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ECTOMYCORRHIZAL FUNGAL COMMUNITY STRUCTURE OF PINYON PINES GROWING IN TWO ENVIRONMENTAL EXTREMES

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Abstract. We used molecular techniques to examine the ectomycorrhizal fungal community associated with pinyon pine (*Pinus edulis*) growing in two soil types in a semiarid region of northern Arizona: cinder soils low in nutrients and moisture, and sandy-loam soils with higher moisture and nutrient levels. Pinyon performance (e.g., growth, reproduction, water stress) has been shown to be markedly lower in cinder than in sandy-loam environments. Fungal community composition and richness were determined using RFLP (restriction fragment length polymorphism) analysis of ectomycorrhizal root tips collected from three sites within each soil type.

Several patterns emerged from these analyses. First, communities in both cinder and sandy-loam soils were dominated by one or a few abundant ectomycorrhizal types, a species abundance pattern common to many plant and animal communities. Second, unlike the pattern for many other organisms, ectomycorrhizal fungal type (species) richness was not correlated with measures of ecosystem productivity such as soil nutrient and moisture levels; cinder and sandy-loam soils had similar numbers of ectomycorrhizal fungal types (range of 15–19 fungal types for both soil types). Third, soil type and fungal community composition were linked, as cluster analysis demonstrated greater similarity of fungal communities from sites within soil types than between them. Differential amplification using primers with enhanced specificity for basidiomycetes indicated that an average of 85% of the ectomycorrhiza found at the sandy-loam sites were members of the subphylum Basidiomycotina, whereas over half (mean = 52%) of the ectomycorrhiza at the cinder sites were formed by members of other fungal groups, probably the subphylum Ascomycotina. Fourth, a preliminary survey of 14–45 ectomycorrhizal root tips from each of 20 trees at one cinder site indicated that trees were dominated by one or a few ectomycorrhizal RFLP types. However, the same RFLP types did not dominate on all trees, and dominant types showed considerable spatial variation. Fifth, the RFLP patterns of some fungal sporocarps matched those of ectomycorrhizal root tips, but many did not, indicating that many of the ectomycorrhizal fungi at these sites fruit infrequently, whereas other fungi with more abundant sporocarps may not form ectomycorrhiza. This emphasizes the need to characterize the ectomycorrhizal communities formed on the plant roots themselves, rather than characterization based on sporocarps alone, particularly in arid environments. Finally, the differences in ectomycorrhizal fungal communities we observed between soil types supported the concept that conserving fungal diversity requires conservation of host plant species over their entire range, not just typical sites. If future studies corroborate these patterns, our results suggest that abiotically stressful environments are important to include in these conservation efforts.

Key words: community composition; conservation; diversity; ectomycorrhizal fungi; environmental stress; RFLP analysis; species richness; sporocarp surveys.

INTRODUCTION

One of the major types of mycorrhiza, the ectomycorrhiza, involves an estimated 5000 species of fungi in the subphyla Ascomycotina and Basidiomycotina (Kendrick 1992, Molina et al. 1992) and ~2000 species of plants, including important components of forest ecosystems worldwide, e.g., members of the Pinaceae, Fagaceae, and Myrtaceae (Kendrick 1992). Knowledge of which fungi form ectomycorrhizal associations with which host plants is important for at least two reasons. First, different fungal species/strains vary in their ability to form ectomycorrhiza or to enhance plant nutrient uptake when grown in association with different plant species and under different environmental conditions (e.g., Parke et al. 1983, Finlay 1989, Tonkin et al. 1989, Molina et al. 1992, Burgess et al. 1994). Second, the species diversity of ectomycorrhizal fungi can be high in areas where plant species diversity is relatively low (Bledsoe 1992, Bruns 1995). Estimates of aboveground diversity may thus be poor predictors of belowground diversity. This has implications for the conservation of biodiversity, and may be particularly important if high fungal diversity affects ecosystem function, such as

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TABLE 1. Comparisons of soil and pinyon performance parameters for cinder and sandy-loam environments.

	Cinder	Sandy-loam
Soil conditions		
Phosphate (μg/g)†	4.45	12.20
Soil moisture (% water)†	5.61	9.39
NO_3 mineralization ($\mu g \cdot g^{-1}$ soil$\cdot d^{-1}$)†	0.015	0.147
NH_4 mineralization ($\mu g \cdot g^{-1}$ soil$\cdot d^{-1}$)†	−0.021	0.062
Silt : clay fraction (%)‡	5.1	42.9
pH‡	7.75	7.90
Pinyon performance		
Growth (1990 shoot length; mm)†	23.20	28.90
Conelet production (mean no. cones per tree per site)§	185.00	300.00
Water stress (xylem pressure; MPa)‖	−2.830	−2.380
Mortality during 1996 drought (%)¶	7.00	0.700

† Data represent the mean values for four cinder and four sandy-loam sites, including those used in this study (adapted from Gehring and Whitham 1994).

‡ Data from three cinder and sandy-loam sites described in Cobb et al. (1997).

§ Mean of total conelet production per tree in 1988, based on a minimum of 9 trees per site (extrapolated from Christensen and Whitham 1991: Fig. 1).

