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Inoculated biocrust cover and functions diverged over a gradient of soil textures and water availability

Running Head: Environmental conditions influence inoculated biocrust outcomes

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KEY, SCR, MM, MAB created and implemented the experiment, KEY, SCR, MAB wrote the manuscript

Implications for Practice:

- Following biocrust inoculation in restoration settings, variations in soil texture and moisture can substantially affect the composition and trajectory of biocrust communities, making these drivers important to consider when setting restoration goals.
- When planning for biocrust inoculation, locations with fine-textured soils and higher soil moisture may be more suitable for supporting moss cover, while coarser-textured soils with lower soil moisture may better support cyanobacteria cover.
- Restoration practitioners wanting to increase soil stability and nutrient cycling should explore the feasibility of including moss in biocrust inoculum when restoring in wetter, finer textured soils, or

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should consider creating site conditions that increase soil moisture retention for moss on drier, coarser textured soils, such as by adding shade elements.

Abstract

Restoring biological crust (biocrust) in disturbed drylands is challenging due to the difficult environmental conditions, such as limited soil moisture, low soil nutrients, and extreme temperatures, that impede growth. Understanding how the key components of biocrust—mosses, lichens, and cyanobacteria—react to different environmental factors is crucial for determining the optimal timing, locations, and species composition for biocrust reintroduction, thereby increasing the likelihood of establishment. Here, we inoculated soils with a diverse range of biocrust organisms, analogous to seeding an area with diverse vascular plant seeds, and varied environmental conditions to observe how these changes influenced the development and functions of reintroduced biocrust. We found that by manipulating soil texture and time spent wet, we can change the proportional cover of biocrust within a restoration-like setting. Specifically, we found that 4-months after inoculation, finer textured soils that received more water become dominated by moss cover, while coarser textured soils with less water remained dominated by cyanobacteria cover, and the interactions between texture and time spent wet strongly influenced cover. We found biocrust morphological group cover had a small, but detectable, effect on ecosystem functions (soil stability and nitrogenase activity, a proxy for nitrogen fixation), but that environmental conditions had a stronger impact on the functions we measured. Manipulative experiments in controlled environments, like this one, can help elucidate the mechanisms underlying the establishment rate and patterns of biocrusts post-inoculation, and guide implementation of inoculations in the field.

Keywords: drylands, soil rehabilitation, biological soil crust, reclamation, soil texture, ecosystem functions

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Introduction

Dryland ecosystems make up over 40% of Earth's land surface and serve as home to about 2.7 billion people (Pravalie et al. 2016; Stavi et al. 2021). These expansive regions are experiencing high rates of land conversions and are exhibiting large responses to land use and climate change (e.g., reduced net primary productivity [NPP], Zika et al. 2009), resulting in substantial areas of land in need of restoration and/or rehabilitation (Reynolds et al. 2007; Cowie et al. 2011). However, restoration in dryland ecosystems is constrained by low and variable precipitation, extreme temperature, and low soil fertility (Shackelford et al. 2021). Due to these constraints, restoration efforts such as seeding have a high failure rate and, when plants do establish, seeded species consistently decline over time (Shackelford et al. 2021). Despite relatively low success rates, dryland restoration is increasingly expensive, averaging \$46,198/km^2, for example, in the semi-arid and arid American Southwest (Copeland et al. 2018). In light of these costs and challenges, improving restoration success is a primary goal of researchers and practitioners in this socially and ecologically important biome.

One emerging restoration practice with considerable potential to increase restoration success in drylands is the assisted recovery of biological soil crusts (biocrust). Biocrusts are communities comprised to varying degrees by lichens, mosses, and cyanobacteria that live on the top few centimeters of the soil surface in areas where soils have direct access to sunlight (e.g., drylands; Weber et al. 2022). Biocrusts can serve as primary producers alongside vascular plants and, in restoration settings, can improve soil stability/erosion resistance, build and maintain fertility (e.g., through carbon [C] and nitrogen [N] fixation and nutrient cycling), influence movement and storage of soil moisture, and shape other biotic communities (e.g., plants, microbiota; Bowker et al. 2022; Zhao et al. 2016). Because of the many ecological services biocrusts provide and the potential for biocrusts to improve conditions for plant germination and establishment, restoring biocrusts is a sought-after restoration goal (Antoninka et al. 2020a).

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Biocrust restoration efforts typically apply biocrust as inoculum, which involves collecting or growing biocrust organisms and spreading them over surface areas in need of restoration, similar to the process of seeding with vascular plant species (Giraldo-Silva et al. 2019). However, just as with seeding, successfully establishing biocrust in the field has been challenging due to the extreme temperatures, low precipitation, high erosivity, and heterogenous resources inherent to dryland areas that need restoration (Antoninka et al. 2020a). To overcome environmental stressors, biocrust restoration efforts have focused on hardening biocrust organisms by exposing inoculum to extreme conditions before inoculation or softening the environment by applying amendments such as water or soil binding agents and physical barriers that reduce soil erosion, increase moisture retention, and reduce UV exposure (Giraldo-Silva et al. 2020; Bowker et al. 2020; Fick et al. 2020; Schultz et al. 2022). These trials have had mixed success and the most consistent success has occurred in cool deserts when water was applied in the days following inoculation (Wang et al. 2009; Wu et al. 2013; Faist et al. 2020).

A complementary approach that may help overcome difficult field conditions is to match biocrust inoculum to the environment (Faist et al. 2020). This approach involves matching the growth requirements of the biocrust organisms being used in an inoculum to environmental conditions at a restoration site, such as the soil texture, pH, water availability, and microsite C:N ratios (Rozenstein et al. 2014; Román et al. 2018; Rosentreter 2020; Zhao et al. 2023a). While most restoration field trials use native biocrust organisms that are presumed to be adapted to site conditions (Giraldo-Silva et al. 2019), sites that have been disturbed can have markedly different soil conditions than pre-disturbance conditions and varying degrees of microsites that may favor some biocrust organisms over others (Chambers et al. 2000). Additionally, increasing climate variability makes understanding water and temperature-controlled growth requirements of inoculated biocrust organisms important for restoration planning (Butterfield et al. 2017). This approach includes, but is not limited to, designing inoculum mixes for microsites within a restoration site, for example, on different sides of sand dunes (Zhao et al. 2023b) or applying specific inoculum mixes at different times of year that match the climate preferences of the organisms

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(Sorochkina et al. 2018; Giraldo-Silva et al. 2019). Importantly, this type of approach can be achieved while still using biocrust species native to the restoration site to avoid unintended consequences of introducing harmful microorganisms (Bethany et al. 2022). This restoration tactic is similar to the vision outlined by the US National Seed Strategy of providing the "right seed in the right place at the right time" and represents a promising direction for targeted biocrust restoration (Oldfield and Olwell 2015).

Here, we designed an experiment to tease apart how environmental conditions that can exist within a restoration site (i.e., heterogeneity within soil texture, nutrient available, and water availability) influence the growth, composition, and desired ecosystem functions (i.e., soil stability and nitrogenase activity, a proxy for N fixation) of inoculated biocrust organisms in a greenhouse setting. We chose to focus on soil texture, nutrient availability, and water availability, three environmental factors that have been shown to influence the composition and function of inoculated biocrust (Chamizo et al. 2018; Sorochkina et al. 2018; Mugnai et al. 2020). Specifically, we 1. explored the role of soil texture by creating a gradient of soil textures using the same source soils and adding the same inoculum to each soil texture type, then, 2. altered water availability by varying the amount of time that inoculated biocrusts were wet (hereafter called "hydration length") and 3. changed soil nutrient availability by applying fertilizer that contained macro and micro-nutrients. After four months of growth, we measured biocrust percent cover by morphological groups and two metrics of biocrust function, nitrogenase activity and soil stability (Evan & Ehleringer 1993; Barger et al. 2016; Belnap & Budel 2016). We hypothesized that longer hydration, finer texture, and increased nutrient availability would increase biocrust cover and that the combination of these factors would lead to varying compositions, which in turn would influence function.

Methods

Biocrust material & bulk soil collection

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We collected biocrust inoculum from a well-developed biocrust site in Fall 2014 from Frijoles Mesa in Bandelier National Monument in northern New Mexico (35° 51' N, 106° 19' E). We collected biocrust material by scraping biocrusts from the soil surface using a flat-bottomed trowel to a 0.5-1 cm depth and putting the collected material into a ~19 L bucket. Collected biocrusts were dominated by multi-species cyanobacterial communities of lightly pigmented and darkly pigmented cyanobacterial crusts (likely *Microcoleus vaginatus* and *Scytonema spp.*, Garcia-Pichel et al. 2013; Couradeau et al. 2016), in addition to mosses (*Bryum argenteum, Syntrichia caninervis, Bryum kunzei*) and lichens (*Collema coccophorum, Collema tenax, Heppia sp., Peltula spp., Placidium squamulosum, Clavascidium lacinulatum, Cladonia spp., and Acarospora schleicheri*). Mosses and lichen were identified using Rosentreter et al. 2007. During collection, well-developed patches of moss and cyanobacteria were equally targeted, with lichens intermixed within both biocrust types. Biocrust collections occurred when the soil was dry and collected biocrust was brought to Northern Arizona University, Flagstaff, Arizona, USA, where it was kept in a desiccated state and then mixed with a trowel and passed through a 2 mm sieve to disaggregate and homogenize the material. Homogenized inoculum was stored dry in the dark for 6 months, a collection and storage method shown to retain viability of the biocrust organisms (Young et al. 2019). At the same site described above, we collected bulk soil in buckets by digging a < 1.5 m deep hole within ~ 3 km of where the biocrust materials were sourced. The soil type was Hackroy series, order Alfisol, derived from volcanic tuff parent material. Soils were brought back to the lab and autoclaved.

