# Root-associated Microbes in Pinyon Pine: Implications for Management

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**ABSTRACT—**Colorado pinyon pine (*Pinus edulis*) forms mutualistic associations with root-associated ectomycorrhizal (EM) fungi which confer improved fitness under stressful conditions and thus may be critical to the success of this species in response to global climate change. We review studies of the EM fungal associations of *P. edulis* with an emphasis on the literature focused on drought and associated *P. edulis* mortality. We also describe the importance of other soil microbes including nonmycorrhizal fungi and bacteria, and present data describing dominant soil microbial taxa associated with *P. edulis*. Finally, we discuss the relevance of the patterns observed in these studies to the management of pinyon-juniper (*Pinus* spp.-*Juniperus* spp.) ecosystems. We conclude that *P. edulis* reestablishment or restoration following drought-induced mortality may be limited in some areas because of reduced availability of EM fungal inoculum. Genetically based differences in *P. edulis* drought tolerance are also associated with differences in EM fungal species composition. Given that the diversity of mycorrhizal fungi can be directly tied to host plant performance, loss of EM fungal diversity after differential *P. edulis* mortality may negatively affect recruitment in natural populations or in restoration efforts. Yet drought-tolerant *P. edulis* genotypes and their associated EM fungi may be critical to survival of both host and fungus during ongoing drought.

**KEYWORDS—**climate change, drought, ectomycorrhizal fungi, Pinus edulis, pinyon pine, rhizosphere

## INTRODUCTION

Plants and fungi frequently form intimate associations with one another that can be mutually beneficial, neutral, or parasitic. Among the most widespread of these associations are the mycorrhizas, mutualistic associations between soil fungi and the fine roots of plants in which plants exchange carbon fixed through photosynthesis for soil resources (Smith and Read 2008). In addition to promoting plant survival and growth, mycorrhizal fungi can influence larger scale processes such as plant species richness (Teste et al. 2017; van der Heijden et al. 1998), the abundance and species composition of soil organisms (Smith and Read 2008), nutrient cycling (Phillips et al. 2013), and soil stability (Wilson et al. 2009). There are several types of mycorrhizal associations (Johnson and Gehring 2007). The most widespread are the arbuscular mycorrhizas (AM), in which the fungi involved are limited in diversity (~300 species in a single phylum), and the plants are highly diverse (~85 percent of land plants) and widespread (Wang and Qiu 2006). Arbuscular mycorrhizas generally produce hyphae without septa. They also produce characteristic exchange structures (arbuscules) and storage structures (vesicles) that occupy the cortex of host roots and must be stained to be observed.

Ectomycorrhizal (EM) fungi form a diagnostic morphological structure within roots, the Hartig net, but

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also envelope root tips within a sheath or mantle of fungal hyphae. Ectomycorrhizas are also widespread because they dominate on plants that occur in many temperate and boreal forest ecosystems. In this case, fewer plant taxa are involved with thousands of species of fungi from two major phyla, the Basidiomycota and the Ascomycota (Tedersoo et al. 2008, 2011). Prominent EM host trees include pine (*Pinus* spp.) and spruce (*Picea* spp.) (Wang and Qiu 2006), which have broad distributions across the forest ecosystems of the northern hemisphere and are functionally obligately dependent on EM fungal associations (Molina et al. 1992). Importantly, members of only a few plant families are able to form associations with both AM and EM fungi; this specificity can reduce mycorrhiza formation on plants in some settings (Chen et al. 2000).

Numerous studies demonstrate the importance of mycorrhizal fungi to their host plants, but there also is significant evidence of functional variation among fungal species. For example, the species composition of EM fungi associated with plants varies with environmental variables such as precipitation (Karst et al. 2014), soil nitrogen (N) availability (Lilleskov et al. 2011), and disturbance such as fire (Hewitt et al. 2016; Kipfer et al. 2011). Similarly, species of EM fungi vary in their ability to access different forms of N (Smith and Read 2008) and to promote drought tolerance in their host plants (Peay et al. 2007). The AM fungi also vary in distribution and function (Kivlin et al. 2011); recent studies suggest that many AM fungal species are distributed across six continents (Davison et al. 2015). However, for both AM and EM fungi, it is important to understand not only whether inoculum is available at a site, but also whether that inoculum consists of suitable fungal species for a given environment. Associated fungal species composition can vary among both species (Molina et al. 1992) and genotypes of plant hosts (Lamit et al. 2016), and some fungal taxa demonstrate considerable host specificity (Molina et al. 1992), such as between members of the EM fungal family Suillaceae and members of the Pinaceae (Bruns et al. 2002).

Although many forested ecosystems contain plant species that form either AM or EM associations, pinyon-juniper (*Pinus* spp.-*Juniperus* spp.) woodlands are unusual in that the codominant trees that characterize the ecosystem form different types of mycorrhizal associations, AM for juniper and EM for pinyon. In addition, the grasses and shrubs that form most of the remainder of the vegetation in pinyon-juniper woodlands form AM associations (Haskins and Gehring 2005). This distribution means that pinyons are often the only host for EM fungi across a substantial portion of their geographic range (Gehring et al. 2016). Colorado pinyon pine (*Pinus edulis*) has experienced significant mortality across the southwestern United States, with models projecting further contraction of its range (Cole et al. 2008; Rehfeldt et al. 2006). In this paper, we discuss what is known about the mycorrhizal associations of *P. edulis*, with particular emphasis on applications for management given its sensitivity to drought and its recent drought-induced mortality across much of the Southwest (Breshears et al. 2005; Garrity et al. 2013; Mueller et al. 2005a). We also describe research on other microbes associated with *P. edulis* and their potential importance to pinyon ecology.

## PINYONS, EM FUNGI, AND PLANT NEIGHBORS

Given that across much of the range of pinyon-juniper woodlands, pinyons are often the sole host for EM fungi, available EM inoculum and successful pinyon reestablishment could be limited following drought-induced mortality. Gehring et al. (2016) estimated that *P. edulis* is the only host for EM fungi across nearly 60 percent of its geographic range. At sites where junipers dominate, pinyons show reduced overall abundance of EM fungi, and seedlings are less likely to have any EM fungal associations when grown in soil from juniper-dominated areas (Haskins and Gehring 2005). Pinyons experimentally isolated from neighboring junipers respond with a rapid doubling of fine-root biomass; EM fungi respond similarly, also doubling in abundance (Haskins and Gehring 2004). Allen et al. (2010) observed that the EM associations of *P. edulis* were more sensitive to N deposition than the AM associations of juniper. These results indicate that junipers and their associated soil microbial communities (including AM fungi) may influence the environment through competitive or antagonistic effects (or both) that render *P. edulis* less able to establish and survive.

A similar competitive release was seen when AM-associated shrubs, principally *Fallugia paradoxa*, were

removed from beneath mature pinyons in the field, resulting in increases in root biomass, needle length, and EM abundance (McHugh and Gehring 2006). Associations with this species of shrub help juvenile pinyons to survive and grow in stressful environments (Sthultz et al. 2007). At low-stress sites, this relationship becomes more competitive, with both aboveground and belowground factors contributing to the variation, though additional research is necessary to clarify mechanisms (Sthultz et al. 2007). The dynamics of these relationships are likely to change as climate continues to warm and dry, with increased likelihood that relationships between shrubs and pinyons will be beneficial when trees are young and detrimental as they age.

Research from other forested ecosystems also demonstrates the importance of EM plants to one another. A large-scale study of northern temperate forest trees showed that species that form EM associations are generally positively influenced by soils in which conspecifics had grown previously and negatively influenced by soil in which heterospecifics had grown previously. The formation of mycorrhizas in EM-dependent trees was more efficient when seedlings were grown in soil collected beneath a conspecific tree rather than beneath a heterospecific tree, and this difference was further reflected in the overall performance of the seedling as a function of biomass (Bennett et al. 2017). This result could be due to decreased or less-compatible EM fungal inoculum from heterospecific soil, or an increase in pathogen load, or both. Combined with previous studies of *P. edulis*, these results suggest that EM seedlings establishing in areas lacking conspecifics will perform poorly, potentially due to lack of EM inoculum or other complex differences in local soil communities. Inoculation with live soil or other sources of EM inoculum may remedy inoculum limitation.

An experiment conducted in *Pinus patula* demonstrated that soil collected from a *P. patula* plantation offered an effective source of EM fungal inoculum, as did mixed cultures of certain EM fungal species (Restrepo-Llano et al. 2014). Similarly, de Oliveira et al. (2006) isolated and cultured EM fungi from a loblolly pine (*P. taeda*) plantation which was then successfully used to inoculate greenhouse seedlings. It therefore may be possible to supplement soil with ectomycorrhizal inoculum where pine restoration is desired following high-mortality events. Because culturing techniques for most EM fungal taxa associated with *P. edulis* have yet to be developed, inoculation with soil from nearby areas with living pinyons is more likely to provide inoculum-limited pinyons with beneficial EM fungal taxa than inoculation with soil fungal cultures or commercially produced generalist EM fungi that may not be native to the area (Schwartz et al. 2006).

