SEE COMMENTARY

Tree genetics defines fungal partner communities that may confer drought tolerance

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Plant genetic variation and soil microorganisms are individually known to influence plant responses to climate change, but the interactive effects of these two factors are largely unknown. Using long-term observational studies in the field and common garden and greenhouse experiments of a foundation tree species (*Pinus edulis*) and its mutualistic ectomycorrhizal fungal (EMF) associates, we show that EMF community composition is under strong plant genetic control. Seedlings acquire the EMF community of their seed source trees (drought tolerant vs. drought intolerant), even when exposed to inoculum from the alternate tree type. Drought-tolerant trees had 25% higher growth and a third the mortality of drought-intolerant trees over the course of 10 y of drought in the wild, traits that were also observed in their seedlings in a common garden. Inoculation experiments show that EMF communities are critical to drought tolerance. Drought-tolerant and drought-intolerant seedlings grew similarly when provided sterile EMF inoculum, but drought-tolerant seedlings grew 25% larger than drought-intolerant seedlings under dry conditions when each seedling type developed its distinct EMF community. This demonstration that particular combinations of plant genotype and mutualistic EMF communities improve the survival and growth of trees with drought is especially important, given the vulnerability of forests around the world to the warming and drying conditions predicted for the future.

climate change | drought | ectomycorrhizal fungi | host genetics | plant-soil feedback

Feedback between plants and soil microbes influences plant productivity and diversity (1, 2), with the strongest evidence of their importance coming from studies comparing plant species. However, trait variation within plant species also alters the abundance, species composition, and diversity of microbes (3, 4). Variation in the efficacy of different microbial communities among plant genotypes may lead to ecological and evolutionary feedback between microbial communities and their host plants (4, 5). This feedback could influence how plant species respond to climate change (6, 7), and it is critical to understand in long-lived, foundation species that define habitat structure and ecosystem function (8). The mycorrhizal fungi that occur in association with the roots of most plant species may have particularly important feedback effects on the climate change responses of their host plants because they facilitate soil resource uptake and improve the ability of plants to tolerate moisture and temperature stress (6, 7). Plants and their mycorrhizal fungal communities also can be locally adapted to one another and their environment, potentially maximizing their combined performance (9–11). However, it is difficult to link the results of the highly controlled studies necessary to isolate the effects of differing communities of mycorrhizal fungi to patterns of vegetation change in nature.

We combined greenhouse and common garden experiments with two decades of observations in the field to examine the importance of the interaction between within-population variation in drought tolerance and ectomycorrhizal fungal (EMF) species composition in pinyon pine (*Pinus edulis* Engelm), a widespread foundation tree species of the southwestern United States. Pinyon pine has suffered large-scale mortality resulting from climate change (12), and its range is expected to contract this century as the climate continues to change (13). Pinyons are ideal to test the influence of plant genetics on complex mycorrhizal fungal–plant–climate interactions for several reasons. First, forest and woodland biomes around the world are increasingly vulnerable to drought as a result of climate change (14), and studies of pinyon–juniper woodlands have already provided important insights (12). Second, *P. edulis* is often the only plant species colonized by EMF in its woodland habitat, facilitating study of the importance of these fungi to their host plant and the surrounding ecosystem (15). Third, drought-tolerant and drought-intolerant trees can be distinguished in the field, enabling study of their traits and those of their offspring. Drought tolerance and susceptibility to chronic herbivory by a stem-boring moth that attacks adult trees (*Dioryctria albovittella*) are genetically controlled and inversely related in *P. edulis* (16, 17). Although the two tree types grow side by side, adult moth-resistant trees had threefold higher mortality during extreme drought than adult moth-susceptible trees (17). Chronic moth herbivory alters tree architecture and is thus a phenotypic marker for drought tolerance in the field (17) (see icons in Fig. 1). Fourth, studies conducted over the course of 16 y show that moth-susceptible (drought tolerant) and moth-resistant (drought intolerant) trees differ consistently in EMF species composition (18), and moth removal experiments demonstrate that these differences are not the result of herbivory (19).

Significance

Soil microbes influence plant performance and may be critical to the persistence of some plants with climate change. However, microbes are highly diverse, and individuals of the same plant species often associate with different microbes. We examined the importance of soil microbes to the growth and survival of a widespread pine under drought conditions. We found that specific beneficial fungus–host genotype combinations promoted drought tolerance in field and greenhouse studies. Drought tolerance, and associations with particular fungi, were passed from mother trees to their offspring, indicating the traits are genetic. These results demonstrate the interlinked importance of the genetics of a tree and its microbiome, which can be used to restore forest losses resulting from drought.

Author contributions: C.A.G., C.M.S., A.V.W., and T.G.W. designed the study; C.A.G., C.M.S., and L.F.-R. conducted the greenhouse experiments; C.A.G., A.V.W., and T.G.W. collected field data and established the common garden; C.A.G. analyzed the data; C.A.G. wrote the manuscript; and all authors contributed to revisions.