Experimental design

Our experiment took place in the Northern Arizona University Research Greenhouse Facility. Photosynthetically active radiation (PAR) measurements averaged ~ 173.8 μmol/m^2/s in the greenhouse and the temperature averaged 18.9˚C in April, 22.2˚C in May, 27.1˚C in June, 22.3˚C in July, and 21.6˚C in August. We grew homogenized biocrust inoculum for 4 months (April, May, June, July) on varying soil textures, with different hydration length and fertilizer amounts. These experimental treatments were

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applied in a full-factorial design with 5 replicates for each treatment combination (n = 120). We describe each experimental treatment in detail below.

Soil Texture

We created four different soil texture classes by passing the soils through a series of sieves in the following order: 4 mm, 2 mm, 250 µm and 75 µm. Because we did not want to break up soil aggregates and release naturally unavailable nutrients, we passed the soils through each sieve one time and did not grind or shake the soil. We then mixed soils from the different sieves together to attain the following texture classes: silt loam with 20% sand, 68% silt, 11% clay; sandy loam with 61% sand, 30% silt, 7% clay; loamy sand 85% sand, 11% silt, 3% clay; gravelly sandy loam with 63% sand, 8% silt, 2% clay, 25% gravel. Additional information about soil texture creation and bulk soil nutrient values can be found in Table S1. Bulk soil nutrient concentrations and soil textures were measured in the Colorado Plateau Analytical Laboratory at Northern Arizona University.

Hydration Length

We administered three different watering treatments: 4 days, 7 days, and 14 days hydration length every ~16 days. Because biocrusts are active only when wet, the length of the hydration period is perhaps the most important aspect of water supply. Hydration for all treatments started at the same time and continued for their designated length. The 14 days treatment was given ~2 days to dry at which point a new hydration period was initiated for all experimental units, repeating the above pattern for 4 months (April, May, June, July). Water was administered through a wicking system described by Doherty et al. (2015). Briefly, plastic containers were placed within basins of the same size. The bottom of the container holding the soil contained holes through which charcoal filtered water was wicked up to the soil surface from the lower basin. Water was administered until it filled ¼ of the basin and passively wicked up to the soil surface. After watering periods ended, water drained from each container leaving the soil near field capacity.

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Fertilization Addition

Nutrient treatments included two levels: the application of 8 ml of Knop's fertilizer once per month, or no additional nutrients. The Knop's solution consisted of a dilute solution of macro and micronutrients (KH_2PO_4 (250 mg/l), KCl (250 mg/l), $MgSO_4 \cdot 7\ H_2O$ (250 mg/l), $Ca(NO_3)_2 \cdot 4\ H_2O$ (1000 mg/l), $FeSO_4 \cdot 7\ H_2O$ (12.5 mg/l)) (Reski & Abel, 1985), and has been shown to positively influence biocrust growth (Doherty et al. 2015). We applied fertilizer using a spray bottle, and fertilizer was sprayed evenly across the entirety of the soil surface from 10 cm away, as was done in Doherty et al. 2015.

Applying biocrust inoculum

We added homogenized biocrust inoculum to each container to cover 40% of the soil surface in a layer equal to approximately 0.2 cm thick at the beginning of April. Inoculum was scooped into a measuring cup and spread evenly over the soil surface within each container. The volume of biocrust inoculum to add was determined by calculating 40% of the surface area of the container and multiplying that by 0.2 cm to achieve a volume.

Biocrust Community Measurements

We visually estimated the percent cover of macroscopic organisms (lichens and mosses) and cyanobacterial colonies (multiple species assemblages detectable by biological structures and pigmentation) at roughly one-month intervals during our four-month experiment: April 2nd - April 25th (1st Month), April 25th - May 29th (2nd Month), May 29th - July 3rd (3rd Month), July 3rd - August 6th (4th Month). We placed 25 cm by 25 cm gridded quadrats broken into 5 cm x 5 cm grids over the samples to aid in cover estimates. We used a pin-flag to test for cyanobacterial presence by pushing the pin-flag gently into the soil surface to see if an indent remained. An indent indicated that the soil was held together by cyanobacteria. We corroborated this method by looking for visual signs of cyanobacterial filaments in the soil through the sides of the clear plastic containers the samples were located in. If filaments were not visible, we lifted a small portion of the biocrusted surface to check for filaments. We sorted our data into

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the broad groups of mosses, lichens, and cyanobacteria to simplify analysis and data presentation (Eldridge & Rosentreter 1999; Belnap et al. 2008). Grouping organisms into morphological groups is an established practice when analyzing biocrust cover (Belnap et al. 2003). The experiment ended when total cover reached ~ 100%, which was four months after applying inoculum. At the conclusion of the experiment, we measured the average depth of the cyanobacterial filaments by removing a cross section of soil from each container and measuring the average depth of visible filaments with a ruler.

Functional Measurements

We measured nitrogenase activity (a proxy for N_2 fixation, Belnap 2003; Barger et al. 2016), penetration resistance, and shear strength at the end of the experiment. We used the acetylene reduction assay (Hardy et al. 1968 as modified by Reed et al. 2007) to estimate nitrogenase activity. Briefly, two biocrust plugs were collected from each sample and sealed in 2.54 cm diameter clear acrylic tubes with a 25 ml headspace. Pure acetylene gas was injected into each sealed tube through a self-sealing septum, so acetylene occupied 10% of the headspace volume. The headspace was vented to the atmosphere to ensure atmospheric pressure within the tubes. We accounted for ethylene presence in the acetylene and for production of ethylene by the samples in our calculations: these ethylene sources were consistently low relative to the ethylene produced by the nitrogenase enzyme. Samples were incubated for 24 hours in the greenhouse while receiving natural variation in daylight and nighttime conditions. After incubation, sample headspaces were mixed, removed, and injected into pre-purged Vacutainer tubes (Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA). Ethylene concentrations of headspace were measured using a Shimadzu 14-A Gas Chromatograph equipped with a flame ionization detector (330 °C) and a Poropak N column (110°C; Supelco, Bellefonte, Pennsylvania, USA). Rates of acetylene reduction were calculated using the unit nanomoles (10^9 mol) of acetylene reduced per cm^2 area of biocrust surface per hour.

To explore biocrust community effects on soil stability, we measured two soil stability metrics, penetration resistance and shear strength. We collected two readings of compression resistance using a 0-

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5 kg/cm^2 penetrometer, holding the penetrometer vertically and pressing into the soil at a depth of 6 mm (Zaady & Bouskila, 2002). The duplicate measurements were averaged for each experimental unit prior to analysis. We also collected two readings of shear strength resistance using a shear vane (Torvane, Durham Geo Slope Indicator) by pressing the shear vane into the soil surface and twisting until resistance stopped, then recorded maximum resistance in kg/cm^2 (Brunori et al. 1989).

Statistical analysis

Statistics were performed using R (R Core Team 2022). We used a Permutational Multivariate Analysis of Variance (PERMANOVA) to determine how biocrust cover types changed over time under different treatments. We chose to use a PERMANOVA because the data did not meet model assumptions of more commonly used repeated measures analyses. Because PERMANOVA makes no distribution assumptions but also does not specifically incorporate repeated measures models, we also performed a repeated measures multivariate analysis of variance (MANOVA) to determine if the results from both statistical tests were similar. Both analyses indicated similar results and we present the PERMANOVA results here. Over the course of the experiment, some samples were lost due to being disturbed during cover measurements. The few missing values were imputed using a linear regression approach with the R package MICE.

To assess differences in the proportional cover of individual biocrust morphological groups and average filament depth among treatments at the final time point of the experiment, we conducted a three-way ANOVA and Tukey's HSD. Cover values for each morphological group were transformed into relative abundances to determine differences between groups when overall cover was low. Data were checked for model assumptions using a Shapiro-Wilk test (Shapiro & Wilk, 1965) and, when non-normal, the data were transformed to achieve normality. Lichen cover could not be transformed to achieve normality and was not analyzed for cover due to the large number of zeros.

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To determine the correlation between the proportional cover of mosses or cyanobacteria and functions (penetration resistance, shear strength, and N_2 fixation), we used linear regression after checking for model assumptions. We did not perform a linear regression on the lichen cover because residuals were not normally distributed. We used structural equation modeling (SEM) methods to determine the relative influence of biocrusts *and* abiotic treatments on functions. By using SEMs, we can understand both the net effect of treatments on response variables, and the influence of the response variables on one another, as well as partitioning direct and indirect effects (Bowker et al. 2008). Details of our SEM can be found in Supplement S1. While SEM has major value in elucidating causal network structure, and partitioning effects that one variable may have on another via multiple pathways, it is not optimal to test and estimate interactions in multifactor experiments.

Results

Overall, the proportional cover of mosses and cyanobacteria changed with hydration length and soil texture, and minimally with fertilizer addition, throughout the experiment (Figure 1, Figure 2, Table S2). Functional properties increased with moss cover relative to cyanobacteria cover (Figure 3) but were predominantly influenced by texture and hydration length (Figure 4).

