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YOUNG VOICES AND VISIONS FOR THE UN DECADE OF RESTORATION

RESEARCH ARTICLE

Postrestoration colonization suggests slow regeneration, plant translocation barriers, and other host/symbiont lessons during the United Nations' Decade on Ecosystem Restoration

Lisa M. Markovchick[1,2,3,4], Elena A. Schaefer[2,5], Tessa Deringer[2], Zsuzsi I. Kovacs[1,2], Ron J. Deckert[1,2], Jamie Yazzie[2], Aalap Dixit[6], Jeffrey R. Propster[7], Adair Patterson[1,8], Kevin R. Hultine[9], Kevin Grady[10], Gerard J. Allan[1,2], Thomas G. Whitham[1,2], Catherine A. Gehring[1,2]

Mycorrhizal restoration benefits are widely acknowledged, yet factors underpinning this success remain unclear. To illuminate when natural regeneration might be sufficient, we investigated the degree mycorrhizal fungi would colonize *Populus fremontii* (Fremont cottonwood) 2 years after the restoration of a riparian corridor, in the presence of an adjacent source. We compared colonization levels across plant populations and ecotypes, and from trees in the planted area to those in natural source populations. Four findings contribute to the theory and application of host–symbiont interactions. (1) Median ectomycorrhizal colonization of trees in the planted area was less than one-tenth of that within natural source populations ($p < 0.05$), suggesting that even with adjacent intact habitat, sluggish regeneration would make proactive mycorrhizal restoration beneficial. (2) Within the planted area, median ectomycorrhizal and arbuscule colonization of trees sourced from greater distances were less than one-third of that for trees sourced locally ($p < 0.05$), suggesting translocation poses barriers to symbioses. (3) Changes in colonization did not align with plant ecotypes, suggesting that geographic scales of selection for plants and fungi differ. (4) Slight increases in median mycorrhizal colonization (from 0% to 5%) were strongly correlated with increased survival for the plant provenance with lowest survival ($r^2 = 46\%$ and $r_s = 48\%$, $p < 0.05$), suggesting mycorrhizae are particularly beneficial when plants are under stress (including translocation-induced stress). This study is novel in demonstrating that mycorrhizal regeneration is slow even in the presence of adjacent intact habitat, and that when colonization could seem negligible, it may still have biological significance.

Key words: assisted migration, cottonwood, ecosystem restoration, mycorrhiza, restoration, riparian, translocation

Implications for Practice

- Mycorrhizal restoration is often recommended for highly degraded areas isolated from intact native habitat, yet our results suggest that it would be helpful following disturbance even when intact habitats are nearby.
- Assisted migration of plants is increasingly considered as a strategy to boost restoration outcomes under climate change, but our results indicate that the success of this approach may be limited if appropriate fungal symbionts are not available.
- Even small increases in colonization may improve plant survival under stress.
- As a result, restoration of diverse, native mycorrhizal fungi, paired with plants and site conditions, could meaningfully increase restoration success during the UN's Decade on Ecosystem Restoration, even in situations when it might have been considered unnecessary.

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[1] Center for Adaptable Western Landscapes, Northern Arizona University, Flagstaff, AZ 86011, U.S.A.
[2] Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011, U.S.A.
[3] Conservation Team, WildEarth Guardians, 87501, Santa Fe, NM, U.S.A.
[4] Address correspondence to L. M. Markovchick, email lisa_markovchick@nau.edu
[5] Department of Renewable Resources, University of Alberta, T6G 2R3, Edmonton, Alberta, Canada
[6] Department of Forestry, New Mexico Highlands University, Las Vegas, NM 87701, U.S.A.
[7] Center for Ecosystem Science and Society, Northern Arizona University, Flagstaff, AZ 86011, U.S.A.
[8] Research Greenhouse, Northern Arizona University, Flagstaff, AZ 86011, U.S.A.
[9] Department of Research, Conservation and Collections, Desert Botanical Garden, Phoenix, AZ 85008, U.S.A.
[10] School of Forestry, Northern Arizona University, Flagstaff, AZ 86011, U.S.A.

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Introduction

As the United Nations' Decade on Ecosystem Restoration begins, climate change related increases in natural disturbances such as drought, heat waves, and wildfire are amplifying the need for restoration and regeneration of natural ecosystems (Parks & Abatzoglou 2020; National Academies of Sciences, Engineering, and Medicine 2020; Fargione et al. 2021). However, appropriate planting material resources have been declining, limiting the ability to scale up restoration activities (Wheeler et al. 2015; National Academies of Sciences, Engineering, and Medicine 2020; Fargione et al. 2021). We examine strategies for addressing these challenges which are rarely considered in tandem: mycorrhizal symbioses, the assisted migration of plants, and the interaction between them.

Multiple studies show that proactive restoration of diverse, native mycorrhizal fungal communities boosts restoration outcomes (e.g. Wubs et al. 2016; Neuenkamp et al. 2019; Policelli et al. 2020), particularly following disturbances known to negatively impact mycorrhizal fungi, such as pollution, land-use changes, invasion by certain exotic species, and even management activities such as pesticide application (e.g. Egerton-Warburton & Allen 2000; Meinhardt & Gehring 2012; Helander et al. 2018). Yet, many questions about the application of this method, and the principles underlying its success, remain. For example, few studies examine the conditions under which the proactive restoration of mycorrhizal fungi is unnecessary. It could be reasonably assumed, e.g. that in at least some cases, mycorrhizal fungi would either naturally regenerate from nearby habitat remnants, or be available on planting material, or both. Although few studies address these questions, Pankova et al. (2018) found that the natural regeneration of mycorrhizal fungi after a single fungicide application had not restored the mycorrhizal inoculum potential of the soil, or corresponding plant outcomes, to reference levels even after 5 years. Similarly, two studies specifically examining mycorrhizal fungal communities on planting material versus those in natural ecosystems suggested that greenhouse plants have different and less diverse mycorrhizal communities than those growing in natural populations (Sykorova et al. 2007; Southworth et al. 2009). Neither study explicitly examined the implications of this for field transplantation. However, since every change in plant host, fungal partner, and environment can elicit changes in symbiotic outcomes (e.g. Rillig & Mummey 2006), it seems likely that greenhouse mycorrhizal communities may not provide the same suite of benefits that fully diverse, native mycorrhizal communities found in natural environments would. Some studies suggest that forest thinning may decrease and shift ectomycorrhizal fungi (EMF) if gaps are 5–6 m (e.g. Parsons et al. 1994). However, the effects could be due to other factors, such as the conditions created by forest thinning, rather than to dispersal limitations (Varenius et al. 2017), making the implications for replanting and restoration projects unclear without studies that specifically address this question. We could find no studies specifically addressing how proximate mycorrhizal source populations in the field would need to be in order to sufficiently provide natural regeneration of mycorrhizal fungi in a restored or replanted area within the short term (a year or two).