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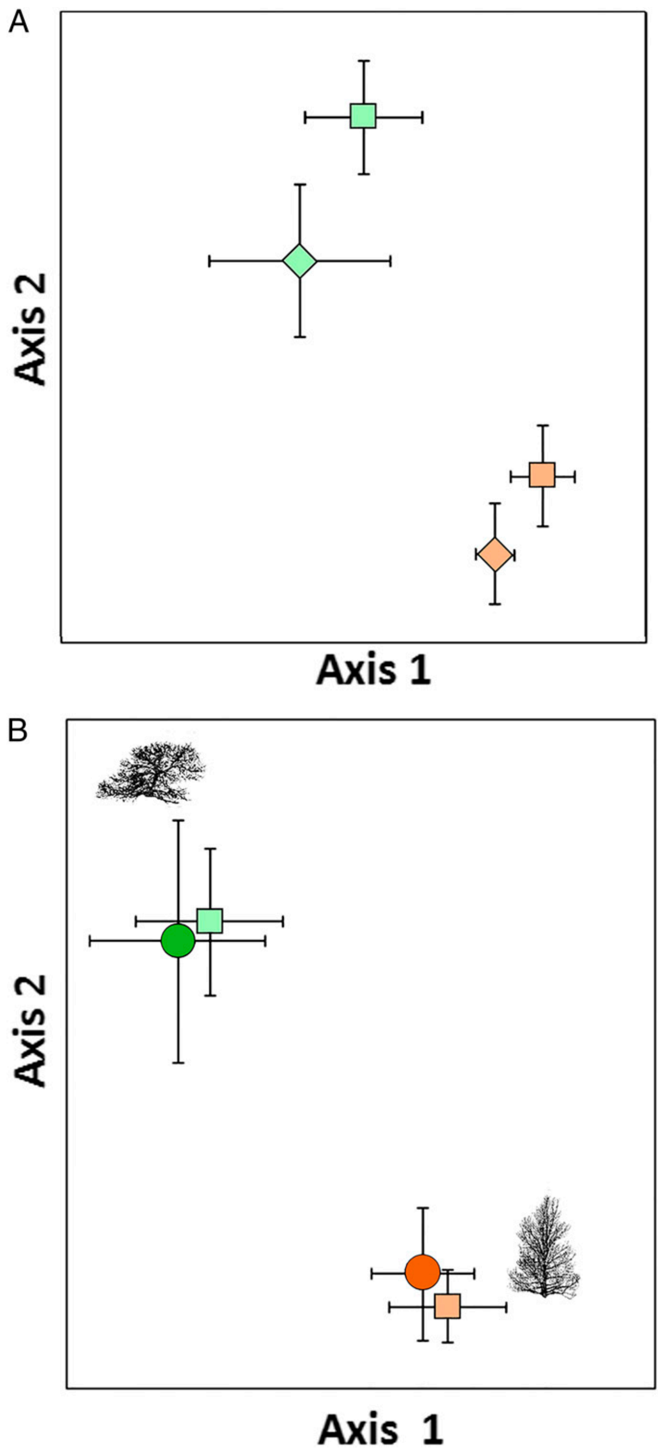

Fig. 1. (*A*) Nonmetric multidimensional scaling ordination of *P. edulis* EMF community composition showing that seed source (symbol color), but not inoculum source (symbol shape), influenced the EMF community composition of *P. edulis* seedlings. Light orange symbols represent seeds from drought-intolerant mothers, and light green symbols represent seeds from drought-tolerant mothers. Squares represent inoculum from drought-tolerant mothers and diamonds represent inoculum from drought-intolerant mothers. Points are the centroid of the EMF community, with lines depicting ±1 SE. The closer centroids are to one another, the more similar the EMF community. (*B*) Nonmetric multidimensional scaling ordination of the EMF communities of drought-tolerant and drought-intolerant adult trees in the field and seedlings grown from the seeds of drought-tolerant and drought-intolerant trees. The communities of adult trees are shown by large circles (dark green, drought tolerant; dark orange, drought intolerant), and the communities of seedlings are indicated by smaller squares (light green, seeds from drought-tolerant trees; light orange, seeds from drought-intolerant trees). Points are centroids, with lines depicting ±1 SE. Icons show the morphology of adult drought-intolerant (tall, upright) and drought-tolerant (prostrate, shrub-like) trees.

These previous studies of adult trees demonstrate important interrelationships among tree genetics, drought tolerance, and fungal mutualist communities. However, in the absence of carefully controlled greenhouse experiments and common gardens, it has not been possible to determine whether *P. edulis* can pass traits of drought tolerance and EMF community composition to their offspring, or to directly establish the importance of differing EMF communities to drought tolerance. We integrated greenhouse and common garden experiments with long-term field studies to partition the effects of host genetics and EMF communities on plant performance during drought. This combination of tests is crucial, as the collective results determine whether fungal mutualists contribute to the drought tolerance of a foundation tree species that defines a much larger ecosystem. They also evaluate the importance of plant genetic variation to climate change response, acting either directly through plant traits or indirectly through root-associated microbes.

