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RESEARCH ARTICLE

Inoculation and habitat amelioration efforts in biological soil crust recovery vary by desert and soil texture

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As dryland degradation continues, it is increasingly important to understand how to effectively restore biocrust communities. Potential techniques include the addition of biocrust inoculum to accelerate biocrust recovery. Enhanced erosion typical of degraded environments creates a challenge for these approaches, due to loss by wind or water and burial by saltating particles. To retain and protect added inoculum, the inclusion of habitat-amelioration techniques can improve recovery rates. This study tested three different types of inoculum (field-collected, greenhouse-cultivated, and laboratory-cultivated biocrust) coupled with two treatments to augment soil stability and ameliorate habitat limitations: soil surface polyacrylamide additions and installation of straw barriers. This was done across two deserts (Great Basin and Chihuahuan) and separated into generally coarse- or finer-textured soils in each desert, with results monitored for 3 years (2015, 2016, 2017). While the inoculum type, coupled with habitat ameliorations, occasionally enhanced biocrust growth across years and treatments, in other cases, it made no difference compared to natural recovery rates. Rather, the desert location and soil texture groupings were the most prominent factors in determining recovery trajectories. Recovery proportions were similar in the finer-textured sites in both the Great Basin and the Chihuahuan deserts, while the coarser-textured site in the Great Basin did show some recovery over time and the Chihuahuan coarser-textured site did not. This study demonstrates the importance of understanding site potential and identifying key limitations to biocrust recovery for successful restoration projects.

Key words: biocrust, Chihuahuan, cyanobacteria, Great Basin, polyacrylamide, soil texture

Implications for Practice

- Application of biocrust inoculum did not consistently hasten recovery in these active restoration efforts.
- Soil binding polyacrylamide may provide initial stabilization but if biocrust does not recover before the natural degradation process, soil stability may return to original degradation levels irrespective of restoration.
- Soil texture and other inherent site-level differences may be more important than current active intervention efforts in determining biocrust recovery.

Introduction

Drylands cover over 40% of the terrestrial land surface globally (Reynolds et al. 2007a; Prăvălie 2016) and, with accelerated climate change, their extent is projected to increase (Huang et al. 2016). Alongside increases in land surface area, dryland degradation is also increasing (Reynolds et al. 2007b). This degradation can lead to loss of ecosystem productivity, enhanced soil erosion, and increased dust emissions, as well as a suite of other altered ecosystem processes that negatively impact human well-being (Zika & Erb 2009). As a response to this loss of ecosystem function, novel restoration and intervention techniques are being developed for highly degraded landscapes to promote soil health and recovery of a broad range of impaired ecosystem services. One such emerging field is that of biological

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soil crust (biocrust) restoration. Biocrust encompasses a suite of photosynthetic and heterotrophic communities that colonize the soil surface, including cyanobacteria, lichens, and/or mosses (Belnap & Lange 2003). While found in many environments globally (Rodríguez-Caballero et al. 2018), biocrusts are most prevalent in drylands and can dominate plant interspaces in some ecosystems (Belnap 2003).

Biocrusts can provide multiple ecological benefits to the areas they inhabit. For instance, early colonizing bundle-forming filamentous cyanobacteria (Belnap et al. 2008; Giraldo-Silva et al. 2019*a*; Rodríguez-Caballero et al. 2018) can quickly stabilize soil through mechanisms such as exudation of exopolysaccharides that bind surface soils (Mager & Thomas 2011; Chock et al. 2019), or through direct mineral particles interlocking into larger soil aggregates or crusts resistant to erosion (Garcia-Pichel & Wojciechowski 2009). During large rain events, the presence of well-developed biocrust in cooler deserts, with the underlying soils potentially more prone to physical crusting, sediment loss, and runoff, can decrease (Rodríguez-Caballero et al. 2015; Chamizo et al. 2017; Faist et al. 2017) wherein hot deserts, often with highly permeable soils, runoff from cyanobacteria-dominated biocrust can provide water to downslope vegetation (Kidron et al. 2003). In addition to the direct benefits of soil stability, biocrust can provide carbon and nutrients to the soil through photosynthesis and nitrogen fixation (Cantón et al. 2014; Tucker et al. 2019).

The most successful and widely tested biocrust restoration technique to date is the use of field-collected inoculum (e.g. from a neighboring undisturbed area). However, this technique has a major barrier to broad-scale application by resource managers because it requires availability of undisturbed biocrusts for harvesting and thus has a low feasibility for large-scale restoration due to this constraint on source material at a small scale. In response, methods have been developed to cultivate small amounts of native biocrust under optimal greenhouse conditions to increase the biocrust biomass for larger-scale restoration projects (Antoninka et al. 2016, 2018; Velasco-Ayuso et al. 2017; Doherty et al. 2018). A major challenge in this approach is maintaining similar biocrust community composition to what is found in the natural environment (Velasco-Ayuso et al. 2017). Alternatively, one can use laboratory-cultured biocrust cyanobacteria, or grown in lab conditions, for restoration efforts (Wang et al. 2009; Giraldo-Silva et al. 2019a), but this requires specialized techniques and identification of optimal species compositions for the inoculated cultures.

While greenhouse and lab cultivation of biocrust inoculum for restoration has been successful (Velasco-Ayuso et al. 2017), inoculation of degraded soils without significant modification of the habitat has been met with variable success (Antoninka et al. 2018; Bowker et al. 2019; Fick et al. 2019a, 2019b). In addition, cultivation occurs under conditions of high-water availability and less environmental stress compared to the natural environment. Thus, cultivated biocrust material may be maladapted to the harsh conditions found in drylands, which may require additional "hardening" procedures to be carried out (Bowker et al. 2019).

