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ECTOMYCORRHIZAL ABUNDANCE AND COMMUNITY COMPOSITION SHIFTS WITH DROUGHT: PREDICTIONS FROM TREE RINGS

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Abstract. Mycorrhizae play a key role in ecosystem dynamics, and it is important to understand how environmental stress and climate change affect these symbionts. Several climate models predict that the intercontinental western United States will experience an increase in extreme precipitation events and warming temperatures. In 1996, northern Arizona, USA, experienced a 100-year drought that caused high local mortality of pinyon pine (*Pinus edulis*), a dominant tree of the southwest. We compared trunk growth, water potentials, and ectomycorrhizal dynamics for surviving trees at three high-mortality sites and adjacent low-mortality sites. Four major patterns emerged. First, surviving trees at sites that suffered high mortality exhibited reduced long-term growth and increased water stress relative to adjacent sites where little or no mortality occurred. Second, surviving trees from high-mortality sites had 50% lower ectomycorrhizal colonization and showed a pronounced shift in fungal community composition relative to low-mortality sites. Third, in support of an intermediate-host plant stress hypothesis, trees that experienced intermediate levels of stress supported two-fold greater ectomycorrhizal colonization than trees at the high or low end of a stress gradient. Fourth, we observed a strong correlation between trunk growth and ectomycorrhizal colonization and validated the resulting regression model with independent data. This relationship suggests that tree rings can be used to reconstruct past and predict future ectomycorrhizal colonization. Overall, our findings suggest that predicted climate changes might be accompanied by both qualitative and quantitative changes in ectomycorrhizal dynamics that could affect ecosystems by altering nutrient cycling, carbon dynamics, and host-plant performance.

Key words: Arizona, USA (northern); climate-change effects; community structure; drought effects; ectomycorrhizal fungi; intermediate-host plant stress hypothesis; Pinus edulis; *tree rings predict ectomycorrizal colonization.*

INTRODUCTION

Soil microbes play important roles in ecosystems through their effects on plant performance, soil structural stability, and nutrient availability. For example, the benefits that ectomycorrhizal fungi confer on their host plants can lead to improved growth, increased survival, greater drought tolerance, and resistance to disease (Molina et al. 1992, Smith and Read 1997). In addition, by altering nutrient dynamics (Smith and Read 1997) and carbon flow (Rygiewicz and Andersen 1994) these fungi may play an important role in ecosystem processes. These effects are likely to be widespread, as ectomycorrhizal fungi colonize the roots of >2000 species of woody plants in habitats ranging from boreal forests to semi-arid woodlands (Smith and Read 1997). Over 5000 species of fungi form ectomycorrhizal associations (Molina et al. 1992), but little is known about the importance of this diversity of ectomycorrhizal fungal associates. However, accumulating evidence suggests that different species of ectomycorrhizal fungi vary both in their responses to environmental variables and in their relationship with host plants (e.g., Godbold and Berntson 1997, Cairney 1999, Saikkonen et al. 1999).

While the importance of ectomycorrhizal fungi in ecosystems is becoming increasingly appreciated, we have a poor understanding of how changes in climate, such as increased drought stress, might quantitatively or qualitatively influence these fungi. In previous studies, quantitative ectomycorrhizal responses to water stress have been mixed, with some researchers finding declines in ectomycorrhizal colonization with moisture stress (e.g., Worley and Hackskaylo 1959, Runion et al. 1997, Nilsen et al. 1998), while others observed a positive relationship (Gehring and Whitham 1994, 1995, Swaty et al. 1998) or no significant association (Meier et al. 1990). Ectomycorrhizal fungal community composition also may shift in response to drought and soil warming (Gehring et al. 1998, Rygiewicz et al. 2000, Kernaghan and Harper 2001, Shi et al. 2002). For example, the ectomycorrhizal fungal community composition of pinyon pine (*Pinus edulis*) differed among semi-arid sites that varied in soil properties including soil moisture, temperature, and fertility (Gehr-

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ing et al. 1998). Shi et al. (2002) observed that the ectomycorrhizal fungal community composition of beech trees (*Fagus sylvaticus*) exposed to three months of experimental drought differed significantly from control trees, demonstrating rapid responses to climate change. While the results of these studies suggest that the effects of drought on ectomycorrhizal fungi could be important, little research has been done on the ectomycorrhizal fungi associated with trees experiencing large-scale drought in the field.

Recent drought events in northern Arizona (USA) revealed areas of high stress and provided us with the opportunity to examine the responses of trees and their associated ectomycorrhizal fungi to severe drought. Global warming has been predicted to result in increasingly arid continental interiors that experience more frequent and intense droughts, with semiarid ecosystems among the most sensitive (Risser 1995, Allen and Breshears 1998). Regionally, Palmer drought severity index values indicate that northern Arizona has experienced moderate to extreme drought for five of the last seven years.[2] Furthermore, these drought events have resulted in significant dominant-tree mortality in major vegetation zones. For example, in northern Arizona, the 100-year drought of 1995–1996 was followed by up to 70% mortality in mature pinyon pines (*P. edulis*) at some sites (Ogle et al. 2000). By pairing sites that suffered high pinyon mortality with adjacent sites where few trees died (within 650 m) we quantified the factors associated with extreme pinyon mortality without the complication of extensive spatial variation among sites that differed in their responses to drought.

To examine how pinyon pine and its ectomycorrhizal fungal associates responded to drought, we addressed the following questions: (1) How do ectomycorrhizal colonization and community structure vary between sites of high and low host-plant mortality? (2) Does ectomycorrhizal colonization vary linearly or nonlinearly with host-plant stress? The nature of the relationship between ectomycorrhizal fungi and their hosts is important as it may provide insights into the factors that regulate the mutualism. (3) Is there a predictable relationship between tree-ring expansion, a measure of host-plant vigor, and ectomycorrhizal colonization? By addressing these questions at adjacent sites that varied primarily in host-plant stress due to drought, we hoped to gain a better understanding of the responses of ectomycorrhizal fungi to predicted climate change.

METHODS

Description of the study sites

We addressed the above questions at three high-mortality sites distributed across the pinyon distribution in north-central Arizona (USA). The sites were on average 14.8 km apart from one another and had rates of mortality that ranged from 50% to 70% in mature (trunks with a basal diameter >10 cm) pinyon pines (Table 1). High-mortality sites were paired with nearby low-mortality sites in which trees of the same size experienced 0–5% mortality (average distance between members of a pair = 450 m) (Table 1). Hereafter the high-mortality–low-mortality site *pairs* are referred to as sites 1, 2, and 3. The sites were dominated by *Pinus edulis* and one-seed juniper (*Juniperus monosperma*). The soil parent material at all of the sites was basaltic/cinder and the soils were classified as Vitrandic Ustocrepts for site 1, Typic Ustorthents for site 2, and Typic Haplustalfs for site 3 (Miller et al. 1995). Site slope measurements were taken with a bubble protractor at five random points within a site and means are presented in Table 1. For 2000 and 2001, primary data collections were done during the month of June, the hottest, driest time of the year. Mean daily ambient temperatures during June were 29.8° for 2000 and 27.8°C for 2001, while total monthly precipitation was 0.28 mm for 2000 and 0.007 mm for 2001 for the area that encompassed the sites.

