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RESEARCH ARTICLE

From farm to field: testing different biocrust cultivation approaches and application techniques in the Sonoran Desert

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Abstract

Drylands are among the most degraded ecosystems globally and are difficult to restore due to limited water availability. Biocrusts are a key component of maintaining soil stability and function in these systems. Since biocrust salvage opportunities are limited, cultivating salvaged biocrusts is a promising method. Previously, biocrusts were cultivated in three different ways: in a greenhouse, in situ layered with shade cloth ("quesadilla"), or in situ with a hoophouse. Our current research objective is to field test methods for establishing the previously cultivated biocrusts. The goals were to (1) compare the efficacy of stabilization treatments for biocrust establishment, (2) test establishment when cultivated biocrusts are transferred with jute compared to scraped-off jute, (3) evaluate field survival and community composition, and (4) investigate plant-biocrust interactions. Psyllium outperformed other stabilization treatments, but all treatments improved biocrust cover compared to the no biocrust control in the first season. Increased cover of biocrusts resulted in higher levels of colonization outside the treated area over time. We found no whole community fungal or bacterial differences across cultivation treatments. Seedling establishment was reduced when applied under biocrust sods but improved under jute without biocrust. Cultivating biocrusts to increase the availability and application of salvaged biocrusts is a promising method for restoration.

Key words: biocrust, citizen science, cultivation, cyanobacteria, lichen, moss, restoration, seeding, Sonoran Desert

Implications for Practice

Adding psyllium with biocrust is a promising treatment for increasing biocrust establishment in the field compared to pellets or watering. Cultivating biocrusts on jute and transferring them to the field as sods has promise for biocrust establishment. Cultivating biocrusts in the field compared to the greenhouse may confer drought resistance and improve field establishment. Separating plant and biocrust restoration in space may help to minimize competition in later stages of establishment. Jute is useful for improving seed and biocrust establishment in the field.

Introduction

Drylands constitute approximately 45% of earth's terrestrial surface, are an important part of the global climate cycle (Prăvălie 2016), and support a disproportionate number of ecosystem services (Reynolds et al. 2007). Erosion, dust, loss of fertility and water holding capacity, and exotic plants impede the recovery of ecosystem function and services (Burrell et al. 2020). Biological soil crusts (biocrusts), a community of mosses, lichens, and cyanobacteria, are a dominant living soil cover that fills spaces between vascular plants and binds the

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top millimeters of soil (Weber et al. 2022). Biocrusts can withstand long periods of drought and high ultraviolet (UV) (Oliver et al. 2005; Adessi et al. 2021) and provide critical ecosystem functions, including binding and stabilizing soil (Belnap & Büdel 2016; Garcia-Pichel et al. 2016), enhancing nutrient capture and fixing C and N (Elbert et al. 2012; Torres-Cruz et al. 2018), influencing soil hydrology (Eldridge et al. 2020), and interacting with vascular plants (Havrilla et al. 2019). In drylands, where land degradation is prevalent, biocrusts are often lost from the system (Rodriguez-Caballero et al. 2018). Because of these factors, there has been a concerted effort to develop methods to use biocrusts alone and in conjunction with plants to restore degraded drylands (Antoninka et al. 2020; Li et al. 2021; Bowker et al. 2022; Malešević et al. 2024).

Cultivating biocrusts for restoration, rather than salvaging and reapplying elsewhere, has been proposed (Tucker et al. 2020). Theoretically, a small amount of inoculum can be expanded to treat larger areas with less disturbance. Various approaches have been tried, including focusing on early colonizers (Giraldo-Silva et al. 2019; Ayuso et al. 2020), assessing impacts of specific functional groups like mosses (Doherty et al. 2015; Antoninka et al. 2016; Bowker et al. 2017), or using whole community approaches (Antoninka et al. 2018; Jech et al. 2023). Challenges include contamination (Bethany et al. 2019) and poor establishment (Antoninka et al. 2018; Faist et al. 2020). Attempts at hardening inoculum by simulating drought or higher UV prior to field application (Antoninka et al. 2018; Giraldo-Silva et al. 2019) or softening the environment by providing shade or irrigation after inoculation (Bowker et al. 2020) have been trialed with mixed success. Alternatively, biocrust can be cultivated in the field, which may allow acclimation to field conditions and long-term establishment (Jech et al. 2023).

Establishment of vascular plants in drylands is critical for restoring ecosystem function (Reynolds et al. 2007) and is similarly challenging (Ramón Vallejo et al. 2012; Hoover et al. 2020). Biocrusts and plants have complex interactions dependent on life stage, plant traits, and resource limitations (Havrilla et al. 2019; Bowker et al. 2022). Most plant restoration studies have been conducted at the germination stage. Evidence suggests that biocrusts may facilitate plant germination and establishment (Li et al. 2002; Ferrenberg et al. 2018; Slate et al. 2019) or have no effect (McIntyre et al. 2021). Alternatively, biocrusts can selectively facilitate some plants (Zhang et al. 2016; Bowker et al. 2022) or exclude plants when biocrusts are mature and intact (e.g. Zhang et al. 2016; Li et al. 2021). These complexities are poorly understood, but seeds germinating in biocrusts may gain access to limiting nutrients and water (Chen et al. 2018; Ferrenberg et al. 2018; Havrilla & Barger 2018).

This project evaluated approaches to reliably establish cultivated biocrusts in the field and how cultivated biocrusts and native seeds interact in a restoration context. Previously, we cultivated local biocrusts either in a greenhouse or Sonoran Desert field conditions with the following layers (bottom to top): weed cloth, sand or soil, jute or no jute, salvaged biocrust, irrigation tubing, and shade cloth either layered directly over the biocrust or hooped over PVC pipe (Antoninka et al. 2024). In the current study, we established three field experiments to test the establishment of these cultivated biocrusts. We tested the effects of different stability treatments (experiment 1 or E1), transfer methods (experiments 2 and 3 or E2–3), and vascular plant seeding (experiment 3 or E3) on biocrust establishment. Our specific objectives were to determine the following: (1) which stability treatments (psyllium, watering, or pellets) work best for establishing cultivated biocrust in the field (E1); (2) if sods improve establishment (E2–3); (3) if differences in initial cultivation method lead to biocrust establishment or community composition differences in the field (E1–3); (4) how well biocrust colonizes adjacent areas (E2–3); and (5) if approaches applied in this study have a facilitative or competitive relationship with plants (E1–3).

Methods

Site Description

Our study site was near Granite Mountain in the northern region of McDowell Sonoran Preserve (henceforth "the Preserve," 33.7707, −111.78996) at the northeastern edge of the Phoenix metropolitan area in Scottsdale, Arizona, U.S.A. The area was chosen as a restoration site based on past recreational disturbance (camping, vehicle use) and low vegetation cover. The site elevation is 776 m with a mixed scrub plant community dominated by *Ericameria laricifolia* and *Parkinsonia microphylla* (Brown et al. 1979). The USDA-NRCS ecological site classification is R040XA120AZ—Clay Loam Hills 10–13″ p.z., with deep and moderately deep soils that formed in clayey alluvium of mixed origins in Sonoran Desert Upland habitat (Soil Survey Staff 2024).

During this study, December 2019–April 2022, the monthly mean temperatures ranged from 11.4 to 33.8°C (PRISM Climate Group 2022). Rainy seasons in the Sonoran Desert occur in winter (October–March) and summer (monsoon, June–September). Rainfall during the study was significantly below average in 2020 and winter 2020–2021 and above average in the 2019–2020 winter and 2021 monsoon (Table 1). In this region, winter rains typically deliver more overall precipitation, but this pattern was not seen in 2021 (Table 1).

Previous Work: Biocrust Salvage and Cultivation

Biocrusts were salvaged in July 2018 before construction of the Fraesfield Trailhead in the Preserve (33.741528, −111.7882963), which is 4.2 km from the study site with the same classification (R040XA120AZ), mixed with R040XA114AZ—Loamy Upland 10–13″ p.z. The Fraesfield Trailhead plant community is a mix of *Ambrosia deltoidei–Parkinsonia microphylla*-mixed scrub association and *Ericameria laricifolia–Parkinsonia microphylla*. We cultivated salvaged biocrusts at Scottsdale Community College as a fully replicated experiment reported in Antoninka et al. (2024). Briefly, biocrusts were grown with the following layers on each 0.25 m^2 plot: (1) synthetic light gray landscape fabric, (2) 1 L of sand or native soil substrate, (3) 0.25 m^2 jute

Table 1. Site precipitation and temperature (*t*) normal and study period means. Data were downloaded from PRISM, May 2024 (PRISM Climate Group 2022). Winter months include October–December of the previous year and January–March of the year labeled in the first column. Summer months include April–September.