At highly degraded sites, restoration efforts may be strongly limited by abiotic processes, particularly accelerated erosion (Duniway et al. 2015). Efforts to aid in plant establishment in drylands have been effectively implemented using low stature barriers (Rachal et al. 2015; Fick et al. 2016). To curtail erosion in biocrust restoration efforts, a broad range of soil stabilizing and habitat amelioration strategies have been tested, such as increasing surface roughness to reduce wind erosivity and provide protected microsites (Li et al. 2010; Xhao et al. 2016), synthetic soil stabilization products (e.g. polyacrylamides [PAMs]) (Antoninka et al. 2019), and other strategies meant to curb accelerated erosion and allow biocrust organisms to establish (Fick et al. 2019a, 2019b).

The general objectives of this study were to test our ability to promote rapid biocrust recovery on disturbed dryland soils, comparing three inoculum types (field-collected, greenhouse-cultivated, and lab-cultivated biocrust), as well as two strategies for habitat amelioration (straw barriers and PAMs). We expected that responses to biocrust restoration would not be uniform across climatic regions and by soil types, and thus established biocrust restoration studies in contrasting soil textures (coarser and finer texture) in a hot North American desert (Chihuahuan) as well as a cooler North American desert (Great Basin). Our questions were: (1) Do different inoculum types (i.e. field-collected, greenhouse-cultivated, and lab-cultivated) differ in their biocrust recovery trajectories? and (2) Do different habitat amelioration measures (straw barriers and soil surface stabilizing PAMs) influence biocrust recovery? We hypothesized that, regardless of inoculum type, the addition of propagules would increase biocrust biomass over the observation period (2015–2017) and consequently increase soil stability. We also hypothesized that the habitat amelioration implementations with the inclusion of PAMs or straw borders would further promote biocrust recovery above the natural recovery rate. We ultimately predicted that the best restoration outcome would include the combination of inoculum with soil stability infrastructure to increase biocrust cover and soil stability over time, regardless of desert type and soil texture.

Methods

Site Description

We conducted this study in a hot and a cold desert in the western United States (Fig. 1). The cold desert site was located west of Salt Lake City at the Hill Air Force Base Utah Testing and Training Range (hereafter referred to as UTTR). The UTTR location in the Great Basin Desert experiences a mean annual precipitation (MAP) of 200 mm. Mean monthly maximum UTTR temperatures range from 3°C in January to 34°C in July. The hot desert location was in southern New Mexico, United States, on the Jornada Experimental Range (hereafter referred to as Jornada). The Jornada is in the northern Chihuahuan Desert and has monsoonal-driven precipitation patterns with 64% of its 282 mm of MAP occurring between June and September. Mean maximum annual temperatures range from 14°C in January

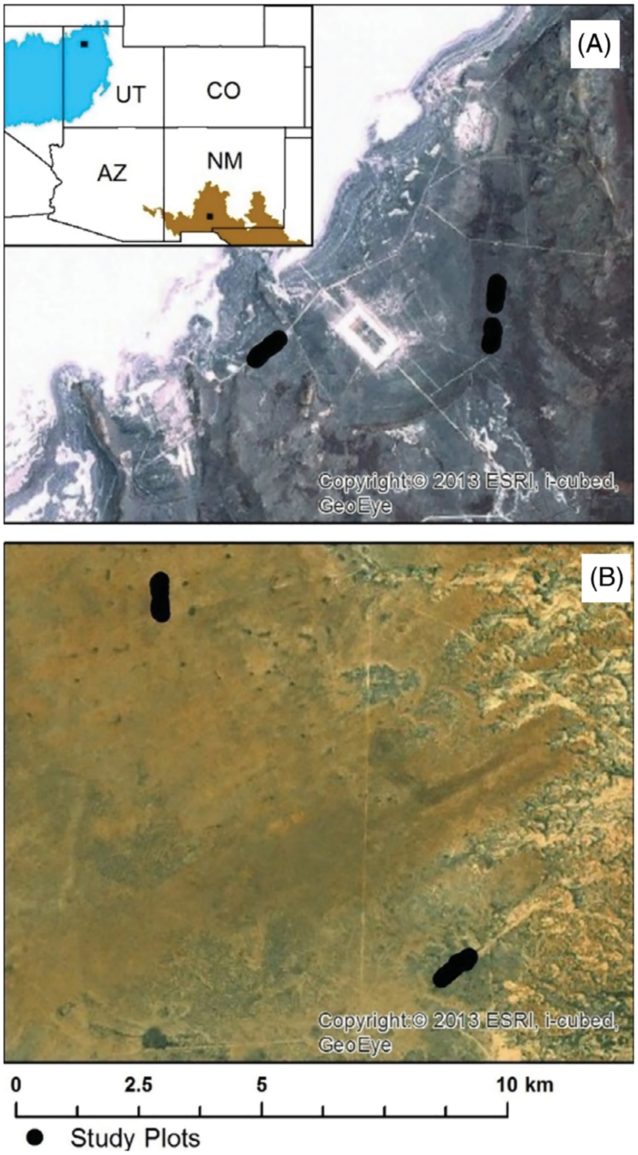

Figure 1. Location of study areas within the southwestern United States (inset) and study plot distribution within study areas at Utah Testing and Training Range (UTTR) (A) and Jornada Experimental Range (B). The UTTR base site is in northwestern Utah and in the eastern extent of the Central Basin and Range (blue). The Jornada site is in south-central New Mexico and the northern reaches of the Chihuahuan Desert (brown; Omernik & Griffith 2014). Locations of the study plots are shown as black circles atop the satellite site maps.

to 36°C in July. Within each desert, two sites were chosen with contrasting soil textures, resulting in a total of four sites (Table 1). The UTTR (Great Basin) sites, due to their lacustrian parent materials, were generally higher in their silt content than the alluvial sediments of the Jornada (Chihuahuan sites). The Jornada "fine" site had the highest within-site variability in soil texture, and the Jornada "coarse" site was the coarsest soil of all sites, with very low amounts of silt or clay-sized soil separates (Table 1). For simplicity, the sites within deserts (e.g. Great Basin or Chihuahuan) are hereafter referred to as "coarse" or "fine" although variability does exist both within, and outside of, the different sites and deserts.

