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 tech-

function by providing armor that reduces weed seed

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.

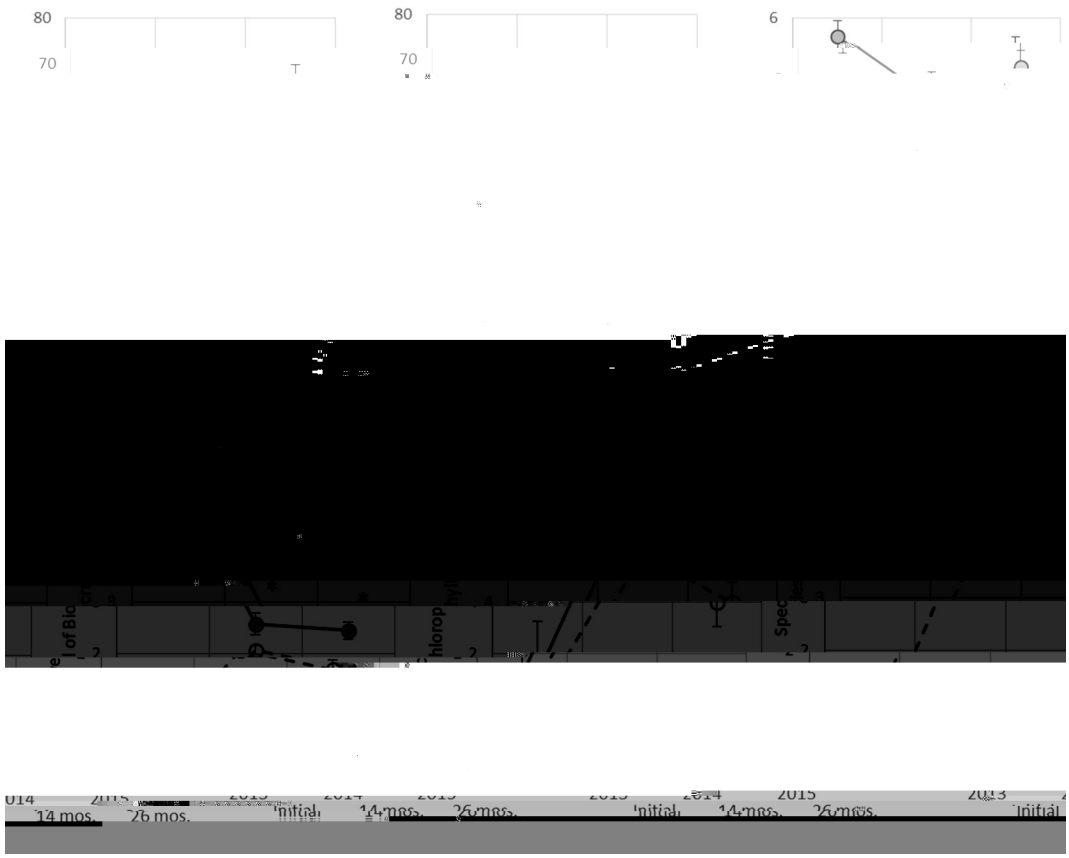
H

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 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 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

E[illegible] 1. F[illegible] B[illegible] 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.

E[illegible] 2. C[illegible] B[illegible] 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



. 1 Change in biocrust metrics from initial to 26 months are given for Experiment 1 (field-collected inoculum, = 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 ≤ 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.

E 2. *C* *B* 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

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 and soil aggregate stability were only measured after 12 months. Chlorophyll 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.

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.

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 aggre-

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

(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

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

Belnap J, Weber B, Büdel B (2016) Biocrusts as an organizing principle in drylands. In: Weber B, Büdel B, Belnap J (eds) Biological soil crusts: an organizing principle in drylands. Ecological Studies Series. Springer-Verlag, Berlin, pp 3–14

Bowker MA (2007) Biological soil crust rehabilitation in theory and practice: an underexploited opportunity. Restor Ecol 15:13–23

Bowker MA, Antoninka AJ (2016) Rapid ex situ culture of N-fixing soil lichens and biocrusts is enhanced by complementarity. Plant Soil 408:415–428

Bowker MA, Maestre FT, Escolar C (2010) Biological soil crusts as a model system for examining the diversity-ecosystem function relationship in soils. Soil Biol Biochem 42:405–417

Bu C, Wu S, Zheng M (2014) Identification of factors influencing restoration of cyanobacteria-dominated biological soil crusts. PLoS One. doi:10.1371/journal.pone.0090049

Castle SC, Morrison CD, Barger NN (2011) Extraction of chlorophyll a from biological soil crust: a comparison of solvents for spectrophotometric determination. Soil Biol Biochem 43: 853–856

Chaudhary VB, Bowker MA, O'Dell TE, Grace JB, Redman AE, Rillig MC, Johnson NC (2009) Untangling the biological contributions to soil stability in semiarid shrublands. Ecol Appl 19:110–122

Chen YQ, Zhao YG, Ran MY (2009) Experimental research on artificial culture method of moss crust in hilly loess Plateau region. Acta bot Boreal-Occident sin 29:586–592

Chiquoine LP, Abella SR, Bowker MA (2016) Rapidly restoring biological soil crusts and ecosystem functions in a severely disturbed desert ecosystem. Ecol Appl 26:1260–1272

Condon LA, Pyke DA (2016) Filling the interspace- restoring arid land mosses: source populations, organic matter and overwintering govern success. Ecol Evol 0:1–10. doi:10.1002/ece3.2448

Davidson DW, Bowker M, George D, Phillips SL, Belnap J (2002) Treatment effects on performance of N-fixing lichens in disturbed soil crusts of the Colorado Plateau. Ecol Appl 12: 1391–1405

Delgado-Baquerizo M, Maestre FT, Eldridge DJ, Bowker MA, Ochoa V, Gozalo B, Berdugo M, Val J, Singh BK (2016) Biocrust-forming mosses mitigate the negative impacts of increasing aridity on ecosystem multifunctionality in drylands. New Phytol 209:1540–1552. doi:10.1111/nph.13688

Doherty KD, Antoninka AJ, Bowker MA, Velasco Ayuso S, Johnson NC (2015) A novel approach to cultivate biocrusts for restoration and experimentation. Ecol Restor 33:13–16

Escolar C, Maestre FT, Martínez I, Bowker MA (2012) Warming reduces the growth and diversity of lichen-dominated biological soil crusts in a semi-arid environment: implications for ecosystem structure and function. Proc R Soc B 367:3087–3099

Faist AM, Herrick JE, Belnap J, Van Zee JW, Barger NN (2017) Biological soil crust and disturbance controls on surface hydrology in a semi-arid ecosystem. Ecosphere 8(3):e01691

Ferrenberg S, Reed SC, Belnap J (2015) Climate change and physical disturbance cause similar community shifts in biological soil crusts. Proc Nat Acad Science 112:12116–12121

Gao Q, Garcia-Pichel F (2011) Microbial ultraviolet sunscreens. Nat Rev Microbiol 9:791–802. doi:

Weber B, Bowker M, Zhang Y, Belnap J (2016) Natural recovery of biological soil crusts after disturbance. In: Weber B, Büdel B, Belnap J (eds) Biological soil crusts: an organizing principle in drylands. Ecological Studies Series. Springer-Verlag, Berlin, pp 479–498

Xiao B, Wang QH, Zhao YG et al (2011) Artificial culture of biological soil crusts and its effects on overland flow and infiltration under simulated rainfall. Appl Soil Ecol 48:11