Plant Soil
DOI 10.1007/s11104-017-3300-3

REGULAR ARTICLE

Maximizing establishment and survivorship of field-collected and greenhouse-cultivated biocrusts in a semi-cold desert

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Received: 31 March 2017 / Accepted: 25 May 2017

Abstract
Aims Biological soil crusts (biocrusts) are soil-surface communities in drylands, dominated by cyanobacteria, mosses, and lichens. They provide key ecosystem functions by increasing soil stability and influencing soil hydrologic, nutrient, and carbon cycles. Because of this, methods to reestablish biocrusts in damaged drylands are needed. Here we test the reintroduction of field-collected vs. greenhouse-cultured biocrusts for rehabilitation.
Methods We collected biocrusts for 1) direct reapplication, and 2) artificial cultivation under varying hydration regimes. We added field-collected and cultivated biocrusts (with and without hardening treatments) to bare field plots and monitored establishment.
Results Both field-collected and cultivated cyanobacteria increased cover dramatically during the experimental period. Cultivated biocrusts established more rapidly than field-collected biocrusts, attaining ~82% cover in only one year, but addition of field-collected biocrusts led to higher species richness, biomass (as assessed by chlorophyll *a)* and level of development. Mosses and lichens did not establish well in either case, but late successional cover was affected by hardening and culture conditions.
Conclusions This study provides further evidence that it is possible to culture biocrust components from later successional materials and reestablish cultured organisms in the field. However, more research is needed into effective reclamation techniques.

Keywords Biological soil crust · Drylands · Hardening · Field establishment · Ecological restoration · Ecological rehabilitation · Soil erosion resistance

Abbreviations
LOD Level of biocrust development
UTTR Utah Test and Training Range

Responsible Editor: Jayne Belnap.

Electronic supplementary material The online version of this article (doi:10.1007/s11104-017-3300-3) contains supplementary material, which is available to authorized users.

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Published online: 09 June 2017

Introduction

Biological soil crusts (biocrusts) are a critical component of semiarid and arid ecosystems, providing foundational structure and function in numerous ways; for example, influencing plant establishment, controlling the inputs and cycling of nutrients and carbon into soils, stabilizing soil surfaces, and impacting hydrology (reviewed in Belnap et al. 2016). Further, the dryland ecosystems where biocrusts are common are among the most degraded on Earth due to pressures such as grazing, cropland extensification, and climate change (Reynolds et al. 2013). While local consequences of biocrust loss are obvious (e.g., increased soil erosion and loss, exotic plant species invasion), the regional and global effects can be equally important. For example, loss of biocrust in disturbed drylands in the US Southwest enhances dust emissions which, by accelerating snowmelt, can reduce input to major rivers (Painter et al. 2010). A recent study also suggests that a loss of late-successional biocrust could have such an extensive influence it could directly affect the Earth's energy balance via changes to dryland surface albedo (Rutherford et al. 2017).

Although an improved capacity to rehabilitate biocrusts could provide many benefits to drylands, only limited progress has been made in rehabilitation technology. An effective and simple method to reestablish biocrusts is to collect biocrusts from areas with high cover and redistribute the collected material to areas with limited or no biocrusts (Belnap 1993; Chiquoine et al. 2016; Condon and Pyke 2016). However, the harvesting of intact biocrust communities can lead to significant secondary disturbance. In addition, the disturbed environment itself may present multiple barriers to successfully establishing reintroduced biocrusts. Rehabilitation methods have to effectively address the ecological constraints that may hinder reestablishment of a functioning biocrust community (Bowker 2007). For example, spreading inoculum on unstable soils will likely be ineffective because both soils and added biocrusts will eventually be transported away from the rehabilitation site by wind or water. Instead, it may be necessary to first address the highest order barrier (e.g. stabilizing soils) before addition of inoculum will be successful. Other challenges, such as climate suitability, selection of target species, and determination of restoration goals are important to consider in planning rehabilitation (Zhao et al. 2016).

A recent key advance has been the cultivation of biocrust components in the lab, which when used in place of the field grown biocrust significantly reduces the amount of field disturbance required for inoculum. This process involves harvesting small amounts of material in the field and then expanded under laboratory or greenhouse conditions, either as individual components or as a community (eg. Lan et al. 2013; Bu et al. 2014; Ayuso Velasco et al. 2016). This is an important step because cultivation of biocrusts enhances the amount of biocrust that can be used for a given problem without causing a new disturbance by harvesting intact biocrusts as an inoculum source. Using light pigmented cyanobacteria cultures has been the most common practice for a variety of reasons: because they are early- to mid-successional in most drylands, among the first biocrust colonizers following disturbance; because they can be grown rapidly and in large quantity in liquid culture; and because they provide the ecosystem benefit of increased soil stability (Bu et al. 2014; Weber et al. 2016). There are two important disadvantages to these methods. First, successful establishment generally requires irrigation, which is not feasible for many arid and remote regions where water delivery is not possible (Zhao et al. 2016). Second, other organisms such as mosses and lichens, which contribute additional benefits to ecosystem structure and function and even attain dominance in many drylands, are not contained within these inocula.

