

THESIS

EXPLORING INTERACTIONS AMONG BIOLOGICAL SOIL CRUSTS, PLANT  
GERMINATION, AND MORPHOLOGICAL SEED TRAITS: IMPLICATIONS FOR PLANT  
COMMUNITY ASSEMBLY AND DRYLAND RESTORATION

Submitted by

John Bacovcin

Department of Forest and Rangeland Stewardship

In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Fall 2023

Master's Committee:

Advisor: Caroline Havrilla

Troy Ocheltree  
Kelly Wrighton  
Mark Paschke

Copyright by Jonathon Joseph Bacovcin 2023

All Rights Reserved

## ABSTRACT

### EXPLORING INTERACTIONS AMONG BIOLOGICAL SOIL CRUSTS, PLANT GERMINATION, AND MORPHOLOGICAL SEED TRAITS: IMPLICATIONS FOR PLANT COMMUNITY ASSEMBLY AND DRYLAND RESTORATION

Arid and semi-arid (dryland) ecosystems make up over 40% of our planet's terrestrial surface and are incredibly vulnerable to land degradation. To combat dryland degradation, active plant and soil restoration is often needed and the role of plant-soil microbe interactions can be key to dryland restoration trajectories. Within drylands, biological soil crusts (biocrusts), collections of cyanobacteria, algae, lichen, and moss are key surface communities that influence soil processes (e.g., stability, nutrient cycling, hydrology) and can thereby strongly influence recruitment of dryland plants. These biocrusts may interact with plant functional traits (i.e., seed morphological traits), and these interactions can influence germination. However, much is still unknown about mechanisms that underlie these interactions and how plant functional traits mediate effects of biocrusts on plant germination. To investigate these knowledge gaps, I conducted two studies: (Chapter 1) a global meta-analysis of the role of morphological seed traits in determining biocrust effects on germination, and (Chapter 2) a full-factorial greenhouse study examining the effects of biocrust inoculum cover treatments and plant functional traits on plant recruitment to investigate questions about how biocrust heterogeneity and biotic components of biocrusts in the context of restoration.

To explore effects of morphological seed traits on plant germination responses to biocrusts (Ch. 1), we compiled a global database of 491 studies of biocrust effects on plant germination encompassing 101 unique plant species and their associated morphological seed traits. For the greenhouse study (Ch. 2) we seeded two seed mixes on three different inoculum cover treatments (i.e., 0%, 30%, and 100%) using both biologically active (live) and autoclaved biocrust inoculum, to assess effects of cover heterogeneity, biological biocrust activity, and plant functional traits on percent germination. Results from the meta-analysis showed that morphological seed traits do mediate plant germination responses to biocrusts, and that, in general, germination of smaller seeded species with appendages was increased by biocrusts. Results from the greenhouse study showed that, in a restoration context, increasing cover of biocrust inoculum increases plant germination, and that these effects were explained by physical rather than biotic effects of inoculum on germination. As in Chapter 1, we found that biocrusts effect on germination differed across plant functional groups and that seed traits also influenced germination responses to biocrust inoculum cover treatments. Together, both studies showed that morphological seed traits mediate effects of biocrusts on plant germination. These findings increase understanding of the role of biocrusts in determining dryland plant community assembly and have implications for dryland restoration.

## ACKNOWLEDGEMENTS

I would like to thank so many people for their help while I wrote this thesis and completed my research. Without their support I would not have been able to complete this. First, I would like to thank my advisor, Dr. Carrie Havrilla, whose patience and guidance have been invaluable in my growth not only as a scientist but as a student, teacher, and writer. Second, I would like to thank all of the current and past members of the Dryland Ecology and Management Lab; Louisa Kimmell, Amy Gill, Ember Bradbury, Nora Bales, Alyssa Johnson, Mabruka Hadya and Emily Swartz. Your support and encouragement, not to mention help with these projects was truly inspiring and you are quite frankly the greatest cohort, lab, and friends a person could ask for. I would also like to thank the members of my committee, Dr. Troy Ocheltree, Dr. Kelly Wrighton, and Dr. Mark Paschke, whose feedback and assistance have only strengthened my research and my knowledge.

## TABLE OF CONTENTS

ABSTRACT.....	ii
ACKNOWLEDGMENTS.....	iv
INTRODUCTION.....	1
CHAPTER 1- Morphological seed traits mediate the effect of biocrusts on plant germination: a meta-analysis.....	6
Introduction.....	6
Methods.....	10
Original Database of Biocrust-Plant Germination Studies from Havrilla et al. (2019).....	10
Candidate Morphological Seed Trait Data Acquisition.....	11
Original Germination Model from Havrilla et al. (2019).....	14
Preliminary Data Exploration and Candidate Morphological Seed Trait Variable Selection.....	15
Mixed Effects Meta-Regression.....	16
Results.....	17
Database Summary.....	17
Candidate Variable Selection.....	18
Effects of Biocrust and Key Moderators on Plant Germination.....	19
Seed Mass Interacts with Biocrust Community Type, Soil Reference State, and Plant Origin to Control the Effects of Biocrusts on Germination.....	23
Seed Appendages Control the Effects of Biocrusts on Germination for Different Biocrust Types and Native vs. Non-Native Species.....	27
Seed Morphological Traits May Affect C4 Grass Interactions with Biocrusts.....	29
Discussion.....	30
Seed Traits Influences Germination Outcomes on Different Biocrust Community Types.....	32
Seed Traits Influences the Effects of Biocrusts on Native vs. Non-Native plant germination.....	34
Seed Traits may Mediate the Germination Responses of Different Plant Functional Groups.....	36
Limitations and Further Research Directions.....	38
Seed Traits Improve Predictions of Outcomes of Biocrust Plant Interactions and Plant Community Assembly Processes in Drylands.....	40
CHAPTER 2- Biocrust inoculum and seed traits interact to shape native plant community assembly.....	42
Introduction.....	42
Methods.....	48
Biocrust Collection Methods.....	48
Experimental Setup.....	48
Seed Mix Creation and Considerations.....	50
Data Acquisition and Analysis.....	52
Results.....	54

	Biocrust Inoculum Cover Influences Percent Germination.....	54
	Biocrust Sterilization Does Not Influence Percent Germination.....	56
	Plant Functional Groups Influence Percent Germination in Response to Differing Biocrust Inoculum Cover Percentages.....	58
	Community Weighted Mean of Seed Mass Influences Percent Germination Response to Differing Biocrust Inoculum Cover Percentages.....	61
Discussion.....		63
	Biocrust Inoculum Cover Mediates Plant Germination.....	64
	Biocrust Inoculum Sterilization Does Not Influence Germination.....	67
	Plant Functional Group Interacts with Biocrust Inoculum Cover to Influence Germination.....	68
	Seed Mass Interacts with Biocrust Inoculum Cover to Influence Germination.....	70
	Future Research Directions and Limitations.....	72
	Biocrust Inoculum Cover, Sterilization, and Plant/Seed Traits Interact to Influence Germination.....	74
CONCLUSIONS.....		76
REFERENCES.....		79
APPENDIX 1.....		88
APPENDIX 2.....		91

## INTRODUCTION

Arid and semi-arid ecosystems (drylands) comprise over 40% of Earth's landmass and support the cultures and livelihoods of almost 38% of the world's population (Právělie, 2016). Yet, global drylands are highly susceptible to land degradation with ~70% of drylands currently degraded (Funk et al. 2017). Land degradation negatively affects dryland plant and soil microbial communities (Ye, Fei et al. 2022) as well as other abiotic factors like soil health (Právělie, 2016) thereby resulting in declines in ecosystem services including plant productivity, forage availability, and plant diversity (Zaddy, Eldridge, and Bowker, 2016). To restore these ecosystem services land managers often engage in active restoration efforts, defined as the process of applying active management techniques to overcome abiotic and biotic barriers that limit ecosystem recovery (Gann et al. 2019). These efforts often target restoration of degraded plant communities through seeding (Palma and Laurance, 2015). Unfortunately, dryland plant restoration efforts are often unsuccessful due to harsh growing conditions and soil degradation (D'Odorico et al. 2007; Turnbull et al. 2012; Gann et al. 2019; Palma and Laurance, 2015). Improved understanding of plant-soil interactions will be important for improving our understanding of plant community assembly processes in drylands (Harris, 2009; Potthoff et al. 2006) and plant restoration outcomes (Cross and Aronson, 2023).

The role of plant-soil interactions may be critical to plant community assembly processes in drylands (Mahmoudi et al. 2021). There are many factors that affect plant community assembly ranging from abiotic factors including climate, soil texture and health, and resource availability to plants (HilleRisLambert et al. 2012). Such physical factors can determine what plants initially germinate and establish at a site (Kraft et al. 2015). Biotic factors can directly influence plant

recruitment (Copeland and McDonald, 2012) and subsequently can control which established plants continue to survive to establish into a long-term community (Amat, Cortina, and Zubcoff, 2015). For example, plant interactions with the soil microbial community can play important roles in plant community assembly processes (Bruno, Stachowicz, and Bertness, 2013, Bowker et al. 2022). The first filter that needs to be surpassed is seed dispersal into the site (Copeland and McDonald, 2012). Germination is the next step in the plant community assembly process. To effectively germinate, seeds need three basic things: sufficient water, sufficient nutrients, and adequate temperatures (Copeland and McDonald, 2012). In dryland ecosystems, the main limiting factor is the availability of water (Naorem et al. 2023; Husein et al. 2021). Dryland ecosystems, in North America often get relatively low amounts of precipitation often getting pulses through monsoonal, or seasonal rains (Kipkemoi et al. 2021). Drylands generally have low water retention rates (Kipkemoi et al. 2021) and are relatively low in nutrients (Kaushal & Wani, 2016) posing additional barriers to seed germination. Soil microbial communities can aid in both water retention (Eldridge et al. 2020) and soil nutrient availability (Barger et al. 2016), and may thus be important for alleviating barriers to germination in drylands.

In drylands, biological soil crusts (biocrusts), a collection of cyanobacteria, algae, lichen, and mosses, inhabit the top few centimeters of mineral soil surface (Weber et al. 2022). Biocrusts are key microbial communities within drylands and alter soil resources important to plant germination and recruitment. For example, biocrusts alter soil moisture by increasing infiltration time extending access to water (Eldridge et al. 2020). This increased soil moisture can be a signal to seeds that there is sufficient water to germinate and can promote establishment (Copeland and McDonald, 2012). These biocrust communities can also affect soil temperature (Rutherford et al. 2017; Xiao and Bowker 2020). Biocrusts also modify soil nutrient availability, for example, some

biocrust organisms (e.g., cyanobacteria and lichens) are capable of fixing dinitrogen ( $N_2$ ) and can thereby increase soil nutrients (Barger et al. 2016). Increased soil N can promote germination of some plant species (Leghari et al. 2016) and promote plant growth and establishment (Kaushal & Wani, 2016). Biocrust communities can also modify soil temperature. For example, dark pigmented biocrusts dominated by lichens and cyanobacteria often increase soil surface temperatures by decreasing soil surface albedo (Ruthherford et al. 2017; Xiao and Bowker 2020). This increase in temperature could be a key signal in seed germination (Copeland and McDonald, 2012).

Biocrust effects on plant germination have been shown to be mixed, whereby biocrusts can have positive, negative, or neutral effects on germination depending on biocrust and plant characteristics (Havrilla et al. 2019). A recent meta-analysis by Havrilla et al. (2019), showed, for example, that lichen dominated biocrusts tended to inhibit the germination of plants while other biocrust communities (i.e., cyanobacteria dominated, moss dominated, and mixed communities) had no significant effect on germination, and varied depending on plant functional type and origin. Specifically, biocrusts inhibited the germination of C4 grasses and non-native plant species overall (Havrilla et al. 2019). A different study also found that biocrusts inhibit the germination of non-native plant species as well (Slate, Callaway, and Pearson, 2019). While these studies revealed patterns in biocrust mediation of plant germination, we continue to lack deeper knowledge of the mechanisms underlying these interactions. One primary hypothesis posited by the researchers was that morphological seed traits (e.g., seed mass, seed shape, presence of appendages) mediate outcomes of germination responses to biocrusts (Havrilla et al. 2019; Slate, Callaway, and Pearson, 2019), but this hypothesis has yet to be rigorously tested.

Understanding mechanisms underlying the effects of biocrusts on germination will be critical to increasing understanding of dryland plant community assembly processes as well as reassembly processes during dryland restoration projects in which biocrusts and plants are being restored in tandem. Seeding of native plant species in drylands is a common strategy in dryland restoration (Palma and Laurance, 2015) but is often unsuccessful, resulting in low rates of seedling recruitment due in part to soil degradation (Shackleford et al. 2021; Funk et al. 2017). Biocrust inoculation, often achieved by salvaging biocrusts from disturbed areas, homogenizing them into smaller crumbles, and spreading inoculum on degraded areas to promote biocrust recolonization, is an increasingly common restoration practice in drylands that can accompany plant seeding efforts, (Antoninka et al. 2020). Due to the complex interactions between biocrusts and plant germination and establishment in drylands, work is needed to understand how joint biocrust and plant restoration treatments may affect plant recruitment and community assembly. Further, understanding how morphological seed traits affect plant responses to biocrust inoculum treatments could assist with predicting community reassembly processes in the context of dryland restoration.

To address the knowledge gaps of how morphological seeds traits influence plant germination responses to biocrusts, and how plant functional traits interact with biocrust inoculum during restoration, this thesis includes two studies:

1. A global meta-analysis of how morphological seed traits influence the plant germination responses to biocrusts, and
2. A full-factorial greenhouse experiment that examines the interactions between biocrust inoculum cover, the relevance of an intact biological vs physical component of biocrust

inoculum on plant germination, and the interactions between seed/plant traits and biocrust inoculum and how they influence seed germination.

# CHAPTER 1 - MORPHOLOGICAL SEED TRAITS MEDIATE THE EFFECTS OF BIOCRUSTS ON PLANT GERMINATION: A META-ANALYSIS

## **Introduction**

Understanding the drivers of community assembly remains a consistent goal in plant ecology. A number of abiotic and biotic factors control which plant species establish and persist in a given environment (Kraft et al. 2015; Amat, Cortina, and Zubcoff, 2015; Balazs et al. 2020). Abiotic factors such as climate, soil condition, and availability of key resources (i.e., soil moisture, nutrient availability, and space; HilleRisLambert et al. 2012) first control which species out of a regional species pool can germinate and establish at a site (Kraft et al. 2015). Secondly, biotic factors including competitive and facilitative plant-plant interactions also affect the ability of plants to persist at the site and ultimately determine plant community composition (Amat, Cortina, and Zubcoff, 2015). Plant-microbe interactions may also influence plant community assembly processes (Bruno, Stachowicz, and Bertness, 2013, Bowker et al. 2022). Soil microbial communities play a critical role in shaping the physical soil environment by modifying the soil structure and the availability of resources important to plant germination and development (Yang, HilleRisLambers, and Ruesink, 2016). Plant-soil microbial interactions can result in positive (Eldridge et al. 2021) and negative (Hoose et al. 2022) effects on plant germination and recruitment depending on species and ecological context. For example, plant pathogens can attack seed when they are dormant and limit potential germination (Hoose et al. 2022). Conversely, facilitative plant-microbe interactions (e.g., mycorrhizal fungi) can benefit plant recruitment and performance. For example, arbuscular mycorrhizal fungi (AMF) often form mutually beneficial relationships with host plants and can allow the plant to access nutrients that would otherwise be unavailable, and thereby, can increase plant growth (Trivedi et al. 2020).

Plant-microbe interactions may be particularly important for determining plant germination and/or recruitment in resource-limited environments such as Earth's arid and semiarid (dryland) regions (Mahmoudi et al. 2021). All seeds require adequate moisture, ~~nutrients~~, and temperature to successfully germinate, with the amounts of these resources varying by plant species and ecological context (Baskin and Baskin, 1998). In drylands, water scarcity is the main factor limiting plant recruitment (Naorem et al. 2023, Husein et al. 2021). Soil moisture affects the rate at which soil microbes respire (Kaushal & Wani, 2016). This respiration affects the rate that nitrogen and other soil nutrients enter the soil to become available for plants (Kaushal & Wani, 2016). Soil temperature too affects the respiration of soil microbes (Dacal et al. 2019). Soil microbes can adapt to higher soil temperatures (Dacal et al. 2019) and higher soil temperatures can affect the germination rates of seeds (Baskin and Baskin, 1998).

In drylands, surface dwelling biocrusts mediate key ecosystem functions and soil resources important to plant recruitment (Havrilla et al. 2019). Biocrusts are an interaction between the soil surface and photoautotrophic (e.g., cyanobacteria, algae, lichens, and bryophytes) and heterotrophic (e.g., bacteria, fungi, and archaea) organisms, which reside on the surface and in the top millimeters of the soil (Weber et al. 2022). Biocrusts can affect plant germination by modifying the physical and biotic conditions important for germination for example, biocrusts increase physical soil stability by exopolysaccharides or EPS which function like glue, holding the soil together (Delgado-Baquerizo et al. 2013) and providing protection against wind and water erosion (Parwani, Bhatt, and Singh, 2021). Biocrusts also influence soil hydrology, reducing water runoff and increasing moisture storage (Eldridge et al. 2020). Soil nitrogen is also often increased by the presence of biocrusts. Biocrusts play a key role in nitrogen cycling within dryland communities as some cyanobacteria and lichen taxa within biocrusts can fix atmospheric dinitrogen (Barger et al.

2016, Belnap, Prasse, and Harper, 2001). Soil temperature and surface albedo is also impacted by biocrusts. For example, dark pigmented biocrusts often increase soil surface temperature by decreasing soil surface albedo (Ruthherford et al. 2017; Xiao and Bowker 2020). Biocrusts may also influence germination through direct biotic mechanisms. For example, microorganisms within biocrusts may be critical in breaking the seed dormancy of some plant species (Eldridge et al. 2021). Given the physical and biological modifications of the soil environment, biocrusts may be important mediators of plant recruitment (Havrilla et al. 2019) and community assembly processes (Bowker et al. 2022) in drylands.

A recent global meta-analysis performed by Havrilla et al. (2019) found that germination of dryland plant species is affected by the presence of biocrusts, with variable effects based on the composition of the biocrust community (i.e., cyanobacteria, lichen, moss, or mixed dominated crust communities), plant origin (i.e., native versus non-native to the study region), and plant functional group (i.e., C3 grasses, C4 grasses, Nitrogen-fixing forbs, Nitrogen-fixing woody plants, Non-nitrogen-fixing forbs, and Non-nitrogen-fixing woody plants). For example, overall lichen dominated biocrusts decreased plant germination, while other biocrust types did not affect germination. Biocrust effects on plant germination also differed across plant functional groups with the strongest inhibition of germination in C4 grasses. The study also found that biocrusts decreased germination of non-native species but had neutral effects on native species.

Havrilla and colleagues (2019) suggest that one potential explanation for observed species-specific and group-specific effects of biocrusts on germination could be the physical interactions between morphological seed traits (e.g., size, shape, and structure) and the biocrust community (Havrilla et al. 2019). Morphological seed traits, physical characteristics of seeds (e.g., shape, mass, and structure, Saatkamp, et al. 2019) may be particularly important for determining

outcomes of biocrust effects on germination since seed form closely moderates interactions with the biocrust surface and its resources. For example, seed mass could control seed contact with the biocrust surface and associated resources. Seed mass is often positively associated with increased germination rates in bare soil in many species across different environmental conditions (Larson et al. 2015). Yet, this may not be the case in systems with biocrusts. As biocrust cover increases seed contact with the mineral soil surface often decreases. Larger seeded species in particular may have limited contact with the mineral soil surface, and may be less likely to be situated within favorable microsites on soils occupied by biocrusts. In contrast, smaller seeds may be more likely to fall into biocrust cracks (Havrilla and Barger, 2018), increasing contact with the mineral soil surface and/or favorable, shaded microsites with greater soil moisture and lower surface temperature. As such, we might predict that biocrusts may favor germination of smaller seeded species. Seed appendages or awns, a bristle-like extension from the lemma in the floret (Ntakirutimana and Xie, 2019), may also affect seed capture and positioning on the biocrust surface (Havrilla and Barger, 2018). While seed morphological traits may provide a framework for understanding interspecific variation in germination responses to biocrusts, seed traits have not yet been integrated into synthesis efforts exploring these interactions (Havrilla et al. 2019).

To address this knowledge gap, we conducted a quantitative meta-analysis to explore the role of morphological seed traits in mediating biocrust effects on plant germination. We integrated seed trait data from publicly available databases (i.e., TRY Plant database, Kattage et al. 2020), KEW Botanical database (Royal Botanic Gardens Kew. 2020), and the Encyclopedia of Life (Parr et al. 2014) into a previously published database of studies of biocrust effects on plant germination (Havrilla et al. 2019). We then used mixed-effects meta-regression models to explore the relationship among morphological seed traits (i.e., seed mass, shape, and the presence of seed

appendage) and other ecological factors important for determining outcomes of biocrusts on plant germination (i.e., biocrust type, plant functional group, and plant origin; Havrilla et al. 2019). We hypothesized that (1) morphological seed traits influence the effects of biocrusts on plant germination, and specifically (a) germination of larger seeds is inhibited by biocrusts due to reduced contact with the mineral soil surface, while germination of smaller seeds will be increased, and (b) germination of seeds with appendages is inhibited by biocrusts while germination of seeds without appendages would be unaffected because the topography of biocrusts will serve as a barrier to seeds with appendages. We also hypothesized that (2) seed traits mediate the effects of biocrusts on germination of native vs exotic plants and plants belonging to different plant functional groups, for example, decreased germination of non-native plants and C4 grasses (Havrilla et al. 2019). Results of our synthesis will support an improved understanding of the interactions between biocrusts and plant germination. This information can be used to support a predictive understanding of the role of biocrusts in determining plant community assembly and/or outcomes of restoration in global drylands.

## **Methods**

### ***Original Database of Biocrust-Plant Germination Studies from Havrilla et al. (2019)***

Our study leverages an existing, multilingual database of published literature on biocrust-plant interactions containing 491 unique comparisons (“studies”) of plant germination on biocrust versus controls lacking intact biocrusts (i.e., bare soil, disturbed biocrust, removed biocrust, or filter paper) across global drylands published by Havrilla et al. (2019). The database included germination responses for 101 unique plant species across six continents (Figure 1).

We extracted the following data from the Havrilla et al. (2019) database to construct the germination specific database used in our study: (1) plant species information (i.e., genus and species), (2) effect sizes showing the effect of biocrust on germination relative to bare soil (i.e., log response ratio (LnRR; Equation 1) (3) the estimate of within-study variance (“ESTVAR3”; Equation 2; Hedges et al. 1999, Havrilla et al., 2019), and (4) metadata for all plant and biocrust covariates found to be predictive in the meta-regression model for plant germination responses to biocrust presence (i.e., Biocrust Type, Plant Functional Group, Plant Origin, and Soil Reference State; described in Table 1; Havrilla et al. 2019).

Equation 1:  $\ln = (X_{\text{crust}}/X_{\text{ctrl}})$

Where  $X_{\text{crust}}$  is the mean plant response in the biocrust treatment, and  $X_{\text{ctrl}}$  is the mean plant response in the biocrust-absent control.

Equation 2:  $\sigma^2 = [SD_{\text{crust}}^2 / (n_{\text{crust}})(X_{\text{crust}}^2)] + [SD_{\text{ctrl}}^2 / (n_{\text{ctrl}})(X_{\text{ctrl}}^2)]$

Where  $X_{\text{crust}}$  and  $X_{\text{ctrl}}$  are the mean plant response with and without biocrust,  $SD_{\text{crust}}$  and  $SD_{\text{ctrl}}$  are the standard deviation of the treatment and control means, and  $n_{\text{crust}}$  and  $n_{\text{ctrl}}$  are the number of replicates within biocrust and biocrust-absent soil treatments.

### ***Candidate Morphological Seed Trait Data***

For each of the unique plant species contained in the Havrilla et al. (2019) germination database (n = 101 species total), we compiled morphological seed trait data from publicly available plant trait databases; KEW Royal Botanical Gardens Trait Database (Royal Botanic Gardens Kew, 2020), TRY Plant Database (Kattage et al. 2020), and the Encyclopedia of Life (Parr et al. 2014). For a full description of our data collection process see Appendix 1. From these databases, we extracted values for seven candidate morphological seed traits of interest (Table 1). Seed mass (g/1000 seeds) was selected to indicate a general measure of seed size and potential effects on germination (Fenner and Thompson, 2005, Larson et al. 2015). A series of candidate traits

reflecting seed architecture were also added to the database to explore potential interactions among seed physical structure and the biocrust surface to determine germination outcomes. These included seed shape (i.e., ovate versus linear) and appendage presence (i.e., yes/no), appendage length (mm), appendage hygroscopicity (i.e., yes/no), and whether the seed has a mucilaginous seed coat (i.e., yes/no). Hygroscopic appendages are specialized seed structures that allow seeds to drill into the soil surface when the appendage is exposed to water (Elbaum, Gorb, and Fratzl, 2008). As such, we hypothesized that seeds with appendages may help seeds overcome physical barriers posed by biocrusts to germination (e.g., soil surface hardness) may increase germination of these seeds on soils with biocrusts. Mucilaginous seed coats are an adaptation that takes the form of a mucilage layer when wetted (Yang et al. 2012). This mucilage coat is often adhesive and may aid with seed positioning and retention within favorable microsites, and may provide lubricant for the plant radicle once germination has occurred (Yang et al. 2012). Continuous variables (i.e., seed mass, seed length, and seed appendage length) were converted to categorical variables (Table 1) prior to data analysis (Zheng and Casari, 2018).