Measurements of tree condition

To determine if levels of host-plant stress varied among the high- and low-mortality sites, we measured trunk growth and predawn water potential for 20 mature trees at sites 1, 2, and 3–10 trees at each high-mortality site and 10 trees at each low-mortality site (total: 60 trees). The trees were similar in size (15–25 cm basal trunk diameter) among sites and were free of obvious infestation by herbivores or parasites. This size range was selected because trees within this size class showed equal levels of mortality during the 1996 drought in northern Arizona (T. G. Whitham, *unpublished data*). The trees within a site pair were also similar in age (t test $P > 0.05$) as determined by the tree-ring analyses described below (Table 1). To measure trunk growth we collected one core per tree at $\sim$25 cm above the base of the tree. Trees on slopes were cored parallel to the slope to account for reaction and tension wood (Ogle et al. 2000) and prepared as described in Swetnam et al. (1988). We measured rings laid down between 1986 and 2000 and cross dated the cores using 1989 and 1996 rings as markers because rings from those years were characteristically narrow due to severe drought (see Stokes and Smiley 1968). To compare ring widths between high- and low-mortality sites, we performed a repeated measures ANOVA in SPSS 10.0 for Windows (referred to as ‘‘SPSS’’ hereafter; Norusis 1999) with ring width as the dependent variable and time and degree of mortality (high or low) as within-subject factors. Rings were counted from the entire core to estimate tree age.

We measured the predawn water potentials of all trees because this measure indicates water deficits within a plant and predicts overall tree performance (Myers 1988, Bandara et al. 1999). We compared the water relations of trees in the high- and low-mortality sites

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[2] URL: ⟨www.noaa.gov⟩

TABLE 1. Tree and soil characteristics at three pairs of high-mortality–low-mortality pinyon pine (*Pinus edulis*) sites in northern Arizona, USA.

	Site	
	1	
Tree and soil parameters	High	Low
Pinyon mortality (%)†	70.1	3.2
Distance between paired high- and low-mortality site (m)	630	
Slope, Aspect	37°, east	7°, east
Tree age (yr)	72.3 ± 18.0	62.2 ± 5.1
Tree water potentials (−MPa)		
2000	4.1 ± 0.21	3.1 ± 0.18
2001	4.7 ± 0.14	4.3 ± 0.13
pH	7.3 ± 0.15	7.1 ± 0.20
NH_4 (ug/g dry soil)	3.5 ± 0.61	3.3 ± 0.52
NO_3 (ug/g dry soil)	5.5 ± 0.91	5.3 ± 0.71
PO_4 (ug/g dry soil)	123.9 ± 13.1	94.1 ± 29.2
Soil composition (% sand, silt, clay)‡	37.4, 49.9, 12.7	36.6, 50.9, 12.5
2-mm fraction (%)§	56.8 ± 2.11	52.1 ± 2.35
Soil moisture (%)‖	4.8 ± 0.12	5.2 ± 0.21
Soil temperature (°C)‖	16.6 ± 3.54	16.3 ± 3.78

Note: Data are either means or means ± 1 SE.

† Trees with trunk diameters at breast height (1 m) >10 cm.

‡ Mean sand, silt, and clay fraction based on 10 samples per site.

§ Percentage of soil particles >2 mm.

‖ Mean values calculated from five sample periods (see *Methods*: *Soil parameters* for details).

by measuring the predawn water potential of each of the study trees during June, in both 2000 and 2001. Using a pressure chamber (PMS Instrument, Corvallis, Orogon, USA) we measured water potentials of two fascicles from the previous year's growth randomly collected from different areas of each tree. All trees from one site pair were sampled in one morning to minimize temporal variation. The measurements from the two fascicles per tree were averaged and the data were analyzed in SPSS using a two-way ANOVA with water potential as the dependent variable and site and degree of mortality (high or low) as fixed factors.

Estimating ectomycorrhizal colonization and community structure

We compared the ectomycorrhizal colonization and community structure of pinyons growing in high- vs. low-mortality sites by collecting root samples (250 cm of roots) from the eastern side of the drip line of each tree to a maximum depth of 30 cm. Our studies on pinyon pine indicate that ectomycorrhizal colonization and community composition vary with aspect and distance from the trunk (C. A. Gehring, *unpublished data*), so we standardized our sampling to reduce these sources of variation. We collected all of the roots for trees located within a site pair on the same day during midsummer of both 2000 and 2001 and sampled the three site pairs on consecutive days to minimize temporal variation. Within three days of collection, we used the techniques of Gehring and Whitham (1991) to distinguish ectomycorrhizal from non-ectomycorrhizal and living from nonliving root tips and calculated percentage ectomycorrhizal colonization for each tree as the number of active ectomycorrhizal root tips divided by the total number of short roots available for colonization (Gehring and Whitham 1991, 1994, Swaty et al. 1998). We used this measure because we have observed that pinyon ectomycorrhizae in these semi-arid ecosystems senesce during dry times and are replaced at their bases by fresh tips when conditions improve. Our measure looks at only those root tips that appear active (turgid, varying in mantle color, and with turgid hyphae) during the time of collection. Previous studies demonstrate that this measure correlates well with environmental variables such as ambient precipitation and temperature (Swaty et al. 1998) as well as with pinyon seedling performance (Gehring and Whitham 1994). We analyzed the ectomycorrhizal colonization data in SPSS using a two-way ANOVA with ectomycorrhizal colonization as the dependent variable and site and degree of mortality as fixed factors.

To compare the ectomycorrhizal fungal communities across sites, we categorized all of the living ectomycorrhizal tips from each tree into morphological types (morphotypes) using a dissecting microscope at 40× magnification. We employed the morphotyping techniques described by Horton and Bruns (2001) in which ectomycorrhizal root tips were categorized by branching pattern, color, and texture along with the presence, color, and texture of emanating hyphae and rhizomorphs. For the 2001 samples, we placed three tips of a given morphotype per tree in microcentrifuge tubes and stored them at −70°C for subsequent molecular analyses. DNA was extracted from three root tips per morphotype per tree using the techniques described by Gardes and Bruns (1993) and modified by Gehring et al. (1998) for pinyons. The internal transcribed spacer (ITS) region of the fungal genome, located between the

TABLE 1. Extended.

