

THE EFFECTS OF BIOLOGICAL SOIL CRUSTS ON ROOT-
ASSOCIATED FUNGI COLONIZATION RATES OF VASCULAR
PLANTS ACROSS SOUTHWESTERN DESERTS

by

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Abstract

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The Effects of Biological Soil Crusts on Root-associated Fungi Colonization Rates of Vascular Plants Across Southwestern Deserts

Thesis directed by Associate Professor Nichole Barger

Biological soil crusts (“biocrusts”) are communities of cyanobacteria, moss, lichen, and fungi that grow in arid/semiarid soils. Biocrusts perform several ecosystem functions including increased soil stability, water/nutrient retention and aid in plant growth. Root associated fungi (RAF) form symbioses with plant roots by bringing them nutrients and water in exchange for photosynthates. This study aimed to understand if the biocrust type had an influence on the RAF under a field setting. Plant roots were collected throughout the US Southwest from four common crust types (bare ground, light cyanobacterial, dark cyanobacterial, and cyanolichen (lichen or moss present)) which represent increasing level of development. The dominant grass and shrub were sampled across four desert sites (Great Basin, Colorado Plateau, Sonoran, Chihuahuan) from each crust type, and the RAF colonization rates were measured. The results indicate that the roots from bare ground soils had the highest colonization rate, which steadily decreased through crust type maturation. With bare ground roots having 5.6%, 7.9%, and 13% higher than the light cyanobacterial, dark cyanobacterial, and cyanolichen crusts respectively.

Dedication

I would like to dedicate this thesis to Sierra Jech who has been encouraging me since my first day in lab in 2019, taught me many field and laboratory techniques, helped me with data analysis, and has overall become one of my closest friends.

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Background

Biological soil crusts (“biocrusts” hereafter) are communities comprised of cyanobacteria, other bacteria, algae, lichens, fungi, and bryophytes that inhabit the top few millimeters of the soil surface (Weber et al. 2016). Biocrusts cover roughly 12% of the Earth’s land area and are especially ubiquitous across dryland ecosystems (Rodríguez et al. 2018). As plant cover declines with increasing aridity of ecosystems, biocrusts cover soil surfaces in plant interspaces (Belnap et al. 2001). Biocrusted soils are often sites for enhanced soil water (Abed et al. 2013, Chen et al) and nutrients (Abed et al. 2013, Barger et al. 2016, Belnap and Lange. 2003, Havrilla et al. 2020, Weber et al. 2016) and may partially explain the consistently higher vascular plant growth on biocrusts relative to their bare ground counterparts (Havrilla et al. 2019).

Although higher plant growth on biocrusts is often explained by higher water and nutrient availability, other biotic factors may influence these patterns. Despite extensive research into the role of cyanobacteria, lichens, and bryophytes in nutrient cycling and water availability within biocrusts (Evans and Lange. 2001, Rosentreter and Belnap. 2001, Seppelt et al. 2016), biocrust fungal communities have been largely ignored. Many fungal species are present in biocrusts including root-associated fungi (RAF) such as arbuscular mycorrhizal fungi (AMF) and dark septate endophytic fungi (DSE) (Bates and Garcia-Pichel. 2009, States and Kinter. 2001). Both AMF and DSE colonize vascular plant roots and are shown to improve the host’s biomass and survival (Jumpponen. 2001, Mandyam and Jumpponen. 2005, Newsham. 2011). In arid ecosystems, DSE is more dominant than AMF (Knapp et al. 2012, Maier et al. 2016) as the dark melanization of DSE from polymerized phenolic compounds aids in UV protection and desiccation tolerance (Maier et al. 2016, Spagnoletti and Giacometti. 2020), while AMF is more sensitive to arid conditions (Aanderud et al. 2018, Maier et al. 2016). A majority of vascular

plants form RAF associations and benefit from them (Willis et al. 2013) and the symbiosis can be particularly advantageous in an arid context as nutrients and water are often scarce.

We know that RAF are prevalent within biocrusts and that fungal community diversity has large overlap between biocrusts and rhizosphere soils (Porrás-Alfaro et al. 2009, Steven et al. 2014) and fungal diversity increases with biocrust maturity (Bates et al. 2012, Xiao and Veste. 2017). Although another study found higher fungal diversity on subsurface soils than biocrusts (Pombubpa et al. 2020). There are mixed results about the abundance of fungal populations between biocrust and bare soil. Some studies indicate fungal abundance does not significantly differ between biocrusts and rhizosphere soils (Steven et al. 2014) while others claim the RAF abundance to be higher in biocrusts (Maier et al. 2016) and increases over biocrust development (Maier et al. 2018). Looking specifically at RAF populations: the fungal community within dryland systems is dominated by *Pleosporales*, which are particularly abundant within the rhizosphere, and are known to form root associations (Abed et al. 2013, Bates et al. 2012, Green et al. 2008, Porrás-Alfaro et al. 2009, Steven et al. 2014). However, overall, the RAF abundance and diversity is higher within biocrusts than uncrusted soil (Belnap et al. 2001, Harper and Belnap. 2001, Harper and Pendleton. 1993, Maier et al. 2016) although fungal diversity and abundance can vary in biocrusts on small spatial scales (Steven et al. 2015).