In cases where *P. edulis* is extirpated from an area due to drought or other disturbance, reestablishment may occur with EM fungal inoculum that persists in the soil. However, reestablishment requires that EM fungal communities persist in the absence of a host plant. Little is known about how long existing EM fungal inoculum remains viable or which taxa persist after disturbance events. Long-term experimental data on the ability of dormant EM fungal spores to colonize bishop pine (*P. muricata*) yield mixed results. Several *Rhizopogon* species (Basidiomycota) show an improved potential to colonize roots over time (Bruns et al. 2009), whereas other EM fungal taxa were undetectable after several years (Nguyen et al. 2012).

Though these data indicate that at least some EM fungal propagules will persist and facilitate growth of pines over time, they also imply an eventual loss of EM fungal diversity in the absence of the EM host. This may help to explain the result of Haskins and Gehring (2005), who observed decreased colonization by EM fungi among pinyon seedlings grown in soil collected from juniper-dominated stands. This observation implies a density-dependent relationship as stands previously shared by pinyons and junipers shift toward a juniper-only woodland following drought-induced pinyon mortality events. Further, the effect of persistent juniper dominance may result in an altered soil microbial community that specifically favors the growth of junipers over pinyons as soil communities become locally adapted to the juniper-dominated regime (Revillini et al. 2016).

Besides examining roots directly to assess mycorrhizal colonization and EM fungal species composition, another approach to describing EM fungal communities (or other microbes) found in pinyon-juniper woodlands is the use of next-generation DNA sequencing

technologies. These techniques can provide data on the relative abundance of all fungi or bacteria present in a soil sample (e.g., Caporaso et al. [2012]). In a preliminary survey, we used this technology at Sunset Crater National Monument (SCNM) in northern Arizona, which revealed differences in EM and AM fungal composition between root-associated soil collected beneath live, adult *P. edulis* (n = 31), and interspace zones devoid of vegetation for a diameter of 6 m (n = 26, collected in four transects within the population of adult trees). Taxa were identified from polymerase chain reaction amplicon sequencing of the ITS2 rDNA gene for each sample using fungal-specific primers (Taylor et al. 2016) on an Illumina® MiSeq Desktop Sequencer (Illumina Inc., San Diego, California). Paired t-tests in which the data were matched by sample were used for comparing relative abundances of AM and EM taxa within each group (interspace or adult trees). These tests revealed no difference between AM and EM abundances in the interspace soil ($t = 1.4488$, $p = 0.1536$), and a strong difference between AM and EM abundances in soil from adult trees ($t = 14.4659$, $p < 0.0001$).

Unpaired t-tests were used to compare AM or EM taxa abundances separately between each group, revealing that differences exist for both AM ($t = 4.3331$, $p < 0.0001$) and EM ($t = 3.8931$, $P = 0.0003$) fungal relative abundances. Pinyon-associated soil was rich with EM fungal symbionts at this site (12.1 percent relative abundance), consisting mostly of the genera *Geopora* and *Rhizopogon*, while there was a marked absence of AM fungi (0.8 percent relative abundance) (fig. 1). These EM taxa have been observed consistently at this site in conjunction with pinyon roots (Gehring et al. 1998, 2014b; Gordon and Gehring 2011; Sthultz et al. 2009b). EM taxa were also abundant in the interspace soils (8.1 percent relative

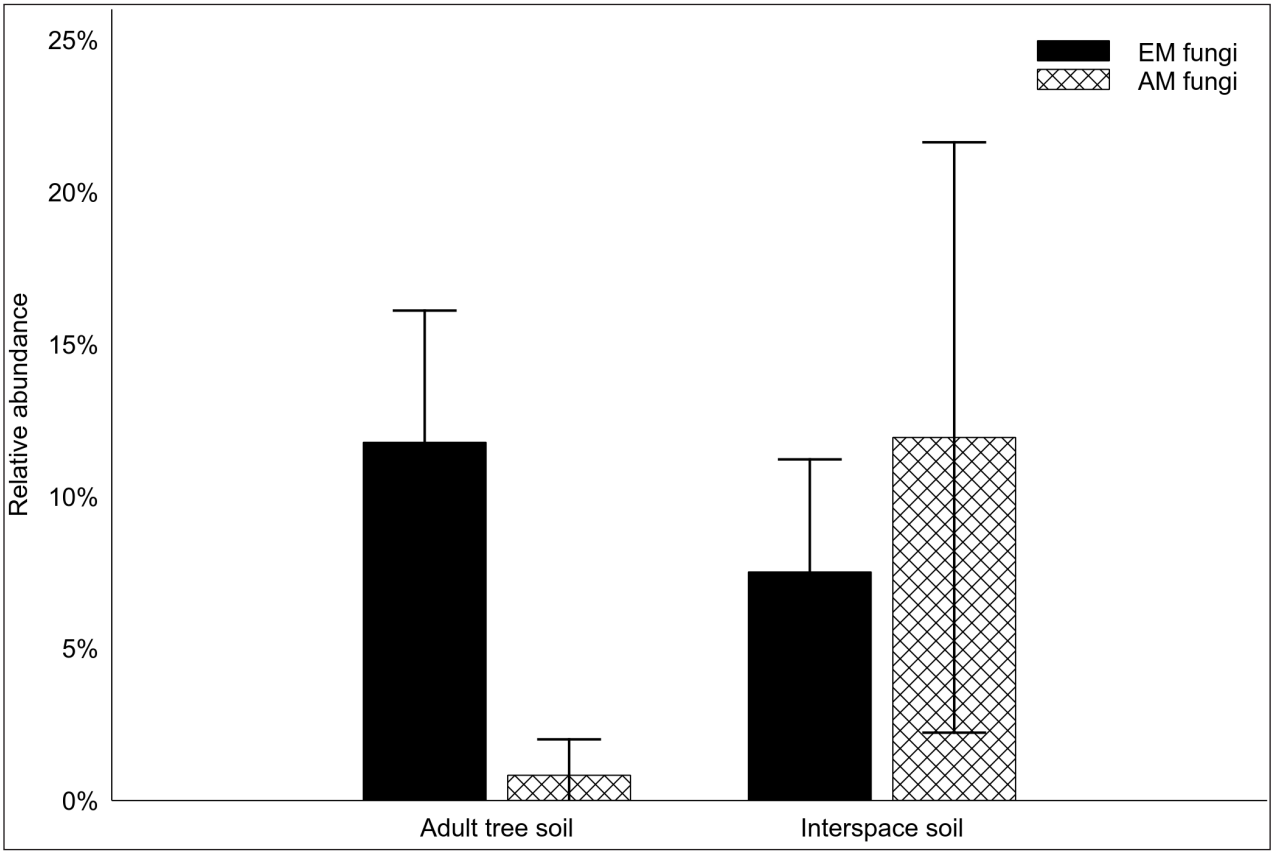


**Figure 1**—Average relative abundances of total ectomyccorhizal (EM) or arbuscular myccorhizal (AM) fungal taxa observed by DNA sequencing of root-associated soil associated with adult *Pinus edulis* (n = 31) or from vegetation-free interspaces (n = 26) at Sunset Crater National Monument, Arizona. Error bars represent +/- one standard deviation. *Pinus edulis* hosted a significantly larger fraction of EM than AM fungi ($p < 0.0001$), in contrast with interspaces ($p = 0.1536$). *Pinus edulis* hosted a larger fraction of EM fungi than observed in interspaces ($p = 0.0003$) while the opposite was true for AM fungi ($p < 0.0001$).

abundance), locations where it would not have been possible to observe them in the absence of pinyon roots without the use of this DNA sequencing technique. We note the presence of both AM (12.4 percent relative abundance) and EM fungal taxa in the interspace soils, which implies that mycorrhizal symbionts are readily available for establishing seedlings of AM- and EM-associated plant species in this ecosystem. Though these data represent just a single time point for a single site, they serve as a useful illustration of the challenges that EM hosts may face during seedling recruitment in heterospecific soils.

