The Influence of Biological Soil Crust Inoculum on Dryland Vascular Plant Establishment in a Greenhouse Experiment

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Abstract

Biocrusts influence vascular plant species performance by modifying soil stability, hydrology, and fertility. Multiple studies suggest that biocrusts have species-specific effects on plant species performance depending on plant characteristics and ecological context and that biocrust inoculum could be used in restoration. Therefore, by promoting the performance of some plant species, but inhibiting others, biocrusts may influence vascular plant community structure. In this study, I compared emergence and establishment of C3 and C4 perennial grasses and annual and perennial forbs grown in biocrust-inoculated versus bare-soil mesocosms in a greenhouse. I hypothesized that biocrust inoculum will increase germination, growth and species richness of a plant community composed of multiple plant functional types based on increased resource availability of soil moisture due to biocrusts soil binding effects that trap moisture and decrease evaporation as well as enhanced soil N availability through biocrusts fixing atmospheric N2. A seed mix containing five plant species was sown into 32 mesocosms containing full factorial crosses of two soil treatments (biocrust-inoculated versus bare-soil) and two watering levels (high-water versus low-water). To accept or refute my hypothesis, I tested parameters incorporating plant germination, growth, and species richness. Overall plant growth results indicate biocrusts did not benefit vascular pant species with neutral effects on germination (high-water) and plant species richness but negative effects on germination (low-water) and plant growth. However, the neutral effects that biocrust inoculum has on vascular plants shows that there may be few negative effects overall, allowing restoration to occur in a holistic way that restores both vascular plants and biocrusts.
Introduction

Biological Soil Crusts (biocrusts) are soil surface communities consisting of lichens, mosses, cyanobacteria, green algae, fungi, and bacteria that reside in the top few millimeters (mm) of the soil surface and are widespread across dryland landscapes (Williams et al. 2012). Biocrusts coexist within a matrix of vascular plant species in dryland ecosystems worldwide making up approximately 12% of global terrestrial cover (Rodriguez-Caballero et al. 2018). These soil surface communities have mixed effects on plant germination and growth, that are highly dependent on plant species in addition to biocrust type (Zhang et al. 2016; Havrilla et al. 2019). Recent research suggests that biocrusts may be beneficial to vascular plant species, increasing the performance of native dryland species while inhibiting invasive species (Havrilla et al. 2019), influencing soil hydrology (Warren 2001; Chamizo et al. 2016), and increasing soil nutrients (Delgado-Baquerizo et al. 2013; Barger et al. 2016; Evans and Ehleringer 1993; Evans and Lange 2003) thus, making them a strong candidate for increasing restoration success.

While biocrust inoculum strategies have received interest as a means of restoration in dryland ecosystems (Zhang et al. 2016, Havrilla et al. 2019) the present knowledge of the effects of biocrust inoculum on native plant establishment is limited. Since the topsoil layer microbiome is important to plant growth by providing nutrients, its degradation poses challenges to restoration (Bateman et al. 2018; Golos and Dixon et al 2014) as most restored regions can be depleted of soil nutrients. Thus, it is essential to restore the microbiome itself, not just plant species. Soil microbiota have the ability to increase root efficiency (amount of mineral uptake per root unit over time (Hunt 1973)) by improving nutrient uptake, increasing root surface area via the presence of fungal hyphae, and making soil nutrients available to plants in general (Mengual et al. 2014). Biocrust inoculum has the potential to facilitate plant germination, growth, and establishment.
while improving soil function in terms of soil structure and nutrients which makes biocrust inoculum useful in restoration (Munoz-Rojas et al. 2018) as it will benefit both the soil microbial community and fertility (Xiao and Maik 2017). The addition of cyanobacteria to soil used in restoration has shown a positive outcome without inhibiting the plant establishment with carbon content increasing in degraded soils 3-fold three months after the soil was inoculated (Munoz-Rojas et al. 2018). All of these processes are an integral component of drylands and due to the millions of hectares of degraded drylands, restoration solutions need to support plant establishment and survival (Keesstra et al. 2018). Long term success is more important to restoration and the best solutions are those that will incorporate natural ecosystem functioning already present on the landscape such as interactions between the physical effects of biocrusts and vascular plant species.