Biocrust cover change over time

Moss cover increased through time, with the highest median values starting at around 25% cover and reaching around 75% cover by the end of the experiment (Time: $F = 158.6$, $p = 0.001$). Moss cover increased on different textures at different rates, sandy loam texture showing the fastest and largest increase and gravelly sandy loam texture showing the slowest and smallest increase (Time x Texture: $F = 3.4$, $p = 0.02$). The rate of cover increased with different watering levels, with the fastest increase in cover occurring with the longest hydration time, and the slowest with the shortest hydration time (Time x Hydration: $F = 8.4$, $p = 0.001$). Moss cover increased most over time on the finest textured soil, under the longest hydration length (Time x Texture x Hydration: $F = 2.7$, $p = 0.01$). Fertilization alone did not

strongly affect moss cover (Fert: F = 0.9, p = 0.3). There was a slight interaction between hydration length and fertilizer addition (Hydration x Fert: F = 3.2, p = 0.04), where moss cover in month two of the experiment was slightly higher in fertilized pots than unfertilized pots, however a post-hoc test did not show strong evidence of statistical differences (Table S3, Figure S1). Fertilizer addition did not significantly affect soil available nutrients, apart from available nitrate, which was higher in soils with fertilizer addition (Figure S2).

Cyanobacterial cover initially increased, and then began to decrease with time (Time: F = 11.6, p = 0.001). Cover changed with texture over time, in some cases starting around twice as high on the silt loam and sandy loam textures than the loamy sand and gravelly sandy loam textures, but ending with near equal cover across textures (Time x Texture: F = 13.1, p = 0.001). Cyanobacterial cover also changed with hydration length, beginning with higher cover under longer hydration lengths, then transitioning to higher cover with shorter hydration lengths, and finally ending with similar cover levels across hydration lengths (Time x Hydration: F = 12.9, p = 0.001). Time, hydration length, and soil texture influenced cover, where cover was highest on fine textured soils with longer hydration lengths to relatively equal cover across textures and hydration lengths (Time x Texture x Hydration: F = 13.7, p = 0.001). Fertilization had a weak effect on cyanobacteria cover (Fert: F = 5.5, p = 0.02), mainly in the second month where cover was lower in unfertilized pots than fertilized pots, but post-hoc analyses indicated the difference were not statistically significant (Table S4, Figure S3)

The highest lichen cover overall was 14% total cover or 12% proportional cover however, the majority of samples through time had lichen cover values between 0 - 2% (Figure S4). Lichen cover values were frequently zero, especially under the 14-day hydration length (Figure S4). Because of this we chose not to conduct analyses on lichen cover and instead focused on cyanobacteria and moss cover.

Proportional biocrust cover at the final timepoint

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At the final time point, the proportional cover of biocrust morphological groups was strongly influenced by the watering treatment and textures (Figure 5, Table S5, Table S6, Table S7). Both texture class ($F = 9.8$ $p < 0.0001$) and hydration length ($F = 37.2$, $p < 0.0001$) influenced moss cover, however there was no evidence to support an interaction between these two treatments ($F = 0.5$, $p = 0.8$). Fertilizer addition had no detectable influence on moss cover ($F = 0.2$, $p = 0.7$). Moss cover was an average of 2.7 times higher on silt loam texture soil with the longest hydration length (median cover value of 75%) than on the gravelly sandy loam texture with the shortest hydration length (median cover value of 28%) (Figure 5).

Texture class ($F = 7.5$, $p = 0.0002$) and hydration length ($F = 31.9$, $p < 0.0001$) also strongly influenced cyanobacterial cover, while there was no evidence of an interaction between these variables ($F = 0.4$, p-value = 0.9) or an impact of fertilizer addition ($F = 0.2$, $p = 0.7$). Cyanobacterial cover was an average of 3 times lower on silt loam texture soil with the longest hydration length (median cover value of 22%) than on the gravelly sandy loam texture with the shortest hydration length (median cover value of 66%) (Figure 5). The proportional cover of liches never reached higher than 1.3% (Figure 5).

Filament Depth

Texture strongly influenced the average depth of cyanobacteria filaments ($F = 40.8$, p-value = <0.0001), with greater depths on coarser textured soils. Hydration length influenced filament depth ($F = 6.9$, p-value = 0.002), but the effect was largely interactive between hydration length and texture ($F = 4.6$, p-value = 0.0004). Filament depth was 2.7 times deeper on the two coarser textured soils than the two finer textured soils under the longest hydration time This pattern was still present but more muted under the shortest hydration time, where filament length was ~ 1.3 times longer on the two coarser textured than the two finer textured soils (Figure 6, Table S5, Table S8).

Functional results

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All functions measured were positively correlated with moss cover and negatively correlated with cyanobacterial cover (Figure 3). Penetration resistance (R^2 = 0.10, p-value = 0.0004), shear strength (R^2 = 0.17, p-value = <0.0001), and nitrogenase activity (R^2 = 0.02, p-value = 0.08) were positively correlated with proportional moss cover and negatively correlated with proportional cyanobacterial cover (penetration resistance, R^2 = 0.08, p-value = 0.003, shear strength R^2 = 0.15, p-value = <0.0001, and nitrogenase activity, R^2 = 0.02, p-value = 0.09). Because lichen cover was non-normal, we did not conduct correlation tests between lichen cover and functions.

Ecosystem functions were most affected by abiotic conditions and less so by biocrust attributes (Figure 4, Table S9). Some notable effects of treatments on function were the effect of texture on nitrogenase activity (r=|.30|) and penetration resistance (r=|.46|) and the effect of hydration length on shear strength (r=.65) and penetration resistance (r=.54) (Figure 4, Table S9). Composite effects of biocrust exerted weaker effects on function, namely resistance to penetration (r=|.24|), nitrogenase activity (r=|0.22|), and shear strength (r=|0.13|) (Figure 4, Table S9). The results from the biocrust composite and texture composite represent the absolute value of the effect because different indicators of the composite may exert differently signed effects.

Discussion

Successful restoration requires a nuanced understanding of how seeded or inoculated species will respond to environmental conditions that can affect establishment and growth (Copeland et al. 2021). While interactions between seeded species and the abiotic environment have been studied for decades (e.g., Temperton et al. 2004; Shaw et al. 2020), our understanding of how interacting environmental factors influence biocrust growth after inoculation is limited. This study attempts to quantify how environmental conditions influence biocrust growth, composition, and function after inoculation, providing a foundation that can be built upon to introduce biocrust inoculum more successfully in restoration settings under field conditions. Within our study, our results reveal that hydration length and soil texture significantly influence the growth and composition of inoculated biocrust and that the abiotic

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environment, and to a lesser degree, biocrust composition, influence soil stability and fertility via N_2 fixation (i.e., nitrogenase activity). We discuss each of these findings in detail below.

How texture and hydration affected biocrust composition

In our restoration-like setting, we observed that water availability and soil texture strongly influenced the cover and composition of inoculated biocrust on bare soil. These findings align with previous studies demonstrating the abiotic environment can restrict the distribution of biocrust morphological groups to specific soil types (Pietrasiak et al. 2011) and impact biocrust community structure through variations in nutrient availability, porosity, temperature, susceptibility to disturbance, pH, electrical conductivity, and water availability (Rozenstein et al. 2014; Fischer & Subbotina, 2014; Lalley et al. 2014). For example, coarser-textured soils can support highly mobile, large filamentous cyanobacteria more effectively than finer-textured soils, likely due to the morphology of cyanobacteria that can take advantage of the large pore spaces in coarse soils (Belnap, 1993; Mugnai et al. 2020). Consistently, we observed greater filament depth on the coarsest textured soils at all hydration lengths, highlighting the influence of soil texture on cyanobacterial growth. Hydration length also significantly influenced the cover of biocrusts in our experiment. Field studies have shown that adequate hydration can rapidly increase inoculated moss cover and moss cover has been observed to increase along precipitation gradients (Belnap et al. 2003; Xiao et al. 2015; Zhou et al. 2020). Interestingly, cover type showed similar successional trajectory across the treatments from cyanobacteria-dominated to moss-dominated. However, the speed of succession and the relative cover reached in the finite time period of our experiment were strongly affected by interacting environmental factors. This finding suggests that interacting abiotic factors can determine the composition of biocrusts and that cover type is not determined by a single environmental parameter (Kidron & Xiao, 2023).

Added nutrients had little effect on biocrust cover

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The influence of soil nutrients on biocrust cover is not well understood. Biocrusts have been shown to be positively correlated with nutrients such as N, P, Mn, Zn, K, and Mg in nature (Bowker et al. 2005, 2006; Moreno-Jimenez et al. 2020) and after inoculation (Antoninka et al. 2020b; Zhao et al. 2023a). However, these correlations are not always easy to interpret because they may imply limitation of biocrusts by those nutrients, or modification of nutrient availability by biocrusts or both. Our experiment did not show a large effect of nutrient addition on biocrust morphological group cover. Similar lack of strong response to nutrient addition has been observed in other biocrust studies where the addition of Mn, P, K, and Zn had little effect on growth (Bowker et al. 2008). This may be due to the method we used to add fertility to the soil, which involved a single type of fertilization and did not test other elements, concentrations, or stoichiometries that could have resulted in a stronger biocrust response (but see Antoninka et al. 2016 who saw a positive response from a similar fertilization method). Fertilizer addition is also an imperfect method for adding mobile nutrients due to the potential for gaseous loss and leaching (McHugh et al. 2017). Background differences in nutrient retention across soil textures may have played a role in biocrust community structure, as finer textured soils retain nutrients more readily than coarser textured soils (Brady & Weil, 2008; Osborne et al. 2022), suggesting that differences in cover type between soil textures may be due in part to the higher background nutrient levels within fine soil textures that were not overcome with our method of nutrient addition. Unraveling the complex relationships between biocrusts and the many essential nutrients derived from soil is beyond the scope of our current study, but could lead to great insights explaining biocrust distribution, improving cultivation practice and elevating restoration outcomes.