Mixed cultures of later successional biocrust (including dark-pigmented cyanobacteria, moss and lichens) and reintroduction have received less research attention. Experimental culturing of biocrust mosses has been successful in a variety of systems, and with a variety of species (e.g. Chen et al. 2009; Xiao et al. 2011; Antoninka et al. 2015). Mosses can make up 30% or more of the biocrust cover in many drylands, providing significant ecosystem services in terms of dust capture, soil stability, water holding capacity and carbon fixation (reviewed by Seppelt et al. 2016). Moss also adds fertility to the soil by supporting the soil food web and housing N_2-fixers on their leaves (Wu et al. 2009). Although less studied, some lichens have been successfully cultured (Antoninka et al. 2015; Bowker and Antoninka 2016), and efforts to understand their needs in field transplant experiments have also occurred (Davidson et al. 2002). Lichens add ecosystem

function by providing armor that reduces weed seed germination, further stabilizes soil, alters soil albedo and, in many cases enhances N_2-fixation (reviewed by Rosentreter et al. 2016).

Early successional species, such as cyanobacteria in this case, are commonly used for rehabilitation under the assumption that they are the best equipped to survive and may facilitate colonization by later successional species. However, this assumption remains untested for biocrusts. Another strategy might be to introduce several species with differing successional preferences and divergent functional traits, and to allow the environment to select which are most likely to be successful. Thus, applying a mixed inoculum of multiple functional groups may optimize the chances of biocrust establishment and may enhance ecosystem multi-functionality due to complementarity of functional traits (Delgado-Baquerizo et al. 2016; Bowker et al. 2010; Bowker and Antoninka 2016). Here we test two methods of reestablishing mature, mixed species biocrusts in the field: 1) immediately re-applying field-collected biocrusts (experiment 1), and 2) inoculating with greenhouse-cultivated biocrusts made up of cyanobacteria, mosses and lichens, that were grown under a range of conditions (Experiment 2). Field-collected biocrusts were assumed to be "field-hardened". In contrast, growing crust organisms in the greenhouse provides an opportunity to prepare large amounts of inoculum with minimum site disturbance, yet these biocrusts are not hardened. To determine how best to establish greenhouse-cultivated mixed moss, lichen and cyanobacteria biocrusts under field conditions, we cultivated biocrusts in the greenhouse under conditions varying in the number of days continuously watered per week. We then conditioned each of these cultured communities with one of three hardening treatments and subsequently applied them back to the field site from which the biocrusts were originally collected (Supplemental Fig. 1). We expected field-collected biocrusts to reestablish most successfully, given that they had been exposed to field growing conditions the longest. Likewise, we expected our cultured biocrusts with shorter hydration periods over the growing period, and with the harshest hardening conditions, to have the greatest survival and establishment in the field as they would be most accustomed to field-like conditions.

Methods

Experiment 1. Field-collected Biocrust trial

In April of 2013, we collected intact biocrusts from a site within the Utah Test and Training Range (UTTR, 41°0625.60″ N 113°30′04.24 W, elevation ~1295 m) located in the Great Basin, Great Salt Lake Area. Soils collected from this site were characterized by silt loam soils in the Skumpah series. To collect field grown biocrust material, we scraped biocrusts from the surface of the soil to a depth of 0.5-1 cm using metal dust pans. Adhering soil below biocrusts was also removed by scraping. We crumbled biocrusts by hand to fragments no larger than 0.5 cm, with the majority in the range of 0.1–0.2 cm, and homogenized the inoculum. The collected inoculum was a mix of light-pigmented cyanobacteria (dominated by *Microcoleus vaginatus*; 28.6% ±6.5 cover), dark-pigmented cyanobacteria (a mixture of *Nostoc* sp., and *Scytonema* sp.; 45.8% ± 6.9 cover), lichens (primarily *Collema* spp., *Placidium* spp., and some *Psora decipiens;* 17.2 ± 6.0 cover), and moss (primarily *Syntrichia caninervis*: 8.4% ± 2.1 cover). In the same location, we created 10 replicate 25 cm × 25 cm plots, with randomly selected control (uninoculated) and inoculated plots ($n = 5$). We selected plots that were level, free of vascular plant vegetation, and a minimum distance of 1 m away from the nearest shrub. To prepare the plots for treatment, we scraped away the top 2 cm of biocrusts and soil with a metal dust pan, carefully removed all surface materials, and evened out the soil surface by breaking up soil aggregates and smoothing by hand. We treated inoculated plots by hand-broadcasting 125 ml of crumbled inoculum to cover about 10% of the surface area.

Experiment 2. Cultivated and hardened Biocrust trial

Collection and cultivation conditions

We used the greenhouse cultivated biocrusts collected from the experiment described in Antoninka et al. (2015) to cultivate a mixed species biocrust community in the greenhouse. In brief, we collected the common desert mosses *Syntrichia caninervis* and *S. ruralis* from the UTTR on Murray Mesa (41°0222.80″ N 112°5939.10, elevation ~1450 m, Amtoft, dry-rock outcrop complex, 30 to 70% slopes) in April 2013. We treated collected mosses by washing, breaking into small fragments, and cultivating on autoclaved sand (Bowker

and Antoninka 2016), with a watering system that wicks water from below to the moss at the surface (Doherty et al. 2015). While moss was the initial focus of the collection, our methods resulted in the growth of a mature biocrust community that included light and dark pigmented cyanobacteria, lichens, and mosses (Antoninka et al. 2015). All biocrusts were cultivated for six months in one of four watering treatments (5, 4, 3, or 2 days per week of continuous hydration, followed by a weekly drying event). Weekly drying events were used in an effort to maintain the biocrust community dominance over potential weedy species coming in from the greenhouse environment, which do best in continuous hydration.