Across approximately 40 percent of its geographic range, *P. edulis* occurs with other hosts for EM fungi, such as ponderosa pine (*P. ponderosa*) and a variety of species of oaks (*Quercus* spp.) (Gehring et al. 2016). *Pinus edulis* and *P. ponderosa* habitats overlap near the upper elevational limit of *P. edulis*. When the rhizospheres of *P. edulis* and *P. ponderosa* overlapped, they shared 88 percent of the fungal taxa observed (Hubert and Gehring 2008) (fig. 2). The EM taxa shared between *P. edulis* and *P. ponderosa* were dominated by what were then defined as unknown Pezizalean (Ascomycete) fungi (Hubert and Gehring 2008), and are probably what we now recognize as a group of EM fungi in the genus *Geopora* of particular significance to arid *P. edulis* ecosystems (Flores-Renteria et al. 2014; Gordon and Gehring 2011). Such zones of host-species overlap may provide important refugia for EM fungal taxa important to *P. edulis*.

Less is known about overlap with other EM hosts such as oaks. To our knowledge, the EM community composition of *P. edulis* and *Quercus* species has not been examined in areas where their distributions overlap. However, data from other systems demonstrate the importance of host-overlap zones for maintenance of EM taxonomic diversity. For example, Horton et al. (1999) observed that in mixed stands of Douglas-fir (*Pseudotsuga menziesii*) and manzanita (*Arctostaphylos* spp.), most trees were colonized by

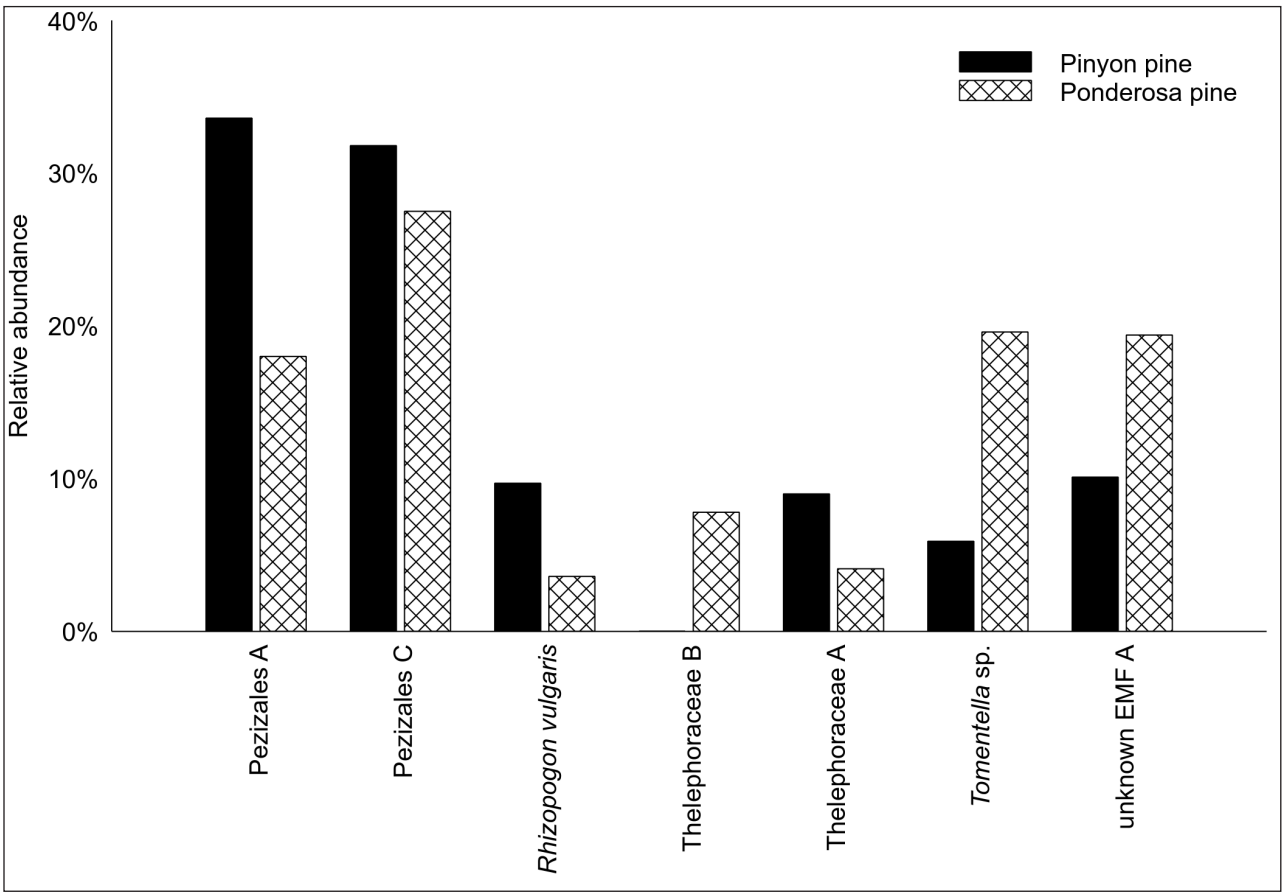


**Figure 2**—Average relative abundance of ectomyccorhizal fungal taxa observed on roots of *P. edulis* and *P. ponderosa* when the rooting zones of the two species overlapped. The two pine species shared six of seven EM fungal taxa with similar relative abundance (redrawn from Hubert and Gehring [2008]).

EM fungal taxa which regularly colonize the roots of either host, illustrating an ecological linkage between gymnosperm and angiosperm populations. Likewise, Cullings et al. (2000) examined a mixed forest of Engelmann spruce (*Picea engelmannii*) and lodgepole pine (*P. contorta*), and discovered that 95 percent of the observed EM fungi colonized both hosts. These results demonstrate little evidence for host specificity in their respective systems and imply that the presence of one host tree species may have benefits for maintaining EM fungal diversity that could benefit other tree species dependent on EM fungi.

## HOST GENETICS, DROUGHT TOLERANCE, AND EM FUNGI

*Pinus edulis* is especially vulnerable to drought stress relative to other regional forest tree species, and experiences high rates of mortality under prolonged periods of drought (Gitlin et al. 2006). This vulnerability can be magnified by the effects of climate change, which are predicted to include a continued increase in mean average temperature for the present range of *P. edulis* (Adams et al. 2009) and a greater likelihood of more frequent and severe drought events across the southwestern United States (Seager et al. 2007). Indeed, projections of future plant distribution based on climate modeling data suggest the current range of *P. edulis* may no longer be suitable habitat for these trees by the end of this century (Rehfeldt et al. 2006). An extreme drought event from 2002 to 2003 resulted in high rates of pinyon mortality across the range of the species (Breshears et al. 2005). Later observations revealed that survival or death of P. edulis during this event was correlated with lower precipitation and vapor pressure levels (Clifford et al. 2013).

*Pinus edulis* mortality can be highly variable among stands; dead and surviving trees may occur side by side, suggesting individual variation in drought tolerance (Mueller et al. 2005a; Ogle et al. 2000). Sthultz et al. (2009a) showed that at several sites near SCNM, genetic differences across the population correlated with survival or demise during the 2002–2003 drought, indicative of a genetic basis for drought tolerance or intolerance. At these sites, drought-intolerant trees, initially present as a high proportion of the population, were reduced to a similar proportion to that of drought-tolerant trees, even as the overall population density contracted (Sthultz et al. 2009a).

The genetic basis of drought tolerance and intolerance in these trees was distinguishable because of variation in susceptibility to a stem-boring moth whose chronic herbivory on mature trees alters their architecture. Susceptible trees chronically attacked by the moth have a prostrate, shrub-like architecture in contrast to the normal upright architecture of moth-resistant trees (Whitham and Mopper 1985). Subsequent studies demonstrated a genetic basis to moth resistance or susceptibility (Mopper et al. 1991). Sthultz et al. (2009a) observed that moth-resistant trees were approximately three times more likely than moth-susceptible trees to perish under drought stress, indicating that this phenotypic differentiation is indicative of drought tolerance in the moth-susceptible group. Seedlings of moth-susceptible trees also survived longer under drought conditions experimentally imposed in a greenhouse, demonstrating the genetic basis of drought tolerance (Sthultz et al. 2009a). Such phenotypically dimorphic populations present important opportunities for study as not every population will provide such a simple diagnostic tool for examining genetic effects on ecological interactions, and resources for studying the genetic composition of pinyon pine populations remain limited (Krohn et al. 2013; Lesser et al. 2012).