Results

Two lines of evidence showed that EMF community composition was strongly influenced by host plant genetics. First, a reciprocal transplant greenhouse experiment of seed source (seeds from drought-tolerant and drought-intolerant adult trees, representing the influence of plant genetics) and inoculum source (EMF roots and adhering soil from drought-tolerant and drought-intolerant trees, representing the influence of the root/rhizosphere community) showed that the EMF communities colonizing the roots of *P. edulis* seedlings were influenced significantly by seed source (Pseudo-$F_{1,20}$ = 6.78; P = 0.001), but not by inoculum source (Pseudo-$F_{1,20}$ = 1.04; P = 0.378), and there was no significant interaction between seed source and inoculum source (Pseudo-$F_{4,45}$ = 0.72; P = 0.536; Fig. 1*A*; species list in Table S1). The EMF communities of seedlings from drought-tolerant and drought-intolerant seed sources had different indicator species; the basidiomycete, *Rhizopogon roseolus*, characterized seedlings from drought-intolerant mothers [indicator value (IV) = 74.0; P = 0.0002], and two members of the ascomycete genus, *Geopora*, characterized seedlings from drought-tolerant mothers [IV = 24.9 (P = 0.032) for *Geopora pinyonesis* and IV = 34.2 (P = 0.011) for *Geopora* 2].

The second line of evidence in support of the importance of plant genetics to EMF communities comes from the similarity between the EMF communities of seedlings from the reciprocal transplant experiment and the EMF communities of adult trees of the same drought tolerance class growing in the field (Fig. 1*B*). Seedlings and adult trees had similar mean species richness, ranging from 2.1 to 2.5, comparable to values reported previously during drought (18). The EMF community composition of the seedlings and trees were influenced by drought tolerance class (Pseudo-$F_{1,38}$ = 7.48; P = 0.001), but not by tree age class (seedling vs. adult; Pseudo-$F_{1,38}$ = 0.72; P = 0.560), and there was no significant interaction between drought tolerance class and age class (Pseudo-$F_{1,38}$ = 0.55; P = 0.707). The EMF communities of drought-tolerant adult maternal trees and their seedlings were dominated by the same two species of *Geopora*, making up, on average, 77% and 78% of their communities, respectively. Drought-intolerant trees and their seedlings were dominated by *R. roseolus*, making up, on average, 41% and 50% of their communities, respectively. Taken together, the findings of the reciprocal transplant experiment show that EMF community composition represents a heritable plant trait.

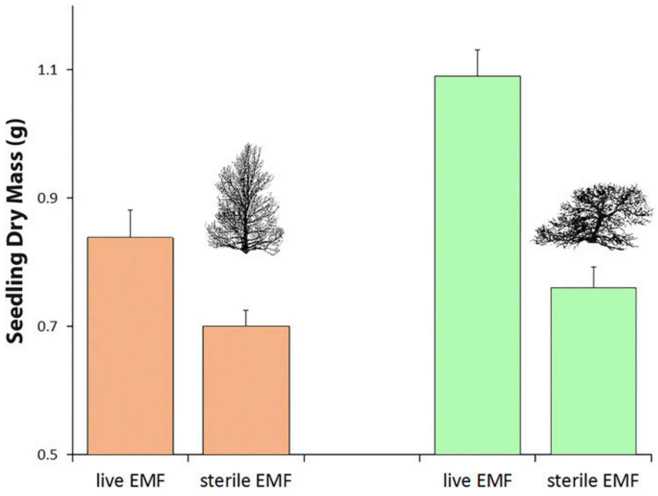

Fig. 2. Mean (+1 SE) dry biomass of seedlings from drought-intolerant (light orange bars; tree icon with upright architecture) and drought-tolerant (light green bars; tree icon with shrub-like architecture) mothers grown with live EMF or sterilized EMF inoculum in drought conditions. Seedlings grown from drought-tolerant seeds received inoculum from drought-tolerant trees (live vs. sterile), and seedlings grown from drought-intolerant seeds received inoculum from drought-intolerant trees (live vs. sterile).

The genetically based differences in seedling associations with EMF had important consequences for seedling growth. In a second greenhouse experiment (EMF drought experiment), seedlings from drought-tolerant and drought-intolerant mothers were grown under drought conditions with (live inoculum) and without (sterile inoculum) their respective EMF communities. The use of sterile versus live inoculum allowed us to determine the importance of differing EMF associations to host plant performance during drought. The live EMF of drought-tolerant and drought-intolerant trees promoted growth relative to sterile inoculum ($F_{1,32} = 50.42$; $P < 0.0001$; Fig. 2), indicating that both EMF associations functioned as mutualists. However, seedlings of drought-tolerant mothers grew larger than seedlings of drought-intolerant mothers ($F_{1,32} = 20.32$; $P < 0.0001$), but only in the presence of live EMF inoculum (seed source × inoculum interaction, $F_{1,32} = 4.83$; $P = 0.030$). Seedlings of drought-tolerant and drought-intolerant mothers were similar in size in the presence of sterile inoculum, but in the presence of live inoculum, seedlings from drought-tolerant mothers grew 25% larger (Fig. 2).

