

Responses of biological soil crusts to rehabilitation strategies

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ABSTRACT

Biological soil crusts (biocrusts) are common to dryland ecosystems and can influence a broad suite of soil ecological functions including stability and surface hydrology. Due to long recovery times following disturbance, there is a clear need for rehabilitation strategies to enhance the recovery of biocrust communities. Essential to biocrust recovery are exopolysaccharides (EPS): secretions comprised mainly of high molecular weight polymers that protect cyanobacteria and other biocrust organisms from harsh environmental conditions. We examined whether biocrust rehabilitation strategies (combinations of inoculation with surface shading and artificial soil stabilization) promote EPS production. To test if responses varied by soil texture, we measured biocrust recovery on two fine-textured soils (clay and sandy clay loam) in a cool desert ecosystem. Shade coupled with inoculum addition resulted in the highest biocrust recovery, especially on clay soils. Independent of rehabilitation strategies, natural recovery of biocrusts occurred more rapidly on clay soils, reflected by greater increases in chlorophyll *a* (chl *a*). Chl *a*, a proxy for cyanobacterial biomass, was correlated to EPS amounts, suggesting that cyanobacteria are significant contributors to EPS production in biocrust development. Despite the role of EPS in biocrust establishment, EPS amounts had negligible effects on soil stability on the fine soil texture.

1. Introduction

Biological soil crusts (biocrusts) are comprised of mosaics of cyanobacteria, algae, fungi, moss, and lichen species on the soil surface, and are important functional components of dryland ecosystems (Weber et al., 2016a). Biocrusts influence a broad range of ecological processes by enhancing soil stability (Belnap and Büdel, 2016), increasing moisture retention (Chamizo et al., 2012), contributing to soil fertility (Chen et al., 2014), and reducing nutrient loss and oftentimes runoff (Barger et al., 2006; Faist et al., 2017). Soil surface disturbances from human activities can damage biocrusts and significantly alter many of these processes (Weber et al., 2016a). Natural recovery of biocrusts to a late successional state can take several decades to millennia (Belnap and Warren, 2002; Weber et al., 2016b). However, recovery of some key ecological functions such as soil stability may occur on shorter time scales from months to years following small scale disturbances (Dojani et al., 2011; Weber et al., 2016b).

Since natural recovery of full biocrust communities can be slower than desirable, there is keen interest in developing assisted biocrust

recovery or rehabilitation strategies to facilitate growth (Bowker, 2007; Zhao et al., 2016). Depending on the level of soil degradation and site-specific characteristics, barriers to restoration success such as available biocrust propagules, resource limitation (e.g., water and nutrients), and soil stability may significantly slow the natural recovery of biocrust communities (Bowker, 2007). Thus, rehabilitation efforts that target site-specific barriers to recovery are more likely to be successful.

Essential to biocrust function and surface soil matrix structure are exopolysaccharides (EPS), which are secreted by cyanobacteria, microalgae, microfungi, and other biocrust organisms (Rossi et al., 2017). EPS facilitate desiccation tolerance in water-limited environments (Wingender et al., 1999), assist in cell locomotion, and stabilize soils (Belnap and Gardner, 1993). For these reasons, EPS are critical for primary cyanobacterial establishment on disturbed soils to provide the foundation for biocrust succession and establishment, and are also of great interest to rehabilitation efforts.

EPS in the soil system are operationally defined by their extraction methods into two main fractions: tightly-bound EPS (TBEPS) and colloidal EPS (CEPS) (Rossi et al., 2017). CEPS comprise the soluble

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fraction and are loosely-bound, lower-molecular weight “slime” exudates secreted in copious amounts (Chen et al., 2014). Chemical affinities to clay and calcium compounds allow CEPs to create micro-aggregates with soil particles, which can assist in soil stabilization (Belnap and Büdel, 2016; Belnap and Gardner, 1993). In contrast, TBEPS require a stronger EDTA extraction method and are generally comprised of thicker, more complex gelification polysaccharides (Rossi et al., 2017; Wingender et al., 1999). EPS envelop soil particles, creating soil macroaggregates (Belnap and Gardner, 1993; Chen et al., 2014), structuring the soil (Mager et al., 2011), and protecting soil surfaces from wind and water erosion (Barger et al., 2006; Belnap and Büdel, 2016; Faist et al., 2017; Hu et al., 2002). Soils stabilized by EPS can also collect fine soil particles and nutrients (Mazor et al., 1996; Van den Ancker et al., 1985; Williams et al., 1995), which can further promote cyanobacterial colonization (Rozenstein et al., 2014) and in turn, greater soil stability. Because of EPS's close association with biocrust establishment, our research focused on how EPS responded to biocrust rehabilitation efforts and how changes in EPS may influence soil stability.