Sthultz et al. (2009b) observed that the differential phenotypes of the pinyon population previously described associate with specific communities of EM fungi. Specifically, the drought-tolerant group hosted more Pezizalean EM fungi, including the genus *Geopora* (Ascomycota), and this pattern held whether data were from a relatively wet or dry year. Gehring et al. (2014a) explored the EM fungal communities of drought-tolerant and -intolerant trees over time and observed that drought-tolerant trees hosted a consistent community as the regional climate became more arid, whereas the drought-intolerant trees exhibited a significant shift in EM community during the same period. In removing shrubs from beneath mature *P. edulis*, a competitive release was observed in which the focal pinyon exhibited increased growth (Gehring et al. 2014a). This competitive release was more significant in the drought-tolerant trees, which maintained a consistent EM fungal community, than in the drought-intolerant trees, whose EM fungal community

also shifted following shrub removal. The improved performance of the drought-tolerant trees after shrub removal is most likely due to added benefits provided from the associated EM community, implying that the EM community commonly observed among drought-tolerant trees is composed of mutualists superior to those observed among drought-intolerant trees (Gehring et al. 2014b, 2017). These superior mutualists may confer upon drought-tolerant trees the opportunity to perform more consistently under variable climatic conditions, though members of the genus *Geopora* have increased in abundance with drought both within and among sites (Gehring et al. 2014b; Gordon and Gehring 2011), possibly benefiting drought-intolerant trees as well.

Given the evolutionary patterns of *P. edulis* mortality following recent drought, in which climatic pressure selected against drought-intolerant genotypes (Sthultz et al. 2009a), it is possible that drought-intolerant genotypes will be extirpated during subsequent drought events. A potential consequence of this differential mortality is the loss of EM fungal diversity across local or regional scales due to the differences in EM fungal communities among the different genotypes. It is unclear how the loss of *P. edulis* genetic diversity and EM fungal biodiversity may affect recruitment of seedlings in natural settings or in restoration efforts.

The idea that it may be important to maintain intraspecific diversity of either the host plant or the fungal symbiont is not a new one (Johnson et al. 2012), and may be particularly relevant to the *P. edulis* system. While exploring the effect of AM fungal diversity on plant communities, van der Heijden et al. (1998) observed that increased AM fungal species richness yielded greater hyphal mass in the soil, increased plant biomass, and increased plant species richness. These effects corresponded with a depletion of available phosphorus in the soil, implying that the increase in AM fungal hyphae provided an improved mechanism for foraging nutrients from the environment (van der Heijden et al. 1998). More recently, Teste et al. (2017) demonstrated the importance of diverse soil biota to maintaining taxonomic and functional diversity in Australian shrublands. While experimental data showed that different resource acquisition strategies by plants (e.g., EM-dependent or AM-dependent) resulted in variability in plant response to soil microbial content, simulations indicated that the presence of these different strategies combined with their associated soil microbial communities contributed strongly to maintenance of plant species and functional diversity across the environment (Teste et al. 2017). Although comparable data for pinyon-juniper woodlands are not available, these studies speak to the importance of maintaining soil microbial diversity in order to benefit plant communities. Because *P. edulis* is considered a foundation species, negative environmental effects on this species may exert a disproportionate effect on many dependent organisms (Whitham et al. 2003).

## OTHER ROOT AND SOIL MICROBES

Many organisms besides mycorrhizal fungi inhabit the soil among plant roots, but our overall understanding of their species composition and interactions remains limited (Ingleby and Molina 1991). In addition to pine-associated EM fungal communities, non-EM fungi and bacteria are present and participate in ecological processes in the soil. Bacteria are most abundant by cell count, regardless of host type (Neal et al. 1964; Rouatt and Katznelson 1961), and represent the bulk of soil genetic diversity, which may contain thousands of genomes per gram of soil (Torsvik and Øvreås 2002). The contribution of soil bacteria to host tree performance was first observed by comparing the growth of *P. radiata* in fumigated and nonfumigated soils, though it was unclear at the time which microbial group was mediating this effect (Ridge and Theodorou 1972). Later, it was recognized that various bacteria may either benefit the host plant directly as plant growth-promoting rhizobacteria (PGPR) (Kloepper and Schroth 1978) or indirectly by promoting the interaction between the host plant and potential mycorrhizal associates as mycorrhizal helper bacteria (MHB) (Garbaye 1994). Archaea are less well studied than bacteria due to their relative scarcity and a lack of available tools for examining these organisms. However, recent work indicates that although they represent just a small percentage of soil microbial communities, they may exert a powerful influence on nutrient-cycling processes such as ammonia oxidation (Leininger et al. 2006).

Nonmycorrhizal fungi represent a class of potent decomposers which release the bulk of present but unavailable nutrients into the ecosystem (Schlesinger and Bernhardt 2013). Much of the matter consumed

during decomposition consists of various plant tissues, so these taxa rely on the plant community even as they make nutrients available to that community. The apparent relationship between plants and microbial taxa gets even more complicated when we consider that, despite the well-known symbiotic mutualisms such as mycorrhizal associations in such communities, microbes and plants effectively compete for available nutrients (Harte and Kinzig 1993), implying a relationship that is simultaneously competitive and mutualistic. It is likely that the relationships among plant and microbial community members, combined with the abiotic characteristics of the surrounding soil, are the main influences that define the community structure in plant ecosystems.

Besides EM fungi, heterotrophic fungi and bacteria have been examined in pinyon-associated soils (Kuske et al. 2003). Patterns were observed with differences in host genetics, tree age, and soil type for drought-tolerant and -intolerant *P. edulis*. Tree age consistently affected bacterial content with older (ca. 150 years old) trees hosting about three times that of younger (ca. 60 years old) trees, whereas fungi do not correlate with age (Kuske et al. 2003). These results demonstrate that bacterial communities may become denser as the trees age, which could improve survival beyond a certain age if those communities possess adequate levels of PGPR or MHB taxa. Conversely, because some soil bacteria may be pathogens (Revillini et al. 2016), the host may instead require more consistent associations with EM fungi to better survive increased antagonistic pressure through the protective effects of EM (Laliberté et al. 2015).

DNA sequencing data that we have collected for the microbial content of pinyon-associated soils describe the nonmycorrhizal fungi (based on ITS2 rDNA gene sequencing) (fig. 3) and bacteria (based on 16S v4 rDNA gene sequencing) (fig. 4) in finer detail than previously possible. Importantly, the largest observed

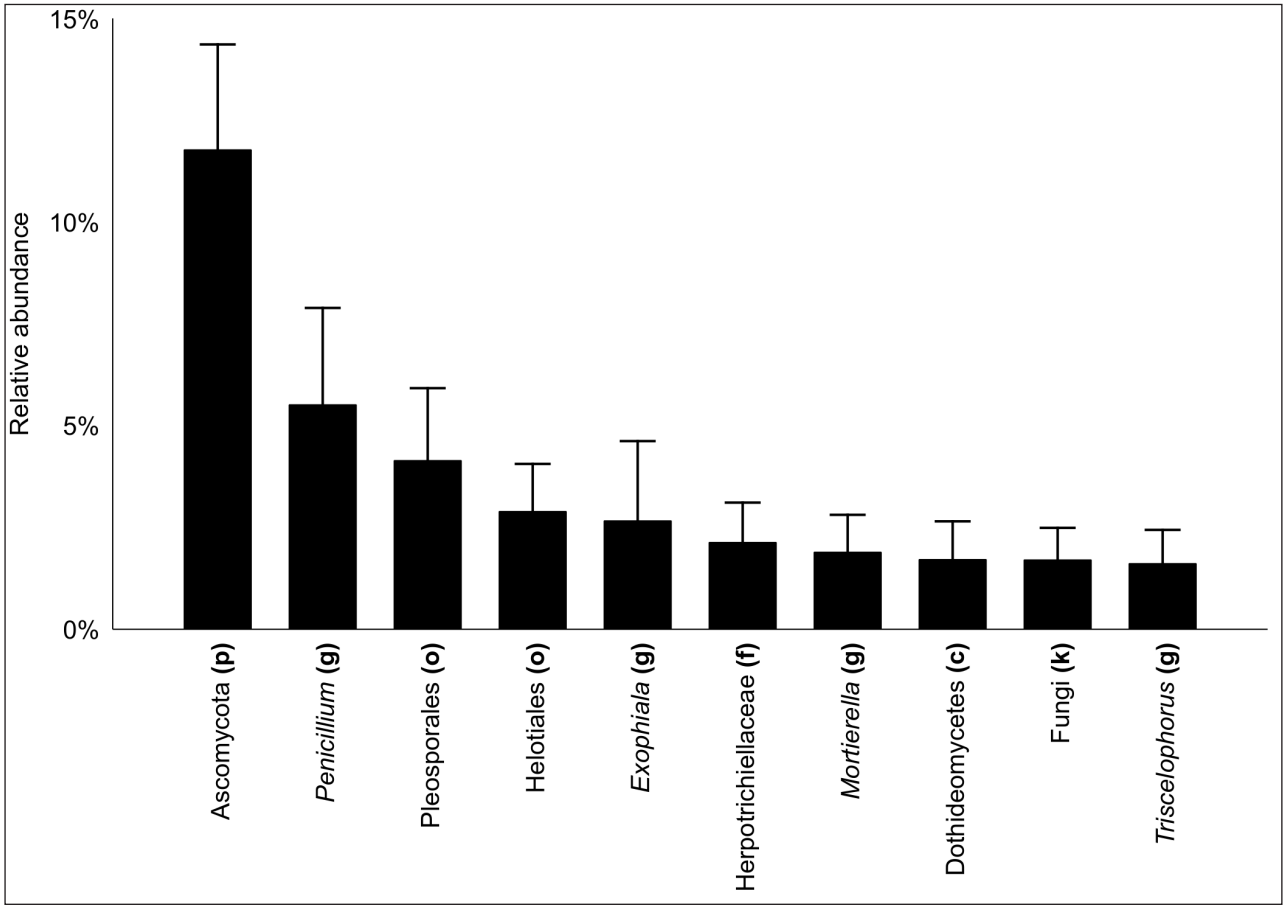


**Figure 3**—Average relative abundance of nonmycorrhizal fungal taxa observed by DNA sequencing of soil from roots of *P. edulis*. Data are summarized to the deepest confident taxonomic designation for each observation. Hierarchical taxonomic depth for each classification is indicated in bold parentheses (k = kingdom, p = phylum, c = class, o = order, f = family, g = genus). Error bars represent +/- one standard deviation.