The greater growth of seedlings from drought-tolerant mothers than drought-intolerant mothers after inoculation with EMF could be a result of differences in EMF abundance or species richness, EMF community composition, or responsiveness to EMF inoculum. Of these possibilities, we could not test the third alternative because the tight linkage between seedling genetics and EMF community composition shown here made the critical reciprocal inoculum experiments impossible. Of the remaining possibilities, our data do not support the first alternative hypothesis because colonization by EMF, a measure of fungal abundance, was similar among seedlings from drought-tolerant and drought-intolerant maternal trees, ranging from 40% to 44% in seedlings receiving live inoculum. Similarly, average per seedling EMF species richness ranged from 2.2 to 2.5.

In contrast, two lines of evidence support our second alternative hypothesis, that EMF community composition contributed significantly to differences in the growth of the two seedling types. First, the EMF communities of seedlings of drought-tolerant and drought-intolerant mothers differed significantly in the EMF drought experiment (Pseudo-$F_{1,17} = 9.08$; $P = 0.0004$). Two species of *Geopora* were indicators of the communities of seedlings of drought-tolerant mothers [IV = 65.1 ($P = 0.044$) for *G. pinyonensis* and IV = 62.4 ($P = 0.050$) for *Geopora* 2; combined average of 81% of the community], and *R. roseolus* was an indicator for seedlings of drought-intolerant mothers (IV = 100; $P = 0.002$; average of 50% of the community). These community differences, including indicator species, were similar to those observed in the reciprocal transplant experiment, demonstrating their consistency. Second, although the EMF communities of the seedlings of drought-intolerant trees in the greenhouse drought experiment were generally dominated by *R. roseolus*, many of them were colonized to some extent by *G. pinyonensis* and *Geopora* 2, the dominant EMF on drought-tolerant trees and their seedlings. Importantly, the growth of drought-intolerant seedlings was significantly positively associated with the combined relative abundance of members of the genus *Geopora* ($R^2 = 0.367$; $F_{1,8} = 5.65$; $P = 0.05$; Fig. S1), a result also observed in drought-intolerant adult trees in the field (18) during drought ($R^2 = 0.465$; $F_{1,19} = 13.93$; $P = 0.002$). In combination, these data suggest members of the genus *Geopora* are associated with higher growth in drought conditions.

Studies of adult drought-tolerant and drought-intolerant trees in the field and their seedlings in a common garden support and expand our greenhouse studies in two ways. First, a reversal in performance of the two types of adult trees in the field as climate changed from semiarid to drought conditions suggests the EMF communities of drought-intolerant trees are more beneficial during wetter times, whereas the EMF communities of drought-tolerant trees are more beneficial during drought. During the 10 y before the onset of drought (1986–1995), the mean annual average stem growth of drought-intolerant adult trees exceeded that of drought-tolerant adult trees by an average of 15% (Fig. 3). The EMF communities of these trees during this predrought period (1994) showed dominance by *Geopora* in drought-tolerant trees and *R. roseolus* and other basidiomycetes in drought-intolerant trees (18). Over the course of 10 y of drought (1997–2006), the mean annual average stem growth of both tree types declined ($F_{1,36} = 45.99$; $P < 0.0001$), but the growth of drought-intolerant trees declined 45%, whereas that of drought-tolerant trees declined only 12% (drought by tree phenotype interaction, $F_{1,36} = 13.67$; $P = 0.001$). The EMF communities during this drought period (2004) again showed dominance by *Geopora* in drought-tolerant trees, with *Geopora* abundance also increasing on drought-intolerant trees (18). Although the abundance of basidiomycetes on drought-intolerant adult trees was lower in 2004 than 1994,

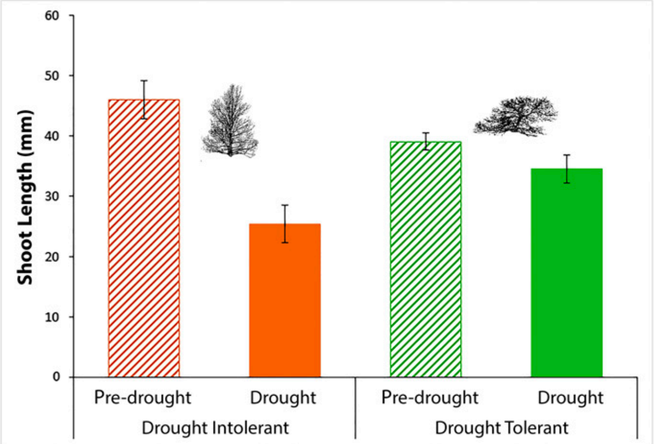

Fig. 3. Mean shoot (±1 SE) growth of drought-intolerant (orange bars) and drought-tolerant adult trees (green bars) during a 20-y period ($n = 11$ trees/group). Predrought bars illustrate 10-y mean shoot growth from 1986 to 1995, when conditions were relatively moist, and drought bars represent the 10-y mean shoot growth from 1997 to 2006, when drought conditions prevailed.

these fungi, including *R. roseolus*, made up ∼40% of the community (18).