Within dryland ecosystems, various physical characteristics of biocrusts create an environment beneficial to plant communities. Firstly, biocrusts can alter landscapes physically through their microtopography and effects on local hydrology (Pissolito et al. 2019; Zhang et al. 2016; Thiet et al 2014), and thereby affect rates of plant germination, growth, and survival. In natural landscapes, biocrusts can trap seeds via their physical microtopographies. It is assumed that pinnacled or rolling crusts are more effective at trapping seeds. However, increased roughness does not always benefit seed germination as it increases difficulty for seeds to root within the substrate beneath the biocrust (Zhang et al. 2016). As seed germination is dependent on the availability of water and the temperature of the soil, these microtopographies could have great effect on seedling germination (Zhang et al. 2016). Differences in the beneficial effects of a biocrust-plant relationship could result in various levels of diversity in arid landscapes (Zhang et al. 2016). These different levels of diversity can also be affected by biocrusts influence on soil moisture.
Biocrusts increase soil moisture in dryland ecosystems (Chamizo et al. 2016b). Since water availability is the most limiting resource in dryland ecosystems, biocrust effects on soil moisture could be key characteristics (Reynolds et al. 2004; Chamizo et al. 2016b). Studies show that 65% of biocrust research on soil hydrology found evaporation is reduced in biocrust compared to bare soil (Chamizo et al. 2016). When soil surface water content is high, there is a reduction in evaporation and soil moisture is retained longer due to biocrusts clogging soil pores from the establishment of cyanobacterial, moss, and lichen communities (Chamizo et al. 2013). It is clear that biocrusts improve water resource availability in drylands and this benefits both biocrusts and vascular plants as biocrusts are only physically active in contributing to soil nutrients when wet (Barger et al. 2016).

The soil nutrient effects of biocrust that pertain to this study are primarily those that alter soil nitrogen (N) content. Communities of biocrusts influence processes of N-fixation and mineralization rates (Delgado-Baquerizo et al. 2013) and contribute N inputs through biological fixation and dust capture, which they can then transform through N-fixation into N compounds that can be taken up by plants (Barger et al. 2016). Fixation of atmospheric N2 in biocrusts is a ubiquitous biogeochemical transformation, a major source of N nutrients in drylands (Evans and Ehleringer 1993, 2003) and as N is a necessary plant nutrient its presence in soil is important to plant growth and survival. These soil nutrient resources are released after the process of N-fixation in biocrusts, known as extracellular release, in which 5-70% of fixed-N are released by N-fixing species (Magee and Burris 1954; Silvester et al 1996) including N compounds that can be taken up by plants. Biocrusts thus have a positive effect on soil nutrient availability, providing more fixed-N to propagating species. Due to this, I predict that biocrust inoculation will enhance soil N availability.
Research Questions and Hypothesis

In order to better understand how biocrust restoration strategies influence native plant establishment, I addressed the question of how biocrust inoculum influences plant germination, growth, and species richness. I hypothesized that biocrust inoculum will increase germination, growth, and species richness of a plant community composed of multiple plant functional types based on increased resource availability of soil moisture due to biocrusts soil binding effects that trap moisture and decrease evaporation as well as enhanced soil N availability through biocrusts fixing atmospheric N2.