In addition to the planting material shortages and restoration challenges posed by climate change, climate changes may also outpace the ability of plants to adapt or migrate to appropriate climate envelopes via natural dispersal (Whitham et al. 2020). Thus, translocating plant species or populations from warmer locations has been suggested as a tool to boost restoration success and promote more rapid adaptation under climate change (Etterson et al. 2020; Gomory et al. 2020; Sáenz-Romero et al. 2020). However, translocation of plant populations can still result in neutral to negative outcomes compared to those for local populations (Tiscar et al. 2018; Cooper et al. 2019; Simler et al. 2019) if the traits of the translocated populations are not well-matched to the planting site (Ikeda et al. 2017; Cooper et al. 2019; Blasini et al. 2021). Regional intraspecific plant adaptations, such as tolerance of frost or drought, can be of particular concern, due to increased variability in the temperature extremes (at both ends of the spectrum) associated with climate change (Montwe et al. 2018). For example, Fremont cottonwoods have developed contrasting physiological strategies, known as ecotypes, to survive extreme heat (Sonoran Desert or SD ecotype) or frost (Mogollon Rim or MR ecotype; Hultine et al. 2020; Blasini et al. 2021, 2022) that could interact with, or pose challenges to translocation.

These two restoration considerations (mycorrhizal symbioses and translocation) are rarely examined in tandem: there is an urgent need for multi-species studies to address the implications of assisted migration for interspecies interactions (Bucharova 2017; Remke et al. 2020, 2022). An organism's ability to adapt is likely dependent on the traits of the organism and its microbiome (sometimes referred to as the holobiont, a discrete ecological unit; Zilber-Rosenberg & Rosenberg 2008; Bordenstein & Theis 2015; Whitham et al. 2020). Research shows the importance of interactions between plant, fungi, and soil, emphasizing the need to consider mycorrhizal symbioses and soil sources in conjunction with plant source populations (Johnson et al. 2010, 2014; Rua et al. 2016), and demonstrates that complete reversals of results are possible when additional factors are considered in studies (Bailey & Whitham 2007). Yet, host–symbiont relationships are largely absent in studies of assisted migration (e.g. Etterson et al. 2020; Cooper et al. 2019; Sáenz-Romero et al. 2020), leaving important questions about how assisted migration influences host–symbiont interactions, including the geographic scales relevant to both plants and fungi, unanswered.

To address these knowledge gaps, we utilized a common garden experiment in a replanted riparian corridor after land conversion to agriculture. Riparian areas in the southwestern United States are of special conservation concern because they support 60–75% of the wildlife on less than 2% of the land area (Poff et al. 2012) and have suffered extreme losses of at least 90% (Zaimes 2007). Fremont cottonwoods (*Populus fremontii*) are a useful study species due to the extensive knowledge of their ecotypes, genetics, and eco-physiology (Ikeda et al. 2017; Cooper et al. 2019; Blasini et al. 2022), their strong associations

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with both ectomycorrhizal and arbuscular fungi from a young age (EMF and AMF, respectively; Brundrett et al. 1996; Gehring et al. 2006; Meinhardt & Gehring 2012), and their rapid growth and foundational nature in riparian ecosystems (Whitham et al. 2020, 2020).

We investigated the timeliness and effectiveness of natural mycorrhizal regeneration with an adjacent intact habitat that could serve as a source of mycorrhizal fungal propagules, the impact of assisted migration, and how these factors interacted to affect plant performance. Specifically, we examined whether mycorrhizal colonization in replanted areas approximated that in two natural populations, 2 years after replanting. Studies suggest that appropriate mycorrhizal inoculation is beneficial and needed after restoration from agriculture (Koziol & Bever 2017), but typically do not address whether nearby habitat remnants could act as sufficient mycorrhizal sources in the short term. Thus, we investigated whether natural recruitment of mycorrhizal fungi from an adjacent habitat remnant (and planting material source) could provide replanted trees with mycorrhizal colonization levels similar to those in the reference sites (planting material source populations) within 2 years. One of the planting material source populations was adjacent to the experimental site and water flowing through this extant natural population was used to irrigate the replanted site (for convenience, since water sources are rare in this remote, arid region), providing an additional potential vector for mycorrhizal regeneration. We also investigated whether the establishment of mycorrhizal associations would differ by transfer distance or ecotype and evaluated the correlation of colonization results with growth and survival. Transfer distance was measured by changes in temperature between source populations and planting sites to reflect changes experienced with assisted migration, and for consistency with studies comparing phenological and ecophysiological changes along the same temperature change gradient (e.g. Cooper et al. 2019; Blasini et al. 2021, 2022). We examined differences by ecotype to be consistent with studies showing that adaptive syndromes associated with ecotypes determine major plant physiological differences (Blasini et al. 2021, 2022). We hypothesized that: (1) mycorrhizal colonization of planted trees would be comparable to colonization in the natural source populations due to the adjacent remnant habitat and source; (2) colonization of planted trees would be highest for trees sourced from the adjacent natural population and lowest for trees sourced from the contrasting ecotype; and (3) plant survival and growth in the planted area would be positively correlated with levels of EMF and AMF colonization. Results from this study have important implications for both the fundamental scientific understanding of host–symbiont interactions and translocation, and to restoration applications.