Our objectives in this study were to 1) determine whether biocrust rehabilitation strategies (inoculum addition, shading, and artificial soil stabilization) enhanced EPS content in soils and 2) evaluate the relationship between soil EPS content and biocrust recovery and soil stability metrics. We predicted that biocrust inoculum additions would increase soil EPS levels by enhancing the growth of early successional cyanobacteria and other EPS-producing organisms present in biocrusts (e.g., microfungi, microalgae), while habitat modification using soil surface shading would alleviate environmental stressors and promote biocrust recovery. We also hypothesized that artificial soil stabilization, which can provide a stable surface for cyanobacterial growth, would facilitate establishment of biocrust organisms.

2. Methods

2.1. Site description

The project site is in the Great Basin Desert at Hill Air Force Base,

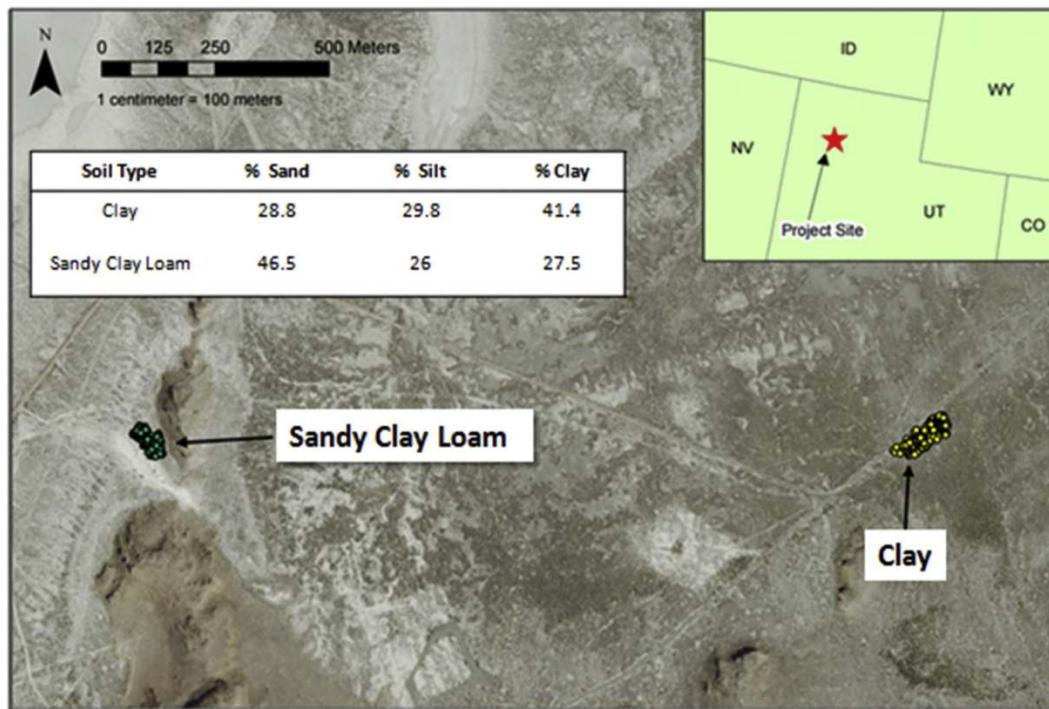


Fig. 1. Hill Air Force Base Utah Test and Training Range project location, displaying plot locations on clay and sandy clay loam soil types.

Table 1

Soil properties of the top 1 cm of clay and sandy clay loam (SCL) soil types.

Soil property	Clay	SCL
pH	8	7.76
EC (mS/cm)	0.66	1.02
TN (%)	0.17	0.13
TC (%)	5	7.1
TOC (%)	3.43	5.45
CEC (meq/100 g)	10.46	8.95
Total P ^a (μ g P/g soil)	19.52	17.56

*Total N was determined in a Perkin Elmer 2400 elemental analyzer after combustion according to standard methods of analyses (AOAC, Method 972.43, 1997).

**Total P was measured using the ammonium molybdate-ascorbic acid method and a Lachat QC8000 continuous flow colorimeter according to Mehlich (1978); samples were extracted into a bicarbonate solution which was then neutralized prior to analysis of total P.

^a From Velasco Ayuso et al. (2016).