**Table 1.** The 11 candidate categorical predictor variables used within the mixed-effects meta-regression models.

Explanatory Variable	Number of levels	Original (from Havrilla et al. 2019). or new variable	Description of variable levels	Selected Variable	Included in the final meta-regression model
BIOCRUST_TYPE	4	Original	Cyanobacteria, Moss, Lichen, Mixed; Classified by the dominant biocrust taxonomic group in the biocrust community as reported in the study. 'Mixed' biocrust are communities containing substantial cover of both mosses and lichens.	Yes	Yes

PLANT_FUNCTIONAL_GROUP	7	Original	C3 grass, C4 grass, N-fixing forb, Non-N-fixing forb, N-fixing woody plant, Non-N-fixing woody plant, and Community; Plant functional group as designated in herbarium record for plant species. 'Community' designates multiple plant species belonging to multiple plant functional groups.	Yes	Yes
PLANT_ORIGIN	3	Original	Native or Non-Native; Corresponding to the native status of the plant in the study region. Non-Native species include any species not native to the study region	Yes	Yes
SOIL_REFERENCE_STATE	4	Original	Bare soil, Biocrust removal, Biocrust disturbance, or Filter paper; Experimental control soil substrate for comparison to biocrust treatment as recorded in the study. 'Biocrust removal' controls are those in which biocrust organisms have been removed from the soil surface while 'biocrust disturbance' controls are those that have been mechanically disturbed or trampled.	Yes	Yes
SEED_MASS	3	New	Small, Medium, and Large; Originally collected as continuous variables but were binned into the three above based on seed mass. Breakdown was done in 0.5 g increments 0-0.5, 0.5-1, 1+.	Yes	Yes
SEED_LENGTH	3	New	Short, Medium, and Long; Originally collected as a continuous variable but was binned into the three above categories based on seed length. Breakdown was done in 2 mm increments 0-2, 2-4, 4+.	No	No
SEED_APPENDAGE	2	New	Yes or no; Corresponding to the presence or absence	Yes	Yes

			of an awn or appendage for any particular plant species.		
SEED_APPENDAGE_LENGTH	3	New	Small, Medium, and Large; Originally collected as a continuous variable but was binned into the three above categories based on seed appendage length. Breakdown was done in 10 mm increments 0-10, 10-20, 20+	No	No
SEED_APPENDAGE_HYGROSCOPICITY	2	New	Yes or no; Corresponding to if the present awn is hygroscopic or not.	No	No
SEED_SHAPE_SIMPLE	2	New	Linear or Oval; A simplified classification of seed shape. Seeds that were roughly linear were assigned the Linear category, seeds that were more rounded or elliptic were assigned Oval.	No	No
SEED_MUCILAGINOUS	2	New	Yes or No; Corresponding to if the seed produces a mucilaginous coating or not.	No	No

***Original Germination Model from Havrilla et al. (2019)***

The original germination meta-regression model from Havrilla et al. (2019) served as a base for our new model which incorporated morphological seed traits. Variables carried over from the original model were: BIOCROUST\_TYPE, SOIL\_REFERENCE\_STATE, PLANT\_FUNCTIONAL\_GROUP, PLANT\_ORIGIN, and STUDY\_ID (Table 1). BIOCROUST\_TYPE, SOIL\_REFERENCE\_STATE, PLANT\_FUNCTIONAL\_GROUP, and PLANT\_ORIGIN, variables describing biocrust plant characteristics, were included in the model as fixed effects. These variables were mainly populated with the information contained in study papers and assessments by Havrilla and colleagues (2019). For example, when the study paper did not report PLANT\_ORIGIN of a given species the authors assessed plant origin by determining

whether the plant species was native or naturalized to the region or continent in which the study was conducted using records in the USDA Plants Database (for North American studies) and/or the Kew Gardens plant database. The residual between study variance ('STUDY\_ID'; Havrilla et al. 2019) for each unique study included as a random effect. This original germination meta-regression model, and all subsequent statistical analyses in this study were conducted in R (version 4.3.0; R Core Development Team, 2021).

### ***Preliminary Data Exploration and Candidate Morphological Seed Trait Variable Selection***

To explore the relative importance of the candidate morphological seed trait moderators and their potential interactions with one another and original moderators from the Havrilla et al. (2019) model, we used a three-step variable selection process. First, we examined the sample size of the various variables. This examination was used to inform if any variables needed to be excluded from the final model. Second, we explored potential correlations among candidate morphological seed trait predictor variables using correlation analysis via the *corrplot* package (Wei, Simko. 2017). If two variables had a correlation statistic  $r = +/- 0.70$ , based on the cutoff levels suggested by Hinkle, Wiersma, and Jurs (2003), then those variables could not be included in the same model. Second, after reducing highly correlated variables down to a list of relatively orthogonal morphological seed traits, we used boosted regression tree (BRT) data exploration using the *gbm* package (Greenwell et al. 2022), to explore the relative importance of the candidate moderators and their potential interactions in explaining variation among plant responses to biocrusts. Boosted regression tree analysis additively fits and combines multiple trees using a forward stepwise procedure, thus improving accuracy (De'Ath, 2007). BRT analysis is ideal for complex data and unidentified distributions (De'Ath, 2007), and additionally, can accommodate missing values in moderators (De'Ath, 2007, Elith, Leathwick, & Hastie, 2008). We performed

BRTs using the ‘gbm.step’ function in the *gbm* (Ridgeway, Southworth, & Runit, 2013), and *dismo* packages (Hijmans, Phillips, Leathwick, & Elith, 2007) as in Elith and Leathwick (2017). In each BRT model, we included only those moderators that had sufficient representation in the dataset and corresponded to meaningful a priori hypotheses (Figure S1a); we then weighted each analysis according to the within-study variance. Models were simplified using the ‘gbm.simplify()’ function suggested by Elith and Leathwick (2017). Simplified BRT models for each analysis included the most influential moderators and ranked them according to their relative contributions (which are scaled to sum to 100% within each model – i.e., a particular moderator explains X% of the variation explained by the fitted BRT) to the explanation of variation in effect size. Relative variable influences were derived as an average of variable influence in all trees in each BRT model (Friedman & Meulman, 2003). Potential interactions between moderators in final BRT models were explored using the ‘gbm.interaction()’ function (Elith & Leathwick, 2017). If BRT identified significant interactions among candidate predictor variables, we included these interactions in our initial meta-regression models.

### ***Mixed Effects Meta-Regression***

Following the selection of candidate moderators, meta-analysis was performed by fitting meta-regression models using the *metafor* package (Viechtbauer 2010) with restricted maximum likelihood estimation of parameters. We first used the *rma()* function to fit a pure random effects model to estimate the overall weighted mean effect size for the model (i.e., the weighted, overall log response ratio of the germination of biocrust presence), with effect size weighted by within-study variance and the residual between-study variance component (‘STUDY\_ID’) as a random-effect. Then, we investigated the relative importance of the categorical fixed-effect moderators (Table 1) included in the model by analyzing a series of mixed-effect multiple meta-regression

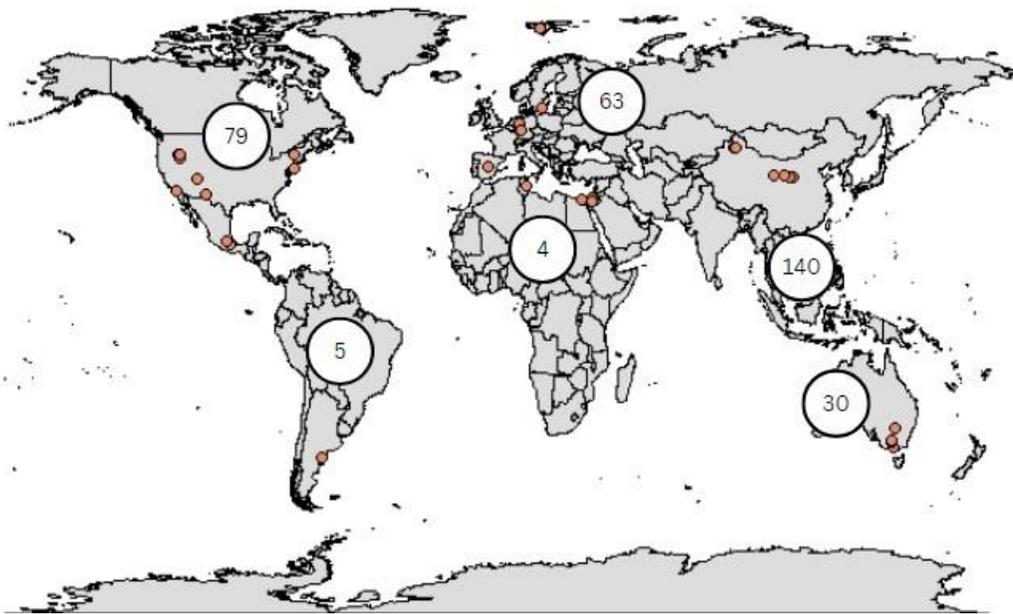
models using the *rma.mv()* function, including a global model containing all the fixed factors (moderators) and candidate interaction terms being considered for that dataset and each of the nested subset models containing one more fixed factor. The model also contained the random effect STUDY\_ID variable to account for residual between-studies variation. When categorical moderators were significant (Q statistic < 0.05), differences in moderator levels were detected using planned contrasts with the *ghlt()* function from the multcomp package (Hothorn, Bretz, and Westfall. 2008). To explain residual heterogeneity and understand the potential effect of contextual factors on plant responses to biocrusts, we ran a series of separate univariate and bivariate interaction meta-regression models for each analysis that included single significant moderators (Hoeksema et al. 2010, Havrilla et al. 2019). We chose to analyze the univariate and/or bivariate interaction models because this allowed us to maximize the number of studies that could be analyzed as not all of the moderator variables were reported in every study. This also allowed us to maximize the studies used to calculate the intercept and slope or mean effect size values that described the relationship between each moderator variable and its effect on the log response of biocrust on germination. This approach allowed us to calculate these values while still taking into account the effects of all moderator variables to ensure that each moderator variable analyzed still had a significant effect on LnRR in the presence of other moderators.

## **Results**

### ***Database Summary***

Our final database contained 321 (64.6%) unique studies of plant germination response to biocrust presence that were retained from the original 491 germination studies within the original Havrilla et al. (2019) germination database. Studies included in this integrated database spanned

12 different countries and all continents except Antarctica (Figure 1) and encapsulated the responses of 101 plant species from 27 families. Most (n = 248, 78.2%) were studies of germination responses of native plant species, while 75 (21.8%) were of non-native species. Studies contained a variety of biocrust community types: cyanobacterial biocrusts made up 25.9% (n = 82), lichen made up 18.9% (n = 60). Data for all morphological seed traits were not available for all plant species. SEED\_MASS (g/1000 seeds) was available for 314 (99.1%) studies, SEED\_APPENDAGE (yes/no) was available for 250 (78.9%) studies, and SEED\_SHAPE data was available for only 133 (42.0%) studies. SEED\_APPENDAGE\_LENGTH (mm) was accounted for in 60 (18.9%) studies. Seeds with hygroscopic awns accounted for only 2.5% of studies (n = 8) while the remaining 97.2% either lacked hygroscopic awns or lacked information on if the species had hygroscopic awns (n = 308). Seeds with mucilaginous seeds accounted for only 7.6% of studies (n = 24) while most seeds studied were not mucilaginous (n = 292).



**Figure 1:** Map of locations of studies incorporated into the model analysis.

### *Candidate Variable Selection*

From the correlation analysis we determined that SEED\_MASS and SEED\_SHAPE were highly correlated ( $r = -0.71$ ; Supplementary Figure 1), and SEED\_APPENDAGE and SEED\_LENGTH were also highly correlated ( $r = +0.78$ ; Supplementary Figure 1). As such, we elected to retain SEED\_MASS and SEED\_APPENDAGE in our models since they were assumed to represent relative orthogonal morphological seed characteristics, there was a greater number of studies with this trait information available, and these traits were more directly relevant to our hypotheses. BRT data exploration showed SOIL\_REFERENCE\_STATE explained the greatest amount of variation in the responses of plant germination response to biocrusts  $\sim 29.5\%$ , Supplementary Figure 2), while BIOCRUST\_TYPE explained  $\sim 20.2\%$  (Supplementary Figure 2), PLANT\_FUNCTIONAL\_GROUP  $\sim 19.3\%$  (Supplementary Figure 2), SEED\_MASS  $\sim 17.0\%$  (Supplementary Figure 2), and finally SEED\_APPENDAGE explained  $\sim 10.4\%$  (Supplementary Figure 2) of variation. SEED\_APPENDAGE\_HYGROSCOPY and SEED\_MUCILAGINOUS were not significantly influential. BRT analysis found that there were interactions between BIOCRUST\_TYPE and SEED\_MASS as well as SOIL\_REFERENCE\_STATE and SEED\_MASS. The full final list of candidate variables included in meta-analysis can be found in Table 1.

## **Meta-analysis**

### ***Effects of biocrust and key moderators on plant germination***

Meta-analysis showed that while the pure random effect model showed that overall there were no significant effect of biocrust on plant germination overall ( $p = 0.444$ , Table 2), germination responses varied depending on a variety of ecological factors including morphological seed traits (list relevant figs here). Each of the individual fixed effect variables (i.e.,

BIOCRUST\_TYPE, PLANT\_ORIGIN, SOIL\_REFERENCE\_STATE, PLANT\_FUNCTIONAL\_GROUP, SEED\_MASS, and SEED\_APPENDAGE; Table 2) significantly influenced plant germination responses to biocrust interacted with several other variables to determine the effects of biocrusts on germination. For example, SEED\_MASS moderated the effects of BIOCUST\_TYPE, PLANT\_ORIGIN, and SOIL\_REFERENCE\_STATE on germination, and SEED\_APPENDAGE interacted with BIOCUST\_TYPE and PLANT\_ORIGIN to influence the effects of biocrust on germination (Table 2). Together, results from the meta-regression model revealed overarching effects of moderators on the effect of biocrust on plant germination.

First, as in Havrilla et al. (2019), we found different biocrust community types had differential effects on plant germination overall. Relative to bare soil, cyanobacteria decreased plant germination by 52.6% ( $p < 0.0001$ , Table 2), lichen decreased plant germination 4.2-fold ( $p < 0.001$ , Table 2), moss increased plant germination by 39.3% ( $p = 0.033$ , Table 2), and mixed crusts increased germination by 21.3% ( $p = 0.037$ , Table 2). Similarly, germination responses to biocrust varied across different plant functional groups (PLANT\_FUNCTIONAL\_GROUP) and between native and exotic species (PLANT\_ORIGIN). Overall, biocrusts increased germination of C3 grasses by 147.6% ( $p = 0.045$ , Table 2), decreased germination of C4 grasses 3.1 fold ( $p = 0.045$ , Table 2), decreased germination of Nitrogen-fixing forbs by 1.9 fold ( $p < 0.0001$ , Table 2), Nitrogen-fixing woody plants increased germination by 4.9 fold ( $p < 0.0001$ , Table 2), Non-nitrogen-fixing forbs germination were decreased by 143.3% ( $p < 0.0001$ , Table 2), and Non-nitrogen-fixing woody plants increased germination by 21.5% ( $p = 0.013$ , Table 2).

PLANT\_ORIGIN also played a role in determining germination responses to biocrust presence. Overall, native species germination was increased 2.3-fold ( $p < 0.001$ , Table 2) and non-

native species had no effect on their germination ( $p = 0.005$ , Table 2). SOIL\_REFERENCE\_STATE also influenced germination responses; while there was no effect of intact biocrust on germination relative to filter paper and disturbed biocrust controls ( $p = 0.772$  and  $0.123$  respectively, Table 2), biocrusts increased germination 2.6-fold relative to bare soil ( $p < 0.001$ ), and by 14.2% relative to biocrust removed controls ( $p = 0.029$ , Table 2).

SEED\_MASS also affected germination responses to biocrusts. Overall, small seeded (0.00-0.50 g/1000 seeds) and medium seeded (0.51-1.00 g/1000 seeds) species experienced a 3.6-fold and 3.8-fold increases on biocrust respectively ( $p < 0.0001$  each, Table 2), whereas germination of large seeded species (1.1+ g/1000 seeds) decreased germination by 3.0-fold ( $p < 0.0001$ , Table 2).

Finally, SEED\_APPENDAGE denoting the presence or absence of morphological appendages, also influenced the effects of biocrusts on germination. Overall, seeds with appendages experienced a 6.3-fold decrease in germination on biocrust relative to controls ( $p < 0.0001$ ; Table 2), while species lacking appendages experienced a more minor but significant decrease of 16.3% in germination ( $p < 0.0001$ , Table 2).

**Table 2:** Results of the multivariate mixed-effects model that was used to analyze the candidate variables and their interactions.

Germination Response	Fixed effects	Multivariate Model					
		Est	SE	z-value	p-value	ci.lb	ci.up
	<i>Intercept</i>	0.119	0.142	0.838	0.402	-0.160	0.398
	<b>BIOCRUST_TYPE</b>						
	Cyanobacteria	-0.840	0.0775	-10.843	<.0001	-0.992	-0.688
	Lichen	-0.383	0.0409	-9.363	<.0001	-0.464	-0.303
	Moss	0.0724	0.0339	2.137	0.0326	0.00600	0.139
	Mixed	0.0939	0.0449	2.0906	0.0366	0.00590	0.1820
	<b>PLANT_FUNCTIONAL_GROUP</b>						
	C3 Grass	0.262	0.131	2.00330	0.0452	0.00570	0.519

C4 Grass	-0.262	0.131	-2.00330	0.0452	-0.519	-0.00570
N-fixing Forb	-0.107	0.00810	-13.120	<.0001	-0.123	-0.0909
N-fixing Woody Plant	0.700	0.0662	10.585	<.0001	0.571	0.830
Non-N-fixing Forb	-0.0516	0.00970	-5.350	<.0001	-0.0706	-0.0327
Non-N-fixing Woody Plant	0.0937	0.0470	2.180	0.0133	0.0195	0.168
<b>SOIL_REFERENCE_STATE</b>						
Filter Paper	-0.0991	0.341	-0.290	0.772	-0.768	0.570
Bare Soil	0.885	0.180	4.919	<.0001	0.532	1.237
Biocrust Disturbance	0.0879	0.0569	1.545	0.123	-0.0237	0.200
Biocrust Removal	0.102	0.0468	2.391	0.0293	0.0103	0.195
<b>PLANT_NATIVEIVENESS</b>						
Native	0.735	0.0560	13.114	<.0001	0.625	0.845
Non-Native	-0.176	0.0632	-2.790	0.00530	-0.300	-0.0525
<b>SEED_APPENDAGE</b>						
No	-0.640	0.0833	-7.686	<.0001	-0.803	-0.477
Yes	-0.638	0.0737	-8.655	<.0001	-0.782	-0.493
<b>SEED_MASS</b>						
0.00 – 0.50 (S)	1.464	0.206	7.113	<.0001	1.0608	1.868
0.51-1.0 (M)	0.577	0.0493	11.688	<.0001	0.480	0.674
1.1(1+)	-0.247	0.0508	-4.852	<.0001	-0.346	-0.147

**Interactions**

**PLANT\_ORIGIN x SEED\_MASS**

Native x 0.00-0.5 (S)	0.107	0.0839	1.280	0.201	-0.0571	0.272
Native x 0.51-1.0 (M)	0.366	0.0847	4.316	<.0001	0.200	0.532
Native x 1.1+ (L)	-0.326	0.0836	-3.900	<.0001	-0.490	-0.162
Non-Native x 0-0.5 (S)	0.116	0.0400	2.902	0.00370	0.0376	0.194
Non-Native x 0.51-1.0 (M)	-0.837	0.0532	-15.719	<.0001	-0.941	-0.733
Non-Native x 1.1+ (L)	-0.176	0.0464	-3.800	0.0001	-0.267	-0.0854

**PLANT\_ORIGIN x SEED\_APPENDAGE**

Native x Yes	-0.0449	0.0785	-0.572	0.567	-0.199	0.109
Native x No	-0.0936	0.0779	-1.202	0.229	-0.246	0.0590
Non-Native x Yes	0.0582	0.0476	1.223	0.221	-0.0350	0.151
Non-Native x No	-0.103	0.0399	-2.587	0.0097	-0.182	-0.0250

**BIOCRUST\_TYPE x SEED\_MASS**

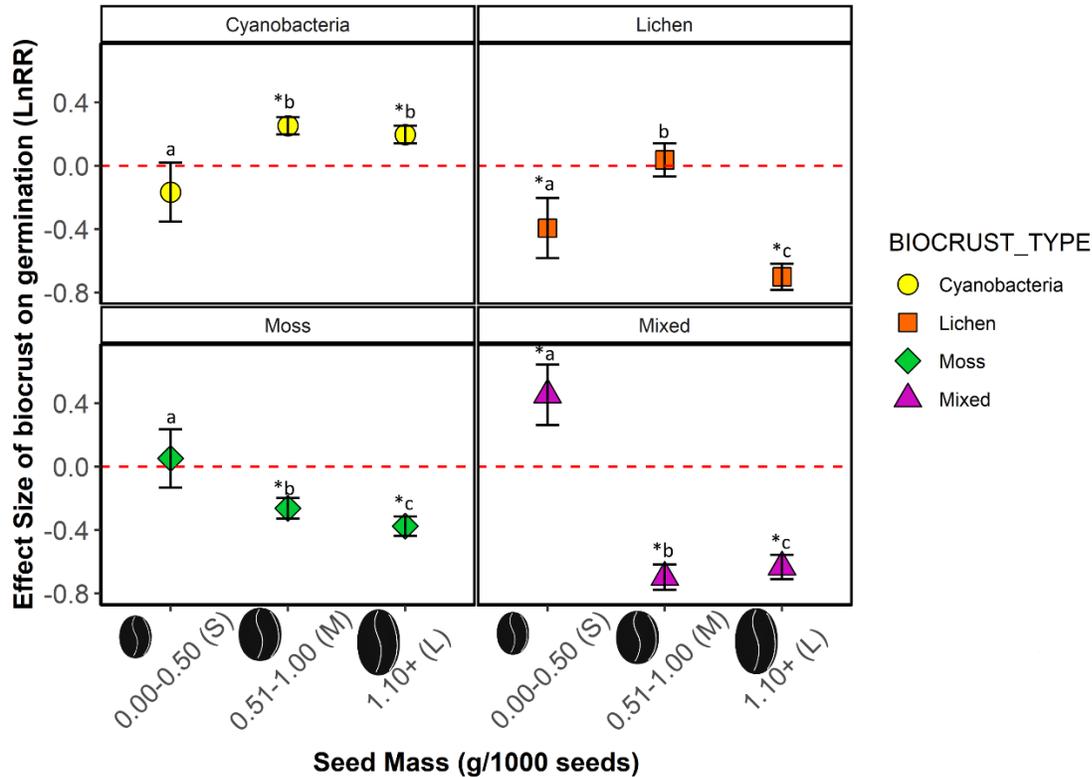
Cyanobacteria x 0-0.5 (S)	-0.167	0.0952	-1.752	0.0797	-0.353	0.0198
Cyanobacteria x 0.51-1.0 (M)	0.253	0.0279	9.0674	<.0001	0.198	0.308
Cyanobacteria x 1.1+ (L)	0.197	0.0281	6.997	<.0001	0.142	0.252
Lichen x 0-0.5 (S)	-0.393	0.0967	-4.0651	<.0001	-0.583	-0.204
Lichen x 0.51- 1.0 (M)	0.0370	0.0532	0.695	0.487	-0.0673	0.141
Lichen x 1.1+ (L)	-0.701	0.0422	-16.622	<.0001	-0.784	-0.619
Moss x 0.-0.5 (S)	0.0521	0.0940	0.554	0.580	-0.132	0.236
Moss x 0.51-1.0 (M)	-0.263	0.0334	-7.876	<.0001	-0.328	-0.197
Moss x 1.1+ (L)	-0.375	0.0313	-12.0106	<.0001	-0.437	-0.314
Mixed x 0-0.5 (S)	0.453	0.0975	4.650	<.0001	0.262	0.644
Mixed x 0.51- 1.0 (M)	-0.698	0.0411	-16.999	<.0001	-0.778	-0.617
Mixed x 1.1+ (L)	-0.633	0.0395	-16.0496	<.0001	-0.711	-0.556
<b>SOIL_REFERENCE_STATE x SEED_MASS</b>						
Filter Paper x 0- 0.5 (S)	0.0464	0.235	0.197	0.844	-0.414	0.507
Filter Paper x 0.51-1.0 (M)	0.277	0.0643	4.307	<.0001	0.151	0.403
Filter Paper x 1.1+ (L)	-0.286	0.0824	-3.471	0.0005	-0.447	-0.125
Bare Soil x 0- 0.5 (S)	-0.120	0.0994	-1.206	0.228	-0.315	0.0749
Bare Soil x 0.51-1.0 (M)	0.115	0.0987	1.166	0.243	-0.0783	0.309
Bare Soil x 1.1+ (L)	0.0571	0.0987	0.578	0.563	-0.136	0.251
Biocrust Disturbance x 0- 0.5 (S)	0.0475	0.0519	0.915	0.3602	-0.0543	0.149
Biocrust Disturbance x 0.51-1.0 (M)	-0.545	0.0734	-7.431	<.0001	-0.689	-0.402
Biocrust Disturbance x 1.1+ (L)	-1.0415	0.0464	-22.466	<.0001	-1.132	-0.951
Biocrust Removal x 0-0.5 (S)	0.121	0.0432	2.808	0.00500	0.0367	0.206
Biocrust Removal x 0.51- 1.0 (M)	-0.444	0.0531	-8.365	<.0001	-0.548	-0.340

Biocrust Removal x 1.1+ (L)	-0.753	0.0561	-13.425	<.0001	-0.862	-0.643
<b>BIOCRUST_TYPE x SEED_APPENDAGE</b>						
Cyanobacteria x No	-0.0340	0.102	-0.332	0.740	-0.234	0.166
Cyanobacteria x Yes	-0.392	0.0503	-7.795	<.0001	-0.491	-0.294
Lichen x No	-0.417	0.105	-3.987	<.0001	-0.622	-0.212
Lichen x Yes	0.366	0.0569	6.426	<.0001	0.254	0.477
Moss x No	-0.169	0.102	-1.656	0.0977	-0.369	0.0311
Moss x Yes	0.581	0.0591	9.817	<.0001	0.465	0.696
Mixed x No	-0.0601	0.102	-0.588	0.557	-0.260	0.140
Mixed x Yes	1.238	0.0554	22.370	<.0001	1.130	1.347

***Seed mass interacts with biocrust community type, soil reference state, and plant origin to control the effects of biocrusts on germination***

Seed mass also controlled the effects of biocrust community composition (i.e., cyanobacteria, lichen, moss, and mixed communities) on plant germination. Overall, on cyanobacteria-dominated biocrusts, medium and large-seeded species experienced over 2-fold increases in germination respectively ( $p < 0.0001$  and  $p < 0.0001$  respectively, Fig. 2, Table 2) while small-seeded species were not significantly affected ( $p = 0.080$ , Fig 2, Table 2). On lichen-dominated biocrusts, germination of both small-seeded (-35.7%;  $p < 0.0001$ , Fig 2, Table 2) and large seeded species decreased, with these effects greater for large seeded species, which experienced a ~3-fold decrease in germination in the presence of biocrust ( $p < 0.0001$ , Fig. 2, Table 2). Germination of medium-seeded species was unaffected by lichen dominated biocrusts ( $p = 0.487$ ; Fig. 2, Table 2). On moss-dominated biocrusts, germination of medium- and large-seeded species was decreased by biocrust presence (-57.6%,  $p < 0.0001$  and -125.1%,  $p < 0.0001$  respectively; Fig 2, Table 2), while germination of small-seeded species was unaffected ( $p = 0.580$ , Fig 2, Table 2). Finally, in mixed biocrust communities small-seeded species experienced a nearly 5-fold increase in germination ( $p < 0.0001$ , Fig 2, Table 2), while germination of medium and

large-seeded species was decreased nearly ~3-fold in response to biocrust presence (3.2-fold,  $p < 0.0001$  and -2.8 fold,  $p < 0.0001$  respectively; Fig 2, Table 2).