Field observations and common garden experiments support our greenhouse results in a second way. Patterns of seedling growth in the EMF drought experiment were similar to patterns of shoot growth of both adult trees under drought conditions in the field and the offspring of those adult trees growing in a nearby common garden, where seedlings of drought-tolerant and drought-intolerant maternal trees were planted randomly (common garden experiment) (Fig. 4). Average shoot growth during a drier-than-average 10-y period from 1997 to 2007 was ∼25% higher in adult drought-tolerant trees than in adult drought-intolerant trees (Fig. 4; statistical analysis in previous paragraph). Seedlings of these drought-tolerant trees grown in a common garden for 2 y, also in drier-than-average conditions, grew 50% larger than seedlings of drought-intolerant trees ($t = 4.70$; $P < 0.0001$; Fig. 4). Cumulative 2-y mortality in the common garden was also higher in seedlings from drought-intolerant mothers than drought-tolerant mothers ($t = 2.681$; $P = 0.017$), similar to the mortality in adult trees during drought (Fig. 4). The close correspondence among greenhouse, common garden, and field results argues that plant genotype–EMF community interactions contribute to the dramatic performance differences observed in response to drought in adult *P. edulis* in nature.

Discussion

Our findings demonstrate that EMF community composition is strongly influenced by host plant genetics. A handful of other studies have documented that plant genotypes vary in their EMF communities (i.e., community specificity at the genotype level) (3, 20), but they do not combine long-term measurements with experimental manipulations that separate genetic and environmental effects. Our previous studies of natural populations of *P. edulis* conducted over the course of ∼20 y show that differences in the EMF community composition of drought-tolerant and drought-intolerant genotypes in the field are consistent, long-term (18, 19), and observed in multiple populations of trees (18). The current study experimentally demonstrates that genetic effects outweigh environmental effects as seedlings acquire the EMF community of their seed source trees, regardless of inoculum source. These data greatly strengthen the evidence that host genotype is an important force structuring biodiversity, both above- and belowground (3, 20).

Our study documents the importance of community specificity at the genotype level to plant performance during climate stress. We show that interactions between plant genetics and EMF fungi affect drought tolerance. Further, our experiments show that the propensity to support different EMF communities is both genetically based and heritable. Ectomycorrhizal fungi are known for their role in promoting tolerance to drought (21) and other plant stressors (6), supporting the hypothesis that these fungi could be a critical mechanism allowing plants and their associated communities to survive climate change (6, 7, 22). A recent study across widely separated populations also shows the importance of plant genetic–soil fungal interactions; EMF community dissimilarity from the local population resulted in 15% lower growth of *Pseudotsuga menziesii* seedlings transplanted up to 400 km northward (23). These results show the importance of matching host populations with appropriate EMF mutualists across large spatial scales (23). Our study shows that plant genotype-driven EMF community variation and its feedback effects on plant growth can operate at a much finer scale, within a population, among trees growing intermixed within a stand. There also is growing evidence that the use of climate change-appropriate plant genotypes has economic value in forestry and restoration (24, 25), and our results argue that particular plant genotype–soil microbe combinations also could be critical to forest management and restoration.