Utah Test and Training Range (41.104198° N, -113.023194° W) (Fig. 1). This is a cool desert system that receives an average annual precipitation of ~250 mm (ranging from ~240 mm to 350 mm) (WRCC, 2017). We conducted experiments at two sites that had well-developed biocrust communities but differed in soil texture. The first site was a clay soil consisting of 28% sand, 30% silt, and 42% clay in the top 1 cm, with the majority of biocrust composition comprised of the lichen *Collema tenax* and cyanobacteria *Microcoleus* spp. and *Scytonema* spp. (Velasco Ayuso et al., 2016; Supplementary Fig. S1). The second site was sandy clay loam (SCL) which consisted of 46% sand, 26% silt, and 28% clay in the top 1 cm, with the majority of biocrust composition comprised of the cyanobacteria *Microcoleus* spp. and *Tolyphothrix* spp. (Velasco Ayuso et al., 2016; Supplementary Fig. S1) and *Collema tenax*. Other soil properties are described in Table 1. Experiments mirrored one another and were established in June 2013 on each soil type.

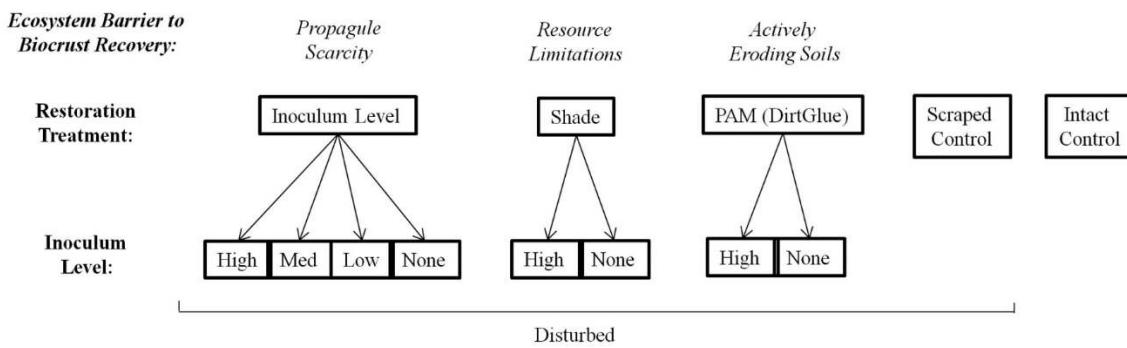


Fig. 2. Schematic of experimental design showing restoration treatment experiments corresponding to ecosystem barriers to biocrust recovery. Each experiment was conducted on clay and sandy clay loam soils. PAM: polyacrylamide.

Table 2

Repeated measures linear mixed effect model results for chl a, CEPS, and TBEPS response variables. The *F* value is followed by the *p* value in parentheses. Bolded *p* values indicate *p* < 0.05. Chl a reported in ($\mu\text{g chl a/g soil}$), CEPS and TBEPS reported in ($\mu\text{g glucose/g soil}$).

Experiment	Response Variable	Fixed Effects						
		Treatment	Soil	Year	Treatment:Soil	Treatment:Year	Soil:Year	Treatment: Soil:Year
Inoculum Level	CEPS	2.6 (0.46)	3 (0.08)	120 (< 2.2e-16)	9.3 (0.03)	6.1 (0.41)	57 (4.2e-13)	6.4 (0.38)
	TBEPS	7.2 (0.06)	2.5 (0.16)	19 (7.2e-5)	0.74 (0.86)	11 (0.09)	7.8 (0.02)	8.3 (0.21)
	chl a	12 (0.008)	0.63 (0.43)	248 (< 2.2e-16)	4.5 (0.21)	5.0 (0.54)	12 (0.002)	18 (0.007)
Shade (+ Inoculum)	CEPS	17 (0.0002)	11 (0.001)	124 (< 2.2e-16)	7.8 (0.02)	18.9 (0.0008)	36 (1.5e-8)	9 (0.06)
	TBEPS	11 (0.005)	3.6 (0.06)	19 (7.3e-5)	1.1 (0.58)	6.5 (0.16)	12 (0.002)	9.4 (0.05)
	chl a	34 (3.4e-8)	13 (0.0003)	301 (< 2.2e-16)	5 (0.08)	18 (0.001)	1.3 (0.53)	5.2 (0.27)
PAM (+ Inoculum)	CEPS	0.9 (0.64)	5.6 (0.02)	28 (7.0e-7)	0.72 (0.70)	4.3 (0.37)	20 (5.4e-5)	4.4 (0.36)
	TBEPS	1.5 (0.48)	25 (5.8e-7)	2.7 (0.26)	0.26 (0.88)	9.3 (0.05)	6.9 (0.03)	1.9 (0.76)
	chl a	5.0 (0.08)	4.6 (0.03)	217 (< 2e-16)	2.3 (0.31)	1.2 (0.87)	0.94 (0.63)	4.6 (0.33)

2.2. Biocrust rehabilitation experiments

We implemented three experiments to test the effects of inoculum level, soil surface shading, and addition of an artificial soil stabilizer (Fig. 2). To remove the influence of the previously established biocrust community, we scraped the top 1 cm of biocrust from the soil surface. The biocrust material from all plots was bulked, crumbled into pea-sized fragments, homogenized, and then used as inoculum for our experiments. To ensure that all surface biocrust was removed, we scraped the soil surface down to 2 cm below ground surface. Natural recovery (NR) plots were scraped and then left untreated and the inoculum level was left at “none” (Fig. 2). We monitored plots annually for three years following treatment implementation (2014–2016).