All four sites (UTTR-fine, UTTR-coarse, Jornada-fine, Jornada-coarse) received the same experimental treatments. To create similar conditions upon which to conduct restoration experiments, the top approximately 2.5 cm of soil was cleared in a 3 × 5 m area to create a distinct plot. The top 1 cm of biocrust propagules that were captured during the scraping were removed and stored for use as field-collected inoculum type. After the top layer of soil was removed, the soil was trampled such that the entire area of each 3 × 5 m plot received one footstep. After these initial disturbances, 1 × 3 m plots (3 m^2) were established in the center of the disturbance leaving a 1-m biocrust-free buffer surrounding each plot. Two types of control plots, with intact biocrusts, were maintained throughout the experiments as a "target" for biocrust restoration: (1) a 1 × 3 m control plot where the 1-m area surrounding the plot was left intact and (2) a control that included a 1 m disturbance surrounding the 1 × 3 m intact control plot. This two-control approach was included to address any unintentional effects on biocrust caused by the presence of a nearby disturbance. We hereafter refer to the two controls as "Con-no-disturb" and "Con-buffer-disturb" where the control buffer-disturb is surrounded by a 1-m disturbance. Outside of the intact controls, to allow for similar plot/treatment comparisons, a uniform disturbance was implemented on all treated plots (Fig. 2).

Treatments (11 in total, including the controls) were randomly assigned to plots evenly distributed across the landscape (see following sections for details), each replicated eight times within each soil type ($n = 88$ plots/soil type within a desert location). The 11 treatments included an inoculum treatment with four levels (no inoculum, field-collected, greenhouse-cultivated, and lab-cultivated) with two levels of a habitat amelioration effort (straw or PAM) plus the two intact controls and disturbance only (Fig. 2).

Inoculum Addition

To test the contribution of propagule addition to biocrust recovery we added three biocrust inoculum types. The field-collected soil inoculum was taken from the top 1 cm of the soil surface prior to the plot-level disturbance. This inoculum type represents the full biocrust community that was locally adapted to the site-level conditions. Due to the lack of adequate biocrust cover at the Jornada-coarse soil site, this site did not have a field-collected inoculum treatment. The second inoculum type was greenhouse-cultivated, to assure adequate time for inoculum growth in the greenhouse prior to the broad level experiment, biocrust samples were collected from an associated field site (or nearby sites) with similar edaphic and biocrust communities as our experimental sites. After collection, inoculum was then spread and grown on a base substrate under controlled greenhouse conditions, with a cooler greenhouse for the UTTR site and a warmer greenhouse for the Jornada site, as described in Velasco-Ayuso et al. (2017) with the watering regime for both UTTR and Jornada biocrust material following Doherty et al. (2015). A comparison of greenhouse-grown inoculum to intact

Table 1. Site-level surface substrate and soil properties. Mean substrate percentages obtained over entire site using a series of 50-m transects (±1 SE), with point observations every 1 m, and bulk density measurements collected two times along each site-level transect. Mean soil particle size measurements (±1 SE) collected directly on a subset of plots ($n = 3$ per 11 treatments for a total of 33 observations per desert and specific soil type).

	UTTR		*Jornada*	
Metric	*Coarse*	*Fine*	*Coarse*	*Fine*
Bare ground (%)	4 ± 0.9	4 ± 1.1	54 ± 4.7	1 ± 0.4
Biocrust (%)	62 ± 3.1	72 ± 3.3	1 ± 0.5	75 ± 5.9
Litter (%)	20 ± 2.6	8 ± 2.0	21 ± 3.0	5 ± 2.4
Rock (%)	0	1 ± 0.4	1 ± 0.5	0
Grass (%)	2 ± 0.6	0	13 ± 2.6	7 ± 0.7
Forb (%)	4 ± 1.81	1 ± 0.4	4 ± 1.3	1 ± 0.5
Shrub (%)	8 ± 2.2	15 ± 2.9	7 ± 1.7	11 ± 3.9
Bulk density (g/cm^3)	1.07	1.12	1.44	1.19
Sand (%)	22.71.0	16.9 ± 1.1	90.5 ± 0.5	34.3 ± 5.1
Silt (%)	64.5 ± 0.9	69.5 ± 1.1	3.6 ± 0.3	44.6 ± 3.7
Clay (%)	12.8 ± 0.5	13.6 ± 0.6	5.9 ± 0.3	21.0 ± 1.9

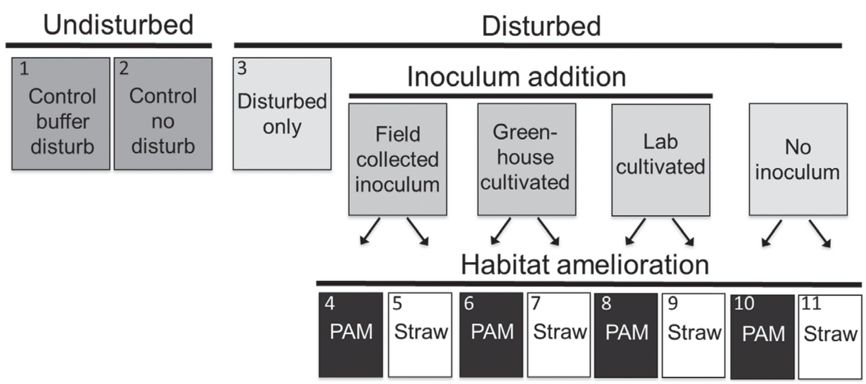

Figure 2. Experimental design showing all 11 treatments ($n = 8$ replicates/site, $n = 88$ plots/site) as numbered in the top left corner of the boxes. All 11 treatments, including inoculum addition and habitat amelioration efforts, were mirrored across desert locations and soil types.