Site			
2		3	
High	Low	High	Low
56.5	4.7	60.1	2.2
400		320	
30°, south	0°, ···	39°, south	0°, ···
158.9 ± 16.5	171.3 ± 12.52	61.5 ± 5.7	57.6 ± 2.8
3.3 ± 0.21	2.3 ± 0.11	3.5 ± 0.11	2.7 ± 0.11
5.3 ± 0.18	4.7 ± 0.21	4.6 ± 0.23	4.4 ± 0.28
6.8 ± 0.22	6.9 ± 0.24	7.2 ± 0.21	7.4 ± 0.27
3.0 ± 0.43	3.4 ± 0.67	2.8 ± 0.49	2.5 ± 0.29
4.8 ± 0.73	5.3 ± 0.51	2.5 ± 0.13	3.4 ± 0.22
76.6 ± 17.1	100.2 ± 11.7	106.3 ± 18.1	110.4 ± 21.7
79.8, 16.3, 3.9	81.5, 13.7, 4.8	52.1, 35.4, 13.2	51.6, 35.2, 13.1
25.6 ± 1.89	30.3 ± 1.95	38.7 ± 2.21	41.9 ± 2.51
3.5 ± 0.22	5.0 ± 0.30	3.8 ± 0.19	4.1 ± 0.21
17.8 ± 3.33	15.1 ± 3.37	17.7 ± 3.77	14.5 ± 4.01

18S and 28S rRNA, was amplified using PCR (Polymerase chain reaction) with the ITS1F and ITS4 primer pair (Gardes and Bruns 1993). The amplified ITS region was characterized using restriction-enzyme digestion with *Hin*fI and *Mbo*I (Promega brand [Madison, Wisconsin, USA]) followed by visualization using agarose gel electrophoresis. Digestion with two enzymes frequently distinguishes among ectomycorrhizal fungal species (Gardes and Bruns 1996, Dahlberg et al. 1997) and these two enzymes have been used successfully to discriminate among fungal species in *Pinus edulis* (Gehring et al. 1998). Digital images of gels were recorded and analyzed using a Kodak EDAS 2900 gel documentation system and accompanying software.

We used nonmetric multi-dimensional scaling (NMDS), an ordination technique, to statistically analyze the RFLP (restriction fragment length polymorphism) community of the 2001 data from each tree using the program DECODA (Fensham et al. 2000). We used analysis of similarity (ANOSIM; Clarke 1993, Dungey et al. 2000) to test for significant differences in ectomycorrhizal community composition. First, we compared within site-pair (high mortality vs. low mortality) RFLP communities for 2001 for the three sites. Second, we pooled the RFLP data from high-mortality sites and compared that to low-mortality sites to determine if there were consistent differences between low- and high-mortality sites independent of location. ANOSIM operates directly from the dissimilarity matrix and yields a test statistic, R, that measures the difference of rank similarities between and within groups. An R value of 1 indicates that all replicates within groups are more similar to each other than to any replicates from different sites. An R value of 0 indicates no difference in the rank of similarities (no consistent difference exists in species composition between the two areas) (Clarke 1993). We also calculated species richness (S) for the 2001 RFLP data and compared these values between high- and low-mortality sites in SPSS with a paired t test.

To determine the nature of the relationship (linear vs. nonlinear) between host-plant condition and ectomycorrhizal colonization, we established a gradient of host-plant condition at one of the high-mortality sites, site 2 (O'Neil Crater). We chose 50 trees that seemed to represent a stress continuum based on physical appearance and divided the trees evenly into ranks 1–5, with trees in rank 1 experiencing the lowest levels of stress and trees in rank 5 the highest. We then quantified levels of tree stress in each rank by visually estimating the percentage of dead branches within a tree, counting the number of years of needles retained, and collecting tree cores to estimate ring-width expansion over the past 15 years. Data on foliage retention and percentage dead branches were analyzed in SPSS first using a multivariate analysis of variance (MANOVA) with number of years of needles retained and percentage dead branches as the dependent variables and tree rank as the fixed factor. A univariate ANOVA (Johnson 1998) and a Tukey's hsd test to locate significant differences followed a significant MANOVA. Ring width was analyzed using a repeated-measures ANOVA as described above for the high- and low-mortality sites followed by a Tukey's hsd test. Once tree selection was complete, we sampled and scored roots for ectomycorrhizal colonization as described above.

To examine the relationship between 2001 ectomycorrhizal colonization and 2001 tree-ring expansion, we used SigmaPlot 6.0 (SPSS 2000), to fit ectomycorrhizal colonization data from the host-plant stress gradient with regression models, using the model that yielded the highest adjusted R (or r^2) for subsequent analyses. Then, to validate the model, we used the regression equation and 2000 ring-width data from O'Neil Crater (Site 2) high- and low-mortality sites to predict the 2000 ectomycorrhizal colonization of those trees. This represents an independent test because a separate set of trees was sampled during a different year. To determine the accuracy of these predictions, we tested the significance of the correlation between the predicted and observed ectomycorrhizal values (Kleinbaum et al. 1988).

Soil parameters

To determine if variation in soil texture, nutrients, temperature, or moisture availability was associated with differences in tree and ectomycorrhizal parameters between high- and low-mortality sites, we measured soil particle-size distribution, soil pH, and available NH_4, NO_3, and PO_4 at all three site pairs. For each site, a 50-m transect was established that bisected the area of sample trees. Ten samples were collected per site, one at a random lateral distance every 5 m along the transect. On 19 March 2001 we collected 100 mL of soil in the root zone (15–30 cm) of the pinyon pine closest to the transect. To prevent possible changes in exchangeable NH_4^+-N or NO_3-N during transport, soils to be analyzed for available N were sieved and extracted in the field with pre-weighed 2 mol/L KCl-filled bottles as outlined in Overby and Perry (1996) and Hart and Firestone (1989). Separate non-sieved subsamples were analyzed for pH, PO_4, and particle size. Ammonium concentration was determined using the phenolate method, which is based on the Berthelot reaction where ammonia reacts with alkaline phenol then with sodium hypochlorite to form indophenol blue (Keeney and Nelson 1982, Lachat Instruments 2001*a*). Soil NO_3 was analyzed by diazotiation after reduction to NO_2 by zinc (Keeney and Nelson 1982, Lachat Instruments 2001*b*). All N analyses were completed on a Lachat flow-injection analyzer. Available phosphorus was determined using the sodium-bicarbonate extraction method (Olsen and Sommers 1982). After extraction the subsequent colorimetric analysis was performed on a Technicon autoanalyzer. Soil pH was determined using a calcium chloride solution as described in McLean (1982). Particle-size distribution was determined using the hydrometer method after chemical dispersion with Na-hexametaphosphate and physical dispersion with an electric mixer (Gee and Bauder 1982). In addition to the gravimetric soil moisture and root-zone (15–30 cm) temperature measurements taken at the time of soil collection for the preceding analyses, we also measured morning soil temperatures of the individual study trees with a VRS soil thermometer and collected soils under the sample trees for soil-moisture analysis during July, September, and November 2000 and February and June 2001.