2.2.1. Inoculum additions

To address if biocrust inoculation increased soil EPS, we inoculated 50 cm × 50 cm plots with low (500 cubic centimeters [cc] inoculum: 10% cover), medium (1000 cc inoculum: 20% cover), and high (2000 cc inoculum: 40% cover) amounts of field-collected inoculum spread evenly over the plot surface, similar to the methods of Chiquoine et al. (2016). Each inoculum treatment (NR, low, med, high) was replicated in 5 plots across an approximately 1 ha area on each soil type (Fig. 2).

2.2.2. Shading

To modify biocrust habitat to promote more favorable soil moisture and light conditions, we placed shade structures over 50 cm × 50 cm plots (shade plots). Shade cloth, which reduced ~50% of the incoming UV, was mounted on PVC pipes 15 cm above the ground. Shade structures were erected between June 2013 and June 2015 but removed during the winter months to avoid damage from snowfall. To address the possibility of propagule scarcity and resource limitations, we shaded plots and added field-collected inoculum at 40% cover

(shade + inoc plots). Each treatment (NR, shade, shade + inoc) was replicated in five plots on each soil type (Fig. 2).

2.2.3. Artificial soil stabilization

To test whether artificial soil stabilization in combination with biocrust inoculation enhanced soil EPS levels, we applied a photo-degradable water-soluble polyacrylamide, hereafter referred to as PAM (Dirtglue Enterprises® Salem, NH), to scraped 1 m² plots. Polyacrylamide was sprayed in a 1:8 PAM:water dilution (40% cover) with and without inoculum (PAM + inoc and PAM, respectively). Each treatment (NR, PAM, PAM + inoc) was replicated in seven plots on each soil type (Fig. 2).

2.3. Biocrust recovery responses

We monitored plots one (June 2014), two (June 2015), and three (May 2016) years following treatment implementation (referred to as Years 1, 2, 3, respectively). Soils from the plots were collected for EPS and chlorophyll a (chl a) content, and their soil aggregate stability was recorded. During the final year of monitoring, we also took measurements of newly scraped plots (scraped control) and intact biocrust plots (intact control) to serve as reference points for recently disturbed and intact soil surfaces, respectively.

In each test plot, we collected three soil samples from standardized locations and pooled them. Soil samples were collected using a 1.5 cm diameter core to a depth of 10 mm, and stored in the dark at -20 °C until they were analyzed. We homogenized samples with a mortar and pestle and portioned them into approximately 50 mg and 1 g subsamples using the cone and quarter method (Gerlach et al., 2002) for EPS and chl a analyses, respectively.