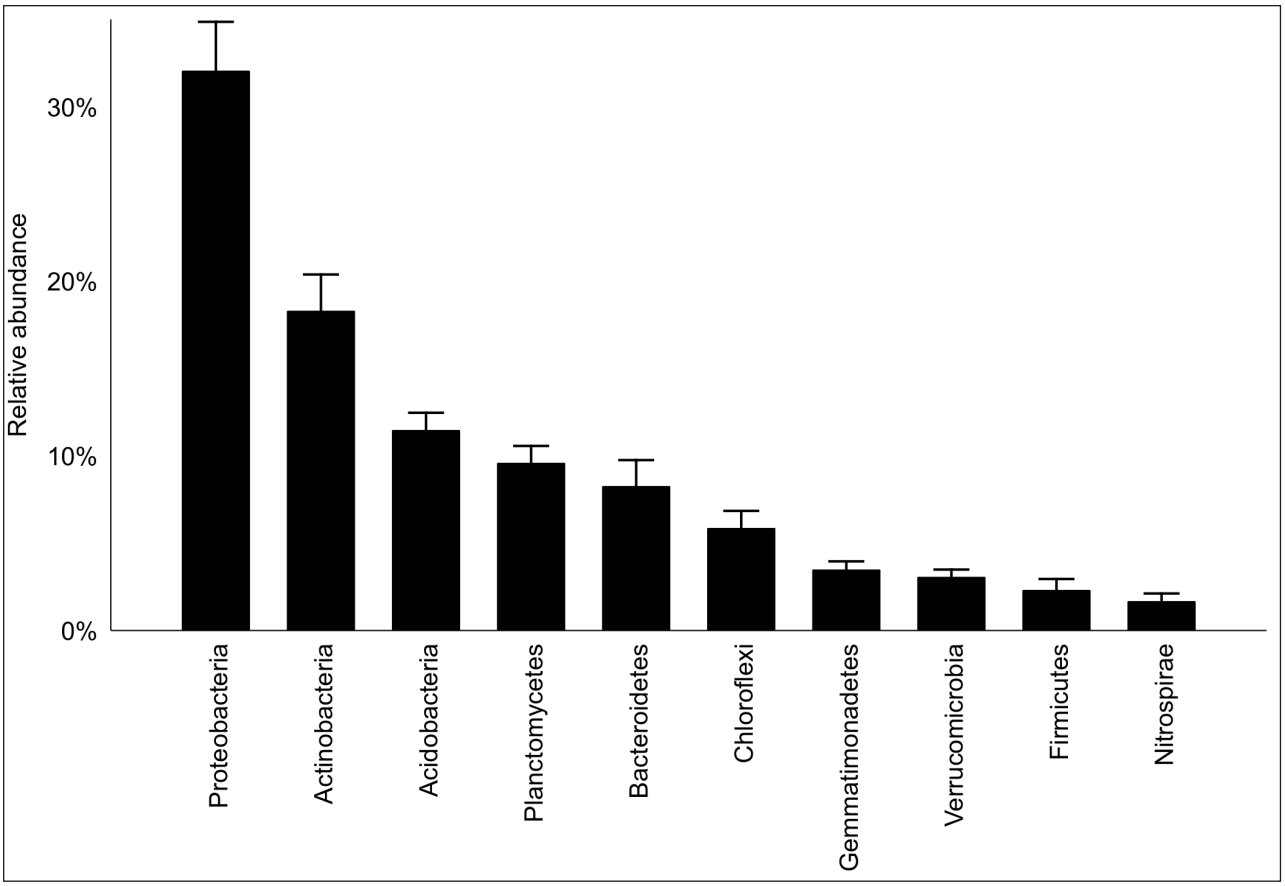


**Figure 4**—Average relative abundance of bacterial taxa observed by DNA sequencing of soil from roots of *P. edulis*. Data are summarized to the phylum level for all observations. Error bars represent +/- one standard deviation.

component of the nonmycorrhizal fungal community includes undescribed taxa in the phylum Ascomycota, highlighting the need for continued basic research describing the biodiversity of fungi in the environment. High proportions of fungi from the genus *Penicillium* indicate ample presence of saprotrophs (Kirk et al. 2008), as well as potential pathogen pressure as various species of Penicillium may also cause disease in plants (Ballester et al. 2015; Louw and Korsten 2013). Intriguing is the considerable proportion of fungi from the order Pleosporales, taxa that generally belong to the less well understood class of plant-symbiotic fungi known as dark septate endophytes (DSE) (Jumpponen and Trappe 1998). These fungi may turn out to be important players, as a meta-analysis revealed that associations by a variety of plant species with DSE generally confer an increased growth response in a host plant (Kivlin et al. 2013). Identification of soil bacteria using short DNA sequences generally fails to provide taxonomic information deeper than family, and we present only phylum-level classifications here (fig. 4). In terms of the dominant phyla, these data are generally consistent with previous sequencing-based analyses from other forest soil bacteria (Roesch et al. 2007).

Numerous studies have implicated specific nonmycorrhizal taxa as either direct or indirect facilitators, including in various Pinus systems (e.g., Kataoka et al. [2009]). While studying the grass *Brassica rapa*, Lau and Lennon (2011) observed that more complex soil microbial communities facilitated an increase in plant biomass, leaf number, and flower count, due in part to the bacterial community composition. They later found that soil microbial communities adapted to environmental conditions, offering an alternative adaptive strategy to the host plant, which can utilize the benefits of a well-adapted soil microbial community as a proxy for evolutionary adaptation in order to better survive under stressful conditions (Lau and Lennon 2012). Such effects emphasize that beneficial soil microbes include those besides mycorrhizal fungi, and that these microbes can also have a profound impact on their plant hosts.

## MANAGEMENT IMPLICATIONS

At sites where *P. edulis* has been lost due to drought and EM fungal inoculum may be limited, provision of EM fungal inoculum during restoration may be necessary. Although there are few data on the direct effect of fire on pinyon EM fungi, a recent review showed that fire and drought have similar consequences for EM fungal populations and communities, particularly when they represent a loss of pines from the landscape (Karst et al. 2014). Because pines may exhibit specificity for certain EM taxa and vice versa (Bruns et al. 2002), commercially available EM fungal inoculum may be ineffective. A better option may be to grow plants in soil inoculated with live soil from surviving pinyons. Data on variation in drought tolerance among *P. edulis* genotypes (Sthultz et al. 2009a) indicate that it may also be important to manage pinyon ecosystems to maximize diversity of individual trees, which would be expected to translate into improved genetic diversity. In turn, greater genetic diversity of the *P. edulis* host is likely to contribute to maintenance of a more complete resource of belowground EM fungal propagules which will facilitate recruitment of new seedlings in the future as well as improve survival during restoration or conservation projects.

A feasible strategy for restoration of *P. edulis* in drought-stressed areas could make use of drought-tolerant genotypes, at least initially. These trees would better survive additional drought stress (Sthultz et al. 2009a), and may help to establish an adequate reservoir of EM fungal inoculum to eventually expand the genetic diversity of the host in order to further improve the resident EM inoculum potential. However, this strategy may have additional challenges. For instance, the drought-tolerant genotypes at SCNM often produce significantly fewer female cones (Mueller et al. 2005b), implying that collection of seeds from the desired genotypes could be more difficult. However, annual application of the systemic insecticide Cygon (Drexel Chemical Co., Memphis, Tennessee) is sufficient to control insect herbivory such that insect damage on drought-tolerant trees becomes equivalent to that observed on drought-intolerant trees, and cone production is similarly restored after just 4 years of treatment (Mueller et al. 2005b). Not all drought-tolerant genotypes will share the same ecological pressures as those observed at SCNM that result in easily identifiable drought-tolerant genotypes. Adequate study of individual populations at different sites will be required to identify trees that possess drought-tolerant traits, and additional tools will need to be developed to better understand the genetics behind such traits.