Methods

I conducted a greenhouse experiment in the University of Colorado Boulder 30th St. Greenhouse using biocrust inoculum and soil sourced from Canyonlands National Park. For this experiment, I selected five plant species based on their plant functional types and their existence within the regional species pool of the semi-arid ecosystems of southeastern Utah, from where my biocrust was sourced. I conducted germination trials on 15 possible species to determine if the seeds were viable and which showed promising rates of germination. The finalized selections were made from these original species based on data provided by the USGS Plant Database and their availability with native seed companies that provide seeds that are not fertilized and harvested in the United States. The final five species used in my experiment were two perennial grasses, *Leymus cinereus* (C3) and *Aristida purpurea* (C4); two perennial forbs, *Sphaeralcea grossulariifolia* (C3) and *Astragalus convallarius* (C4); and one summer annual, *Helianthus annuus* (C3).
Experimental Design

To address the research questions, I devised four treatments in total, two soil treatments of biocrusts versus bare-soil at two watering levels of high water versus low water to ensure germination. These treatments were applied to 32 mesocosms (2x1’ plots) with 8 replicates per treatment. Soil moisture was recorded using soil moisture probes placed beneath the soil surface in four of the mesocosms in treatments A, B, C and D. Soil moisture probes took readings every four hours for the duration of the greenhouse stage of the experiment. These readings were transferred from the device into a spreadsheet. For readability in the figures, one reading was selected from every day to include in the mesocosm. In order to not include times directly after watering – as these would have a high biased towards high soil moisture content – the reading selected was in the late afternoon as watering occurred in the morning or early afternoon. These values were plotted on a line graph that included both bare-soil and biocrust-inoculated mesocosm values for the high-water treatment mesocosms over the course of one month per mesocosm.

Each mesocosm contained 75 seeds in total, 15 of each of the five species selected. Seeds were randomly placed in the mesocosm by hand. The inoculum and the seeds were placed on the soil within two days of each other with the inoculation of the selected soils occurring first.

Plant Responses

Plant Germination and Soil Moisture

Following the placement of seeds and inoculation of soils, germination (%) and survivorship was tracked by recording germination for the first month of experimentation and survivorship of seeds germinated for the duration of the experiment. The low-water mesocosms were watered on every fifth day and the high-water mesocosms on every third day beginning the
day of seeding. Once germination of a seed occurred, the date was recorded. If a seed died after germination, the date it died was also recorded. Once the germination term of one month (30 days) ended, misting was stopped, and the soil moisture probes were placed in each mesocosm (so that misting would not contribute to water treatment levels once germination occurred). Survivorship was monitored throughout the experiment but the official surviving species in each mesocosm were those alive at the end of the greenhouse experiment.

*Plant Growth and Survival*

Height (cm) was recorded every other week beginning the week the month-long germination period ended at the four-week, six-week, and eight-week point for each live individual plant using a ruler. Height was the measurement from the base of the plant at the soil surface to the tip of all plant organs (stem, leaves, or flowers).

The surviving plants at 10 weeks were harvested and separated into their roots and shoots. The roots and shoots were placed in individually labeled coin envelopes and these envelopes and their contents was dried in a Thermo Scientific model oven at 60 degrees Celsius for 48 hours in total. Once dried, roots and shoots were removed from their envelopes, weighed, and the root-to-shoot ratio was calculated as:

\[
\text{Root} \div \text{Shoot} = R:S
\]

Species richness was recorded after each surviving species was collected following the greenhouse stage of experimentation and calculated as:

\[
\frac{\text{Number of Species}}{\text{Mesocosm}} = \text{Species Richness}
\]
Total biomass (g) was determined for all surviving species per mesocosm. These weights include all plant material and are not split by root or shoot. Drying occurred after harvesting for 48 hours.

Plant density per mesocosm was determined for all surviving species at the end of the greenhouse stage of experimentation and calculated as:

\[
\text{Number of Individuals/Area} = \text{Density}
\]

**Statistical Analysis**

Statistical analysis was conducted using R Studio under the version of 3.5.2 “eggshell igloo.” For each figure, the mean and standard error were calculated for error bars using the dataSE function. Packages used to create these figures were ggmesocosm, ggmesocosm2, and plyr packages.