Hardening conditions

During cultivation, the biocrusts were grown under reduced UV and milder climate conditions than they would experience in the field. Thus, we also tested whether "hardening" them would increase survival and growth. The biocrust experimental trays of greenhouse-cultured material (Antoninka et al. 2015) were allowed to slowly dry in the greenhouse for one week. To consolidate the number of units per hardening treatment, we pooled units from each experimental watering treatment and collected the cultivated biocrusts. To this we tipped each unit over a 1 mm mesh sieve to separate the biocrusts from the underlying sand. The surface biocrusts generally remained intact, while the sand particles passed through the sieve with some light shaking. We then disaggregated the harvested biocrusts with a 2 mm sieve and homogenized gently by mixing. We placed 1 cm of autoclaved sand into each of 12–0.4m^2 plastic basins (3 × 4 watering treatments) with 16–0.3 cm holes drilled in the bottom and covered with cotton cloth to allow for drainage and to keep the sand in place. We then sprinkled 400 ml of inoculum evenly over the surface of each basin.

We applied three hardening conditions to the four inoculum types (Supplemental Fig. 1): 1) no hardening: kept in the greenhouse and provided luxury water, 2) moderate hardening: kept outside with 50% of natural UV and given low water conditions, or 3) severe hardening: kept outside with full UV and low water conditions. This resulted in 12 separate inocula treatments (i.e., four initial watering conditions and three subsequent hardening conditions). The unhardened treatment units (control) were placed in basins in the greenhouse, and hydrated daily with DI water using a pump sprayer from above to achieve full hydration of the biocrust organisms lasting 24 h per day. This was achieved by timed spraying equivalent to ~2 L water per day, which saturated the soil without pooling. We placed the remaining units outside adjacent to the greenhouse in an area that receives no natural shading. In both cases, we hydrated basins for 2–3 h per day by watering from above until the surface was moist with a timed spray, resulting in ~0.5 L per unit per day. We created the "moderate" treatment by covering the basins with a shade cloth that removes 50% of incoming solar radiation to separate the effects of exposure to short hydration periods and the effect of UV light exposure. No precipitation occurred during this time and daily outside average high temperature was 26C and average low temperature was 3C, compared to inside the greenhouse at 25 ± 3C. We applied all treatments for 21 days, and allowed three days for complete drying before we harvested and homogenized as described above.

Field application

We located our experimental plots near to where the inoculum material was initially collected (41°1129″ N 112°99,646, elevation ~1295 m, Skumpah silt-loam, 0 to 2% slopes). We designated 78, 50 cm × 50 cm plots in October 2015 that were level, free of vascular plant vegetation, and no closer than 1 m to the nearest shrub. We scraped the surface and removed all biocrust materials. In the center of each plot, we designated a 25 cm × 25 cm area, surrounded by a 12.5 cm × 12.5 cm buffer area, marked on the corners with nails. The buffer areas were intended to decrease biocrust colonization from the plot edge. We randomly assigned treatments and created six replicate plots for 12 treatment types (four watering by three hardening combinations, plus controls). Each inoculated plot received 125 ml of crumbled inoculum to cover ~10% of the surface area. Constituents of the greenhouse-grown inoculum varied, depending on the watering treatment under which they were grown, but in all cases they were strongly dominated by dark pigmented cyanobacteria and contained a mix of early-, mid- and late-successional biocrust members (Supplemental Table 1).

Measurements

We monitored the two experiments at different intervals. Experiment 1 was monitored at 14 months (June 2014)

and 26 months (June 2015) after inoculation, and Exp. 2 was monitored six months (April 2015) and 12 months (October 2015) after inoculation. We assessed each plot for biocrust cover, biomass, and stability. We used the point intercept method with 20 points to estimate biocrust cover (Jonasson 1983). Species not captured by the points were noted at 2.5% cover. We assessed the biocrust level of development (LOD) using methods described in Belnap et al. (2008). This method correlates well with biocrust maturity on a scale of 1–6, where 1 represents an early successional light cyanobacteria crust, and 6 represents a fully developed, mature biocrust dominated by dark cyanobacteria, lichens, and mosses. Species richness was calculated by summing the number of cyanobacteria, moss and lichen species recorded in each plot. We used chlorophyll *a* concentrations as a proxy for phototrophic biomass. From each plot we collected and pooled five soil cores (1 cm diameter by 0.5 cm depth) from the randomly selected points. We extracted chlorophyll *a* using the methods of Castle et al. (2011). We measured soil aggregate stability using a field-based test kit based on immersion and wet sieving (Herrick et al. 2001). We obtained climate data from the Utah Climate Center from a weather station on the UTTR (Station Network: GHCN:COOP; Station ID: USC00428987; 41°0497 N, 112°9370 E, 1353 m; https://climate.usurf.usu.edu/).

Statistical approach

Experiment 1. Field-collected Biocrust trial We used one-way repeated measures MANOVA to analyze differences in biocrust cover, composition, and soil stability through time with and without inoculum additions. Post-hoc, we also used one-way ANOVA to test for differences in response variables based on inoculation within a sampling date when time was a significant factor, after checking for homogeneity of variance and normal distribution using SAS-JMP 14.0.