			Seasonal Precipitation (mm)				
Year	*Annual Precipitation (mm)*	*Deviation from Normal*	*Winter*	*Summer*	t_{min} (°C)	t_{mean} (°C)	t_{max} (°C)
1991–2020 Normals	*328.32*		*221.44*	*106.9*	*14.7*	*21.2*	*27.7*
2020	191.95	−136.37	279.64	27.66	15.4	22.3	29.1
2021	426.23	97.91	70.9	269.96	15.6	21.8	28
2022	280.94	−47.38	144.66		15.6	21.7	27.9

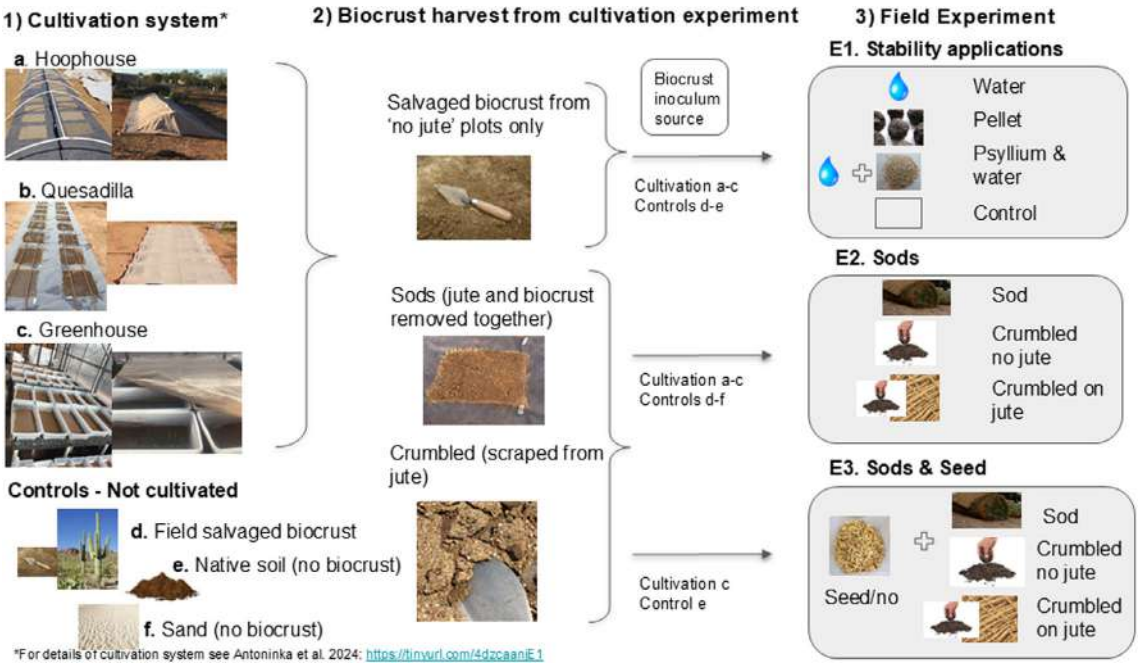

Figure 1. Conceptual diagram of the three field experiments shown in three stages as follows. (1) Cultivation system: In a previous experiment (Antoninka et al. 2024), we grew biocrusts on layers of weedcloth, jute or no jute, sand or native soil, inoculated with biocrust, then (A) covered with shadecloth in a hoophouse, (B) covered flat with shadecloth (quesadilla), (C) covered with shadecloth in a greenhouse. For this experiment, we also collected (D) field-salvaged biocrust as a positive control or (E) soil with no biocrust as a negative control. (2) Biocrust harvest from the cultivation experiment: Biocrusts were harvested from the three cultivation systems with different methods. For Experiment 1, we salvaged biocrust from all plots in the three cultivation systems that were grown on native soil and no jute. For Experiments 2 and 3, we harvested biocrust grown on jute with native soil from all three cultivation systems by either scraping from the jute (crumbled) or removing the jute with the biocrust as a sod. In Experiment 2, we also harvested biocrust grown on jute with sand from the quesadilla cultivation system then scraped or removed the biocrusts as sods. (3) Field experiments: In Experiment 1, we applied the salvaged biocrust from all cultivation systems and both controls (d, e) with either (1) psyllium and water, (2) water alone, (3) added the crumbled biocrust in pelletized form, or (4) no additives. In Experiment 2, we applied all cultivated biocrusts harvested in the previous step plus the three controls (d-f) into plots on new jute, on no jute, or as sods. In Experiment 3, we added the seed treatment (seed/no seed) first, then added either the sods or the crumbled or control biocrust on jute or no jute, using only greenhouse cultivated biocrust and native soil control (e). For specific cultivation treatments used in Experiments 2 and 3, see Table 2.

material with burlap weave (hereby jute) or no jute, and (4) biocrust inoculum spread at a density of 20% cover. These layers were grown in three cultivation systems: (1) "*Quesadilla*" or layered method, with fabric on the bottom, shade cloth on top, a filling of soil and biocrust, and inline drip irrigation applied directly on the surface; (2) "*Hoophouse*" method, with microjet spray irrigation above the biocrust and a shade structure created from 0.5-inch PVC pipe bent to a 1 m height and covered by shade cloth; and (3) greenhouse method with a nested basin irrigation system covered by shade cloth (see diagram, Fig. 1). The cultivation ran for 11 weeks (January–April 2019). Field-salvaged biocrust not used in the cultivation experiment was stored in dark, ambient temperatures.

Harvesting the Cultivated Biocrust

We harvested cultivated biocrust from plots with no jute using the same approach as the original salvage by removing only the top 0.5-cm layer of biocrust with cement trowels and passing it through a 0.5-cm sieve. This biocrust was mixed within cultivation type (greenhouse, hoophouse, or quesadilla). We

Table 2. Treatments included in experiments 2 and 3. Column 2 specifies the source of the biocrust for this experiment: either the original cultivation method from the previous experiment or controls (not cultivated, "none"). Column 3 indicates whether the cultivated biocrusts were sod (biocrust transferred with the jute on which it was grown) or crumbled (scraped off the jute) or controls (positive biocrust control salvaged from the field or a negative control of native soil or sand without biocrusts). The biocrust-free controls included a total of 450 mL of native soil or sand to mimic the original amount of soil (360 mL) and biocrust inoculum (90 mL) applied in the prior cultivation experiment (Antoninka et al. 2024). Column 4 indicates whether inoculum was placed as sod or on new jute (jute) or not on jute (no jute) in the field plots. Column 5 refers to the previous cultivation experiment and indicates whether the sods or crumbles were grown on a layer of native soil or sand. Column 6 refers to whether the plots were seeded and column 7 indicates the number of plots of each treatment.

Experiment	*Original Cultivation Method*	*Biocrust Type and Amount (mL)*	*Jute*	*Cultivation Base*	*Seeded*	n
2	None	Field salvage 550	Jute	NA	No	5
2	None	Field salvage 550	No jute	NA	No	5
2	Greenhouse	Sod	Jute	Native soil	No	5
2	Hoop	Crumbled 550	Jute	Native soil	No	5
2	Hoop	Crumbled 550	No jute	Native soil	No	5
2	Hoop	Sod	Jute	Native soil	No	5
2	Quesadilla	Crumbled 600	Jute	Native soil	No	5
2	Quesadilla	Crumbled 500	Jute	Sand	No	5
2	Quesadilla	Crumbled 600	No jute	Native soil	No	5
2	Quesadilla	Crumbled 500	No jute	Sand	No	5
2	Quesadilla	Sod	Jute	Native soil	No	5
2	Quesadilla	Sod	Jute	Sand	No	5
2	None	No biocrust control, native soil 450	No jute	NA	No	5
2	None	No biocrust control, sand 450	No jute	NA	No	5
3	Greenhouse	Crumbled 600	Jute	Native soil	Seed	5
3	Greenhouse	Crumbled 600	No jute	Native soil	Seed	5
3	Greenhouse	Sod	Jute	Native soil	Seed	5
3	None	No biocrust control, native soil 450	Jute	NA	Seed	5
3	None	No biocrust control, native soil 450	No jute	NA	Seed	5
3	None	No biocrust control, native soil 450	No jute	NA	No	5

harvested biocrust grown on jute by randomly assigning 15 jute treatment plots within cultivation type to one of two harvest methods: five by separating the "sod" (jute and biocrust together) from the underlying sand or native soil and 10 by scraping the biocrust off the jute ("crumbled") and mixing it within cultivation type.

