa pine Ecosystems Restoration and Conservation: Steps Toward Stewardship, Flagstaff, Arizona, 25–27 April 2000. *Compiled by* R.K. Vance, C.B. Edminster, W.W. Covington, and J.A. Blake. USDA For. Serv. RMRS-P-22. pp. 36–42.

Kjøller, R., and Bruns, T.D. 2003. *Rhizopogon* spore bank communities within and among California pine forests. Mycologia, **95**: 603–613.

Korb, J.E., Johnson, N.C., and Covington, W.W. 2003. Arbuscular mycorrhizal propagule densities respond rapidly to ponderosa pine restoration treatments. J. Appl. Ecol. **40**: 101–110.

Kranabetter, J.M., and Wylie, T. 1998. Ectomycorrhizal community structure across forest openings on naturally regenerated western hemlock seedlings. Can. J. Bot. **76**: 189–196.

Langston, N. 1995. Forest dreams, forest nightmares: the paradox of old growth in the inland West. University of Washington Press, Seattle, Wash.

McCullagh, P., and Nelder, J.A. 1991. Generalized linear models. 2nd ed. Chapman & Hall, London.

Magurran, A.E. 1988. Ecological diversity and its measurement. Princeton University Press, Princeton, N.J.

Magurran, A.E., and Henderson, P.A. 2003. Explaining the excess of rare species in natural species abundance distributions. Nature (London), **422**: 714–716.

Mah, K., Tackaberry, L.E., Egger, K.N., and Massicotte, H.B. 2001. The impacts of broadcast burning after clear-cutting on the diversity of ectomycorrhizal fungi associated with hybrid spruce seedlings in central British Columbia. Can. J. For. Res. **31**: 224–235

Massicotte, H.B., Molina, R., Luoma, D., and Smith, J.E. 1994. Biology of the ectomycorrhizal genus, *Rhizopogon*. II. Patterns of host-fungus specificity following spore inoculation of diverse hosts grown in monoculture and dual culture. New Phytol. **126**: 677–690

Massicotte, H.B., Molina, R., Tackaberry, L.E., Smith, J.E., and Amaranthus, M.P. 1999. Diversity and host specificity of ectomycorrhizal fungi retrieved from three adjacent forest sites by five host species. Can. J. Bot. **77**: 1053–1076.

MathSoft. 1988–1999. S-Plus version 2000 [computer program]. Data Analysis Products Division, MathSoft, Seattle, Wash.

Miller, S.L., Torres, P., and McLean, T. 1993. Basidiospore viability and germination in ectomycorrhizal and saprotrophic basidiomycetes. Mycol. Res. **97**: 141–149.

Miller, S.L., Torres, P., and McLean, T. 1994. Persistence of basidiospores and sclerotia of ectomycorrhizal fungi and *Morchella* in soil. Mycologia, **86**: 89–95.

Molina, R., Massicotte, H., and Trappe, J.M. 1992. Specificity phenomena in mycorrhizal symbiosis: community-ecological consequences and practical implications. *In* Mycorrhizal functioning: an integrated plant-fungal process. *Edited by* M.F. Allen. Chapman & Hall, London. pp. 357–423.

Monleon, V.J., Cromack, K., Jr., and Landsberg, J.D. 1997. Short- and long-term effects of prescribed underburning on nitrogen availability in ponderosa pine stands in central Oregon. Can. J. For. Res. **27**: 369–378.

Oregon Climate Service. 1971–2000. Online weather data. Available from http://www.ocs.orst.edu [updated 29 January 2004; cited September 2000].

Perry, D.A., Amaranthus, M.P., Borchers, J.G., Borchers, S.L., and Brainerd, R.E. 1989. Bootstrapping in ecosystems. Bioscience, **39**: 230–237.

Prieto-Fernandez, A., Vilar, M.C., Carballas, M., and Carballas, T. 1993. Short-term effects of a wildfire on the nitrogen status and its mineralization kinetics in an Atlantic forest soil. Soil Biol. Biochem. **25**: 1657–1664.