When managing land for wildlife, land management agencies are often concerned with maintaining healthy plant populations that serve as food and shelter for game and nongame animals. The studies and data presented here suggest that soil micro-organisms contribute in important ways to plant and soil health. Of particular importance are the mycorrhizal fungal communities on which plant communities and their dependent organisms rely for efficient nutrient extraction from the soil (Smith and Read 2008) and for efficient deposition and storage of belowground carbon (Treseder and Holden 2013). For a recent, comprehensive review of how management practices relate to the management of mycorrhizal fungi, see Tomao et al. (2017). It is important to recognize that this review, which focuses on silvicultural practice, relates more directly to forest stands of limited composition and relatively uniform age. The authors specifically highlight the need for more research into the growth of mushrooms (which generally constitute available EM fungi in forest ecosystems) under forest conditions more typical of pinyon-juniper woodlands.

Another review by Karst et al. (2014) describes findings directly relevant to EM fungi and pines in general with respect to fire, drought, and herbivory, but again concludes by highlighting the many gaps in our understanding of the dynamics of EM fungal communities and their hosts. Based on the data available, we suggest that land managers regard effects of stand-reducing events (e.g., fire, drought, insect infestation) in pinyon pine and EM fungi similarly. Such reductions in host populations alter EM abundance, species richness, or community composition, thus decreasing the resiliency of the landscape to recover, at least temporarily. However, though fire will have an indiscriminate effect across a population, drought or insect infestation is likely to target certain host plant genotypes with greater consequences than others, reducing available genetic diversity of forest trees, and in turn, altering the composition of available EM fungi across an affected area. Including soil micro-organisms, such as mycorrhizal fungi that link aboveground and belowground portions of ecosystems, in future research and management of pinyon-juniper woodlands could improve outcomes for host plants, soils, and associated wildlife. Given these

challenges, we implore forest management agencies to direct available funding specifically to exploration of EM fungal communities in both healthy and affected P. *edulis* ecosystems.

## CONCLUSIONS

Associations between plants and microbes are increasingly recognized as key to plant performance and ecosystem function. The studies we have described here demonstrate the importance and complexity of microbial associations in pinyon-juniper woodlands, with an emphasis on mycorrhizal fungi. The mycorrhizal associations of pinyon-juniper woodlands are unique in ways that may affect their management, including the potential for EM fungal inoculum limitation following *P. edulis* mortality and the tight association between pinyon genotype and a genus of poorly described EM fungi. As studies of microbial communities remain limited spatially and temporally, much remains to be learned, though several recent studies have begun to explore the geographic distribution of mycorrhizal fungi on broad scales (Davison et al. 2015; Kivlin et al. 2011; Opik et al. 2010; Swaty et al. 2016; Tedersoo et al. 2011). Newer, culture-independent DNA sequencing methods are making characterization of microbial communities more rigorous and less costly (e.g., Caporaso et al. [2012]). A host of emerging methods are also elucidating the complexities of microbial function. These techniques may improve our understanding of the drivers of pinyon-juniper ecosystem function and better inform management of these ecosystems in an era of rapid environmental change.

## REFERENCES


Adams, H. D.; Guardiola-Claramonte, M.; Barron-Gafford, G.A.; Villegas, J.C.; Breshears, D.D.; Zou, C.B.; Troch, P.A.; Huxman, T.E. 2009. Temperature sensitivity of drought-induced tree mortality portends increased regional die-off under global-change-type drought. Proceedings of the National Academy of Sciences of the United States of America. 106: 7063–7066.

Allen, M. F.; Allen, E.B.; Lansing, J.L.; Pregitzer, K.S.; Hendrick, R.L.; Ruess, R.W.; Collins, S.L. 2010. Responses to chronic N fertilization of ectomycorrhizal pinon but not arbuscular mycorrhizal juniper in a pinon-juniper woodland. Journal of Arid Environments. 74: 1170–1176.

Ballester, A.-R.; Marcet-Houben, M.; Levin, E.; Sela, N.; Selma-Lázaro, C.; Carmona, L.; Wisniewski, M.; Droby, S.; González-Candelas, L.; Gabaldón, T. 2015. Genome, transcriptome, and functional analyses of *Penicillium expansum* provide new insights into secondary metabolism and pathogenicity. Molecular Plant-Microbe Interactions. 28: 232–248.

Bennett, J. A.; Maherali, H.; Reinhart, K.O.; Lekberg, Y.; Hart, M.M.; Klironomos, J. 2017. Plant-soil feedbacks and mycorrhizal type influence temperate forest population dynamics. Science. 355: 181 LP-184.

Breshears, D. D.; Cobb, N.S.; Rich, P.M.; Price, K.P.; Allen, C.D.; Balice, R.G.; Romme, W.H.; Kastens, J.H.; Floyd, M.L.; Belnap, J.; Anderson, J.J.; Myers, O.B.; Meyer, C.W. 2005. Regional vegetation die-off in response to global-change-type drought. Proceedings of the National Academy of Sciences of the United States of America. 102:15144–15148.

Bruns, T. D.; Bidartondo, M.I.; Taylor, D.L. 2002. Host specificity in ectomycorrhizal communities: What do the exceptions tell us? Integrative and Comparative Biology. 42: 352–359.

Bruns, T. D.; Peay, K.G.; Boynton, P.J.; Grubisha, L.C.; Hynson, N.A.; Nguyen, N.H.; Rosenstock, N.P. 2009. Inoculum potential of *Rhizopogon* spores increases with time over the first 4 yr of a 99-yr spore burial experiment. New Phytologist. 181: 463–470.

Caporaso, J. G.; Lauber, C.L.; Walters, W.A.; Berg-Lyons, D.; Huntley, J.; Fierer, N.; Owens, S.M.; Betley, J.; Fraser, L.; Bauer, M.; Gormley, N.; Gilbert, J.A.; Smith, G.; Knight, R. 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. The ISME Journal. 6: 1621–1624.

Chen, Y. L.; Brundrett, M.C.; Dell, B. 2000. Effects of ectomycorrhizas and vesicular-arbuscular mycorrhizas, alone or in competition, on root colonization and growth of *Eucalyptus globulus* and *E. urophylla*. New Phytologist. 146: 545–556.

Clifford, M. J.; Royer, P.D.; Cobb, N.S.; Breshears, D.D.; Ford, P.L. 2013. Precipitation thresholds and drought-induced tree die-off: Insights from patterns of *Pinus edulis* mortality along an environmental stress gradient. New Phytologist. 200: 413–421.

Cole, K. L.; Ironside, K.E.; Arundel, S.T.; Duffy, P.; Shaw, J. 2008. Modeling future plant distributions on the Colorado Plateau: An example using *Pinus edulis*. The Colorado Plateau III: Integrating research and resources management for effective conservation. 12: 319–330.

Cullings, K. W.; Vogler, D.R.; Parker, V.T.; Finley, S.K. 2000. Ectomycorrhizal specificity patterns in a mixed *Pinus contorta* and *Picea engelmannii* forest in Yellowstone National Park. Applied and Environmental Microbiology. 66: 4988–4991.

Davison, J.; Moora, M.; Opik, M.; Adholeya, A.; Ainsaar, L.; Ba, A.; Burla, S.; Diedhiou, A.G.; Hiiesalu, I.; Jairus, T.; Johnson, N.C.; Kane, A.; Koorem, K.; Kochar, M.; Ndiaye, C.; Partel, M.; Reier, U.; Saks, U.; Singh, R.; Vasar, M.; Zobel, M. 2015. Global assessment of arbuscular mycorrhiza fungus diversity reveals very low endemism. Science. 349: 970–973.

de Oliveira, L. P.; Rossi, M.J.; Júnior, A.F.; Filho, G.N.S.; de Oliveira, V.L. 2006. Viability and infectivity of an ectomycorrhizal inoculum produced in an airlift bioreactor and immobilized in calcium alginate. Brazilian Journal of Microbiology. 37: 251–255.

Flores-Renteria, L.; Lau, M.K.; Lamit, L.J.; Gehring, C.A. 2014. An elusive ectomycorrhizal fungus reveals itself: A new species of *Geopora* (Pyronemataceae) associated with *Pinus edulis*. Mycologia. 106: 553–563.

Garbaye, J. 1994. Helper bacteria: A new dimension to the mycorrhizal symbiosis. New Phytologist. 128: 197–210.