Whether RAF abundance and density in the soil influences the colonization rate is often species specific (Vannette and Hunter. 2013); however, often the abundance of fungi present in the soil is positively correlated with colonization rates (Barceló et al. 2020, Karliński et al. 2010 van Aarle et al. 2002, van Aarle et al. 2003). This would suggest that biocrusts should have higher colonization rates than bare soil as shown by some studies (Harper and Pendleton. 1993, Pendleton and Warren. 1995). Other, more recent, studies have found an opposite pattern, where

plants grown on biocrusts had lower RAF colonization (Chaudhary et al. 2019, Havrilla et al. 2020). These mixed results may be partially explained by how the older studies only measured AMF colonization, which we now know make up very little of the RAF populations in arid systems (Knapp et al. 2012) while in recent studies, DSE has been included (see Table B in Appendix).

However, many aspects of the biocrust-RAF relationship remain unclear. Specifically, whether biocrust may influence RAF's ability to infect the roots of vascular plants. One recent greenhouse study found that RAF root colonization was lower in plants growing on biocrusts than on a bare substrate; however, the plant biomass was higher in biocrusts indicating that vascular plants are still benefiting from the crust (Havrilla et al. 2020). Another recent inoculum study found similar results where plants grown on biocrusts had ~50% less AMF colonization than those grown without biocrusts (Chaudhary et al. 2019). A field study conducted on restored biocrusts found that as percent biocrust increased, the percent mycorrhizal colonization decreased (Bi et al. 2022) but does not include the effects on DSE. It can be inferred from these results that RAF colonization on plants occurring on biocrusted soils have a lower affinity for RAF colonization (Havrilla et al. 2020), but the studies do not address if the full suite biocrust community types across different desert systems play a role in the RAF colonization.

Many studies have shown that RAF greatly improves their host's drought tolerance (Dennison and Kiers. 2011, Jamiolkowska et al. 2018, Li et al. 2019, Liu and Wei. 2021, Wu and Zou. 2017, Zhang et al. 2017). AMF can transport water to the host plant and therefore greatly increases the water uptake via the mycelial network as the hyphae can grow 100x the length of the host root (Dennison and Kiers. 2011). Roots colonized with AMF grown in drought stress had significantly greater root growth in both length, diameter, and branching than plants

without AMF. AMF can also influence the phenology of its host to alleviate drought stress by improving the plant's epicuticular wax and root morphology and decreasing the leaf's osmotic potential to reduce loss in turgor pressure (Wu and Zou. 2017). DSE colonization appears to have similar effects where the plant's root architecture is improved for drought tolerance through increased root length and decreased root diameter. The altered root morphology improved plant growth overall when grown under water-deficit stress (Li et al. 2019). Greenhouse studies have also found DSE to help protect plant's organelles from damage (Liu and Wei. 2021) and increase photosynthesis efficiency during drought stress (Zhang et al. 2017).

Aspects of biocrusts may also provide stress relief to plants during times of drought as they retain water (Colica et al. 2014, Menon et al. 2011) by improving infiltration and reducing soil evaporation (Chamizo et al. 2015). These effects can be significant as some mature biocrusts can have a 7.6% increase in soil moisture compared to uncrusted soil (Xiao et al. 2016). And a greenhouse study found plant communities growing on biocrust had a 46% improvement in drought tolerance (Chaudhary et al. 2020). As biocrusts develop through successional stages, there are significant community composition changes that subsequently influences the biogeochemistry of the soil. The youngest stage of development is mostly made up of pioneer cyanobacteria like *Microcoleus* which begin to fix nitrogen (N) and bind the soil particles together (Belnap. 2002). As soil conditions improve, more organisms can begin to colonize (Maier et al. 2019). With the increased diversity and abundance of cyanobacteria, the later stage dark cyanobacteria crust is fixing more N, allowing for larger and more complex organisms to colonize such as lichens and bryophytes. The more mature stages of biocrust have the highest rates of N fixation and available N (Barger et al. 2013, Barger et al. 2016, Maier et al. 2018) and

are able to retain more water (Chen et al. 2019). These trends demonstrate how the changes in biocrust community composition impact the fertility and hydrological function of the soil.

Various abiotic factors, like climate and soil chemistry, can influence the relationship between the plant host and its RAF (Jamiolkowska et al. 2018, Mack and Rudgers. 2008, Soudzilovskaia et al. 2015). However, the symbiosis can be largely dependent upon the plant host's deficiencies. When nutrients are scarce, colonization by fungi promotes plant nutrient uptake, transpiration and photosynthetic rates. However, when there are ample nutrients in the soil available to the plant, less photosynthates are available to the fungi which therefore reduces colonization (Willis et al. 2012). Other studies have shown that adding N to soil reduces AMF populations (Blanke et al. 2005). However, plants under nutrient or water stress actively seek out fungal associations by releasing phytohormones to attract RAF and enhance colonization (Nagahashi and Douds. 2000).

If plants benefit from RAF in drought conditions, then they may be more likely to form associations with RAF under drought stress (Jamiolkowska et al. 2018). Plants under nutrient stress (typically N) are also more likely to form RAF associations as they may rely on the fungi to gain nutrients (Smith and Read. 1997), as plants with RAF have higher N and P levels in their tissues than they would without RAF (Newsham. 2011).