biocrusts at the site showed that the greenhouse grown maintained a similar community composition to the intact biocrusts in the field (Velasco-Ayuso et al. 2017). The third inoculum type deployed was a lab-cultivated inoculum (Giraldo-Silva et al. 2019a). Here, the five most common cyanobacteria for each desert location and soil type were identified and isolated from a subset of site-specific field-collected biocrusts. After isolation, the strains were scaled-up and mixed with a low level of sterile sand to match the relative abundance of cyanobacteria found originally at each of the restoration sites. For further detail on the lab-cultivated methods see Giraldo-Silva et al. (2019a). Additionally, both inoculum cultivation techniques were conditioned to increasing temperature and solar radiation and recurrent wet-dry cycles to potentially increase survival rate and speed up recovery, with the lab-cultivated efforts explicitly stated in Giraldo-Silva et al. (2019b). While the Jornada coarse site did not have adequate biocrusts available for field-collected inoculum, a nearby site with similar characteristics and community structure on Fort Bliss Air Force base was used as the collection site for the greenhouse- and lab-cultivated inocula. After cultivation and collection, inoculum was air-dried at room temperature and stored in a cool, dark, and dry location prior to field inoculation.

To create uniform inoculum distributions across all plots, each individual inoculum type was sieved through a 0.56-cm sieve, homogenized, and evenly divided to allow for an approximate 10% cover of inoculum "crumbles" (1,500 cc/plot) in the 1 × 3 m treatment plots. This cover value was established from previous field trials that demonstrated a higher inoculum rate was observed to be no more effective in establishing recovery (Antoninka et al. 2018; Chock et al. 2019). This level of inoculum, while assuring an even coverage of the biocrust species across the entire plot, was added to maintain only a thin layer to not bury the live strains of inocula and was added after habitat amelioration efforts were installed.

Habitat Amelioration Treatments

Two habitat amelioration treatments (straw barrier and a soil binding PAM) were implemented to examine whether biocrust recovery is inhibited by unstable soil surfaces. Having been shown to stabilize mobile sand dunes in China (Li et al. 2003; Zhao et al. 2016), straw barriers can increase surface roughness, obstruct movement of saltating sands, and thus provide protection for biocrust recovery. A similar treatment was implemented (also see Antoninka et al. 2019) by placing a vertical straw border approximately 7–12 cm in height around the border of the 1 × 3 m plot, inserted 2 cm below the soil surface.

Our second habitat amelioration treatment was an application of a thin layer of PAM. PAM binds the soils at the surface to limit erosion and thus has the potential to facilitate biocrust recovery through the reduction in soil movement. PAMs have been proven effective at surface soil stabilization efforts inroad-side stabilization and other restoration efforts. However, PAMs degrade after repeated precipitation events and thus the goal of using this method in conjunction with biocrust inoculum to provide initial stabilization allowing the biocrust to establish. The PAM (Dirt Glue Polymer, Global Environmental Solutions, Salem, NH, U.S.A.) was mixed to a 1:9 glue to water ratio, as described by the industry standards for application and was evenly sprayed using a fine mist on the 1 × 3 m plots for a total of 1 L of solution per m^2 of plot surface. The intent of the PAM application was to test its ability to facilitate biocrust recovery, and not address if it meets intended usages in other applications.

Monitoring

To understand the photosynthetic potential of the soil, as well as serve as a proxy for biocrust photosynthetic biomass, we extracted chlorophyll-*a* from soil samples at the plot level through three pooled 3 cm length × 3 cm width × 0.5 cm depth soil surface collections to provide a single homogenized sample for each plot. To understand photosynthetic potential over time, we measured chlorophyll-*a* in all plots following the same sampling season (late May–early June) on dry soils across years (2015, 2016, and 2017). Our chlorophyll-*a* extraction protocols followed standard soil extraction and analysis methods as described in Ritchie (2006) and Castle et al. (2011), and then further refined in Chock et al. (2019) using acetone as the solvent and classified chlorophyll-*a* wavelengths using a Spectrophotometer (Ocean Optics CHEMUSB4-VIS-NIR). Cyanobacteria were our dominant group and the chlorophyll-*a* extraction methods were chosen to best represent this group. We calculated μg of chlorophyll-*a* g^{-1} of soil rather than area due to the fact we did not collect bulk density in the top 5 mm of the soil surface (Lan et al. 2011) and samples were needed to be comparable across years. Thus, our reported numbers should be considered a conservative estimate. As observed in other studies (Bowker et al. 2002), chlorophyll-*a* concentrations varied temporally in our two control plot types (Figure S1). To address this, when looking at recovery over time, and across treatments, we divided chlorophyll-*a* in individual plots and multiplied by 100 to obtain a percent by corresponding control plot average in a given year, providing a more realistic recovery value.

Soil aggregate stability in water was also assessed by collecting six predetermined 6–8 mm diameter soil surface aggregates to a depth of 2–3 mm from each plot. After collection, we measured a stability index in the field using a test kit (Herrick et al. 2001). The six measurements within each plot were averaged to provide a single value for each plot (index scale of 1 to 6 with greater value indicating greater stability in water). Biocrust ground cover was also assessed with point intercept methods (Supplement S1, Table S1).