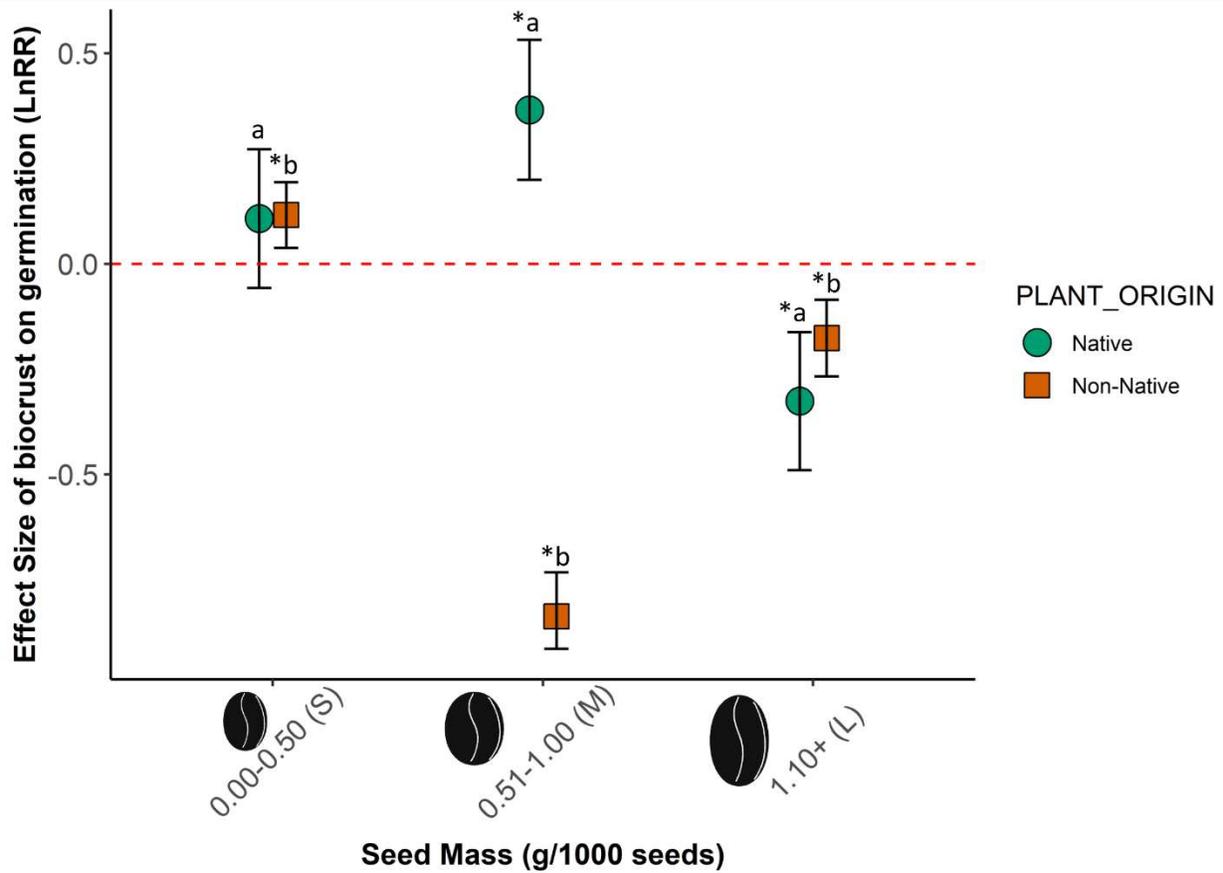


**Figure 2.** Effects of SEED\_MASS on germination responses to different biocrust types. Lowercase letters denote significantly significant pairwise differences ( $p < 0.05$ ) within BIOCRUST\_TYPE while “\*” indicates significant differences from the red-dashed line of no effect.

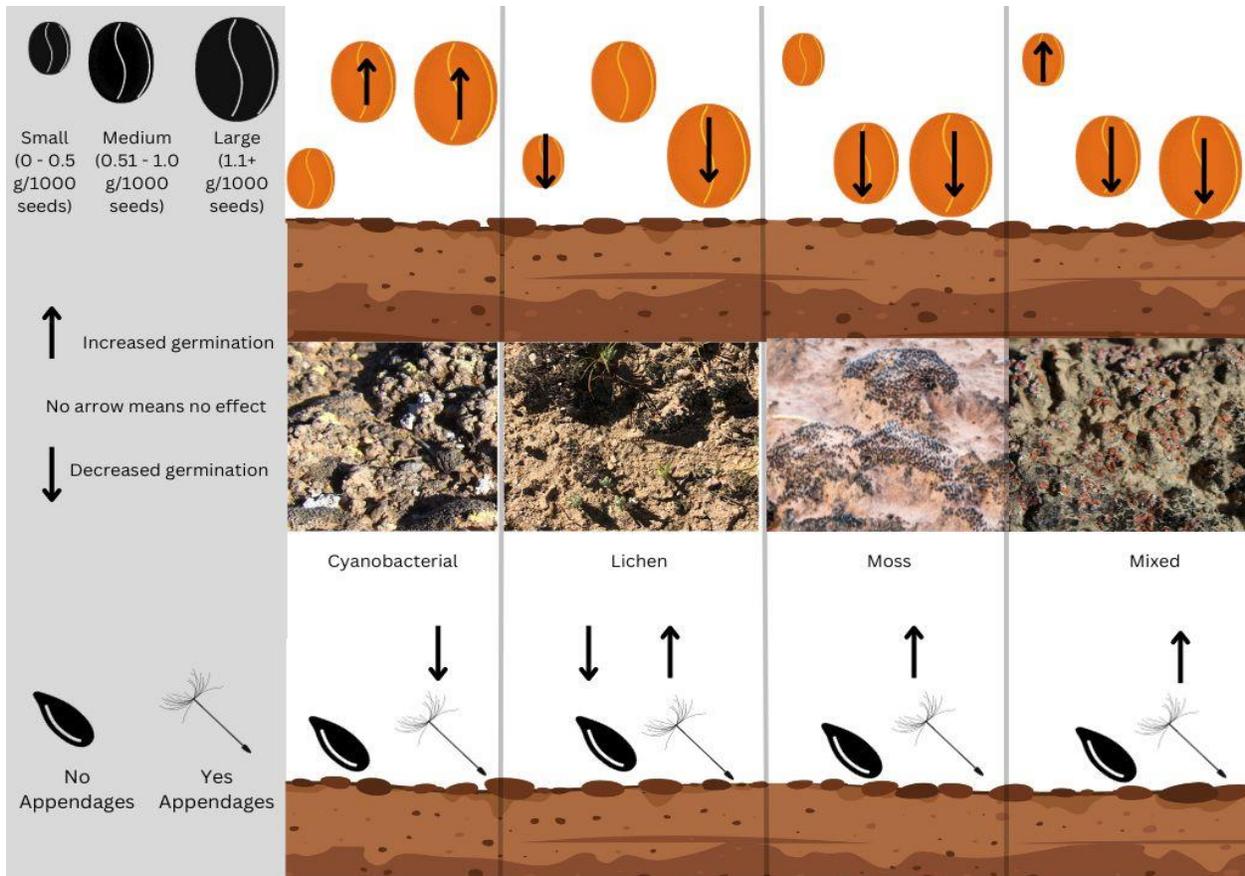
SEED\_MASS also mediated the effects of biocrusts on germination across different SOIL\_REFERENCE\_STATES (i.e., the control substrate to which intact biocrusts are being compared in each study) When compared to germination on filter paper, biocrusts increased germination of medium-seeded species 8-fold ( $p < 0.0001$ , Supplementary Fig. 3, Table 2). Conversely, germination of large-seeded species was decreased by half (-48.3%,  $p = 0.0005$ , Supplementary Fig. 3, Table 2) while germination of small-seeded species was unaffected ( $p = 0.844$ , Supplementary Fig. 3, Table 2) on biocrust relative to filter paper controls. Interestingly, there was no significant effect of biocrust on germination within any of the seed

mass categories relative to bare soil controls ( $p = 0.228$  (S),  $p = 0.243$  (M), and  $p = 0.563$  (L) respectively, Table 2). However, relative to disturbed biocrust controls medium and large-seeded species both experienced decreased germination (10.2-fold,  $p < 0.0001$  and -9.3 fold,  $p < 0.0001$  respectively, Supplementary Fig. 3, Table 2), while small-seeded species experienced no effect ( $p = 0.360$ , Supplementary Fig. 3, Table 2). Finally, compared to biocrust removal controls, we found that small-seeded species experienced a 2-fold increased germination on intact biocrust (2.0-fold,  $p = 0.005$ , Supplementary Fig. 3, Table 2). Both medium (8.8-fold) and large-seeded (5.3-fold) species showed negative responses to their germination (both  $p < 0.0001$  respectively, Supplementary Fig. 3, Table 2).

SEED\_MASS also mediated the differential effects of biocrusts on the germination of native versus non-native plant species (PLANT\_ORIGIN). Overall, germination responses of small and large seeded species were similar in native versus non-native plant species, while medium species displayed divergent responses depending on plant origin (Fig. 3). Small seeded species experienced either slightly increased germination if non-native 7.8%,  $p = 0.004$ , Fig. 3, Table 2), or did not have their germination affected if native ( $p = 0.200$ ; Fig. 3, Table 2). Medium seeds had the starkest difference. Native species experienced increased germination 140.5%,  $p < 0.0001$ ; Fig. 3, Table 2), while non-native species experienced decreased germination (8.8-fold,  $p < 0.0001$ ; Fig. 3, Table 2). Large seeded species experienced decreased germination regardless of plant origin with native species experiencing a 5-fold decrease in germination on biocrust ( $p < 0.0001$ ; Fig. 3, Table 2), and non-native species experiencing a 2.6-fold decrease in germination ( $p = 0.0001$ ; Fig. 3, Table 2).



**Figure 3.** Effects of SEED\_MASS on germination responses to biocrust between native versus non-native plant species (PLANT\_ORIGIN). Lowercase letters denote significant pairwise differences among SEED\_MASS \* PLANT\_ORIGIN levels while “\*” indicates a significant change in plant germination relative to the red-dashed line of no effect.

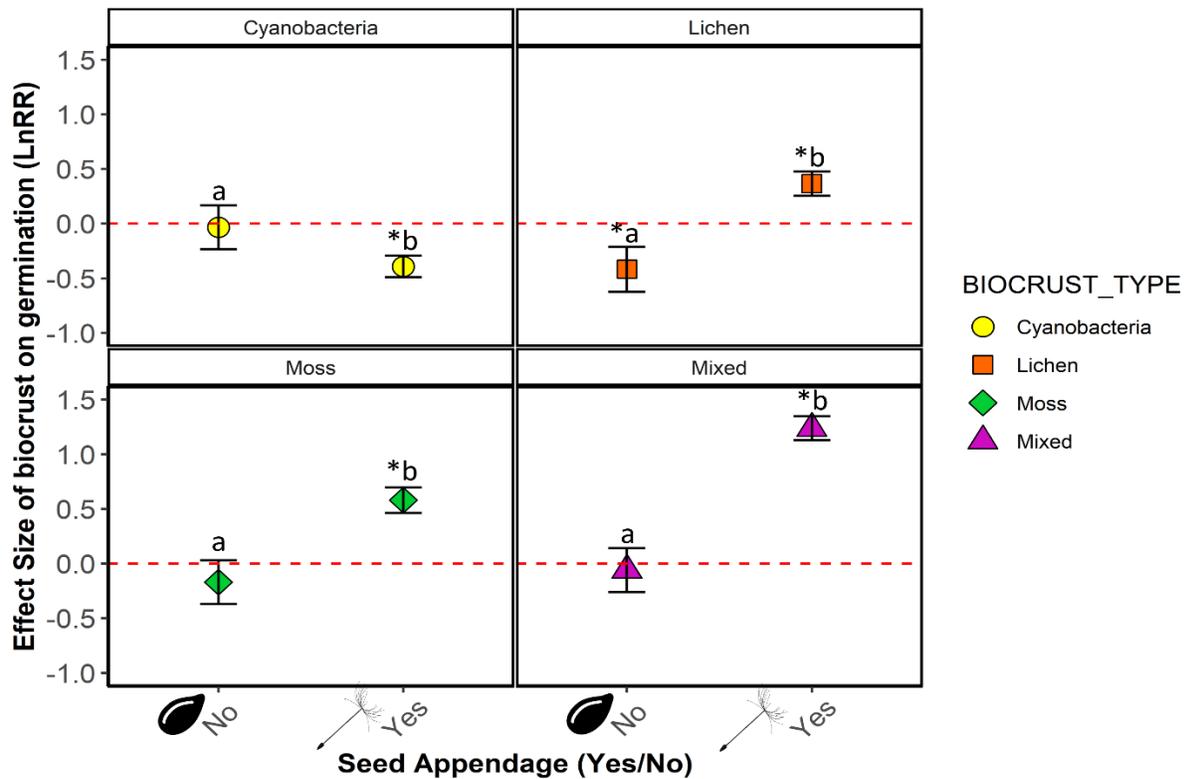


**Figure 4.** Conceptual diagram showing results for the overall direction of the effects of SEED\_MASS (top) and SEED\_APPENDAGE (bottom) on plant germination responses to biocrust presence across different biocrust community types (i.e., cyanobacteria, lichen, moss, and mixed community types).

***Seed appendages control the effects of biocrusts on germination for different biocrust types and native vs. non-native species***

Seed appendages also mediated the effects of biocrusts on germination across different biocrust types (Fig. 4). Germination of seeds with appendages was decreased on cyanobacteria dominated biocrusts 16- fold ( $p < 0.0001$ , Fig. 5, Table 2) while cyanobacteria dominated biocrusts had no effect on the germination of seeds without an appendage ( $p = 0.740$ , Fig. 5, Table 2). Lichen dominated crusts increased the germination of seeds with appendages by 11- fold ( $p < 0.0001$ , Fig 5, Table 2) and decreased the germination of seeds without by 10- fold ( $p < 0.0001$ , Fig 5, Table 2). Moss dominated crusts increased germination of seed with appendages by 18- fold ( $p < 0.0001$ ,

Fig 5, Table 2), but had no effect on seeds without an appendage ( $p = 0.031$ , Fig 5, Table 2). Finally, mixed biocrust communities increased the germination of seeds with appendages by 37-fold ( $p < 0.0001$ , Fig 5, Table 2), but had no effect on seeds without an appendage ( $p = 0.557$ , Fig 5, Table 2).



**Figure 5.** Effects of SEED\_APPENDAGE on germination responses to biocrust on different biocrust community types (BIOCRUST\_TYPE). Lowercase letters denote significantly significant pairwise differences among SEED\_APPENDAGE \* BIOCRUST\_TYPE levels while “\*” indicates a significant change in plant germination relative to the significant differences from the red-dashed line of no effect.

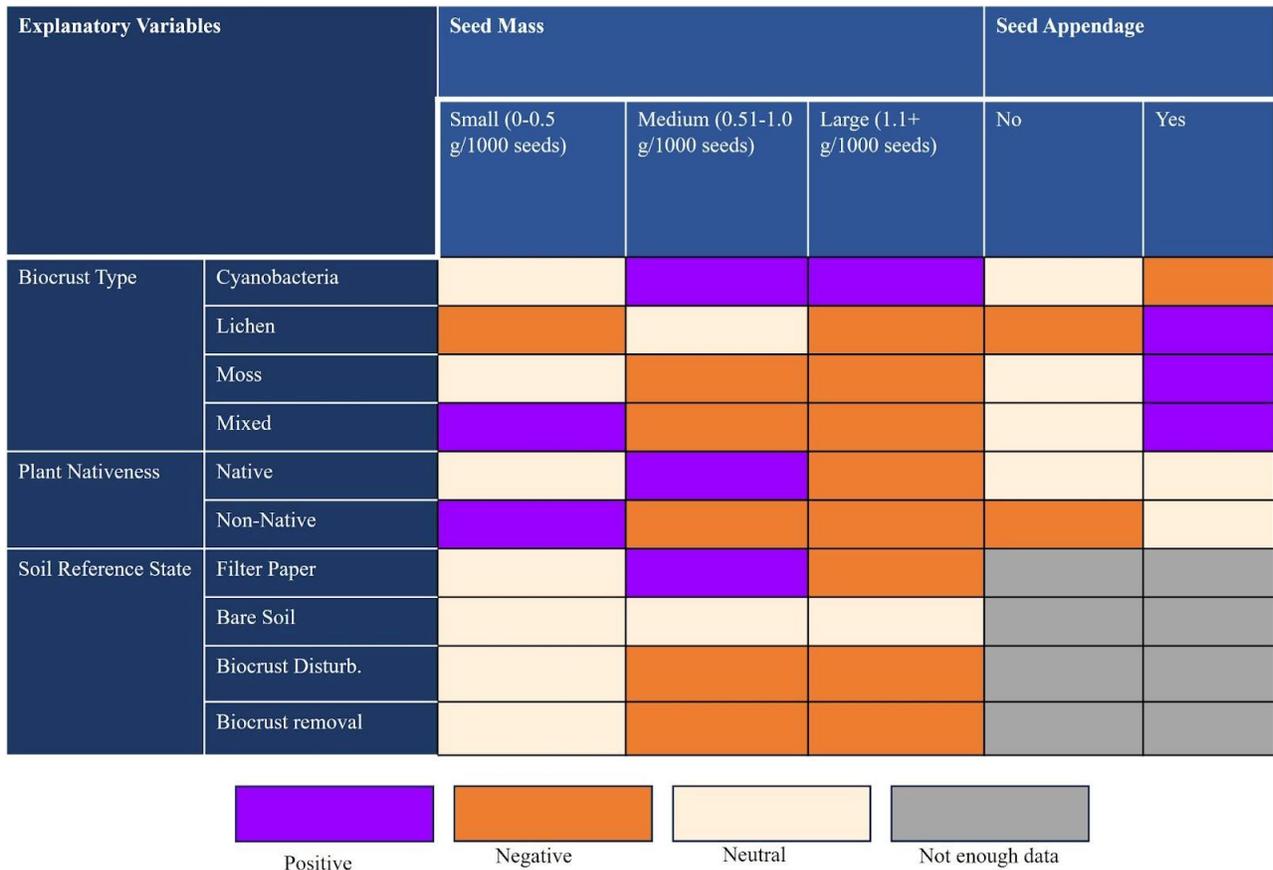
The effects of PLANT\_ORIGIN on germination responses to biocrust also varied between seeds with and without appendages. Germination of non-native species lacking appendages decreased slightly in the presence of biocrusts (-18.8%,  $p = 0.0097$ ; Supplementary Fig 4, Table 2) while germination of non-native seeds with appendages was unaffected ( $p = 0.221$ ; Supplementary Fig 4, Table 2). As in Havrilla et al. (2019), overall native species germination was

unaffected by biocrust presence regardless of whether seeds had appendages ( $p = 0.229$  for natives without appendages and  $p = 0.089$  for natives with appendages, Supplementary Fig 4, Table 2).

***Seed morphological traits may affect C4 grass interactions with biocrusts, but patterns remain unclear***

PLANT\_FUNCTIONAL\_GROUP (i.e., C3 Grass, C4 Grass, Nitrogen-fixing Forb, Non-Nitrogen-Fixing Forb, Nitrogen-Fixing Woody Plant, and Non-Nitrogen-Fixing Woody Plant) influenced overall germination responses to biocrust presence (Table 2). Among plant functional types, only C4 grasses overall experienced significant changes in germination on biocrust ( $p = 0.0002$ , Table 2). While we aimed to explore whether morphological seed traits explained this pattern, due to small sample sizes for some plant functional groups (Supplementary Table 1) and within subsets of interactions among functional traits of interest (e.g., PLANT\_FUNCTIONAL\_GROUP  $\times$  SEED\_MASS), we were unable to incorporate these interactions into our meta-regression model to formerly test our hypothesis that seed traits mediate the effects of biocrusts on the germination of plants from different plant functional groups. However, we separately explored potential relationships among morphological seed traits and C4 grass responses to biocrust presence. Our analysis included evaluation of eight unique C4 grass species that occurred in one or more studies. Of the eight C4 grass species, five (62.5%) have seeds with appendages and six are native species. Four of the species have small seeds, one has medium seeds, and 3 have large seeds. These proportions were not reflected in the individual studies. The majority of the C4 grass studies have small seeds (S; 0.00-0.50 g/1000 seeds) with 71.1% of studies ( $n = 27$ , Supplementary Table 1), while 10.0% of have medium seeds (M; 0.51-1.00 g/1000 seeds; Supplementary Table 1) and 18.4% have large seeds (L; 1.1+ g/1000 seeds; Supplementary Table 1). Most C4 grass studies had seeds that lack appendages (68.4%,  $n = 26$  studies, Supplementary

Table 1), while only 31.6% had appendages. The majority of C4 grass studies used native species (88.9%; n = 40, Supplementary Table 1) while 11.1% were non-native. When examining the interaction between C4 grasses and biocrust types cyanobacteria dominated biocrusts made up 17.8% (n = 16) of studies, while moss dominated biocrusts made up 38.9% of studies (n = 35), lichen dominated biocrusts made up 21.1% of studies (n = 19), and mixed biocrust communities made up 22.2% of studies (n = 20).



**Figure 6.** Summary diagram showing the effects of moderator variables SEED\_MASS, SEED\_APPENDAGE, BIOCRUST\_TYPE, PLANT\_NATIVENESS, and SOIL\_REFERENCE\_STATE and their interactions on the effect of biocrust on plant germination. Purple boxes denote a significant positive effect on germination ( $p < 0.05$ ), orange boxes denote a significant decrease in germination ( $p < 0.05$ ), beige boxes indicate that there was no significant influence on germination, and gray boxes indicate that there was no analysis or not enough data to perform an analysis of the interaction.

## Discussion

Understanding drivers of plant community assembly will be key to predicting ecosystem responses to global change and developing strategies to combat land degradation in the future (Larson et al., 2015 and Delgado-Baquerizo et al., 2020). In dryland ecosystems, biocrusts are ecosystem engineers (Weber et al. 2022) that can have significant impacts on plant recruitment (synthesized in Havrilla et al. 2019), though the mechanisms of these interactions have remained uncertain. Building upon the Havrilla et al. (2019) meta-analysis of germination responses to biocrust presence, we used meta-regression to analyze 321 published studies of plant germination responses to biocrusts within and found that morphological seed traits including seed mass and structure influence the effects of biocrusts on plant germination. First, overall, we found that seed mass consistently influenced plant germination response to biocrusts. Specifically, seed mass interacted with biocrust community type, soil reference state, and plant origin to control the effects of biocrusts on germination. Second, seed appendages differentially influenced germination responses to biocrusts in native versus non-native plant species and moderated the effects of different biocrust community types on germination. Finally, we explored potential links between seed traits and previously observed differences in germination responses to biocrusts across different plant functional groups. We found that C4 grasses experienced overall decreased germination on biocrusts, however we were unable to determine if morphological seed traits were responsible for this decrease. Based on the traits that are typical for C4 grasses (i.e., small seeds and the lack of appendages; Cavanagh, Godfree, and Morgan, 2019), we would have expected to see increased germination instead of the decrease in germination that was shown here and by Havrilla et al. (2019). It is possible that different plant traits or other morphological seed traits that were not tested here could be responsible for this negative impact on germination. Taken as a whole, these results provide a more mechanistic understanding of how morphological seed traits

influence germination responses to biocrusts, and as such, may advance understanding of community assembly processes in dryland ecosystems.