The aim of this project is to examine the impact of biocrusts of RAF colonization overall within field conditions and how the different types of biocrusts influence the RAF colonization of their plant host among various desert types. Climatic gradients, geography, and desert type can dictate the community composition of both plant and microbial communities. We know RAF can adapt to various climatic conditions and therefore may be more influenced by their plant

host's success in those environments. We want to see if the pattern of decreased RAF colonization within plants grown on biocrust mesocosms demonstrated by Havrilla et al. 2020, hold within a range of field conditions (plant species, location factors). I hypothesize that this trend will remain within the field across the range of factors. We also want to understand if plant RAF colonization rates vary in a predictable way within different biocrust types. Given that biocrusts increase nutrient and water availability, I hypothesized that RAF colonization rates on vascular plants will be highest in bare ground soils that are characterized by low water and nutrient availability and will decrease with increasing level of development of the biocrust.

Methods

Sites

Samples were collected from four locations in the Southwest part of the United States in May 2021 (Map 1). The first location was the Canyonlands Research Center in SE Utah on the Colorado Plateau (38°05'40"N, -109°34'36"W). The mean annual temperature ranges from 4.5 - 18°C and the mean annual precipitation is 152.72 mm (PRISM Climate Group). The area falls within the Semidesert Sand (Four-wing Saltbush) ecological site description with a loamy fine sand soil type (USDA NRCS. 2009). Root samples were sampled from the dominant shrub: fourwing saltbush (*Atriplex canescens*) and the dominant grass: indian ricegrass (*Achnatherum hymenoides*).

The second location was Bureau of Land Management land Southwest of Provo, UT— West Desert District— in the Great Basin Desert (40°15'09"N 112°13'22"W). The mean annual temperature ranges from 0.5 – 18.1°C and the mean annual precipitation is 170.6 mm. The soil type is described as Semidesert gravelly loam, with a Semidesert Gravelly Loam ecological site

description (USDA NRCS. 2009). Root samples were collected from the dominant shrub: greasewood (*Sarcobatus vermiculatus*) and the dominant grass: big galleta (*Hilaria rigida*). Both the Colorado Plateau and the Great Basin are cool deserts, where precipitation is evenly distributed throughout the year with 50% occurring in the winter and 50% occurring in the summer (Daly et al. 2008).

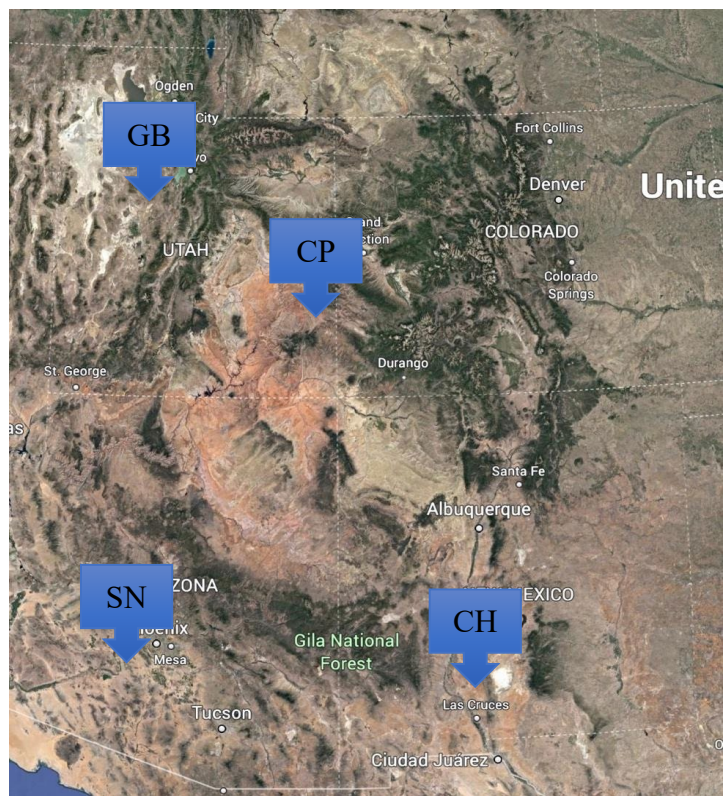
The third location was Bureau of Land Management land outside of Phoenix, AZ—Lower Sonoran District—in the Sonoran Desert (33°11'32"N 112°16'13"W) with an annual precipitation of 140 mm and an annual temperature ranging from 16.1 – 30.4°C (PRISM Climate Group). This area has a loamy fan soil type and the roots sampled were from the dominant shrub: creosote (*Larrea tridentata*), and the heavily dominant and invasive Mediterranean grass (*Schismus barbatus*).

The fourth and final location was at the Jornada Experimental Range in the Chihuahuan Desert (32°31'34"N 106°44'47"W) with an annual temperature ranging from 7.5-25.7°C and an average annual precipitation of 207.43 mm (PRISM Climate Group). The plant community had a low diversity, and all of the plants appeared to be dead or dormant. The shrubs were unidentifiable due to lack of leaves, and the only grass present was burro grass (*Scleropogon brevifolius*) which was sampled.