‖ Mean water stress for pinyons growing in one cinder and one sandy-loam site (extrapolated from Mopper et al. 1991: Fig. 2).

¶ K. Ogle and T. G. Whitham, *unpublished data.*

providing a buffer against disturbance (Perry et al. 1987).

Although plant species composition (e.g., Bills et al. 1986, Brunner et al. 1992, Nantel and Neumann 1992) and host plant/forest age (e.g., Deacon et al. 1983, Mason et al. 1983, Richter and Bruhn 1993, Visser 1995) have important influences on the community of ectomycorrhizal fungi found in a given environment, abiotic factors may have independent effects on host plants and ectomycorrhizal fungi. For example, Nantel and Neumann (1992) found that the ectomycorrhizal Basidiomycete associates of a particular tree species were associated with that tree species over only a portion of its distribution. As a result, host plant community composition may not accurately reflect fungal community composition, especially across environments that experience different abiotic conditions. Studies of abiotic effects on fungal community structure may be confounded by the concomitant changes in plant communities that can occur across abiotic gradients. An alternative approach is to follow changes in the fungal community associated with one plant species across environmental extremes. However, this requires that the fungal species sampled are, in fact, associated with the plant species under study.

Because most studies on ectomycorrhizal community structure have focused on counts of fungal sexual reproductive structures (i.e., fruiting bodies or sporocarps) rather than assessing the fungal community actually present on the root system, it is not always possible to determine which of several plant species is the host for a given ectomycorrhizal fungus. Sporocarp studies have several other limitations as well, including: (1) greater emphasis on fungi that produce obvious aboveground sporocarps rather than fungi that produce belowground sporocarps (i.e., some fungi, such as truffles, fruit belowground and are dispersed by mammals; Fogel 1981; reviewed by Johnson 1996); (2) the assumption that the species that fruit abundantly are also the same species found on the root tips below ground (Gardes and Bruns 1996); (3) the requirement for long-term monitoring because of the irregular nature of sexual reproduction in many species (Vogt et al. 1992); and (4) limitation of these studies to mesic sites where conditions are favorable for fungal fruiting (Gardes and Bruns 1996).

Approaches independent of fungal sporocarp production are increasingly being used to assess ectomycorrhizal fungal communities. These include characterization of ectomycorrhiza based upon morphology (e.g., Danielson 1984, Agerer 1987–1993, Visser 1995) and, more recently, based upon restriction fragment length polymorphisms (RFLPs; e.g., Gardes et al. 1991, Gardes and Bruns 1993, Erland et al. 1994, Kraigher et al. 1995). In the latter case, RFLP patterns generated from single ectomycorrhizal root tips can be matched to patterns from known fungal sporocarps, thereby allowing identification of the fungal associate. Even if the ectomycorrhizal associates cannot be identified by comparison with sporocarps, estimates of species richness and overlap between communities can be obtained by comparison of RFLP types alone.

In order to better understand how abiotic conditions that negatively affected plant performance affected the community of mutualists associated with the plant's roots, we compared the ectomycorrhizal fungal community found on pinyon pines (*Pinus edulis* Engelm) growing in two extreme environments. Productivity, as measured by soil fertility, soil moisture, and pinyon growth, mortality, and reproduction, was dramatically lower in one of these environments than the other (Table 1). Previously, we demonstrated that ectomycorrhizal colonization and ectomycorrhizal responses to simulated herbivory differed between pinyon pines

growing in these two different environments (Gehring and Whitham 1994, 1995). We used RFLP analysis to compare ectomycorrhizal communities, because fungal fruiting was infrequent in these semiarid environments and, thus, was unlikely to be a good estimator of ectomycorrhizal fungal community structure.

METHODS

Description of the study sites

We compared the ectomycorrhizal communities of pinyon pines growing in two types of environments in northern Arizona, United States. The first environment consisted of cinder soils resulting from volcanic eruptions that began in AD 1064 and lasted for 200 years (Krutch 1974). The second environment consisted of sandy-loam soils derived from limestone parent material (Hendricks 1985). Three replicates of each environmental type were sampled in April of 1995 within a 20-km^2 region of pinyon–juniper woodland. Each site within a soil type was separated from other sites by $\geq$3.5 km.

In this area of northern Arizona, the majority of the precipitation occurs during the winter months and during the summer monsoonal season (July–September). Annual precipitation data for 1995 were available for areas adjacent to two of the six sites (one cinder and one sandy-loam site) from Walnut Canyon and Sunset Crater National Monument staff. Annual precipitation in 1995 was 28.2 cm for the cinder site and 27.5 cm for the sandy-loam site. Mean annual temperature for 1995 was also similar in the two environments, averaging 9.0°C for the cinder site and 10.4°C for the sandy-loam site.

Plant communities at the six sites were dominated by pinyon pine and one-seed juniper (*Juniperus monosperma*). Other than pinyon pine, the only known ectomycorrhiza-forming plant species were ponderosa pine (*Pinus ponderosa*) and Gambel's oak (*Quercus gambelli*). Ponderosa pine was found at all of the sites, whereas a single clone of Gambel's oak occurred in the study area at one of the sandy-loam sites. Grasses were present at the sandy-loam sites, but not at the cinder sites.