Experiment 2. Cultivated and hardened Biocrust trial We used two-way repeated measures MANOVA to analyze differences in the community composition by culture and hardening conditions. Inoculated plots required a separate test in order to compare them to uninoculated controls, because controls were not replicated across all treatment combinations. To do this, we used one-way repeated measures MANOVA. We also used one-way ANOVA to look for differences at each time point post-hoc when time was a significant factor in the MANOVA, after testing data for assumptions of homogeneity of variance and normal distribution. Post-hoc Tukey's HSD were performed for pairwise comparisons and to determine which treatments were different when interaction terms were significant.

Data availability The datasets generated during and/or analyzed during the current study are not publicly available because collaborative research results have not all been published at this time, but are available from the corresponding author on reasonable request.

Results

Experiment 1. Field-collected Biocrust trial Overall, we observed increases in chlorophyll *a* and LOD with inoculation, and increased species richness with time, regardless of inoculation (Fig. 1; Supplementary Table 2). Chlorophyll *a* increased more than 7X after 14 months, and more than 4X after 26 months, compared to inoculum addition; however the values at 26 months were lower in both inoculated and control plots compared to 14 months (Supplemental Table 2), which might be explained by seasonal differences in our sampling. LOD has not approached the level of the surrounding biocrusts (LOD = 6), but we did see an increase with inoculation (Supplemental Table 2). Species richness also increased with time, regardless of inoculation, exceeding the initial inoculum species count by nearly two species at 26 months in both inoculated and control plots (Supplemental Table 2). Light cyanobacteria cover, species richness, and chlorophyll *a* were greater in inoculated plots compared to controls after 14 months, but not at 26 months (Supplemental Table 2; Fig. 1). Soil aggregate stability was not different at any time point between inoculated and control plots. Control plots and inoculated plot cover were similar after 26 months for all measures except LOD, which remained elevated in inoculated plots. Field-collected biocrusts established well in our inoculated plots, with a light cyanobacteria cover of ~55% and late successional cover of ~14.5% at 14 months. However, recovery slowed, and even reversed in some cases by 26 months, such that controls equaled inoculated plots, and in some cases had less than controls (Supplementary Table 2). We observed a 250% positive change in light cyanobacteria after 14 months, followed by a 190%

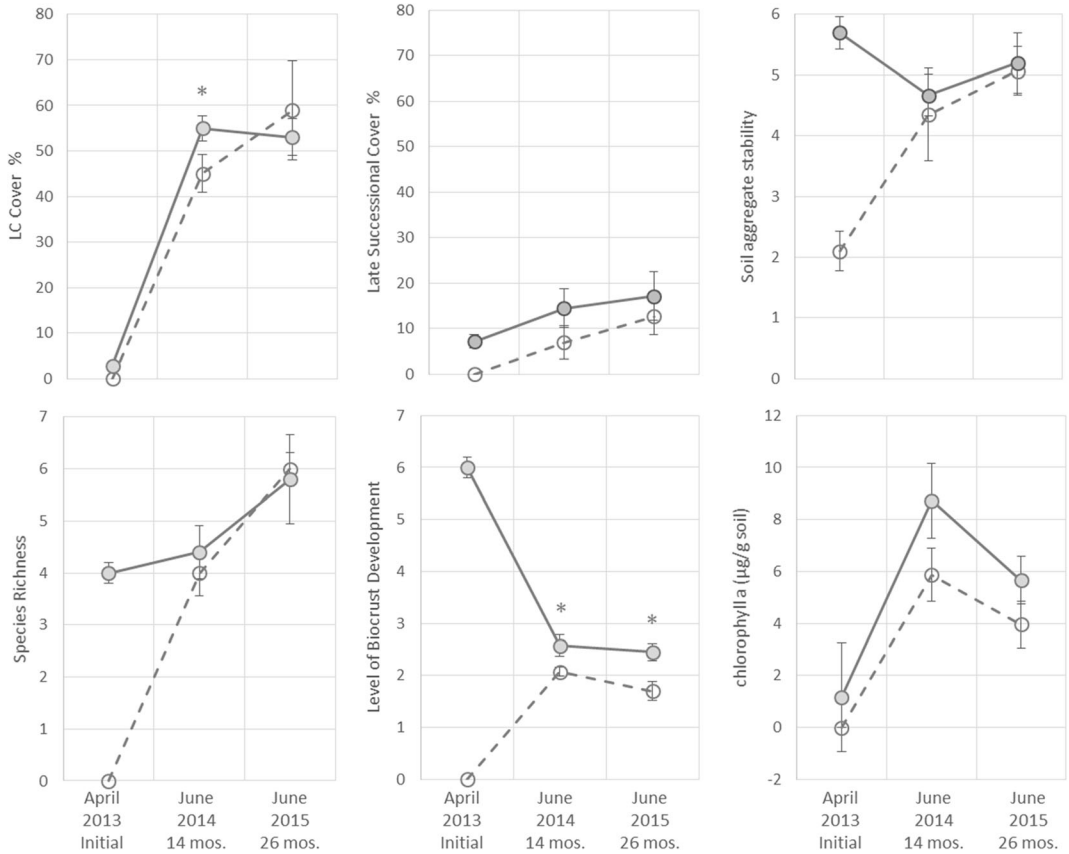

Fig. 1 Change in biocrust metrics from initial to 26 months are given for Experiment 1 (field-collected inoculum, $n = 5$). Open symbols with dashed lines represent control plots, whereas closed symbols with solid lines represent inoculated plots. Error bars are one SE of the mean. (* = significant differences at $p \leq 0.05$) determined by one-way ANOVA at a given time point. Differences were not tested for the initial inoculation. LC = light pigmented cyanobacteria

negative change after 26 months. We did observe increases in lichens and mosses over time, but decreases in dark pigmented cyanobacteria when accounting for initial inoculation and the cover in control plots (Table 1).