Germination results were analyzed using R and standard error was calculated using the dataSE function. Significance was calculated using a two-way ANOVA and Tukey test. Ending growth in terms of density, root-to-shoot ratio, and biomass was analyzed using R and standard error was calculated using the dataSE function. Significance for growth was calculated using a two-way ANOVA. Parameters associated with soil moisture were analyzed using R with standard error calculated using the dataSE function. Significance was calculated using a one-way ANOVA. For ending mesocosm species richness, results were analyzed using R and statistical analysis were performed using a one-way ANOVA. Standard error was calculated using the dataSE function.
Results

Germination

In the high-water mesocosms, biocrust inoculation did not increase the percent of germination relative to the bare-soil mesocosms ($p = 0.366$) and there was no significance within species by treatment ($p = 0.866$) (Figure 1). Thus, biocrust inoculum did not increase percent of germination in inoculated mesocosms. For the low water mesocosms, biocrust-inoculated mesocosms were significantly lower in percent germination than bare-soil ($p = 0.000737$) and there was a significant difference within species between treatments ($p = 0.0061$) with *A. purpurea* showing lower germination rates in the biocrust-inoculated mesocosms than the bare-soil ($p = 0.0003$).

![Figure 1](image)

Percent germination per species for the high water mesocosms (percent germination mean per species ± SE) shows no significance between biocrust-inoculated vs. bare-soil mesocosms ($p = 0.336$) and no significance within species by treatment ($p = 0.866$).
Plant Growth and Survival

Plant height after eight weeks were significantly lower in the biocrust-inoculated versus the bare-soil mesocosms (p = 1.58e-08). However, there is not a significant difference between treatments within species (p = 0.055).
Eight week species height per treatment by species (height mean per treatment by species ± SE) shows a significant difference between biocrust-inoculated vs. bare-soil mesocosms ($p = 1.58e-08$) with lower heights in biocrust-inoculated mesocosms than bare-soil. However, there is no significant difference within species between treatments ($p = 0.055$).

Root-to-shoot ratio showed no significant difference between the biocrust-inoculated and bare-soil mesocosms ($p = 0.462$) and no significant difference in root-to-shoot ratio between treatments within species ($p = 0.961$) (Figure 4).
Root to shoot ratio per treatment by species (root:shoot mean per treatment by species ± SE) shows no significant difference between the biocrust-inoculated vs. bare-soil mesocosms ($p = 0.462$) and no significant difference within species between treatments ($p = 0.961$).
Species richness are not significantly greater for the biocrust-inoculated versus the bare-soil mesocosms with a p-value of $p = 0.199$ (Figure 5).

![Species Richness per Treatment](image)

Species richness (Number of Species / Mesocosm = Species Richness) by treatments (species richness mean per treatment ± SE) shows that there is no significant difference between biocrust-inoculated and bare-soil mesocosms ($p = 0.199$)

Total, root, and shoot biomass were all significantly lower biomass in biocrust mesocosms in comparison to bare-soil mesocosms. Total biomass showed a significant difference between biocrust-inoculated versus bare-soil mesocosms ($p = 1.18e-06$) and a significant difference in total biomass within species between treatments ($p = 1.98e-11$) (Figure 6). *H. annuus* showed the largest difference in total biomass with biomass in the biocrust-inoculated mesocosms being lower than the bare-soil ($p < 0$). Root biomass results showed a significant difference between biocrust-inoculated and bare-soil mesocosms ($p = 0.00144$) and
between treatments within species \( (p = 0.036) \) (Figure 7). \textit{H. annuus} again shows a significantly lower root biomass in the biocrust-inoculated mesocosms than the bare-soil \( (p = 0.025) \). This shows that biocrust inoculum does decrease root biomass (as this would correlate to a smaller root-to-shoot ratio) but it does not in turn increase shoot biomass with shoot biomass results showing that biocrust-inoculated mesocosms have significantly less shoot biomass than bare-soil mesocosms \( (p = 1.34e-06) \). Additionally, there is a significant difference in shoot biomass between treatments within species \( (p = 4.15e-13) \) (Figure 8) with \textit{H. annuus} having significantly lower biomass in the biocrust-inoculated mesocosms than bare-soil \( (p < 0) \). In relation to density, there is not a negative correlation between biomass and density but a weak positive correlation \( (R^2 = 0.558) \) (Figure 10) and thus the negative effects seen are not a result of greater plant density increasing competition.