The two site types differed markedly in soil parameters that significantly affected pinyon performance. Cinder soils were low in nutrients and moisture (Mopper et al. 1991, Gehring and Whitham 1994, Cobb et al. 1997), and pinyons growing in this environment experienced chronic, severe herbivory by two insect species that resulted in substantial foliage loss and an altered tree architecture (Whitham and Mopper 1985, Cobb and Whitham 1998). Pinyons growing in the sandy-loam soils experienced less insect herbivory, had lower levels of water stress, higher growth rates, higher cone production, and lower mortality than their cinder counterparts (Christensen and Whitham 1991, Mopper et al. 1991, Gehring and Whitham 1994; K. Ogle and T. G. Whitham, *unpublished data*). Comparisons of soil parameters and pinyon performance in these two environments are summarized in Table 1. Levels of an additional 10 micro- and macronutrients in soils from the two site types were reported in Cobb et al. (1997). In all cases, the concentration of these nutrients was higher in the sandy-loam than in the cinder soils (Cobb et al. 1997). With supplemental water and nutrients, pinyons growing in cinder soils achieved increased growth, increased resin defense production, and decreased herbivory. This experiment demonstrated that nutrient and moisture stress were at least partially responsible for the lower pinyon performance observed at the cinder sites (Cobb et al. 1997).

Laboratory analysis of ectomycorrhizal fungal community structure

To establish if there were differences between the ectomycorrhizal fungal communities associated with pinyon pines growing in cinder and sandy-loam soils, we collected ectomycorrhizal root samples from 50 mature pinyon pines (trunk diameter 25–30 cm at 10 cm above ground level) covering an area of $\sim$0.5 km^2 at each of the six sites. Root samples were collected during the last two weeks of April 1995. At each site, one-half of the samples were collected from under the tree canopy and the other one-half were collected from outside the tree canopy beyond the dripline. Samples were collected from the four cardinal points around trees in roughly equal proportions. This protocol maximized the number of potentially different environmental microsites that were sampled, because these could have been occupied by different ectomycorrhizal fungi (Bruns 1995). Because of the difficulty of coring cinder soils, all samples were collected using a trowel and digging to a maximum depth of 20 cm. C. Gehring has extensive experience working with pinyon roots in these environments, and sorted all of the samples to ensure that roots from plants other than pinyon pine were not included.

In the laboratory, root samples were washed gently with tap water and stored at 4°C. Within one week of collection, four living ectomycorrhizal root tips per sample were removed with forceps, placed into individual microcentrifuge tubes, and frozen at -70°C. We classified the roots as ectomycorrhizal if they possessed a fungal mantle when examined under a dissecting microscope. We have verified several times that roots classified in this way possess a Hartig net (Gehring and Whitham 1991, 1994, 1995), and we did not section the root tips used for RFLP analysis because of the potential for loss of tissue. Viability of ectomycorrhizal root tips was based on turgescence and color, as described by Harvey et al. (1976).

One of the four frozen ectomycorrhizal root tips was selected at random for RFLP analysis. If RFLP analysis failed with that tip, another tip from the same tree was randomly drawn until an unambiguous RFLP pattern

was obtained. In some cases, none of the four tips from a tree yielded unambiguously scorable RFLP patterns. Therefore, our final sample sizes ranged from 43 to 48 ectomycorrhizal root tips per site, with each ectomycorrhizal root tip coming from a different tree.

In order to maximize sample independence, we chose to sample one ectomycorrhizal root tip per tree for 43–48 trees rather than analyzing multiple tips from a smaller number of trees. Ectomycorrhizal root tips collected from the same tree are unlikely to represent independent samples, as they experience many of the same environmental conditions. Thus, multiple samples from a single tree may not represent the range of variation found in the ectomycorrhizal fungal community at a given site. We tested this hypothesis by performing a pilot study at one of the three cinder sites in April 1994. This study involved RFLP analysis of 560 ectomycorrhizal root tips collected from 20 pinyon pines (15–45 ectomycorrhizal tips per tree, with an average sample size per tree of 28 ectomycorrhizal root tips). Ectomycorrhizal root tips were collected and handled as described for the main study. We plotted the distribution of RFLP types obtained from the 20 trees and determined whether there was a relationship between the number of root tips sampled and the percentage of root tips that were of a dominant type, using regression analysis.

We compared the RFLP patterns obtained from ectomycorrhizal root tips to those obtained from fungal sporocarps collected at the six sites since 1992 in an attempt to identify the fungi forming ectomycorrhizal associations with pinyon pine. Sporocarp collections were made in the spring and fall of 1992–1995 at the time of ectomycorrhizal collections. In 1992, 1993, and 1995, few fungi fruited in either of the two environmental types. Increased moisture in the fall of 1994 stimulated increased fungal fruiting; to enlarge our pool of identified species, we collected fungal sporocarps every 7–10 d from August through the end of October. In many cases, only a single individual of a species was observed to fruit. Although these rare sporocarps could be distinguished as different from others found during the collections, many could not be identified to species level because all of the characters necessary for species identification were not present on the single specimen.