Statistical Analyses

All statistical analyses were performed in R (R Core Development Team, 2017 version 3.3.4). For the main effect analysis, soil type was nested as a discrete grouping within desert location ("fine" or "coarse" with the knowledge that variability in texture classes may exist within sites). Dependent variables were chlorophyll-*a* levels, plot-level soil stability index average, and ground cover observations (Tables S1, S2, and S3). None of the data met assumptions of normality required for parametric statistical tests and were not able to be transformed. To address this, permutational ANOVAs were used with the lmPerm R package (Wheeler et al. 2016) to test for treatment differences across year, desert location, or soil type. If differences were observed in the main effects year × desert × soil type (nested within desert) × treatment level permutations ($n = 999$), we separated variables and applied post hoc pairwise comparisons using the RVAideMemoire Package (Herve 2019). The pairwise comparisons included testing differences within a treatment across years or across treatments within a year. To control for Type I error inflation that can occur from repeat pairwise testing, we used a False Discovery Rate correction (Benjamini & Hochberg 1995) and an alpha of 0.05 to denote significance.

Results

Biocrust Recovery

The UTTR-fine site generally had higher mean chlorophyll-*a* levels (18.7 ± 1.3 μg/g soil for intact controls and 4.7 ± 0.5 μg/g soil for all treatments with a disturbance) than its partnered coarser-textured site (15.8 ± 2.4 μg/g soil for intact controls and 3.0 ± 0.2 μg/g soil for disturbed treatments). In the final observation year of 2017, the Jornada-fine site had higher chlorophyll-*a* concentrations (10.9 ± 2.3 μg/g soil in intact controls and 5.0 ± 0.5 μg/g soil in disturbed treatments) than the Jornada coarser- textured site (0.5 ± 0.1 μg/g soil in intact controls) and treatment were consistently higher, although not significant, in the field-collected than the greenhouse-cultivated or lab-cultivated inoculations as well as uninoculated disturbed sites across sites (Table 2). The effects of PAM and straw additions were not distinctly different from one another and effects relative to disturbed controls depended upon desert and soil texture (Table 2). The full main effects model on chlorophyll-*a* concentrations demonstrated that desert type ($p < 0.0001$) and treatment ($p < 0.0001$) were both significant variables; however, multiple significant interactions occurred (Table S2).

In considering chlorophyll-*a* concentrations and possible recovery across years, the percent chlorophyll-*a* (as determined by its relationship to associated control concentrations) generally increased from 2015 to 2017 across all sites except for the Jornada-coarse site (Fig. 3A–D). The increase in percent chlorophyll-*a* within individual deserts and soil types was not uniform across applied treatments. For instance, the UTTR finer-textured site in 2017 had an average of 25 ± 2.5% chlorophyll-*a* concentration as compared to the intact control, whereas the UTTR coarser-textured site had a slightly lower percentage (19 ± 1.2%). All treatments in the UTTR-fine site

Table 2. Mean chlorophyll-*a* concentrations and soil stability index values from the final observation period (2017) across desert and soil type for inoculum and habitat amelioration treatments. The Jornada-coarse site did not have field-collected inoculum and is represented by na. Differences represented by 1 ± SE.

		Inoculum			*Habitat Amelioration*		
Desert	*Soil type*	*Inoculum trtmnt*	*chl* a	*Soil stab*	*Habitat trtmnt*	*chl* a	*Soil stab*
UTTR	Coarse	None	2.9 ± 0.3	4.5 ± 0.2	None	1.9 ± 0.3	4.1 ± 0.5
		Field collected	3.9 ± 0.5	5.2 ± 0.2	PAM	2.8 ± 0.2	5.0 ± 0.1
		Greenhouse cultivated	2.8 ± 0.3	4.6 ± 0.3	Straw	3.5 ± 0.3	4.6 ± 0.2
		Lab cultivated	2.5 ± 0.3	4.8 ± 0.2			
	Fine	None	4.0 ± 0.6	4.3 ± 0.3	None	4.7 ± 1.0	4.9 ± 0.7
		Field collected	7.1 ± 1.6	4.7 ± 0.4	PAM	5.0 ± 0.8	4.7 ± 0.3
		Greenhouse cultivated	3.8 ± 0.6	5 ± 0.3	Straw	4.4 ± 0.7	4.4 ± 0.3
		Lab cultivated	4.2 ± 0.7	4.6 ± 0.4			
Jornada	Coarse	None	0.3 ± 0.0	2 ± 0.2	None	0.2 ± 0.1	1.4 ± 0.3
		Field collected	na	na	PAM	0.2 ± 0.0	2.6 ± 0.3
		Greenhouse cultivated	0.1 ± 0.0	1.9 ± 0.3	Straw	0.2 ± 0.0	1.5 ± 0.2
		Lab cultivated	0.2 ± 0.0	1.9 ± 0.3			
	Fine	None	4.9 ± 0.7	5.5 ± 0.1	None	3.5 ± 0.6	5.5 ± 0.2
		Field collected	6.2 ± 1.9	5.4 ± 0.2	PAM	5.0 ± 0.8	5.3 ± 0.2
		Greenhouse cultivated	4.5 ± 1.0	5.3 ± 0.2	Straw	5.4 ± 1.0	5.5 ± 0.1
		Lab cultivated	4.1 ± 0.7	5.3 ± 0.2			