Study Design and Treatments

We established three experiments (E1–E3) to compare cultivated biocrust establishment. Specific objectives were as follows: E1 (Stability Applications) compared additives for securing biocrusts to the soil surface; E2 (Sods) compared field establishment of biocrust applied with jute (sod) or scraped from jute (crumbled); and E3 (Sods and Seed) explored adding biocrust and seed together (Fig. 1). For all experiments, we compared divergence among fungal and bacterial community composition between the cultivated and field-salvaged crusts.

E1 Stability Applications had a full factorial randomized complete block design with the following treatments: five levels of biocrust inoculum all collected or cultivated on native soil (no biocrust control, field-salvaged control, greenhouse cultivated, hoophouse cultivated, quesadilla cultivated) applied at a rate of 100 mL per plot crossed with four levels of additives (psyllium and water, pelletized biocrust, water alone, no additive control). We expected that broadcasting biocrust with psyllium would stabilize the biocrust and that pelletizing biocrust would protect the organisms, similar to the benefits of seed pellets (Gornish et al. 2019). We randomly assigned treatments to twenty 30 × 30–cm plots within six replicate blocks with a minimum of 30 cm distance between plots (120 plots total). The control inoculum was native soil without biocrusts collected from the same area as the original salvaged soil. For the psyllium treatment, we mixed 15 g psyllium with biocrust inoculum and watered for 1 minute with a backpack sprayer. The water treatment was similarly applied, with biocrust crumbles sprinkled on the ground and then watered with a backpack sprayer for 1 minute. Biocrust pellets for each plot consisted of 100 mL of the harvested, sieved, and mixed cultivated biocrusts, 100 mL of diatomaceous earth, mixed with water to make 2–10 mm diameter pellets, and air dried. The control included only biocrust inoculum (Fig. 1).

Experiments 2 and 3 were designed to assess transfer of cultivated biocrusts on jute ("sod"), crumbled and added to new jute ("crumbled on jute"), or no jute ("crumbled no jute"; Fig. 1). In E3, we seeded eight species of native grasses and forbs (Armenta Seed Company, Gilbert, Arizona, U.S.A.; Table 3). We marked 100 - 50 × 50–cm plots (70 for E2; 30 for E3) on disturbed, unvegetated soil, ensuring a minimum distance of 50 cm between plots. In the center 30 × 30 cm area of each plot, we applied biocrust crumbled on jute, crumbled no jute, or sod and marked the inner plot with corner nails (see Table 2 for the full list of treatments for E2 and E3). The treatments were not fully crossed due to limited harvested biocrust. The amount of crumbled biocrust inoculum applied per plot differed among cultivation type due to differential growth rates by cultivation method (Antoninka et al. 2024). However, we standardized the application rate to the amount of

Table 3. Seeded species list, descriptions, and rates. Seeding rate for each species was based on the higher end of the recommended rate by seed producers and adjusted by purity from the label. Species in bold established in more than one plot. Growth forms were as follows: G = grass, S = shrub, F = forb. Seedling number is the total number of seedlings established over the course of the study.

Species Name	*Common Name and Growth Form*	*Recommended Seeding Rate (lb/Acre)*	*Purity*	*Adj. Seed Rate (lb/Acre)/Purity)*	*Seed Weight (g)*	*Seedling Number*
Bouteloua curtipendula	Sideoats grama (G)	9	0.964	9.333	4.193	0
Encelia farinosa var. farinosa	Brittlebush (S)	2	0.857	2.333	1.048	1
Aristida purpurea var. purpurea	Purple threeawn (G)	6	0.913	6.574	2.954	0
Plantago ovata	Desert indianwheat (F)	10	0.982	10.181	4.574	33
Senna covesii	Coues' senna (L)	14	0.999	14.01	6.295	72
Calliandra eriophylla	Fairyduster (L)	5	0.999	5.001	2.247	0
Bouteloua barbata var. rothrockii	Rothrock Grama (G)	2	0.944	2.118	0.952	364
Bouteloua aristidoides	Needle grama (G)	3	0.982	3.055	1.373	12

biocrust grown on one plot in the cultivation experiment to be comparable with sods within cultivation type by dividing the amount of biocrust harvested within cultivation type by the number of plots harvested.

For E3, plots were seeded first before applying jute and biocrust treatments at species rates recommended by the seed company (Table 3). For both E2 and E3, we monitored the outer plot (the area between the inner plot and the outside 50 × 50 cm plot) to assess biocrust colonization.

Measurements

We estimated percent biocrust cover of each unit in each experiment at installation and in April 2020, April 2021, October 2021, and April 2022 using a point intercept method. We recorded the ground cover underlying each point on a 30 × 30 cm gridded frame as cyanobacteria, moss, lichen, plant, bare ground, or rock cover at 25 points per plot. Any biocrust taxa not intercepted but present in the plot was recorded as an additional 0.25 point. We converted the points to estimated percent cover by subtracting the number of rock points from the total number of points to equal available habitat points. Biocrust (cyanobacteria, moss, and lichen) points were summed and divided by the number of available habitat points and multiplied by 100 to estimate biocrust cover percentage. Biocrust richness was calculated as the number of biocrust taxa (cyanobacteria, moss, and lichen) in each plot. We visually estimated plant cover in all plots to provide a better estimate of plant influence compared with the point intercept data using the following cover classes: 0%, 0–0.1%, 0.1–1%, 1–2%, 2–5%, 5–10%, 10–25%, 25–50%, 50–75%, 75–95%, greater than 95% (Peet et al. 1998).

In the outer plots of E2 and E3, we used the same point intercept approach at 25 points in a 50 × 50 cm gridded frame, with a set number of points on the top, bottom, and sides of the inner plot. We estimated plant cover separately for inner and outer plots using the cover classes described above. For E3, we counted the number of individual seedlings of each seeded species that emerged in the inner and outer plots and summed the number of individual seedlings from all seeded species that established in more than one plot.

Biocrust Community Composition: Molecular Sampling and Processing

DNA Extraction. To assess biocrust community composition, we extracted DNA from a subset of plots, across a crust cover gradient from all experiments. We randomly selected one plot in each of eight classes of total biocrust cover (0%, 1–5%, 6–10%, 11–15%, 16–25%, 26–50%, 51–75%, 75–100%) within two biocrust cultivation treatments (greenhouse, quesadilla) and two controls (no biocrust native soil, field salvaged) for a total of 32 plots. We collected and aggregated 1–2 g of biocrust and subsoil to 2 cm deep with a spoon from within each corner of the plot. Soil was stored in sterile specimen containers at room temperature as these are all from surface dry soil. The DNeasy® PowerSoil® Pro kit was used to extract DNA twice from 250 mg of homogenous soil and crust sample following the manufacturer's protocol (QIAGEN, Valencia, CA, U.S.A.). DNA was stored in a −20°C freezer.

Amplicon Sequencing. To examine the fungal and bacterial communities, Illumina-based amplicon sequencing was performed on fungal and bacterial specific targets. We used fungal-specific ribosomal DNA primers targeting the ITS2 region of the genome to examine differences in fungal communities as described in Taylor et al. (2016). Briefly, two rounds of amplification generated the amplicon pools for sequencing using locus-specific PCR primers with a 5′ universal tail (5′-CCTA TGTGGAGAGCCAGTAAGCGATGCTATGGT-AACTTTYR RCAAYGGATCWCT-3′, 5′ GTCAACGCTCACTACTGCGA TTACCCAAGTCAG-AGCCTCCGCTTATTGATATGCTTA ART-3′) in 25 µL PCR reactions containing 1 × Phusion Green Hot Start II High-Fidelity PCR Master Mix (Thermo Fisher Scientific, Inc., Waltham, MA, U.S.A.), 3.0 mM $MgCl_2$, 200 nM each primer, and 2 µL each normalized DNA sample or 5 µL each dilute DNA sample. Cycling conditions were 95°C for 2 minutes, 35 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 60°C for 4 minutes. We checked PCR products on a 1% agarose gel, purified by bead-prep, and diluted tenfold. Indexing sequences were added in the second round of amplification using the following primers: 5′-AATGATACGG CGACCACCGAGATCTACAC-NNNNNNNN-CCTATGTG GAGAGCCAGTAA-3′, 5′-CAAGCAGAAGACGGCATA

CGAGAT-NNNNNNNN-GTCAACGCTCACTACTGCGA-3′.-PCR conditions were as above. Pooled sequences were run on MiSeq Desktop Sequencer (Illumina, Inc., San Diego, CA, U.S.A.) in 2 × 300 bp mode. To examine the bacterial communities, we used the 515F and 806R primers to amplify the bacterial specific 16s ribosomal RNA Variable region 4, as described in Caporaso et al. (2011). Briefly, PCR reactions were completed in 25 µL total volume, which included 10 µM of each primer, 1 µL of genomic DNA, 12.5 µL of Taq 2X DNA Polymerase (Thermo Fisher Scientific Inc., Waltham, MA, U.S.A.) and 9.5 µL of nuclease-free water (Sigma-Aldrich, St Louis, MO, U.S.A.). PCR conditions were as follows: initial denaturation at 93°C for 3 minutes; 35 cycles of denaturation at 95°C for 45 seconds, annealing at 50°C for 1 minute, extension at 72°C for 90 seconds, and a final extension at 72°C for 10 minutes. We processed and sequenced the pools as above without the second PCR amplification.