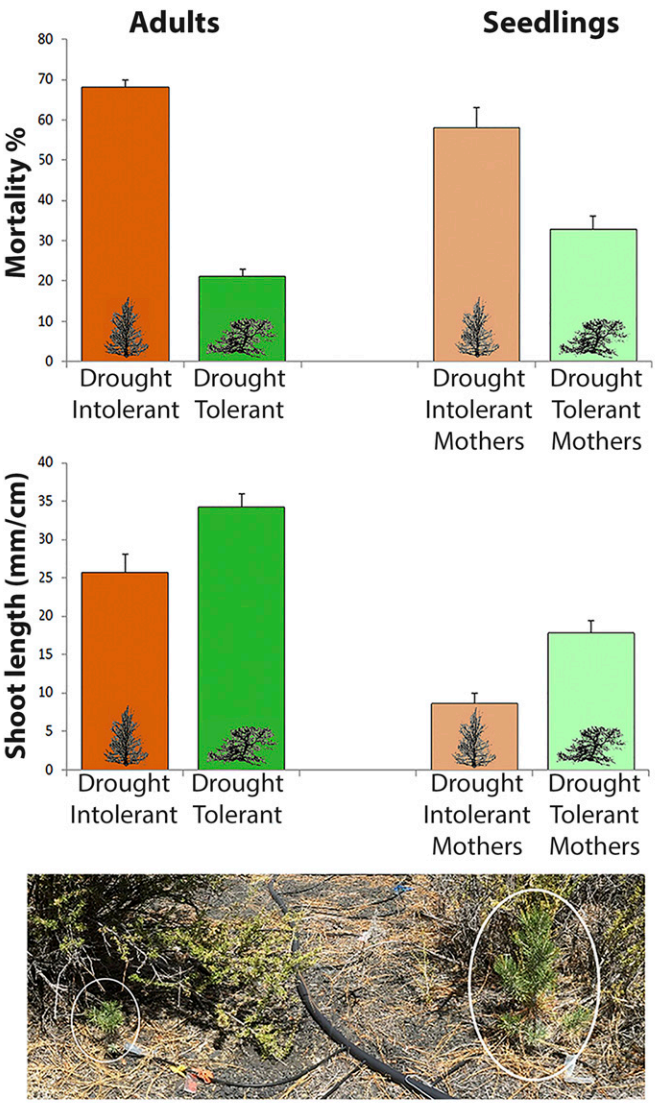

Fig. 4. Similar patterns of mortality and growth of adult drought-intolerant (dark orange bars) and drought-tolerant (dark green bars) trees at the field site and of seedlings of these trees in a common garden (light orange bars, seedlings from drought-intolerant mothers; light green bars, seedlings from drought-tolerant mothers). Mortality data for adult trees resulted from extreme drought in 2002 and were previously published (17). Growth data for adult trees are mean shoot length in millimeters (+1 SE) of drought-tolerant and drought-intolerant trees over the course of 10 y of drought (1997–2006). These data are shown in Fig. 3 but are presented here for comparison with seedling data. Seedling mortality data are mean mortality (±1 SE) over the course of 2 y, and seedling growth is mean cumulative total shoot growth in centimeters (±1 SE) during the same 2 y. The photograph shows common garden seedlings from a drought-intolerant mother (within circle on left) and a drought-tolerant mother (within circle on right) to illustrate size differences.

Our data suggest that the EMF communities of drought-tolerant and drought-intolerant trees contribute to the differing performance of these two tree types in wetter versus drier conditions, yet we know little about the fungal traits that contribute to this functional divergence. Drought-tolerant pinyons are associated primarily with ascomycetes of the genus *Geopora* that frequently occupy stressful or early successional environments (18), but are among a large number of ascomycete taxa only recently described as ectomycorrhizal based on molecular characterization of root tips (26, 27). Members of the genus *Geopora* produce short-distance explorative hyphae (27), a trait observed in relatives of these fungi that preferentially associated with pines on a desert island (28). Members of the genus *Rhizopogon* are also observed in early successional environments (29), but unlike *Geopora* spp., their effects on seedling performance have been assessed in inoculation studies. Consistent with our findings, the growth of *P. muricata* seedlings was improved by three species of *Rhizopogon* in wetter, but not drier, conditions (30). However, the dominant *Rhizopogon* we observed, *R. roseolus*, did not promote growth of *P. sylvestris* seedlings in wet or dry conditions (31), although in vitro studies suggest it tolerates low moisture (32).

The consistent results we observed across greenhouse, common garden, and field studies allows us to link interactions among plant genotype, EMF communities, and drought to landscape-scale changes in dominant plants and their associated communities. Before drought, drought-intolerant pinyons and their associated EMF communities had higher performance than drought-tolerant pinyons and their EMF communities, but these patterns reversed during drought, resulting in rapid shifts in the genetic structure of the adult *P. edulis* population. Drought-intolerant trees dominated on the landscape before drought, but differential mortality resulted in near equality of the two tree types (17). Importantly, the shift in the genetic structure of the *P. edulis* population is associated with changes in the composition of hundreds of other species including insects, birds, and mammals whose survival may depend on the dynamics of the EMF mutualism (33). Likewise, members of the genus *Geopora* have increased with drought within and among pinyon–juniper woodlands (18, 34), a potentially favorable shift for *P. edulis* if drought persists (13). However, ongoing drought is associated with a reduction in the ecosystem's already low EMF diversity (18, 19), which could ultimately negatively affect *P. edulis*, particularly as it is the only host for EMF across much of its range (15).

In conclusion, our studies demonstrate intimate relationships among plant genetics, fungal communities, and drought tolerance in *P. edulis*. The existence of a strong genetic component to the establishment of mycorrhizal partnerships argues that evolutionary relationships among plants and mutualists will play a role in determining the fate of foundation plant species with climate change. The association we observed among host genotype, drought tolerance, and EMF community composition became evident only after an extreme drought, suggesting the cascading effects of plant genetics may become stronger as the effects of climate change increase. Genetically based traits that were less important in benign environments may become crucial as more foundation species live in environments on the edge of their physiological and ecological tolerances.

Methods

We studied drought-tolerant and drought-intolerant *P. edulis* and their associated EMF in northern Arizona (35° 23′ 25″N, 111° 25′ 40.8″W). Soils were 1,200-y-old cinder deposits that include basaltic ash, cinders, and lava flows that belong in the US Department of Agriculture Soil Taxonomic Sub-Group of Typic Ustorthents (35). These soils have low water-holding capacity and low levels of several nutrients (35). The site was dominated by *P. edulis* and one-seed juniper (*Juniperus monosperma* Engelm). This region of northern Arizona is semiarid, averaging ∼320 mm precipitation/year (35). It has experienced drought for most of the last 20 y (1996–2015) according to the Palmer Drought Severity Index (PDSI; National Climate Data Center; https://www.ncdc.noaa.gov/temp-and-precip/drought/historical-palmers/).

Greenhouse Reciprocal Transplant Experiment. We determined the importance of seed source (plant genetics) and soil inoculum source (environment) to EMF community composition, using a reciprocal transplant experiment in the greenhouse. We collected seeds from open pollinated drought-tolerant and drought-intolerant pinyons and planted them in steam-sterilized field soil inoculated with roots and adhering soil collected beneath drought-tolerant and drought-intolerant trees in a factorial design. We predicted that if plant genetics were more important than inoculum source to EMF community composition, seedlings grown from drought-tolerant mothers would have similar EMF communities regardless of the inoculum they received (from drought-tolerant or drought-intolerant trees).