DNA was extracted from ectomycorrhizal root tips and fungal sporocarps using the miniprep method of Gardes and Bruns (1993), with the following modification: the alcohol precipitation step was replaced with precipitation using glass fines, (GeneClean, BIO101, Vista, California, USA), and samples were subsequently eluted from the fines in 50 μL of double-distilled water and then diluted 10-fold before amplification, using polymerase chain reaction (PCR). The internal transcribed spacer region (ITS) of each sample was amplified using primers ITS1-F and ITS4, following the protocol of Gardes and Bruns (1993). The ITS region is located between the 18S and 28S rRNA genes in the nuclear genome and consists of a coding region and two highly variable noncoding regions (Gardes and Bruns 1993). The ITS amplicons were digested for 2 h using *Hin*fI and *Mbo*I enzymes, and the resulting RFLP patterns were photographed under UV illumination. After the initial screening, samples from different sites that showed similar RFLP patterns for both enzymes were reamplified, digested, and electrophoresed side-by-side to confirm whether the patterns were identical. We classified ectomycorrhizal root tips as the same type if their RFLP patterns were identical for both enzymes. Putative matches with fruiting-body RFLP patterns were verified in the same way. The ITS1-F and ITS4 primer combination amplifies a wide array of fungal taxa, whereas the ITS1-F and ITS4-B primer set is more restrictive, amplifying primarily DNA from fungi classified in the subphylum Basidiomycotina (Gardes and Bruns 1993). We tested whether the samples we amplified using primers ITS1-F and ITS4 could also be amplified using primers ITS1-F and ITS4-B, by adding DNA from samples representing each RFLP pattern to identical PCR reactions that varied only in which primer set was present. These sets were then run at the same time on the same thermocycler, and the presence or absence of amplicons was determined.

To determine whether the ectomycorrhizal fungal communities of pinyons occupying cinder and sandy-loam soils varied systematically, we performed a cluster analysis on the data from the six sites using TWINSPAN (Hill 1979). TWINSPAN is a polythetic, divisive classification program that uses two-way indicator species analysis to produce hierarchical classifications (Gauch 1982). TWINSPAN uses both the presence or absence of species and their abundance in its classification. TWINSPAN divisions were stopped at the second level.

Results

RFLP discriminatory ability and sampling strategy pilot study results

RFLP analysis of identified sporocarps indicated that digestion with two restriction enzymes allowed us to distinguish among genera, and frequently among species within a genus. For example, we examined 14 genera of fungal sporocarps (Table 2) and each genus had a unique RFLP pattern. Two species in the genus *Amanita* and four species in the genus *Russula* could also be distinguished from one another using RFLP analysis based on two enzymes. However, we were unable to distinguish two species within the genus *Lactarius.* We conclude from these findings that the ectomycorrhizal types we observed that did not match with known sporocarps are also likely to represent different species, or at least different genera. Gardes and Bruns (1996) also detected differences at the species level using two restriction enzymes, and Kraigher et

TABLE 2. Identity of sporocarps collected from cinder and sandy-loam sites, combined with the identity (from Fig. 4) and location of occurrence of ectomycorrhizal root tip RFLP patterns that matched RFLP patterns generated from the sporocarps.

Cinder		Sandy-loam		Both site types	
Sporocarp	RFLP type and occurrence	Sporocarp	RFLP type and occurrence	Sporocarp	RFLP type and occurrence
Geopora cooperi	type 1, all cinder sites	*Amanita pantherina*		*Cortinarius* sp.	
Rhizopogon rubescens	1 cinder site	*Amanita* sp.		*Lactarius deliciosus*	type 5; all sandy-loam, 2 cinder sites
Russula sp. 1		*Armillaria straminea*		*Rhizopogon pinyonensis*	
Russula sp. 2	2 cinder sites	*Chalciporus* sp.		*Rhizopogon* sp.	
Suillus subolivaceous		*Chroogomphus leptocystis*		*Russula rosaceae*	
Suillus kaibabensis		*Hebeloma* sp.		*Tricholoma terreum*	type 10, all sites
		Hygrophorus sp.	1 sandy-loam site		
		Inocybe fastigiata			
		Lactarius barrowsii			
		Russula sp. 3	1 cinder site		
No sporocarp match	15 RFLP types	No sporocarp match	17 RFLP types	No sporocarp match	12 RFLP types

al. (1995) were able to distinguish two species within the same genus using only one enzyme.

The results of our pilot-study analysis of 560 ectomycorrhizal root tips from 20 pinyon trees supported our hypothesis that sampling multiple root tips from an individual tree was not the best way to assess the ectomycorrhizal fungal community of pinyon pines across sites. We found that a mean of 55.6% of the ectomycorrhizal root tips on a tree were of a single dominant RFLP type (Fig. 1). An average of 82% of the ectomycorrhizal root tips on a tree were of two dominant types (Fig. 1). Furthermore, the percentage of ectomycorrhizal root tips that were of the dominant type on a tree was not significantly correlated with the number of ectomycorrhizal root tips sampled from that tree ($r^2 = 0.192$, $F_{1, 18} = 4.288$, $= P > 0.05$), suggesting that increased sampling did not result in a greater percentage of root tips of the nondominant types. Although each tree in the pilot study was dominated by a single ectomycorrhizal type, the same type did not dominate on all trees, and dominant types exhibited substantial spatial variation. Therefore, the ectomycorrhizal fungal community associated with pinyon pines across sites would best be described by sampling a large number of trees, but collecting only a small number of samples from each tree.