Garrity, S. R.; Allen, C.D.; Brumby, S.P.; Gangodagamage, C.; McDowell, N.G.; Cai, D.M. 2013. Quantifying tree mortality in a mixed species woodland using multitemporal high spatial resolution satellite imagery. Remote Sensing of Environment. 129: 54–65.

Gehring, C.; Flores-Rentería, D.; Sthultz, C.M.; Leonard, T.M.; Flores-Rentería, L.; Whipple, A.V.; Whitham, T.G. 2014a. Plant genetics and interspecific competitive interactions determine ectomycorrhizal fungal community responses to climate change. Molecular Ecology. 23: 1379–1391.

Gehring, C.A.; Mueller, R.C.; Haskins, K.E.; Rubow, T.K.; Whitham, T.G. 2014b. Convergence in mycorrhizal fungal communities due to drought, plant competition, parasitism, and susceptibility to herbivory: Consequences for fungi and host plants. Frontiers in Microbiology. 5: 306.

Gehring, C. A.; Theimer, T.C.; Whitham, T.G.; Keim, P. 1998. Ectomycorrhizal fungal community structure of pinyon pines growing in two environmental extremes. Ecology. 79: 1562–1572.

Gehring, C.A.; Swaty, R.L.; Deckert, R.J. 2016. Mycorrhizas, drought and host plant mortality. In: Johnson, N.C.; Gehring, C.; Jansa, J., eds. Mycorrhizal mediation of soil: Fertility, structure, and carbon storage. Amsterdam: Elsevier: 279–298.

Gehring, C.A.; Sthultz, C.M.; Flores-Renteria, L.; Whipple, A.V.; Whitham, T.G. 2017. Tree genetics defines fungal partner communities that may confer drought tolerance. Proceedings of the National Academy of Sciences of the United States of America. 114 (142): 11169-11174.

Gitlin, A. R.; Sthultz, C.M.; Bowker, M.A.; Stumpf, S.; Paxton, K.L.; Kennedy, K.; Muñoz, A.; Bailey, J.K.; Whitham, T.G. 2006. Mortality gradients within and among dominant plant populations as barometers of ecosystem change during extreme drought. Conservation Biology. 20:1477–1486.

Gordon, G. J.; Gehring, C.A. 2011. Molecular characterization of pezizalean ectomycorrhizas associated with pinyon pine during drought. Mycorrhiza. 21:431–441.

Harte, J.; Kinzig, A.P. 1993. Mutualism and competition between plants and decomposers: Implications for nutrient allocation in ecosystems. The American Naturalist. 141:829–846.

Haskins, K. E.; Gehring, C.A. 2004. Interactions with juniper alter pinyon pine ectomycorrhizal fungal communities. Ecology. 85:2687–2692.

Haskins, K. E.; Gehring, C.A. 2005. Evidence for mutualist limitation: The impacts of conspecific density on the mycorrhizal inoculum potential of woodland soils. Oecologia. 145:123–131.

Hewitt, R. E.; Hollingsworth, T.N.; Chapin, F.S., III; Taylor, D.L. 2016. Fire-severity effects on plant–fungal interactions after a novel tundra wildfire disturbance: Implications for arctic shrub and tree migration. BMC Ecology. 16: 25.

Horton, T. R.; Bruns, T. D.; Parker, V.T. 1999. Ectomycorrhizal fungi associated with *Arctostaphylos* contribute to *Pseudotsuga menziesii* establishment. Canadian Journal of Botany. 77:93–102.

Hubert, N. A.; Gehring, C.A. 2008. Neighboring trees affect ectomycorrhizal fungal community composition in a woodland-forest ecotone. Mycorrhiza. 18: 363–374.

Ingleby, E.; Molina, R. 1991. Interactions among mycorrhizal fungi, rhizosphere organisms, and plants. In: P. Barbosa, V. Krischik, and C. Jones, editors. Microbial mediation of plant-herbivore interactions. New York, NY: John Wiley & Sons: 169–197.

Johnson, D.; Martin, F.; Cairney, J.W.G.; Anderson, I.C. 2012. The importance of individuals: intraspecific diversity of mycorrhizal plants and fungi in ecosystems. New Phytologist. 194:614–628.

Johnson, N. C.; Gehring, C.A. 2007. Mycorrhizas: Symbiotic mediators of rhizosphere and ecosystem processes. In: Cardon, Z.G.; Whitbeck, J.L., eds. The rhizosphere: An ecological perspective. Amsterdam: Elsevier: 73–100.

Jumpponen, A.; Trappe, J.M. 1998. Dark septate endophytes: A review of facultative biotrophic root-colonizing fungi. New Phytologist. 140: 295–310.

Karst, J.; Randall, M.J.; Gehring, C.A. 2014. Consequences for ectomycorrhizal fungi of the selective loss or gain of pine across landscapes. Botany. 92: 855–865.

Kataoka, R.; Taniguchi, T.; Futai, K. 2009. Fungal selectivity of two mycorrhiza helper bacteria on five mycorrhizal fungi associated with *Pinus thunbergii*. World Journal of Microbiology and Biotechnology. 25: 1815–1819.

Kipfer, T.; Moser, B.; Egli, S.; Wohlgemuth, T.; Ghazoul, J. 2011. Ectomycorrhiza succession patterns in *Pinus sylvestris* forests after stand-replacing fire in the Central Alps. Oecologia. 167: 219–228.

Kirk, P.M.; Cannon, P.F.; Minter, D.W.; Stalpers, J.A. 2008. Ainsworth & Bisby's dictionary of the Fungi. Page Dictionary of The Fungi. 10th edition. CABI Europe.

Kivlin, S. N.; Emery, S.M.; Rudgers, J.A. 2013. Fungal symbionts alter plant responses to global change. American Journal of Botany. 100: 1445–1457.

Kivlin, S. N.; Hawkes, C.V.; Treseder, K.K. 2011. Global diversity and distribution of arbuscular mycorrhizal fungi. Soil Biology and Biochemistry. 43: 2294–2303.

Kloepper, J.; Schroth, M. 1978. Plant growth promoting rhizobacteria on radishes. In: Proceedings of the 4th International Conference on Plant Pathogenic Bacteria. INRA, Angers 879–882.

Krohn, A. L.; Flores-Rentería, L.; Gehring, C.A. 2013. Microsatellite primers in the foundation tree species *Pinus edulis* and *P. monophylla* (Pinaceae). Applications in Plant Sciences. 1: 1200552.

Kuske, C. R.; Ticknor, L.O.; Busch, J.D.; Gehring, C.A.; Whitham, T.G. 2003. The pinyon rhizosphere, plant stress, and herbivory affect the abundance of microbial decomposers in soils. Microbial Ecology. 45: 340–352.

Laliberté, E.; Lambers, H.; Burgess, T.I.; Wright, S.J. 2015. Phosphorus limitation, soil-borne pathogens and the coexistence of plant species in hyperdiverse forests and shrublands. New Phytologist. 206: 507–521.

Lamit, L. J.; Holeski, L.M.; Flores-Rentería, L.; Whitham, T.G.; Gehring, C.A. 2016. Tree genotype influences ectomycorrhizal fungal community structure: Ecological and evolutionary implications. Fungal Ecology. 24: 124–134.

Lau, J. A.; Lennon, J.T. 2011. Evolutionary ecology of plant-microbe interactions: Soil microbial structure alters selection on plant traits. New Phytologist. 192: 215–224.

Lau, J. A.; Lennon, J.T. 2012. Rapid responses of soil microorganisms improve plant fitness in novel environments. Proceedings of the National Academy of Sciences of the United States of America. 109: 14058–14062.

Leininger, S.; Urich, T.; Schloter, M.; Schwark, L.; Qi, J.; Nicol, G.W.; Prosser, J.I.; Schuster, S.C.; Schleper, C. 2006. Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature. 442: 806–809.

Lesser, M. R.; Parchman, T.L.; Buerkle, C.A. 2012. Cross-species transferability of SSR loci developed from transcriptome sequencing in lodgepole pine. Molecular Ecology Resources. 12: 448–455.

Lilleskov, E. A.; Hobbie, E.A.; Horton, T.R. 2011. Conservation of ectomycorrhizal fungi: Exploring the linkages between functional and taxonomic responses to anthropogenic N deposition. Fungal Ecology. 4:174–183.

Louw, J. P.; Korsten, L. 2013. Pathogenic *Penicillium* spp. on apple and pear. Plant Disease 98: 590–598.

McHugh, T. A.; Gehring, C.A. 2006. Below-ground interactions with arbuscular mycorrhizal shrubs decrease the performance of pinyon pine and the abundance of its ectomycorrhizas. New Phytologist. 171: 171–178.