Both the Sonoran and the Chihuahua are hot deserts where they receive 80% of their precipitation during the monsoon summer months, and then the other 20% is received throughout the rest of the year (Daly et al. 2008). It is important to note that all of the locations are experiencing a prolonged drought since the early 2000s (NOAA. 2021), and low precipitation conditions had been occurring more within the hot deserts (Table A. in Appendix). Because of

poor conditions of the roots and difficulty sampling, the shrub roots for the Great Basin and the Chihuahua were removed from the study (Table 1)

Map 1



Sampling locations indicated on map. CP=Colorado Plateau, GB=Great Basin, SN=Sonoran, CH=Chihuahua

Table 1. General Characteristics of Sampling Sites

Desert	Soil Texture	Ecological Site Group	Plants Sampled	Annual Precipitation	Average Annual Temperature
Great Basin	Gravelly loam	Semidesert Gravelly Loam (Wyoming Big Sagebrush) South	Grass: <i>H. rigida</i>	170.6 mm	9.3°C
CO Plateau	Loamy fine sand	Semidesert Sand (Four-wing Saltbush)	Grass: <i>A. hymenoides</i> Shrub: <i>A. canescens</i>	152.7 mm	11.2°C
Sonoran	Gravelly loam	Sandy Wash 7"-10" p.z.	Grass: <i>S. barbatus</i> Shrub: <i>L. tridentata</i>	140 mm	23.27°C
Chihuahua	Fine sandy loam	Sandy	Grass: <i>S. brevifolius</i>	207.4 mm	16.6°C

Sampling Design

At each location, 3 sites were selected that contained all 4 of the biocrust successional stages. These were identified based on coloration and the presence/absence of bryophytes or lichens. Early stage biocrusts contain the cyanobacteria *Microcoleus vaginatus* and *Microcoleus steenstrupi* which do not alter the color of the soil, whereas later stages of biocrusts appear darker due to colonization of pigmented cyanobacteria: *Scytonema myochrous* and *Nostoc commune* (Belnap. 2002). We categorized them as: bare ground (no biocrust present), light cyano (early stage cyanobacterial biocrust light in appearance), dark cyano (late stage cyanobacterial biocrust dark in appearance), and cyanolichen (a mature biocrust that has either or both lichen and bryophytes present).

At each site, 5 root samples from two plant species were collected from every biocrust type. The dominant grass and shrub of each location were (Table 1). Grass roots were sampled by carefully uprooting the entire plant by digging around with a spade and uplifting the entire root system and storing it in a plastic bag on ice. Shrub roots were sampled by taking a 3cm wide soil core along the base of the shrub and then storing it in a plastic bag on ice. In the Colorado Plateau and Great Basin, the cores were 10cm deep, whereas in the Sonoran, where the soil was more compacted and rocky, core depth only reached 7cm. Shrubs that had other plants growing near the base were not sampled to remove the possibility of other species' roots being collected. The samples were kept on ice until being placed in a 4° C refrigerator where they were stored before processing.

RAF Colonization

For grass samples, the roots were isolated from the rest of the above ground biomass of the plant. The shrub roots were picked out of the soil sample. All roots were cleaned with tap water to

remove excess soil and left to dry in an oven at 60° C for 48 hours. The roots were then stored at room temperature until further processing. The roots were cleared of pigments in a boiling 10% KOH solution. Once cleared of melanization, the roots were stained following the methods described in Vierheilig et al. (1998). The roots were soaked in a boiling 5% ink-vinegar solution for 3-5 minutes using Schaeffer's black ink and household vinegar (5% acidity) and then mounted onto slides. Using a compound microscope (400x), we counted the number of fungal structures which included hyphae, vesicles, spores, arbuscules, hyphal coils, or microsclerotia per root section. It was difficult to distinguish some mycorrhizal fungal features from the dark septate endophytes, therefore all fungal structures were recorded without specifying the fungal type. Rate of RAF colonization was calculated by following the methods of McGonigle et al. 1990. Finding the percentage of colonization was calculated as:

$$\left(\frac{\text{Number of Fungal Structures Observed}}{\text{Number of Roots that Intersected Gridlines}} \right) \times 100$$

Data Analysis

All statistical analyses were conducted in R (R Core Development Team 2019 version 3.5.2). We assessed the effects of the predictor variables: biocrust type, sampling location, and plant species on RAF colonization percentage (Percent RAF) to test the variety of factors included in this study. We used general linear models (GLM) with the *lme4* package (Bates et al. 2014) with the gamma family for error distribution (log link). Chi-squared (X^2) and p-values were calculated with the analysis of variance (Anova) function using the *car* package (Fox et al. 2012). Tukey tests were used as *post-hoc* contrasts on all GLMs using the *emmeans* package

version 1.3.4 (Lenth et al. 2019). The Tukey tests were used to circumvent Type I errors from performing multiple comparisons by creating Holm-adjusted p -values.

Results

We found that every plant species formed associations with RAF (Fig. 2, Fig. 3). The results support both of our hypotheses as biocrust type had a significant effect on RAF colonization (Table 2, Table 3) and biocrust overall had 16.2% lower colonization rates than the bare ground. The plants growing in bare ground had the highest percentage of RAF with a mean of 41.4%. As the biocrust went through developmental succession, we saw a decline in colonization, where light cyano had 5.6% less than bare ground, dark cyano had 7.9% less than bare ground, and cyanolichen had the lowest colonization of 13% less than bare ground. This pattern of colonization was found across all sites (Fig. 1) as well as in each plant species (Fig. 3).