Read, D.J. 1998. The mycorrhizal status of Pinus. *In* Ecology and biogeography of *Pinus. Edited by* D.M. Richardson. Cambridge University Press, Cambridge. pp. 324–340.

Robbins, W.G., and Wolf, D.W. 1994. Landscape and the intermontane northwest: an environmental history. USDA For. Serv. Gen. Tech. Rep. PNW-GTR-319.

Rosling, A., Landeweert, R., Lindahl, B.D., Larsson, K.-H., Kuyper, T.W., Taylor, A.F.S., and Finlay, R.D. 2003. Vertical distribution of ectomycorrhizal fungal taxa in a podzol soil profile. New Phytol. **159**: 775–783.

Sandberg, D.V., Ottmar, R.D., and Cushon, G.H. 2001. Characterizing fuels in the 21st century. Int. J. Wildl. Fire, **10**: 381–387.

Scott, D.W. 2002. Review of the prescribed fire program on Emigrant Creek Ranger District – 2001. USDA For. Serv. Pacific Northwest Region, Wallowa-Whitman Nat. For. Rep. BMPMSC-02–03.

Sims, H.P. 1976. The effect of prescribed burning on some physical soil properties of jack pine sites in southeastern Manitoba. Can. J. For. Res. **6**: 58–68.

Smith, J.E., Johnson, K.A., and Cázares, E. 1998. Mycorrhizal colonization of seedlings of Pinaceae and Betulaceae after spore inoculation with *Glomus intraradices*. Mycorrhiza, **7**: 279–285.

Smith, J.E., Molina, R., Huso, M.M.P., Luoma, D., McKay, D., Castellano, M.A., Lebel, T., and Valachovic, Y. 2002. Species richness, abundance, and composition of hypogeous and epigeous ectomycorrhizal fungal sporocarps in young, rotation-age, and old-growth stands of Douglas-fir (*Pseudotsuga menziesii*) in the Cascade Range of Oregon, U.S.A. Can. J. Bot. **80**: 186–204.

Smith, S.E, and Read, D.J. 1997. Mycorrhizal symbiosis. 2nd ed. Academic Press, San Diego, Calif.

Stendell, E.R., Horton, T.R., and Bruns, T.D. 1999. Early effects of prescribed fire on the structure of the ectomycorrhizal fungal community in a Sierra Nevada ponderosa pine forest. Mycol. Res. **103**: 1353–1359.

Swezy, D.M., and Agee, J.K. 1991. Prescribed fire effects on fine-root and tree mortality in old-growth ponderosa pine. Can. J. For. Res. **21**: 626–634.

Swofford, D.L. 1993. PAUP: phylogenetic analysis using parsimony. Version 3.1. Computer program distributed by the Illinois Natural History Survey, Champaign, Ill.

Swofford, D.L. 1999. PAUP*: phylogenetic analysis using parsimony (*and other methods). version 4. Sinauer Associates, Sunderland, Mass.

Taylor, A.F.S. 2002. Fungal diversity in ectomycorrhizal communities: sampling effort and species detection. Plant Soil, **244**: 19–28.

Taylor, D.L., and Bruns, T.D. 1999. Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: minimal overlap between the mature forest and resistant propagule communities. Mol. Ecol. **8**(11): 1837–1850.

Thies, W.G., Niwa, C.G, and Westlind, D.G. 2001. Impact of prescribed fire in ponderosa pine stands in the southern Blue Mountains on various components of the ecosystem—three years post fire. *In* Proceedings of the 49th Annual Western International Forest Disease Work Conference. *Edited by* K. Marshall. 16–20 September 2001, Carmel, CA. USDA For. Serv., Central Point, Ore. pp. 108–112.

Torres, P., and Honrubia, M. 1997. Changes and effects of a natural fire on ectomycorrhizal inoculum potential of soil in a *Pinus halepensis* forest. For. Ecol. Manage. **96**: 189–196.

Visser, S. 1995. Ectomycorrhizal fungal succession in jack pine following wildfire. New Phytol. **129**: 389–401.