Molina, R.; Massicotte, H.; Trappe, J.M. 1992. Specificity phenomena in mycorrhizal symbioses: community-ecological consequences and practical implications. In: Allen, M.F., ed. Mycorrhizal functioning: An integrative plant-fungal process. New York, NY: Chapman and Hall: 357–423.

Mopper, S.; Mitton, J.; Whitham, T.; Cobb, N.S.; Christensen, K.M. 1991. Genetic differentiation and heterozygosity in pinyon pine associated with resistance to herbivory and environmental stress. Evolution. 45: 989–999.

Mueller, R. C.; Scudder, C.M.; Porter, M.E.; Trotter, R.T., III; Gehring, C.A.; Whitham, T.G. 2005a. Differential tree mortality in response to severe drought: evidence for long-term vegetation shifts. Journal of Ecology. 93: 1085–1093.

Mueller, R. C.; Wade, B.D.; Gehring, C.A.; Whitham, T.G. 2005b. Chronic herbivory negatively impacts cone and seed production, seed quality and seedling growth of susceptible pinyon pines. Oecologia. 143: 558–565.

Neal, J., Jr.; Bollen, W.; Zak, B. 1964. Rhizosphere microflora associated with mycorhizae of Douglas-fir. Canadian Journal of Microbiology. 10: 259–265.

Nguyen, N. H.; Hynson, N.A.; Bruns, T.D. 2012. Stayin' alive: Survival of mycorrhizal fungal propagules from 6-yr-old forest soil. Fungal Ecology. 5: 741–746.

Ogle, K.; Whitham, T.; Cobb, N. 2000. Tree-ring variation in pinyon predicts likelihood of death following severe drought. Ecology. 81: 3237–3243.

Opik, M.; Vanatoa, A.; Vanatoa, E.; Moora, M.; Davison, J.; Kalwij, J.M.; Reier, U.; Zobel, M. 2010. The online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). New Phytologist. 188: 223–241.

Peay, K. G.; Bruns, T.D.; Kennedy, P.G.; Bergemann, S.E.; Garbelotto, M. 2007. A strong species-area relationship for eukaryotic soil microbes: Island size matters for ectomycorrhizal fungi. Ecology Letters. 10: 470–480.

Phillips, R. P.; Brzostek, E.; Midgley, M.G. 2013. The mycorrhizal-associated nutrient economy: A new framework for predicting carbon-nutrient couplings in temperate forests. New Phytologist. 199: 41–51.

Rehfeldt, G. E.; Crookston, N.L.; Warwell, M.V.; Evans, J.S. 2006. Empirical analyses of plant-climate relationships for the western United States. International Journal of Plant Sciences. 167: 1123–1150.

Restrepo-Llano, M.; Osorio, N.W.; León, J.D. 2014. Assessment of the effectiveness of ectomycorrhizal inocula to promote growth and root ectomycorrhizal colonization in *Pinus patula* seedlings using the most probable number technique. Applied and Environmental Soil Science. 2014: 870616.

Revillini, D.; Gehring, C.A.; Johnson, N.C. 2016. The role of locally adapted mycorrhizas and rhizobacteria in plant-soil feedback systems. Functional Ecology. 30: 1086–1098.

Ridge, E. H.; Theodorou, C. 1972. The effect of soil fumigation on microbial recolonization and mycorrhizal infection. Soil Biology and Biochemistry. 4: 295–305.

Roesch, L. F. W.; Fulthorpe, R.R.; Riva, A.; Casella, G.; Hadwin, A.K.M.; Kent, A.D.; Daroub, S.H.; Camargo, F.A.O.; Farmerie, W.G.; Triplett, E.W. 2007. Pyrosequencing enumerates and contrasts soil microbial diversity. The ISME Journal. 1: 283–290.

Rouatt, J.; Katznelson, H. 1961. A study of bacteria on the root surface and in the rhizosphere soil of crop plants. Journal of Applied Bacteriology. 24: 164–171.

Schlesinger, W. H.; Bernhardt, E.S. 2013. Biogeochemistry: An analysis of global change. 3rd edition. Oxford, UK: Academic Press. 672 p.

Schwartz, M. W.; Hoeksema, J.D.; Gehring, C.A.; Johnson, N.C.; Klironomos, J.N.; Abbott, L.K.; Pringle, A. 2006. The promise and the potential consequences of the global transport of mycorrhizal fungal inoculum. Ecology Letters. 9: 501–515.

Seager, R.; Ting, M.; Held, I.; Kushnir, Y.; Lu, J.; Vecchi, G.; Huang, H.-P.; Harnik, N.; Leetmaa, A.; Lau, N.-C.; Li, C.; Velez, J.; Naik, N. 2007. Model projections of an imminent transition to a more arid climate in southwestern North America. Science. 316: 1181–1184.

Smith, S. E.; Read, D. 2008. Mycorrhizal symbiosis. 3rd edition. Amsterdam: Academic Press. 800 p.

Sthultz, C. M.; Gehring, C.A.; Whitham, T.G. 2007. Shifts from competition to facilitation between a foundation tree and a pioneer shrub across spatial and temporal scales in a semiarid woodland. New Phytologist. 173: 135–145.

Sthultz, C. M.; Gehring, C.A.; Whitham, T.G. 2009a. Deadly combination of genes and drought: Increased mortality of herbivore-resistant trees in a foundation species. Global Change Biology. 15: 1949–1961.

Sthultz, C. M.; Whitham, T.G.; Kennedy, K.; Deckert, R.; Gehring, C.A. 2009b. Genetically based susceptibility to herbivory influences the ectomycorrhizal fungal communities of a foundation tree species. New Phytologist. 184: 657–667.

Swaty, R.; Michael, H.M.; Deckert, R.; Gehring, C.A. 2016. Mapping the potential mycorrhizal associations of the conterminous United States of America. Fungal Ecology. 24: 139–147.

Taylor, D. L.; Walters, W.A.; Lennon, N.J.; Bochicchio, J.; Krohn, A.; Caporaso, J.G.; Pennanen, T. 2016. Accurate estimation of fungal diversity and abundance through improved lineage-specific primers optimized for Illumina amplicon sequencing. Applied and Environmental Microbiology. 82: 7217–7226.

Tedersoo, L.; Bahram, M.; Jairus, T.; Bechem, E.; Chinoya, S.; Mpumba, R.; Leal, M.; Randrianjohany, E.; Razafimandimbison, S.; Sadam, A.; Naadel, T.; Kõljalg, U.. 2011. Spatial structure and the effects of host and soil environments on communities of ectomycorrhizal fungi in wooded savannas and rain forests of Continental Africa and Madagascar. Molecular Ecology. 20: 3071–3080.

Tedersoo, L.; Jairus, T.; Horton, B.M.; Abarenkov, K.; Suvi, T.; Saar, I.; Kõljalg, U. 2008. Strong host preference of ectomycorrhizal fungi in a Tasmanian wet sclerophyll forest as revealed by DNA barcoding and taxon-specific primers. New Phytologist. 180: 479–90.

Teste, F. P.; Kardol, P.; Turner, B.L.; Wardle, D.A.; Zemunik, G.; Renton, M.; Laliberté, E. 2017. Plant-soil feedback and the maintenance of diversity in Mediterranean-climate shrublands. Science. 355: 173–176.

Tomao, A.; Bonet, J.A.; de Aragón, J.M.; de-Miguel, S. 2017. Is silviculture able to enhance wild forest mushroom resources? Current knowledge and future perspectives. Forest Ecology and Management. 402: 102–114.

Torsvik, V.; Øvreås, L. 2002. Microbial diversity and function in soil: From genes to ecosystems. Current Opinion in Microbiology. 5: 240–245.

Treseder, K. K.; Holden, S.R. 2013. Fungal carbon sequestration. Science. 339: 1528–1529.

van der Heijden, M. G. A.; J. N. Klironomos; M. Ursic; P. Moutoglis; R. Streitwolf-Engel; T. Boller; A. Wiemken; and I. R. Sanders. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396:69–72.

Wang, B.; Qiu, Y.-L. 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza. 16: 299–363.

Whitham, T.; Young, W.; Martinsen, G.; Gehring, C.; Schweitzer, J.; Shuster, S.; Wimp, G.; Fischer, D.; Bailey, J. ; Lindroth, R.; Woolbright, S.; Kuske, C. 2003. Community and ecosystem genetics: a consequence of the extended phenotype. Ecology. 84: 559–573.

Whitham, T.G.; Mopper, S. 1985. Chronic herbivory: Impacts on architecture and sex expression of pinyon pine. Science. 228: 1089–1091.

Wilson, G.W.T.; Rice, C.W.; Rillig, M.C.; Springer, A.; Hartnett, D.C. 2009. Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: results from long-term field experiments. Ecology Letters. 12: 452–461.