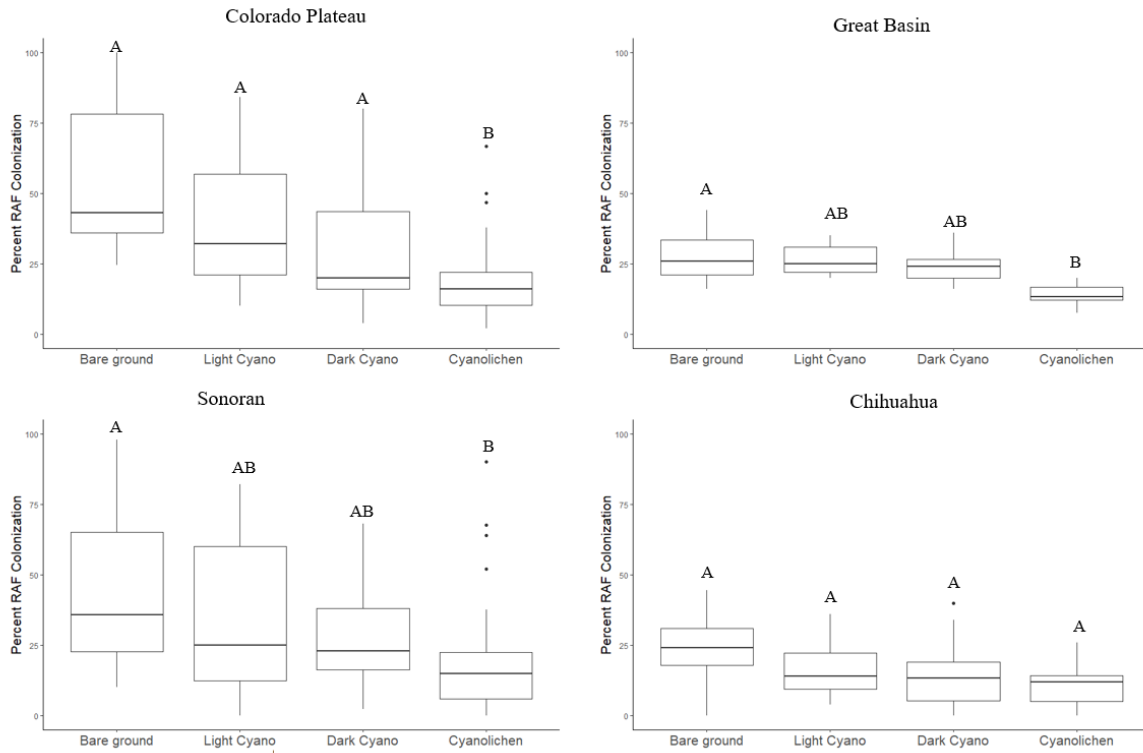
RAF colonization of all plant species differed significantly across desert types (Table 2). The Colorado Plateau had the highest overall colonization; however, there was no statistical significance between it and the Sonoran ($p=0.078$). The Great Basin and the Chihuahua had significantly lower values with 25.6% ($p<0.0001$) and 35.7% ($p<0.0001$) lower than the Colorado Plateau respectively.

Table 2.

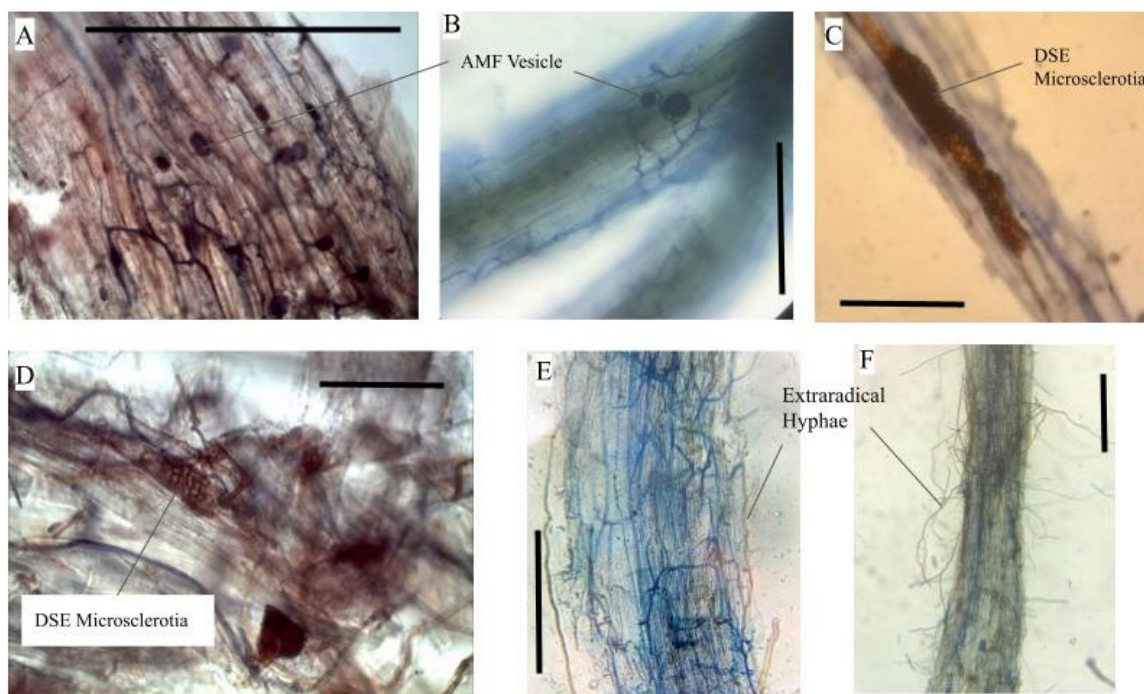
	X²	df	p-value
Location	61.456	3	<0.005
Type	75.111	3	<0.005
Location*Type	2.814	9	0.971

Results from GLM Anova used to evaluate the effects of study location and biocrust type on RAF colonization

Fig. 1



The effects of biocrust type, ranging from least developed (bare ground) to most developed (cyanolichen), on RAF colonization across all sampling locations. Boxes that share a letter are not significantly different.