Precipitation was also different between the sampling points, with greater total precipitation in the second sampling period compared to the first, but the majority falling as rain. During the first 14 months, the plots received 168.4 mm of precipitation, with 90.4 mm received as snow and 78.0 mm as rain. In the second sampling period (month 15–26) plots received 335.1 mm of precipitation, with 74.3 mm in snow and 260.8 mm in rain (Supplemental Fig. 1). In the two weeks leading up to sampling, there were zero rain events at 14 months and daily rain events at 26 months.

Experiment 2. Cultivated and hardened Biocrust trial Surprisingly, there was little response to culture or hardening conditions (Supplementary Table 3). The exception to this was late successional cover (the sum of dark pigmented cyanobacteria, lichens and mosses), which responded to an interaction of time, culture conditions and hardening (Supplementary Table 3). The highest late successional cover was observed with two or three days continuous hydration during cultivation and moderate hardening (outdoor with 50% shade and low water), compared to the lowest cover with three days continual hydration with no hardening, or extreme hardening with two or five days of continuous hydration during cultivation.

For the remaining results, we pool inoculated plots, and compare to control plots because culture conditions and hardening conditions had little effect on biocrust establishment. Light cyanobacteria, dark pigmented cyanobacteria, lichens and total late successional cover increased over the sampling period. Late successional cover increased 35% at 12 months compared to the 6 month sampling point after accounting for cover in

Table 1 Percent change in biocrust cover is given from initial to the first sampling point, and from the first sampling point to the second for each experiment. Values are mean percent change in biocrust functional groups

	Exp. 1: Field-collected		Exp. 2: Greenhouse-cultivated	
	14 months*	26 months≠	6 months*	12 months≠
LC	249.7	−190	16,828.8	-102.9
DC	-13.0	−100	−59.2	−13.1
Lichen	45.3	16.3	741.6	121.1
Moss	−100	212.5	−60.4	20.0
Late successional cover	−8.7	−30.8	−40.1	35.2

*%Change$_{T1}$ = ((Cover$_{T1}$-Cover Control$_{T1}$) -Cover$_{T0}$)/Cover$_{T0}$)*100

≠%Change$_{T2}$ = (Cover$_{T2}$-Cover Control$_{T2}$)-Cover$_{T1}$)/Cover$_{T1}$)*100

control plots (Table 1; Fig. 2). Inoculation had effects on light and dark cyanobacteria, lichens, mosses, late successional cover and LOD (Supplementary Table 4; Fig. 2). Likewise, most measures were affected by time, and light cyanobacteria and late successional cover were affected by an interaction of time and inoculations (Supplementary Table 4; Fig. 2). Mosses decreased 60% over the initial inoculum, accounting for control cover after 6 months, but recovered with an increase of 20% from 6 months to 12 months (Table 1). Lichen cover increased 741% over the initial inoculum and control cover at 6 months, and an additional 20% from 6 months to 12 months (Table 1).

Chlorophyll *a* and soil aggregate stability were only measured after 12 months. Chlorophyll *a* was not different between inoculated and control plots after 12 months, which is not surprising because cover was also not different after 12 months ($F = 0.2$, $p = 0.4$). However, soil aggregate stability was still slightly higher after 12 months in inoculated plots ($F = 7.1$, $p = 0.01$, control: 4.3 ± 0.2, inoculated: 5.0 ± 0.2). Similarly to the field trial, we saw strong differences between control and inoculated plots in most response variables at our first measurement point (6 months), but those differences disappeared by our second measurement point (12 months; Fig. 2; Supplemental Table 4).

A total of 97.3 mm of precipitation, with 74.3 mm received as snow occurred in the first sampling period from October 2014–April 2015 (6 months; Supplementary Fig. 2). In the second period, 249.3 mm of precipitation (all rain) was received between May 2015 and October 2015 (Supplemental Fig. 2), which is 48% greater than the amount received in one year of Exp. 1, and nearly equivalent with the amount received in the second 13 months of Exp. 1. In both sampling periods, there were daily rain events in the two weeks prior to sampling.

Discussion

Biocrust inoculum successfully established in the field

This study provides evidence that we can successfully reestablish biocrusts in the field using both field-collected and cultured biocrust inoculum. Biocrust cover increased dramatically for plots treated with both field collected and greenhouse gown inoculum at our first monitoring point (6 months or 14 months from inoculation), with the majority of the cover by early successional light cyanobacteria. Natural recovery times can range from a few years to millennia in various ecosystems, and typically span a decade or more (Belnap 1993; Weber et al. 2016). We might have seen rapid recovery in control and inoculated plots because of the size and nature of the disturbance, where surrounding crusts existed nearby, as opposed to a larger, landscape-scale removal of biocrust. Even with a short natural recovery time, inoculum addition increased recovery early on (noted at 14 months with field-collected and 6 months with cultured biocrust inoculum), and early colonization can be critical in soil stabilization following disturbance events (USDI-BLM 2007).