How biocrust composition and abiotic conditions influenced function

Biocrust composition can have a strong (Bowker et al. 2013) or weak (Maestre et al. 2012) influence on function, depending on the specific function being considered. In our experiment, we observed that biocrust composition was correlated with every function that was measured, however, our SEM indicated that those correlations were driven more by the abiotic environment rather than by biocrusts.

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Shear resistance and penetration resistance were most strongly influenced by abiotic factors, and to a lesser degree by biocrust cover. Soil particle size is known to influence soil stability (Williams et al. 1983), but the direct effect of hydration time on soil stability was surprising. One explanation may be that water amounts change underground soil community composition and abundance, for example, the presence of cyanobacteria or/and fungi, both of which can increase soil stability (Chaudhary et al. 2009). It is important to note that the methods of measurement can be imprecise and may be influencing our functional results (Mallen-Cooper et al. 2020). For example, we measured penetration resistance and shear resistance to 6 mm but may have observed a greater biocrust effect if we have measured to a shallower depth. Further, our treatments may have influenced the microbial community in a way that muted functional responses. For example, N addition can change the abundance of soil bacteria, fungi, and green algae in ways that will likely influence biocrust-mediated nutrient availability (Ochoa-Hueso et al. 2016). Identifying and quantifying the microbial community would improve our ability to differentiate the relative impacts of the abiotic environment and biocrust community in mediating function.

While abiotic conditions appeared to exert the most control over ecosystem functions in our experiment, the dominance of mosses relative to cyanobacterial biocrust cover did exert a measurable effect on functions. In many systems, including our experiment, cyanobacteria are earlier successional biocrust formers which may be replaced by mosses through succession, if abiotic conditions allow, resulting in greater function (Housman et al. 2006; Torres-Cruz et al. 2018). Although cyanobacterial biocrusts would be expected to be more highly functional than bare soils, our data suggest that moss biocrusts are more functional than cyanobacterial biocrusts. Mosses are morphologically more complex than cyanobacteria, as they possess root-like structures known as rhizoids that attach to soil particles and form moss crusts (Bao et al. 2019). These morphological features likely contribute to increased penetration resistance and shear strength compared to filamentous cyanobacteria. While both cyanobacteria and moss dominated crusts can increase erosion resistance relative to bare soil, field observations have observed biocrust communities dominated by cyanobacteria can be less resistant to soil erosion compared to later-successional communities

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dominated by mosses (Gao et al. 2017). Mosses are often associated with higher microbial biomass and more diverse microbial communities compared to cyanobacteria-dominated communities, many of which may fix N (Xiao & Veste, 2017; Bao et al. 2019; Delgado-Baquerizo et al. 2016). A larger and more diverse microbial community beneath biocrust mosses may help explain the weak positive relationship between proportional moss cover and ethylene production.

Restoring biocrust in field settings

Within restoration settings, our findings suggest that even subtle changes in soil texture and water availability can change biocrust composition and function after inoculation. During restoration, site conditions and the desired ecosystem functions should be carefully considered before adding costly biocrust inoculum. Fine-textured soils and higher soil moisture may be more suitable for supporting moss cover as an end goal, while coarser-textured soils with lower soil moisture may preclude a complete succession to moss dominance, potentially making cyanobacterial cover a better target. When attempting to increase soil stability and N cycling quickly, it may be important to include moss in biocrust inoculum. In more arid locations, if mosses are desired, it may be necessary to create site conditions that favor the growth of moss on drier, coarser textured soils, such as by adding slash pieces, jute cloth, or sun barriers to create microsites with higher soil moisture (Antoninka et al. 2020b). In drier, more coarse-textured soils, cyanobacteria that are both N-fixers and soil stabilizers may overcome the limited soil stability and nutrient availability of those soil types (Roncero-Ramos et al. 2022). When a specific biocrust cover type is desired, it may be possible to manipulate the soil texture to favor a given cover type by adding fine texture soils, such as clay, or by manufacturing topsoil (as in after post-mining or extractive reclamation, Schultz et al. 2022). If irrigation is an option, even temporarily, lengthening the hydration time may be a way to achieve moss cover more quickly, if moss cover is the desired goal. It should be noted, however, that we used field-collected biocrust for this experiment, which may behave differently from cultivated biocrust inoculum. Additionally, environmental factors not explored here, such as light availability, temperature, and interactions with vascular plants, will have to be considered when choosing biocrust

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inoculum. Further experimentation with cultivated biocrust inoculum is necessary to determine how cultivated biocrust responds to varying abiotic settings. Taken together, these findings underscore the importance of understanding and manipulating barriers in the abiotic environment that will result in desired biocrust composition and functions.

Biocrust restoration is a globally growing field of research that seeks to use inoculated biocrust to increase soil stability and fertility to rehabilitate degraded soils (Zhao et al. 2016; Antoninka et al. 2020a). Yet, consistently restoring biocrust in degraded regions requires a comprehensive understanding of how abiotic factors influence inoculated biocrust cover and function, similar to what exists for vascular plant seeds (Bach et al. 2010; Hulvey & Aigner, 2014; Shaw et al. 2020). Untangling the influence of environmental conditions on inoculated biocrust can inform a quantitative and comprehensive understanding of the barriers to restoration success and can improve ways to overcome these barriers across restoration sites and time (Bowker et al. 2007; Copeland et al. 2021). This work and continued experimentation exploring ways to achieve the desired biocrust cover and functions are necessary to achieving consistently successful restoration in global drylands.

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Literature Cited

Antoninka A, Bowker MA, Reed SC, Doherty K (2016) Production of greenhouse-grown biocrust mosses and associated cyanobacteria to rehabilitate dryland soil function. *Restoration Ecology* 24(3):324-335

Antoninka A, Faist A, Rodriguez-Caballero E, Young KE, Chaudhary VB, Condon LA, Pyke DA (2020a) Biological soil crusts in ecological restoration: emerging research and perspectives. *Restoration Ecology* 28:S3-S8

Antoninka A, Bowker MA, Barger NN, Belnap J, Giraldo-Silva A, Reed SC, Garcia-Pichel F, Michael C. Duniway MC (2020b) Addressing barriers to improve biocrust colonization and establishment in dryland restoration. *Restoration Ecology* 28:S150-S159

Bach EM, Baer SG, Meyer CK, Six J (2010) Soil texture affects soil microbial and structural recovery during grassland restoration. *Soil Biology and Biochemistry* 42:2182–2191

Bao T, Zhao Y, Gao L, Yang Q, Yang K (2019) Moss-dominated biocrusts improve the structural diversity of underlying soil microbial communities by increasing soil stability and fertility in the Loess Plateau region of China. *European Journal of Soil Biology* 95:103120

Barger NN, Weber B, Garcia-Pichel F, Zaady E, Belnap J (2016) Patterns and controls on nitrogen cycling of biological soil crusts. Pages 257-287 In: Weber B, Budel B, Belnap J (eds) Biological soil crusts: an organizing principle in drylands. Springer, Cham, Switzerland

Belnap J (1993) Soil microstructure in soils of the Colorado Plateau: The role of the cyanobacterium *Microcoleus vaginatus*. *Great Basin Naturalist* 53:40–47

Belnap J (2003) Factors influencing nitrogen fixation and nitrogen release in biological soil crusts. Pages 241-261. In: Belnap J & Lange OL (eds) Biological Soil Crusts: Structure, Function, and Management. Springer-Verlag, Berlin, Germany

Author Manuscript

Belnap J, Büdel B, Lange OL (2003) Biological Soil Crusts: Characteristics and Distribution. Pages 3-30. In: Belnap J & Lange OL (eds) Biological Soil Crusts: Structure, Function, and Management. Springer-Verlag, Berlin, Germany

Belnap J, Phillips SL, Witwicki DL, Miller ME (2008) Visually assessing the level of development and soil surface stability of cyanobacterially dominated biological soil crusts. *Journal of Arid Environments* 72:1257–1264

Belnap J & Büdel B (2016) Biological soil crusts as soil stabilizers. Pages 305-320. In: Weber B, Budel B, Belnap J (eds) Biological soil crusts: an organizing principle in drylands. Springer, Cham, Switzerland

Bethany J, Johnson SL, Garcia-Pichel F (2022). High impact of bacterial predation on cyanobacteria in soil biocrusts. *Nature communications* 13(1):4835

Bowker MA, Belnap J, Davidson DW, Phillips SL (2005) Evidence for micronutrient limitation of biological soil crusts: Importance to arid-lands restoration. *Ecological Applications* 15:1941–1951

Bowker MA, Belnap J, Davidson DW, Goldstein H (2006) Correlates of biological soil crust abundance across a continuum of spatial scales: support for a hierarchical conceptual model. *Journal of Applied Ecology* 43:152–163

Bowker MA (2007) Biological soil crust rehabilitation in theory and practice: an underexploited opportunity. *Restoration Ecology* 15(1):13-23

Bowker MA, Koch GW, Belnap J, Johnson NC (2008) Nutrient availability affects pigment production but not growth in lichens of biological soil crusts. *Soil Biology and Biochemistry* 40:2819–2826

Bowker MA, Maestre FT, Mau RL (2013) Diversity and patch-size distributions of biological soil crusts regulate dryland ecosystem multifunctionality. *Ecosystems* 16:923–933

Bowker MA, Antoninka A, Chuckran P (2020) Improving field success of biocrust rehabilitation

1526100x, 2024, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/rec.14125 by Office Of Scientific And Technical Information, Wiley Online Library on [22/08/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Author Manuscript

materials: hardening the organisms or softening the environment? *Restoration Ecology* 28: S177-S186.