We collected seeds from drought-tolerant and drought-intolerant trees that have been studied since the mid- to late 1980s (18, 19, 36). To help account for maternal effects, we used similarly sized seeds that represented the most common range of seed size (0.30–0.35 g) from 18 trees. We collected roots and associated soil from nine drought-tolerant and nine drought-intolerant trees during the wettest month of a severe drought year, as EMF abundance peaks at this time (37). We collected six soil cores (20 cm depth, 6 cm diameter) from each tree and removed roots from one core per tree and stored them at −20 °C until they could be analyzed for EMF community composition. We analyzed adult tree EMF communities so we could directly compare the EMF communities of adult trees to the EMF communities of experimental seedlings. We filled pots (656 mL) with steam-sterilized field soil (two successive nights at 95 °C) collected from an intercanopy space at the study site, and then added live inoculum (∼25 g EMF colonized roots and adhering soil) from one of the soil cores. We added two viable seeds from the same maternal tree each into each pot and thinned to one seedling as needed. Low germination of some seeds resulted in a final design of 24 pots; six replicates of each of four treatments in which seeds from drought-tolerant trees received inoculum from either drought-tolerant or drought-intolerant trees. Seeds from drought-intolerant trees received the same inoculum treatments. We maintained pots in a greenhouse, under 12:12 supplemental light, with temperatures of 24–27 °C during the day and 13–16 °C at night, and watered them every 3 d, creating dry conditions (∼3.8% soil moisture) (37). We harvested seedlings after 12 mo and measured their EMF communities (see below).

Greenhouse EMF Drought Experiment. We tested whether genetically based linkages between plants and EMF influenced seedling performance during drought with a second greenhouse experiment. We compared the biomass of seedlings inoculated with live roots and adhering soil to those inoculated with sterilized inoculum to assess the importance of EMF originating from drought-tolerant versus drought-intolerant trees to seedling growth. Seed source was the only significant predictor of the EMF community composition of seedlings in the reciprocal transplant experiment (Fig. 1*A*), so we did not incorporate a reciprocal inoculum design. We used 18 seed sources, nine drought tolerant and nine intolerant, and collected inoculum from 12 drought-tolerant and 12 drought-intolerant trees as described earlier. We sterilized half the roots/adhering soil from each tree to create a sterile inoculum treatment by microwaving moistened roots and soil in a covered glass dish at high power for 6–8 min (38). We planted seeds of similar size (0.30–0.35 g) of each maternal seed source in 656-mL pots containing steam-sterilized field soil with 25 g of either live or sterilized inoculum ($n = 36$, nine each in four treatments). We did not match inoculum source tree identity to seed source identity, but assigned them to one another randomly within a treatment. Growing conditions were the same as in the reciprocal transplant experiment except that after 30 d, seedlings were placed in a low-water treatment (∼2.5% soil moisture) that simulated moderate drought, but did not result in mortality (17, 37). Soil moisture in these soils ranges from <1% to 10%, depending on season of the year (37). We harvested seedlings after 7 mo and measured their dry biomass, EMF colonization, and EMF community composition.

Observational Field Studies and Common Garden Experiment. We explored the relationships between EMF communities and pinyon growth in drought and less arid conditions by measuring the shoot growth ($n = 10$ shoots/tree) of a set of drought-tolerant ($n = 11$) and drought-intolerant adult trees ($n = 11$) over the course of two 10-y periods: one before the onset of drought in 1996 (1986–1995) and one during drought (1997–2006). We linked patterns in these data with the EMF community composition of a subset of these trees ($n = 9$) collected before drought (1994) and during drought (2004) and published previously (18, 19). The average annual PDSI for northern Arizona for the predrought 10-y period included 5 moist or very moist years and 1 y of drought. The average annual PDSI for the 10-y drought period included 6 y of drought: 3 y classified as severe and 3 y classified as extreme (National Climate Data Center; https://www.ncdc.noaa.gov/temp-and-precip/drought/historical-palmers/).

We related greenhouse experiment results to patterns in the field by qualitatively comparing the growth of seedlings in the greenhouse under simulated drought conditions with the growth of adult trees in the field and seedlings in a common garden. We used the shoot length data described here for the drought period to characterize adult trees and established a common garden near the field site (common garden experiment) and measured the growth and mortality of replicate seedlings ($n = 5$–8) from 10 drought-tolerant and 10 drought-intolerant maternal trees. Seedlings were grown in the greenhouse for 1 y and then transplanted to a common garden in their native habitat in 2010. Nurse plants are essential for establishment in the wild (39), so all pinyon seedlings were planted within 0.5 m of a nurse shrub (*Fallugia paradoxa*). We added supplemental water during the first two dry seasons (May–June, October) to further facilitate establishment. We report data on mortality and cumulative shoot length for 2 y after the cessation of watering (2013–2014) when average climate based on PDSI was moderate and severe drought, respectively. *P. edulis* grows very slowly, so we did not assess EMF community composition on common garden seedlings because it would have required whole seedling harvests, reducing the future use of the garden. However, we collected roots from several seedlings to verify they had formed ectomycorrhizas morphologically similar to those observed previously.