Exp. 1. Field-collected biocrust trial With very little effort we were able to harvest and reapply crumbled biocrust at a relatively low cover (10%) to bare soil and

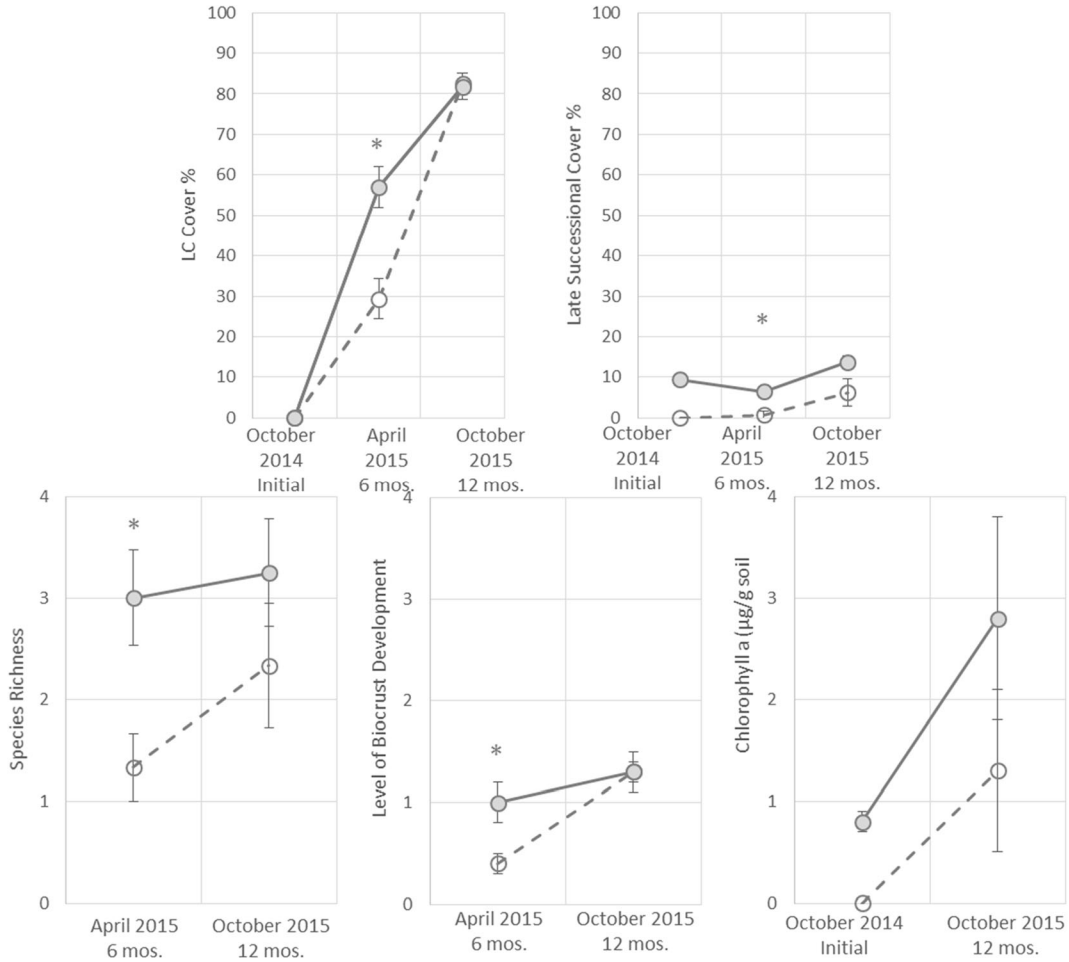

Fig. 2 Change in biocrust metrics from initial to 12 months are given for Experiment 2 (cultivated and hardened inoculum, $n = 6$). Open symbols with dashed lines represent control plots whereas closed symbols with solid lines represent inoculated plots. Error bars are one SE of the mean. (* = significant differences at $p \leq 0.05$) by one-way ANOVA at a given time point. Differences were not tested for the initial inoculation. Note: some panels have only two points because measurements were either not taken at the initial time point (species richness and LOD) or at the 6 month time point (Chlorophyll *a*). LC = light pigmented cyanobacteria

observed a 249% positive change in light cyanobacteria and 45% positive change in lichen cover in only 14 months, although net loss was observed for dark cyanobacteria and mosses. This is a simple and relatively low-effort option for land managers for speeding up biocrust recovery rates in small areas of disturbance, particularly when salvageable biocrust is available. While inoculation enhanced LOD, the levels were substantially lower than the background surface and we saw no benefit of inoculation to soil aggregate stability. Others have tested similar methods in a variety of dryland ecosystems with similar results, suggesting this is a viable method in a variety of ecosystems where a disturbance can be treated once and left to recover (Belnap 1993; Chiquoine et al. 2016; Condon and Pyke 2016). However, collecting on-site for a 10% cover reapplication can translate into a relatively large new disturbance depending on the area requiring rehabilitation. Additionally, late-successional biocrust of this spatial extent may not be available for many sites. Thus, caution and a cost-benefit analysis is warranted. For example, if erosion control is needed quickly, the benefit of inoculation might be greater than the cost of causing a secondary disturbance.