Amplicon Data Processing. We demultiplexed ITS2 samples in Quantitative Insights Into Microbial Ecology 2 v2023.7 (Bolyen et al. 2019), requiring 95% of each read to have a minimum *q*-score of 20 with no exceptions (*-q* 19 *-r* 0 *-p* 0.95). We trimmed forward reads of primer sequence, followed by quality control filtering by DADA2 (Callahan et al. 2016), and screened demultiplexed, trimmed sequences for chimeras using VSEARCH (Rognes et al. 2016) and screened for fungal ITS2 sequences with ITSx (Bengtsson-Palme et al. 2013). We assigned operational taxonomic units (OTUs) taxonomy using BLAST (Van Nguyen & Lavenier 2009) against the UNITE reference database (Hibbett et al. 2016). We used the same bioinformatic procedure for 16S sequences; however, we assigned taxonomy against the SILVA reference database (Quast et al. 2012).

Statistics

E1 Stability Applications. We analyzed E1 data with a linear mixed effects model (Mixed Model personality in JMP) with the following fixed effects: inoculum source (five levels: greenhouse, hoop, quesadilla, field, control), stability (water, psyllium, pellet, and control), and season (spring 2020, spring 2021, fall 2021, spring 2022) in a full factorial. The response variables included total biocrust cover, richness, and plant cover. Plot was included as the random effect variable to account for repeated measures. We included plant cover as a covariate in the biocrust models and biocrust cover as a covariate in the plant model to account for competition. For the final models, the three-way interaction was removed if not significant. If a covariate was significant, we added an interaction term of season and examined post hoc differences. We checked model assumptions with residuals. Biocrust and plant cover were square root transformed to meet assumptions of homoscedasticity. Analyses were completed with and without five outlier plots with no plant cover with no change in model interpretation, so we retained outliers in the final model.

E2 Sods. We analyzed E2 treatments as combinations (14 levels of combined treatment, 5 replicates, Table 2) because the use of sods made it difficult to separate individual treatment factors. Using combinations allowed for treatment comparisons because the treatments were not fully crossed due to limitations in the cultivated crust. E2 data were analyzed using the same mixed model with treatment and season and their interaction as the fixed factors and inner plot biocrust cover and richness and inner plot plant cover as response variables. Baseline total biocrust cover and total plant cover were fixed factor covariates to control for initial variation in biocrust level and plant competition in the biocrust model. We included inner plot biocrust in the plant model as a covariate to account for competition and different initial biocrust levels. We included plot as the random factor to control for repeated measures across seasons.

To determine whether the initial application of sand or native soil in the cultivation stage led to differences in biocrust cover in the field, we applied contrast statements between native soil and sand to the quesadilla treatments within sod, crumbled on jute, and crumbled no jute (three separate statements) for the first and last seasons.

To test whether biocrust colonization of outer plots differed by inner plot treatment, we used the same model as above with a full factorial of treatment, inner biocrust cover, and season with outer plot plant cover as a covariate, outer biocrust cover as the response variable, and plot as a random factor to control for repeated measurements. The three-way interaction was removed if not significant. We checked model assumptions with residuals; inner and outer biocrust cover were square root transformed to normalize the data.

E3 Sods and Seed. In E3, we tested six levels of treatments for field application: biocrust cultivated in the greenhouse, sod versus crumbled on jute and crumbled no jute, combined with seeding (Table 2). Models in E2 Sods were applied to E3 Sods and Seed to analyze differences in inner plot biocrust cover, richness, and plant cover and the colonization of outer plot biocrust cover. The sum of seeded individuals was analyzed using a mixed model with treatment and season as a full factorial and inner plot plant cover and biocrust cover as covariate fixed factors. Model assumptions were checked with residuals, and the seeded individuals and inner plot biocrust cover were square root transformed to normalize the data. All above analyses were conducted in JMP 18.0, and Tukey HSD was used to analyze post hoc differences among significant effects for categorical variables (JMP 18.0).

Molecular Biocrust Community Composition. OTU and taxonomy tables were analyzed using R (R Core Team 2014). Microbial community data were analyzed using the Phyloseq R package version 3.5.1 (McMurdie & Holmes 2013). Fungal and bacterial data were rarefied to the lowest coverage depth sample (2,111 and 11,756 reads, respectively) coverage. OTU richness and Shannon diversity index were used to calculate alpha diversity, and the Jaccard distance matrix was used for beta diversity between treatment groups. Differences in alpha diversity were evaluated with ANOVA followed by a Tukey post hoc pairwise

multiple comparison. Differences in beta diversity were evaluated with PERMANOVA followed by post hoc pairwise PERMANOVA tests using the Jaccard dissimilarity distance matrix, which accounted for effects of low abundance taxa on beta diversity between treatments as it is calculated from taxa presence/absence, not abundance. Nonmetric multidimensional scaling (NMDS) ordination plots were created to visualize microbial community dissimilarities between treatments.

Results

E1 Stability Applications

For the biocrust cover model, differences in biocrust cover among stability treatments depended on the season (stability × season: $F_{[9,330.5]} = 5.69$, $p < 0.0001$; Table S1). Biocrust cover was highest in the psyllium treated plots in the first season, but after the 2020 drought year, biocrust cover was not different across the stability treatments (Fig. 2B; Table S2). Biocrust richness did not differ by stability treatment (Table S2).

Biocrust cover and richness differed by cultivation method, modified by season (Fig. 2A & 2C; Table S1). In the first season, all cultivated biocrust and field biocrust had more cover and higher richness than the control. However, in the second year, after two seasons of drought (monsoon and winter drought), only the field-salvaged and hoophouse cultivated plots had higher biocrust cover than the controls without biocrust (Fig. 2A; Table S3). In fall 2021, all biocrust treatments had higher biocrust cover than the no biocrust control (Fig. 2A; Table S3), but only greenhouse and hoophouse cultivated plots had higher biocrust richness than controls (Fig. 2C; Table S4). The field, hoophouse, and greenhouse cultivated biocrust had higher richness than controls without biocrust, and biocrust richness was higher in field-salvaged plots compared with quesadilla cultivated plots (Fig. 2C; Table S4). In spring 2022, differences between treatments and the controls disappeared for both biocrust richness and cover (Fig. 2A & 2C; Tables S3 & S4).