Bowker MA, Doherty KD, Antoninka AJ, Ramsey PW, DuPre ME, Durham RA (2022) Biocrusts influence vascular plant community development, promoting native plant dominance. *Frontiers in Ecology and Evolution* 10:840324

Brady NC, Weil RR (2008) Soil Water: Characteristics and Behavior. Pages 173-217. In: Anthony VR, Kottcamp S (eds) The Nature and Properties of Soils. Prentice-Hall, Upper Saddle River, New Jersey, USA

Brunori F, Penzo MC, Torri D (1989) Soil shear strength: Its measurement and soil detachability. *Catena* 16:59–71

Butterfield BJ, Copeland SM, Munson SM, Roybal CM, Wood TE (2017) Prestoration: using species in restoration that will persist now and into the future. *Restoration Ecology* 25:S155-S163

Chambers JC (2000) Seed movements and seedling fates in disturbed sagebrush steppe ecosystems: implications for restoration. *Ecological Applications* 10(5):1400-1413

Chamizo S, Mugnai G, Rossi F, Certini G, De Philippis R (2018) Cyanobacteria inoculation improves soil stability and fertility on different textured soils: gaining insights for applicability in soil restoration. *Frontiers in Environmental Science* 6:49

Chaudhary VB, Bowker MA, Dell TEO, Grace JB, Redman E, Rillig MC, Johnson NC (2009) Untangling the biological contributions to soil stability in semiarid shrublands. *Ecological Applications* 19: 110–122

Copeland SM, Munson SM, Pilliod DS, Welty JL, Bradford JB, Butterfield BJ (2018) Long-term tren ds in restoration and associated land treatments in the southwestern United States. *Restoration Ecology* 26(2):311-322

1526100x, 2024, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/rec.14125 by Office Of Scientific And Technical Information, Wiley Online Library on [22/08/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Author Manuscript

Copeland SM, Baughman OW, Boyd CS, Davies KW, Kerby J, Kildisheva OA, Svejcar T (2021) Improving restoration success through a precision restoration framework. *Restoration Ecology* 29(2):e13348

Couradeau E, Karaoz U, Lim HC, da Rocha UN, Northen T, Brodie E, Garcia-Pichel F (2016) Bacteria increase arid-land soil surface temperature through the production of sunscreens. *Nature Communications* 7:10373

Cowie AL, Penman TD, Gorissen L, Winslow MD, Lehmann J, Tyrrell TD, Twomlow S, Wilkes A, Lal R, Jones JW, Paulsch A, Kellner K, Akhtar-Schuster M (2011) Towards sustainable land management in the drylands: scientific connections in monitoring and assessing dryland degradation, climate change and biodiversity. *Land Degradation & Development* 22(2):248-260

Delgado-Baquerizo M, Maestre FT, Eldridge DJ, Bowker MA, Ochoa V, Gozalo B, Berdugo M, Val J, Singh BK (2016) Biocrust-forming mosses mitigate the negative impacts of increasing aridity on ecosystem multifunctionality in drylands. *New Phytologist* 209(4):1540-1552

Doherty K, Antoninka A, Bowker MA, Ayuso SV, Johnson NC (2015) A novel approach to cultivate biocrusts for restoration and experimentation. *Ecological Restoration* 33:13–17

Eldridge DJ, Rosentreter R (1999) Morphological groups: A framework for monitoring microphytic crusts in arid landscapes. *Journal of Arid Environments* 41:11–25

Evans ARD, Ehleringer JR (1993) A break in the nitrogen cycle in aridlands? Evidence from δ 15N of soils. *Oecologia* 94:314–317

Faist AM, Antoninka AJ, Belnap J, Bowker MA, Duniway MC, Garcia-Pichel F, Nelson C, Reed SC, Giraldo-Silva A, Sergio Velasco-Ayuso S, Barger NN (2020). Inoculation and habitat amelioration efforts in biological soil crust recovery vary by desert and soil texture. *Restoration Ecology* 28:S96-S105

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Author Manuscript

Fick SE, Day N, Duniway MC, Hoy-Skubik S, Barger NN (2020) Microsite enhancem ents for soil stabilization and rapid biocrust colonization in degraded drylands. *Restoration Ecology* 28:S139-S149

Fischer T, Subbotina M (2014) Climatic and soil texture threshold values for cryptogamic cover development: a meta analysis. *Biologia* 69:1520–1530

Gao L, Bowker MA, Xu M, Sun H, Tuo D, Zhao Y (2017) Biological soil crusts decrease erodibility by modifying inherent soil properties on the Loess Plateau, China. *Soil Biology and Biochemistry* 105:49-58

Garcia-Pichel F ,Loza V, Marusenko Y, Mateo P, Potrafka RM (2013) Temperature drives the continental-scale distribution of key microbes in topsoil communities. *Science* 340:1574–1577

Giraldo-Silva A, Nelson C, Barger NN, Garcia- Pichel F (2019) Nursing biocrusts: isolation, cultivation, and fitness test of indigenous cyanobacteria. *Restoration Ecology* 27(4):793-803

Giraldo-Silva A, Nelson C, Penfold C, Barger NN, Garcia- Pichel F (2020) Effect of preconditioning to the soil environment on the performance of 20 cyanobacterial strains used as inoculum for biocrust restoration. *Restoration Ecology* 28:S187-S193

Hardy RWF, Holsten RD, Jackson EK (1968) The acetylene-ethylene assay for N2 fixation: laboratory and field evaluation. *Plant Physiology* 43:1185–1207

Housman DC, Powers HH, Collins AD, Belnap J (2006) Carbon and nitrogen fixation differ between successional stages of biological soil crusts in the Colorado Plateau and Chihuahuan Desert. *Journal of Arid Environments* 66:620–634

Hulvey KB, Aigner PA (2014) Using filter-based community assembly models to impro ve restoration outcomes. *Journal of Applied Ecology* 51(4):997-1005

Kidron GJ, Xiao B (2023) A false paradigm? Do biocrust types necessarily reflect 'successional stages'?

15261100x, 2024, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/rec.14125 by Office Of Scientific And Technical Information, Wiley Online Library on [22/08/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

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Lalley JS, Viles HA, Copeman N, Cowley C (2006) The influence of multi-scale environmental variables on the distribution of terricolous lichens in a fog desert. *Journal of Vegetation Science* 17(6):831-838

Mallen-Cooper M, Bowker MA, Antoninka AJ, Eldridge DJ (2020) A practical guide to measuring functional indicators and traits in biocrusts. *Restoration Ecology* 28:S56-S66

Maestre FT, Castillo-Monroy AP, Bowker MA, Ochoa-Hueso R (2012) Species richness effects on ecosystem multifunctionality depend on evenness, composition and spatial pattern. *Journal of Ecology* 100:317–330

McHugh TA, Morrissey EM, Mueller RC, Gallegos-Graves LV, Kuske CR, Reed SC (2017) Bacterial, fungal, and plant communities exhibit no biomass or compositional response to two years of simulated nitrogen deposition in a semiarid grassland. *Environmental Microbiology* 19:1600–1611

Moreno-Jiménez E, Ochoa-Hueso R, Plaza C, Aceña-Heras S, Flagmeier M, Elouali FZ, VOchoa V, Gozalo B, Lázaro R, Maestre FT (2020) Biocrusts buffer against the accumulation of soil metallic nutrients induced by warming and rainfall reduction. *Communications Biology* 3(1):325

Mugnai G, Rossi F, Chamizo S, Adessi A, De Philippis R (2020) The role of grain size and inoculum amount on biocrust formation by *Leptolyngbya ohadii*. *Catena* 184:104248

Ochoa-Hueso R, Delgado-Baquerizo M, Gallardo A, Bowker MA, Maestre FT (2016) Climatic conditions, soil fertility and atmospheric nitrogen deposition largely determine the structure and functioning of microbial communities in biocrust-dominated Mediterranean drylands. *Plant and Soil* 399:271-282

Oldfield S, Olwell P (2015) The right seed in the right place at the right time. *BioScience* 65(10):955-956

Osborne BB, Roybal CM, Reibold R, Collier CD, Geiger E, Phillips ML, Weintraub MN, Reed SC

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1526100x, 2024, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/rec.14125 by Office Of Scientific And Technical Information, Wiley Online Library on [22/08/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

(2022) Biogeochemical and ecosystem properties in three adjacent semi-arid grasslands are resistant to nitrogen deposition but sensitive to edaphic variability. *Journal of Ecology* 110(7):1615-1631

Pietrasiak N, Johansen JR, Drenovsky RE (2011) Geologic composition influences distribution of microbiotic crusts in the Mojave and Colorado Deserts at the regional scale. *Soil Biology and Biochemistry* 43:967–974

Prăvălie R (2016) Drylands extent and environmental issues. A global approach. *Earth-Science Reviews* 161:259-278

Reed SC, Seastedt TR, Mann CM, Suding KN, Townsend AR, Cherwin KL (2007) Phosphorus fertilization stimulates nitrogen fixation and increases inorganic nitrogen concentrations in a restored prairie. *Applied Soil Ecology* 36:238–242

Reynolds JF, Smith DMS, Lambin EF, Turner BL, Mortimore M, Batterbury SP, Downing TE, Dowlatabadi H, Fernández RJ, Herrick JE, Huber-Sannwald E (2007) Global desertification: building a science for dryland development. *Science* 316(5826):847-851

Reski R, Abel WO (1985) Induction of budding on chloronemata and caulonemata of the moss, Physcomitrella patens, using isopentenyladenine. *Planta* 165:354–358

Román JR, Roncero-Ramos B, Chamizo S, Rodríguez- Caballero E, Cantón Y (2018) Restoring soil functions by means of cyanobacteria inoculation: importance of soil conditions and species selection. *Land Degradation & Development* 29(9):3184-3193

Roncero-Ramos B, Román JR, Acién G, Cantón Y (2022) Towards large scale biocrust restoration: Producing an efficient and low-cost inoculum of N-fixing cyanobacteria. *Science of The Total Environment* 848:157704

Rosentreter R, Bowker M, Belnap J (2007) A Field Guide to Biological Soil Crusts of Western U.S.