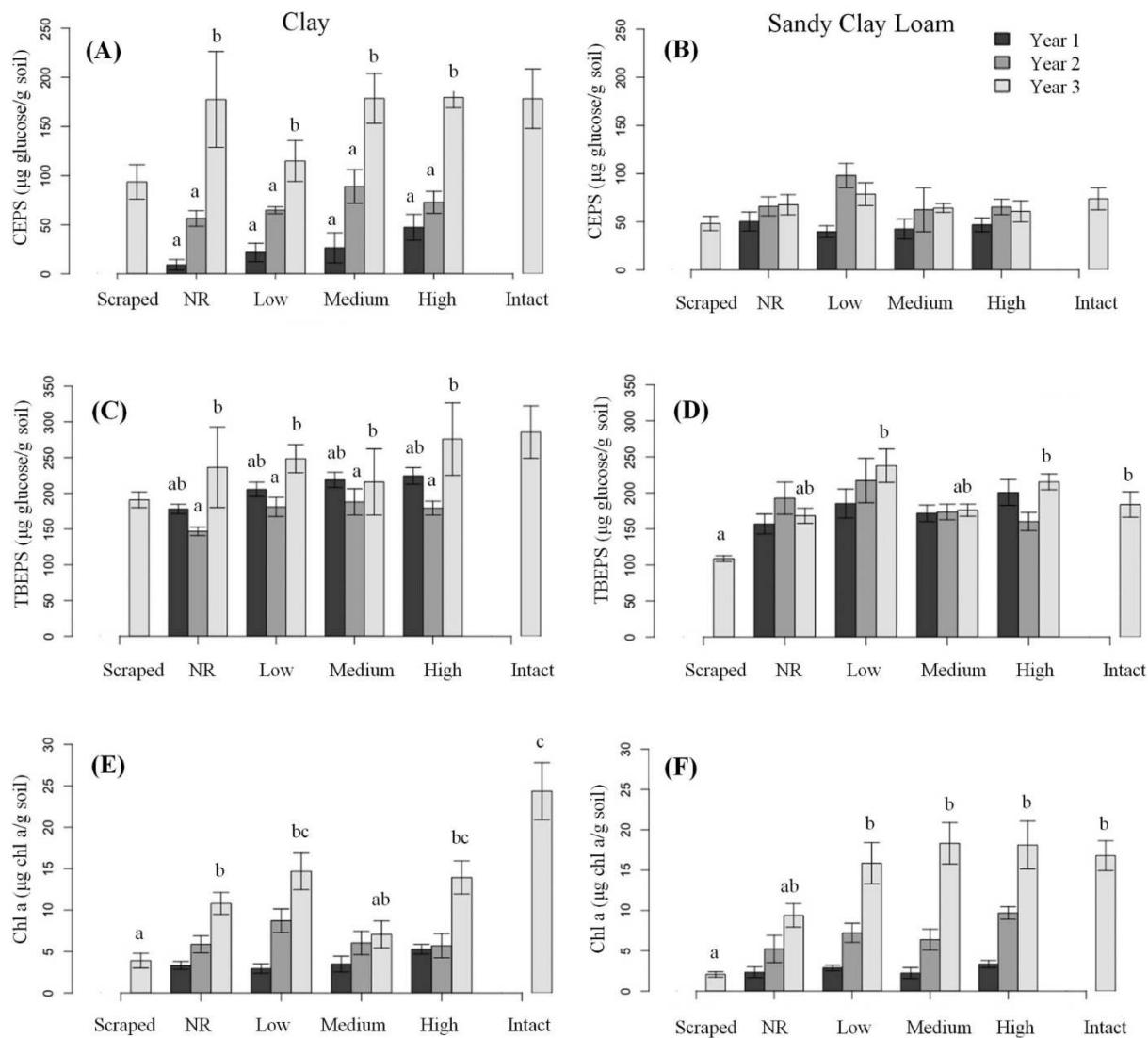


Fig. 3. Results from inoculum additions on clay and sandy clay loam soils. Bars show mean \pm 1 SD. Letters denote significant differences ($p < 0.05$) between years (A, C) and between treatments in Year 3 (D, E, F). (A) CEPS responses on clay soil; (B) CEPS responses on sandy clay loam soil; (C) TBEPS responses on clay soil; (D) CEPS responses on sandy clay loam soil; (E) Chl a responses on clay soil; (F) Chl a responses on sandy clay loam soil. NR: natural recovery. Scrapped: scraped control. Intact: intact control. Scrapped and intact controls were established in Year 3.

2.3.1. EPS quantification

CEPS and TBEPS fractions were extracted from a 50 mg soil sample. 400 µL of DI water was added to the soil sample, vortexed, and placed on a shaker at room temperature (RT) for 15 min. We centrifuged samples at 8000 $\times g$ for 6 min. 200 µL of supernatant was retained for CEPS quantification. The remaining supernatant was discarded, and the remaining soil was retained for TBEPS analysis (de Brouwer and Stal, 2001). We added 500 µL of 100 mM Na₂EDTA to the soil, then vortexed the sample and placed it on a shaker for 16 h at RT. Samples were centrifuged at 8000 $\times g$ for 6 min. 200 µL of EDTA supernatant was retained for TBEPS quantification.

To determine EPS amounts, we used the phenol-sulfuric acid assay (Dubois et al., 1956) with a glucose standard curve. 200 µL of each EPS fraction was combined with 200 µL of 5% w/v phenol and 1 mL of sulfuric acid. The mixture was vortexed and then incubated for 45 min at RT before the absorbance was read on an Ocean Optics CHEMUSB4-VIS-NIR Spectrophotometer (400–950 nm). We took the absorbance at 490 nm along with a 1000 nm background reading which subtracted as the baseline.

2.3.2. Chlorophyll a

To obtain chl a levels, we ground 1 g of soil with a mortar and pestle in 3 mL of 90% acetone for 3 min. The sample and solvent were transferred into a 15 mL centrifuge tube, and the total volume was brought up to 10 mL with 90% acetone. The sample was vortexed for 2 min and incubated in the dark at 4 °C for 24 h. After incubation, we centrifuged the sample for 12 min at 4000 RPM at 15 °C and removed the supernatant. The absorbance of the supernatant was recorded at 663 nm using an Ocean Optics CHEMUSB4-VIS-NIR Spectrophotometer (400–950 nm) for chl a content and at 1000 nm for background adjustment. We used the adjusted absorbance and soil sample mass to determine chl a content using calculations outlined in Ritchie (2006).