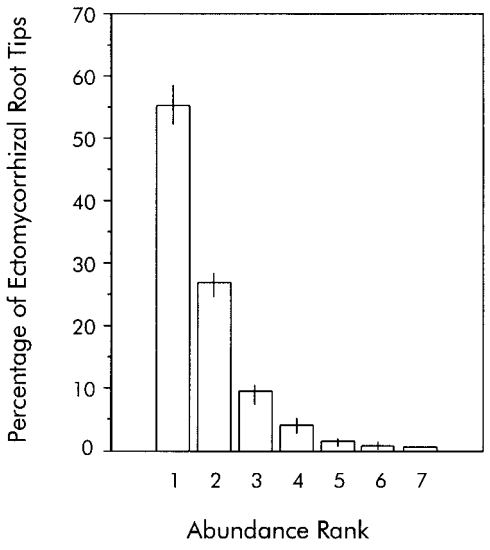

FIG. 1. The frequency of ectomycorrhizal RFLP types (mean ± 1 SE) on individual pinyon pine trees, demonstrating that one ectomycorrhizal type dominated on each tree. This figure is based on analysis of 560 ectomycorrhizal root tips obtained from 20 mature pinyon pine trees (n = 15–45 root tips per tree).

Contrasting RFLP and sporocarp-based estimates of ectomycorrhizal fungal communities

Our results emphasized that ectomycorrhizal fungal surveys based upon sporocarp censuses may be particularly poor indicators of the ectomycorrhizal fungal community actually colonizing plant root systems in arid environments. We analyzed DNA from a total of 272 ectomycorrhizal root tips from the six sites (136 from the three cinder sites and 136 from the three sandy-loam sites) and found 51 distinct RFLP patterns. Only seven of these (14%) matched RFLP patterns obtained from the 22 species of fungi observed to fruit at these sites over several years, accounting for 26% of the 272 ectomycorrhizal tips (Table 2). The remaining 44 RFLP types did not match with sporocarps.

Some of the species in which sporocarp and ecto-

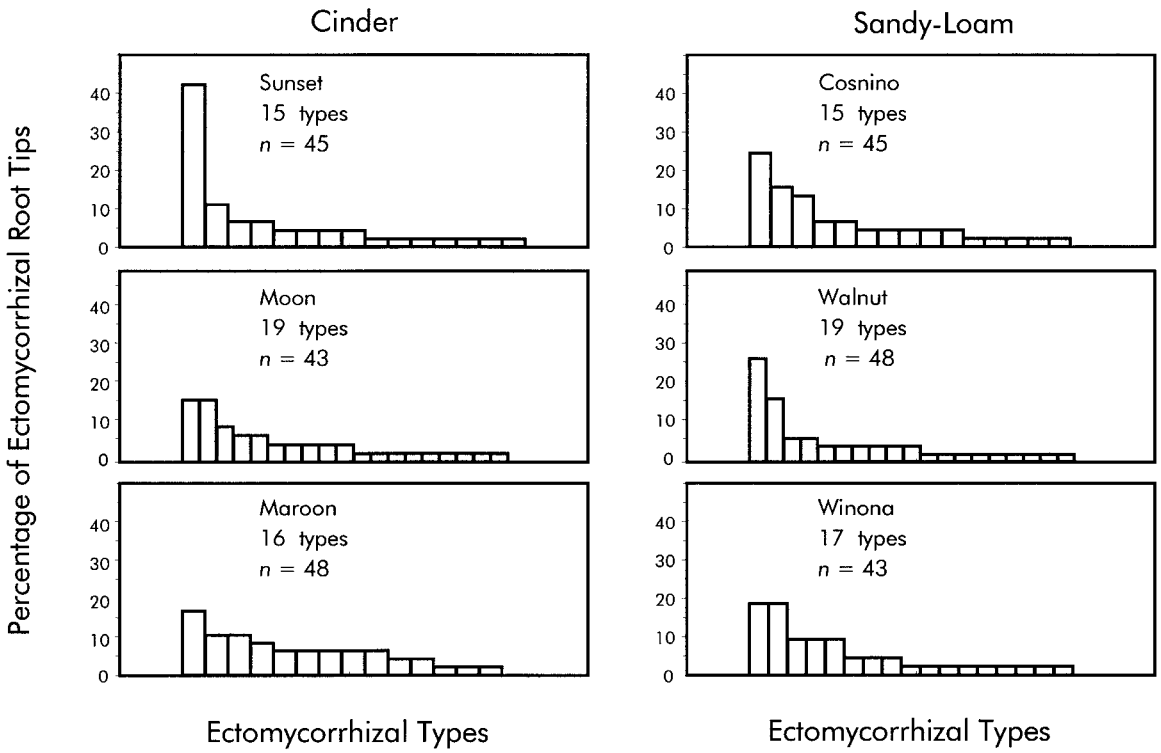

FIG. 2. Patterns of abundance of the ectomycorrhizal types associated with the three cinder sites and the three sandy-loam sites, demonstrating that the sites were characterized by one or two dominant types and a larger number of rare types.