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15261100x, 2024, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/rec.14125 by Office Of Scientific And Technical Information, Wiley Online Library on [22/08/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Drylands. Bureau of Land Management. U.S. Government Printing Office, Denver, Colorado

Rosentreter R (2020) Biocrust lichen and moss species most suitable for restoration projects. *Restoration Ecology* 28:S67-S74

Rozenstein O, Zaady E, Katra I, Karnieli A, Adamowski J, Yizhaq H (2014) The effect of sand grain size on the development of cyanobacterial biocrusts. *Aeolian Research* 15:217–226

Shackelford N, Paterno GB, Winkler DE, Erickson TE, Leger EA, Svejcar LN, Breed MF, Faist AM, Harrison PA, Curran MF, Guo Q (2021) Drivers of seedling establishment success in dryland restoration efforts. *Nature Ecology & Evolution* 5(9):1283-1290

Schultz NL, Sluiter IR, Allen GG, Machado-de-Lima NM, Muñoz-Rojas M (2022) Biocrust amendments to topsoils facilitate biocrust restoration in a post-mining arid environment. *Frontiers in Microbiology* 13:882673

Shapiro SS, Wilk MB (1965) An analysis of variance test for normality (complete samples). *Biometrika* 52(3/4):591-611

Shaw N, Barak RS, Campbell RE, Kirmer A, Pedrini S, Dixon K, Frischie S (2020) Seed use in the field: delivering seeds for restoration success. *Restoration Ecology* 28:S276-S285

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

Stavi I, Paschalidou A, Kyriazopoulos AP, Halbac-Cotoara-Zamfir R, Siad SM, Suska-Malawska M, Savic D, Roque de Pinho J, Thalheimer L, Williams DS, Hashimshony-Yaffe N (2021) Multidimensional food security nexus in drylands under the slow onset effects of climate change. *Land* 10(12):1350

Temperton VM, Hobbs RJ (2004) The search for ecological assembly rules and its relevance to restoration ecology. Assembly rules and restoration ecology: bridging the gap between theory and

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practice 5:34

Torres-Cruz TJ, Howell AJ, Reibold RH, McHugh TA, Eickhoff MA, Reed SC (2018) Species-specific nitrogenase activity in lichen-dominated biological soil crusts from the Colorado Plateau, USA. *Plant and Soil* 429:113-125

Wang W, Liu Y, Li D, Hu C, Rao B (2009) Feasibility of cyanobacterial inoculation for biological soil crusts formation in desert area. *Soil Biology and Biochemistry* 41(5):926-929

Weber B, Belnap J, Büdel B, Antoninka AJ, Barger NN, Chaudhary VB, Darrouzet-Nardi A, Eldridge DJ, Faist AM, Ferrenberg S, Havrilla CA et al. (2022) What is a biocrust? A refined, contemporary definition for a broadening research community. *Biological Reviews* 97(5):1768-85

Williams JR, Renard KG, Dyke PT (1983) EPIC: A new method for assessing erosion's effect on soil productivity. *Journal of Soil and Water Conservation* 38(5)381-383

Wang W, Liu Y, Li D, Hu C, Rao B (2009) Feasibility of cyanobacterial inoculation for biological soil crusts formation in desert area. *Soil Biology & Biochemistry* 41:926-929

Wu Y, Rao B, Wu P, Liu Y, Li G, Li D (2013) Development of artificially induced biological soil crusts in fields and their effects on topsoil. *Plant and Soil* 370:115-124

Xiao B, Zhao Y, Wang Q, Li C (2015) Development of artificial moss-dominated biological soil crusts and their effects on runoff and soil water content in a semi-arid environment. *Journal of Arid Environments* 117:75-83

Xiao B, Veste M (2017) Moss-dominated biocrusts increase soil microbial abundance and community diversity and improve soil fertility in semi-arid climates on the Loess Plateau of China. *Applied Soil Ecology* 117:165-177

Young KE, Bowker MA, Reed SC, Duniway MC, Belnap J (2019) Temporal and abiotic fluctuations may be preventing successful rehabilitation of soil-stabilizing biocrust communities. *Ecological*

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Applications 29:1908

Zaady E, Bouskila A (2002) Lizard burrows association with successional stages of biological soil crusts in an arid sandy region. *Journal of Arid Environments* 50:235–246

Zhao Y, Bowker MA, Zhang Y, Zaady E (2016) Enhanced recovery of biological soil crusts after disturbance. Pages 499-523. In: Weber B, Budel B, Belnap J (eds) Biological soil crusts: an organizing principle in drylands. Springer, Cham, Switzerland

Zhou X, Zhao Y, Belnap J, Zhang B, Bu C, Zhang Y (2020) Practices of biological soil crust rehabilitation in China: experiences and challenges. *Restoration Ecology* 28:S45-S55

Zhao Y, Zhao Y, Xu W, Wang N (2023a) Soil C: N and C: P ratios positively influence colonization and development of incubated biocrusts in a sandy desert environment. *Land Degradation & Development* 34(17):5171-9

Zhao Y, Xu W, Zhao Y, Pan Y, Su J, Zhang Z (2023b) Micro-topography dominated microhabitat significantly influences colonization and development of incubated-cyanobacteria biocrusts in a harsh sandy desert environment. *Applied Soil Ecology* 188:104874

Zika M, Erb KH (2009) The global loss of net primary production resulting from human-induced soil degradation in drylands. *Ecological Economics* 69(2):310-8

Figures

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15261100x, 2024, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/rec.14125 by Office Of Scientific And Technical Information, Wiley Online Library on [22/08/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

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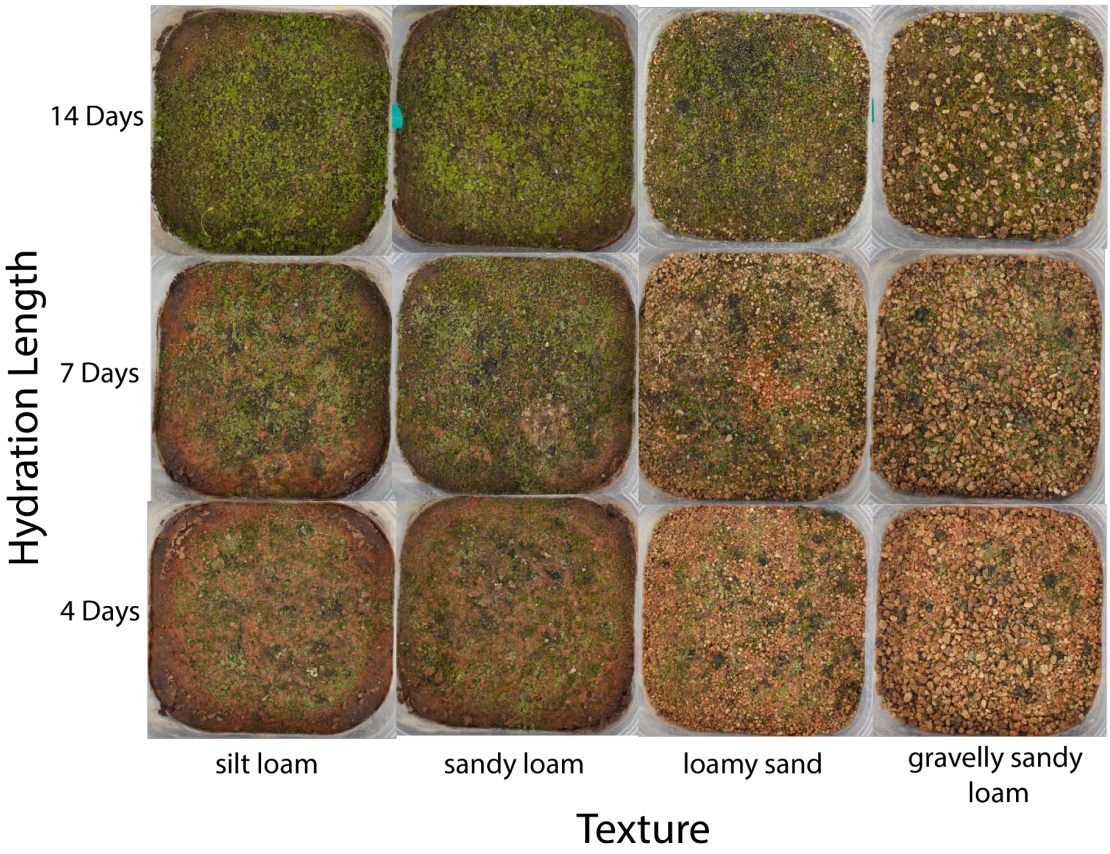

Figure 1. Representative photos showing biocrust growth across different treatments at the final time point of the experiment (4th Month). The different texture classes (silt loam, sandy loam, loamy sand, gravelly sandy loam) are shown along the bottom row and the number of days of continuous hydration (14 days, 7 days, 4 days) are shown with numbers on the left column. Mosses are the mostly green biomass growing in the brown mineral soil matrix, while darker colored biomass is either lichens or darkly-pigmented cyanobacteria.