### **Seed traits influence germination outcomes on different biocrust community types**

The effects of biocrusts on plant germination may partially be explained by physical interactions between seeds of different sizes and structures across different biocrust community types. Biocrust community types often differ in their physical (e.g., topographic) structure (Rosentreter, Bowker, and Belnap, 2007; Colesie et al, 2016). Biocrust surface roughness is often associated with climate, disturbance, and/or community composition (Weber et al. 2022; Caster et al. 2021). In hot desert ecosystems or recently disturbed areas biocrusts are often smooth, with low roughness and may have heights of only ~1cm (Rosentreter, Bowker, and Belnap, 2007). Over the course of biocrust succession, or with varying climate, biocrusts may also form small (1-3 cm tall) patchy or rugose bumps on the soil surface (Rosentreter, Bowker, and Belnap, 2007). Rugose bumps are often separated and have cyanobacteria or exposed mineral soil surface in between. In cooler deserts that experience freeze-thaw cycles and frost-heaving (Chen et al. 2023) and at later stages of biocrust succession the biocrusts often form more rolling microtopography and/or form pinnacles reaching heights of 5-15cm with biocrusts with very few sections of open mineral soil surface. These different biocrust morphologies often have an associated community composition. The smooth biocrusts are typically made up of mostly cyanobacteria with the rugose biocrusts beginning to have patches of lichen and moss. Pinnacled and rolling biocrusts are made up of larger percentages of lichen and moss, sometimes having up to 40% (Rosentreter, Bowker, and Belnap, 2007).

Observed patterns in germination responses to biocrust types may suggest interactions between biocrust surface topography and seed mass and morphological structure. We found that

biocrust communities that typically have rougher surfaces (i.e., lichen, moss, and mixed communities, Rosentreter, Bowker, and Belnap, 2007) decreased the germination of large seeded species and minorly decreased the germination of medium seeded species. This could suggest that the rougher microtopography of these biocrusts may pose a barrier to larger seeds as supported by Zhang et al. (2016) and Li et al. (2005). Biocrusts with smoother microtopography (e.g., recently disturbed biocrusts or cyanobacteria dominated biocrusts Rosentreter, Bowker, and Belnap, 2007) however displayed opposite results. Large and medium seeded species experienced increased germination on cyanobacterial biocrusts. Smoother, early development, or more disturbed crusts usually are more patchy and have larger portions of the mineral soil surface exposed (Rosentreter, Bowker, and Belnap, 2007). These larger gaps in the biocrusts, which leave more access to the mineral soil surface, may allow for germination of the larger seeded species to get germination benefits without being inhibited due to the increased potential access to the mineral soil surface. Larger seeds have more resources stored for germination (Baskin and Baskin, 1998) which improves their germination chance in harsher conditions as shown in Mian and Nafziger (1994). On bare soil these seeds would have to contend with the typical lack of water and other key resources that is common in dryland ecosystems (Naorem et al. 2023, Husein et al. 2021). With the presence of cyanobacteria dominated biocrust communities, water and nitrogen limitations would be alleviated somewhat (Eldridge et al. 2020) leading to the observed increase in germination of larger seeded plant species on cyanobacteria dominated biocrusts relative to bare soil controls or soils lacking intact biocrust.

Interactions between biocrust type and seed appendage may also be explained by seed interactions with biocrust microtopography (Zhang et al. 2016). Overall, relative to seeds lacking appendages, germination of seeds with appendages was increased on lichen, moss, and mixed

biocrust communities, which generally have rougher microtopography and/or greater presence of cracks (Rosentreter, Bowker, and Belnap, 2007). As such, it may be that biocrust communities with greater surface roughness and/or occurrence of cracks may provide more favorable microsites and/or opportunities for seeds with appendages to be caught and/or retained within the biocrust surface until germination (Havrilla and Barger, 2018; Zhang and Belnap, 2015). The cracks within the biocrusts may also give access to the soil subsurface (Weber et al. 2022). In contrast, germination of seeds with appendages was decreased on cyanobacterial biocrusts. This may be because early successional cyanobacterial biocrusts, or those in hot deserts, are often smoother and less topographic. As such, for these seeds with appendages there is less to catch on with the lack of ‘bumpy’ topography from the biocrust and the bare soil which could be leaving seeds exposed to predation and desiccation on or above the soil surface (Song et al. 2020) leading to the observed decrease in germination.

### **Seed traits influence the effects of biocrusts on native versus non-native plant germination**

Seed mass and appendage presence also controlled how native versus non-native plant species responded to biocrust presence. As in Havrilla et al. (2019), we found that biocrusts decreased germination of non-native plant species but had neutral effects on native species overall, suggesting that biocrusts may act as a biotic control of non-native plant invasion in drylands (Bowker et al. 2022; Havrilla and Barger 2018; Slate, Callaway, and Pearson, 2019). Interestingly, our analyses showed that seed mass may partially explain these differential effects. While germination responses of small (0.00-0.50 g/1000 seeds) and large (1.1+ g/1000 seeds) seeded species to biocrust presence were similar in native versus non-native species (slightly positive in small seeds, negative for large seeds; Fig. 3), responses of medium-seeded species that weigh between 0.51-1.00 g/1000 seeds, diverged dramatically in native vs non-native species. Native

medium-seeded species experienced increased germination on biocrusts, while non-native medium-seeded species experienced substantial decreases in germination. It is possible that medium-seeded species are the perfect candidates to show how coadaptation and plant origin influences the response of plant germination on biocrusts. Native plants that coevolved alongside biocrusts may be more adapted to the presence of biocrusts and so may have higher germination rates, relative to non-native species that may not be as well adapted (McTavish et al. 2021). Medium sized seeds may be the middle ground between large seeds that can be inhibited by the biocrust community and small seeds that seem to be able to germinate regardless of plant origin. Copeland and McDonald (2012) stress the need for a larger seed size in arid environments to have increased chance of moisture uptake. A study by Morgan (2006) found that smaller seeded native species experienced less germination inhibition by biocrusts than larger non-native species. It could be argued that native plants with medium sized seeds are coadapted to existence with biocrusts and can make the most of the moisture and nutrient benefits that biocrusts provide while non-native plants do not have these characteristics and so are outcompeted by natives.

Similarly, seed appendages also mediated the effects of biocrusts on the germination of native versus non-native species. While germination of native seeds was unaffected by the presence of appendages, non-native seeds lacking appendages had decreased germination on biocrusts while germination of non-native seeds with appendages was unaffected. This result could be partially explained by the fact that some dryland plant species possessing awns may be specially adapted to promote seed movement and allow seeds to burrow into the soil surface. Hygroscopic awns, an adaptation that allows a seed to move across the surface into a favorable microsite (Peart 1979; Larsen 1995) or to “drill” into the soil when the awn is wetted (Briggs and Morgan, 2011), may help some seeds overcome physical barriers posed by biocrusts to germination. While

hygroscopic awns may be a beneficial adaptation, our database only had 3.2% of studies with hygroscopic awns which is an unfortunate limitation of our database.

### **Seed traits may mediate the germination responses of different plant functional groups**

Morphological seed traits may also offer additional insights into the previously observed pattern that biocrust effects on germination differ across plant functional groups (Havrilla et al. 2019). As in Havrilla et al. (2019), we found that overall, across different plant functional types, biocrusts only significantly decreased germination of C4 grasses. While we were unable to include interaction terms for plant functional groups and seed traits of interest in our meta-analysis due to small sample size in some plant functional groups, we explored seed traits of the C4 grasses in our database. Specifically, we examined mean morphological trait values (i.e., seed mass, seed appendage, and plant origin), and compared them with the germination, survival, and overall performance model results from Havrilla et al. (2019) and our results depicting the interaction between seed size/biocrust type, and seed appendage/biocrust type. Unexpectedly, we found that the C4 grasses in the study generally possessed traits that we found to promote germination on biocrusts. The majority of C4 species included in the data set were small seeded and had appendages. As such, based on these traits and results from our meta-analysis, we would expect that C4 grasses would experience increased germination on biocrusts, however this was not the case. This may be because the eight C4 grasses species were not represented proportionally within our database, where most of the C4 grass studies lacked appendages. While this unequal representation in our database may have masked the influence of appendages, C4 grasses are more likely to lack appendages compared to C3 grasses (Cavanagh et al. 2019). Additionally, there may be other morphological seed traits not measured here, or otherwise other ecological factors driving these effects.

Other functional traits of C4 grasses may explain negative effects of biocrusts on C4 germination. For example, reflective of their physiological adaptations, C4 grasses are often more prevalent in warmer environments (Edwards et al. 2008) such as warm and hot desert ecosystems and/or warmer seasons. However, the C4 grasses within our data set were interacting with mostly lichen and moss dominated biocrust communities which often occur in cooler climates. We found that while not inhibitory, cyanobacterial biocrusts do not promote small seeded species (i.e., C4 grasses), but could be supporting other species that the C4 grasses would be in competition with (i.e., medium and large seeded species) which are being promoted. The biocrusts may also be creating a more inhospitable environment for C4 grasses than other species. C4 grasses often go to seed during the warmer months (Balazs et al. 2020), which when interacting with the albedo decreasing biocrust (Couradeau et al. 2016) would lead to hotter surface temperatures for any seed that is trying to germinate in the same year it fell. Balazs et al. (2020) found that in general, larger seeded dryland plant species are better suited to germinate in high-temperature conditions, and so with the addition of the facilitative effects that we found of large-seeded species by cyanobacteria dominated biocrusts, this may be a reasonable explanation of why the generally smaller seeded C4 grasses are being inhibited. Additionally, Balazs et al. (2020) found that small-seeded species often rely on a seed bank to promote/increase germination rates. If there is biocrust cover over much of the mineral soil it would be difficult for any seed that does not germinate the first growing season to enter the mineral soil and establish a seed bank (Li et al, 2005). Between the promotion of more competitive large-seeded species, the increased effect of temperature on fallen seeds, and the inhibition of seedbank establishment it may be that cyanobacteria dominated biocrust interactions with C4 grass species are responsible for the inhibitory response that both we and Havrilla et al.

(2019) observed despite having traits that would be expected of a species that would be promoted by most biocrust communities.

### **Study limitations and future research directions**

Our study demonstrates that morphological seed traits can be used to predict the effects of biocrusts on plant germination across dryland ecosystems, but our study is also limited in several ways. These limitations allow for consideration of future research directions that could improve understanding of biocrust controls on plant germination and community assembly.

1. *Availability and quality of trait data* - First, we relied on publicly available trait databases (e.g., TRY Plant database (Kattage et al. 2020), KEW Botanical database (Royal Botanic Gardens Kew. 2020) to add traits to prior studies of germination responses to biocrusts. While we were able to find data for a number of species there was incomplete data for the entire biocrust-plant germination database from Havrilla et al. (2019). Some species had no morphological seed trait data available at all, while others only had data for certain covariates (e.g., having data for seed mass, but not seed length or shape etc.). This issue could be solved by increased reporting of plant and seed trait data for dryland species. Future empirical work could also report the plant/seed trait data of any species used in the supplemental data of published papers to grow the repository of knowledge.
2. *Consistency in data reporting within published studies* - Relatedly, while easily accessible, publicly available plant trait data are pooled averages and, in some cases, can also be misaligned with seeds used in the studies and with traits of local varieties of cultivars (Cordlandwehr et al. 2013). The recording of morphological seed trait data in studies of plant germination responses to biocrusts would aid in the facilitation of future

research that seeks to explore the species-specific effects of biocrusts on plant germination and recruitment.

3. ***Limited set of morphological seed traits considered*** - In part due to limited availability of seed trait data, in our study, we were only able to fully analyze the effects of two relatively orthogonal morphological seed traits (i.e., seed mass and seed appendage) on germination responses to biocrust. There are many other morphological seed characteristics that could also be influencing these interactions (e.g. seed shape, seed length, whether a seed is mucilaginous or has a hygroscopic awn (Zhang et al. 2016; Benard et al. 2019). Future studies should explore these additional morphological traits by potentially narrowing the scope to only a handful of species if additional data is unavailable. Future meta-analyses could be warranted if the additional data was found, and suitable models created. It could be that with larger sample sizes the correlation that we found between certain morphological seed traits (i.e., seed size and seed shape or seed appendage and seed appendage hygroscopicity) may be parsed apart.
4. ***Geographical limitations in the available published literature*** - Biocrusts can be found in every region of the globe, from the arid Southwestern US to the polar regions of Antarctica (Weber et al. 2022). From lower elevations all the way up to the alpine (Weber et al. 2022). As discussed in Havrilla et al., 2019, our data set was geographically limited, and lacked broad representation of studies from South America, Africa, Australia, as well as more polar regions. This was the result of two primary limitations in research; (1) Many of the studies that we found were from the northern portion of the world suggesting a publication bias. (2) A more comprehensive multi-lingual data search process could have improved data selection and coverage (Zenni et al. 2023). The Havrilla et al. (2019) includes only

papers published or translated in English and Chinese; perhaps the incorporation of other language papers could move to more equal study representation across the globe (especially in the Global South).

5. *Exploring biocrust effects on different plant functional groups* - Finally, due to the limited sample sizes future studies should more directly examine the relationships between plant functional groups, plant traits, and biocrusts. Additional empirical studies should examine interactions between different plant functional types (including C4 grasses), their traits, and biocrusts would be of particular interest to parse out the explanations for the interactions that have been observed.

### **Seed traits improve predictions of outcomes of biocrust plant interactions and plant community assembly processes in drylands**

Trait-based approaches are increasingly used in plant ecology to understand mechanisms underlying plant germination and community assembly processes (Larson et al. (2015, 2020), Larson and Funk (2017), Saatkamp et al. (2018)). Results from our study build on a prior meta-analysis by Havrilla et al. (2019) to show that biocrusts can have strong effects on plant germination across global drylands. Ours is the first global synthesis to show that biocrust effects on germination are mediated by morphological seed traits. Understanding biocrust-plant interactions in drylands is critical to understanding community assembly processes in the context of global change and may also assist land managers in planning conservation and restoration activities. Biotic interactions such as these can influence plant community assembly outcomes (HilleRisLambers et al., 2012; Levine et al., 2004; Lortie et al., 2004), and our study suggests that integrating plant functional traits into models of biocrust-plant interactions could improve our predictive understanding of how biocrusts may influence and/or filter plant community assembly

processes in dryland ecosystems. Since biocrusts are highly vulnerable to physical disturbance (Weber et al. 2016) and climate change (Antoninka et al. 2018, Reed et al. 2015, Ferrenberg et al. 2015), it is also possible that decreases in biocrust presence could have indirect effects on plant recruitment and community composition in the face of land degradation and desertification in drylands worldwide (Osborne et al. 2022). Finally, as land managers in drylands prepare to adapt management to these challenges, new understanding of how seed traits mediate biocrust-plant interactions could be key to decision-making about biocrust conservation and restoration. For example, seed trait information may provide guides about which plant species may be successful in degraded systems where biocrusts and plant communities are being restored in tandem (Barger 2018).

## CHAPTER 2 - EXPLORING INTERACTIONS BETWEEN BIOCRUST INOCULUM TREATMENTS AND PLANT FUNCTIONAL TRAITS

### **Introduction**

Over a third of Earth's terrestrial ecosystems are degraded with that proportion predicted to increase to 90% over the next century (FAO, 2020) due in part to biotic disturbances like non-native species invasion (Lake and Michelle, 2004) and/or physical disturbances from recreation, extractive industries, and wildfire (Ramon Vallejo et al. 2012). Land degradation negatively impacts native plant and soil microbial communities and ecosystem functioning (Ye, Fei et al. 2022). Arid and semi-arid ecosystems (drylands), comprise over 40% of Earth's terrestrial surface, and are particularly susceptible to land degradation (Pravalie, 2016). Once degraded, dryland ecosystem functioning often cannot be recovered within the timescale of human lives in the absence of active restoration, broadly defined as the process of applying active management techniques to overcome biogeochemical abiotic and biotic barriers that limit ecosystem recovery (Gann et al. 2019). Conditions in drylands, such as a lack of soil moisture (D'Odorico et al. 2007), and soil degradation (e.g., diminished soil stability; Turnbull et al. 2012) often make restoration particularly challenging.

Most drylands restoration efforts, largely focus on native plant community through revegetation (James et al. 2013). However, degradation often affects both plants and soil communities in tandem, and recovery trajectories of these communities are often linked (Harris, 2009; Wu et al. 2021). Soil microbes can play a crucial role in the establishment and restoration of plant communities (Harris, 2009; Potthoff et al. 2006). These interactions have strong effects on recruitment during various stages of the plant life cycle, from plant germination (Singh et al. 2023), to plant establishment (Codon & Pyke 2018), and plant competition/survival dynamics

(Wang, 2017). When attempting to restore an ecosystem these interactions should be taken into consideration as they may improve (Cross and Aronson, 2023) or hinder (Miller, 1992) the establishment of new plant communities. Soil microbial inoculation has been shown to ameliorate harsh growing conditions in degraded sites (Tran, Ilhan, and Gye-Chun, 2019; Choi, Sasha, and Colin, 2022). For example, in dryland soil microbes affect both soil moisture content, soil nitrogen, and soil temperature (Delgado-Baquerizo et al. 2013; Parwani, Bhatt, and Singh, 2021; Eldridge et al. 2020).

In drylands, biological soil crusts (biocrusts), soil-dwelling assemblages of cyanobacteria, algae, lichens, and mosses inhabit the first few centimeters of the soil surface (Weber et al. 2022), and act as key ecosystem engineers through impacts on soil stability, hydrology, and fertility (Weber et al. 2022). Biocrusts are highly susceptible to physical disturbance (e.g., compressive forces (Durham et al. 2018), fire (Zaddy, Eldridge, and Bowker, 2016), others (Caster et al. 2021)) as well as climate change (Caster et al. 2021; Weber et al. 2016). As such, in the face of escalating land degradation in drylands, biocrusts increasingly require active restoration. While there has been extensive research into the restoration of dryland plant communities (Hulvey et al. 2017; James et al. 2013; Kidisheva et al. 2016) and, increasingly, biocrust communities (Antoninka et al. 2020; Zhou et al. 2020) individually considerably less research has focused on how restoration of both communities in tandem impact restoration outcomes.

Biocrusts can significantly impact the germination and establishment of plants in dryland ecosystems (Havrilla et al. 2019; Bacovcin, McIntyre, & Havrilla, In Prep). Through physical and biotic mechanisms biocrust organisms modify soil resources important for plant recruitment including soil moisture, temperature, and nutrient cycling (Eldridge et al. 2020; Delgado-Baquerizo et al. 2013; Barger et al. 2016; Ruthherford et al. 2017; Xiao and Bowker 2020). For

example, biocrusts often increase soil water retention and infiltration rates leading to increased soil moisture for longer periods (Eldridge et al. 2020), which is the primary limiting factor for dryland plant recruitment (Copeland and McDonald, 2012). Biocrusts also play a large role in both carbon and nitrogen fixation and can thereby promote soil fertility (Delgado-Baquerizo et al. 2013). Soil nitrogen can influence seed germination and is also vital to plant establishment and growth (Leghari et al. 2016). Additionally, biocrusts often increase soil surface temperature by decreasing soil surface albedo (Ruthherford et al. 2017; Xiao and Bowker 2020). Soil temperature serves as a cue for germination with increasing temperatures often serving as a cue for entering a growing season (Copeland and McDonald, 2012). As such, increased surface temperatures on biocrusts could promote germination in certain seasons. Though, high surface temperatures on biocrusts could also limit germination (Ooi et al. 2009; Van der Walt and Witkowski, 2017). Biocrusts may also impact germination and seedling recruitment through biotic mechanisms. For example, some biocrust organisms have been shown to assist in breaking seed dormancy in some plant species (Eldridge et al. 2021).

Due to the crucial roles that biocrusts play in determining soil functioning (Weber et al. 2022) and their concomitant impacts on plant recruitment (Havrilla et al. 2019), increasing understanding of biocrust plant-interactions is vital for predicting outcomes of plant community assembly in the context of dryland restoration. While biocrusts can influence plant recruitment and community assembly processes (Havrilla et al. 2019; Bowker et al. 2018), these effects have been mixed depending on biocrust type and plant characteristics. A global meta-analysis by Havrilla and colleagues (2019) showed that biocrusts have differential effects on the germination of various plant functional groups (i.e., C3 vs C4 grasses, Nitrogen-fixing vs Non-Nitrogen-fixing forbs), and plant origins (i.e., plant species native vs non-native to the location where biocrusts dwell). For

example, Havrilla et al. (2019) found that biocrusts inhibit the germination of C4 grasses while not significantly inhibiting the germination of any of the other plant functional groups. Both Havrilla et al. (2019) and experimental studies (e.g., Slate, Callaway, & Pearson (2018)) have also found that biocrusts inhibit the germination of non-native species. This suggests biocrusts may play a role in inhibiting the germination of non-native plants that are present in communities that contain biocrusts (Song, Li, and Hui, 2020; Rosentreter, Eldridge, and Kaltenecker, 2001). Moreover, a recent meta-analysis by Bacovcin, McIntyre, & Havrilla (In Prep; Chapter 1 of this thesis) showed that morphological seed traits may mediate the context dependency of biocrust effects on plant germination. Specifically, the study found that seed mass interacts with different biocrust community types to affect germination, that cyanobacteria dominated biocrusts promoted the germination of medium and large seeds while lichen, moss, and mixed biocrust communities decreased the germination of seeds with large masses (Bacovcin, McIntyre, and Havrilla, In Prep). The study also showed that seed appendages interact with both biocrust community types and plant origin (i.e., native vs. non-native species), with appendages interacting positively with lichen, moss, and mixed biocrust communities leading to increased germination while the presence or absence of an appendage had no effect on germination when interacting with plant origin. Despite increased understanding of potential biocrust and plant characteristics that mediate outcomes of biocrust effects on plant recruitment, the physical and biotic mechanisms underlying these interactions have remained unclear. Further, few studies have investigated the effects of biocrusts on plant recruitment in the context of concurrent biocrust (i.e., soil inoculation; Rossi, Mugnai, and De Philippis, 2022) and plant (i.e., seeding; Palma and Laurance, 2015) restoration treatments applied in tandem.

A variety of knowledge gaps exist in our understanding of how application of biocrust inoculum might impact the recruitment of native seed mixes. For example, (1) how do seed traits interact with different levels of biocrust inoculum cover, (2) are the effects of inoculation mainly physical or biotic, and finally (3) is there an association between the area of collection for both biocrust inoculum and plant seeds suggesting a co-adaptive response, or is there no effect of co-adaptation? Our study explores the effects of biocrust inoculation on native plant germination and aims to parse out potential physical versus biotic mechanisms whereby biocrust inoculum impacts germination. To achieve this, we performed a full-factorial greenhouse study. We seeded mesocosms with biocrust inoculum and native plant seeds and measured plant germination responses. Specifically, we measured plant responses to biocrust inoculation treatments at different cover levels (i.e., 30% inoculum cover, 100% inoculum cover, relative to a bare soil control). To parse out potential abiotic versus biotic effects of biocrust inoculation on germination, we tested the effects of live vs. sterilized (i.e., autoclaved) biocrust treatments. To explore whether biocrust inoculum effects vary according to whether seed mixes are developed for the area in which the biocrust inoculum was collected, we also tested effects of treatments on two native seed treatments: one developed for the Mojave Desert (where our biocrust inoculum was collected from) and one developed for the more distant Colorado Plateau Desert. Research has shown that increased heterogeneity in a site will lead to increased germination (Tilman, Wedin, and Knops, 1996), by increasing site heterogeneity within our mesocosms with the application of biocrust inoculum there may be a subsequent increase in germination. A paper by Munoz-Rojas et al. (2018) found that biopriming seeds with cyanobacteria led to increased germination. It could be that biologically intact biocrust inoculum could lend these same germination enhancements, instead of the effects being entirely based on the structural components of biocrust inoculum. Finally, some research

has shown that biocrusts can inhibit or promote the germination of non-native or native plant species (McTavish et al. 2021; Havrilla et al. 2019). We created two different seed mixes to test if the distance from the biocrust collection site would impact seed germination.

For each treatment combination we also explored how different plant functional groups and seeds of different masses responded to biocrust treatments to infer potential mechanisms whereby biocrust inoculum impacts plant germination based on plant traits. We hypothesized first that (1) the level of biocrust inoculum cover would significantly influence germination. Specifically, we predicted that bins with moderate (i.e., 30%) biocrust inoculum cover, applied in strips, would have the greatest germination due to an increase in potential ~~recruitment~~ niche space for seeds with different germination requirements (favoring bare soil or biocrust microsites). Second, we hypothesized that (2) application of live biocrust increases germination to a greater degree than autoclaved biocrust due to the potential positive effects that active soil microbes have on germination (e.g., overcoming dormancy barriers (Eldridge et al. 2021) in addition to physical benefits of biocrusts to soil conditions (e.g., water, nutrients)). Third, we hypothesized that (3) the source (i.e., ecoregion) of the seeded plants will influence germination. Specifically, the restoration seed mix sourced closer to the area of biocrust collection (i.e., Mojave Desert) would have greater germination on live biocrust treatments than the seed mix developed for the Colorado Plateau. Finally, we hypothesized that (4) observed effects of biocrust treatments on germination are partially explained by plant functional type and seed mass. Specifically, we predicted that C3 grasses and Non-nitrogen-fixing forbs (as in Havrilla et al. 2019) and smaller seeded species (as in Bacovcin, McIntyre, and Havrilla (In Prep) would experience increased germination in high-cover live biocrust treatments. Taken together, results from this study will advance our understanding of community assembly in drylands and explore potential interactions that may

occur between biocrust inoculum and plant communities when undergoing restoration treatments concurrently.