Exp. 2. Cultivated and hardened biocrust trial Cultivated biocrusts offer a way to increase biocrust biomass, thus requiring less on-site disturbance, and our cultivated biocrusts showed similar reestablishment in the field. After six months, cultivated biocrusts

had dramatically expanded light cyanobacteria cover and increased lichen cover by 741% cover over the initial inoculum and control plot colonization. By one year, our late successional crust cover had a positive change of 35%, covering 14% of the soil surface in inoculated plots. To our knowledge, this is the first successful application of greenhouse-cultured biocrust inoculum containing the full spectrum of early to late-successional species in a field setting. Biocrust cover in uninoculated controls increased from 0% to an average of 29% cover in six months and to 83% after one year. Biocrust cover in inoculated and uninoculated plots also converged by 26 months in Exp. 1 and by 12 months in Exp. 2, suggesting that in this ecosystem, natural recovery of cover would occur without intervention. This leads us to ask if inoculation was not necessary.

Data from this site suggest that propagule limitation is not of concern in this particular location, but recovery would likely be slower where naturally-dispersed propagules were more limited, such as on coarser textured soils or with other barriers to establishment such as active erosion or size or the surrounding disturbance (Belnap and Eldridge 2003; Bowker 2007). Further, even accounting for this significant increase in control plot biocrust cover, inoculated plots showed added benefits related to uninoculated controls. We observed greater late successional cover and species richness after six months, as well as a modest increase in soil aggregate stability that remained after 12 months, even when control and inoculated plot biocrust cover had converged.

These experiments demonstrate that biocrust recovery can be accelerated by inoculation for at least the first 1–2 years. The primary goal of adding biocrust inoculum is to reestablish ecosystem function to damaged systems, and the speed at which functional recovery occurs can be critical (USDI BLM 2007). Adding biocrust inoculum has been shown to enhance soil aggregate stability and to increase nitrogen and carbon fixation (Maestre et al. 2006; Chiquoine et al. 2016). We show here that, even as biocrust cover converged in inoculated and uninoculated plots, those with cultured inoculum addition had greater soil aggregate stability. Our results suggest that removing the disturbance would be enough to allow biocrust recovery to occur, but that we can induce a faster recovery by adding inoculum (Bowker 2007). This type of intervention may have value as a rapid-response technique, for example supplanting soil-disturbing efforts to establish vascular plants in disturbed areas when there is a risk of major erosion (Miller et al. 2012).

Field-collected vs. cultivated biocrust – Is one better?

In the two experiments, we observed faster and higher colonization of plots inoculated with cultivated biocrusts compared to field-collected inoculum. This could be a result of a variety of factors including inter-annual variation in precipitation, where precipitation was lower and temperature varied more greatly initially in Exp. 1, than in the second sampling periods of Exp. 1 or overall for Exp. 2. We also observed daily precipitation in the two weeks leading up to sampling for both periods in Exp. 2, but only one period in Exp. 1 (which could explain the drop off in chlorophyll a at the second sampling point). If climate was less stressful over the course of Exp. 2, it could account for higher colonization of biocrust organisms overall and in inoculated plots.

Other indicators of success, including species richness, late successional cover, LOD, and chlorophyll *a* concentrations were greater with field-collected biocrust compared to cultivated biocrust. This indicates that while cultured biocrusts may have had higher cover (perhaps due to a high precipitation year), they did not develop to the level of diversity and function that field-collected biocrusts did within the timeframes of this study. This could be for a variety of reasons. First, the field-collected biocrusts are already adapted to the local climate and field conditions. In addition, the initial community composition of field-collected vs. cultured inoculum was different: with higher light pigmented cyanobacteria and greater cover and diversity of lichens in field-collected inoculum, and higher dark-pigmented cyanobacteria and moss in cultured inoculum. The greater species richness, and later successional biocrust cover likely contributed to differences in the results between experiments. Both of these factors could contribute greatly to differences in establishment success. Finally, we must consider the duration of the two experiments. Exp. 1 had an additional year to establish and develop compared Exp. 2, potentially giving the field collected inoculum an advantage in biomass, diversity and maturity.

From these results it is unclear if a mixed species inoculum containing late and early successional species is a sound alternative strategy to the addition of field-collected biocrust or cultured early successional species (Zhao et al. 2016). In the field, it was primarily early-

successional species that proliferated. Nonetheless, later successional cyanobacteria, mosses and lichens persisted at low levels in both experiments, and late successional biocrusts play a disproportionately large role in ecosystem function (e.g., Housman et al. 2006; Barger et al. 2013; Faist et al. 2017). The simultaneous inoculation of multiple complementary species may facilitate overall colonization, even if some community members are "winners" and others are "losers." However, more work is needed to test this assumption. What we can say from our results is that biocrust addition, whether cultured or field-collected, worked to re-establish biocrusts more quickly than they would have without inoculation. The trade-offs in terms of reducing the harvest disturbance, versus the effort to grow biocrusts needs to be taken into consideration. For large disturbances in need of inoculation, it will likely be necessary to effectively cultivate biocrusts for reintroduction. However, in systems that will recover naturally, and without risk of immediate erosion, it may be enough to remove the disturbance (Bowker 2007).

Do culture conditions and hardening promote field establishment of biocrusts?