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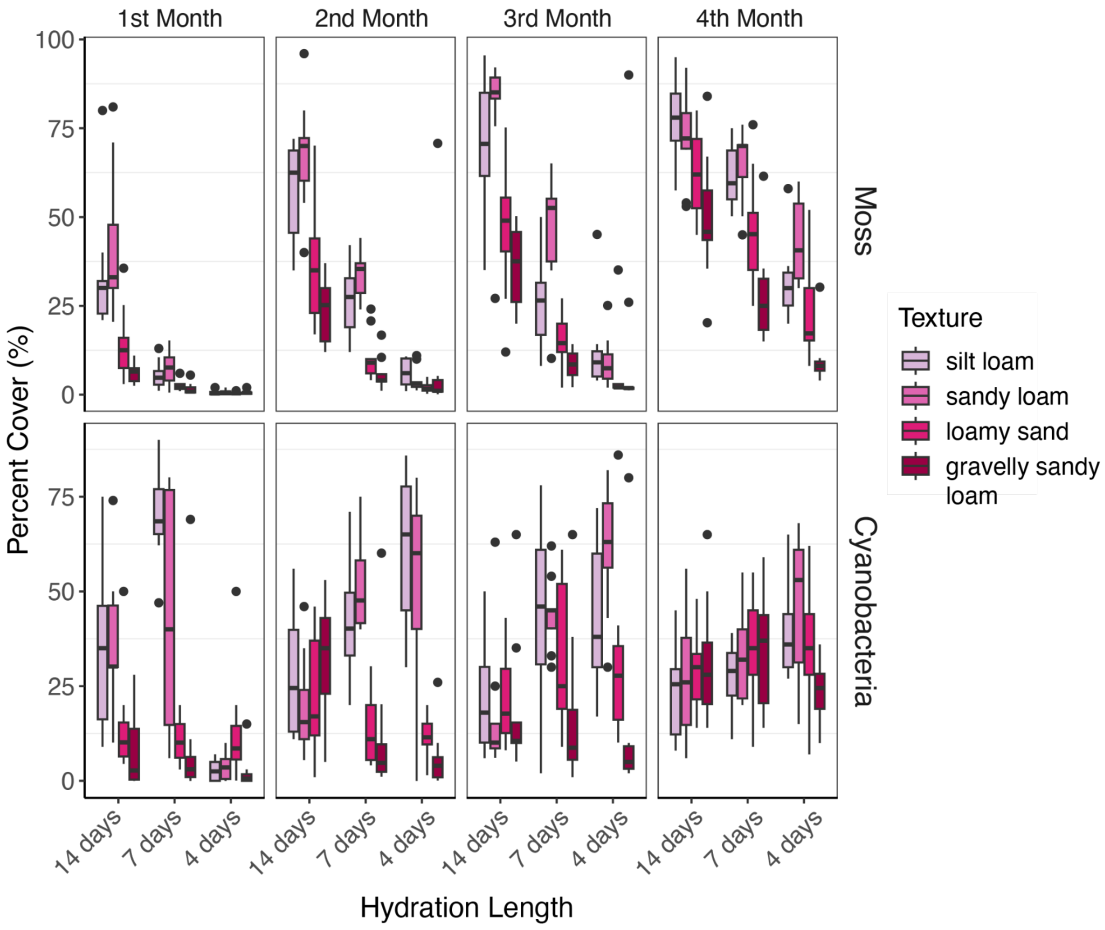

Figure 2: Boxplots showing percent total moss cover (upper panel) and percent total cyanobacteria cover (bottom panel) within different hydration length (14 days hydration, 7 days hydration, and 4 days hydration) and soil textures (silt loam, sandy loam, loamy sand, gravelly sandy loam) over the four measurement time points of the experiment (1st month, 2nd month, 3rd month, 4th month). Hydration length indicates the length of time the biocrusts received water, while soil texture represents the sieved particle size of the different soils the biocrusts were grown on. The fertilization treatment had little effect on cover, and thus is not shown (Table S2, Table S3, Table S4). The box bounds the interquartile range (IQR) divided by the median, and Tukey-style whiskers extend to a maximum of 1.5 × IQR beyond the box. n = 120.

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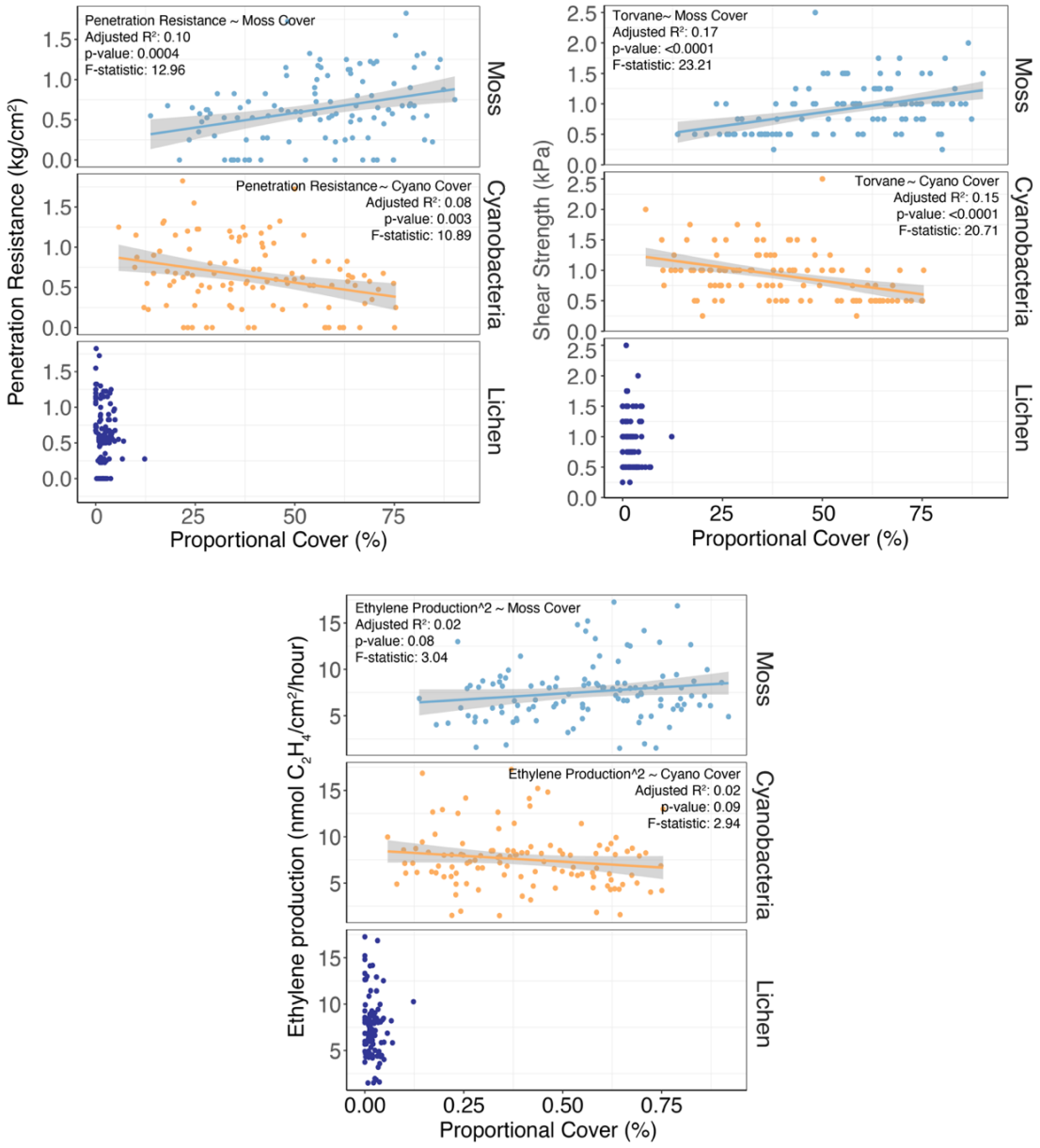

Figure 3. Relationship between proportional cover of biocrust morphological groups (moss, cyanobacteria, and lichen) and different metrics of biocrust function (penetration resistance, shear strength, and nitrogenase activity). A linear model was fit to the data. Lichen morphological group proportional cover had many zeros and did not meet model assumptions, so we did not attempt to fit a model. Grey shading around the lines represents the pointwise 95% confidence interval. The linear model used and the linear model fit are included in panels of each morphological group.