2.3.3. Soil stability

We measured soil aggregate stability using a field slake kit (Herrick et al., 2001) annually for three years following treatment. Three soil surface samples were collected from an average depth of 2 mm from standardized locations on each plot, slake tested, and averaged for each plot. Additional shear strength and soil surface resistance measurements were taken in Year 3. We determined the shear strength of the soil surface using a hand-held torsional vane shear tester (Humboldt H-

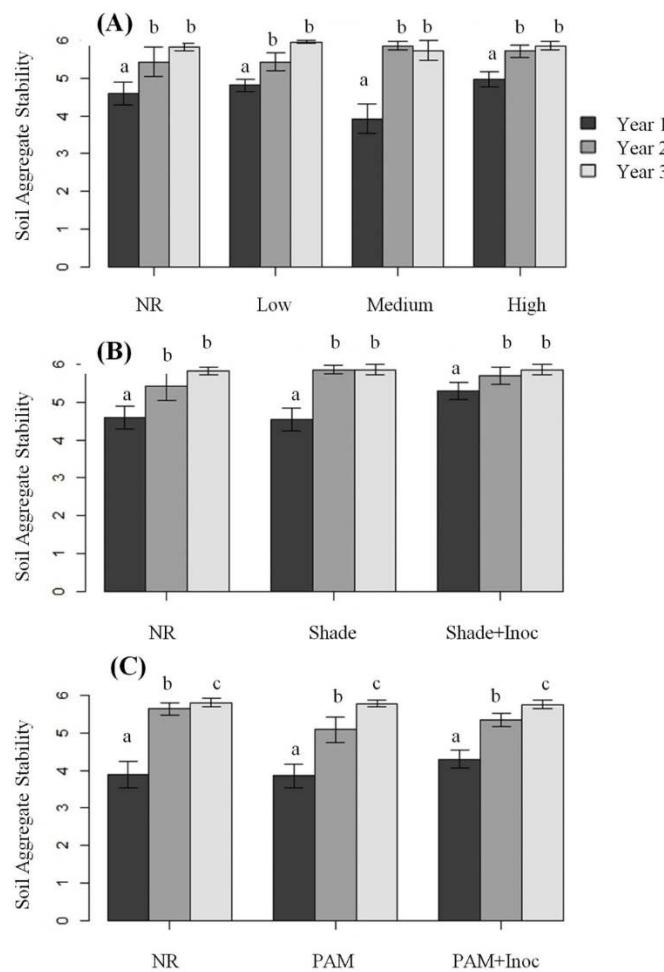


Fig. 4. Average soil aggregate stability for treatments in (A) Inoculum addition experiment; (B) Shading experiment; (C) Artificial soil stabilization using PAM experiment throughout three years following restoration treatment. Letters denote significant differences ($p < 0.05$) between years. Bars show mean \pm SD. NR: natural recovery. PAM: polyacrylamide.

4212MH), and soil surface resistance with a pocket penetrometer (Geotest).

2.4. Data analyses

We performed all data analyses using the statistical program R (R Core Team, 2016), using a linear mixed effect model (Piepho et al., 2003) (R packages: nlme [Pinheiro et al., 2017], and MuMIn [Bartoñ, 2016]). We checked for normality using the Anderson-Darling test and transformed data to meet normality when needed. The few outliers greater than two standard deviations were removed from the dataset. For each response variable (i.e., chl a, CEPS and TBEPS), soil type, year, and treatment were treated as fixed effects, while plot was treated as a random effect. We used the ‘car’ package in R to calculate the Wald Chi-Square test statistic to determine the contribution of each explanatory fixed effect (Fox and Weisberg, 2011). Tukey post-hoc honest significant difference tests were used for pairwise comparisons when data met normality assumptions.

In Year 3, we compared experimental treatment plots to scraped and intact controls using two-way ANOVAs to look at the effects of soil type and treatment on chl a, CEPS, and TBEPS response variables. Tukey post-hoc tests were used to evaluate differences between plot treatments and controls. Simple linear regressions were used to evaluate how much variation in CEPS, TBEPS, and total EPS was explained by chl a amounts.

Using the community ecology “vegan” package in R (Oksanen et al., 2017), we created a soil stability response variable based on Bray-Curtis dissimilarities of soil aggregate stability, shear strength, and soil surface resistance using a data-reduction non-metric multidimensional scaling (NMDS) method. We used a non-parametric permutational analysis of variance (PERMANOVA) approach (Anderson, 2001) to determine the impact of CEPS, TBEPS, and soil type on overall stability.

For all statistical tests, an alpha of ≤ 0.05 was used to determine significance.