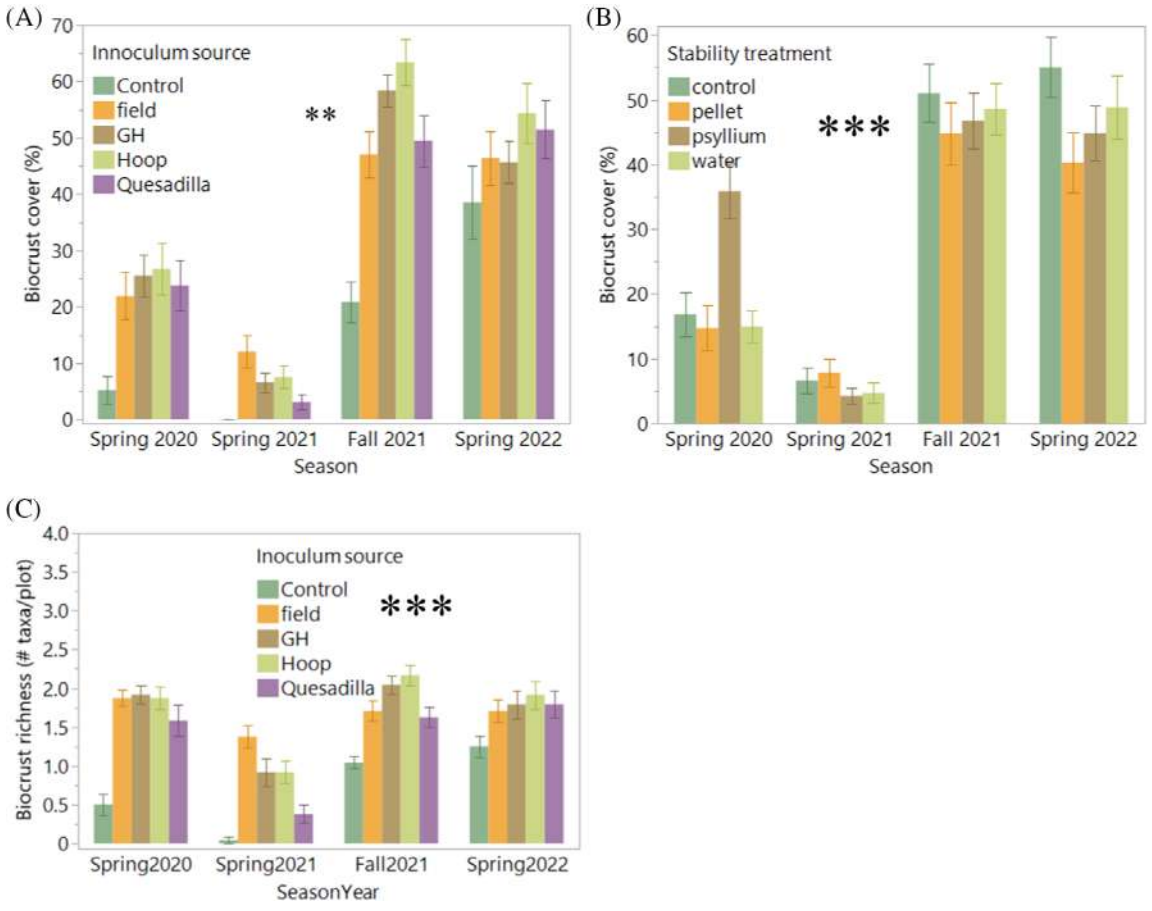

Figure 2. In the E1 Stability experiment, biocrust cover by (A) inoculum source, season, and (B) stability treatment and season and biocrust richness differed by (C) inoculum source and season. Asterisks indicate a two-way significant interaction among treatment and season as follows: ***$p < 0.0001$, **$p < 0.001$. Treatments were applied in fall 2019. Error bars indicate ±1 SE. Data was analyzed with a mixed model with inner plot biocrust as the response variable and inoculum source, stability treatment, and season and their interactions as fixed factors and plot as a random factor. Plant cover was added as a fixed factor to control for competition effects.

The relationship between biocrust cover and plant cover depended on season (plant cover × season: $F_{[1,376.8]} = 2.86$, $p = 0.037$; Table S1; Fig. 1). The relationship between biocrust and plant cover was negative in the final season of the experiment (Fig. S1). There were no differences in total plant cover among stability or cultivation treatments, but plant cover differed by season ($F_{[3,362.1]} = 83.16$, $p < 0.0001$, Table S5); the highest plant cover was in fall 2021 (Fig. S2).

E2 Sods

Biocrust cover and richness in the inner plots differed by treatment, moderated by season (cover: $F_{[39,166.8]} = 4.42$, $p < 0.0001$, richness: $F_{[39,167.7]} = 3.15$, $p < 0.0001$, Table S6). For the first two seasons, the greenhouse cultivated sod had the highest biocrust cover compared with the no biocrust control and a few other treatments within the season (Fig. 3A; Table S7). However, biocrusts on the greenhouse

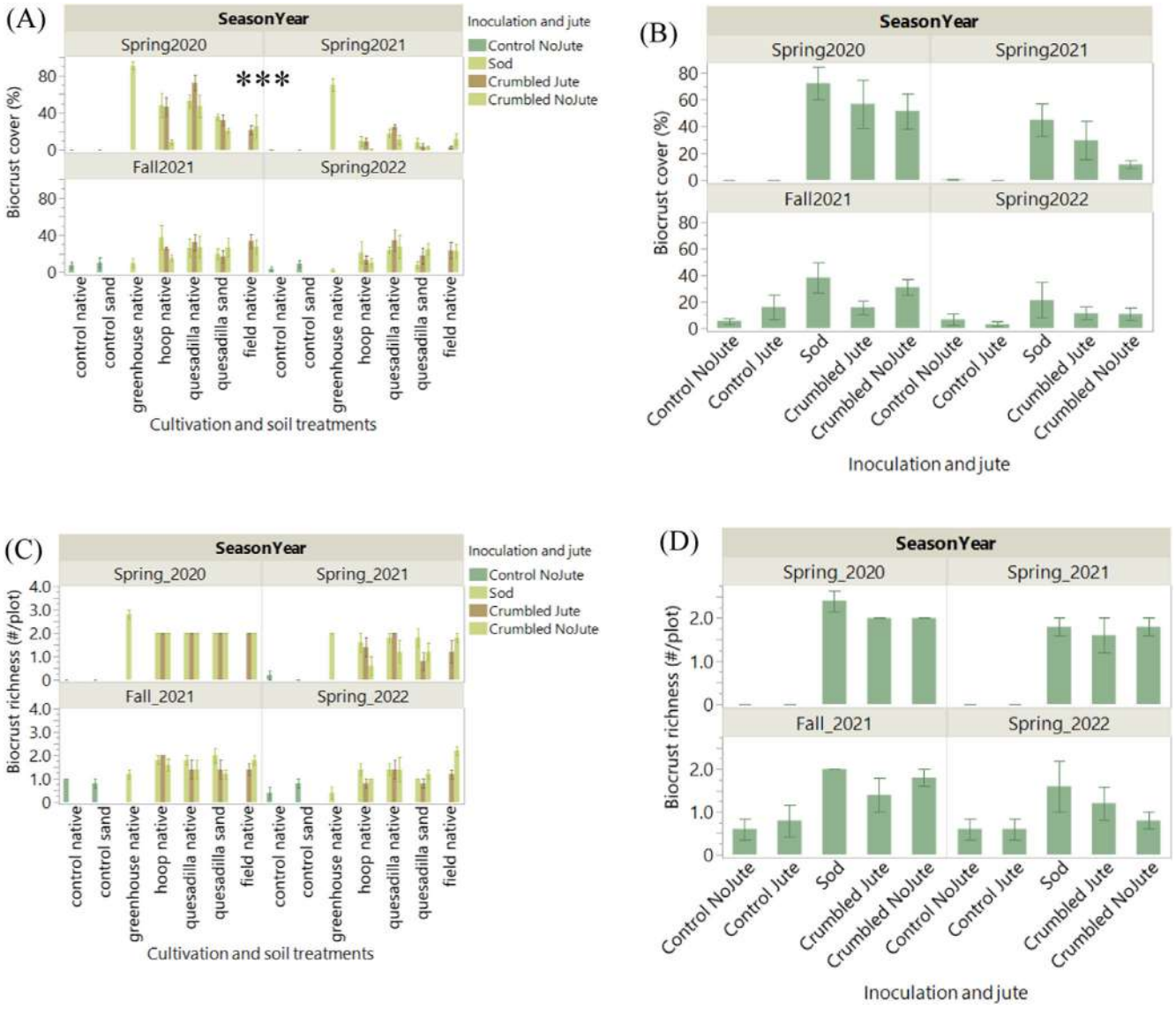

Figure 3. Biocrust cover of inner plots differed by treatment and season in the (A) E2 Sods experiment and (B) E3 Sods and Seed experiment. Biocrust functional group richness also differed by treatment and season in the (C) E2 Sods experiment and the (D) E3 Sods and Seed experiment. Inoculation and jute treatments include the method of inoculation (crumbles were scraped from the jute on which they were cultivated and sprinkled either on top of jute or no jute; in the sod treatment, the biocrust and the jute on which they were cultivated were moved together and stapled to the plot, control treatments received no biocrust addition). Cultivation and soil treatment refer to the cultivation environment (greenhouse, layered or "quesadilla," hoophouse or "hoop," field-salvaged biocrust, or control with no biocrust added) and the soil treatment (cultivation treatments included a base of either native soil or sand). E3 treatments were either cultivated in the greenhouse (crumbled and sod) or no biocrust controls with and without jute. Asterisks indicate significant interaction of season and treatment as follows: ***$p < 0.0001$, **$p < 0.001$. Treatments were applied in fall 2019. Error bars indicate ±1 SD. Data was analyzed with a mixed model with inner plot biocrust as the response variable and overall treatment, season, and their interaction as fixed factors and plot as a random factor. Plant cover and baseline biocrust cover were added as a fixed factor to control for competition and differences in initial condition.

sods reduced over time such that, in the final season, they had lower cover than on the quesadilla sods (Fig. 3A; Table S7). In the third season, there were no detectable differences among treatments (Fig. 3A; Table S7). The trends in biocrust richness were different from biocrust cover (Fig. 3C). Of the sod treatments, only the greenhouse sod had higher biocrust richness than controls and only in the first season (Fig. 3C; Table S8). By the final season, the field-salvaged biocrusts sprinkled on no jute had the highest richness (Fig. 3C; Table S8).