The rationale behind "hardening" is to condition organisms to a harsher environment than the one in which they were cultivated. Field conditions have higher UV, more variation in temperature and relative humidity, and a lower frequency and predictability of water. Our three hardening conditions were chosen in an effort to maximize feasibility for land managers, and offer conditions that might benefit different groups of biocrust organisms. Different biocrust organisms are known to have variable sensitivities to environmental conditions (e.g., Grote et al. 2010), and thus biocrust populations may require different hardening treatments to achieve optimal establishment and growth. In addition, we know that some mosses require a period of "dehardening" where plants are given luxury conditions in order to build up all of their protective systems to minimize damage caused by desiccation events (Stark et al. 2012.). This suggests that mosses might establish best in the field when cultured with long hydration periods and treated to luxury greenhouse conditions, or no hardening. Dark pigmented cyanobacteria and the dominant lichens of our study system have protective UV pigments that are inducible by UV exposure (Gao and Garcia-Pichel 2011). We also know that lichens, mosses and dark pigmented cyanobacteria are sensitive to warming, and particularly warming with water stress (Belnap et al. 2006; Escolar et al. 2012; Ferrenberg et al. 2015). This might suggest that these late successional groups could benefit by hardening to temperature fluctuation and water stress, as given with shorter hydration culturing and exposure to outdoor conditions. Light pigmented cyanobacteria without UV protective pigments have different strategies to avoid stress, retreating under the soil surface for protection from UV, and to track moisture (Garcia-Pichel and Pringault 2016). It is possible that light pigmented cyanobacteria need no hardening because of their avoidance strategy, but instead, would benefit from being added to the field with the physical cover of soil, another substrate, or dark pigmented, later successional biocrust organisms.

Our general conclusion is that the hardening treatments did not strongly affect field establishment. This finding could be perceived as encouraging for rehabilitation applications, because an additional effort to harden biocrusts may not be needed. Instead, cultured materials could be directly transferred from greenhouse environment to the field. However, there are significant caveats. We did not attempt an exhaustive array of hardening conditions and we had low establishment of all late successional organisms. Further, the only effect of hardening or culture conditions that we detected was on these late-successional organisms. Thus, it would be valuable to more thoroughly study whether some benefits of hardening exist, even though they were scarcely detected in our study.

Late successional cover was greater when hardened outdoors under 50% shade cloth following culture with shorter hydration events (2 or 3 days hydration per week) compared to the lowest cover with three days continual hydration with no hardening, or extreme hardening with two or five days of continuous hydration during cultivation. It is desirable to have late successional biocrust cover because of the substantial ecosystem benefits conferred by later-successional biocrust communities (e.g., Barger et al. 2005; Housman et al. 2006; Chaudhary et al. 2009). This result highlights a crucial trade-off in the production of biocrusts for rehabilitation. On the one hand, longer hydration periods can result in the fastest growth of total biomass, and efficient production of inoculum

(Antoninka et al. 2015). On the other, they may not optimize the survivorship of later successional elements when applied to the field. Additional efforts to optimize culturing and hardening methods are clearly needed.

It is interesting that we had similar results with our field-collected inoculum in comparison to our cultured biocrust inoculum. The field-collected inoculum was certainly "hardened" to field conditions as it was collected, crumbled and reapplied to its home site within a few days. However, the late successional cover was low in these plots as well, especially in comparison to the initial ratios of the inoculum, where late successional cover dominated. Instead, the colonization of biocrusts was dominated by the early successional light pigmented cyanobacteria in plots treated with both field-collected and cultivated biocrust. This suggests that the successional development of biocrusts from light pigmented cyanobacteria, to dark pigmented cyanobacteria and then mosses and lichens dominates the process, regardless of the organisms added (Belnap and Lange 2003). It is also possible that light pigmented cyanobacteria have an advantage to establishment when crumbled and applied, because their biological preference is to be buried, whereas mosses, lichens and dark pigmented cyanobacteria need to land upright and above the soil to be physiologically active.

Conclusions

The results of these two experiments demonstrate that we can successfully add and establish biocrusts to damaged ecosystems. Although control and inoculated cover converged over time, inoculation allowed initial recovery to occur more quickly than if plots were not inoculated. A cost-benefit analysis is important to consider in determining management decisions. The need for rapid recovery versus the desire to reduce additional disturbance weigh into choosing whether or not to add inoculum, and whether to use field-collected or greenhouse-cultivated inoculum. Plots treated with field-collected biocrusts had less overall cover, but were generally more mature than plots treated with cultivated biocrusts. The late successional community members were collectively sensitive to hardening, suggesting that we should refine our effort to optimize hydration and hardening conditions for maximum establishment. We also noted effects of inter-annual variation in precipitation on our results, and this warrants further study. Seasonal patterns in precipitation are important to consider in planning an ideal timing for inoculum addition. While we have not yet optimized our methods to attain mature biocrusts from cultivated biocrusts, we believe this first field trial is an important advance, and may be a critical option when disturbances exceed the amount of salvage biocrust that can be collected.

Acknowledgements This work was partially supported by the Strategic Environmental Research and Development Program (Grant number RC- 2329; Department of Defense, Department of Energy, and Environmental Protection Agency). We also gratefully acknowledge facilitation of field site permitting and access at the Utah Test and Training Site by Russell Lawrence. We appreciate the help with field collections, greenhouse cultivation, preparation and field data collection by Akasha Faist, Dustin Kebble, Channing Laturno and Brook Stamper. We are also grateful for additional review provided by Akasha Faist, Kara Gibson and two anonymous reviewers that greatly improved the manuscript. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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