RESULTS

Surviving pinyons at high-mortality sites suffer chronic stress

Rates of trunk growth over the past 15 years indicated that pinyons growing in high-mortality sites suffered chronic stress (Fig. 1). We observed two patterns. First, ring-width expansion of surviving pinyons at the three high-mortality sites were significantly lower (30% on average) than those of pinyons at the three low-mortality sites ($F_{2,40} = 12.012$, $P < 0.001$). These results suggest that trees growing at high-mortality sites experienced chronic stress that reduced trunk growth. Second, tree growth varied significantly over time at all site pairs, ($F_{14,40} = 12.220$, $P < 0.001$), with the lowest values of ring expansion observed during 1996, the year of a 100-year record drought in the region. These results demonstrate significant respon-

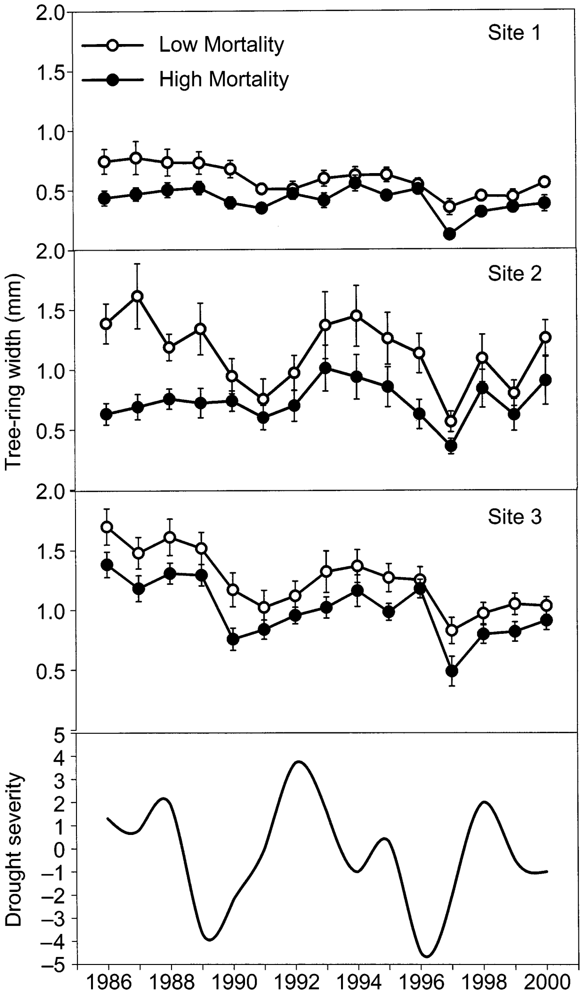

FIG. 1. Trunk growth of pinyon pines at three high- and low-mortality sites in northern Arizona, USA. Ring-width expansion is greater at low-mortality sites. The top three panels show that ring-width expansion was significantly lower at high-mortality sites than at low-mortality sites. Each circle represents the ring width for 10 trees at each site during 1986–2000; data are means ± 1 SE. The bottom panel shows values for the Palmer drought severity index (PDSI; Palmer 1965) for northern Arizona, demonstrating severe drought in 1989 and 1996. PDSI values >0 indicate rainfall surplus, and values <0 indicate deficit compared to climatological norms for the region.

siveness of pinyon growth to temporal changes in climatic conditions. These temporal patterns are corroborated by values of the Palmer drought severity index, a measure that incorporates relative humidity, temperature, soil moisture, and precipitation in a single index that describes the relative wetness or dryness compared to a regional overage (Palmer 1965) (Fig. 1).

Our water-potential measurements provide support for the hypothesis that reduced ring widths at high-mortality sites were associated with higher levels of water stress (Table 1). We found that water potentials were significantly lower (on average 31% and 16%, respectively for 2000 and 2001) for pinyons growing in the high-mortality sites ($F_{5,68} = 47.921$, $P < 0.001$ for 2000; $F_{5,66} = 6.983$, $P < 0.001$ for 2001) for both years and all three site pairs. We did not find a significant site × treatment interaction ($F_{5,68} = 0.446$, $P = 0.642$), indicating that water-potential patterns were similar among sites. Although ring widths were not measured in 2001 because the growing season had not yet finished at the time cores were collected, we found that water potentials for 2000 predicted 29% of the ring-width expansion for 2000 ($r^2 = 0.292$, $P = 0.007$). This finding demonstrates a significant link between water-potential measurements and tree growth.

Ectomycorrhizal colonization and community structure

Although the high- and low-mortality sites were on average less than 500 m apart, surviving trees from high-mortality sites supported 34% and 52% less ectomycorrhizae for 2000 and 2001, respectively, than trees from low-mortality sites (Fig. 2). These significant differences demonstrated a strong association between host-plant stress and ectomycorrhizal colonization ($F_{1,56} = 42.5$, $P < 0.001$ for 2000; $F_{1,56} = 79.5$, $P < 0.001$ for 2001). There were also significant differences among sites in both years ($F_{2,56} = 4.31$, $P = 0.018$ for 2000; $F_{2,56} = 12.01$, $P = 0.003$ for 2001), but no significant site × mortality interaction ($F_{2,56} = 0.043$. $P = 0.958$ for 2000 and $F_{2,56} = 2.73$, $P = 0.07$ for 2001). The lack of a significant interaction between site and stress indicates that the relationship between ectomycorrhizal (EM) colonization and host-plant stress was similar across sites.

We found a total of 34 putative EM fungal species as indicated by unique RFLP (restriction fragment length polymorphism) types across the six sites. Although some morphotypes represented multiple RFLP types as described by Jonsson et al. (1999), the same RFLP types were consistently represented by a given morphotype. Furthermore, within a given study tree each morphotype tended to have only one RFLP type, so that sampling of three root tips per morphotype per tree was adequate. Four RFLP types matched those observed in previous studies of pinyon pine in northern Arizona, and only one of these matched a sporocarp, *Tricholoma terreum* (Gehring et al. 1998). Ectomycorrhizal fungal RFLP type richness was lower in high-mortality sites than low-mortality sites. RFLP richness averaged 8.30 ± 0.17 types for the low-mortality sites and 5.32 ± 0.85 types for the high mortality sites (mean ± 1 SE; $t = -3.385$, $P = 0.04$).