3. Results

3.1. Inoculum additions

Overall visual observations of the plots included biocrusts getting darker (more dark cyanobacteria and *Collema tenax*) and containing more microtopography as time following restoration treatments increased. Plots at the clay site generally had higher levels of development (i.e., more later successional species such as mosses and lichens) over the same amount of recovery time compared to those at the SCL site, which contained more patches of light cyanobacterial biocrusts.

Overall, soil EPS amounts were not strongly influenced by biocrust inoculation additions. Only colloidal EPS (CEPS) responses showed a significant effect of treatment, but those treatment effects were dependent on soil type (Table 2) as CEPS increased more with inoculum additions on clay soil (Fig. 3A and B). However, CEPS amounts across both soil types did not increase with increasing amounts of inoculum as we had predicted (Fig. 3A and B; Table 2). CEPS and tightly-bound EPS (TBEPS) amounts were affected by an interaction of soil type and year (Table 2). On clay soils, both CEPS and TBEPS increased from Year 2–3 (Fig. 3A, C). On SCL soils, CEPS and TBEPS amounts were similar across all years (Fig. 3B, D). By Year 3 on SCL soil, all treatment plots had similar TBEPS levels to the intact controls (Fig. 3D), which suggests that TBEPS amounts had fully recovered over this time period.

Similar to EPS observations, there was no clear pattern of soil chl a content in response to biocrust inoculation treatments (Fig. 3E and F; Table 2). Although chl a content was influenced by inoculation level, the response was also dependent on year and soil type (treatment x soil type x year) (Table 2). By Year 3 on SCL, chl a amounts in treatment plots were similar to intact control amounts (Fig. 3F). However, NR and medium plots contained significantly less chl a amounts than intact control plots on clay soil (Fig. 3E).

Soil aggregate stability across all treatments and soil types increased from Year 1–2 and stabilized at the highest level after Year 2 (Fig. 4).

3.2. Shading

Shade treatment effects on CEPS were dependent on interactions with year (treatment x year), and with soil type (treatment x soil type) (Fig. 5A and B; Table 2). Shading increased soil CEPS in clay soils, especially in Year 2, but had less of an effect in SCL soils. TBEPS amounts responded to shading and by Year 3, shaded plots had more TBEPS than scraped plots and comparable TBEPS amounts to intact plots (Fig. 5C and D).

Chl a responded positively to shading, but this effect was also dependent on year (Table 2). In Year 3, shade plus inoculum addition resulted in higher chl a amounts compared to NR and shade only treatments. Soil type continued to display the greatest difference in chl a amounts, where levels were 41% higher in clay soil than in SCL (Fig. 5E and F).

Across all treatments (NR, shade, shade + inoc) and in both soil types, soil aggregate stability increased from Year 1 to Year 2, and then achieved the highest level of stability by Year 3 (Fig. 4).