mycorrhiza RFLP patterns matched were widespread both as sporocarps and ectomycorrhiza (e.g., *Tricholoma terreum* and *Lactarius deliciosus*), whereas others were widespread as sporocarps, but were never observed as ectomycorrhiza (e.g., *Rhizopogon pinyonensis*). The reverse was also true; RFLP patterns of many of the common ectomycorrhizal types did not match patterns generated from known sporocarps. In our pilot study at one of the cinder sites ($n = 560$ root tips), we found an additional four rare (<3% of the sample) ectomycorrhizal RFLP types that were not observed in this study. These RFLP patterns also did not match with RFLP patterns generated from any of the sporocarps. Similarly, Lane (1995) compared the ectomycorrhizal basidiomycete community of pinyon pines at one cinder site and one sandy-loam site and found that 60% of the ectomycorrhizal types did not match with basidiocarps found at the same sites. These data suggested that sporocarp surveys were unlikely to be informative in arid areas where fruiting was infrequent and potentially biased toward only a few species.

Comparison of richness and dominance of ectomycorrhizal types between cinder and sandy-loam soils

Across soil moisture and nutrient extremes, we found that richness of ectomycorrhizal fungal types remained nearly constant. The cinder and sandy-loam soil types had similar numbers of ectomycorrhizal types; cinder sites averaged 16.7 ± 1.20 types (mean ± 1 SE; range 15–19 types), and sandy-loam sites averaged 17.0 ± 1.16 types (range 15–19; Fig. 2). Of the 51 ectomycorrhizal types, 28 occurred at only one of the six sites, and 16 of these were represented by only one ectomycorrhiza. The number of unique ectomycorrhizal types per site was similar for pinyons growing in both soil types, averaging 5.0 ± 1.73 types for the sandy-loam soil type and 4.3 ± 1.86 types for the cinder soil type.

The pattern of abundance of ectomycorrhizal types within a site was similar at all six sites, with one to three dominant ectomycorrhizal types accounting for 33–53% of the ectomycorrhiza (Fig. 2). The remaining ectomycorrhizal types were comparatively rare. This dominance by a limited number of ectomycorrhizal types was particularly evident at one of the three cinder sites, Sunset Crater, where a single ectomycorrhizal type accounted for 42.2% of the ectomycorrhiza. Similar species abundance patterns have been observed in studies of ectomycorrhizal fungal communities using sporocarp censuses (e.g., Bills et al. 1986, Villeneuve et al. 1989).

Differences in ectomycorrhizal community composition of pinyons growing in cinder and sandy-loam soils

Based upon our analyses of root tips, pinyons at cinder and sandy-loam sites had similar values for ec-

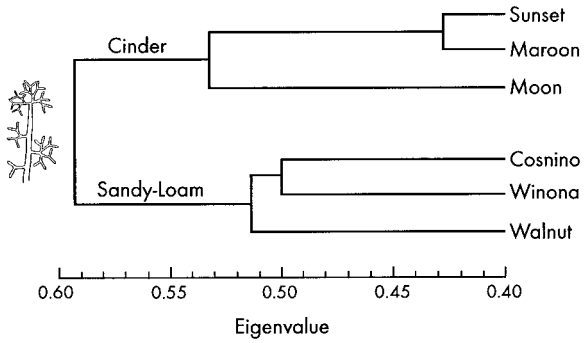

FIG. 3. Dendrogram illustrating the results of a TWINSPAN analysis of the ectomycorrhizal fungal types occurring at the six sites. Similarity between sites in ectomycorrhizal fungal communities increases from the left to the right. The first TWINSPAN division separated the cinder soil type ectomycorrhizal types from the sandy-loam soil type ectomycorrhizal types, indicating that differences across soil types (cinder vs. sandy-loam) were greater than differences within soil types.

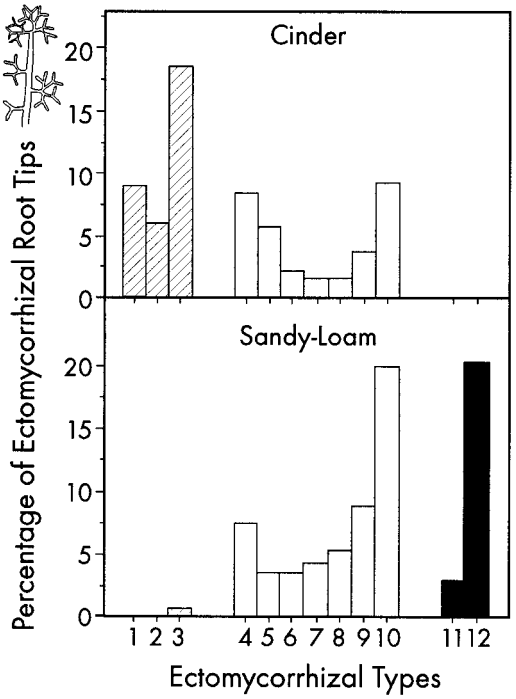

FIG. 4. Abundance in cinder and sandy-loam soils of the 12 most common ectomycorrhizal types, showing that five of the common ectomycorrhizal types were found only in one of the soil types, whereas seven occurred in both soil types. The most common ectomycorrhizal types are those that were found at more than one site and that represented ≥5% of the ectomycorrhiza at at least one site.