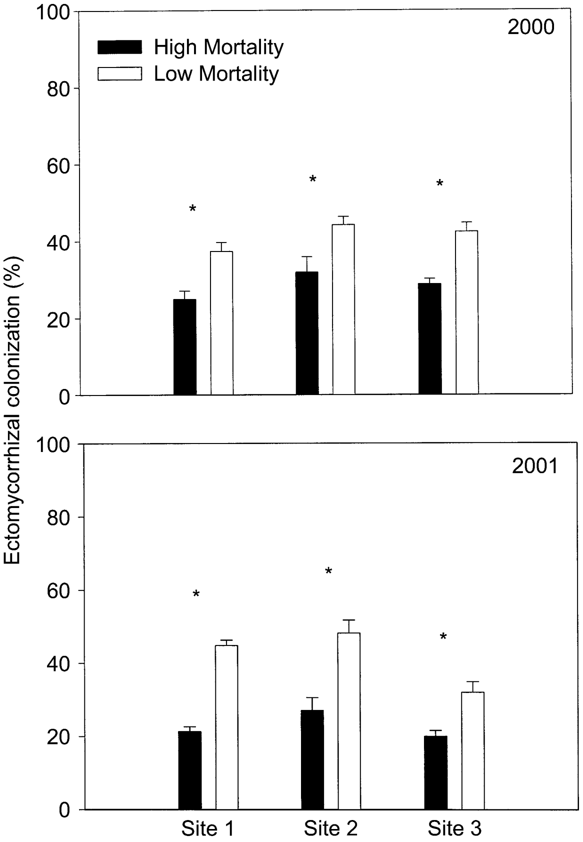

FIG. 2. Ectomycorrhizal colonization is reduced at high-mortality sites. Ectomycorrhizal colonization was significantly lower at high mortality sites for both 2000 and 2001. Data are means and 1 SE; asterisks (*) above bars indicates significant differences at the $P < 0.05$ level.

Ectomycorrhizal community composition differed significantly between the high- and low-mortality sites in a comparison within site pairs (see Fig. 3 for illustration and test statistics). These differences were due both to differences in the EM types present and in the abundance of shared EM types between high- and low-mortality sites. Across the three site pairs, on average, 47% of EM types were observed only within a high- or a low-mortality site. In addition, 50% of the species that were shared by paired high- and low-mortality site pairs showed two-fold or greater variation in relative abundance at the high-mortality site relative to the low-mortality site.

Although we observed greater overlap in species composition within high- and low-mortality site pairs than across sites, species that were shared among sites showed similar patterns with respect to host-plant mortality. Only four species were found at more than one

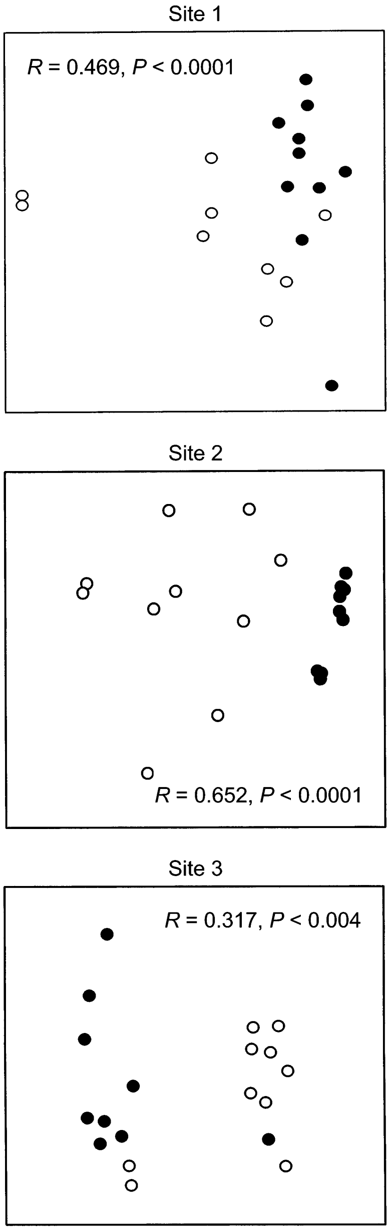

FIG. 3. Nonmetric multidimensional scaling ordinations of ectomycorrhizal EM fungal communities at the three high- and low-mortality site pairs for 2001. High- and low-mortality sites had different EM fungal communities based on RFLP (restriction fragment length polymorphism) analyses. Each circle represents the community of one tree; open circles = trees from low-mortality sites, and solid circles = trees from high-mortality sites. In the plot, circles are placed in a configuration where the distances between circles are in rank order with their ecological dissimilarities using the Bray-Curtis index. Because any rotation of this configuration that preserves the distance–dissimilarity relationship is equally good, the axes themselves have no real meaning. The R and P values presented in each panel are the results of the ANOSIM analyses described in *Methods*: *Estimating ectomycorrizal colonization. . . .*

of the three main sites, yet high-mortality and low-mortality sites differed from one another in a pooled analysis ($R = 0.09$, $P = 0.013$). For example, the only fungal species that the high-mortality area of site 2 shared with another site was found in the high-mortality area of site 3.

Host-plant stress gradient and ectomycorrhizal abundance

Trees that visually appeared to be in the poorest condition (rank 5) had the lowest trunk growth, highest percentage dead branches, and lowest foliage retention, while those that appeared to be in the best condition (rank 1) showed the opposite patterns. Foliage retention and percentage dead branches varied significantly with tree rank (Wilks' $\text{lambda}_{2,62} = 18.963$, $P < 0.0001$). Foliage retention was 62% higher in trees ranked 1 than in trees ranked 5, while trees ranked 2, 3, and 4 were intermediate (number of years of foliage retained for trees ranked 1 = 8.4 ± 0.852 yr, ranked 2 = 7.5 ± 0.186 yr, ranked 3 = 5.0 ± 0.610 yr, ranked 4 = 3.2 ± 0.395 yr, and ranked 5 = 2.72 ± 0.533 yr [mean ± 1 SE]; $F_{4,32} = 41.447$, $P < 0.001$). Tukey's test results indicated that trees ranked 1, 2, and 3 differed significantly from one another and from group 5. Trees ranked 1 and 2 also differed significantly from trees ranked 4, but trees ranked 3 did not.