Methods

Source Material and Study Sites

The Agua Fria common garden, previously described by Cooper et al. (2019) and Hultine et al. (2020), comprises 1.2 ha at Horseshoe Ranch within the Agua Fria National Monument in Black Canyon City, Arizona, U.S.A. The common garden was planted after an agricultural legacy of at least 50 years (Cornerstone Environmental 2015), and is near the climatic and elevational mid-point of Fremont cottonwoods. Cuttings from 16 populations across the climatic gradient of Fremont cottonwoods within Arizona were collected from trees at least 20 m apart during the 2013–2014 winter, propagated in the greenhouse, and planted at the Agua Fria common garden when approximately 1 year old, in October 2014. Replicates of genotypes were planted randomly within population plots, which appear in random order within each of four blocks (Fig. S1). Blocks of population plots were replicated four times. Due to the aridity of the region and remote location, trees were drip-irrigated during the growing season with river water that first flowed through the adjacent Agua Fria natural cottonwood population.

For this study, we focused on a subset of living trees in the common garden sourced from three natural populations representing two contrasting ecotypes (Agua Fria [SD], Jack Rabbit [MR], and Cibola [SD]) and compared them to a subset of trees located within two of the planting material source populations, one from each of the ecotypes (Agua Fria [SD] and Jack Rabbit [MR]; Table 1; Fig. 1). To inform understanding of how tree age and time since planting impact on results, older trees planted adjacent to the common garden (within the historic ranch house compound) were also sampled (Fig. 2).

Trees in the common garden were approximately 3 years of age and approximately 2 m tall at the time of root sampling (almost 2 years after planting), while trees located within the natural populations were of unknown age and approximately 6 m tall. Trees from within the ranch house compound were at least 30 years of age and approximately 6 m tall. No trees were inoculated with mycorrhizal fungi.

Climate and soil data for the common garden and source populations can be found in Table 1. The ecotypes (Sonoran Desert [SD] and Mogollon Rim [MR]) and the absolute difference in mean annual temperature between tree source and planting locations (MAT transfer distance) are used in graphs to depict scales of selection on plant adaptations that might be expected to affect mycorrhizal symbiosis (Cooper et al. 2019; Blasini et al. 2022).

Soil Samples

To understand whether any differences in mycorrhizal colonization among sites might be related to soil properties, soil samples were collected to provide descriptive information on soil characteristics. Soil texture, electrical conductivity (EC), pH, %C, %N, and %P measurements were conducted on soil samples collected from the soil surface (0–5 cm depth) in the common garden ($n = 6$), and at the two natural populations representing both ecotypes, Agua Fria (Sonoran Desert; SD ecotype) and Jack Rabbit (Mogollon Rim; MR ecotype; $n = 2$ and $n = 3$, respectively). Details of soil characterization methods can be found in Supplement S1.

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Table 1. Climate and soil characteristics for the common garden experimental site and planting material natural source populations. [a]Ecotype abbreviations for planting material source populations are as follows: trees from the warmer Sonoran Desert (SD) ecoregion and trees from the cooler Mogollan Rim (MR) ecoregion (Blasini et al. 2021, 2022). Trees planted at the Agua Fria common garden experiment experienced climatic conditions within the range of the SD ecotype, but consisted of trees sourced from both ecotypes. [b]Climate data for 2016–2018 is from PRISM Climate Group (2020). Climate abbreviations are as follows: mean annual temperature (MAT), precipitation (PPT), and vapor pressure deficit (VPD). [c]MAT transfer is calculated as the absolute difference between the MAT at the tree's source population and the MAT where the trees were planted at the common garden. [d]USDA soil series, soil content, and soil pH is from USDA, Natural Resources Conservation Service (2020). [e]Salinity, pH, %C, %N, %P, and ribbon test results are from soil samples collected during the study, as described in the Methods section. [f]Roots and soil samples were not available for the Cibola natural population.

Population Type	*Common Garden Experiment*		*Natural Populations*	
Population	*Agua Fria*	*Agua Fria*	*Jack Rabbit Natural*	*Cibola*[f]
Ecotype[a]	—	SD	MR	SD
MAT (°C)[b]	18.4	18.4	14.2	22.1
MAT transfer (°C)[c]	—	0	4.2	3.7
Latitude	34.259688	34.257657	34.989867	33.36077
Longitude	−112.057859	−112.064022	−110.622894	−114.69856
Elevation (m)	988	988	1,507	70
Mean PPT (cm)[b]	35	35	15.7	7.4
Max VPD (hPa)[b]	18.4	18.4	27.6	34.1
Soil series[d]	Gila Soils	Barkerville Cobbly Sandy Loam	Ives Soils	Lagunita Loamy Sand
Soil content[d]	39% sand, 37% silt, 24% clay	68% sand, 20% silt, 13% clay	71% sand, 17% silt, 13% clay	81% sand, 17% silt, 3% clay
pH[d]	—	7	8.2	8.2
pH[e]	7.40–8.48	6.81–6.95	6.75–7.98	—
Salinity (μS)[e]	103–166	99–375	104–441	—
% C[e]	1.39–1.69	1.61–3.46	0.8–2.86	—
% N[e]	0.14–0.15	0.12–0.26	0.01–0.12	—
% P[e]	0.08–0.17	0.08–0.10	0.03–0.06	—
Ribbon test[e]	Silt loam; loam	Silt loam; loam; loamy sand	Silt loam; silt clay loam	—

Figure 1. Map and photos of the three natural populations across the climatic and elevational gradient from which trees in this study were sourced. Sonoran Desert ecotype locations, Cibola and Agua Fria, are in shown in red and pink. The Mogollon Rim ecotype location is shown in blue. The mid-elevation location of Agua Fria is also the location of the Agua Fria common garden. Photos by Lisa Markovchick and Kevin Grady.