Sand vs. native soil: Using a priori contrast statements, we found that biocrust cover was greater on the plots cultivated using native soil compared to sand within quesadilla crumbled treatments (2020 crumbled on jute native soil vs. sand [difference ± SE]: 2.75 ± 1.12, $p < 0.05$; 2020 crumbled on no jute native soil vs. sand: 2.27 ± 1.12, $p < 0.05$) but not on quesadilla sods in the first season (2020 quesadilla sods native soil vs. sand: 1.34 ± 1.14, $p > 0.05$; Table S9). By the last season, biocrust cover was higher with native soil than with sand only on sods (2022 quesadilla sods native soil vs. sand: 2.54 ± 1.14, $p < 0.05$, Table S9) but not on quesadilla crumbled treatments (Table S9).

Biocrust colonization of outer plots: In the final 2 years of the study, biocrusts in the inner and outer plots had a strong positive linear relationship, but this trend was not seen in the first 2 years of the study (Fig. 4A; season: $F_{[3,176]} = 32.21$, $p < 0.0001$; Table S10A). Biocrust colonization of the outer plots did not depend on treatment (treatment: $F_{[3,77]} = 1.11$, $p < 0.367$; Table S10A). The covariate total outer plot plant cover also increased with outer plot biocrust cover ($F_{[1,204.9]} = 12.32$, $p < 0.001$; Table S10A).

Total inner plot plant cover varied by season (Fig. S3A; $F_{[3,176.4]} = 99.91$, $p < 0.0001$; Table S11A) but not by treatment ($F_{[3,56.8]} = 1.19$, $p = 0.314$; Table S11A).

E3 Sods and Seed

Four of the eight seeded plant species established in more than one plot (Table 3). Seedling establishment differed by treatment, depending on season ($F_{[15,71]} = 5.96$, $p < 0.0001$; Table S12). In fall 2021, the sum of seeded individuals was highest in treatments with jute and no biocrust or crumbled biocrust. Seedling emergence was lowest under sod and unseeded plots (Fig. 5). All treatment combinations with seed had higher seed establishment than the unseeded control plot with no jute or biocrust in fall 2021 (Fig. 5). The other seasons had very low seedling establishment (Fig. 5).

The inner plot biocrust cover and richness differed by treatment, mediated by season (Fig. 3B & 3D, treatment × season for cover: $F_{[39,167.7]} = 3.15$, $p < 0.0001$; for richness: $F_{[15,71.5]} = 3.26$, $p < 0.0001$; Table S6). In the first season, the crumbled treatment plots, but not the sod treatments, had higher biocrust cover than the controls with no biocrust (Fig. 3B; Table S13). By the final year, there were no differences among treatments (Fig. 3B; Table S13). Biocrust richness in the treatments with biocrust was higher than the no added biocrust controls in the first two seasons, but this difference disappeared in the last two seasons (Fig. 2D; Table S14).

Biocrust cover colonization of the outer plots increased with inner plot biocrust cover, moderated by season, but not by treatment effects (Fig. 4B; Table S10B). The relationship between outer and inner plot biocrust cover was strongest in spring 2021 and spring 2022 (Fig. 4B).

Total plant cover differed by treatment moderated by season (Fig. S3B; season × treatment: $F_{[15,72.1]} = 2.50$, $p = 0.005$; Table S11B).

Fungal and Bacterial Community

The fungal and bacterial communities showed no significant differences among biocrust cultivation methods. There were no statistical differences in OTU richness (Fig. S4A) or Shannon

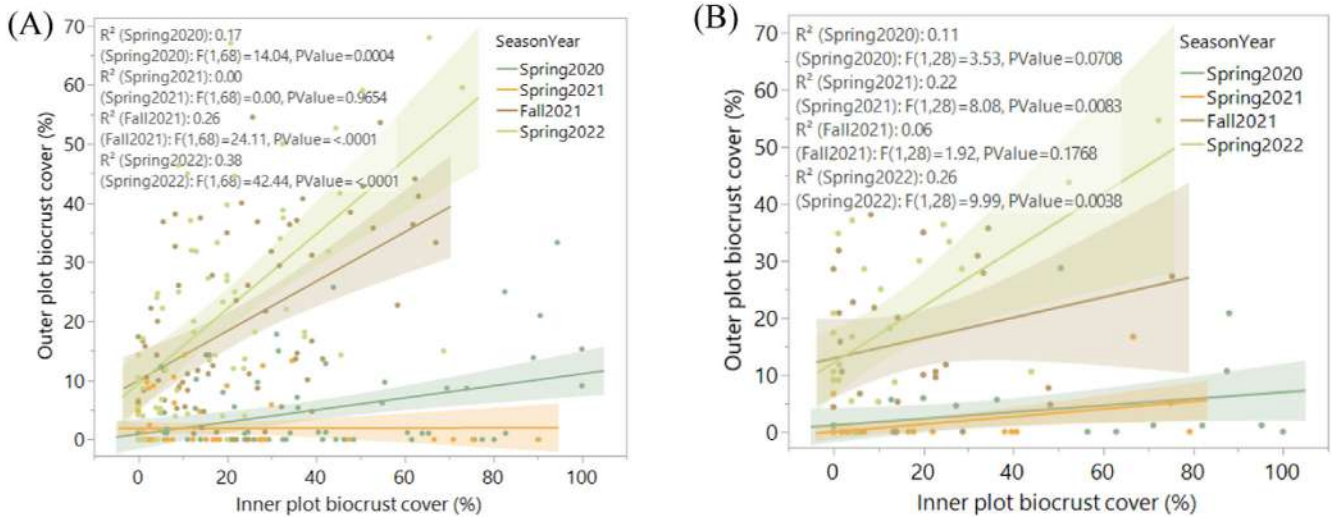

Figure 4. Colonization of biocrust in the uninoculated outer plots was associated with inner plot biocrust and time in the (A) E2 Sods experiment and (B) E3 Sods and Seed experiment. Treatments were applied in fall 2019. Line indicates the mean fit. Shading indicates the 95% CI. Data was analyzed with a mixed model with outer plot biocrust as the response variable and overall treatment, season, and their interaction as fixed factors and plot as a random factor. Plant cover and baseline biocrust cover were added as fixed factors to control for competition and differences in initial condition.

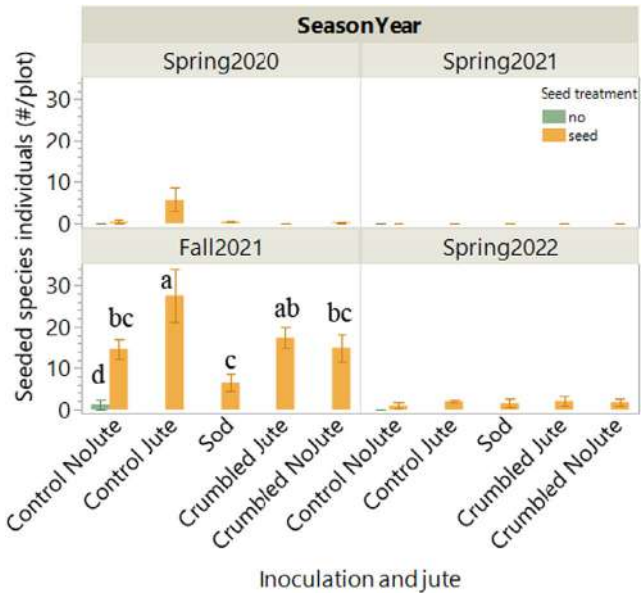

Figure 5. Seed establishment of the four species (*Senna covesii*, *Bouteloua aristidoides*, *Bouteloua rothrockii*, *Plantago ovata*) that established from the eight species seed mix in more than one plot by season and treatment. Inoculation and jute treatments include the method of inoculation (crumbles were scraped from the jute on which they were cultivated and sprinkled either on top of jute stapled to the ground or no jute; in the sod treatment, the biocrust and the jute on which they were cultivated were moved together and stapled to the plot; control treatments received no biocrust addition). Crumbled and sod treatments were cultivated in the greenhouse. Lowercase letters indicate differences among treatments within season. Bars indicate one standard error. Data was analyzed with a mixed model with the number of seeded species established as the response variable and overall treatment, season, and their interaction as fixed factors and plot as a random factor. Plant cover and baseline biocrust cover were added as fixed factors to control for competition and differences in initial condition.

diversity (Fig. S4B) of fungi among the cultivation methods. There were also no statistical differences in OTU richness (Fig. S4C) or Shannon diversity (Fig. S4D) of bacteria among treatments. Beta diversity did not differ by cultivation method in either the fungal or bacterial communities. No significant clustering in NMDS ordination was observed in the fungal or bacterial communities based on cultivation method (Fig. S5A & S5B, respectively).