## **Methods**

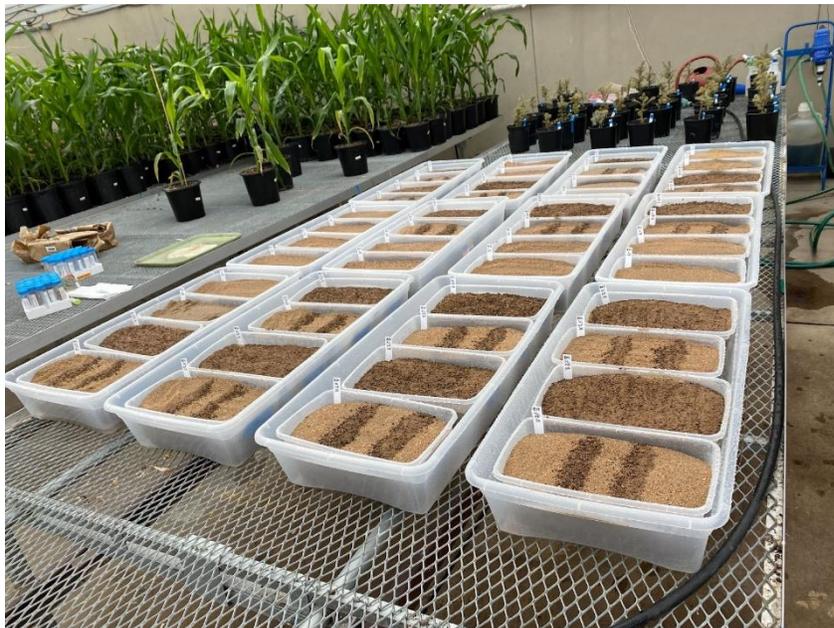
### ***Biocrust Collection Methods***

Biocrusts were salvaged in fragments or “crumbles” from areas slated for development within the Gemini Solar Project (36°29'32.7"N 114°44'53.3"W) approximately 25 miles northeast of Las Vegas, Nevada within the Mojave Desert Ecoregion. Vegetation at the sampling sites was predominantly black sagebrush (*Artemisia nova*) shrubs with occasional stands of grasses and cacti though much of the ground surface was bare soil (Supplementary Table 2). Both sites were dominated by cyanobacteria biocrust communities ~60% at Site 1 and ~50% at Site 2, (Supplementary Table 2), moss communities made up ~15% of the communities at both sites, and lichen communities made up ~20% at Site 1 and ~25% at Site 2 with bare soil and rocks making up the rest of the ground cover. To collect the biocrust inoculum we followed the salvage collection method laid out in Tucker et al. (2020). Specifically, we used a small garden trowel to collect small patches of dark cyanobacterial biocrusts. Biocrusts were harvested dry and were placed in large 5-gallon buckets that were left open for ventilation. Once collected, the biocrust inoculum was transported directly to storage at Colorado State University storage, and stored dry, shaded, vented, and at room temperature (roughly 20-22°C) as suggested in the Tucker et al. (2020) biocrust inoculum salvage protocol. To collect enough inoculum salvage for the experiment we sampled at two sites and then pooled the salvaged “crumbles” together.

### ***Experimental Design***

Our experiment was conducted within a greenhouse at the Plant Growth Facility on the Colorado State University campus located in Fort Collins Colorado, USA (approximately 40.5762°N, 105.0808°W). Each experimental mesocosm consisted of one (34.8cm wide, 20.3cm deep, and 12.4cm high) plastic bin (hereafter “mesocosm”) which was filled with 100% play sand substrate. Prior to experimentation, mesocosms were treated with one of three biocrust inoculum cover treatments (bare soil/0% inoculum, 30% inoculum, 100% inoculum; Fig. 7). Treatments that received inoculum (i.e., 30% and 100% cover) received biocrust inoculum with one of two treatments: live biocrust and autoclaved inoculum (autoclaved biocrust inoculum was prepared by autoclaving batches of live biocrust at 350° C and 25 PSI for 1.5 hours) for a total of 5 biocrust inoculum treatments. Each treatment combination was repeated with one of two seed mixes: Colorado Plateau Desert and Mojave Desert for a total of 10 treatment combinations (5 biocrust inoculum treatments x 2 seed mixes x 4 reps = 40 mesocosms). We used a random number generator to randomly assign each experimental bin to one of four sections of the growth bench where it remained for the entirety of the experiment. Treatments were implemented by spreading appropriate biocrust inoculum treatments over the relevant experimental units at ~30% cover and ~100% cover. Once treated, each mesocosm began to receive watering treatments to begin exhausting the local seed bank. The watering protocol was to mist and sub-irrigate mesocosms twice a week. This continued for twenty-three days until the native seed bank was exhausted. Each mesocosm was checked daily during this period. Upon emergence, plants were carefully removed with forceps and disposed of. After the 23-day seed bank exhaustion period, mesocosms were allowed to completely dry down prior to seed mix application for a week and a half. Appropriate seed mix packets were applied to each mesocosm with care taken to spread seeds evenly across the mesocosm soil surface. Then, a new watering protocol was established with subirrigation once

weekly and misting twice per week to allow for seed germination. This protocol was continued for sixty days until the end of the experiment.



**Figure 7.** Photograph of experimental setup. In the foreground of the first row are 30% biocrust inoculum cover mesocosms with the inoculum spread in two strips. In the second row, 100% biocrust inoculum cover mesocosms with inoculum covering the entire mesocosm surface. In the third row from the front are bare soil mesocosms lacking biocrust cover.

During the germination phase of the experiment (days 60-83) we collected species-level germination data once per week. Each mesocosm was examined and individual species germination was recorded along with taking percent volumetric water content data (% VWC). Percent VWC data was taken with the Campbell Scientific HydroSense II (Campbell Scientific) in three separate areas of each bin, one spot on each end and one in the middle. These were then averaged to get the total %VWC of each bin. While not used in the analysis the %VWC was taken in the event that the study wanted to test the effects of biocrust inoculum levels on soil moisture.

### ***Seed Mix Creation and Seeding Treatments***

Seed mix treatments consisted of two separate 12 species mixes, one developed for the Colorado Plateau Desert Ecoregion and one for the Mojave Desert Ecoregion. For the creation of

the seed mixes we began by using the species listed in the RestoreNet restoration protocol (Laushman et al. 2022), a large, networked restoration project in the Southwestern USA (Havrilla et al. 2020). This gave us a good starting point to develop local seed mixes. However, due to our specific research questions concerning the responses of plant functional groups and seed traits to biocrust inoculum, the given species lists were not sufficient to have three species of each plant functional group (i.e., C3 grasses, C4 grasses, Nitrogen-fixing forbs, Non-nitrogen-fixing forbs) and so we worked to identify additional species to meet our seed mix criteria. This process involved going through multiple species lists for the Mojave and Colorado Plateau Deserts and then checking with the USDA plant database to make sure that they fell within the ecoregion we needed (USDA, 2023). After we had compiled a list of candidate species we then arrived at our final seed mixes for the Mojave and Colorado Plateau Desert (Supplementary Table 3) by which species were available for purchase from local seed vendors. Each mix was broken down into four plant functional groups with three species from each. Within all groups an effort was made to include species with different seed morphological traits. Morphological traits that were considered were seed shape, seed mass, and if a seed had an appendage or not. When acquiring the seeds an effort was made to try and source seeds from as close to their respective ecoregions as possible, and to maintain any present seed appendage.

Once seeds were acquired multiple rounds of germination trials were performed to assess the viability of the seeds (Supplementary Table 4). Germination trials were conducted by rolling 10 seeds of each species into a wet paper towel and placing that into a clear resealable plastic bag. These bags were left within the greenhouse and were checked every other day from the start of the trial with additional water being applied as necessary with a spray bottle. At least 30% germination was used as the cutoff for inclusion for the study. Within germination trials each species was able

to meet or exceed this threshold. After germination trials were completed, we put five seeds for a total of 60 seeds for each treatment into small coin envelopes that were then used to seed the mesocosms.

### ***Data Collection and Calculations***

Seedling germination data was collected once a week before the first watering. Each mesocosm was assigned a number, and a bench ID which consisted of row number, a column number, and a location ID, and a compass coordinate number (NW from rows 1 to 6 and columns 1 and 2, NE from rows 1 to 6 and columns 3 and 4, SW from rows 7 to 12 and columns 1 and 2, SE from rows 7 to 12 and columns 3 and 4, Supplementary Fig.5) on the bench. Within each mesocosm the density of each species was recorded. These records were collected once weekly in weeks 1-4 of the germination phase and then twice more with one week separating the collection dates. The last two collections were to assess which plants were established and survived.

To answer part of our question of how seed traits interact with biocrust inoculum and how this influences germination we utilized the community weighted mean (CWM) of seed mass. CWMs allow us to group certain species/groups by a certain trait and how these species may be sorted across a community (Duarte et al. 2017). To calculate the CWM of seed mass we first calculated the mean seed mass of each plant functional group. Then we utilized equation three to calculate the CWM of each functional group (Shen et al. 2019). Here  $n_{species}$  denotes the number of species in the plant functional group, in this case three,  $\mu_{seed\ weight}$  denotes the average seed mass of the species within that functional group, and  $n_{germination}$  denotes the number of plants that germinated when that data point was collected.

$$\text{Equation 3: } CWM_{\text{Seed Mass}} = (n_{\text{species}}) \times (\mu_{\text{seed weight}}) \times (n_{\text{germination}})$$

This value allowed us to assess the effects that different distributions of seed masses had on the germination of plants when interacting with our different biocrust inoculum treatments.

### ***Data Analysis***

Following the collection of germination and survival data and calculation of community weighted mean seed mass ( $CWM_{seed\ mass}$ ), we tested for the effects of biocrust inoculum cover, sterilization, and seed mix type on the percent germination of each bin using negative binomial general linear mixed effect (*glmer.nb*) models were fitted, using the *lme4* package in R (Bates and Bolker, 2015). We used the *glmer.nb()* function to fit negative binomial general mixed effects regression models due to the large amount of zeros within the data and a non-normal error distribution. Negative binomial distributions can help to off-set zero inflated data (Ismail and Zamani, 2013, Lindén and Mäntyniemi 2011). First, to explore how plant germination responded to treatments (i.e., *Biocrust\_Inoculum\_Cover*, *Seed\_Mix*, *Sterilization*, and *Plant\_Functional\_Group*) and their potential interactions, we constructed a large initial *glmer.nb* model. For each significant interaction from this model, we then created a separate individual models to minimize any potential masking from the high number of zeros in the data (Table 3), while examining its interaction with each seed mix (i.e., Colorado Plateau Desert and Mojave Desert). Second, we used a separate *glmer.nb* model to explore how  $CWM_{seed\ mass}$ , a community weighted average of seed mass within the assembles plant community, responded to treatments (i.e., *Biocrust\_Cover*, *Sterilization*, and *Seed\_Mix*). We again then created bivariate interaction models to analyze any significant interactions. Within each model, when a candidate variable or interactions (i.e., *Biocrust\_Cover*, *Sterilization*, *Seed\_Mix*, *CWM\_Seed\_Mass* and *PFG*) was

found significant, we used the *emmeans()* function from the *emmeans* package (Lenth, 2023) to assess any pairwise interactions. Statistical significance for all analyses was set at  $\alpha = 0.05$ .

**Table 3.** Description of the four predictor variables used within the negative binomial general linear mixed effects models.

Explanatory Variable	Variable Type (Number of Levels)	Description of Variable
Biocrust_Cover	Categorical (3)	0, 30, and 100; Classified by the percent cover of biocrust inoculum ranging from a bare soil control (0) to full coverage (100).
Sterilization	Categorical (2)	Live and Sterile; Classified by if the biocrust inoculum used was sterilized within the autoclave or still had live biotic organisms.
Plant_Functional_Group	Categorical (4)	C3 Grasses, C4 Grasses, Nitrogen fixing forbs, and Non-nitrogen fixing forbs; as assigned by the herbarium records for the various species used within each seed mix.
CWM_Seed_Mass	Continuous	Calculated value of average seed mass multiplied by the community size.

## Results

### *Plant germination increased with increasing biocrust inoculum cover*

Biocrust inoculum cover was not found to individually influence the germination ( $p = 0.371$  and  $p = 0.125$  for the 30% inoculum and 100% inoculum cover treatments respectively, Table 4) and seed mix significantly interacted to determine plant germination outcomes ( $p < 0.001$ , Table 4).

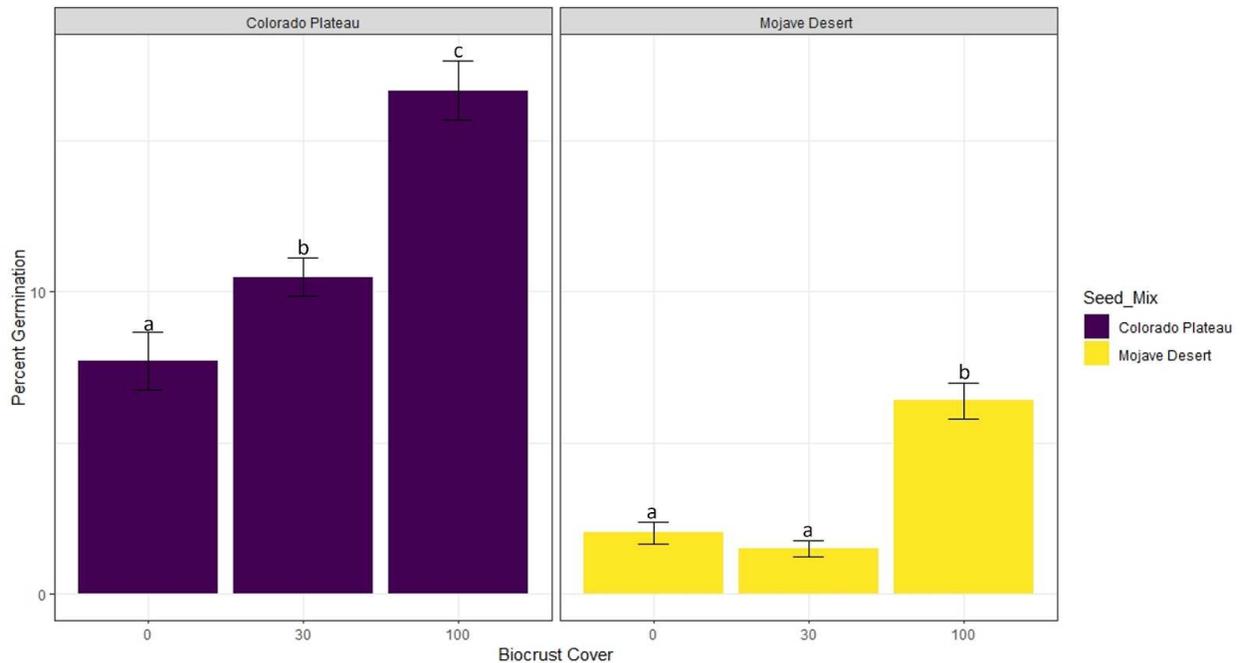
**Table 4:** Results of the negative binomial general linear mixed effects regression model used to analyze the individual candidate variables and their interactions.

Germination Interaction	Plant Functional Group and Biocrust Cover Model			
	Estimate	Standard Error	Z value	P value
Intercept	1.907	0.414	4.600	<0.0001

Biocrust_Cover 30	-0.313	0.349	-0.895	0.371
Biocrust_Cover 100	0.4980	0.324	1.536	0.125
Seed_Mix Mojave Desert	-1.649	0.321	-5.112	<0.0001
Sterilization Live Biocrust Inoculum	0.06149	0.216	0.284	0.776
PFG C4 Grasses	0.214	0.314	0.680	0.496
PFG Nitrogen Fixing Forbs	-21.536	27.768	-0.776	0.438
PFG Non-Nitrogen Fixing Forb	0.224	0.342	0.656	0.512
Biocrust_Cover (30) : Seed_Mix Mojave Desert	-0.922	0.363	-2.539	0.0111
Biocrust_Cover (100) : Seed_Mix Mojave Desert	0.703	0.3480	2.018	0.0435
Biocrust_Cover (30) : PFG C4 Grasses	0.621	0.3887	1.596	0.110
Biocrust_Cover (100) : PFG C4 Grasses	-0.169	0.356	-0.475	0.635
Biocrust_Cover (30) : PFG Nitrogen Fixing Forbs	21.233	27.769	0.765	0.445
Biocrust_Cover (100) : PFG Nitrogen Fixing Forbs	21.0717	27.768	0.759	0.448
Biocrust_Cover (30) : PFG Non-Nitrogen Fixing Forb	0.819	0.424	1.931	0.0535
Biocrust_Cover (100) : PFG Non-Nitrogen Fixing Forb	0.846	0.403	2.101	0.0357
Seed_Mix Mojave Desert : Sterilization Live Biocrust Inoculum	0.103	0.222	0.463	0.643
Sterilization Live Biocrust Inoculum : PFG C4 Grasses	-0.139	0.274	-0.507	0.612
Sterilization Live Biocrust Inoculum : PFG Nitrogen Fixing Forbs	-1.796	0.0102	0.103	0.918
Sterilization Live Biocrust Inoculum	-0.210	0.286	-0.734	0.463
Seed_Mix Mojave Desert : PFG C4 Grasses	1.394	0.251	5.555	<0.0001
Seed_Mix Mojave Desert : PFG Nitrogen Fixing Forbs	-0.683	0.307	-2.227	0.0259
Seed_Mix Mojave Desert : PFG Non-Nitrogen Fixing Forb	-2.0387	0.296	-6.890	<0.0001

Overall, germination was much higher in the Colorado Plateau Desert seed mix relative to the Mojave Desert seed mix (Colorado Plateau Desert percent germination = 37%, Mojave Desert percent germination = 11%, Fig. 8). For the Colorado Plateau seed mix, germination increased with increasing biocrust inoculum cover: relative to the bare soil control, germination was 4.0-fold higher in mesocosms with moderate (30%) biocrust inoculum cover ( $p = 0.0129$ , Fig. 8, Table 5), while germination was increased by 9.6-fold in mesocosms with 100% biocrust inoculum cover ( $p < 0.0001$ , Fig. 8, Table 5). For the Mojave Desert mix, there was a decrease of 25.6% in

germination in mesocosm with moderate (30%) biocrust cover ( $p < 0.0001$ , Fig. 8, Table 5), while the germination in 100% cover increased by 2.5-fold ( $p < 0.0001$ , Fig. 8, Table 5).



**Figure 8.** Effect of biocrust inoculum cover treatments (i.e., 0%, 30%, 100%) on the percent germination of the two seed mixes: Colorado Plateau Desert (dark purple bars) and Mojave Desert (light yellow bars). Lowercase letters (a-c) show significant differences in percent germination among biocrust inoculum cover treatments within seed mixes.

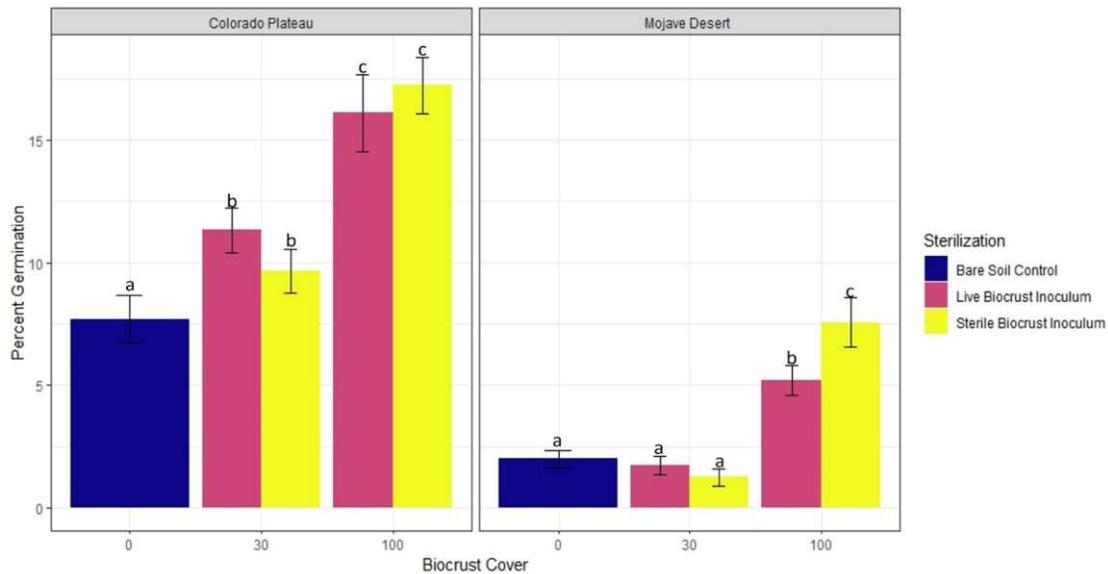
**Table 5.** Results of the negative binomial general linear mixed effects regression model used to analyze the interaction between biocrust inoculum cover and percent germination.

Germination Response	Biocrust Cover Model			
	Estimate	Standard Error	Z value	P value
Intercept	1.767	0.246	7.170	<0.0001
Biocrust Cover (0) : Colorado Plateau	0.0864	0.215	0.402	0.668
Biocrust Cover (30) : Colorado Plateau	0.431	0.173	2.487	0.0129
Biocrust Cover (100) : Colorado Plateau	0.916	0.174	5.271	<0.0001
Biocrust Cover (0) : Mojave Desert	-1.157	0.235	-4.926	<0.0001
Biocrust Cover (30) : Mojave Desert	-1.452	0.183	-7.920	<0.0001

### *Autoclaving of biocrust inoculum had few effects on germination*

Biocrust inoculum sterilization treatments did not significantly influence the germination of either seed mix ( $p = 0.776$ , Table 3). While the results showed that for the Colorado Plateau mix compared to a bare soil control on 30 percent cover there was no significant interaction for

sterile or live biocrust inoculum treatments ( $p = 0.248$  (Live) and  $p = 0.398$  (Autoclaved) respectively, Fig. 9, Table 6). There was no significant difference between the autoclaved and live biocrust treatments. When examining the 100 percent cover bins there was a 51% increase in germination using the autoclaved inoculum ( $p = 0.008$ , Fig. 9, Table 6), and a 43% increase in germination when using the live inoculum ( $p = 0.001$ , Fig. 9, Table 6). For the Mojave Desert seed mix on 30% inoculum cover there was no effect from either of the inoculum treatments ( $p = 0.146$  for live biocrust and  $p = 0.908$  for autoclaved biocrust, Fig. 9, Table 6). When examining the 100 percent cover bins there was a 1.8-fold increase in germination when using the live biocrust inoculum ( $p < 0.0001$ , Fig. 9, Table 5), and a 2.6-fold increase in germination when using the autoclaved biocrust inoculum ( $p < 0.0001$ , Fig. 9, Table 6).



**Figure 9.** Effects of biocrust inoculum cover (i.e., 0%, 30%, 100%) and sterilization (i.e., live vs sterilized) on percent germination of the two seed mixes (i.e., Colorado Plateau Desert and Mojave Desert). Lowercase letters (a-c) show significant differences in percent germination across different biocrust inoculum covers x sterilization treatment combinations relative to bare soil controls within the two seed mixes.

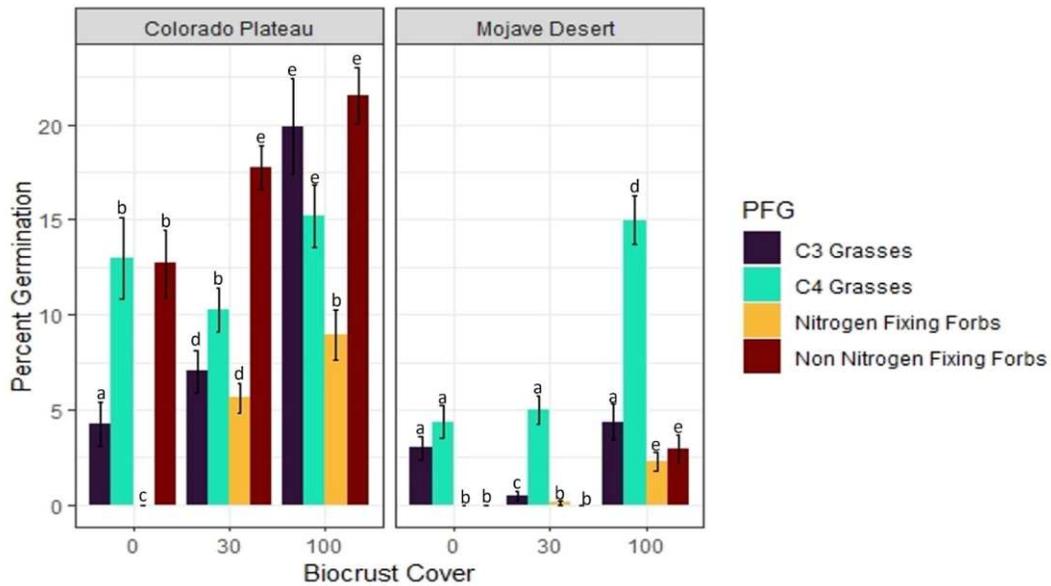
**Table 6.** Results of the negative binomial general linear mixed effects regression model that was used to analyze the interaction between biocrust inoculum sterilization, biocrust inoculum cover, and percent germination.