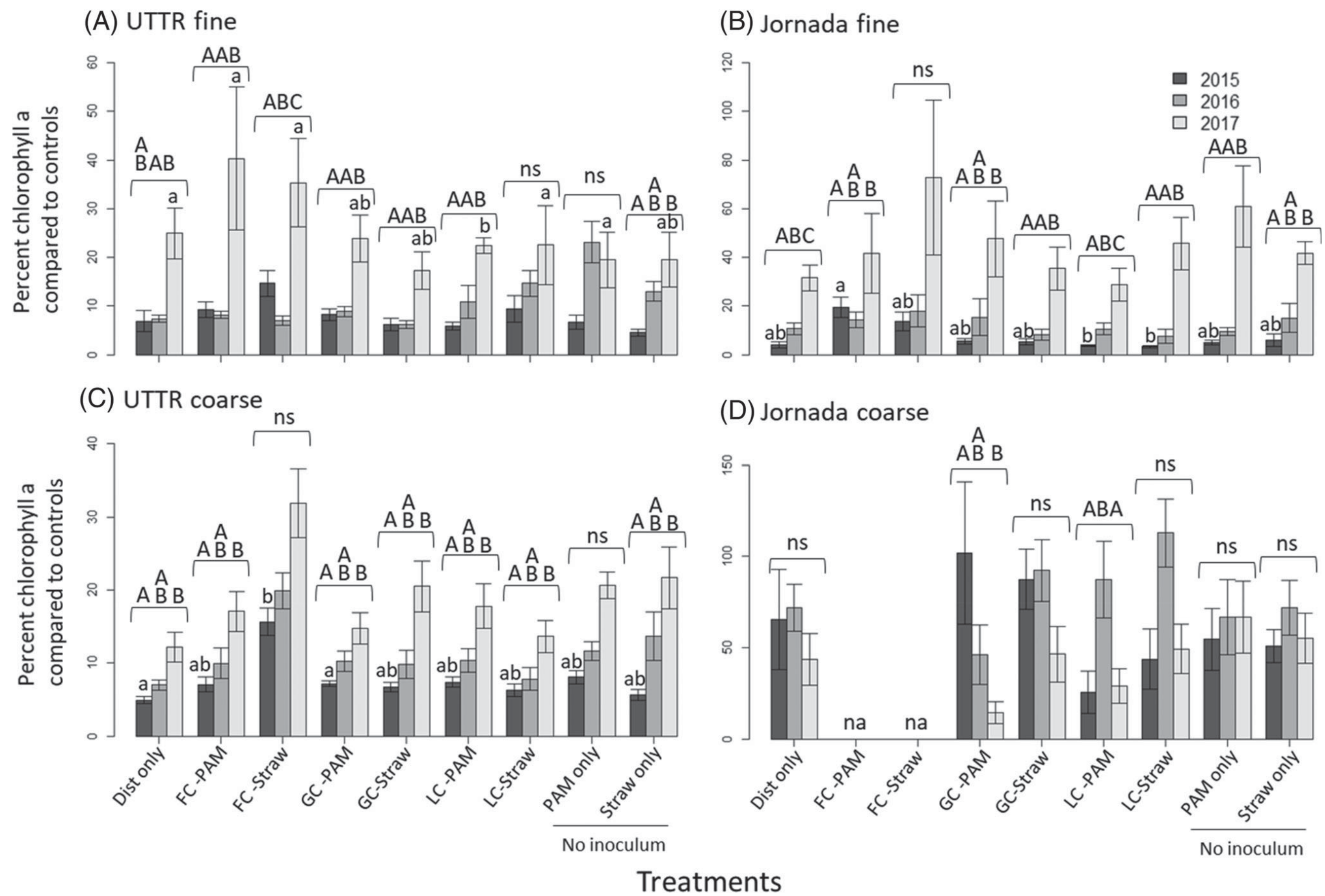

Figure 3. Percent chlorophyll-*a* of nine treatment plots as related to chlorophyll-*a* levels of the control plots for associated year for the four different field sites. Lower case letters indicate post hoc pairwise significant differences across treatments within a year, capital letters indicate significant differences across years within a single treatment (alpha set at 0.05). The Jornada-coarse textured soil site did not have field-collected inoculum available (represented by "na"). All plots were disturbed, Dist are plots that received disturbance and no subsequent inoculum or habitat amelioration treatment, FC are plots that received field-collected inoculum, GC are plots that received greenhouse-cultivated inoculum, LC are plots that received laboratory cultivated inoculum, PAM are plots that received polyacrylamide, and Straw are plots that received straw barriers. Values are means and error bars indicate ±1 SE from the mean.