Similarly, the percentage of tree branches that were dead was 7.5 times lower on trees ranked 1 than on trees ranked 5, while trees visually ranked 2, 3, or 4 had intermediate values (percentage dead branches for trees ranked 1 = 7.5 ± 1.75%, ranked 2 =17.5 ± 4.09%, ranked 3 = 27.5 ± 2.66%, ranked 4 = 44.00 ± 4.18%, and ranked 5 = 56.6 ± 3.333 % [mean ± 1 SE]; $F_{4,37} = 25.663$, $P < 0.001$). Trees ranked 4 and 5 differed significantly from trees ranked 1, 2, and 3, but did not differ significantly from one another. Trees ranked 4 and 5 were also not significantly different from one another.

Our findings regarding the differences among tree ranks in percentage dead branches and years of foliage retention were supported by measures of trunk growth using tree rings. Tree-ring width varied significantly with tree rank ($F_{4,32} = 38.91$, $P < 0.001$) and with time ($F_{4,32} = 22.55$, $P < 0.001$), and there was a significant time × rank interaction ($F_{4,32} = 5.42$, $P < 0.02$). Trees ranked 4 and 5 differed significantly from trees ranked 1, 2, and 3, but did not differ significantly from one another (Fig. 4). These findings indicate that visual estimates of tree condition using simple measures such as foliage retention and percentage dead branches are associated with pinyon trunk growth and host plant stress.

In contrast to the linear relationship between tree rank and the above measures of host-plant stress, we found a curvilinear relationship between tree rank and ectomycorrhizal abundance (Fig. 4). Ectomycorrhizal colonization was significantly higher on trees experi-

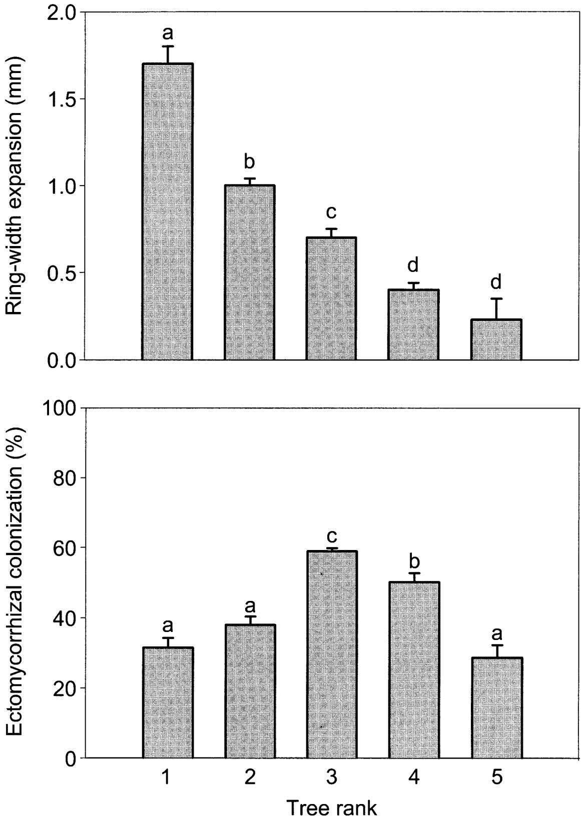

FIG. 4. The relationship between visual rankings of host-plant stress (1 = lowest stress, 5 = highest stress), ring-width expansion, and ectomycorrhizal (EM) colonization. The top panel illustrates that tree-ring-width expansion in 2001 declined as rank of host-plant stress increased. In contrast, EM colonization was highest on trees that were ranked intermediate in terms of plant stress when compared to trees at either end of the plant-stress continuum (bottom panel). The bars represent means (and 1 SE) of 10 trees per rank; within a panel, bars with the same lowercase letter are not significantly different at $P < 0.05$.

encing intermediate stress (i.e., rank 3 with 58% colonization) than trees experiencing either the least stress (i.e., rank 5 with 31% colonization) or the most stress (i.e., rank 1 with 29% colonization)($F_{4,29}$ = 24.12, P < 0.001 for all groups). Trees ranked 2 and 3 were intermediate. Importantly, ectomycorrhizal colonization did not differ significantly between trees experiencing extreme differences in stress (i.e., ranks 1 and 5). These data are consistent with the hypothesis that increasing stress is associated with increased investment into EM mutualists, until a threshold is reached at which further stress prevents plant resources from being allocated to EM mutualists, resulting in declines in EM colonization.

We observed a strong correlation between 2001 trunk growth and 2001 ectomycorrhizal colonization (r^2 = 0.73). The model that best fit the data was a log-normal three-parameter model with the following equation:

$$y = a \exp(-0.5\{[\ln(x) - \ln(x_0)]/b\}^2)$$

where y = percentage ectomycorrhizal colonization, a, b, and x_0 are coefficients developed by Sigma Plot 2000 (SPSS 2000): $a = 56.1122$, $b = 0.9064$, $x_0 = 595.1282$, and x is the ring-width expansion for the year and tree of interest.

To validate this model, we used the above equation to predict the 2000 ectomycorrhizal colonization of 17 trees at site 2 based upon the 2000 ring widths of those trees. The predicted values were highly correlated with observed values, suggesting that tree-ring widths could be useful in predicting ectomycorrhizal colonization (r^2 = 0.5695, P = 0.003)(Fig. 5). These findings suggest a tight link between host-plant trunk growth and EM abundance, allowing the prediction of EM abundance using tree rings.

Importance of soil properties

We examined the potential contribution of variation in soil properties to the differences in ectomycorrhizal communities at high- and low-mortality site pairs. We found that the seven major soil qualities we tested were strikingly similar between the high- and low-mortality site pairs (Table 1). First, the percentage of a soil sample within a particle class size did not vary more than 2% between high- and low-mortality sites for all three site pairs. Average distributions were 53% sand, 33% silt, and 14% clay for the three site pairs. Additionally, there were no differences between the high- and low-

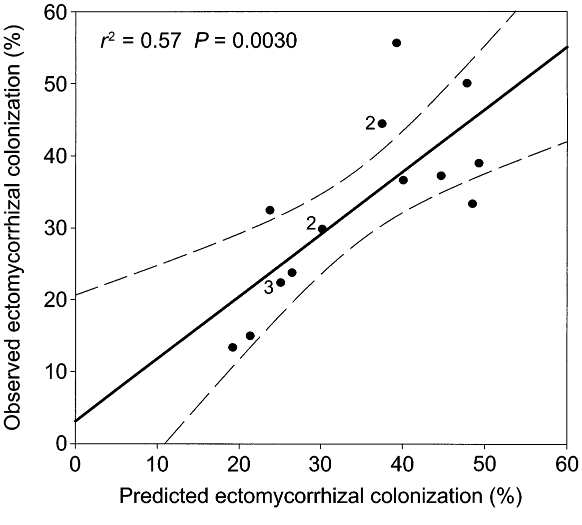

FIG. 5. Results of an independent test of a model predicting ectomycorrhizal (EM) colonization based on trunk growth. The regression model developed based on the relationship between trunk growth and EM colonization for 2001 predicts the relationship between trunk growth and EM colonization of an independent data set collected in 2000. The graph illustrates the correlation between observed EM colonization and that predicted by the model. The dashed lines represent the 95% confidence limits of the correlation. Numbers to the left of circles indicate the number of data points that overlap in that position on the graph.

