Intraspecific variation of root mycorrhizal colonization among genotypes of *Populus fremontii*

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ABSTRACT

In recent years, evidence has been found to support the theory that plant genetics have extended effects on ecosystem processes including nutrient cycling (including carbon sequestration), water-use, resistance to pathogens, and response to environmental stressors such as drought and extreme temperatures. Mycorrhizae may be the hidden link that is selected by genotype and in turn affects ecosystem processes. We hypothesized that total ectomycorrhizae (ECM) abundance varies according to intraspecific genetic variation in *Populus fremontii*. Additionally, we hypothesized that there would be a positive association between mycorrhizal colonization and tree survival and productivity. This study was conducted in a common garden in Cibola, Arizona analyzing 14 genotypes of *P. fremontii* propagated from different sites throughout the southwestern United States. We collected 2 soil and root samples of each of the genotypes, which were then analyzed under microscope for presence and abundance of mycorrhizae as a ratio of total roots. Plant genotype explained over 41% of the variation in abundance of mycorrhizal colonization of the tree roots. However, percentage of ectomycorrhizae did not explain the variation found in tree survival rates or tree productivity.

Keywords: fungi, ectomycorrhizae, ECM, ecosystem processes, genotype, community genetics, ecology, extended phenotype, climate change, mycorrhizal colonization, *Populus*, cottonwood, common garden

INTRODUCTION

Through the flow of biotic and abiotic ecosystem interactions, intraspecific genetic variation of foundation plant species has been found to directly and indirectly affect plant growth morphology and ecosystem processes including aphid-herbivory resistance , metabolic tannins, leaf litter nitrogen content, leaf litter nutrient release, nitrogen mineralization, decomposition rates, aboveground productivity, belowground carbon soil fluxes (Dickson and Whitham 1996, Lindroth et al. 2002, Driebe & Whitham 2000, LeRoy et al. 2007, Madritch et al. 2006, Schweitzer et al. 2004, Driebe & Whitham 2000, Madritch et al. 2006, LeRoy et al. 2007, Lojeweski et al. 2009, 2012). Genetic variation within species has also been found to affect ecological communities, such as soil microbial abundance and diversity, variation in lichen cover and community, arthropod community structure, and endophytic community structure (Madritch and Lindroth, 2011, Schweitzer et al. 2008, 2011, Lamit et al. In Review, Wimp et al. 2007, Lamit et al. 2014).

Mycorrhizal fungi colonize plant roots and exist in a symbiotic, mostly mutualistic, relationship with the plants. Arbuscular mycorrhizal fungi (AM) and ectomycorrhizal fungi (ECM) are the most prevalent types of mycorrhizae. Crop species and grasses most commonly have AM while ECM is commonly found in woody plants. Ectomycorrhizae are very important in carbon cycling, influencing carbon sequestration as well as soil microbial biomass and respiration by facilitating the movement of carbon from trees into soils--62 percent of carbon in the soil organic matter pool was transmitted via mycorrhizae in Populus spp (Godbold et al. 2006). The ECM fungi use carbon to build hyphal networks in the soil, which store carbon longer than roots and were found to decompose slower in a pinon pine forest (Langley et al. 2006). Enzymatic excretions of an ectomycorrhizal fungus, and the mycorrhizal and non-

mycorrhizal roots themselves were found to vary when the fungus was inoculated on many different genotypes of the same species of *Populus* hybrid (Courty et al. 2011). Studies show that ECM fungal community composition varies inter- and intraspecifically and that this fungal compatibility is heritable (Hoeksema and Classen 2012, Velmala et al. 2013). Mycorrhizal communities have been found to change and vary over the course of a long term study in correspondence with differing genotypes of Pinyon Pines (Gehring et al, 2014). Hoeksema and Classen (2012) argue that since mycorrhizal fungal communities vary based on tree genotype, these traits should be factored into consideration as well as aboveground traits in plantation forestry. Mycorrhizal plants are commonly more resistant to diseases (especially microbial soil-borne pathogens), less influenced by high metal concentration in the soil, and benefit from mycorrhizae that facilitate increased water and nutrient absorption.