**Figure 6**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total Biomass by Species and Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>bare soil</td>
<td>bare soil</td>
</tr>
<tr>
<td>biocrust</td>
<td>biocrust</td>
</tr>
</tbody>
</table>

\[ p_{\text{Treatment}} = 1.18e-06^{***} \]
\[ p_{\text{Species:Treatment}} = 1.98e-11^{***} \]
Total root biomass by species per treatment (root biomass mean per species by treatment ±SE) shows that the biocrust-inoculated mesocosms have significantly lower root biomass than the bare-soil mesocosms ($p = 0.00144^{**}$) and a significant difference within species between treatments ($p = 0.036^*$) specifically in *H. annuus* ($p = 0.025$).
Total shoot biomass by species per treatment (shoot biomass mean per species by treatment ±SE) shows that the biocrust-inoculated mesocosms have significantly lower shoot biomass than the bare-soil mesocosms ($p = 1.34e^{-06}$) and a significant difference species within species between treatments ($p = 4.15e^{-13}$) specifically in *H. annuus* ($p < 0$).
Plant density was not significantly different between the inoculated and bare-soil mesocosms ($p = 0.667$) for density in plants per $m^2$ but and shows that there is no significant difference between treatments within species ($p = 0.463$) (Figure 9).

Density (Number of Individuals/mesocosm area = Density) shows density by species per treatment (density mean by species per treatment ± SE) shows that there is no significant difference between the inoculated and bare-soil mesocosms ($p = 0.667$) and shows no significance between treatments within species ($p = 0.463$).
Soil Moisture

Results from soil moisture probes show that soil moisture was not higher in the biocrust-inoculated mesocosms. In the first month, there was significantly higher soil moisture in the bare-soil mesocosms than the inoculated mesocosms (0.00155) (Figure 11). In the second month of soil moisture readings no significant differences were detected between the inoculated and bare-soil mesocosms (p = 0.879) (Figure 12). Additionally, most species germinated around days 15-20 of the first month and it was after this that bare-soil and biocrust-inoculated soil moistures were more similar continuing into month two where there was no significant difference between treatments’ soil moistures.

**Figure 10**

![Correlation Between Biomass and Density](image)

Figure 10 shows that there is not a negative correlation between biomass and density with density slightly increasing as biomass increases.

Soil Moisture

Results from soil moisture probes show that soil moisture was not higher in the biocrust-inoculated mesocosms. In the first month, there was significantly higher soil moisture in the bare-soil mesocosms than the inoculated mesocosms (0.00155) (Figure 11). In the second month of soil moisture readings no significant differences were detected between the inoculated and bare-soil mesocosms (p = 0.879) (Figure 12). Additionally, most species germinated around days 15-20 of the first month and it was after this that bare-soil and biocrust-inoculated soil moistures were more similar continuing into month two where there was no significant difference between treatments’ soil moistures.
Soil moisture in the first month shows that the biocrust-inoculated mesocosms have lower soil moisture as the bare-soil mesocosms trend higher in soil moisture throughout the first month ($p = 0.00155$). Differences between soil moisture decrease once a majority of plants begin to germinate around days 15-20 of the first month.

Soil moisture in the second month of treatment, after the growth of the inoculum, shows that the biocrust-inoculated mesocosms and the bare-soil mesocosms now have no significant difference ($p = 0.879$).
Discussion

This experiment on the effects of biocrust inoculum on the germination, growth, and survival of five dryland plant species in a greenhouse experiment indicates that effects of biocrusts on plants may not be reproducible in a short term, controlled environment experiment. However, the negative results seen could be context-dependent and result largely from various effects on controlled greenhouse conditions such as temperature variability and seedling viability. One could speculate that biocrust’s functional impacts on natural landscapes involve and ability to decrease the establishment of weedy or exotic species (Hernandez and Sndquist 2011; Li et al. 2006; Morgan 2006).