Fig. 2

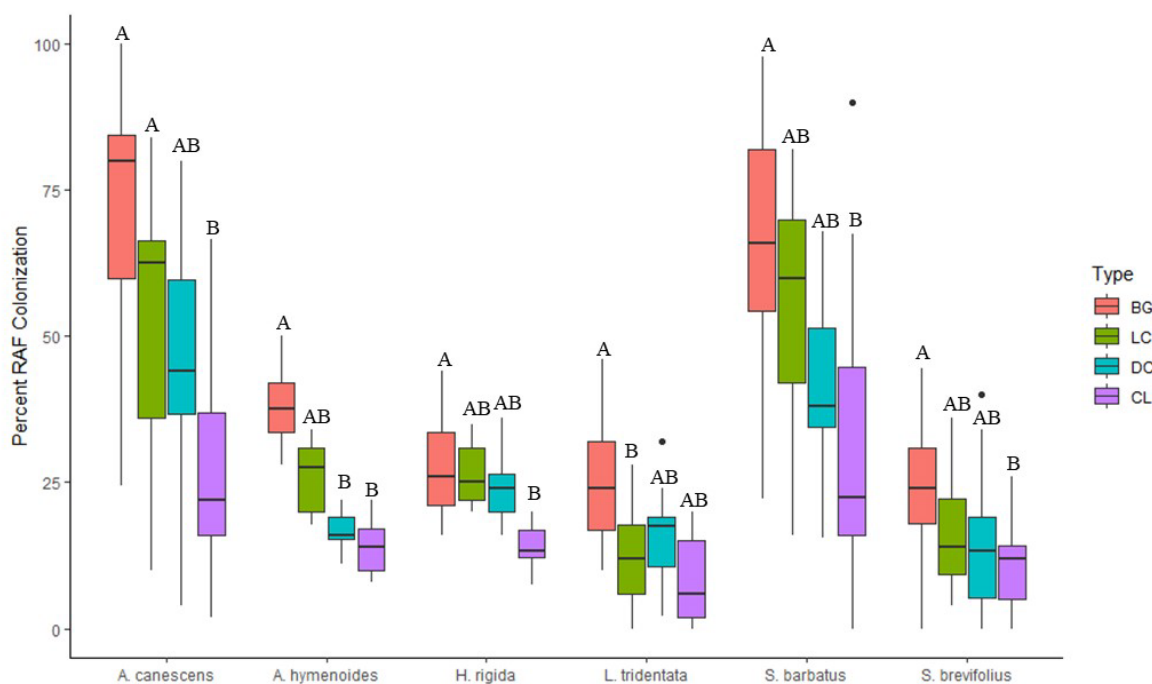
Various structures of root-associated fungi colonized in plant roots. (A) *H. rigida* - AMF and DSE hyphae with AMF vesicles; (B) *S. barbatus* - AMF hyphae with vesicle or spore like structures; (C) *A. canescens* - microscerotia, a dormant structure, of DSE; (D) *H. rigida* - septate hyphae and microscerotia structure of DSE; (E) *S. barbatus* - a highly colonized root with AMF and DSE hyphae; (F) *A. hymenoides* - AMF hyphae and vesicles with DSE extraradical hyphae. All images from a compound microscope at 400x magnification. Bar = 100nm.

The species of the plants had a significant impact on RAF colonization (Table 3, Fig. 3), where some species had higher colonization (*S. barbatus* and *S. canasens*) and some had much lower (*L. tridentata*).

Table 3.

	X²	df	p-value
Species	334.31	5	<0.005
Type	138.77	3	<0.005
Species*Type	16.46	15	0.352

Results from GLM Anova used to evaluate the effects of plant species and biocrust type on RAF colonization

Fig. 3

The RAF colonization for each species across the biocrust types, where BG= Bare ground, LC= Light cyano, DC= Dark Cyano, and CL = Cyanolichen. Species follow a gradient of North to South going left to right. Boxes that share the same letter are not significantly different.

Discussion

We found that the trend of RAF colonization decreasing with increasing biocrust development occurred across all desert types and plant species. This pattern of RAF colonization across the biocrust types is strong as this study contained many unknown factors that are unaccounted for given the different sampling locations as well as different plant species between each location; however, the pattern of the RAF colonization between the various biocrust types persists across plant species and desert type. The consistency of these results may be due to changes in the soil water and fertility between the biocrust types. While the underlying

mechanisms influencing this pattern are unknown, there are a few potential explanations for these results.

RAF Colonization and Biocrust Type

We see a consistent pattern of RAF colonization declining as biocrusts further develop. The plants growing on bare ground with no biocrust had much higher rates of colonization than any of the biocrust types. These results align with a greenhouse study where plants growing on bare soil had a 33.8% increase in colonization as compared to plants grown in biocrust (Havrilla et al. 2020). With the additional variable of biocrust type, we found that light cyano had the highest colonization of any biocrust type, followed by dark cyano, and finally the cyanolichen.