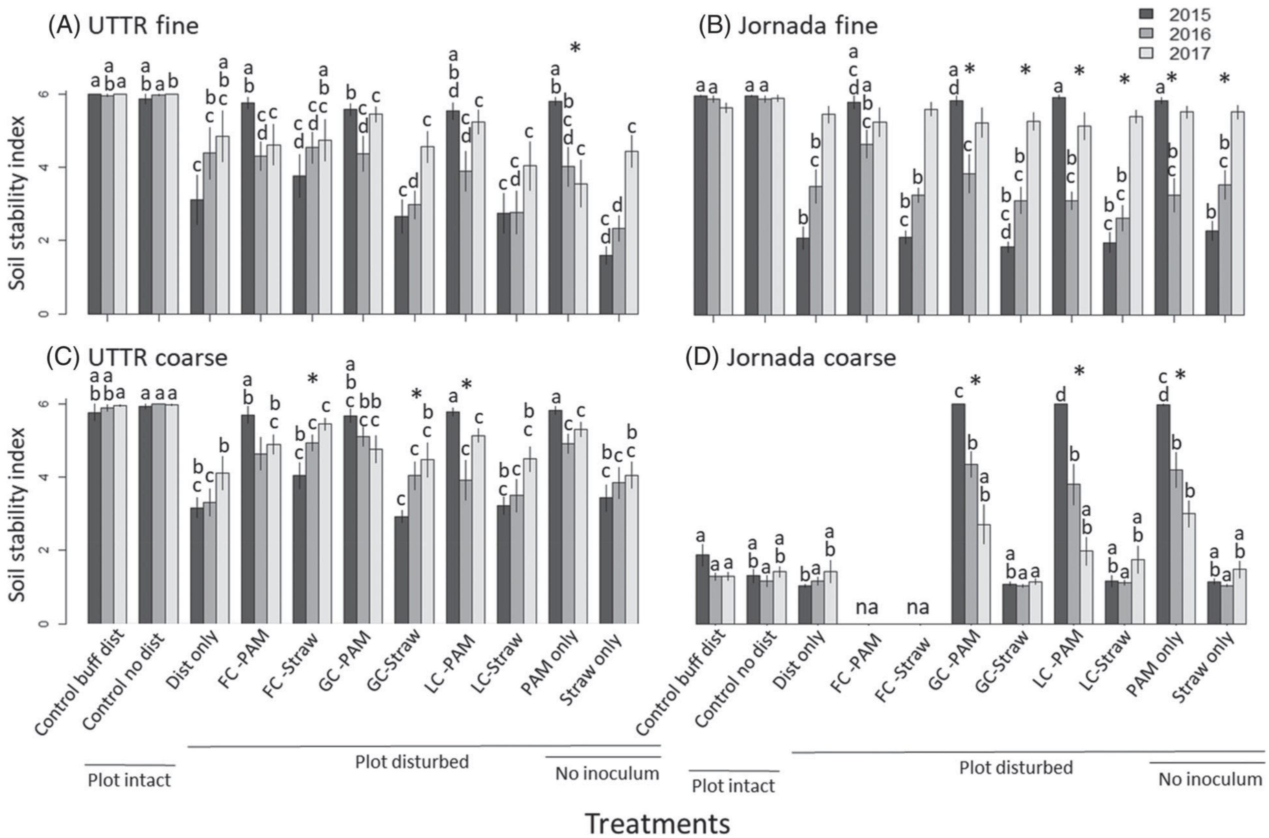

Figure 4. Soil stability index measurements across treatments and years separated by desert and soil type. Letters indicate pairwise post hoc significant differences (alpha set at 0.05) within a single year across treatments and an asterisk indicates a significant difference between years within a treatment.Control buff dist and Control no dist are plots left intact with or without a 1-m disturbance surrounding the primary plot. Dist only are plots that received no subsequent inoculum or habitat amelioration treatment, FC represents field-collected inoculum, GC, greenhouse-cultivated, and LC, lab-cultivated. PAM are plots that received polyacrylamide and Straw are plots that received straw barriers. Jornada-coarse site did not have adequate biocrust for field collection and thus is represented with na. Values are means and error bars indicate ±1 SE from the mean.

demonstrated an increase in chlorophyll-*a* overtime except for the lab-cultivated inoculum with straw and the PAM-only treatment. In the UTTR coarse-textured site, the two treatments that did not show a significant increase over the 3 years were the field-collected inoculum with straw and the PAM-only treatments. The Jornada-fine site generally had increased percent chlorophyll-*a* levels in 2017 as compared to the intact controls (Fig. 3B). The only treatment in the Jornada-fine site that did not have detectable increase over the years was the field-collected + straw treatment, likely due to high among-plot variation. The Jornada coarse-textured site had no clear trends in chlorophyll-*a* either across treatments or years.

Soil Stability

The intact control plots, excluding the Jornada-coarse site, maintained high stability (obtaining near the highest observable score of 6) across years (Fig. 4A–D) suggesting these landscapes are characterized by stable soils when undisturbed (Fig. 4). The different inoculum types did not have a strong influence on aggregate stability at any of the desert locations or soil types in 2017 (Table 2) or across all treatments (except for field-collected inoculum in all sites except for Jornada-coarse), and years (Fig. 4). The full permutation model of the soil stability index showed significant differences for desert and treatment, but not for year, but with multiple significant interactions (Table S3). The PAM addition created an initially high soil stability level (the highest possible soil stability measurement of 6 was commonly observed), even in the highly unstable Jornada-coarse site (Fig. 4D), which decreased over time. However, after 2 years of observation, the soil stability index generally increased across the different disturbed treatments (Fig. 4A–C) with a mean value greater than 4 observed (excluding the low-stability Jornada-coarse site; Fig. 4D). In the Jornada-fine site, the mean soil stability index increased with time and there were no treatment differences in 2017 (Fig. 4B).

Discussion

The need to understand best practices in biological soil crust restoration is growing in importance as drylands are expanding in size and the degradation within their boundaries is increasing (Bowker 2007, and see articles within this special issue). As is true in many ecological restoration projects, identifying the best propagule source or type, as well as altering abiotic conditions to facilitate restoration success, should be considered when attempting biocrust restoration projects (Bowker 2007). This study addressed the need for understanding how different biocrust sources (e.g. field-collected or greenhouse-cultivated) and different concomitant habitat treatments (e.g. straw) can be used to facilitate biocrust restoration over a multi-year observation period. Through field testing this combination, our results suggest that, in some cases, natural recovery may be as effective as current active restoration methods depending on the starting conditions of the site being restored.