Germination

My results indicate that our hypothesis that germination is increased by the presence of a biocrust inoculum was rejected as there was not significantly higher percent of germination in the biocrust inoculate mesocosms in comparison to the bare-soil mesocosms regarding the high-water mesocosms (Figure 1). Additionally, our hypothesis is rejected because the low water treatment exhibited significantly lower rates of germination for biocrust-inoculated mesocosms (Figure 2). This is consistent with past research which has shown that that the overall effects of a biological soil crust on germination were not significant (Havrilla et al. 2019). As observed, biocrusts can benefit vascular plant germination through increasing nutrient levels and water retention (Zhang et al. 2016) However, a reason for the lack of significance between treatments in my research may be the controlled greenhouse environment. Once seeding occurred, daily misting likely kept seeds moist regardless of whether or not a biocrust was present. The influence of biocrusts on vascular plant performance can depend on biocrust type, plant species, and local climate (Zhang et al. 2016). Within this small-scale environment, the biocrust inoculum, species
chosen, and climate were all controlled to exhibit baseline level interactions under such controlled conditions. However, it is possible that research conducted in natural environments could yield different results as there would be larger variation in climate and local heterogeneity in microtopography and plant species. Despite this, it is clear that germination plays a role in soil moisture as it is after a majority of species germinated (Figure 11) that there were fewer differences between the soil moisture levels of the biocrust-inoculated and bare-soil mesocosms and thus the no longer dormant germinating seeds were using more water.

The finding that percent germination was lower in the biocrust versus bare-soil in the low water treatment may have been due to water resources being too low as the high and low water treatments were part of experimental design to ensure survival in at least one set of inoculated mesocosms and bare-soil mesocosms respectively. Additionally, there are differences in biocrust’s soil hydrology effects due to seasonal variation in natural environments (Warren 2001) and when a biocrust is exposed to prolonged dry periods, crusts crack and the crusts lose their ability to bind soil particles (Warren 2001). This results in water retention decreasing as the lack of bound soils would leave them with evaporation rates similar to bare-soil. Moreover, as the biocrust inoculum propagated it required water and both the seeds and inoculum could have been taking up water during the misting period making competition for water high. Had there been more watering via irrigation during misting as there was in the high water mesocosms these effects may not have been seen but due to fewer days of irrigation watering in the low water treatment, the biocrust-inoculated mesocosms experienced significantly lower germination rates than the bare-soil. Additionally, there were lower rates of germination specifically in *A. purpurea* in the biocrust-inoculated mesocosms in comparison to the bare-soil mesocosm. As *A. purpurea* is a C₄ grass species, this was not expected as in past research C₄ grasses were benefited
compared to C3 by the presence of a biocrust (Havrilla et al. 2019). Again, this could largely be due to the biocrust inoculum requiring water to propagate at the same time as the seeds required water for germination and water levels in the low water treatment may have simply been too low.

*Plant Growth and Survival*

Measures of plant growth and survival includes root-to-shoot ratio, total root and shoot biomass, plant height, density, and species richness. For root-to-shoot ratio, there was no significant difference between the biocrust-inoculated and bare-soil mesocosms (Figure 4) and thus the hypothesis that biocrust inoculum decreases root-to-shoot ratio (smaller roots) in the selected species is rejected. Under different resource conditions, root-to-shoot ratio can increase or decrease. Since plants use their roots to take up water, root biomass can be an indicator of soil nutrient levels and water availability (Xu et al. 2015). The findings in my experiment between biocrust and bare-soil suggests that the inoculum did not increase competition. Within ecosystems, resource availability can alter competition. Higher resource availability within soils can result in greater competition with more species able to propagate and survive (Kardol et al. 2012). Furthermore, the role of competition in arid landscapes is relatively important, with competition leading to differences in plant density, growth, and survival with more species in a smaller area resulting in more competition (Fowler et al. 1986). Within arid ecosystems, the interactions between the many plants that may be on the landscape and biocrust correlate with productivity (Dettweiler-Robinson et al. 2018). With no difference between the inoculated and non-inoculated mesocosms, there is evidence that the biocrust inoculum is not increasing competition and impeding growth. More importantly, if the biocrust inoculum propagates with establishing plant communities, it can facilitate growth long term. Though this experiment was short term, literature indicates that biocrust could be a long-term solution to restoration success.
as when in association with a biocrust, native species whose biocrust is removed experience a
decline in growth and thus biomass (Dettweiler-Robinson 2018).