While N or phosphorus are often the limiting factor for plant growth, in these arid systems, water is likely the limiting factor especially during a drought period. Both DSE and AMF have a strong influence on a plant's drought resistance (Dennison and Kiers. 2011, Jamiolkowska et al. 2018, Li et al. 2019, Liu and Wei. 2021, Wu and Zou. 2017, Zhang et al. 2017) and could potentially be necessary for some plants to survive. The plants growing on bare ground are likely to experience the most drought stress, as the soil has less water retention capacity compared to biocrusts (Chamizo et al. 2015, Chen et al. 2019, Colica et al. 2014, Menon et al. 2011, Xiao et al. 2016). These plants are also more likely to experience nutrient limitations and subsequent stressors as there is little N fixation and nutrient availability (Abed et al. 2013, Barger et al. 2016, Belnap and Lange. 2003, Havrilla et al. 2019, Weber et al. 2016). Whereas plants growing on biocrusts are likely reaping the water/nutrient benefits and therefore may be less likely to depend on a fungal symbiosis to provide those resources. And in fact, in soils with high fertility, RAF can become parasitic to the plant (Mack and Rudgers. 2008). We know that the plant-RAF relationship is highly mediated by the plant and in scenarios with a higher nutrient

status, the plant can inhibit the fungi (Vierhelig. 2004) which results in AMF colonization rates decreasing with a lower C:N ratio (Soudzilovskaia et al. 2015). This may be an explanation for why the abundance of Ascomycota fungi is negatively correlated with the higher nutrient content of moss biocrusts (Xiao and Veste. 2017) which could indicate a reduction in DSE in response to increases in soil fertility. Alternatively, another study suggests that due to the high nutrient uptake of mosses and other organisms in mature biocrusts, available N is quickly taken up before RAF can access it (Aanderud et al. 2018). DSE are a diverse group of fungi, and unlike mycorrhizae, are not an obligate plant symbiont (Day and Currah. 2011, Jumpponen. 1998) as they retained their saprobic genes over time (Knapp et al. 2018). Some suggest that DSE may alter its lifestyle depending on environmental conditions (Promputtha et al. 2010) which may partially explain the lower colonization within higher developed biocrusts. Mature biocrusts have twice the C fixation rates than less-developed biocrusts (Housman et al. 2006). If a cyanolichen biocrust has ample carbon (C), DSE may choose to access it saprobically as these crusts have a greater abundance of saprophytic fungi (Omari et al. 2022), whereas in a low C system, it may resort to forming plant symbioses as DSE are facultative biotrophs (Day and Currah. 2011, Jumpponen and Trappe. 1998). It is unclear if these changes in RAF are due to nutrient, water, or other differences between the biocrust types. In the greenhouse study by Havrilla et al. (2020), the decrease in RAF colonization within biocrusts was suggested to be partially attributed to consistent watering as high-water content in soils can reduce the benefits of the fungal symbiosis for the plant (Dastogeer. 2018, Hoeksema et al. 2010). However, while well-developed crusts do increase soil moisture, it is unlikely that it has the high soil water availability as the biocrusts within the greenhouse study, suggesting that other variables are influencing the RAF.

Biocrusts may also negatively influence RAF populations as some lichen taxa release many soluble phenolic compounds as a metabolic pathway to consume C (Miralles et al. 2014). However, lichen phenols are shown to inhibit AMF colonization (Piotrowski et al. 2007, Stark et al. 2007). Thus, the reduction of colonization within cyanolichen crusts may be partially explained by these inhibitory effects.

Returning to microbial community composition changes throughout biocrust succession, and the accompanying biogeochemical changes to the soil, a large range of microhabitat niches can occur (Miralles et al. 2020) and RAF molecular diversity (Weil et al. 2012) and colonization rates can be influenced by microhabitat conditions (Chiapusio et al. 2018). Thus, suggesting that our study's RAF colonization pattern may be influenced at a smaller spatial scale than just biocrust type.

RAF Colonization Across Desert Types

The pattern of RAF colonization decreasing with biocrust development persists across all deserts; however, there is still variation in the colonization percentages between locations. This is to be expected as climate can influence AMF colonization rates, as colonization can increase in areas with cooler winters (Soudzilovskaia, et al. 2015). The Colorado Plateau had a significantly higher overall colonization. This can likely be attributed to the time of sampling in mid-May of 2021. The Colorado Plateau had been experiencing precipitation before and during times of sampling (Table A in Appendix). This was the only location where a majority of the plants in the sampling sites were actively photosynthesizing and did not appear to be experiencing drought stress. However, it is important to note that the high RAF in the Colorado Plateau is largely due to the shrub (*A. canescens*). *A. canescens* is known to form many associations with RAF, particularly DSE (Barrow and Aaltonen. 2001) and other members of the

Atriplex genus are highly mycorrhizal dependent (Plenchette and Duponnois. 2004). Similarly, the Sonoran Desert also had higher overall RAF, but was largely due to the grass (*S.barbatus*). The Great Basin had a lower RAF than the Colorado Plateau, even though had also been experiencing precipitation in the previous months from the time of sampling (Table A in Appendix). The Great Basin site had compacted soil that appeared to have significant water erosion on the surfaces without biocrust. These poor soil conditions may have contributed to lower RAF colonization, as well as having to remove the shrubs from the data set. The Chihuahuan desert had the lowest RAF overall. The Jornada Experimental Range has been experiencing severe drought conditions (Table A in Appendix), and the sampling was conducted during one of the driest months just before the onset of the monsoon season. None of the plants within the sampling sites appeared to be alive or photosynthetically active. Although plants experiencing drought are more likely to have more colonization to help alleviate the stress, plants that are experiencing extreme or prolonged drought stress are likely to inhibit colonization (Wu and Zou. 2017).