mortality sites in amounts of large soil fragments ($>$2 mm; $F_{1,98} = 3.3$, $P = 0.15$). Second, there were no significant differences in gravimetric soil moisture and temperature between the high- and low-mortality sites. There were no clear patterns in gravimetric soil moisture between the high- and low-mortality sites (for averages across all times sampled, $F_{2,40} = 1.800$, $P = 0.17$). For all sites, we found that soil moisture was the highest (9.3% average soil moisture for all sites combined) during the February sampling, and the lowest during June (2.5% average soil moisture for all sites combined). Furthermore, while there were no clear patterns in soil temperature between high- and low-mortality sites ($F_{2,40} = 2.810$, $P = 0.21$), mean soil temperature of the three site pairs followed expected temporal patterns, with the highest temperatures occurring in June (23.2°C) and the lowest temperatures occurring in February (3.7°C). Third, pH and levels of available NH_4, NO_3, and PO_4 did not differ between high- and low-mortality sites ($F_{1,24} = 1.381$, $P = 0.268$ for pH; $F_{1,24} = 0.015$, $P = 0.903$ for NH_4; $F_{1,24} = 3.282$, $P = 0.09$ for NO_3; $F_{1,24} = 0.002$, $P = 0.965$ for PO_4) (Table 1). These similarities in soil nutrients suggest that the differences in tree performance and ectomycorrhizal communities were not related to these soil properties, but rather were the result of plant water relations or other factors.

DISCUSSION

Pinyon stress was greater at high-mortality sites

Although the high- and low-mortality site pairs were on average $<$500 m apart and had similar soil properties, trees that survived the drought at the high-mortality sites experienced reduced growth and lower water potentials than their counterparts at the low-mortality sites. The increased stress in these survivors and the severe mortality were most likely due to water stress that resulted from the slope and/or aspect of the high-mortality sites. We did not find soil moisture and temperature differences in our sampling, but we measured soil temperature and moisture infrequently using comparatively crude methods and thus may have missed important differences between high- and low-mortality sites. Trees with southern exposure may experience higher temperatures and greater rates of evapotranspiration, leading to increased water stress, reduced growth, and increased susceptibility to drought. Our results corroborate those reported by Ogle et al. (2000) who observed rates of pinyon mortality on south-facing slopes that were six-fold greater than the mortality rates found in nearby flat areas. Although these studies suggest that environmental stress is a key contributor to the high rates of tree mortality at the high-mortality sites, plant genetics also may play a role. Pinyon populations at the high-mortality sites may be genetically susceptible to drought stress, while the pinyon populations at the low-mortality sites may be genetically resistant. Several studies with pinyons (Mopper et al. 1991, Cobb et al. 1994, Mitton et al. 1998) have demonstrated that changes in allelic frequency and/or heterozygosity at the glycerate dehydrogenase locus are associated with high-stress sites or increased growth in the face of drought.

The results of both this study and Ogle et al. (2000) suggest that if climate patterns change as predicted (e.g., shifts in spatio-temporal patterns of precipitation), associated changes in the distributions of pinyons will occur. High mortality in pinyon sites on south-facing slopes at mid-to-low elevation will result in a shift upward and northward from their present distribution, resulting in replacement by juniper–grassland ecosystems. Recently this shift has become more apparent, because the worst drought in recorded history (in 2002) has continued to cause extensive mortality, especially in sites that suffered high mortality in 1996 (T. Trotter, *unpublished data*). Furthermore, because little or no juniper mortality occurred in our study sites, stands that were formerly a mixture of pinyon and juniper are becoming juniper dominated. Similarly, during the 1950s drought in New Mexico, high mortality Ponderosa pine (*Pinus ponderosa*) resulted in a 7-km ecotonal shift that persists today (Allen and Breshears 1998).

Ectomycorrhizal colonization and community structure are associated with host-plant stress

Our observations that ectomycorrhizal (EM) colonization was reduced by as much as 50% in high-mortality-site trees and that high-mortality sites consistently differed from low-mortality sites in EM fungal community composition have several potential implications. First, the reduction in ectomycorrhizae at the high-mortality sites may exacerbate water and nutrient stress for the surviving trees. This could result in poorer tree performance and increase the likelihood of mortality in trees that survived the initial drought. Alternatively, reductions in EM colonization during stressful times may be beneficial, as it could reduce the carbon cost of mycorrhizal fungi during times when photosynthesis can be extremely limited due to low water availability. Preliminary surveys of these study sites following a second extreme drought in 2002 support the first hypothesis, with 100% mortality of mature pinyons at one of the three high-mortality sites and far lower mortality at the paired low-mortality site.

Second, lower pinyon ectomycorrhizal colonization at stressful sites could lead to lower levels of EM fungal inoculum in the soil, resulting in less favorable conditions for pinyon seedling establishment. We suggest this as a possibility because sporocarp production is very low in these semi-arid environments, potentially limiting spore-inoculum availability in the soil (Gehring et al. 1998). The fungi that produced abundant sporocarps also were not the same species important belowground (Gehring et al. 1998). In addition, pinyons are the only known EM plant in many of these woodlands, so that inoculum is not provided by other plant species as is observed in some systems (Horton and Bruns 1998, Horton et al. 1999). Preliminary data from an inoculum bioassay study at one of the high- and low-mortality site pairs indicated that seedlings grown in high-mortality soils established poorly and had few ectomycorrhizae (R. Swaty, *unpublished data*).

Third, because different species of ectomycorrhizal fungi vary in drought tolerance and in the benefits they provide to the host plant (Godbold and Berntson 1997, Cairney 1999, Saikkonen et al. 1999), EM community shifts also could affect plant performance. However, we do not know if the community differences we observed are favorable or unfavorable for the stressed plants. In a study on EM responses to herbivory, morphotypes with less extensive hyphal development were more likely to remain following severe herbivory suggesting carbon limitation as a mechanism driving these changes (Saikkonen et al. 1999). The EM variation that we observed in stressed pinyons may also be due to changes in the quantity and/or quality of carbon available to EM fungi.

Finally, given that mycorrhizae can alter the quality, quantity, and retention of carbon allocated belowground (Rygiewicz and Andersen 1994), reductions in EM colonization and changes in EM community composition at high-mortality sites may alter carbon flow. For example, because carbon allocated to EM roots is turned over relatively quickly, declines in EM abundance can slow carbon turnover in ecosystems (Rygiewicz and Andersen 1994).