tomycorrhizal fungal species richness, but there were distinct differences in the communities associated with the two site types. TWINSPAN divided the six sites into two major groups, a sandy-loam group and a cinder group, with an eigenvalue of 0.593 (Fig. 3). The eigenvalue ranges from 0 to 1 and is a rough indicator of the amount of variation in the groups explained by this division, with higher eigenvalues indicating that greater amounts of variation were explained. To divide the ectomycorrhizal types found at cinder and sandy-loam sites, TWINSPAN used differences both in the ectomycorrhizal types present and in the abundance of shared ectomycorrhizal types. For example, ectomycorrhizal type 1 was important in the analysis because it was found at all of the cinder sites, but was absent from all of the sandy-loam sites. Ectomycorrhizal type 10 was found at all of the sites, but was more abundant at the sandy-loam sites and, therefore, was one of the ''species'' used to divide the cinder sites from the sandy-loam sites.

The second TWINSPAN division separated two of the cinder sites (Sunset and Maroon) from the third (Moon), based on the rarity of ectomycorrhizal type 5 at Moon and the presence of an abundant ectomycorrhizal type found only at the Moon site (Fig. 3). The second TWINSPAN division of the sandy-loam sites separated Walnut from Cosnino and Winona, based primarily on the low abundance of ectomycorrhizal type 4 at Walnut relative to the other two sandy-loam sites. Overall, the results of the TWINSPAN analysis indicated that variation in ectomycorrhizal communities was greater between the two soil types than within them.

Five (1, 2, 3, 11, 12) of the 12 most common ectomycorrhizal types (those that were found at more than one site and that represented ≥5% of the ectomycorrhiza at at least one site) were found virtually exclusively at either cinder or sandy-loam sites, whereas the remaining seven fungal types were found at both site types (Fig. 4). Two ectomycorrhizal types (10 and 12) dominated at all three sandy-loam sites, ranging from 37% to 43% of the total number of ectomycorrhiza examined at these sites. The RFLP pattern of one of these common types matched with a sporocarp classified in the subphylum Basidiomycotina, *Tricholoma terreum.* In contrast, each cinder site was dominated by a different ectomycorrhizal type (type 3 for Sunset, type 1 for Moon, and type 10 for Maroon).

Not only were there major differences in the ectomycorrhizal fungal species composition of pinyons growing in cinder and sandy-loam soils, but also there were differences in the subphyla of fungi involved in the symbiosis at the two site types. The three relatively common ectomycorrhizal types in the cinder soils that were virtually absent in the sandy-loam soils (Fig. 4, types 1, 2, 3) all failed to amplify when we used primers designed with enhanced specificity for members of the subphylum Basidiomycotina. Because most ectomycorrhizal fungi are classified in either the Basidiomycotina or the Ascomycotina (Molina et al. 1992), these ectomycorrhizal types are likely to be members of the subphylum Ascomycotina. Consistent with this hypothesis, RFLP pattern 1 matches with a pattern gen-

erated from a hypogeous fruiting body classified within the Ascomycotina (*Geopora cooperi*). Overall, 33% of the ectomycorrhizal root tips examined from both site types did not amplify with the primers specific for the Basidiomycotina: an average of 15% of the ectomycorrhiza at the sandy-loam sites and 52% of the ectomycorrhiza at the cinder sites.

Discussion

Ectomycorrhizal fungal community patterns and comparisons with other communities

All six sites had similar numbers of ectomycorrhizal types (hereafter referred to as species richness) and similar patterns of abundance. The sites were characterized by a few common ectomycorrhizal species and a larger number of rare species. This pattern has been observed in other studies using sporocarp censuses (e.g., Bills et al. 1986, Villeneuve et al. 1989) and in direct censuses of ectomycorrhiza using morphological (e.g., Natarajan et al. 1992, Visser 1995) and molecular techniques (Gardes and Bruns 1996). For example, based upon RFLP analysis, we observed that 42% of the root tips at one site, Sunset Crater, were colonized by a single type of ectomycorrhizal fungus. Similarly, using morphological techniques, Natarajan et al. (1992) found that *Amanita muscaria* colonized 45% of the root tips of 17-yr-old *Pinus patula* grown in plantations in India. These similarities among studies using varying methodologies and sites suggest that dominance by a few species may be a common feature of ectomycorrhizal fungal communities. This pattern is not unique to ectomycorrhizal fungi; similar patterns have also been found in a variety of communities ranging from Amazonian birds (Terborgh et al. 1990) to African dung beetles (Doube 1991).

Although our patterns of species abundance of ectomycorrhizal fungi were in agreement with those observed in other diverse taxa, as previously described, distinct differences also emerged. For example, the lack of an effect on ectomycorrhizal species richness of the environmental differences between cinder and sandy-loam sites contrasted with studies on a variety of other organisms. In many communities, species richness shows a unimodal distribution with productivity; species richness initially increases with increasing productivity, peaks at intermediate levels of productivity, and then declines with further increases in productivity (see reviews by Rosenzweig and Abramsky 1993, Tilman and Pacala 1993). Organisms in which such unimodal distributions have been shown include plants in California (Whittaker 1975) and Australia (Beadle 1966), mammals in tropical Australia (Rosenzweig and Abramsky 1993), and brachiopods in Antarctica (Foster 1974 in Rosenzweig and Abramsky 1993). In these studies, productivity itself was rarely measured, and instead was estimated based upon soil nutrient status, rainfall, and/or biomass. In agreement with these findings, at some of the same study sites used in our ectomycorrhizal community analysis, canopy and litter arthropod species richness and abundance increased dramatically as soil nutrients and moisture increased across soil types (N. S. Cobb and T. G. Whitham, *unpublished data.* K. Morrison and C. A. Gehring, *unpublished data*).