EMF Community Composition and Data Analysis. We determined the EMF community composition of the inoculum source trees in the reciprocal transplant experiment by classifying 75–100 living EM root tips/tree morphologically, followed by molecular analysis, using standard methods (18, 19). We morphologically typed all EM root tips from each seedling in the same way. We extracted DNA from three to five root tips per morphotype per tree/seedling, using a Qiagen DNEasy plant kit (Qiagen), amplified the internal transcribed spacer (ITS) region using PCR with the ITS1F/ITS4 primer pair, and sequenced it using an ABI 3730 Genetic Analyzer. We used BLASTn (40) to query sequences against the NCBI DNA sequence database, using 98% similarity as a cutoff for species identification.

We visualized data on EMF community composition, using nonmetric multidimensional scaling ordinations with a Bray-Curtis distance measure in PC-ORD 5.10 (41). For the reciprocal transplant experiment, we tested the influence of seed source (drought-tolerant vs. drought-intolerant mothers) and inoculum source (drought-tolerant vs. drought-intolerant trees) on EMF community composition with a permutation-based nonparametric multivariate analysis of variance (PerMANOVA), using relative abundance data, also in PC-ORD. We analyzed the main effects of seed source and inoculum source as a two-way factorial, followed by pair-wise tests when main effects were significant ($P \leq 0.05$). We used indicator species analysis in PC-ORD to determine whether particular species contributed to community differences. We combined EMF community composition data from reciprocal transplant experiment seedlings by seed source and compared them with the EMF communities of adult inoculum source trees, using a PerMANOVA with tree category (drought-tolerant or drought-intolerant trees or seeds) and tree age (adult vs. seedlings) in PRIMER version 6.1 (42). We analyzed seedling dry mass in the EMF drought experiment, using a two-way ANOVA with seed source (drought tolerant vs. intolerant), and EMF inoculum (live vs. sterile) as fixed effects in IBM SPSS version 24, and used a PerMANOVA to analyze community composition, excluding the sterile inoculated seedlings, which were not colonized by EMF. We examined associations between the abundance of *G. pinyonensis* and *Geopora* 2 ectomycorrhizas on drought-intolerant seedlings and their growth, using linear regression in SPSS. We used a two-way ANOVA to compare the mean shoot growth of drought-tolerant and drought-intolerant adult trees during predrought and drought 10-y periods, and a Student's *t* test to compare the mean 2-y shoot growth and mortality of the seedlings of drought-tolerant and drought-intolerant trees in the common garden.

Data Availability. Data are available from the corresponding author on request and will be publicly available within 1 y of publication through the Southwest Experimental Garden Array data repository (www.sega.nau.edu/data). Representative DNA sequences of each EMF taxon were previously deposited in the National Center for Biotechnology Information nucleotide database (https://www.ncbi.nlm.nih.gov/) (18) (Table S1).

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Supporting Information

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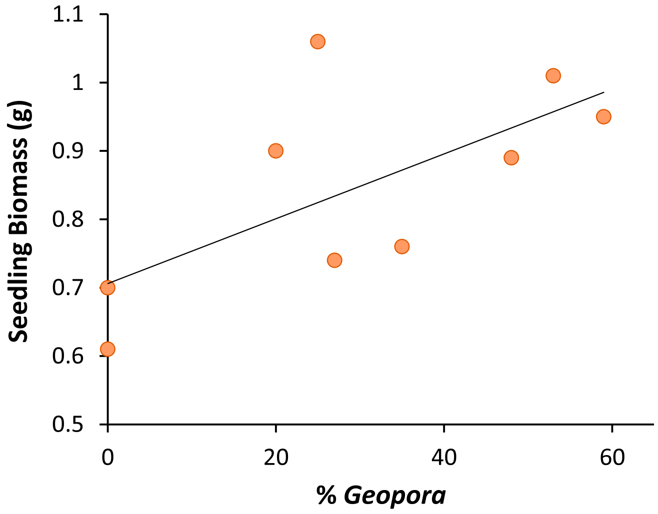

Fig. S1. Positive relationship between the relative abundance of *Geopora* ectomycorrhizas and biomass in seedlings of drought-intolerant mothers from the Greenhouse EMF drought experiment ($R^2 = 0.367$; $F_{1,8} = 5.65$; $P = 0.05$). Please see the text of the manuscript for *Methods*.

Table S1. Identification of EMF observed in this study based on ITS sequences

ID	Best BLAST match*	Accession no.	Percentage identity†
Cortinarius sp.	*Cortinarius sp.**	KF54689	98
G. pinyonensis	*G. pinyonensis**	KF768653.1	100
Geopora 2	*Geopora* sp. K*	HQ630379	99
Geopora 3	*Geopora* sp. J*	HQ630378	100
Geopora 4	*Geopora* sp. C*	HQ630376	99
Lactarius barrowsii	*L. barrowsii**	KF546496	98
R. roseolus	*R. roseolus**	KF546497	100
Russula sp.	Uncultured *Russula**	KF546498	98
Tricholoma terreum	*T. terreum**	KF546500	98

*Closest blast match is to a sequence observed previously in the study system and deposited in GenBank (15, 29).

†Percentage similarity of query and GenBank reference sequences.

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