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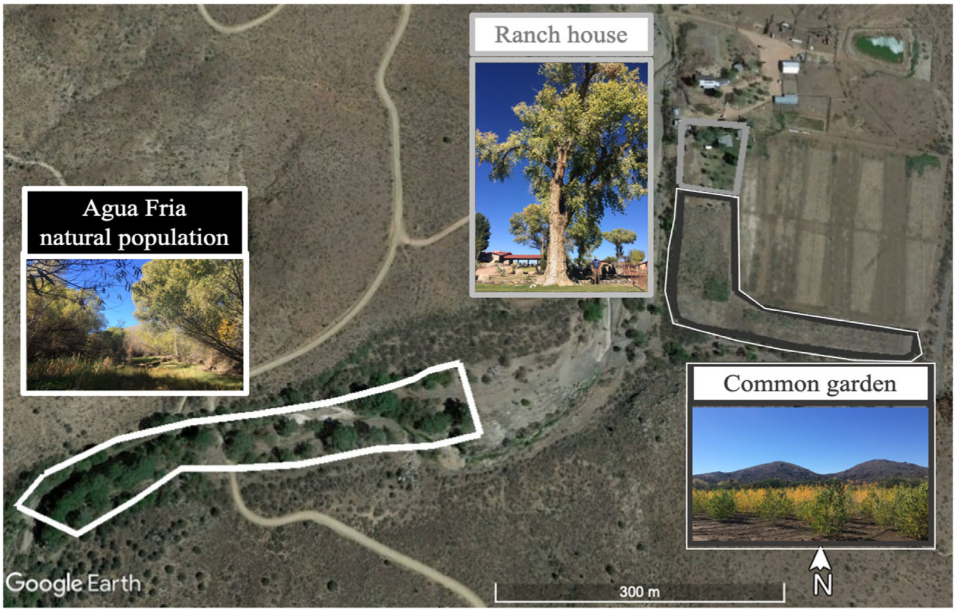

Figure 2. Map and photos of areas compared at Agua Fria: the Agua Fria natural population, the ranch house compound with existing older trees, and common garden experiment. Colors indicate those used in Figure 3 (with the Agua Fria natural population in white, the ranch house in gray, and the common garden in dark gray) (Photos by L. Markovchick).

Survival and Growth Measurements for Planted Trees

Survival and diameter at root crown for trees planted in the Agua Fria common garden were sampled approximately 1 year (January 2016) and 2 years (December 2016) after planting. For these metrics, we focus on trees from the same garden blocks, populations, and genotypes during the same time frame roots were sampled. This yielded 134 trees for whom survival could be evaluated during year 2 (50 from Cibola, 44 from Agua Fria, and 40 from Jack Rabbit).

Growth was assessed for trees that survived from year 1 to year 2 ($n = 109$). Calipers were used to measure diameter at root collar (DRC). Area at root collar (ARC; Supplement S2) was calculated from DRC ($\text{ARC} = 2\pi r^2$, where $r = \text{DRC}/2$). Growth was calculated as the standardized difference in ARC from year 1 to year 2 ([ARC for year 2 − ARC for year 1]/ARC for year 1).

Root Sampling

During the autumn of 2016 (between year 1 and year 2 survival and growth measurements), fine roots within the drip line of each cottonwood tree in the root study were gathered from the four cardinal directions and traced from larger roots radiating from the base of each tree, stored on ice, and frozen at −20°C within 6 hours of collection. In the common garden, three to six replicates of at least four genotypes from each source population were used ($n = 65$, with 31 from Jack Rabbit, 15 from Agua Fria, and 19 from Cibola). To provide reference colonization rates for cottonwood trees growing in the same natural populations from which common garden trees were sourced, and in older trees planted near the garden, roots were sampled in the same manner from cottonwood trees in the Agua Fria and Jack Rabbit natural populations ($n = 4$ each), and trees planted in the ranch house compound adjacent to the common garden ($n = 3$).

Colonization Surveys

EMF root tips were counted under a dissecting scope using the gridline intersection method (Brundrett et al. 1996). Over 7,700 gridline intersections were evaluated and 1,226 mycorrhizal root tips were counted. Representative root tips for each morphotype were placed in extraction tubes for DNA sequencing. Extremely low mycorrhizal colonization in the common garden, where the majority of samples were collected to address translocation questions, resulted in limited EMF community data. Molecular analyses were conducted on roots tips representative of the morphotypes seen for descriptive purposes, but insufficient data were available for a statistical comparison of community data.

Separate root subsamples were cleared and stained, and evaluated for AMF hyphae, vesicles, and arbuscules using a compound microscope and the gridline intersection method (Brundrett et al. 1996). Care was taken to identify dark septate endophytes (DSEs; nonmycorrhizal root fungi that have no specialized exchange structure) separately, as indicated by melanized septate hyphae. Over 7,600 gridline intersections were evaluated and 6,253 AMF and DSE organs were counted. Characterization of AMF colonization can include arbuscules, vesicles, and aseptate hyphae. However, we focus on arbuscule colonization in many of the graphs and statistics because arbuscules are the crucial transfer organ between fungus and plant, more comparable to what is represented by an EMF

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root tip (which harbors the EMF exchange organ, the Hartig net). We followed the methods of Lamit et al. (2016) for morphotyping and sequencing of EMF root tips except that we used a modified primer set to reduce nontarget amplification. Full fungal taxonomic identification details can be found in Supplement S3.

Statistical Analyses

All statistical analyses were conducted in R version 4.0.3 (R Core Team 2020). Individual trees were used as an independent experimental unit (similar to Grady et al. 2011) because plants were randomly planted across the garden, environmental heterogeneity was relatively low within the garden, and in exploratory data analyses both genotype and garden block failed to add significantly to statistical models. We used Bartlett's test for equal variance (also sensitive to non-normal distributions; McDonald 2014) via the bartlett.test() function in base R. Nonparametric tests were used due to small, uneven sample sizes and failed homogeneity of variance tests. Overall differences in colonization and growth were analyzed with Welch's analysis of variance via the oneway.test() function in base R with the var.equal=FALSE setting, and post hoc pairwise comparisons were made using the Tukey–Kramer test using the TukeyHSD() function in base R (McDonald 2014). As our sample size was below 1,000 (McDonald 2014), we utilized Fisher's exact test in base R using the fisher_test() function in the rstatix library (Kassambara 2013) to test if the binomial survival data differed among population groups in the garden. Post hoc Fisher's exact tests with Bonferroni adjustments for multiple tests were used to test pairwise comparisons using the pairwise_fisher_test() function in the rstatix library.