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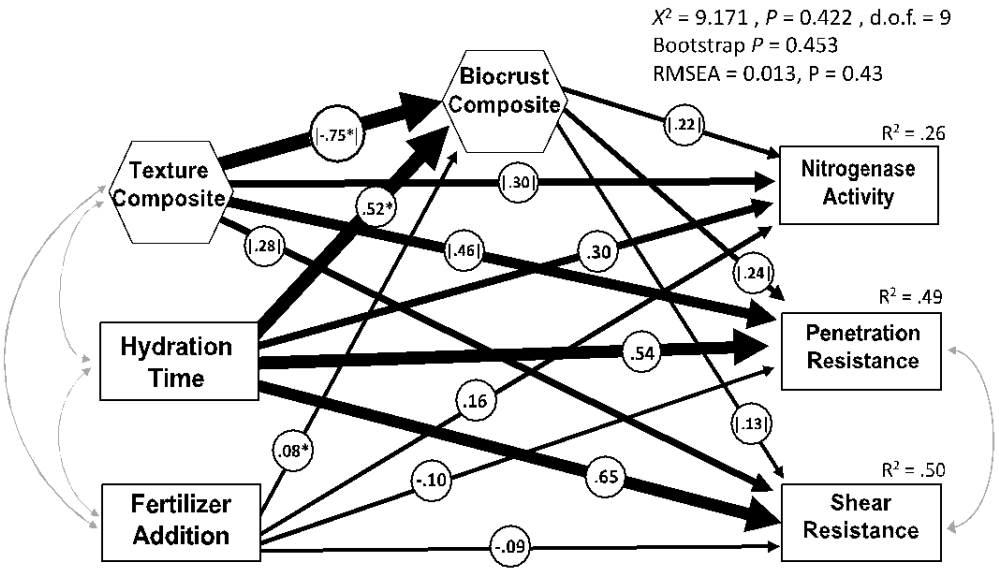

Figure 4: Structural equation model examining the effect of manipulated abiotic factors and a biocrust composite variable on different metrics of function. Numbers on the upper right hand corner of boxes show the R^2 for a response variable. Boxes represent measured variables. Hexagons represent composite variables, used either to pool effects of multiple measured variables, or multiple levels of a categorical predictor upon another variable. The biocrust composite variable is a composite of total biocrust cover, biocrust composition, and filament depth; the texture composite pools the effects of multiple levels of texture. One-way arrows indicate unidirectional, hypothesized causal relationships. Values within white circles indicate standardized path coefficients. Two-way arrows in gray represent covariation between the two variables that are not directional. Arrow width represents the magnitude of the associated path coefficient. Values with a * indicate that treatments had different effects on three different biocrust variables; we chose the largest effect to be portrayed in the figure. The path coefficients for all effects of treatments on biocrust variables are listed in Table S4. The path coefficient of the composited biocrust metrics on function is presented in absolute value, because the sign of this composite is

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uninterpretable. Because the composite effects of texture on other variables are non-linear, the absolute values of path coefficients are presented.

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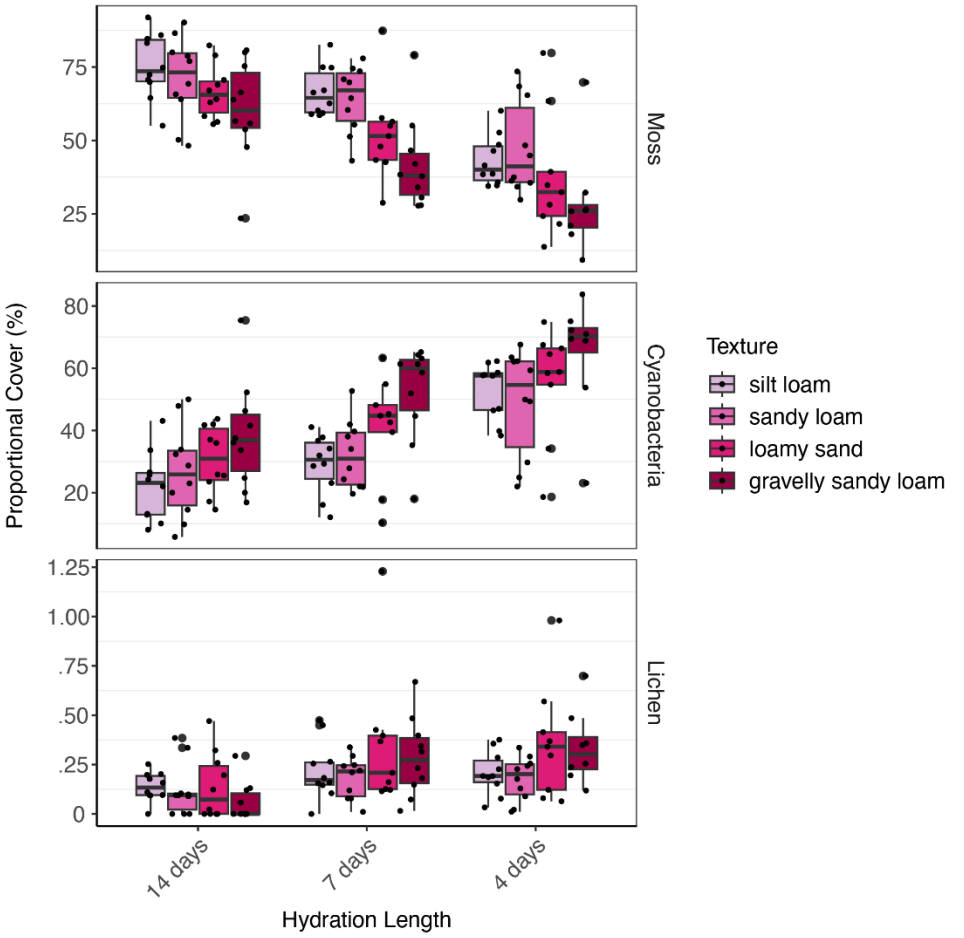

Figure 5. Boxplots showing the proportional covers of the three biocrust types (moss, cyanobacteria, and lichen) after four months of treatments (4th Month) with different hydration length (14 days hydration, 7 days hydration, and 4 days hydration) and texture types (silt loam, sandy loam, loamy sand, gravelly sandy loam). Hydration length indicates the length of time the biocrusts received water, while texture type represents the sieved particle size of the different soils the biocrusts were grown on. The fertilization treatment had little effect on cover at the final time point, and thus is not shown (Table S5). The box bounds the interquartile range (IQR) divided by the median, and Tukey-style whiskers extend to a maximum of 1.5 × IQR beyond the box. Black points are the values from the n = 120 samples. Dots

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represent actual sample values. Full ANOVA model shown in Table S5 and pairwise comparisons shown in Table S7 & Table S8.

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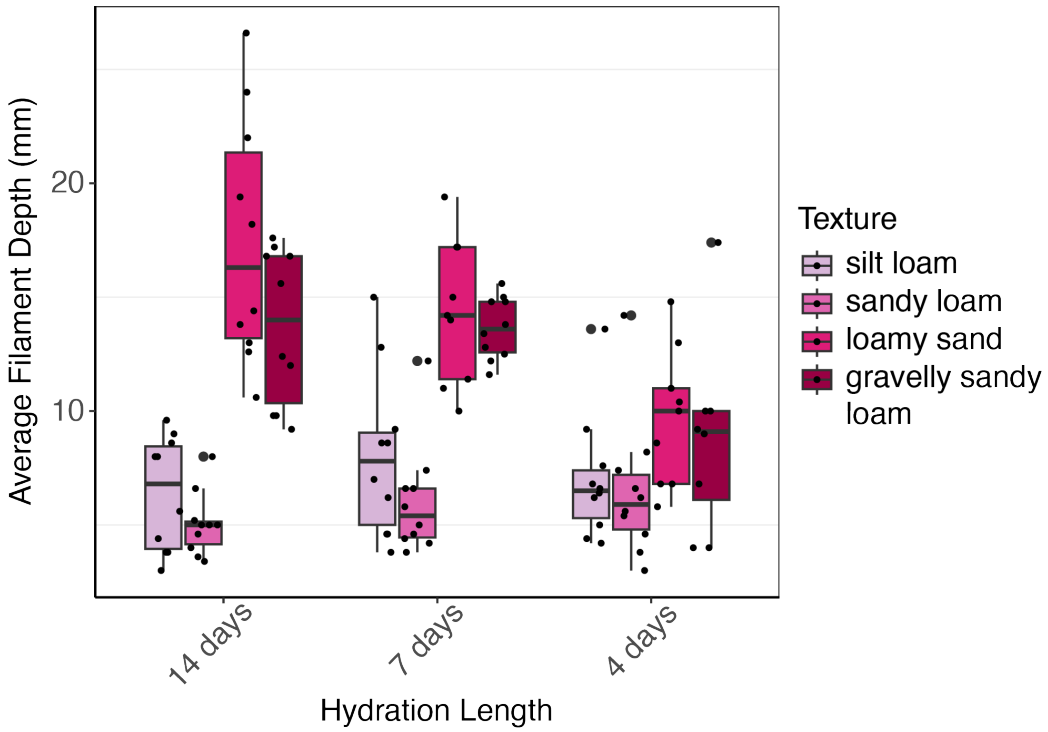

Figure 6. Boxplots showing average filament depth (mm) under the different hydration length (4 days, 7 days, and 14 days) and texture types (silt loam, sandy loam, loamy sand, gravelly sandy loam). Hydration length indicates the length of time the biocrusts received water, while texture type represents the sieved particle size of the different soils the biocrusts were grown on. The fertilization treatment had little effect on filament depth, and thus is not shown (Table S5). The box bounds the interquartile range (IQR) divided by the median, and Tukey-style whiskers extend to a maximum of 1.5 × IQR beyond the box. Black points are the values from the n = 120 samples. Full ANOVA model shown in Table S5 and pairwise comparisons shown in Table S8.

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