Intermediate host-plant stress hypothesis

Our demonstration of a curvilinear relationship between host-plant stress and ectomycorrhizal colonization is important for two reasons. First, it may help to explain the conflicting and diverse relationships observed between EM colonization and water stress in field and laboratory studies (e.g., Meier et al. 1990, Gehring and Whitham 1994, Runion et al. 1997, Nilsen et al. 1998). These conflicting results may be reconciled by an hypothesis about intermediate host-plant stress, which predicts that colonization will be highest when the host plant experiences intermediate levels of stress. If the entire potential host-plant stress gradient is not sampled in a study, or host-plant condition is not quantified, comparisons among studies can be difficult. For example, while we found that EM colonization was lower at the high-mortality (high stress) sites than low-mortality (low stress) sites, Swaty et al. (1998) found the opposite relationship between host-plant stress and EM colonization. We hypothesize that this discrepancy between studies was the result of sampling different portions of the host-plant-stress continuum. Measurements of degree of host-plant stress using techniques such as water potential and tree-ring widths could provide better predictions of the relationship between site water stress and EM colonization across species and time.

Second, our observation of a nonlinear relationship between host-plant stress and ectomycorrhizal colonization in the field suggests that both fungal and plant partners contribute to the responses of the relationship to changing environmental conditions. Our results are consistent with those of a canopy-cover manipulation experiment in which EM colonization was highest in trees growing in environments with intermediate levels of canopy cover (Zhou and Sharik 1997). In both studies the trees with the greatest growth rates did not have the highest levels of EM colonization. Zhou and Sharik (1997) suggested that reduced amounts of soluble carbohydrates limited EM colonization at the low end of the resource gradient, resulting in declines in EM colonization. In contrast, at the upper end of the gradient where resources were abundant for the host plant, Zhou and Sharik (1997) suggested that declines in EM colonization were the result of the fungus becoming ‘‘unnecessary’’ to the host plant. This mechanism implies host-plant ‘‘control’’ of the relationship and suggests that the fungus may act as a parasite under conditions of abundant host-plant resources as discussed by Johnson et al. (1997). The hypothesis that mycorrhizal fungal growth will decline with decreasing carbon allocation from the host plant has been proposed for soil nutrient gradients (Treseder and Allen 2002), and our research suggests that it may apply to plant condition more broadly even when soil nutrients do not vary significantly.

Alternatively, the reduction in ectomycorrhizal colonization in plants experiencing low levels of stress could be due to an imbalance in the growth rates of host plants and EM fungi. In an analysis of models used to describe arbuscular mycorrhizal associations, Allen (2001) proposed that because both fungi and plants seek to maximize their own growth in mycorrhizal relationships, the relationship between them should be modeled with two-organism models that incorporate rates of both fungal and root growth. In our study, host plant root growth may have exceeded fungal growth at the low end of the host-plant stress gradient, leading to reduced levels of colonization, but perhaps no change in the importance of the relationship to either symbiont. Although it is important to test this hypothesis, we believe it is unlikely that root growth exceeds fungal growth at our study sites due to slow rates of root growth, particularly during the driest time of the year when samples were taken.

In addition to quantitative differences in the EM colonization between the high- and low-mortality sites, we found qualitative differences as well. These differences were driven by species composition at the sites and differences in species richness. The differences in community composition were especially interesting because they were not associated with variation in soil factors as frequently reported in the literature (see Edwards and Kelly 1992, Roth and Fahey 1998, Bakker et al. 2000, Conn and Dighton 2000), but appeared to be related to host-plant stress. However, fine-scale variation in soil moisture, temperature, or other parameters that we did not detect in our measurements may have directly and differentially affected the growth and survival of some species of EM fungi. Smith and Read (1997) suggested that different species and strains of EM fungi were likely to vary in their ability to resist exposure to drought, and Lamhamedi et al. (1992) showed significant variation among isolates of *Pisolithus tinctorius* in their ability to influence host-plant water potential during moderate drought. Furthermore, while it is clear that different species of EM fungi can impart variable benefits to their hosts and have different levels of stress tolerance, it is not clear if the greater host-plant condition found at the low-mortality sites is due to the EM fungal types that are present there or vice versa.

Ectomycorrhizal dynamics predicted by tree-ring expansion

Most studies that have examined the relationship between host-plant performance and ectomycorrhizal colonization have done so through controlled-inoculation experiments of seedlings in the greenhouse (Boughter et al. 1990, Edwards and Kelly 1992, Guehl et al. 1992). While this approach is necessary to demonstrate cause-and-effect relationships between EM fungi and plant performance, it leaves us with little understanding of the relationships between EM fungi and their hosts when the host reaches a more mature phase of the life cycle. Here we report, for the first time to our knowledge, a significant relationship between EM colonization and tree-ring widths in mature trees. This relationship was curvilinear and could be used to accurately predict the EM colonization of a previous year based on rates of trunk growth. While we cannot determine whether EM fungi are the drivers of the growth differences or vice versa using this technique, it still allows us to predict rates of EM colonization of the past and to model mycorrhizal relationships under future tree-growth scenarios. If this finding proves to be general, it could allow us to model the responses of EM fungal populations to a variety of environmental perturbations by measuring ring widths. Given the importance of EM fungi in ecosystems, such a tool could improve our understanding of not only these fungi, but also of ecosystem nutrient and carbon dynamics.

Implications

We found that ectomycorrhizal abundance and community structure differed between adjacent sites with similar soil parameters but different levels of host-plant stress. These findings have several implications for long-term climate change. First, if continental interiors experience more frequent and intense droughts as predicted, we may see a shift in EM colonization that may increase or decrease depending on where the host plant is on the stress continuum at the onset of drought. Furthermore, we may see shifts in EM community structure, which could have direct impacts on the plants as different EM fungal types may impart different benefits to their hosts. Second, trees that experienced intermediate levels of stress had two-fold higher EM colonization than trees at either end of the stress continuum. This suggests that host plant–EM relationships at the ends of the host-plant stress gradient may be most sensitive to climate change as EM inoculum levels and abundance may be lowest in these areas. Third, given the relatively tight relationship between EM dynamics and tree-ring width, it may be possible to reconstruct the EM relationships of the past, predict the EM dynamics of the future, model ecosystem dynamics with greater accuracy, and understand the role of climate in influencing nutrient cycling through its effects on EM dynamics. Fourth, because there can also be a strong genetic component to the growth rates of many trees (e.g., Bradshaw and Stettler 1995), it is important to consider how plant genetics and genetic × environmental interactions further affect this fungus–plant association.

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