To investigate if the proportions of EMF root tips and AMF arbuscules were associated with survival, percent survival and median combined colonization (colonization of EMF root tips plus AMF arbuscules) were summarized by garden block, population, and genotype and compared. This was accomplished using both linear regression (the lm() and geom_smooth() functions) and Spearman's rank correlation (cor.test() function with the method='spearman' setting) in base R.

Results

Soil Characteristics

As soil metrics were intended to be descriptive, simple value ranges are reported and qualitatively compared. The ranges of pH, salinity, %C, %N, and %P for the common garden and natural source populations often overlap (Table 1). The largest difference seen was between the common garden and the Jack Rabbit natural population for %P (a metric that other studies suggest can be very high while not indicative of plant available P or AMF growth; Propster & Johnson 2015; Stevens et al. 2018).

Hypothesis 1: Colonization of Planted Trees Versus Natural Populations

EMF colonization of trees significantly differed among the natural population, ranch house compound, and garden ($F = 11.06$, $df = 2, 4.2$, $p < 0.05$; Table S1; Fig. 3). Post hoc pairwise comparisons revealed that EMF colonization in the natural populations was significantly higher than that in either the ranch house area or the common garden ($p < 0.01$ and $p < 0.001$, respectively), while higher colonization in the ranch house compound than in the garden was marginally significant ($p < 0.10$). The hypothesis (1) that mycorrhizal colonization of replanted trees 2 years postplanting would be comparable to that in natural source populations was not supported for EMF (Fig. 3). Differences in overall AMF, AMF arbuscule, and DSE colonization between planting locations were not large enough to be significant (Table S1; Fig. 3).

Hypothesis 2: Colonization of Trees From Different Populations Within the Garden

EMF and arbuscule colonization of trees in the garden differed across populations ($F = 15.31$, $df = 2, 27.05$, $p < 0.001$ and $F = 5.79$, $df = 2, 30.98$, $p < 0.01$, respectively). Post hoc pairwise comparisons revealed that EMF and arbuscule colonization

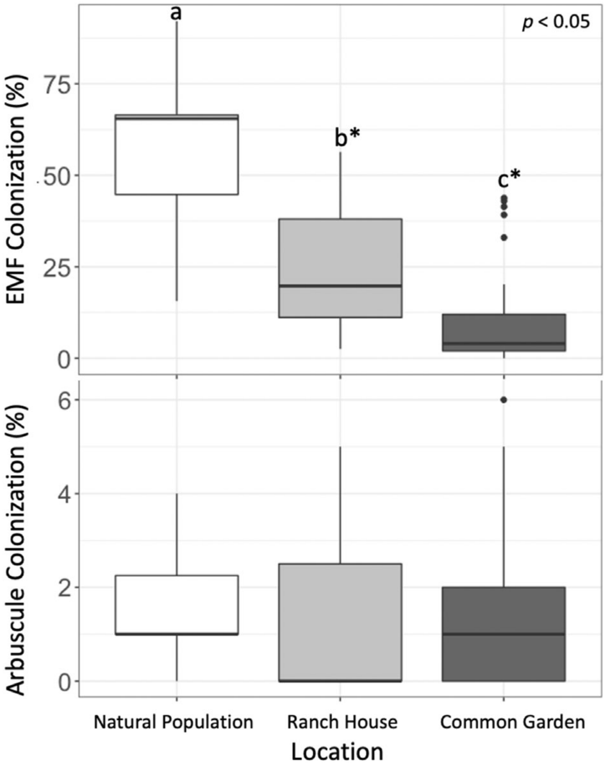

Figure 3. Box and whisker plots showing medians and 25th and 75th percentiles for EMF colonization (top) and arbuscule colonization (bottom) in trees from the natural source populations, ranch house compound, and the common garden. Different letters across box and whisker plots indicate significant differences (typically at $\alpha = 0.05$, with the exception that asterisks [*] between pairs indicate significant differences at $\alpha = 0.10$). Full statistical results can be found in Tables S1 and S2.

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both differed significantly among groups, highest in the locally sourced trees, and significantly lower in trees sourced from more remote locations ($p < 0.05$ and $p < 0.001$, respectively; Table S2; Fig. 4). However, colonization was not lowest in trees from the contrasting ecotype: arbuscule colonization did not differ between trees from the two remote locations, and EMF colonization rates in trees from the Jack Rabbit population (contrasting ecotype to the planting site) were significantly higher than in trees from the Cibola population (same ecotype as the planting site). DSE and overall AMF colonization did not differ significantly among populations in the garden (Table S2; Fig. 4 for AMF). The hypothesis (2) that trees from more remote locations would show reduced colonization was supported for both EMF and AMF arbuscule colonization, but trees sourced from the contrasting ecotype (compared to the planting site) did not show reduced colonization.

Hypothesis 3: Correlation of Survival and Growth With Mycorrhizal Colonization

Survival to year 2 differed significantly between populations ($p < 0.001$; Table S2; Fig. 4). Post hoc pairwise comparisons revealed trees from the warmer source population, Cibola, demonstrated significantly lower survival than the cooler Jack Rabbit or the local Agua Fria populations ($p < 0.001$ for both; Table S2; Fig. 4).

Growth from year 1 to year 2 significantly differed between populations planted in the common garden ($F = 6.80$, $df = 2, 163.33$, $p < 0.01$). Post hoc pairwise comparisons revealed that Jack Rabbit demonstrated significantly higher growth than either the local Agua Fria or the Cibola population ($p < 0.001$ and $p < 0.01$, respectively; Table S2; Fig. 4).

There was a significant and strong relationship between tree survival rates and median colonization rates (EMF + arbuscule; adjusted $r^2 = 46\%$, $F = 4.93$, $df = 5, 18$, $p < 0.01$, and $r_s = 48\%$, $p < 0.05$, for linear regression and Spearman's rank correlation coefficient, respectively). As can be seen in Figure 5, the relationship between survival and colonization for struggling trees from the warmer Cibola population drives this relationship ($t = -3.661$, $p < 0.01$ and $t = 3.21$, $p < 0.01$ for the population and population by colonization interaction, respectively).