Germination Interaction	Sterilization and Biocrust Cover Model			
	Estimate	Standard Error	Z value	P value
Intercept	1.907	0.283	6.731	<0.0001
Bare Soil Control : Colorado Plateau : Biocrust Cover (0)	-0.0974	0.242	-0.402	0.688
Live Biocrust Inoculum : Colorado Plateau : Biocrust Cover (30)	0.288	0.250	1.155	0.248
Sterile Biocrust Inoculum : Colorado Plateau : Biocrust Cover (30)	0.207	0.245	0.844	0.398
Live Biocrust Inoculum : Colorado Plateau : Biocrust Cover (100)	0.676	0.255	2.651	0.0080
Sterile Biocrust Inoculum : Colorado Plateau : Biocrust Cover (100)	0.818	0.2441	3.353	0.0008
Bare Soil Control : Mojave Desert : Biocrust Cover (0)	-1.371	0.274	-5.007	<0.0001
Live Biocrust Inoculum: Mojave Desert : Biocrust Cover (30)	-1.481	0.269	-5.512	<0.0001
Sterile Biocrust Inoculum: Mojave Desert : Biocrust Cover (30)	-1.872	0.261	-7.167	<0.0001
Live Biocrust Inoculum: Mojave Desert : Biocrust Cover (100)	-0.411	0.257	-1.601	0.109

### ***Effects of biocrust inoculum cover on germination differ among plant functional groups***

The effects of biocrust inoculation cover on germination of each seed mix varied across plant functional groups ( $p < 0.0001$ ). For Colorado Plateau seed mix, in mesocosms with moderate biocrust cover (30%) germination of Nitrogen fixing forbs experience a 107% increase in germination ( $p < 0.0001$ , Fig. 10, Table 7). C3 grasses increased 48.7% relative to bare soil controls ( $p < 0.0001$ ), Non-nitrogen fixing forbs experienced a 14% increase in germination ( $p < 0.0001$ ), and finally C4 grasses experienced a 13.2% decrease in germination ( $p < 0.0001$ ). Germination of all plant functional groups increased in response to 100% biocrust inoculation relative to bare soil controls. In response to 100% biocrust inoculum cover, C3 grasses experienced a 133% increase in germination ( $p < 0.0001$ ), C4 grasses experienced a 7% increase in germination ( $p < 0.0001$ ), Nitrogen-fixing forbs experienced a 109% increase in germination ( $p < 0.0001$ ), and finally Non-Nitrogen-fixing forbs experienced a 26% increase in germination ( $p < 0.0001$ ).

For the Mojave Desert seed mix moderate biocrust inoculum cover (30%) resulted in a 2.2-fold decrease in germination on C3 grasses ( $p < 0.0001$ ), a 89.4% increase in germination for Nitrogen-fixing forbs ( $p < 0.0001$ ), there was a 3.5% increase in C4 grass germination ( $p < 0.0001$ ), finally Non-nitrogen- fixing forbs experienced no change in germination ( $p < 0.0001$ ). The 100% biocrust inoculum cover treatment resulted in a 104.8% increase in germination of Non-nitrogen-fixing forbs ( $p < 0.0001$ , Fig. 4, Table 7), a 103.8% increase in germination of Nitrogen-fixing forbs ( $p < 0.0001$ ), an 84.2% increase in C4 grasses germination ( $p < 0.0001$ ), and finally a 52.6% increase in the germination of C3 grasses ( $p < 0.0001$ ).



**Figure 10.** Germination responses of different plant functional groups (i.e., C3 grasses, C4 grasses, Nitrogen-fixing forbs, and Non-Nitrogen-fixing forbs) in the two seed mixes (Colorado Plateau Desert and Mojave Desert) to the three biocrust inoculum cover treatments (i.e., 0 (bare soil control), 30% and 100%). Lowercase letters (a-e) show significant differences of germination of a plant functional group across different biocrust inoculum covers.

**Table 7.** Results of the negative binomial general linear mixed effects regression model that was used to analyze the interaction between plant functional group, biocrust inoculum cover, and percent germination.

Germination Interaction	Plant Functional Group and Biocrust Cover Model			
	Estimate	Standard Error	Z value	P value

Intercept	0.851	0.0102	83.639	<0.0001
C3 Grasses : Colorado Plateau : Biocrust Cover (0)	0.293	0.0102	28.761	<0.0001
C4 Grasses : Colorado Plateau : Biocrust Cover (0)	1.533	0.0102	150.684	<0.0001
Nitrogen Fixing Forbs : Colorado Plateau : Biocrust Cover (0)	-21.763	0.0102	-2137.125	<0.0001
Non-Nitrogen Fixing Forbs : Colorado Plateau : Biocrust Cover (0)	1.553	0.0102	152.600	<0.0001
C3 Grasses : Colorado Plateau : Biocrust Cover (30)	0.841	0.179	4.698	<0.0001
C4 Grasses : Colorado Plateau : Biocrust Cover (30)	1.221	0.0102	120.109	<0.0001
Nitrogen Fixing Forbs : Colorado Plateau : Biocrust Cover (30)	0.574	0.0102	56.437	<0.0001
Non-Nitrogen Fixing Forbs : Colorado Plateau : Biocrust Cover (30)	1.906	0.0102	187.505	<0.0001
C3 Grasses : Colorado Plateau : Biocrust Cover (100)	1.812	0.0102	178.253	<0.0001
C4 Grasses : Colorado Plateau : Biocrust Cover (100)	1.707	0.0102	167.901	<0.0001
Nitrogen Fixing Forbs : Colorado Plateau : Biocrust Cover (100)	1.058	0.0102	104.059	<0.0001
Non-Nitrogen Fixing Forbs : Colorado Plateau : Biocrust Cover (100)	2.180	0.0102	214.399	<0.0001
C3 Grasses : Mojave Desert : Biocrust Cover (0)	-0.0458	0.0102	-4.496	<0.0001
C4 Grasses : Mojave Desert : Biocrust Cover (0)	0.645	0.0102	63.406	<0.0001
Nitrogen Fixing Forbs : Mojave Desert : Biocrust Cover (0)	-20.536	0.0105	-1952.566	<0.0001
Non-Nitrogen Fixing Forbs : Mojave Desert : Biocrust Cover (0)	-20.327	0.0105	-1932.698	<0.0001
C3 Grasses : Mojave Desert : Biocrust Cover (30)	-1.796	0.0102	-176.460	<0.0001
C4 Grasses : Mojave Desert : Biocrust Cover (30)	0.697	0.0102	68.585	<0.0001
Nitrogen Fixing Forbs : Mojave Desert : Biocrust Cover (30)	-3.0283	0.0102	-297.445	<0.0001
Non-Nitrogen Fixing Forbs : Mojave Desert : Biocrust Cover (30)	-20.650	0.0102	-2027.789	<0.0001
C3 Grasses : Mojave Desert : Biocrust Cover (100)	0.3704	0.0102	36.421	<0.0001
C4 Grasses : Mojave Desert : Biocrust Cover (100)	1.903	0.0102	187.142	<0.0001
Nitrogen Fixing Forbs : Mojave Desert : Biocrust Cover (100)	-0.343	0.0102	-33.707	<0.0001

***Biocrust inoculum cover treatments result in plant communities with different mean seed masses***  
***(CWM<sub>seed mass</sub>)***

Upon examination of the plant community assemblages resulting in different biocrust inoculum treatments, we found that biocrust inoculum cover (i.e., 0%, 30%, 100%) significantly affected the resulting plant community's community weighted mean of seed mass with p-values ranging from 0.057 to <0.0001. Both of our seed mixes were predominantly large seeds (1.1+ g/1000 seeds, Table 8).

**Table 8.** Number of species from each seed mix that belong to each of three different seed mass categories (i.e., small (0-0.5 g/1000 seeds), medium (0.51-1.0 g/1000 seeds), and large (1.1+ g/1000 seeds)).

Seed Mix	Seed Size Category	Number of Species
Colorado Plateau	Small (0-0.50g/1000 seeds)	2
	Medium (0.51-1.0 g/1000 seeds)	2
	Large (1.1+ g/1000 seeds)	8
Mojave Desert	Small (0-0.50g/1000 seeds)	1
	Medium (0.51-1.0 g/1000 seeds)	1
	Large (1.1+ g/1000 seeds)	9

**Table 9:** Means and ranges of seed weight (+/- standard deviation) for the two seed mixes.

Seed Mix	Seed Weight Mean (g)	Range
Colorado Plateau	0.0043g +/- 0.0066g	0.0246g +/- 0.0066g
Mojave Desert	0.0046g +/- 0.0044g	0.0162g +/- 0.0044g

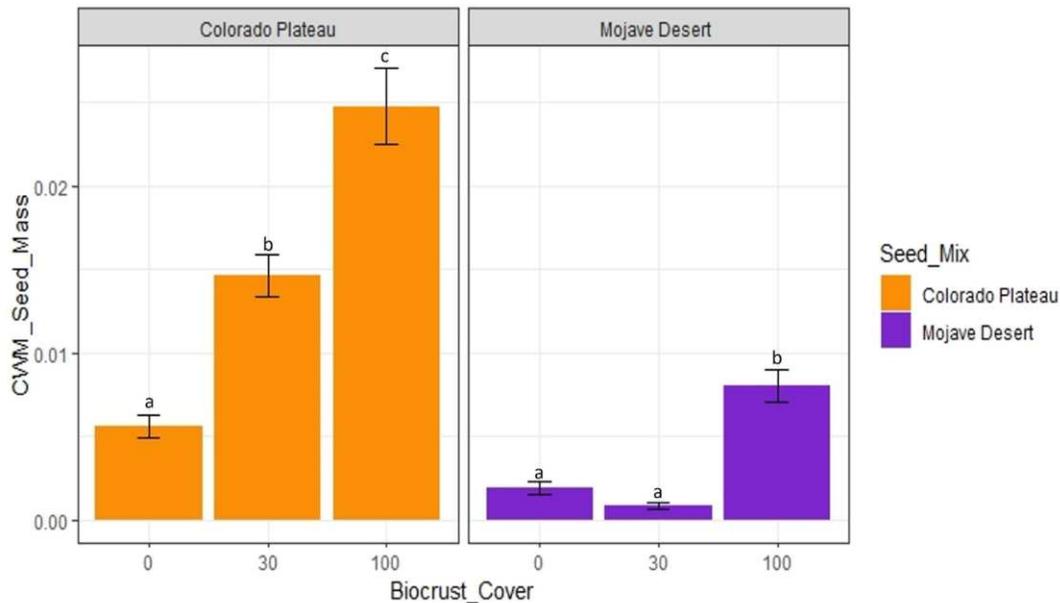
For the Colorado Plateau Desert seed mix there was a 2.5-fold increase in the size of seeds that germinated in the 100% biocrust inoculum cover ( $p < 0.0001$ , Table 10) when compared to bare soil controls, and a 1.5-fold increase in size within the 30% biocrust inoculum cover ( $p = 0.017$ , Table 10) when compared to bare soil controls. For the Mojave Desert seed mix within the

30% biocrust inoculum cover there was an 87.5% decrease in the size of seed that germinated, and the 100% biocrust inoculum cover had a marginally significant effect on the size of seed that germinated ( $p = 0.0566$ , Table 10).

**Table 10:** Results of the negative binomial general linear mixed effects regression model that was used to analyze the interaction between biocrust inoculum cover, seed mix, and CWM

Seed Mass.

Germination Response Interactions	Biocrust Cover Model			
	Estimate	Standard Error	Z value	P value
Intercept	-0.800	0.420	-1.907	0.0566
Biocrust Cover (0) : Colorado Plateau	-0.758	0.279	-2.718	0.00657
Biocrust Cover (30) : Colorado Plateau	0.420	0.176	2.388	0.0170
Biocrust Cover (100) : Colorado Plateau	1.106	0.167	6.612	<0.0001
Biocrust Cover (0) : Mojave Desert	-1.674	0.351	-4.769	<0.0001
Biocrust Cover (30) : Mojave Desert	-3.138	0.476	-6.598	<0.0001



**Figure 11.** Effects of biocrust cover treatments (i.e., 0%, 30%, 100%) on the germinated community's community weighted mean (CWM) seed mass for the two seed mixes (i.e., Colorado Plateau Desert and Mojave Desert).

## Discussion

Globally, ~70% of dryland ecosystems are degraded (Funk et al. 2017) and require innovative active restoration strategies that simultaneously restore plant and soil communities including biocrusts (FAO, 2020; ). While recent research has focused on understanding the

restoration trajectories of plant and soil communities via native seeding restoration treatments (Shackleford et al. 2021; Palma and Laurence, 2015) and, to a lesser degree biocrust inoculation treatments individually (Antoninka et al. 2016, 2020), fewer studies have explored how biocrust inoculation restoration treatments influence native plant community assembly (but see (Havrilla et al. 2019; Bowker et al. 2018; Barger, 2018)). In this greenhouse experiment, we measured plant responses to biocrust inoculation treatments at different levels of applied cover levels (i.e., 30% inoculum cover, 100% inoculum cover, relative to bare soil control) on the recruitment of two native seed mixes from different ecoregions (i.e., Colorado Plateau and Mojave Deserts). To parse out potential abiotic versus. biotic effects of biocrust inoculation on germination, we tested the effects of live vs. sterilized biocrust treatments on germination. To explore whether biocrust inoculum effects vary according to whether seed mixes are developed for the area in which the biocrust inoculum was collected, we also tested effects of treatments on two native seed treatments: one developed for the Mojave Desert (where our biocrust inoculum was collected from) and one developed for the more distant Colorado Plateau Desert. We found that biocrust inoculum application increased germination with these effects greatest in higher biocrust cover treatments. Second, we found that inoculum treatments benefitted germination whether biocrust treatments included live organisms suggesting biocrust inoculum may benefit recruitment through abiotic (e.g., increased nutrients, water) rather than physical mechanisms (i.e., biotic interactions facilitating seeds breaking dormancy). The Colorado Plateau Desert seed mix outperformed the Mojave Desert seed mix overall, and as such there was little evidence of biocrust-plant biotic interactions favoring “locally adapted” seeds. Finally, we explored how important plant traits (i.e., plant functional group (Havrilla et al. 2019) and seed mass (Bacovcin, McIntyre, and Havrilla, In Prep)) interacted with these outcomes. We found in the Colorado Plateau Desert seed mix that all

of the plant functional groups performed best in high biocrust inoculum cover treatments (i.e., 100% cover) with Non-nitrogen fixing forbs performing the best followed by C3 grasses, C4 grasses, and Nitrogen fixing forbs. However, in the Mojave Desert seed mix, C4 grasses performed the best followed by C3 grasses, Non-nitrogen fixing forbs, and finally nitrogen fixing forbs on the 100% cover treatment (which had the highest level of germination (%)). Seed mass also influenced recruitment outcomes. In general, community weighted mean seed mass was a factor in the resulting plant community when interacting with biocrust inoculum. We found that in higher levels of biocrust inoculum communities with higher diversity in seed mass had larger percent germination than lower diversity communities. Taken as a whole, these results provide an increased understanding of how biocrust inoculum treatments may influence plant recruitment and community assembly in drylands.

### ***Plant germination increased with increasing biocrust inoculum cover***

When restoring biocrust communities an often-used tactic is to spread salvage biocrust inoculum or “crumbles” on the soil much like broadcast seeding and then water to help the biocrust establish (Mugnai et al. 2020; Tucker et al. 2020). Recent studies have led to a refinement of biocrust inoculation methods (Bowker, Antoninka, and Chuckran, 2020; Nelson, Giraldo-Silva, and Garcia-Pichel, 2020; Antoninka et al. 2018) and effects of inoculation on soil functioning (Roman et al. 2018). Fewer studies have examined how biocrust inoculation treatments, when applied in tandem with restoration seeding treatments, can influence plant restoration outcomes and community assembly (but see Gao et al. 2023). Biocrusts, and biocrust inoculation, may influence plant germination through a variety of physical and biotic mechanisms. For example, biocrusts can alter the soil moisture and temperature of their ecosystem raising both the available soil moisture (Eldridge et al. 2020) and the soil temperature by decreasing the soil surface albedo

(Rutherford et al. 2017; Xiao and Bowker 2020). Biocrusts also influence the amount of soil nutrients such as carbon and nitrogen (Barger et al. 2016) this increase in nutrients is critical for plants to grow as they break dormancy and begin to grow (Copeland and McDonald, 2012). Biocrust effects on plant germination have been shown to be highly context dependent, and this context dependency can vary according to biocrust composition and plant traits (e.g., plant functional type; Havrilla et al. 2019, and seed size and structure; Bacovcin, McIntyre, and Havrilla, *In Prep* (Chapter 1 of this thesis)). As such, biocrusts can impact plant community assembly processes (Bowker et al. 2022). Based on this rationale, we tested how different biocrust inoculum cover levels (i.e., 30%, applied in strips, and 100% cover) affected plant germination and community assembly outcomes.

We hypothesized that the 30% biocrust inoculum cover treatment would have the greatest percent germination because of the increased niche space that was available for plant taxa with different germination niche requirements (Chase, 2011). Our rationale was that with a significant amount of cover of both bare soil and biocrust sections, species would be able to germinate in the site that best suited them, benefiting overall seed mix germination. However, our results showed that this was not the case. Instead, we found that overall, the 100% biocrust inoculum cover treatment outperformed both the bare soil control and the 30% cover treatments. These results may be explained by the fact that biocrust treatments consisted of biocrust inoculum “crumbles” not intact biocrust communities. While intact, undisturbed biocrust communities often have strong “filtering” effects on plant germination and community assembly and, often negative effects on the germination of some species (Havrilla et al. 2019), use of inoculum may have different effects based on microtopography created by biocrust inoculum. Biocrusts create soil resource heterogeneity. For example, Biocrusts have been shown to alter soil moisture availability and soil

surface microtopography (Eldridge et al. 2020; Rosentreter, Bowker, and Belnap, 2007; Colesie et al, 2016). Biocrust organisms can increase the retention of water within the soil (Eldridge et al. 2020) and water availability is one of the main drivers of plant germination (Copeland and McDonald, 2012). In the field, rough biocrust surfaces can increase seed capture and improve seed retention in favorable microsites for germination (Colesie et al, 2016). While we predicted that the 30% inoculum cover treatment would increase germination by increasing germination niche space by creating a heterogenous matrix composed of bare soil and biocrust inoculum, it is possible that the 100% biocrust inoculum cover treatment had higher heterogeneity of microsites comprised of biocrust crumbles and cracks to the exposed bare soil matrix below (Fig. 1). Conversely, it could be that the high cover inoculum treatment simply had higher resource availability overall, which promoted germination. For example, overall, 100% biocrust inoculum treatments had higher volumetric (%VWC) and took longer to dry down after watering treatments than mesocosms with 30% cover or bare soil controls. This increase in water availability may have increased plant germination in the 100% cover mesocosms. Similarly, although we did not directly measure soil nutrient levels, a greenhouse study by Havrilla and Barger (2018) showed that disturbed biocrust crumbles improved soil nitrogen, which has been shown to promote germination in some plant species (Ahmed et al. 2018).

***Autoclaving biocrust inoculum had few effects on plant germination suggesting effects of biocrust inoculum influences germination mainly through physical mechanisms***

Soil microbial communities can be key to the restoration of an ecosystem's plant community (Lau and Lennon, 2011; Fitzsimons and Miller, 2010; Zhang et al. 2016). Biocrust communities include a variety of organisms that are ecologically important to the functioning of

dryland ecosystems (Xiao et al. 2022) including strong impacts on dryland plant recruitment (Havrilla et al. 2019). Though, the relative importance of physical and biotic mechanisms underlying these interactions has remained uncertain. While biocrusts control resources important for germination (i.e., soil moisture and nutrients, Eldridge et al. 2020; Barger et al. 2016), evidence also suggests that biocrust organisms may directly impact dryland plant germination through biotic interactions such as microbial alleviation of seed dormancy barriers in some species (e.g., Eldridge et al. 2021). We compared the effects of live versus sterile (i.e., autoclaved) biocrust inoculum treatments on germination and found no significant difference in germination between treatments. The only exception to this was the 100% cover Mojave Desert treatment where the sterile biocrust inoculum outperformed the live biocrust inoculum. Given that there was no significant difference between the two inoculation types, our prediction that there would be more germination in the bins inoculated with live biocrust was not supported and suggests that biocrust inoculum benefits germination primarily through physical mechanisms (i.e., increased resource availability to germinating seeds).

This finding conflicts with the results of past studies that have shown different effects of live vs. autoclaved biocrusts on plant germination. For example, Hawkes (2004) found that germination on autoclaved biocrusts was dramatically reduced, when compared to germination on biologically active biocrusts suggesting that the biological community found in the “live” biocrusts does in fact play a role in the germination of plant species. Similarly, isolates of organisms found in biocrusts have been shown to promote germination by breaking seed dormancy (Eldridge et al. 2021). Together, this suggests that there could be contexts in which biocrusts influence germination through biotic means, perhaps in addition to more dominant physical effects. Nonetheless, that biocrust inoculum treatments overall (both live and autoclaved) increased

germination suggests that biocrust inoculum may be an important restoration treatment for improving native plant germination in drylands. Plant recruitment from native plant seeding treatments often fails in drylands due to physical barriers including low moisture (Shackleford et al. 2021). As such, restoration strategies including soil surface treatments (e.g., mulch, soil amendments) are often used to improve germination outcomes (Havrilla et al. 2020; Minnick and Alward, 2012). It has also been shown that seeding species with microbes can help the germination of seeds (Mahmoudi et al. 2021). Song et al. (2022) and Xiao et al. (2022) have shown that biocrusts can fill the role of mulch and other sorts of soil structural additions, particularly moss and lichen and play a crucial role in dryland restoration by alleviating physical constraints on germination at the soil surface.

***Effects of biocrust inoculum cover on germination differed among plant functional groups within the two seed mixes***

Biocrusts can have differential effects on plants depending on plant functional type (synthesized in Havrilla et al. 2019 and Bacovcin, McIntyre, and Havrilla, In Prep). Similarly, here we found that plant functional group played an important role in determining germination responses to biocrust treatments. First, we aimed to compare effects of biocrust treatments on two distinct seed mixes in an effort to explore the hypothesis that biocrusts would have greater benefits on a seed mix comprised of species assumed to be more locally adapted to the same environmental conditions as the biocrusts we collected (i.e., the Mojave Desert) versus a more distantly sourced seed mix from another ecoregion (i.e., the Colorado Plateau Desert) due to the potential of local coadaptation of biocrusts and plants with functional traits adapted to “cope” with biocrust presence (Havrilla et al. 2019). However, our ability to explore this question was hindered by incredibly low germination success within the Mojave Desert seed mix across species.

While there were few discernable differences in biocrust effects on Mojave versus Colorado Plateau Desert seed mixes, we were still able to address the effects of biocrust treatments on the germination of different plant functional groups (i.e., C3 Grasses, C4 Grasses, Nitrogen-fixing forbs, and Non-nitrogen-fixing forbs). Across both seed mixes and biocrust inoculum covers (i.e., 30% inoculum cover and 100% inoculum cover) we found that species from the C4 grass plant functional groups germinated at higher rates on average than C3 grasses (63% vs. 39%). Amongst the forbs Non-nitrogen fixing forbs germinated at higher rates than Nitrogen-fixing forbs (56% vs. 17%). These percentages were found by adding the percent germination numbers across all biocrust covers and seed mixes. Our hypothesis that Non-nitrogen-fixing forbs having the highest germination was supported by our results, while our hypothesis that C3 grasses would have higher germination was not supported.

These results are different from what was found in Havrilla et al. (2019)'s global meta-analysis of the effects of biocrusts on plant germination which found that biocrusts inhibited the germination of C4 grass species as well as marginally inhibited the germination of Nitrogen-fixing forbs. Though, it is important to note that since Havrilla et al. (2019) was a global meta-analysis, that this estimate can be viewed as an average and that many individual studies have shown context dependency in these outcomes depending on plant species, biocrust community composition, and other ecological factors. Further, there could be key differences in the effects of intact biocrusts versus biocrust inoculum on the germination of different plant functional types. Intact biocrusts may impede the germination of certain plant species by stabilizing soils and acting as a physical barrier to seed contact with the mineral soil surface (Deines et al. 2007; Slate, Callaway, and Pearson, 2019 ), whereas biocrust inoculum could benefit germination of the same species by acting as a soil amendment without posing such physical barriers. Bacovcin, McIntyre, and

Havrilla (In Prep, Chapter 1 of this Thesis) found that on areas of biocrust disturbance that small seeded species (<0.50 g/1000 seeds) did not have their germination negatively affected while medium (0.51-1.0 g/1000 seeds) and large (>1.1 g/1000 seeds) seeded species did. It could be that while biocrust inoculum is used for restoration it is providing similar effects to disturbed biocrust communities. C4 grasses are typically small seeded species (Csnontos and Kalapos, 2013) and would have to compete with medium and large seeded species for resources when it comes to germination. Bacovcin, McIntyre, and Havrilla (In Prep) found that on intact cyanobacteria biocrusts, like the community that was sourced for this experiment, medium and large seeded species had their germination promoted. It is possible that breaking up that community for inoculum changed the resulting effects of the plant-biocrust interaction. This would suggest that when disturbed the usual inhibitory effects that small seeded species like many C4 grasses would normally experience is reversed as small seeded species are given enough resources to compete with the medium and larger seeded species.