Further research in the extended effects of tree genetics on ecosystem processes focused on southwest riparian *Populus* species would be beneficial as climate change continues to affect our world and habitat restoration projects must face present-day conditions while, hopefully, also preparing for adaptability to future conditions. This study looks at how percent mycorrhizal colonization on root tips vary in response to different genotypes of *Populus fremontii*. We hypothesized that 1) the amount of observed ectomycorrhizal colonization of root-tips measured in terms of percent colonization varies among different *P. fremontii* genotypes and 2) mycorrhizal colonization positively correlates with tree survival and productivity.

METHODS

Site description

The research site (Fig. 1) for this study is a common garden consisting of 16 genotypes of *P. fremontii* planted in an experimental riparian forest located on the Lower Colorado River

within Cibola National Wildlife Refuge (CNWR) in Arizona, USA (latitude: 33.3117, longitude: -114.6891). *Populus fremontii* is a mid-lower elevation cottonwood species native to riparian zones from central Mexico to the southwestern United States, reaching northern California and Utah. Cottonwood trees are commonly used in habitat restoration projects as a fast-growing foundation plant species influencing ecosystem soil chemistry, understory plants, wildlife populations, insects, microorganisms, and ecosystem processes such as litter decomposition, nutrient cycling, and mineralization.

In winter of 2004, Fremont cottonwood (*P. fremontii*) cuttings were taken at 16 locations from natural populations within the species' geographic range in the Southwest to represent genotypes adapted to varying latitudes, elevations, temperatures, and annual precipitation. Northern Arizona University grew these cuttings out in a greenhouse for two years. The garden was established in fall 2006/winter 2007 on former agricultural (alfalfa) land: 6400 trees were planted in 400 16-tree stands at 4m spacing (Fischer et al. 2013). Blocks of 20 of the 16-tree stands were replicated 20 times to produce at least 321 replicates of each genotype in the garden to minimize site bias and randomize interactions between genotypes, location within tree stands, and location within blocks. The common garden is located in the arid desert region (69 feet elevation) of the southwestern United States and receives less than 7.87 cm of precipitation annually. By using a common garden experiment, we can eliminate environmental variations and study possible correlations between variables (e.g. tree genotype and mycorrhizal colonization of root systems).

Soil sample collection

We used a PVC soil corer (10cm x 15cm) and mallet to obtain 2 fine root samples from within 1 m of the trunk of each tree. Samples were taken in autumn (November 2014) from trees

installed 8 years prior to this study. We collected samples from at least 4 representatives of each genotype analyzed. Trees were previously flagged as healthy, good representatives of their genotype, with preference given to trees in monoculture stands to reduce influence of other genotypes. The canopy cover varies greatly within the common garden as different genotypes vary in their productivity. While 16 genotypes were planted initially, we were only able to collect samples from 14 of the genotypes. We sieved all samples to 2mm and discarded excess soil. We transported the samples on ice back to the lab before keeping them refrigerated prior to analysis.

Assessment of percentage ectomycorrhizal colonization of roots

We used a dissecting microscope to measure root length and mycorrhizal roots using the gridline intersect method (McGonigle et al. 1990) at x40 magnification to assess abundance of ectomycorrhizal colonization of roots. We scored for the presence of ECM fungal structures at a minimum of 100 line intersections per subsample of approximately 7 cm of root, following the methods of Karlinski et al (2009). We recorded the results as percentage of root length colonized (%RLC).

Statistical analysis

We performed a Bartlett's and Spearman's test to assess the data for satisfaction of parametric assumptions. We performed a one-way ANOVA, a resampling ANOVA of tree genotype and percentage ECM colonization of root tips, and a post hoc Tukey's HSD test. To test for a relationship between percentage ECM and percentage surviving trees and for a relationship between percentage ECM and tree productivity, we performed bivariate linear regressions. Statistical analysis was performed in program R.