The hypothesis (3) that mycorrhizal colonization would be significantly correlated with survival and growth, was partially

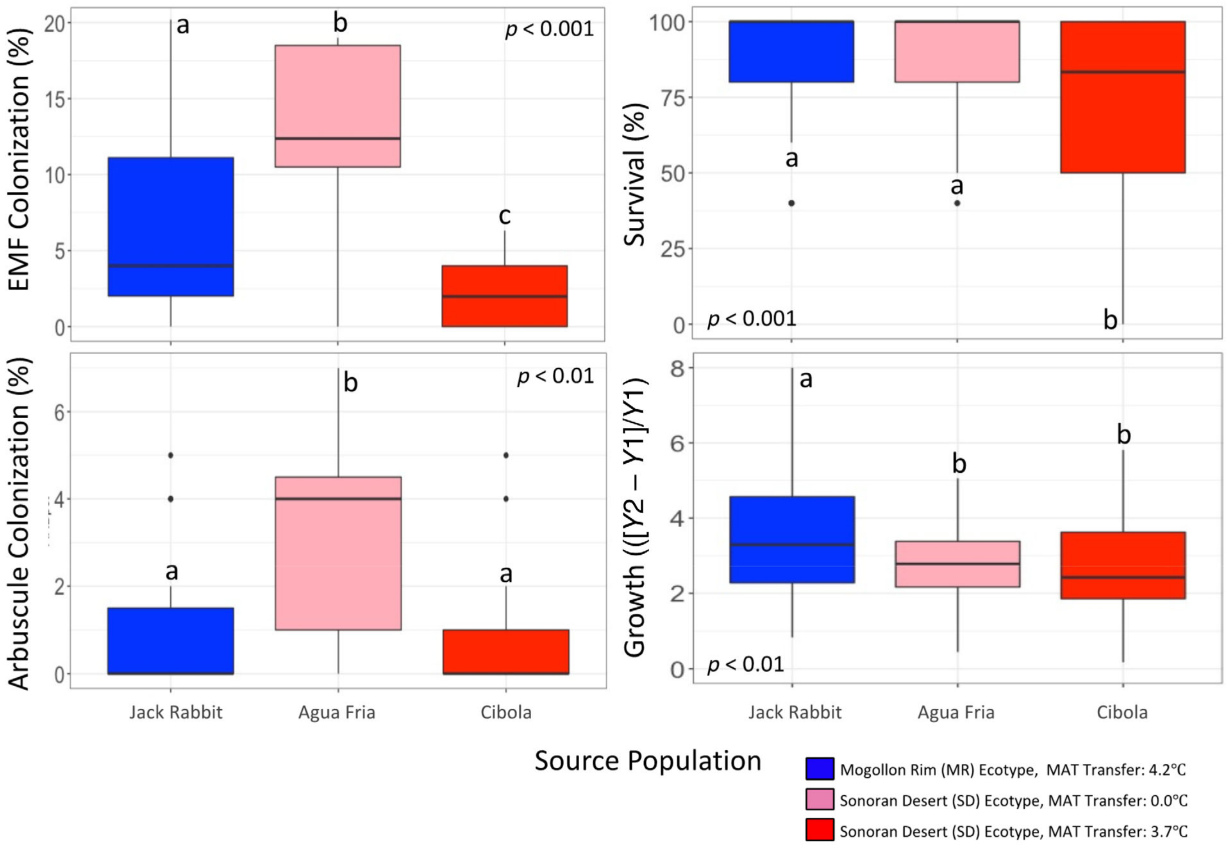

Figure 4. Common garden EMF colonization (top left) and presence of arbuscules (bottom left) of a subset of trees for which roots were sampled; population level survival through the second growing season, and growth of trees during the second growing season after planting (right) for trees sourced from three natural populations, presented by the MAT of the source populations and MAT transfer distance to the common garden. Populations in blue belong to the Mogollon Rim (MR) ecotype and adaptive syndrome described by Blasini et al. (2021), while the populations in red and pink belong to the Sonoran Desert (SD) ecotype and adaptive trait syndrome. Box and whisker plots show the median, 25th and 75th percentiles (boxes). Different letters across source populations represent significant differences between populations. Full statistical results can be found in Tables S1 and S2.

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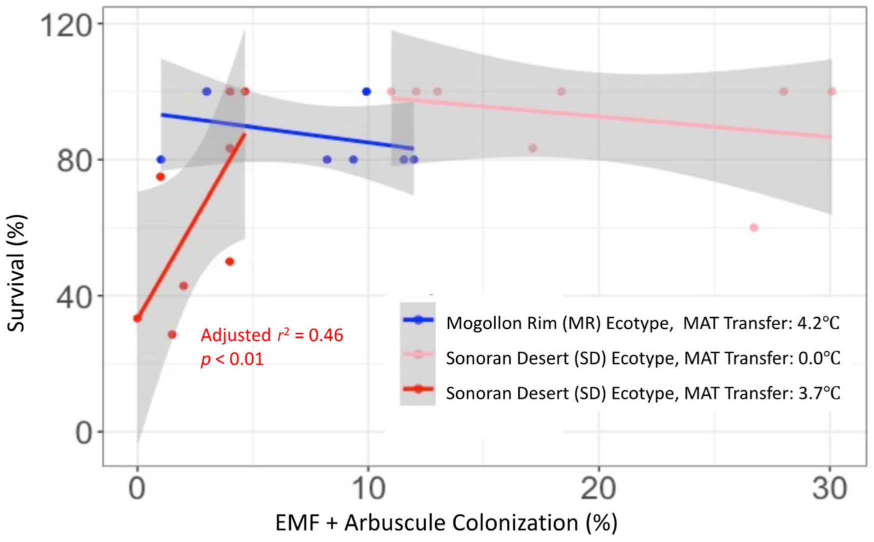

Figure 5. The significant, positive relationship between survival rates and median combined colonization (EMF + AMF arbuscules) for trees in the struggling Cibola population (in red). Although colonization rates were often lowest in this population that was struggling to survive more than the other two populations, even minimal colonization rates were associated with increased survival. The relationship was not significant for the other two populations, which already had quite high survival rates overall. Regression lines are shown in ecotype colors, and SDs for the regressions are shown in gray.

supported. For the population of planted trees struggling most, mycorrhizal colonization was strongly and significantly correlated with survival.