RAF Colonization and Plant Species

The plants sampled in this study were the dominant grass and shrub of each location and we found that every species had RAF colonization. Overall, RAF colonization in grasses was higher compared to shrubs. However, shrub samples from the Great Basin and the Chihuahua Deserts were removed from the study due to poor quality, which may have skewed this result. The Colorado Plateau shrub (*A. canescens*) had significantly higher colonization than the dominant grass, (*A. hymenoides*). In this context, the shrub RAF may be higher due to root architecture. Most of the root biomass for arid shrubs are taproots that grow deep into the soil

matrix to better access water; however, a majority of the N released by biocrusts remain at the top few cm of the soil surface (Johnson et al. 2005, Elbert et al. 2012, Barger et al. 2016). Studies have shown that shrubs take up less P from biocrusts than grasses as their surface layer roots are too short to access it (Belnap. 2011). Shrub roots are also often too short to access the water moisture in biocrust's top layers. In fact, moss dominated crusts can disadvantage shrubs trying to access deeper water as most of it is retained in the upper soil layers disallowing moisture to travel to lower soil layers (Chen et al. 2019, Li et al. 2004). This means that the shrub has far less root biomass within the fertile zone of the soil matrix, and therefore may need the help of RAF to better access those nutrient/water pools. However, we see an opposite pattern within the Sonoran Desert where the grass (*S. barbatus*) had a significantly higher RAF percentage than the shrub (*L. tridentata*). *S. barbatus* is an invasive grass across the American southwest (Beever et al. 2006) and dominated the plant community within the sampling area. Notably, this invasive annual grass had the highest RAF colonization relative to all of the other species within the study. *S. barbatus* has been shown to show strong associations with rhizophilic AMF (AMF that have high root colonization rates) (Weber et al. 2018). Many invasive grasses form strong mutualistic dependencies with AMF as it can aid them in protection from fungal pathogens as they also host more opportunistically parasitic fungi than the native plants (Weber et al. 2019). Exotic grasses are also known to provide more carbon to their fungal symbiont than native species, which can lead to high colonization rates (Hawkes et al. 2006). In addition, invasive species can gain more benefits from RAF through their heightened potential in nutrient exploitation (Willis et al. 2012). This could also work in conjunction with the fact that DSE are non-host specific and can therefore form symbioses with both native and non-native plants (Knapp et al. 2012).

Conclusion

Overall, this study has provided evidence towards the impacts biocrust community composition has on the root-associated fungi-plant symbiosis. We found a consistent pattern of RAF colonization across biocrust types, and that biocrusted soils have lower RAF colonization than bare soils. Root associated fungi deeply influence ecosystem health and are profoundly important for vascular plant success. It is important to understand the potential influences of RAF within arid land ecosystems. Aridlands make up roughly 1/3 of the land area on Earth and are experiencing significant negative impacts from climate change and increased land use, resulting in desertification and decreased soil productivity (Belnap. 1992). It is imperative as conservationists and restoration scientists to plan for more frequent and severe drought conditions. The results provide more evidence to support the restoration methods of biocrust implementation, as well as RAF inoculation (Pendleton et al. 2003) as RAF could be used as a means to improve plant success within restoration of degraded areas. The bare ground areas of sampling from this study are the closest conditions to a degraded soil, indicating that the higher rates of colonization in bare ground soils may aid in plant success within degraded systems in recovery. Future research could be implemented to better understand the underlying mechanisms influencing the consistent pattern of colonization observed in this study.

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Appendix

Table A. Monthly Precipitation (mm) by Location

	November	December	January	February	March	April	May
CO Plateau	7.45	10.64	11.89	15.89	23.31	7.80	6.39
Great Basin	12.34	10.88	16.34	41.42	34.65	27.21	3.92
Sonoran	0.00	22.7	17.22	0.00	11.41	0.00	0.00
Chihuahua	0.00	4.37	3.01	4.68	0.93	2.08	0.00

November & December 2020, January-May 2021 (Data from PRISM Climate Group)

Table B. History of Biocrust and RAF Colonization Studies

Study	Type	Structures Observed	Type of RAF	RAF Colonization Lower with Biocrust	RAF Colonization Higher with Biocrust
Harper and Pendleton. 1993	Field	Arbuscules, Vesicles	AMF		X
Pendleton and Warren. 1995		Arbuscules, Vesicles	AMF		X
Havrilla ET al. 2020	Mesocosm	Arbuscules, Vesicles, Hyphae, Microsclerotia, other RAF structures	AMF and DSE	X	
Chaudhary et al. 2020	Mesocosm	Arbuscules, Vesicles, Aseptate Hyphae	AMF	X	
This Study	Field	Arbuscules, Vesicles, Hyphae, Microsclerotia, other RAF structures	AMF and DSE	X	