Differences in relative abundances within the taxonomic compositions of communities were assessed. For fungal communities, no distinct differences were observed in the relative abundance of the top 35 most abundant genera of fungi (Fig. 6). The top five most abundant genera among all the treatment groups were *Alternaria*, *Curvularia*, *Didymocrea*, *Neodidymelliopsis*, and *Ramimonilia*, and there were minor differences detected between the treatment groups. The abundance of *Curvularia* was significantly higher in the control (no biocrust) treatment than in the greenhouse treatment ($p = 0.018$), and the field salvaged treatment had a significantly higher abundance of *Ramimonilia* than the control no biocrust treatment ($p = 0.05$). For bacterial communities, no distinct differences were observed in the relative abundance of the top 35 most abundant phyla of bacteria (Fig. 6A). There were no statistical differences between treatments within the five most abundant phyla: *Acidobacteria, Actinobacteria, Bacteroidetes, Proteobacteria*, and *Chloroflexi*. We saw differences in the phylum *Cyanobacteria* (Figs. 6C & 7B), with significantly greater OTU richness in the control treatment than the greenhouse treatment ($p = 0.02$; Fig. 7B) and significantly higher Shannon diversity in the control treatment compared to all other treatments ($p = 0.0008$).

Discussion

The goal of this study was to compare field establishment and application methods of biocrusts cultivated with different approaches in the Sonoran Desert. Our primary findings are as follows. First, we found that psyllium had a strong effect on facilitating biocrust establishment in the first season, but this effect disappeared by the second sampling season after below average monsoon and winter rains. Second, biocrust cover and richness were not different on sods compared to biocrusts scraped from jute and crumbled on new or no jute in the field within cultivation method. Third, field cultivated biocrusts may have survived better in the field, but fungal and bacterial communities in biocrust did not differ among the cultivation approaches. Fourth, colonization of outer plots from inoculated inner plots was associated with the interaction of biocrusts in the inner plots and season, but we did not detect differences among application treatments. Fifth, seed establishment was reduced by biocrust sods but enhanced by the protection of jute. Finally, there appeared to be a threshold of plant cover under which biocrusts and plant growth could both grow and over which competition resulted in a negative growth relationship.

Psyllium Helped Establish Cultivated Biocrust in the Field

Psyllium improved biocrust establishment in the first year, compared with pellets, watering, and control, but the treatment did not translate to a long-term advantage due to the drought between the first and second sampling seasons. Psyllium is a tackifier composed of long-chain carbon compounds used for soil stabilization and has previously been tested with moss growth and biocrust restoration. Researchers have found that, compared to water and guar, psyllium increased the growth of mosses, decreased pH, and increased concentrations of P and K (Blankenship et al. 2020). Psyllium helps maintain biocrust inoculum longer than other tackifiers (19 months), increases stability, and improves water infiltration (Fick et al. 2019, 2020). However, including psyllium in a biocrust slurry application may result in soil physical crust rather than increasing biocrust colonization (Schultz et al. 2022). This attribute of holding soil and biocrusts in place long enough for biocrust establishment is an important function in biocrust restoration and, in our study, psyllium performed this function better than pellets. We suggest psyllium addition when a dry period is expected in advance of biocrust activity.

Sods Did Not Improve Establishment

In E2 Sods and E3 Sods and Seed experiments, we applied biocrusts at the same rate within cultivation method to compare

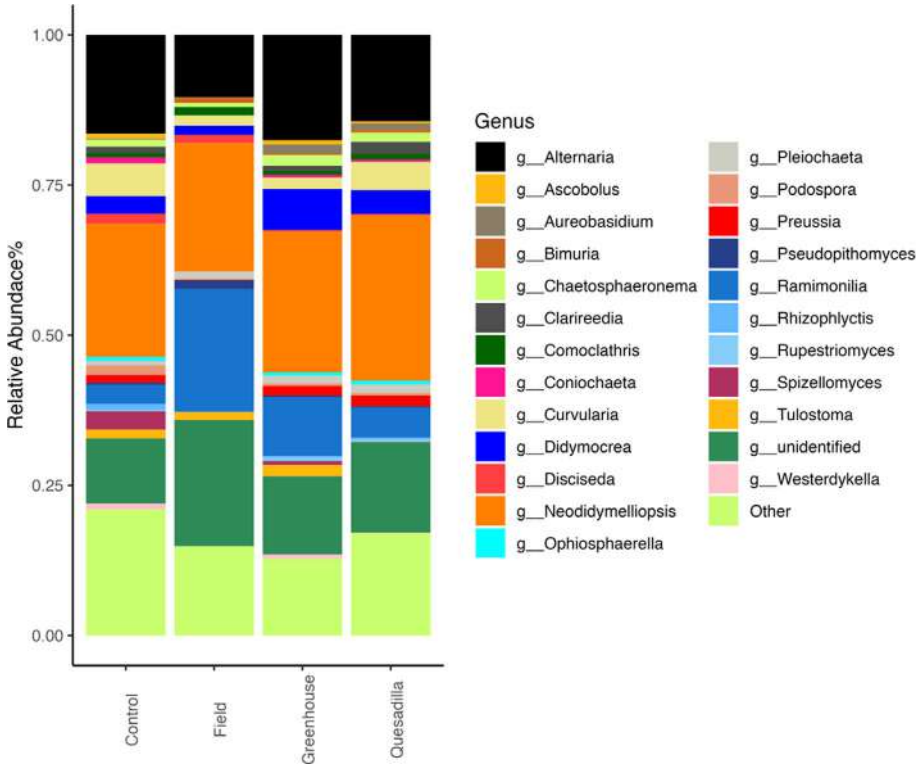

Figure 6. Fungal taxonomic composition bar plots at the genus level by cultivation method. The relative abundance of the top 25 most abundant genera is represented in the boxplots. Treatments are no biocrust control, field salvaged biocrust control, and greenhouse and quesadilla cultivated biocrusts.

sods to crumbled treatments. Within cultivation method, sods did not perform better than the crumbled or field-salvaged biocrusts in either experiment, although sod treatments resulted in higher biocrust cover than controls in both experiments. We thought sods would lead to better biocrust growth and richness by reducing disturbance during transfer from the cultivation farm to the field compared with scraping the biocrust and transferring it to new jute in the field. However, two issues may have

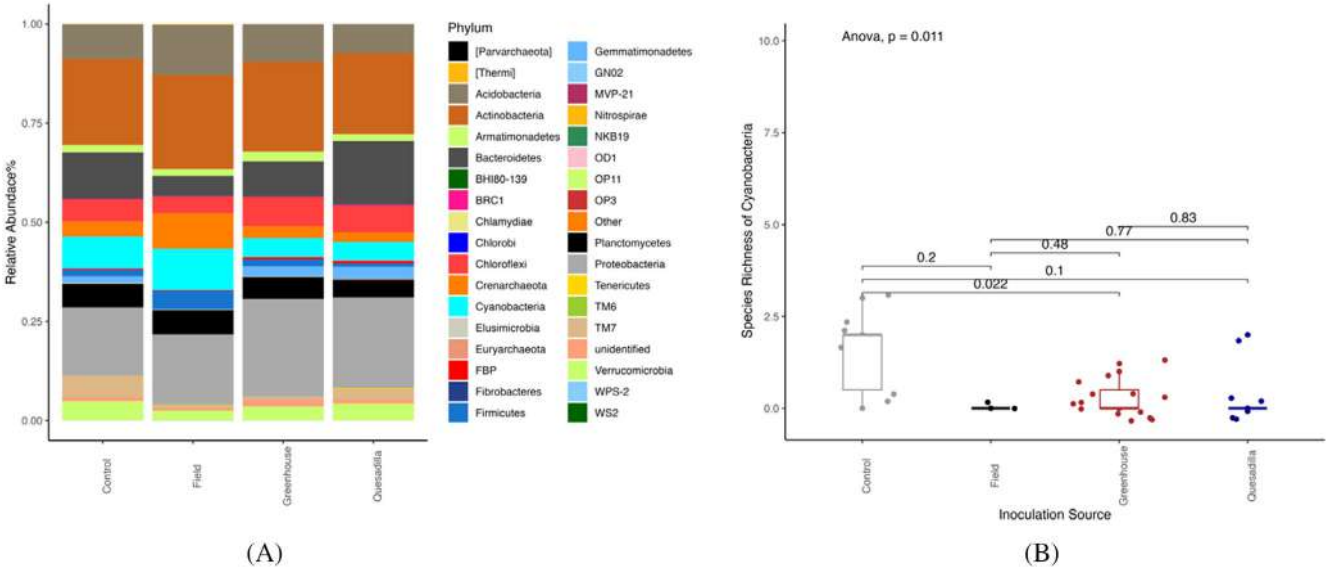

Figure 7. Bacterial taxonomic composition bar plots at the phylum level by cultivation type. (A) The relative abundance of the topmost abundant phyla is represented in the boxplots. (B) ANOVA was used to test differences in the OTU richness of the phylum Cyanobacteria (ANOVA, $p = 0.011$). Treatments are no biocrust control, field salvaged biocrust control, and greenhouse and quesadilla cultivated biocrusts.