Biocrust Recovery

Biocrust community abundance and composition is variable across deserts of North America, with hotter/drier deserts generally having lower amounts of lichens and mosses (Belnap 2006). Nevertheless, biocrust communities are thought to have similar recovery trajectories, albeit at different rates, marked by filamentous cyanobacteria being early colonizers followed by moss and lichen colonization. Moss and lichen colonize more rapidly (and are more common) in cooler/wetter north American deserts (Belnap 2006; Xhao et al. 2016). Within our desert locations, the different soil types were purposely chosen to be close to one another (<15 km) to reduce the effect of climate or weather differences and aid in understanding biocrust recovery across soil types. We hypothesized that biocrust recovery would be enhanced by our efforts and occur over our observation period. Our results found generally higher chlorophyll-*a* levels in our finer-textured sites in the first sampling period and this trend continued across observation years. Finer-textured soils may have faster cyanobacterial recovery rates likely due to smaller soil pore sizes which require less energy to colonize (Zheng et al. 2011; Rosenstein et al. 2014) and which are more readily bound into soil aggregates through exopolysaccharide exudation (Chock et al. 2019).

Limiting exposure to saltating soil particles is also an important component to natural recovery and the potential for restoration success (Gile 1966). Highly mobile soils in the Jornada region in the coarse site are not uncommon, and have been exacerbated with loss of perennial grasses, shrub encroachment, and altering soil textures through dune formation (Gile 1966). This high soil mobility setting is a challenge for biocrust establishment, and it appears our habitat-ameliorating treatments were insufficient to address these harsh abiotic conditions beyond the first year. The Jornada fine-textured site also had visual indicators of surface soil and water movement, but at a lower magnitude. The UTTR sites, both coarse and finer textured, did not appear to have the same soil movement as the Jornada sites, likely due to high-intensity storms in summer rainfall at the Jornada associated with localized convective patterns. Biocrust dispersal mechanisms warrant further studies as they could provide valuable insight into biocrust recovery through passive and active restoration. These processes are especially important to understand for successful biocrust restoration as even when biocrust do establish they are not likely resistant to intense rain or wind for some number of years (Belnap & Büdel 2016; Chamizo et al. 2017).

Finally, while biological inocula and habitat ameliorations had some involvement in structuring outcomes, natural biocrust recovery across the different sites likely helped to drive the observed results (Weber et al. 2016). The background availability in three of the sites likely drove the higher observed chlorophyll-*a* levels and great soil stability, while the Jornada-coarse site did not see the same recovery and did not have the same outside level of propagule availability.

Inoculum and Habitat Amelioration Influences

This study provided a unique perspective of field-testing inoculum-type efficacies coupled with habitat amelioration efforts. While other greenhouse efforts have targeted moss biocrust greenhouse cultivation (Doherty et al. 2015; Antoninka et al. 2016; Bu et al. 2018) the main, and novel, component of the cultivated inoculum additions in this study were composed of lightly pigmented cyanobacteria species specific to the site where they were collected (Velasco-Ayuso et al. 2017; Giraldo-Silva et al. 2019a). The field-collected crusts, however, contained a proportion similar to ambient communities at the time of collection. For instance, the UTTR site had a higher lichen concentration than the Jornada site and thus a higher proportion of lichen was included in the field-collected inoculum. While a greater percent recovery, as suggested by generally higher chlorophyll-*a* levels across years, was observed in the field-collected crusts over the other added biocrust inoculum types, these differences were variable across desert location and soil type. The other two inoculum types, greenhouse cultivated and lab cultivated, did not consistently show recovery differences outside of those seen in disturbed-only plots. Studies in addition to this one support the difficulty in successfully establishing cultivated inoculum (Chandler et al. 2019), although there are also cases in which inoculum successfully establishes (Antoninka et al. 2018; Bu et al. 2018). Our research sought to test different habitat amelioration efforts and the variable outcomes observed in our results point to site-level variation and influence as well as different inoculum types and techniques (e.g. Bowker et al. 2019), all requiring further study.

Coupled with our inoculum additions we also tested habitat amelioration methods that had similar responses over time, regardless of inoculation. Straw barriers have been highly successful at stabilizing dunes and enhancing biocrust recovery in some sites (Xhao et al. 2016). These studies, however, are generally longer term than ours and provide additional time for the stabilized soils to accumulate biomass and diversity. The high initial soil stability measurements observed in the PAM-treated plots were expected, as the PAM is designed to hold the uppermost soil surface in place for a finite amount of time (Wang et al. 2011). Our data suggest that recovery of photosynthetic capacity is occurring in the plots, and that biocrust is replacing the stabilizing function originally provided by the PAM. Although we artificially removed the propagules through our scraping treatment and imposed a 1-m buffer, biocrust propagules were present at the site and could have dispersed into our plots. To better test if sites truly are propagule limited and if our efforts aid in recruitment in the absence of nearby intact biocrust, study sites would need to be in highly degraded areas with no known propagule source directly nearby (e.g. Fick et al. 2019a, 2019b). In summary, although we had limited success in accelerating biocrust recovery beyond natural rates across our different inoculum and habitat amelioration treatments, as hypothesized, we remain optimistic as this study provides a platform for further pursuits in understanding limitations to best biocrust restoration practices, and identifying ways to overcome these limitations.

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CONFLICT OF INTEREST

The authors have no known conflicts of interest to state upon publication of this article.

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Supporting Information

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

Figure S1. Chlorophyll-*a* concentrations in soil (0–0.5 cm depth) of two types of control plots over the 3 years of monitoring across the desert locations and soil type sites.
Supplement S1. Description of methods and results for background ground cover observations
Table S1. Mean 2017 ground cover estimates by desert location, soil type, and biocrust related functional group.
Table S2. Main effects permutation ANOVA output for chlorophyll-*a* concentrations across main factors of year, desert location, soil type, and treatment and associated interactions. Soil type is nested in desert type in the model.
Table S3. Main effects permutation ANOVA output for soil stability index when wet across main factors of year, desert location, soil type, and treatment and associated interactions.

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