My results for total, root, and shoot biomass reject my hypothesis that biomass will be
increased by the presence of a biocrust inoculum because the biocrust-inoculated mesocosms
have significantly lower biomass than the bare-soil mesocosms (Figure 6). In correlation to
density, there was no significant difference between species density in the biocrust-inoculated
mesocosms versus the bare-soil mesocosms (Figure 6). If an increase in species density
correlates to a decrease in biomass these results would be indicative of competition among plant
species as the biocrust would support more plants and thus increase competition among them.
This would be consistent with a recent study by Kidron (2019) in which it was shown biocrusts
can reduce individual plant mass while benefitting cover and plant survival through improving
plant growth conditions in drylands (Chen and Duan 2015) However, as seen in Figure 10, there
is no negative correlation between biomass and density. Thus, the biocrust itself may be the
cause for increased competition. Additional support of this view can be seen in the root and
shoot biomass as both were significantly smaller in the biocrust-inoculated mesocosms than the
bare-soil mesocosms and H. annuus showed significantly lower biomass in the biocrust-
inoculated than bare-soil mesocosms (Figure 7; Figure 8). As for root development, physical and
chemical crusting on soils can inhibit root penetration (Chartres 1992) and a combination of
physical and biological crust effects can be dependent on crust development with most results
being intermediate (Belnap et al. 2001). Results indicating growth impediment by biocrusts
were also seen by Havrilla et al. (2019) as biocrust presence decreased overall plant community
growth by 42%. While the duration of greenhouse experimentation could be lengthened in future
experiments, this data is important in understanding how propagating inoculum affects the early growth stages of vascular plants.

Plant height was not greater in the biocrust-inoculated mesocosms and thus my hypothesis is rejected as results show heights in the biocrust-inoculated mesocosms being significantly lower than the bare-soil mesocosms (Figure 3). The observed significant difference between species in plant height is largely due to the difference in growth rates between species. As for differences between biocrust versus bare-soil treatments, height is clearly impeded in the biocrust-inoculated mesocosms and this corresponds to the lower biomass in inoculated mesocosms. Again, this research further shows the decrease in plant growth as recorded by Havrilla et al. (2019). While there have been multiple studies that test the relationship between biocrusts and vascular plants (Belnap et al. 2001; Zhang et al. 2016) some have shown that the removal of biocrusts can result in an increase in vascular plant growth in the field (Zhang et al. 2016; Beyschlag et al. 2008; Hernandez and Sandquist 2011; Langhans et al 2010; Li et al. 2006). This suggests that while there are benefits to biocrust, there may also be negative effects as biocrusts can slow growth processes and expose seedlings to environmental stress for longer as they require more time to establish themselves and ensure a greater chance of survival (Escudero et al. 2007; Zamfir 2000). Thus, it is clear that negative effects to plant growth were seen as a result of more environmental stress on the establishing research species. Moreover, factors of stress were not alleviated by higher soil moisture as there was no significant difference between soil moisture between inoculated and bare soil mesocosms in the second month of experimentation after the inoculum had propagated (Figure 12).