reduced the efficacy of sods in the field. First, removing sods from the cultivation farm proved to be difficult and resulted in dislodging crusts from the jute, so the transferred sods were not fully intact. Second, wildlife and/or precipitation events disturbed sods and jute in the field. In other studies, jute has shown to be an effective stabilizer that enhances biocrust colonization in the arid southwest (Bowker et al. 2020), semiarid intermountain grasslands (Slate et al. 2020), and the arctic (Ficko et al. 2023). Researchers suggest jute increases soil surface stabilization and resource availability (water), thereby reducing drought stress (Bowker et al. 2020). Improvements in transferring intact sods from farm to field with less disturbance may increase sod performance. Also, increasing the size of sods could reduce disturbance in the field exacerbated by edge effects. Another improvement may be to apply a plasma treatment with dielectric barrier discharge to increase capillary function of jute, resulting in quicker wetting times and enhancing the benefits of jute for biocrust establishment because lack of moisture is a main resource constraint (Ivanovska et al. 2023).

Field Cultivated Biocrusts Established Better Than Greenhouse Cultivated with No Differences in Bacterial and Fungal Communities

In E1 Stability Applications, we applied cultivated biocrust at the same rate without jute or sods so we could test whether the initial cultivation method influenced field establishment. A severe monsoon and winter drought between the first and second sampling period created a natural experiment within the field experiments. Our results show that the hoophouse cultivated and field-salvaged biocrusts grew better than controls after the drought period, indicating a slight improvement in drought tolerance from these treatments. Notably, there were no differences among the cultivated and field-salvaged biocrust cover in any season. The field-salvaged and hoophouse and greenhouse cultivated biocrusts had higher taxa richness compared with controls, which varied with season, but biocrust richness was lower in quesadilla cultivated plots. We do not have a good explanation for this. In the cultivation experiment, quesadilla cultivation was better than hoophouse in terms of cover and late successional biocrust (Antoninka et al. 2024).

In the E2 and E3 experiments, although greenhouse cultivated biocrust sods started with the most biocrust, that biocrust cover declined over the course of the experiment, indicating that the biocrusts may have been poorly adapted to field conditions. In the initial cultivation experiment, biocrusts grew best in the greenhouse and quesadilla environments, compared to the hoophouse. Biocrusts in the greenhouse environment had more mosses, whereas the quesadilla and hoophouse environments were similar in composition with more lichens (Antoninka et al. 2024) and may have been more resilient to the drought stress experienced in the field. In a similar cultivation experiment, researchers sought to cultivate more climate-adapted biocrusts from different environments (Jech et al. 2023). However, they found that biocrusts from different sites converged to the cultivation farm site conditions, indicating a strong potential for adaptation (Jech et al. 2023). Here, we saw the same trend with cultivated biocrust microbial communities, which did not appear different from the home field salvaged biocrust regardless of treatment. In a study in the Great Basin, Utah, U.S.A., when field-salvaged and greenhouse cultivated crusts were applied in the field, the greenhouse cultivated crusts grew faster with more colonization but did not develop as rich a community structure as field-salvaged crusts (Antoninka et al. 2018). In our experiment, the fact that both cover and richness converged among the cultivated and field-salvaged biocrusts in the final year is good news for restoration; cultivation is an important approach for bulking up salvaged biocrust, in lieu of disturbing natural biocrust communities in the field for restoration applications elsewhere.

Biocrust Colonized Well Outside of Treated Plots

By year three, biocrusts had colonized as high as 68% (E2) and 54% (E3) cover of the outer plots and increased with biocrust cover in the inner plot. This finding suggests promise for scaling up by establishing smaller "restoration islands" of dense propagules that would spread and restore biocrusts across a larger site. Study period averages revealed no differences in colonization of the outer plots among any treatment compared to the control over time, which supports this concept. Hulvey et al. (2017) laid out three guiding principles for the restoration island approach: establishment, persistence, and spread, which can also be applied to biocrusts. We have shown that the use of cultivated biocrusts can effectively establish patches of biocrust. Additionally, dense patches of biocrust can proliferate. As with plants, to ensure persistence in arid systems, biocrust patches should not be too small and should be clustered to encourage propagule exchange (Hulvey et al. 2017).

Biocrust Inoculation and Seeding May Be Most Effective If Separated in Space

Biocrusts both facilitate and inhibit plant species performance (Havrilla et al. 2019). In our study, seeds established best under jute without any biocrust and were most inhibited under biocrust sod. As discussed above, jute can provide additional resources (shading, moisture retention) and stability, which improved seed germination. These results suggest that sod biocrusts inhibited plant establishment. Stronger evidence of plant and biocrust competition occurred later in E1 but not the other experiments. Although plants and biocrusts had a neutral relationship early in E1, a negative growth relationship was observed between biocrusts and plants after plants had established in the plots. This is consistent with community theory in which changes in species relationships under stress shift from facilitative or neutral during initial stages of recruitment but transition to competitive later in succession (García-Cervigón et al. 2013). Others have found this trend in restoration of biocrusts and plant interactions shifting from positive to negative over time (Xu et al. 2024).

Biocrust Cultivation Shows Promise as a Restoration Practice

Practitioners can incorporate biocrust with psyllium or jute into restoration to improve soil stability, nutrient cycling, and soil

health in the Sonoran Desert. We found that the cultivated and field-salvaged biocrusts retained their native bacterial and fungal communities and established in the field. Quesadilla cultivated sods showed some promise, but improvements are needed to reduce disturbance in transferring and maintaining sods in the field. Psyllium was an effective addition for short-term establishment, but long-term effects may have been impacted by drought. Jute helped biocrust colonization in the cultivation phase (Antoninka et al. 2024) and aided seedling establishment when not transferred as a sod in the field. Although transferability to other arid contexts is uncertain, these practices may be successful in cooler deserts, where biocrusts grow well. Overall, this field trial of cultivated biocrusts indicates these practices may assist in transferring whole biocrust communities in a restoration context.

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Supporting Information

The following information may be found in the online version of this article:

Figure S1. Biocrust cover depended on plant cover, modified by season in the E1 Stability Applications experiment.
Figure S2. Plant cover differed by season in E1 Stability Applications experiment.
Figure S3. Plant cover of the inner plot differed by season in (A) E2 Sods experiment and by season and treatment in (B) E3 Sods and Seed experiment.
Figure S4. The Alpha diversity of the biocrust microbial communities did not differ between treatment groups.
Figure S5. The Beta diversity of the biocrust microbial communities did not differ between treatment groups.
Table S1. Mixed model output for biocrust cover and richness of for E1 Stability Applications.
Table S2. Post hoc comparison of biocrust cover means for stability treatment and season (E1) using Tukey's HSD test.
Table S3. Post hoc comparison of biocrust cover means for cultivation method and season (E1) using Tukey's HSD test.
Table S4. Post hoc comparison of biocrust richness means for cultivation method and season (E1) using Tukey's HSD test.
Table S5. Plant cover mixed model output for E1 Stability Applications experiment.
Table S6. Mixed model output for E2 inner plot biocrust cover and richness and E3 biocrust cover and richness.
Table S7. Post hoc comparison of biocrust cover means for treatment and season (E2) using Tukey's HSD test.
Table S8. Post hoc comparison of biocrust richness means for treatment and season (E2) using Tukey's HSD test.
Table S9. Contrast statements of inner plot biocrust between native soil and sand within cultivation method for the first and last seasons of the study (spring 2020 and 2022).
Table S10. Outer plot biocrust cover mixed model output for (A) E2 Sods experiment and (B) E3 Sods and Seed experiment.
Table S11. Inner plot plant cover mixed model output for (A) E2 Sods experiment and (B) E3 Sods and Seed experiment.
Table S12. Inner plot seeding establishment mixed model output.
Table S13. Post hoc comparison of biocrust cover means for treatment and season (E3) using Tukey's HSD test.
Table S14. Post hoc comparison of biocrust richness means for treatment and season (E3) using Tukey's HSD test.

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