RESULTS

Hypothesis 1: The amount of observed ectomycorrhizal colonization of root-tips measured in terms of percent colonization varies among different P. fremontii genotypes

The percentage of mycorrhizal colonization of roots varied among tree genotypes in the Cibola garden, supporting hypothesis 1. The genotype SP had the highest mean colonization, at 24%, with the lowest mean colonization recorded for PNWR at 0.95%. Genotype explained over 41% (P=0.0007) of the variation in abundance of ectomycorrhizal root colonization (Fig. 2). A Bartlett's and Spearman's test revealed that our data did not meet the parametric requirements for normality and variability within samples, which led us to perform a resampling ANOVA. This test revealed that after resampling there was still a significant difference in ECM colonization percentages among genotypes, with a p-value of 0.0017. A Tukey's HSD test further explained the drivers behind variation among genotype ECM percentages, grouping the genotype SP by itself in the category A, genotypes HNWR, OV, CNWR, and HP in the category AB and the remaining genotypes in B.

Hypothesis 2: Mycorrhizal colonization positively correlates with tree survival and productivity

Hypothesis 2 was not supported. Greater abundance of ectomycorrhizae (ECM) did not show a positive relationship with higher percentage of tree survival (Tab. 1), explaining only 1.2% of the variation. Percentage ECM root colonization also did not show a positive relationship with higher tree productivity (difference in trunk diameter at 30cm from data collected in 2012 and 2014) with only 2.1% of the variation explained by percentage ECM.

DISCUSSION

Our study implicates that tree genotype plays role in fungal colonization of roots even when host plants are removed from their native habitat and environmental conditions are homogenized in a common garden experiment. Our results show that variation in percentage of ectomycorrhizal (ECM) fungal colonization of tree roots was explained in good part (41%) by intraspecific genetic variation in *Populus fremontii*. However, there was no pattern found between percentage of ECM colonization and the variation in tree survival rates or tree productivity (diameter growth difference between 2012 and 2014). This could indicate that trees forming mycorrhizal relationships with fungi don't gain a significant advantage in terms of growth over those that do not.

Previous studies comparing overall ectomycorrhizal colonization percentages among interspecific genotypes have shown the influence of genetics on these factors to be largely dwarfed by the effects of environmental conditioning, noting genotype as a minor effect, particularly in response to environmental stress (Gehring et al 2006, Karlinski et al 2010). This contrasts with the results found here, which show a difference in ectomycorrhizal colonization of roots among genotypes of *P. fremonti* where environmental factors were kept at a constant across all genotypes. Our results could indicate that intraspecific genetics play a larger role in determining variation in ectomycorrhizal colonization than previously thought.

The arid climate of Arizona is similar to the weather patterns that many future models predict will become prevalent, therefore, knowledge of genetic linkages between mycorrhizae and *Populus* will be useful in determining which individuals are the most favorable to survive in a new world. A better understanding of plant-mycorrhizae interactions could prove extremely useful in the application of habitat restoration and wildlife conservation as well as agricultural

productivity and resilience. With atmospheric CO_2 levels predicted to double by 2020-2075, knowledge of genetics and mycorrhizal influence on carbon sequestration could provide insights into better land management practices.

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SUPPLEMENTAL MATERIAL

TABLES

Table 1. Surviving trees in the common garden by genotype. *B161 and CW were not included in our analyses.

FIGURES

Figure 1. Map showing Cibola National Wildlife Refuge, Arizona, USA where the common garden is located.

Figure 2. Percentage of ectomycorrhizal (ECM) fungal colonization of P. fremontii trees as driven by Genotype. One-way ANOVA with Tukey HSD.