My results show that overall plant density is not higher in the inoculated mesocosms (Figure 9) versus the bare-soil mesocosms and thus, the hypothesis that density will be increased
by biocrust inoculum is rejected. Additionally, there was no difference within species between treatments (Figure 9). Again, this could be a factor of soil moisture (Figure 11; Figure 12) as the biocrust mesocosms (when the inoculum had propagated) are not significantly different in soil moisture. In addition to this, greenhouse conditions may have resulted in optimal seedling germination and survival and thus similar density in both treatments. As shown previously, germination rates from this experiment are not significantly different between treatments and therefore it is expected that density is not greater in correlation with germination in the high-water mesocosms as there was no difference between treatments. Furthermore, the lack of survival in the low water mesocosms and thus lack of cover is indicative of poor plant recruitment in low water conditions. As found by Moreira-Grez et al. (2019), few species performed well on all levels of plant fitness in a semi-arid restoration project using soil microbe inoculum. This suggests that inoculation procedures will need to consider climatological factors such as precipitation frequency at various times of year and or the use of an irrigation system in restoration to establish species.

Species Richness results indicate that the hypothesis that biocrust inoculum will increase the species richness in inoculated mesocosms is rejected as there is no significant difference between the biocrust-inoculated and bare-soil mesocosms (Figure 5). Species abundance and number of species is a mechanism of understanding a community and the drivers of ecosystem functioning (Castillo-Monroy et al. 2011). In addition, the effect of species richness on a given ecosystem’s multifunctionality can depend on the evenness and spatial pattern of species along with species composition (Maestre et al. 2012). While some studies show greater resource availability increases competition (Fowler 1986), it is most prominent when water availability is low. As these figures are based on the high-water mesocosms, water was present and thus may
have resulted in the lack of significant difference between soil moisture once the inoculum propagated (Figure 12).

While survival favored biocrust-inoculated mesocosms in the high-water treatment, there was no survival in the low-water mesocosms for either the biocrust-inoculated or bare-soil mesocosms thus species richness is based on the high-water mesocosms. However, biocrust propagation in low-water mesocosms continued despite water availability being too low for plant recruitment. This suggests, as past research has shown, that biocrusts react to low resource availability differently than vascular plant species (Pissolito et al 2019). In addition, the biocrust inoculum likely had a higher resistance to low-water conditions as it can be propagated from a dormant state as it was in this experiment.

**Conclusion**

This study produced results for a controlled environment and species selection. While overall plant growth results indicate biocrusts did not benefit vascular plant species, further research is needed to determine the best biocrust inoculum type, resource availability, and native vascular plant species to use in restoration in the field. Moreover, any negative effects of biocrusts on early vascular plant growth may be a component of their functionality on arid landscapes in decreasing establishment of weedy and exotic species, thus increasing overall habitat resistance (Hernandez and Sndquist 2011; Li et al. 2006; Morgan 2006). However, those effects that were neutral for germination and species richness show that biocrust inoculum may not have a large number of negative effects overall.

The close association vascular plants in dryland ecosystems have with biological soil crusts is an indication that crust restoration is equally as important as vegetative restoration
(Roncero-Ramos et al. 2019). Thus, biocrust recovery in the form of inoculation in correlation with vegetative plant recovery should be strongly considered as a method of restoration in arid lands. More importantly, current rates of restoration in drylands are low and innovative techniques involving plant restoration, soil rejuvenation, and restoration success are the most important to implement in the coming future (Munoz-Rojas 2018). In addition, if biocrusts are invaluable in ways such as these it is important to note they could decrease by 40% in the next few decades (Havrilla et al. 2019). While climate change can influence vascular plants, it can also greatly affect biocrusts (Chen et al. 2019). Due to the important role biocrusts play in community assemblage in arid ecosystems, their loss due to climate change could greatly affect these ecosystems. Therefore, the most successful restoration techniques are those that incorporate the natural coexistence in drylands between soil microbial communities and plant species taking into account how species interact with one another depending on biocrust type, vascular plant species present, resource availability, and biocrust inoculum type used.

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Literature Cited