Mycorrhizal Taxonomic Identifications

Taxonomic identifications from natural population EMF root tips are shown in Table 2. The two most abundant morphotypes in the root samples (Dark Knobs and Black Fingers) were *Tricholoma populinum* and two species of *Tomentella*, respectively. Other taxa represented in natural populations included a species of *Amanita* and a species of *Sebacina*.

Discussion

In this study, EMF colonization of cottonwood trees 2 years postplanting after historical agriculture was well below that in natural source populations for either ecotype. Yet, trees in the planted area from the local population still showed significantly higher EMF and AMF arbuscule colonization than trees sourced remotely, but decreased colonization did not align with the contrasting plant ecotype as expected. Despite low levels of colonization in trees from the warmer population with the lowest survival rates, colonization and survival demonstrated a strong positive association for this one, struggling population. These results illuminate several underlying concepts of mycorrhizal symbioses and have practical implications for restoration and assisted migration.

Slow Regeneration Makes Mycorrhizal Restoration Important Even Near Adjacent Habitat Remnants

A key element of novelty in this study is our contribution to understanding the limitations of natural mycorrhizal recruitment

Table 2. Operational taxonomic unit information. Examples of some morphotype appearances can be seen in Figure S2. [a]Sequences for OTUs were submitted to GenBank (Sayers et al. 2022) resulting in the listed accession numbers. [b]For all reference names in the table, the *e*-values were zero. [c]This number represents the percentage of the OTU sequence that overlaps the reference sequence. [d]Reference accession selected conservatively, by excluding models and uncultured samples. Full taxonomic identification details can be found in Supplement S3.

Location	*Morphotype Name*	*OTU Name*	*Accession No. of OTU*[a]	*GenBank Reference Name*[b]	*Query Cover*[c]	*Percent Identity*	*Reference Accession No.*[d]
Jack Rabbit Natural Population	Black Finger	Tomentella 1	OQ576093	*Tomentella laterita*	97%	94.77%	KP783474.1
	Black Finger	Tomentella 2	OQ576094	*Tomentella sp. O41*	97%	94.06%	AJ534912.1
Agua Fria Natural Population	White Finger	Sebacina 1	OQ576092	*Sebacina epigaea*	100%	98.52%	KF000427.1
	Dark Knob	Tricholoma 1	OQ576095	*Tricholoma populinum*	97%	100.00%	JN019602.1
	White Mass	Amanita 1	OQ576091	Amanita sp. “sp-AZ28”	97%	98.69%	MN204485.1

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and recovery even with sources nearby. Based on our results, low recruitment of EMF seems to be a factor underlying the success of proactively restoring diverse, native mycorrhizal fungal communities (summarized in Neuenkamp et al. 2019). Our study suggests that dispersal limitations may be operating even when the sources of disturbance are local, and it might reasonably be assumed that mycorrhizal spores could disperse on wind and water to swiftly regenerate postdisturbance. This concurs with decreases in ectomycorrhizal species diversity found with increasing isolation of mycorrhizal tree islands (Peay et al. 2010), reviews of the effects of clear-cut logging (Jones et al. 2003), and results showing that spore dispersal is often limited to less than 5 m correlated with rain rates and events (De-Wei 2005). As drought events become more frequent, prolonged, and widespread in some parts of the world due to climate change, EMF dispersal limitations may become increasingly relevant.

Tree age could be suggested as an alternative cause for reduced EMF colonization levels in the planted area. However, the higher colonization rates found in the natural populations in the current study are similar to those found in young cottonwoods and other *Salicaceae* plants within relatively short time periods when fungi are available or provided (e.g. Gehring et al. 2006; Nara 2006; Meinhardt & Gehring 2012). Also, data from the older trees found in the similarly disturbed, adjacent ranch house compound (30+ years old) show EMF colonization only marginally higher than trees in the common garden (3 years old), and still significantly below those in natural populations. This suggests that while tree age or time since planting could have an effect, it is certainly not the only factor.

Elevated nutrient levels, precluding the need for mycorrhizal nutrient mining services, could be another potential explanation of low EMF colonization rates in the planted area. However, this seems unlikely given the soil nutrient results, which are often roughly comparable between the common garden and the natural populations or similar in the common garden to those found in other studies. In addition, mycorrhizal fungi are known to provide a variety of services besides nutrient mining, such as help with water access and water use efficiency (e.g. Querejeta et al. 2006; Egerton-Warburton et al. 2008) and pest regulation (Reddy et al. 2006; Karst et al. 2015).

The lack of significant differences between common garden and natural population for AMF arbuscule colonization may reflect the agricultural history of the area (Maltz & Treseder 2015; Hart et al. 2017). Yet, proactive restoration of diverse native mycorrhizal communities following agriculture have improved outcomes even in predominantly AMF systems (e.g. Koziol & Bever 2017), and Grünfeld et al. (2022) found distances of 55 and 90 cm (versus 2 cm) between plants reduced AMF colonization in the greenhouse, suggesting similar constraints on native diversity and dispersal may be relevant for AMF, though not revealed here by colonization counts.

Hultine et al. (2020) provides some evidence that EMF colonization of Mogollon Rim ecotype trees in the common garden may have increased to levels more similar to those in natural populations (with average colonization in the range of 50–60% instead of the median 2–12% we found) after 5 years. However, Hultine et al. (2020) had a small sample size ($n = 5$ for each ecotype) and did not provide a direct comparison (e.g. no information on arbuscule colonization was reported, and not all populations in the current study were sampled). This result and results from other literature (Jones et al. 2003; Peay et al. 2010; Pankova et al. 2018) suggest that overcoming mycorrhizal dispersal limitations may take several years and may be largely dependent on whether colonized plants are located close enough to planted or regenerating plants to touch their roots. Future studies could further confirm dispersal limitations as an underlying mechanism leading to the need for mycorrhizal restoration by comparing mycorrhizal inoculation potential and mycorrhizal colonization of trees of the same age planted within natural populations and revegetated areas.