Genotypes	# Surviving Trees in 2012	% Survival
B161*	50	12.5
BD	132	33
CNWR	110	27.5
CW*	141	35.25
FC	198	49.5
FR	67	16.75
GR	58	14.5
HNWR	189	47.25
HP	125	31.25
KT	5	1.25
MC	152	38
NCC	61	15.25
OR	110	27.5
OV	72	18
PNWR	108	27
SP	100	25

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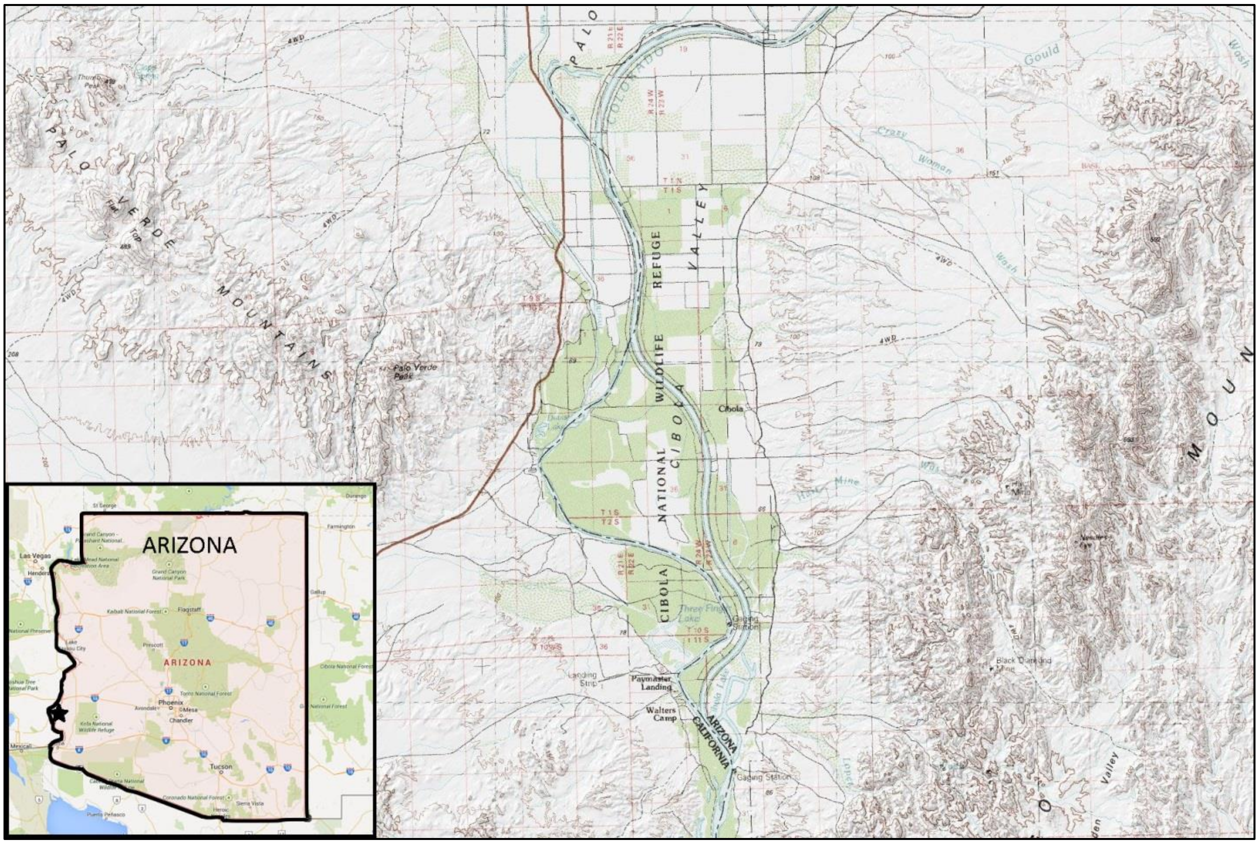
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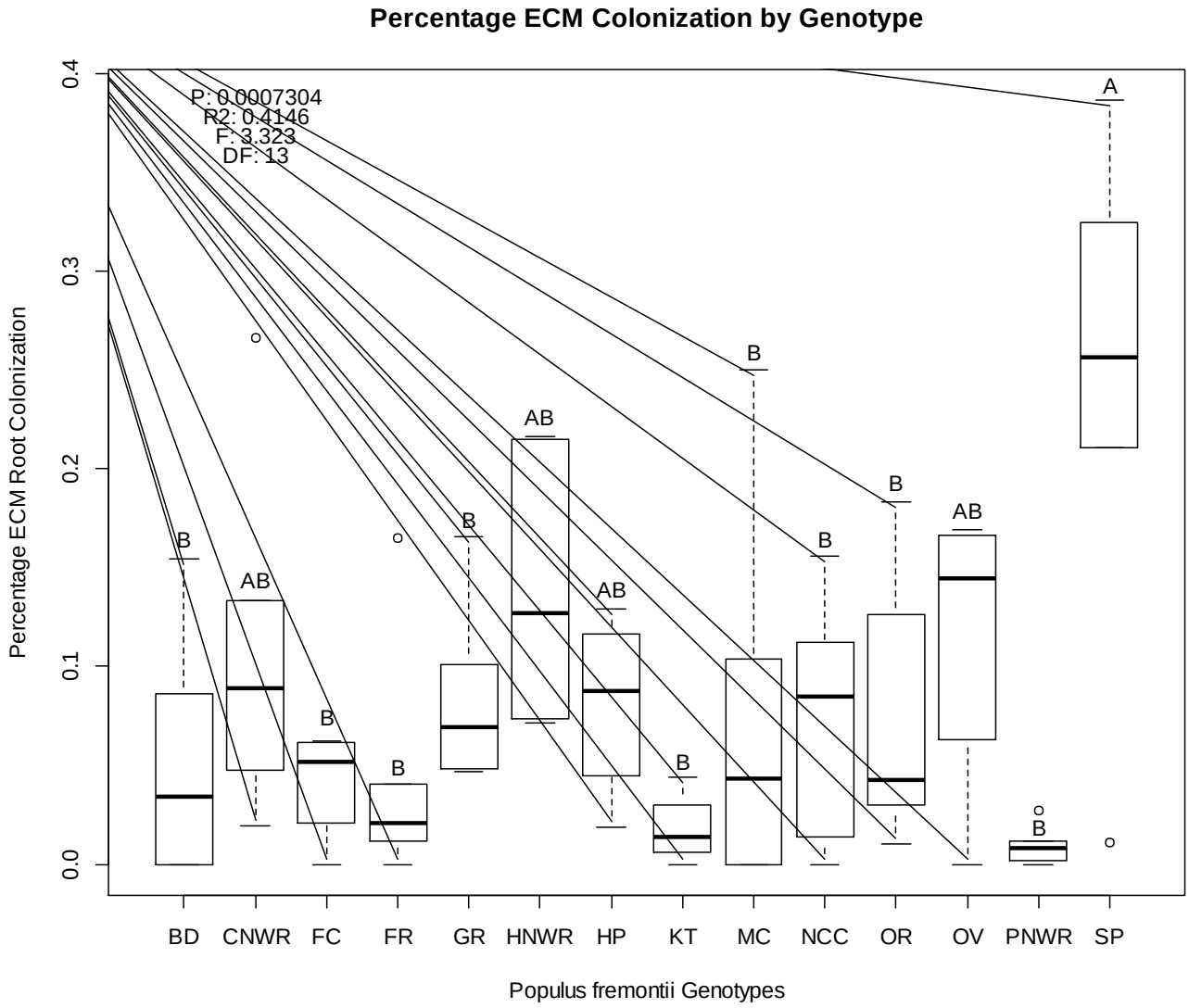

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