***Biocrust inoculum cover treatments result in the germination of plant communities with different mean seed masses (CWM seed mass)***

Morphological seed traits can partially explain germination responses to biocrusts, which was demonstrated in Chapter 1 of this thesis (Bacovcin, McIntyre, and Havrilla, *In Prep*). Specifically, seed mass is a key predictor of germination success on biocrusts (Bacovcin, McIntyre, and Havrilla, *In Prep*). Here, we experimentally demonstrate that seed mass may control plant germination responses to biocrust inoculum treatments. For the Colorado Plateau Desert seed mix, we found that at high levels of biocrust inoculum cover (i.e., 100%), the higher seed masses had larger germination. This suggests that plant community assembly in mesocosms with high biocrust inoculum cover resulted from the promotion of large-seeded plant species. This result

aligns with our earlier assumption that mesocosms with 100% biocrust inoculum cover may have a high diversity of variable microsites for seed germination (e.g., biocrust surfaces, cracks, exposed bare soil below) and aligns with results found for cyanobacteria biocrusts shown in Chapter 1 (Bacovcin, McIntyre, and Havrilla, In Prep). Our seed mixes had predominantly large seeds within the Chapter 1 size category of 1.1+ g/1000 seeds (Table 7) though there was variation within that size category.

This result suggests that high cover biocrust inoculum treatments may promote communities with larger seeds due to the inoculum being predominantly cyanobacteria (Supplementary Table 6). Such differences can have important implications for ecological communities. For example, Levine and HilleRisLambers (2009) showed that increased species differences play a critical role in species diversity. They found that as niche space increased the stability of the resultant plant community also stabilized and that an increase of niche space increased species diversity (Levine and HilleRisLambers, 2009). These findings can be applied to the results found in this research. By increasing diversity of the species, or in this case the diversity of seed mass, and increasing the available niche space (i.e., the 100% inoculum cover treatment) we can promote a more stable and faster growing final plant community. This could be important in the context of dryland ecological restoration as well. When creating a seed mix or preparing a site for restoration land managers should be encouraged to increase plant and niche diversity to encourage a more stable community that might be more resilient to future degradation (Song et al. 2022 ).

### ***Study limitations and future research directions***

Our study shows that interactions among biocrust inoculum cover treatments and plant seed functional traits can affect plant germination and community assembly outcomes. These

results may have important implications for understanding biocrust-plant interactions and community (re)assembly during joint biocrust-plant restoration. Our study also has several key limitations, which have allowed us to identify opportunities for future research and study on the interactions between biocrust inoculum and plant germination.

1. ***Low germination rates limited our ability to test for local coadaptation between native seeds and biocrusts*** - In this study, germination of species within the Colorado Plateau Desert seed mix were relatively high (average = 12.3%), germination of the Mojave Desert seed mix was strikingly low (average = 3.6%). This may be because Mojave Desert species require additional conditions to be met to give the greatest chance of germination (reviewed in Appendix Table X). This limitation made it difficult to test our hypothesis that local seeds gathered from close to the biocrust collection point would have higher germination than seeds sourced from more distant ecoregions. While these additional conditions are easily achievable on a small scale this study also wanted to keep in mind the conditions that may occur during a restoration project. Since many thousands of seeds could be laid down these conditions may become impractical at larger scales and so would not be done by land managers. Future research, however, could perform these additional steps to guarantee increased germination or create different seed mixes to circumvent this issue. Further, we used commercially available seed that may have been sourced from geographic regions distant to the local biocrust sampling locations. In the future, more rigorous tests of ecotypic seed sources with local versus more distant biocrust sources should be used to test for potential coevolutionary and/or ecotype-specific interactions between biocrusts and local plant communities.

2. ***Considerations for seed mix creation*** - Our seed mixes were created with the intention of having an even representation of all plant functional groups of interest (3 species from each group: C3 grasses, C4 grasses, Nitrogen-fixing forbs, and Non-Nitrogen-fixing forbs). There was, however, lower than desired diversity in seed masses within the selected species. Many of our species were small (of low mass) and this lack of broader seed mass diversity within seed mixes may have impacted our evaluation of how seed mass affected plant community assembly on biocrust inoculum. Future studies of the relationships between seed mass and plant community assembly outcomes on biocrust and/or biocrust inoculum could select seed mixes with greater seed mass diversity to investigate the nature of the relationship more directly between seed mass and plant community assembly on biocrust.
3. ***Biocrust “infection” under experimental greenhouse conditions*** - Within our study biocrust inoculum crumbles were watered regularly, one subirrigation per week and misted twice, to allow for plants to germinate. The watering regime had to give ample water to provide resources for seed germination while not allowing outside mosses and algae to infect the biocrust. Antonika et al. (2018) showed that there is an increasing issue with greenhouse grown biocrusts becoming “infected” with foreign mosses and algae, leading to changes in biocrust community composition. Future research could perform a similar experiment under ambient field conditions to allow for more natural environmental moisture conditions to better evaluate biocrust inoculum effects *in situ* without these risks of community contamination.

***Biocrust inoculum cover treatments and plant functional traits interact to shape community assembly outcomes***

Plant functional traits are increasingly utilized to understand outcomes of biotic interactions within communities, particularly in the context of ecological restoration (Larson et al. 2015, 2020; Larson and Funk, 2017; Saatkamp et al. 2018). Research has shown that plant and microbial communities interact in complicated ways to determine the final plant community composition (Bruno, Stachowicz, and Bertness, 2013, Bowker et al. 2022), however a vast majority of this research is done on intact ecosystems (Munson et al. 2011; Butterfield and Munson, 2016). Additional work is needed to explore outcomes of these processes in restoration contexts. Our study examines the effects of biocrust inoculation treatments on native plant recruitment and explores how biocrust inoculum affects different plant functional types and assemblages depending on seed mass, a key morphological seed trait that can determine the effects of biocrusts on germination (Chapter 1; Baovcin, McIntyre, & Havrilla, *In Prep*). In this study, we demonstrate that increased biocrust inoculum cover led to increased plant germination, that the effects of biocrust inoculum on germination may be mostly attributed to physical mechanisms of biocrusts and not biological, and finally that plant and seed traits influence plant germination when interacting with different levels of biocrust inoculum. Together, results suggest that both plant seeding and biocrust inoculum treatments can occur in tandem with positive effects on plant recruitment during community reassembly during restoration. As dryland degradation escalates worldwide (FAO, 2020), knowledge of how plant-microbe, more specifically plant-biocrust interactions, affect plant germination during ecosystem restoration efforts will be key to combating degradation and anticipating dryland restoration trajectories.

## CONCLUSIONS

Drylands are increasingly in threat of degradation, with roughly 90% of the Earth's drylands slated to be degraded in the coming decades (FAO, 2020). In order to effectively restore these degraded communities, we must first understand how they assemble. The process of community assembly has remained a constant goal for ecologists. Understanding the abiotic and

biotic factors that go into the formation of a particular plant or microbial community can give us understanding into how each community works and how they interact with each other (Kraft et al. 2015; Amat, Cortina, and Zubcoff, 2015; Balazs et al. 2020). In dryland ecosystems biocrusts will modify these abiotic factors and can either encourage or inhibit the germination of dryland plant species and can play crucial roles in forming the resultant plant community. Though the mechanism of how this interaction is still little understood, with seed morphological traits being proposed as one possibility of action. However, when drylands are degraded there is a need for active restoration to restore the lost communities. While a great amount of research has been done on the assembly of degraded plant (James et al. 2013) and microbe (Antoninka et al. 2020; Zhou et al. 2020) communities the effect of tandem restoration treatments on community assembly is less understood. This research aims to provide more insight into how biocrusts impact plant community assembly through germination, and how tandem restoration treatments impact the germination and assembly of seeding treatments.

Chapter 1 of this thesis suggests that seed morphological traits interact with biocrusts in a meaningful way when it comes to influencing the germination of plants. Smaller seeded species with appendages experiencing promoted germination on a majority of biocrusts. Chapter 2 suggests that when being restored in tandem applying larger levels of biocrust inoculum cover will led to increased germination, that these effects are primarily due to the physical effects of the biocrust inoculum and not the biological, and that both seed traits (i.e., seed mass) and plant traits (i.e., which functional group the plant species belongs to) also interact with biocrust inoculum to affect germination. All the information gained from this thesis increases our understanding of how plant communities assemble on biocrusts in dryland ecosystems and can be utilized by land managers in restoration efforts. This research will hopefully improve seed

mix creation and restoration practices to increase the success of future dryland restoration projects.

## REFERENCES

- Ahmed, Ahmed Khaled Abdella, et al. "Influences of air, oxygen, nitrogen, and carbon dioxide nanobubbles on seed germination and plant growth." *Journal of Agricultural and Food Chemistry* 66.20 (2018): 5117-5124.
- Antoninka, Anita, et al. "Addressing barriers to improve biocrust colonization and establishment in dryland restoration." *Restoration Ecology* 28 (2020): S150-S159.
- Antoninka, Anita, et al. "Maximizing establishment and survivorship of field-collected and greenhouse-cultivated biocrusts in a semi-cold desert." *Plant and Soil* 429 (2018): 213-225.
- Antoninka, Anita, et al. "Biological soil crusts in ecological restoration: emerging research and perspectives." *Restoration Ecology* 28 (2020): S3-S8.
- Antoninka, Anita, et al. "Production of greenhouse-grown biocrust mosses and associated cyanobacteria to rehabilitate dryland soil function." *Restoration Ecology* 24.3 (2016): 324-335.
- Balazs, Kathleen R., et al. "The right trait in the right place at the right time: Matching traits to environment improves restoration outcomes." *Ecological Applications* 30.4 (2020): e02110.
- Barger, Nichole N., et al. "Patterns and controls on nitrogen cycling of biological soil crusts." *Biological soil crusts: an organizing principle in drylands* (2016): 257-285.
- Barger, Nichole, and University of Colorado at Boulder. "Achieving Dryland Restoration Through the Deployment of Enhanced Biocrusts to Improve Soil Stability, Fertility and Native Plant Recruitment." (2018): 55.
- Bates D, Mächler M, Bolker B, Walker S (2015). "Fitting Linear Mixed-Effects Models Using lme4." *Journal of Statistical Software*, 67(1), 1–48. [doi:10.18637/jss.v067.i01](https://doi.org/10.18637/jss.v067.i01).
- Belnap, J., Prasse, R., & Harper, K.T. (2001). Influence of biological soil crusts on soil environments and vascular plants. In: *Biological soil crusts: Structure, function, and management* (pp. 281-300). Springer Berlin Heidelberg.
- Benard, Pascal, et al. "Microhydrological niches in soils: How mucilage and EPS alter the biophysical properties of the rhizosphere and other biological hotspots." *Vadose Zone Journal* 18.1 (2019): 1-10.
- Bowker, Matthew A., Anita J. Antoninka, and Peter F. Chuckran. "Improving field success of biocrust rehabilitation materials: hardening the organisms or softening the environment?." *Restoration Ecology* 28 (2020): S177-S186.
- Bowker, Matthew A., et al. "Biocrusts influence vascular plant community development, promoting native plant dominance." *Frontiers in Ecology and Evolution* 10 (2022): 151.
- Bowker, Matthew A., et al. "Biocrusts: the living skin of the earth." *Plant and Soil* 429 (2018): 1-7.
- Bruno, John F., John J. Stachowicz, and Mark D. Bertness. "Inclusion of facilitation into ecological theory." *Trends in ecology & evolution* 18.3 (2003): 119-125.
- Briggs, Amber L., and John W. Morgan. "Seed characteristics and soil surface patch type interact to affect germination of semi-arid woodland species." *Plant Ecology* 212 (2011): 91-103.
- Byers, James E., et al. "Using ecosystem engineers to restore ecological systems." *Trends in ecology & evolution* 21.9 (2006): 493-500.
- Caster, Joshua, et al. "Biocrust and the soil surface: Influence of climate, disturbance, and biocrust recovery on soil surface roughness." *Geoderma* 403 (2021): 115369.
- Castillo-Monroy, Andrea P., and Fernando T. Maestre. "Biological soil crusts: recent advances in our knowledge of their structure and ecological function." *Revista Chilena de Historia Natural* 84.1 (2011): 1-21

Cavanagh, A. M., R. C. Godfree, and J. W. Morgan. 2019. An awn typology for Australian native grasses (Poaceae). *Australian Journal of Botany* 67:309-334.

Chase, Jonathan M. "Ecological niche theory." *The theory of ecology* (2011): 93-107.

Chen, Huie, Xiang Gao, and Qing Wang. "Research progress and prospect of frozen soil engineering disasters." *Cold Regions Science and Technology* (2023): 103901.

Chiquoine, Lindsay P., Scott R. Abella, and Matthew A. Bowker. "Rapidly restoring biological soil crusts and ecosystem functions in a severely disturbed desert ecosystem." *Ecological Applications* 26.4 (2016): 1260-1272.

Choi, Ryan T., Sasha C. Reed, and Colin L. Tucker. "Multiple resource limitation of dryland soil microbial carbon cycling on the Colorado Plateau." *Ecology* 103.6 (2022): e3671.

Colesie, Claudia, Vincent John Martin Noah Linus Felde, and Burkhard Büdel. "Composition and macrostructure of biological soil crusts." *Biological soil crusts: An organizing principle in drylands* (2016): 159-172.

Colesie, Claudia, Vincent John Martin Noah Linus Felde, and Burkhard Büdel. "Composition and macrostructure of biological soil crusts." *Biological soil crusts: An organizing principle in drylands* (2016): 159-172.

Condon, Lea A., et al. "Passive restoration of vegetation and biological soil crusts following 80 years of exclusion from grazing across the Great Basin." *Restoration Ecology* 28 (2020): S75-S85.

Copeland, Lawrence O., and Miller F. McDonald. *Principles of seed science and technology*. Springer Science & Business Media, 2012.

Cordlandwehr, Verena, et al. "Do plant traits retrieved from a database accurately predict on-site measurements?." *Journal of Ecology* 101.3 (2013): 662-670.

Cross, Adam T., and James Aronson. "Plant-soil-microbe interactions and drivers in ecosystem development and ecological restoration." *Frontiers in Ecology and Evolution* 11 (2023): 1216016.

Couradeau, Estelle, et al. "Bacteria increase arid-land soil surface temperature through the production of sunscreens." *Nature communications* 7.1 (2016): 10373.

Csontos, P., and T. Kalapos. "More lightweight and isodiametric seeds for C4 than for C3 grasses are associated with preference for open habitats of C4 grasses in a temperate flora." *Grass and Forage Science* 68.3 (2013): 408-417.

Currier, Courtney M., and Osvaldo E. Sala. "Precipitation versus temperature as phenology controls in drylands." *Ecology* 103.11 (2022): e3793.

Dacal, Marina, et al. "Soil microbial respiration adapts to ambient temperature in global drylands." *Nature Ecology & Evolution* 3.2 (2019): 232-238.

De'Ath, Glenn. "Boosted trees for ecological modeling and prediction." *Ecology* 88.1 (2007): 243-251.

Delgado-Baquerizo, Manuel, et al. "Increases in aridity lead to drastic shifts in the assembly of dryland complex microbial networks." *Land Degradation & Development* 31.3 (2020): 346-355.

Delgado-Baquerizo, Manuel, et al. "Biocrusts control the nitrogen dynamics and microbial functional diversity of semi-arid soils in response to nutrient additions." *Plant and Soil* 372 (2013): 643-654.

Deines, Lynell, et al. "Germination and seedling establishment of two annual grasses on lichen-dominated biological soil crusts." *Plant and Soil* 295 (2007): 23-35.

D'Odorico, Paolo, et al. "On soil moisture–vegetation feedbacks and their possible effects on the dynamics of dryland ecosystems." *Journal of Geophysical Research: Biogeosciences* 112.G4 (2007).

Duarte, Leandro DS, et al. "Analyzing community-weighted trait means across environmental gradients: should phylogeny stay or should it go?." *Ecology* 99.2 (2018): 385-398.

Evangelisti, Edouard, Thomas Rey, and Sebastian Schornack. "Cross-interference of plant development and plant–microbe interactions." *Current Opinion in Plant Biology* 20 (2014): 118-126.

Durham, Rebecca A., et al. "Insolation and disturbance history drive biocrust biodiversity in Western Montana rangelands." *Plant and Soil* 430 (2018): 151-169.

Edwards, Erika J., and Christopher J. Still. "Climate, phylogeny and the ecological distribution of C4 grasses." *Ecology letters* 11.3 (2008): 266-276.

Elbaum, Rivka, Stanislav Gorb, and Peter Fratzl. "Structures in the cell wall that enable hygroscopic movement of wheat awns." *Journal of structural biology* 164.1 (2008): 101-107.

Eldridge, David J., et al. "Experimental evidence of strong relationships between soil microbial communities and plant germination." *Journal of Ecology* 109.6 (2021): 2488-2498.

Eldridge, David J., et al. "The pervasive and multifaceted influence of biocrusts on water in the world's drylands." *Global Change Biology* 26.10 (2020): 6003-6014.

Elith, Jane, and John Leathwick. "Predicting species distributions from museum and herbarium records using multiresponse models fitted with multivariate adaptive regression splines." *Diversity and distributions* 13.3 (2007): 265-275.

FAO. 2020. The State of Food and Agriculture 2020. Overcoming water challenges in agriculture. Rome. <https://doi.org/10.4060/cb1447en>

Fitzsimons, Michael S., and R. Michael Miller. "The importance of soil microorganisms for maintaining diverse plant communities in tallgrass prairie." *American Journal of Botany* 97.12 (2010): 1937-1943.

Friedman, J. H., & Meulman, J. J., Multiple additive regression trees with application in epidemiology. *Statistics in Medicine*, 22(9), 1365–1381. (2003).  
<https://doi.org/10.1002/sim.1501>

Funk, Jennifer L., et al. "Revisiting the Holy G rail: using plant functional traits to understand ecological processes." *Biological Reviews* 92.2 (2017): 1156-1173.

Garibotti, Irene A., Marina Gonzalez Polo, and Patricia Satti. "Compost promotes biocrust and plant growth in greenhouse cocultures for dryland restoration." *Restoration Ecology* (2023): e14020.

Gao, Yanhong, et al. "Biocrust and sand burial together promote annual herb community assembly in an arid sandy desert area." *Plant and Soil* (2023): 1-19.

Gill, Richard A., et al. "Niche opportunities for invasive annual plants in dryland ecosystems are controlled by disturbance, trophic interactions, and rainfall." *Oecologia* 187 (2018): 755-765.

Greenwell, Brandon, et al. "GBM: generalized boosted regression models (2020)." *R package version 2.8* (2022).

Hag Husein, Hussam, et al. "A contribution to soil fertility assessment for arid and semi-arid lands." *Soil Systems* 5.3 (2021): 42

Harris, Jim. "Soil microbial communities and restoration ecology: facilitators or followers?." *Science* 325.5940 (2009): 573-574.

Havrilla, Caroline A., et al. "Towards a predictive framework for biocrust mediation of plant performance: A meta-analysis." *Journal of Ecology* 107.6 (2019): 2789-2807.

Havrilla, Caroline A., and Nichole N. Barger. "Biocrusts and their disturbance mediate the recruitment of native and exotic grasses from a hot desert ecosystem." *Ecosphere* 9.7 (2018): e02361.

Havrilla, Caroline A., et al. "RestoreNet: An emerging restoration network reveals controls on seeding success across dryland ecosystems." *Journal of Applied Ecology* 57.11 (2020): 2191-2202.

Hawkes, Christine V. "Effects of biological soil crusts on seed germination of four endangered herbs in a xeric Florida shrubland during drought." *Plant ecology* 170 (2004): 121-134.

Helsen, Kenny, Martin Hermy, and Olivier Honnay. "Trait but not species convergence during plant community assembly in restored semi-natural grasslands." *Oikos* 121.12 (2012): 2121-2130.

Herzberger, Anna J., et al. "Plant-microbe interactions change along a tallgrass prairie restoration chronosequence." *Restoration Ecology* 23.3 (2015): 220-227.

HilleRisLambers, Janneke, et al. "Rethinking community assembly through the lens of coexistence theory." *Annual review of ecology, evolution, and systematics* 43 (2012): 227-248.

Hijmans, Robert J., et al. "Package 'dismo'." *Circles* 9.1 (2017): 1-68.

Hinkle, Dennis E., William Wiersma, and Stephen G. Jurs. *Applied statistics for the behavioral sciences*. Vol. 663. Houghton Mifflin college division, 2003.

Hoose, Benjamin W., et al. "Improving dryland seedling recruitment using fungicide seed coatings." *Ecological Solutions and Evidence* 3.1 (2022): e12132.

Hoeksema, Jason D., et al. "A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi." *Ecology letters* 13.3 (2010): 394-407.

Hothorn, Torsten, et al. "Package 'multcomp'." *Simultaneous inference in general parametric models. Project for Statistical Computing, Vienna, Austria* (2016).

Huber, Jakob K., and Johannes Kollmann. "Recruitment filtering by a moss layer disadvantages large-seeded grassland species." *Basic and applied ecology* 42 (2020): 27-34.

Hulvey, Kristin B., et al. "Restoration islands: a tool for efficiently restoring dryland ecosystems?." *Restoration Ecology* 25 (2017): S124-S134.

Ismail, Noriszura, and Hossein Zamani. "Estimation of claim count data using negative binomial, generalized Poisson, zero-inflated negative binomial and zero-inflated generalized Poisson regression models." *Casualty Actuarial Society E-Forum*. Vol. 41. No. 20. Spring, 2013.

Janzen, Christopher, and Tammy Tobin-Janzen. "Microbial communities in fire-affected soils." *Microbiology of extreme soils*. Berlin, Heidelberg: Springer Berlin Heidelberg, 2008. 299-316.

James, Jeremy J., et al. "A systems approach to restoring degraded drylands." *Journal of Applied Ecology* 50.3 (2013): 730-739.

Kattge, J, Boenisch, G, Diaz, S, et al. TRY plant trait database - enhanced coverage and open access. *Glob Change Biol*. 2020; 26: 119-188. <https://doi.org/10.1111/gcb.14904>

Kaushal, Manoj, and Suhas P. Wani. "Plant-growth-promoting rhizobacteria: drought stress alleviators to ameliorate crop production in drylands." *Annals of Microbiology* 66 (2016): 35-42.

Kildisheva, Olga A., et al. "Setting the scene for dryland recovery: an overview and key findings from a workshop targeting seed-based restoration." *Restoration Ecology* 24 (2016): S36-S42.

Kipkemoi, Isaac, et al. "Climatic expression of rainfall on soil moisture dynamics in drylands." *Hydrology and Earth System Sciences Discussions* (2021): 1-24.

Kiss, Réka, et al. "Grassland seed bank and community resilience in a changing climate." *Restoration Ecology* 26 (2018): S141-S150.

Kraft, Nathan JB, et al. "Community assembly, coexistence and the environmental filtering metaphor." *Functional ecology* 29.5 (2015): 592-599.

Larsen, Kelly D. "Effects of microbiotic crusts on the germination and establishment of three range grasses." (1995).

Larson, Julie E., et al. "Seed and seedling traits affecting critical life stage transitions and recruitment outcomes in dryland grasses." *Journal of Applied Ecology* 52.1 (2015): 199-209.

Lake, Janet C., and Michelle R. Leishman. "Invasion success of exotic plants in natural ecosystems: the role of disturbance, plant attributes and freedom from herbivores." *Biological conservation* 117.2 (2004): 215-226.

Larkin, Daniel J., Gregory L. Bruland, and Joy B. Zedler. "Heterogeneity theory and ecological restoration." *Foundations of restoration ecology* (2016): 271-300.

Larson, Julie E., and Jennifer L. Funk. "Regeneration: an overlooked aspect of trait-based plant community assembly models." *Journal of Ecology* 104.5 (2016): 1284-1298.

Laushman, K. M., McCormick, M. L., Munson, S. M., Balazs, K. R., & Butterfield, B. J. (2022). Protocol for installing and monitoring a RestoreNet restoration field trial network site (No. 2-A18). US Geological Survey.

Lau, Jennifer A., and Jay T. Lennon. "Evolutionary ecology of plant–microbe interactions: soil microbial structure alters selection on plant traits." *New phytologist* 192.1 (2011): 215-224.

Le Bagousse-Pinguet, Yoann, et al. "Testing the environmental filtering concept in global drylands." *Journal of Ecology* 105.4 (2017): 1058-1069.

Laghari, Shah Jahan, et al. "Role of nitrogen for plant growth and development: A review." *Advances in Environmental Biology* 10.9 (2016): 209-219.

Length, Russell V. (2023). "Emmeans: Estimated MArginal Means, aka Least-Squares means." R Package version 1.8.4-1. <https://CRAN.R-project.org/package=emmeans>.

Li, Xin-Rong, et al. "Effects of biological soil crusts on seed bank, germination and establishment of two annual plant species in the Tengger Desert (N China)." *Plant and Soil* 277 (2005): 375-385.

Lindén, Andreas, and Samu Mäntyniemi. "Using the negative binomial distribution to model overdispersion in ecological count data." *Ecology* 92.7 (2011): 1414-1421.

Lipson, David A., Steven K. Schmidt, and Russell K. Monson. "Links between microbial population dynamics and nitrogen availability in an alpine ecosystem." *Ecology* 80.5 (1999): 1623-1631.

Maestre, Fernando T., et al. "Biogeography of global drylands." *New Phytologist* 231.2 (2021): 540-558.

Mahmoudi, Neji, et al. "Arbuscular mycorrhizal traits are good indicators of soil multifunctionality in drylands." *Geoderma* 397 (2021): 115099.

McCluney, Kevin E., et al. "Shifting species interactions in terrestrial dryland ecosystems under altered water availability and climate change." *Biological Reviews* 87.3 (2012): 563-582.

McTavish, M. J., et al. "Sustainable management of grassland soils." *Soils and Landscape Restoration*. Academic Press, 2021. 95-124.

Mian, M. A. R., and E. D. Nafziger. "Seed size and water potential effects on germination and seedling growth of winter wheat." *Crop Science* 34.1 (1994): 169-171.