Although these studies, including two on pinyon pine arthropods in the same habitats we have studied, indicated that species richness was associated with productivity, the results of this study indicated that species richness of the ectomycorrhizal fungal community remained constant across extreme habitats. The different responses of species richness in arthropods and ectomycorrhizal fungi to productivity in the pinyon system may be due to (1) differences in the levels of productivity at which species richness rises and falls in the two types of organisms, because (2) cinder and sandy-loam samples may have represented opposite sides of the unimodal distribution for ectomycorrhizal fungi. A third alternative is also possible; as mutualists that receive most of their energy from the plants with which they form intimate associations, ectomycorrhizal fungi may be buffered against the environmental extremes experienced by their plant associates. Using sporocarp censuses, Villeneuve et al. (1989) found that ectomycorrhizal fungal species richness remained relatively constant (37 to 28 species) along a gradient of increasing environmental rigor and instability. However, the species richness of saprophytic fungi, which did not form intimate mutualistic associations with plants, declined from 125 species to six species along the same environmental gradient (Villeneuve et al. 1989). Thus, it may be important to separate mutualists from other community members when examining general patterns of species richness as a function of ecosystem productivity. Furthermore, these data suggest that it is important to discover how important ectomycorrhizal fungal diversity is to the survival of higher plants and their dependent communities in stressful environments.

Ectomycorrhizal community composition differences and their importance

Our comparisons of the ectomycorrhizal fungal communities of pinyons growing in cinder and sandy-loam soils yielded two potentially important patterns. First, ectomycorrhizal fungi responded to the differences between the soil types in both their patterns of occurrence (presence or absence at a site type; Fig. 4) and their patterns of abundance (frequency of occurrence when they were found at both site types; Fig. 4). Numerous differences between the cinder and sandy-loam sites could explain the variation in ectomycorrhizal fungal community composition. For example, cinder and sandy-loam soils differed in moisture and nutrient content (Table 1) as well as litter depth (C. Gehring, *unpublished data*). Sporocarp censuses have also indicated that the community structure of ectomycorrhizal

Basidiomycetes varied with soil characteristics, including soil parent material (Hering 1966), soil drainage characteristics, mineral soil richness, and humus organic matter content (Nantel and Neumann 1992). However, our results are unusual because they were obtained from direct analyses of ectomycorrhizal roots and, thus, were not complicated by differences in plant species composition or variation in fungal fruiting across environments.

Second, based on differential amplification using basidiomycete-specific primers, most (85%) of the ectomycorrhiza found at the sandy-loam sites were members of the Basidiomycotina, whereas more than half (52%) of the ectomycorrhiza at the cinder sites were most likely to be members of the Ascomycotina. Greater prevalence of members of the Ascomycotina has been observed in the ectomycorrhiza of white (*Picea glauca*) and blue spruce (*P. pungens*) growing in semiarid environments (Danielson and Pruden 1989), and in the early stages of fungal succession on mined sites (Danielson 1991) and on Mount Saint Helens (Carpenter et al. 1987). All of these patterns were consistent with our results. Compared to the sandy-loam sites, the cinder sites represented both a more arid environment (Gehring and Whitham 1994) and a younger one resulting from a geologically recent volcanic event. However, there were some important differences between the aforementioned studies and ours. The studies conducted by Danielson and Pruden (1989) and Danielson (1991) were conducted not in natural environments, but in city parks and coal spoil/oil sands tailings, respectively, and the study on Mount Saint Helens focused on successional changes during the three years immediately following the eruption, when few ectomycorrhizal species were present (Carpenter et al. 1987). Further research is necessary to understand the distribution and importance of ascomycete ectomycorrhizal fungi in natural systems, particularly in abiotically stressful areas.

In conclusion, we found that the ectomycorrhizal fungal communities associated with pinyon pines responded to abiotic environmental conditions not only in terms of putative species associations, but also in the major fungal subphyla involved in the mutualism. Nantel and Neumann (1992) proposed that site selection for conservation based on vegetation mapping and/or classification might miss important fungal species; they argued for the conservation of multiple replicates of the same types of environments. Our data support this view, but we further propose that abiotically stressful locations be considered in conservation efforts for two reasons: (1) these locations may have a different species composition than less stressful areas, as demonstrated in this study, and (2) stressful environments may contain species and/or genotypes of organisms that can better survive human-caused environmental stresses such as global climate change. Although conservation of higher organisms has received much attention, the conservation of fungi is equally important because of their pivotal roles in ecosystem function. This could be especially true for fungi involved in mycorrhizal associations, as these fungi may be crucial to plant survival, especially in stressful and changing environments.

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