Translocation Creates Mismatches Between Host and Symbiont Provenances

Despite low colonization in tree roots from the common garden overall, more locally sourced trees demonstrated significantly higher EMF and AMF arbuscule colonization than their counterparts from more remote locations. These findings are consistent with other literature, predominantly from AMF systems, demonstrating that plants and fungi are coadapted to each other and site conditions (Johnson et al. 1992, 2010, 2014). In tandem with existing literature, our study indicates that even when native mycorrhizal fungi are available, they may be less compatible with translocated plants. Given the ability of mycorrhizal fungi to support plant resiliency to multiple stressors (e.g. Augé et al. 2015; Rivero et al. 2018; Coban et al. 2022), maintaining and enhancing these relationships seems increasingly crucial under climate change, particularly as interest in plant translocation as a tool for climate adaptation increases (Handler et al. 2018).

Geographic Scales of Selection for Plants and Their Mycorrhizal Symbionts Differ

Contributing novelty to this study, results indicate that selection forces on mycorrhizal symbioses are operating at a different scale than for plants. In our study, mycorrhizal colonization results do not align with the idea that the most extreme differences should be found in trees from the contrasting ecotype to the planting location, as would be found for tree physiology (Blasini et al. 2021, 2022). Instead, assisted migrant trees from the same ecotype as the common garden (SD) had even lower median rates of EMF colonization than translocated trees from the contrasting ecotype (MR), and arbuscule colonization rates were similarly low for both remotely sourced populations compared to locally sourced trees. This finding suggests that selection forces on optimal mycorrhizal symbioses are operating at a different geographic scale than for plant adaptive trait syndromes. For instance, primary differences in physiology may be determined at the regional level by climatic differences, followed by smaller differences at the population level (Blasini et al. 2021), while differences in EMF or AMF compatibility may be determined by a suite of factors that vary on a different

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scale, including microclimates and soil variables. This finding affirms the concept that tighter fidelity on ecological time scales would be necessary to consider hosts and microbiomes as holobionts, extends this concept to geographical scales, and suggests that hosts and their microbiomes are better described as ecological communities (Douglas & Werren 2015). This finding also suggests that identifying optimal sources for mycorrhizal restoration should not rely on the factors and geographic scales used to determine plants appropriate for a given restoration site or that determine plant adaptive strategies or ecotypes.

Mycorrhizal Symbioses May Be Most Beneficial to Plants Under Stress, Including Translocation Stress

The strong correlation between survival and colonization rates in trees from the source population with lowest survival rates concurs with the idea that the beneficial effects of mycorrhizal symbioses are particularly large during an "ecological crunch" (Wiens 1977). For example, AMF are generally known to increase stomatal conductance, but this effect increases with the severity of the drought (Ruíz-Sánchez et al. 2011; Worchel et al. 2013; Augé et al. 2015). The magnitude of mycorrhizal benefits to host plants has been similarly correlated with salt stressor severity (Rivero et al. 2018). Our findings suggest that this correlation between plant stressor strength and the strength of mycorrhizal benefits to the plant may extend to stresses induced by assisted migration.

As multiple stressors increase under climate change, findings regarding the correlation between plant stressor strength and the strength of mycorrhizal symbiosis benefits also suggest that mycorrhizal symbioses may become increasingly important. Our study builds on this concept, suggesting that even when colonization levels low and one might be inclined to view symbioses unimportant, they may still have crucial biological significance. Indeed, in concert with previous studies, this finding suggests that inoculating assisted migrant plants with appropriate, diverse mixes of native mycorrhizal fungi could optimize their chances at new locations. Additional research is needed to determine the optimal mixes of native mycorrhizal fungi for this purpose, given studies reflecting the coadaptation of plants and fungi to each other and to site conditions (Johnson et al. 1992, 2010, 2014). For example, optimal mixes might include mycorrhizal fungi from the plant source populations (as in Remke et al. 2020, 2022), from relatively undisturbed habitat remnants near the planting site, from the planting site itself, or a mix.

Importance of Including Interspecies Interactions in Management and Restoration Studies

Soil microbiota are often key to supporting crucial ecosystem services and to the swift adaptation needed under climate change (Wilkinson & Dickinson 1995; Coban et al. 2022). Mismatches in aboveground and belowground biodiversity and conservation needs, and reports of declines in mycorrhizal fungi due to a variety of disturbances (Arnolds 1988; Lilleskov et al. 2002; Meinhardt & Gehring 2012; Helander et al. 2018; Cameron et al. 2019) highlight the need to consider mycorrhizal fungi and microbiota in management, restoration, and climate adaptation (Bucharova 2017). Our findings reiterate this point and highlight the urgency of this need during the climate and biodiversity crises. If the full diversity of the mycobiome and microbiome is not conserved, restored, and maintained resulting in its continued decline (e.g. Baird & Pope 2021; Mueller et al. 2022), many plants may lack their optimal symbionts in the future, resulting in cascading effects on the abilities of ecosystems to adapt to multiple stressors. Conversely, targeting the maintenance and support of the full diversity of the microbiome during land management, regeneration, restoration, and plant translocation could provide ecosystems with the highest chance of adapting at the pace of climate change and optimize the services provided by remaining natural areas. This approach also has the advantage of being aligned with the goals, ethics, and standards of ecological restoration (Gann et al. 2019), and being consistent with United Nations' Principles for Ecosystem Restoration (FAO et al. 2021).

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Supporting Information

The following information may be found in the online version of this article:

Figure S1: Map of Agua Fria common garden showing the four planting blocks.
Supplement S1: Soil characterization details.
Supplement S2: Growth measurement details.
Figure S2: Examples of AMF and EMF morphotypes from this study.
Supplement S3: Fungal taxonomic identification details.
Table S1: Medians, interquartile ranges, Welch's ANOVA results, and post hoc Tukey–Kramer results.
Table S2: Medians, interquartile ranges, and statistical test results.

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