Miller, R. M. "The Application of VA Mycorrhizae to Ecosystem Restoration and Reclamation RM Miller and JD Jastrow." *Mycorrhizal functioning: an integrative plant-fungal process* (1992): 438.

Minnick, Tamera J., and Richard D. Alward. "Soil moisture enhancement techniques aid shrub transplant success in an arid shrubland restoration." *Rangeland Ecology & Management* 65.3 (2012): 232-240.

Mugnai, Gianmarco, et al. "The role of grain size and inoculum amount on biocrust formation by *Leptolyngbya ohadii*." *Catena* 184 (2020): 104248.

Naorem, Anandkumar, et al. "Soil Constraints in an Arid Environment—Challenges, Prospects, and Implications." *Agronomy* 13.1 (2023): 220.

Nelson, Corey, Ana Giraldo-Silva, and Ferran Garcia-Pichel. "A fog-irrigated soil substrate system unifies and optimizes cyanobacterial biocrust inoculum production." *Applied and environmental microbiology* 86.13 (2020): e00624-20.

Ntakirutimana, Fabrice, et al. "Potential effects of awn length variation on seed yield and components, seed dispersal and germination performance in Siberian wildrye (*Elymus sibiricus* L.)." *Plants* 8.12 (2019): 561.

Osborne, Brooke B., et al. "The consequences of climate change for dryland biogeochemistry." *New Phytologist* 236.1 (2022): 15-20.

Palma, Ana Cristina, and Susan GW Laurance. "A review of the use of direct seeding and seedling plantings in restoration: what do we know and where should we go?." *Applied Vegetation Science* 18.4 (2015): 561-568.

Palmer Brianne, Hernandez Rebecca, and Lipson David. "The fate of biological soil crusts after fire: a meta-analysis." *Global Ecology and Conservation* 24 (2020): e01380.

Pandit, Ram, et al. "Summary for policymakers of the assessment report on land degradation and restoration of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services." (2018).

Parr, C. S., N. Wilson, P. Leary, K. S. Schulz, K. Lans, L. Walley, J. A. Hammock, A. Goddard, J. Rice, M. Studer, J. T. G. Holmes, and R. J. Corrigan, Jr. 2014. The Encyclopedia of Life v2: Providing Global Access to Knowledge About Life on Earth. *Biodiversity Data Journal* 2: e1079, doi:10.3897/BDJ.2.e1079

Parwani, Laxmi, Medha Bhatt, and Jaspreet Singh. "Potential biotechnological applications of cyanobacterial exopolysaccharides." *Brazilian Archives of Biology and Technology* 64 (2021).

Peart, Martyn H. "Experiments on the biological significance of the morphology of seed-dispersal units in grasses." *The Journal of Ecology* (1979): 843-863.

Potthoff, Martin, et al. "Soil microbial community composition as affected by restoration practices in California grassland." *Soil Biology and Biochemistry* 38.7 (2006): 1851-1860.

Právālie, Remus. "Drylands extent and environmental issues. A global approach." *Earth-Science Reviews* 161 (2016): 259-278.

Pyšek, Petr, and David M. Richardson. "Invasive species, environmental change and management, and health." *Annual review of environment and resources* 35 (2010): 25-55.

Ramón Vallejo, V., et al. "Perspectives in dryland restoration: approaches for climate change adaptation." *New Forests* 43.5-6 (2012): 561-579.

Reed, Sasha C., et al. "Biocrusts in the context of global change." *Biological soil crusts: an organizing principle in drylands* (2016): 451-476.

Reeve, Sharon, et al. "Facilitating restoration of degraded biological soil crusts using mixed culture inoculation." *Journal of Arid Environments* 208 (2023): 104876.

Ridgeway, Greg, Maintainer Harry Southworth, and Suggests RUnit. "Package 'gbm'." *Viitattu* 10.2013 (2013): 40.

Rivera-Aguilar, V., et al. "Physical effects of biological soil crusts on seed germination of two desert plants under laboratory conditions." *Journal of arid environments* 63.1 (2005): 344-352.

Román, José Raúl, et al. "Restoring soil functions by means of cyanobacteria inoculation: importance of soil conditions and species selection." *Land Degradation & Development* 29.9 (2018): 3184-3193.

Rosentreter, R., D. J. Eldridge, and J. H. Kaltenecker. "Monitoring and management of biological soil crusts." *Biological soil crusts: structure, function, and management*. Berlin, Heidelberg: Springer Berlin Heidelberg, 2001. 457-468.

Rosentreter, R., M. Bowker, and J. Belnap. 2007. A Field Guide to Biological Soil Crusts of Western U.S. Drylands. U.S. Government Printing Office, Denver, Colorado.

Royal Botanic Gardens Kew. (2020) Seed Information Database (SID). Version 7.1. Available from: <http://data.kew.org/sid/>

Roy, Jacques, Martyn M. Caldwell, and Robert P. Pearce. *Exploitation of environmental heterogeneity by plants: ecophysiological processes above-and belowground*. Academic Press, 2012.

R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.

Rutherford, William A., et al. "Albedo feedbacks to future climate via climate change impacts on dryland biocrusts." *Scientific Reports* 7.1 (2017): 44188.

Saatkamp, Arne, et al. "A research agenda for seed-trait functional ecology." *New phytologist* 221.4 (2019): 1764-1775.

Shen, Yong, et al. "Linking aboveground traits to root traits and local environment: implications of the plant economics spectrum." *Frontiers in Plant Science* 10 (2019): 1412.

Singh, Prachi, et al. "Seed biopriming for sustainable agriculture and ecosystem restoration." *Microbial Biotechnology* (2023).

Slate, Mandy L., Ragan M. Callaway, and Dean E. Pearson. "Life in interstitial space: Biocrusts inhibit exotic but not native plant establishment in semi-arid grasslands." *Journal of Ecology* 107.3 (2019): 1317-1327.

Song, Guang, et al. "Biocrusts mediate the plant community composition of dryland restoration ecosystems." *Science of the Total Environment* 844 (2022): 157135.

Song, Guang, Xinrong Li, and Rong Hui. "Biological soil crusts increase stability and invasion resistance of desert revegetation communities in northern China." *Ecosphere* 11.2 (2020): e03043.

Song, Guang, Xinrong Li, and Rong Hui. "Biological soil crusts determine the germination and growth of two exotic plants." *Ecology and Evolution* 7.22 (2017): 9441-9450.

Sorochkina, Kira, Sergio Velasco Ayuso, and Ferran Garcia-Pichel. "Establishing rates of lateral expansion of cyanobacterial biological soil crusts for optimal restoration." *Plant and Soil* 429 (2018): 199-211.

Stiles, Daniel. "Linkages between dryland degradation and migration: a methodology." *Desertification Control Bulletin* (1997): 9-18.

Stohlgren, T. J., Y. Otsuki, C. A. Villa, M. Lee, and J. Belnap. 2001. Patterns of plant invasions: a case example in native species hotspots and rare habitat. *Biological Invasions* 3: 37–50.

Tran, An Thi Phuong, Ilhan Chang, and Gye-Chun Cho. "Soil water retention and vegetation survivability improvement using microbial biopolymers in drylands." *Geomech. Eng* 17.5 (2019): 475-483.

Trivedi, Pankaj, et al. "Plant–microbiome interactions: from community assembly to plant health." *Nature reviews microbiology* 18.11 (2020): 607-621.

Tucker, Colin, et al. "Biological soil crust salvage for dryland restoration: an opportunity for natural resource restoration." *Restoration ecology* 28 (2020): S9-S16.

Turnbull, Laura, et al. "Understanding the role of ecohydrological feedbacks in ecosystem state change in drylands." *Ecohydrology* 5.2 (2012): 174-183.

USDA, NRCS. 2023. The PLANTS Database (<http://plants.usda.gov>, 10/09/2023). National Plant Data Team, Greensboro, NC USA.

Viechtbauer, Wolfgang. "Conducting meta-analyses in R with the metafor package." *Journal of statistical software* 36.3 (2010): 1-48.

Wang, Fayuan. "Occurrence of arbuscular mycorrhizal fungi in mining-impacted sites and their contribution to ecological restoration: Mechanisms and applications." *Critical Reviews in Environmental Science and Technology* 47.20 (2017): 1901-1957.

Weber, Bettina, et al. "What is a biocrust? A refined, contemporary definition for a broadening research community." *Biological Reviews* 97.5 (2022): 1768-1785.

Weber, Bettina, et al. "Natural recovery of biological soil crusts after disturbance." *Biological soil crusts: an organizing principle in drylands* (2016): 479-498.

Wei, Taiyun, et al. "Package ‘corrplot’." *Statistician* 56.316 (2017): e24.

White, Peter S., and A. N. K. E. Jentsch. "Disturbance, succession, and community assembly in terrestrial plant communities." *Assembly rules and restoration ecology: bridging the gap between theory and practice* 5 (2004): 342.

Xiao, Bo, et al. "Biocrusts: Engineers and architects of surface soil properties, functions, and processes in dryland ecosystems." *Geoderma* 424 (2022): 116015.

Xiao, Bo, and Matthew A. Bowker. "Moss-biocrusts strongly decrease soil surface albedo, altering land-surface energy balance in a dryland ecosystem." *Science of the Total Environment* 741 (2020): 140425.

Yang, S., J. HilleRisLambers, and J. L. Ruesink. "Reversal of intraspecific interactions by an ecosystem engineer leads to variable seedling success along a stress gradient." *Marine Ecology Progress Series* 543 (2016): 163-171.

Yang, Xuejun, et al. "More than just a coating: ecological importance, taxonomic occurrence and phylogenetic relationships of seed coat mucilage." *Perspectives in Plant Ecology, Evolution and Systematics* 14.6 (2012): 434-442.

Ye, Fei, et al. "Succession of soil microbial community in a developing mid-channel bar: The role of environmental disturbance and plant community." *Frontiers in Microbiology* 13 (2022): 970529.

Zaady, Eli, David J. Eldridge, and Matthew A. Bowker. "Effects of local-scale disturbance on biocrusts." *Biological soil crusts: An organizing principle in drylands* (2016): 429-449.

Zhang, Chao, et al. "Soil bacterial community dynamics reflect changes in plant community and soil properties during the secondary succession of abandoned farmland in the Loess Plateau." *Soil Biology and Biochemistry* 97 (2016): 40-49.

Zhang, Yuanming, and Jayne Belnap. "Growth responses of five desert plants as influenced by biological soil crusts from a temperate desert, China." *Ecological Research* 30 (2015): 1037-1045.

Zhang, Yuanming, et al. "Interactions of biological soil crusts with vascular plants." *Biological soil crusts: an organizing principle in drylands* (2016): 385-406.

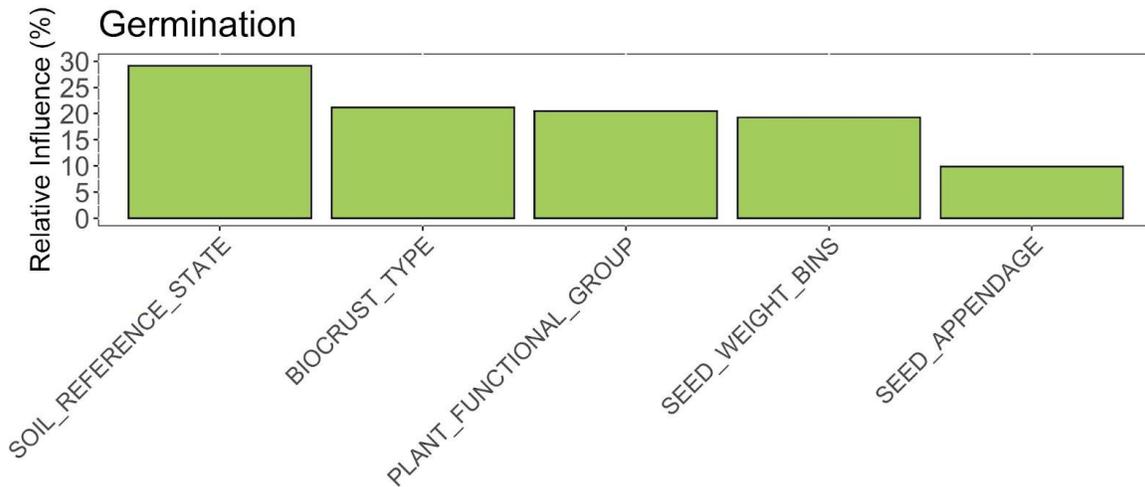
Zheng, Alice, and Amanda Casari. *Feature engineering for machine learning: principles and techniques for data scientists*. " O'Reilly Media, Inc.", 2018.

Zhou, Xiaobing, et al. "Practices of biological soil crust rehabilitation in China: experiences and challenges." *Restoration Ecology* 28 (2020): S45-S55.

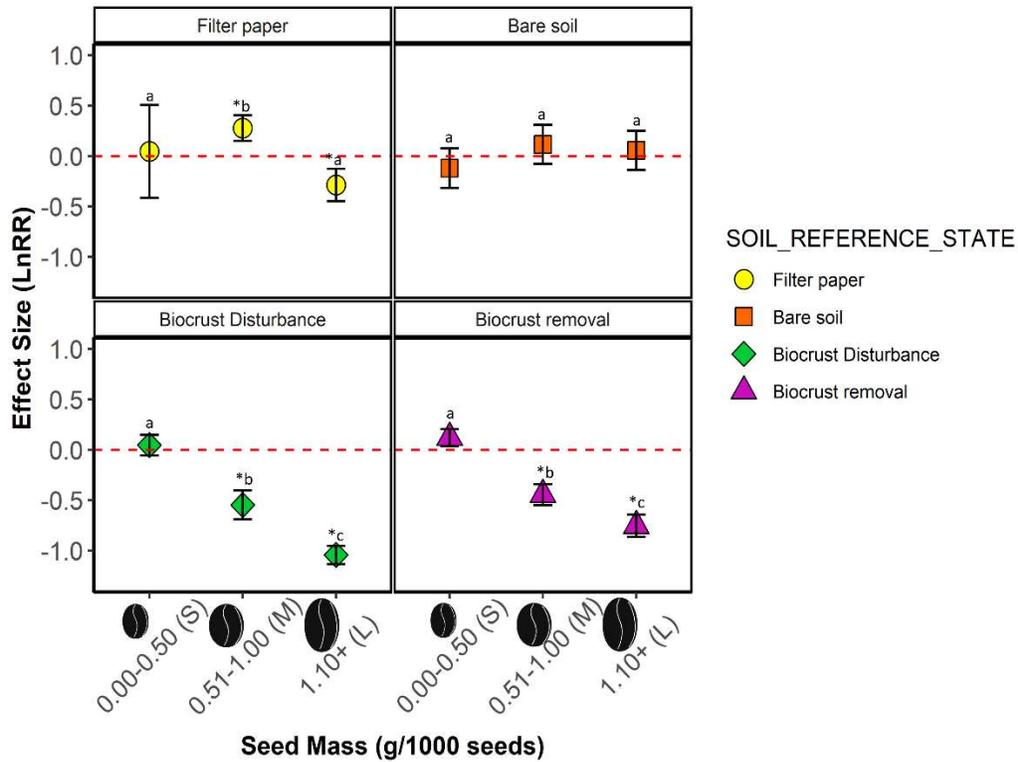
APPENDIX 1



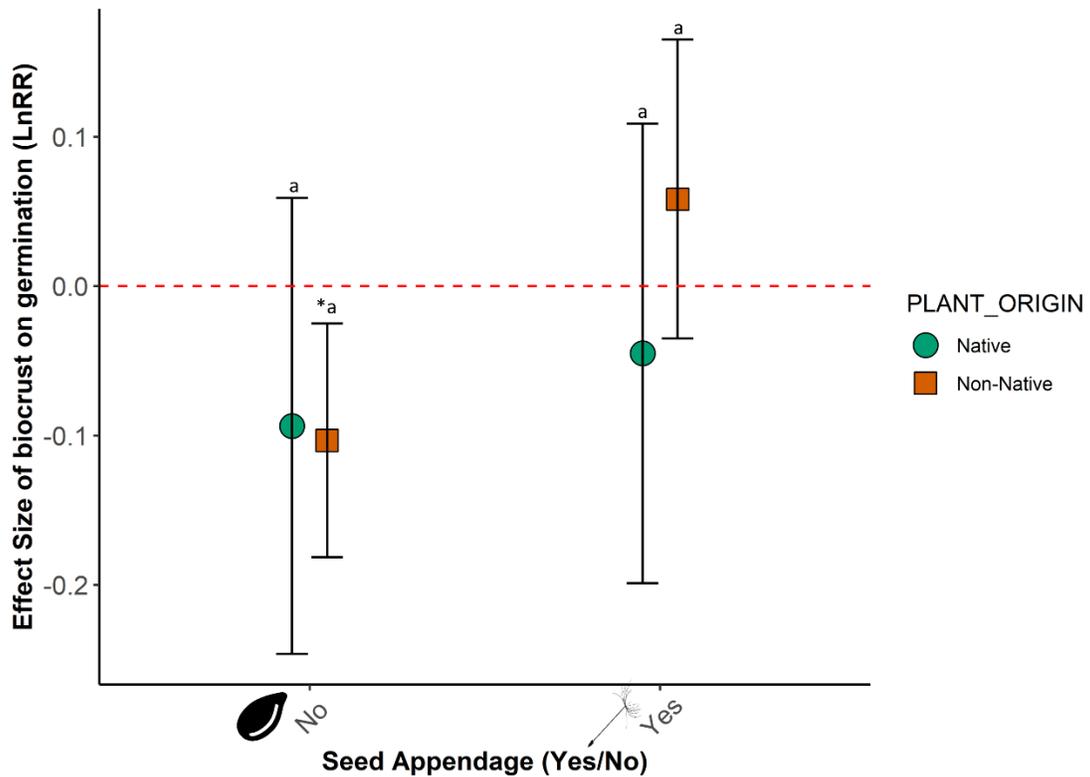
**Supplementary Figure 1.** *Corrplot* depicting the correlation matrix between the variables used within the models for Chapter 1.



**Supplementary Figure 2.** Boosted regression tree model results showing the relative influence of the most influential variables.



**Supplementary Figure 3.** Seed mass influence on germination in response to different soil reference states. Lower case letters denote significantly significant pairwise differences while “\*” indicates a significant difference from zero.



**Supplementary Figure 4.** Seed appendage presence’s influence on germination in response to plant origin and biocrusts. Lower case letters denote significantly significant pairwise differences while “\*” indicates a significant difference from zero.

**Supplementary Table 1.** This table shows a breakdown of the sample sizes of each of the various seed and plant traits for the plant functional group C4 grasses.

<b>Trait</b>	<b>Sample Size</b>
<b>Seed Mass</b>	
Small (0-0.50 g/1000 seeds)	27
Medium (0.51-1.0 g/1000 seeds)	4
Large (1.1+ g/1000 seeds)	7
<b>Seed Appendage</b>	
No	26
Yes	12
<b>Plant Origin</b>	
Native	40
Non-native	5
<b>Biocrust Community</b>	
Cyanobacteria	16
Moss	35
Lichen	19
Mixed	20

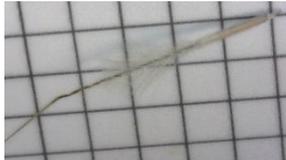
APPENDIX 2

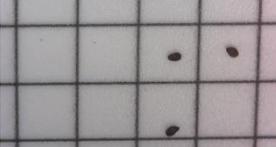
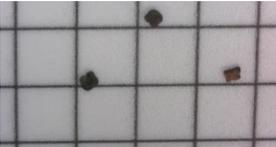
**Supplementary Table 2.** Relative plant cover percentages from the two sites where the biocrust inoculum was collected.

Site	Latitude	Longitude	Percent Cover Grass	Percent Cover Shrubs	Percent Cover Forbs	Percent Cover Cacti	Percent Cover Bare Soil
Site 1	36° 29' 9" N	114° 45' 18" W	3	15	0	2	80
Site 2	36° 29' 11" N	114° 45' 17" W	0	36	0	4	60

**Supplementary Table 3.** Seed mixes used with the scientific name, the USDA database code, a photograph of the seedling (if available), and a photograph of the seed.

**Mojave Desert Species:**

<i>Hilaria rigida (Pleuraphis rigida)</i>	PLRI3	 Seedling	
<i>Achnatherum hymenoides</i>	ACHY		
<i>Achnatherum (Stipa) speciosum</i>	ACSP12	 Seedling	

<i>Distichlis spicata</i>	<b>DISP</b>	 <p>Seedling</p>	
<i>Muhlenbergia microsperma</i>	<b>MUMI</b>		
<i>Sporobolus arioides</i>	<b>SPAI</b>	 <p>Seedling</p>	
<i>Lotus strigosus</i>	<b>LOST</b>	 <p><i>Lotus strigosus</i> <i>Acmispon strigosus</i></p>	
<i>Ottleya rigida (Lotus rigidus, Acmispon rigidus)</i>	<b>OTRI</b>		
<i>Lupinus arizonicus</i>	<b>LUAR</b>	 <p><i>Lupinus arizonicus</i></p>	
<i>Eschscholzia californica</i>	<b>ESCA2</b>		

<i>Abronia villosa</i>	ABVI	 <p><i>Abronia villosa</i> var. <i>villosa</i></p>	
<i>Mirabilis laevis</i>	MILA6	 <p><i>Mirabilis bigelovii</i> var. <i>retrorsa</i> <i>Mirabilis laevis</i> var. <i>retrorsa</i></p>	

**Colorado Plateau Desert Species:**

<i>Poa secunda</i>	POSE		
<i>Leymus cinereus</i>	LECI4	 <p>Seedling</p>	
<i>Pseudoroegneria spicata</i>	PSSP6	 <p>Seedling</p>	

<b><i>Bouteloua gracilis</i></b>	<b>BOGR</b>		
<b><i>Sporobolus cryptandrus</i></b>	<b>SPCR</b>		
<b><i>Hilaria jamesii (Pleuraphis jamesii)</i></b>	<b>PLJA</b>		
<b><i>Dalea candida</i></b>	<b>DACA7</b>	 Seedling	
<b><i>Hedysarum boreale</i></b>	<b>HEBO</b>	 Utah Sweetvetch Seedling	
<b><i>Lupinus sericeus</i></b>	<b>LUSE4</b>		

<i>Achillea millefolium</i>	ACMI2		
<i>Machaeranthera tanacetifolia</i>	MATA2		
<i>Penstemon palmeri</i>	PEPA8		

**Supplementary Table 4.** Results of the germination trials. Species that reached the 30% germination threshold were not carried over into subsequent trials.

<b>Mojave Desert</b>				
Species	Germ Trial 1	Germ %	Germ Trial 2	Germ %
PLRI3	3/10	30	N/A	N/A
ACHY	8/10	80	N/A	N/A
ACSP12	5/10	50	N/A	N/A
DISP	1/10	10	3/10	30
MUMI	6/10	60	N/A	N/A
SPAI	0/10	0	6/10	60
LOST	2/10	20	3/10	30
OTRI	4/10	40	N/A	N/A
LUAR	2/10	20	3/10	30
ESCA2	8/10	80	N/A	N/A
ABVI	0/10	0	3/10	30
MILA6	0/10	0	3/10	30

<b>Colorado Plateau</b>
-------------------------

Species	Germ Trial 1	Germ %	Germ Trial 2	Germ %
POSE	10/10	100	N/A	N/A
LECI4	6/10	60	N/A	N/A
PSSP6	9/10	90	N/A	N/A
BOGR	6/10	60	N/A	N/A
SPCR	0/10	0	3/10	30
PLJA	4/10	40	N/A	N/A
DACA7	10/10	100	N/A	N/A
HEBO	1/10	10	3/10	30
LUSE3	0/10	0	6/10	60
ACMI2	9/10	90	N/A	N/A
MATA2	2/10	20	3/10	30
PEPA8	1/10	10	4/10	40

**North-West**

**North-East**

**Column 1 Column 2**

**Column 3 Column 4**

ACMD 100, 1	ACCP 100, 21	SCMD 100, 29	CCP, 37
SCMD 30, 2	ACCP 30, 22	SCMD 30, 30	CCP, 38
ACCP 100, 3	SCCP 100, 23	CCP, 31	SCCP 100, 39
ACCP 30, 4	SCCP 30, 24	CCP, 32	SCCP 30, 40
CMD, 5	ACMD 100, 17	ACCP 100, 25	SCMD 100, 41
CMD, 6	ACMD 30, 18	ACCP 30, 26	SCMD 30, 42
CCP, 7	CMD, 19	CCP, 27	CMD, 43
CCP, 8	CMD, 20	CCP, 28	CMD, 44
CMD, 9	ACMD 100, 13	ACCP 100, 35	SCCP 100, 45
CMD, 10	ACMD 30, 14	ACCP 30, 36	SCCP 30, 46
SCCP 100, 11	SCMD 100, 15	ACMD 100, 33	SCMD 100, 47
SCCP 30, 12	SCMD 30, 16	ACMD 30, 34	SCMD 30, 48

**Column 1 Column 2**

**Column 3 Column 4**

**South-West**

**South-East**

<b>100</b>	<b>Sterile x Mojave (100)</b>	<b>100</b>	<b>Active x Mojave (100)</b>	<b>Control Mojave</b>
<b>30</b>	<b>Sterile x Mojave (30)</b>	<b>30</b>	<b>Active x Mojave (30)</b>	<b>Control CP</b>
<b>100</b>	<b>Sterile x CP (100)</b>	<b>100</b>	<b>Active x CP (100)</b>	
<b>30</b>	<b>Sterile x CP (30)</b>	<b>30</b>	<b>Active x CP (30)</b>	

**Supplementary Figure 5.** Experimental set up map, with key table beneath.