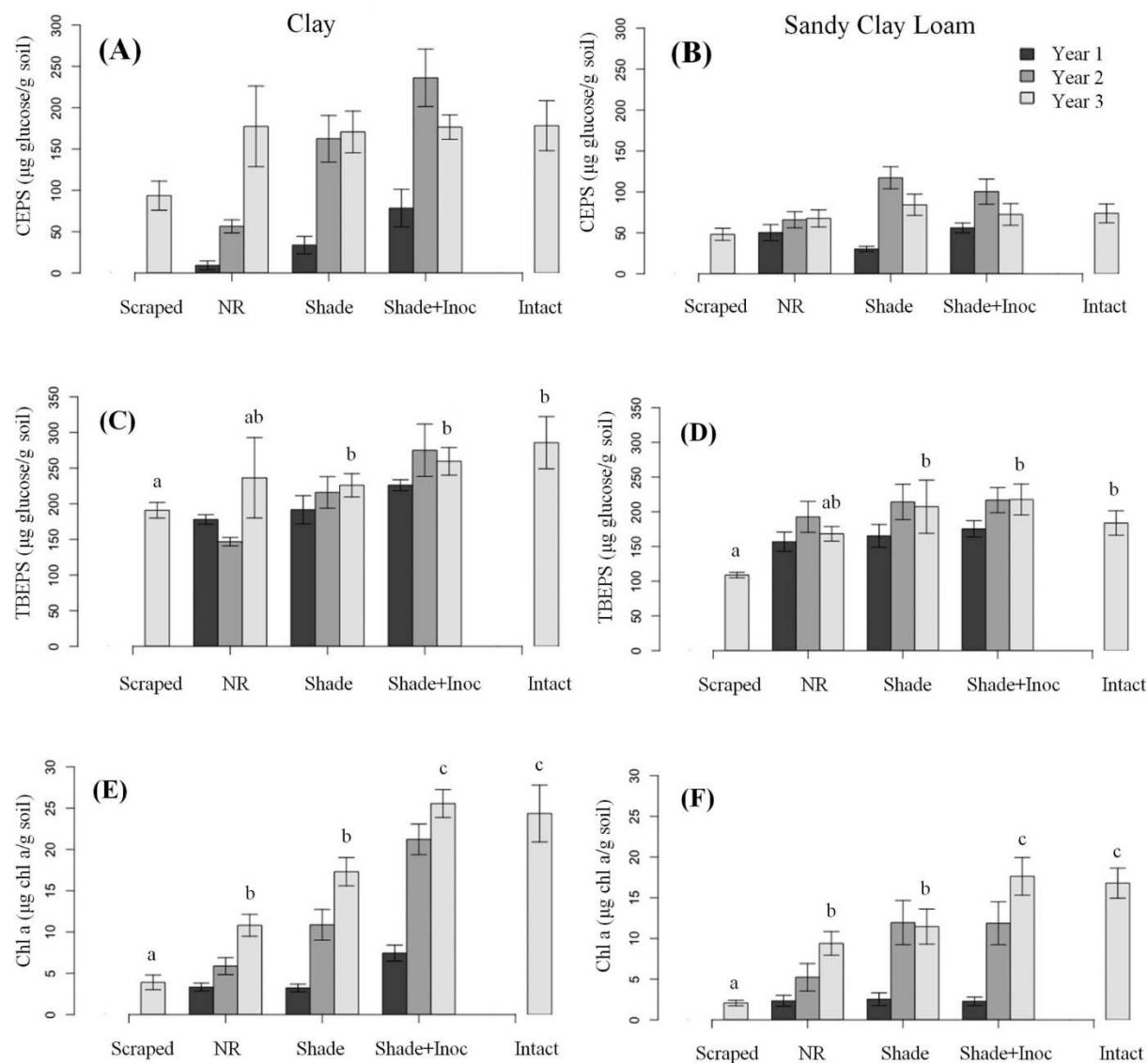


Fig. 5. Results from shading experiment on clay and sandy clay loam soils. Bars show mean \pm 1 SD. Letters denote significant differences ($p < 0.05$) between treatments within Year 3. (A) CEPS responses on clay soil; (B) CEPS responses on sandy clay loam soil; (C) TBEPS responses on clay soil; (D) CEPS responses on sandy clay loam soil; (E) Chl a responses on clay soil; (F) Chl a responses on sandy clay loam soil. NR: natural recovery. Scraped: scraped control. Intact: intact control. Scraped and intact controls were established in Year 3.

3.3. Artificial soil stabilization

The addition of PAM and the addition of PAM + inoc had no effect on CEPS, TBEPS, and chl a on both soil types (Fig. 6A–F, Table 2). Statistical analyses showed that both CEPS and TBEPS were influenced by the interaction between soil and year (Table 2) and that chl a amounts increased with time, especially between Years 1 and 2 (Fig. 6E and F).

Interestingly, PAM additions had no effect on soil aggregate stability across any year or soil type. Soil aggregate stability in all treatment plots and soil types had maximally stabilized by Year 3 (Fig. 4).

3.4. Relationship of EPS to biocrust recovery and soil stability

Approximately 25–34% of the variation in EPS amounts (i.e. CEPS, TBEPS and total EPS) was explained by soil chl a concentrations. (CEPS: $R^2 = 0.27$, $p < 2.2 \times 10^{-16}$, $F = 121.7$; TBEPS: $R^2 = 0.23$, $p < 2.2 \times 10^{-16}$, $F = 100.8$; Total EPS: $R^2 = 0.34$, $p < 2.2 \times 10^{-16}$, $F = 172.4$) (Fig. 7A–C). Total EPS amounts were most correlated with chl a

(Fig. 7C).

Effects of EPS fractions on overall soil stability were mixed and varied by soil type. CEPS and TBEPS had significant but weak positive effects on stability on SCL, while on clay soil, EPS fractions had no effect on overall stability (Supplementary Table S1). Regression analyses between CEPS and TBEPS by individual stability measurements on clay and SCL displayed similar patterns to those corresponding to overall stability, with weak correlations to soil aggregate stability on both soil types. The strongest relationship observed was between TBEPS and soil aggregate stability on SCL ($F = 20$, $R^2 = 0.2$, $p = 3e-5$). However, CEPS correlations to shear strength and soil surface resistance were weak on SCL (Supplementary Table S2).

4. Discussion

The combination of soil surface shading combined with biocrust inoculum addition was the most effective approach to enhance biocrust recovery, especially on clay soils. Shading can be advantageous for recovery because it can reduce UV, temperature, and moisture stressors;

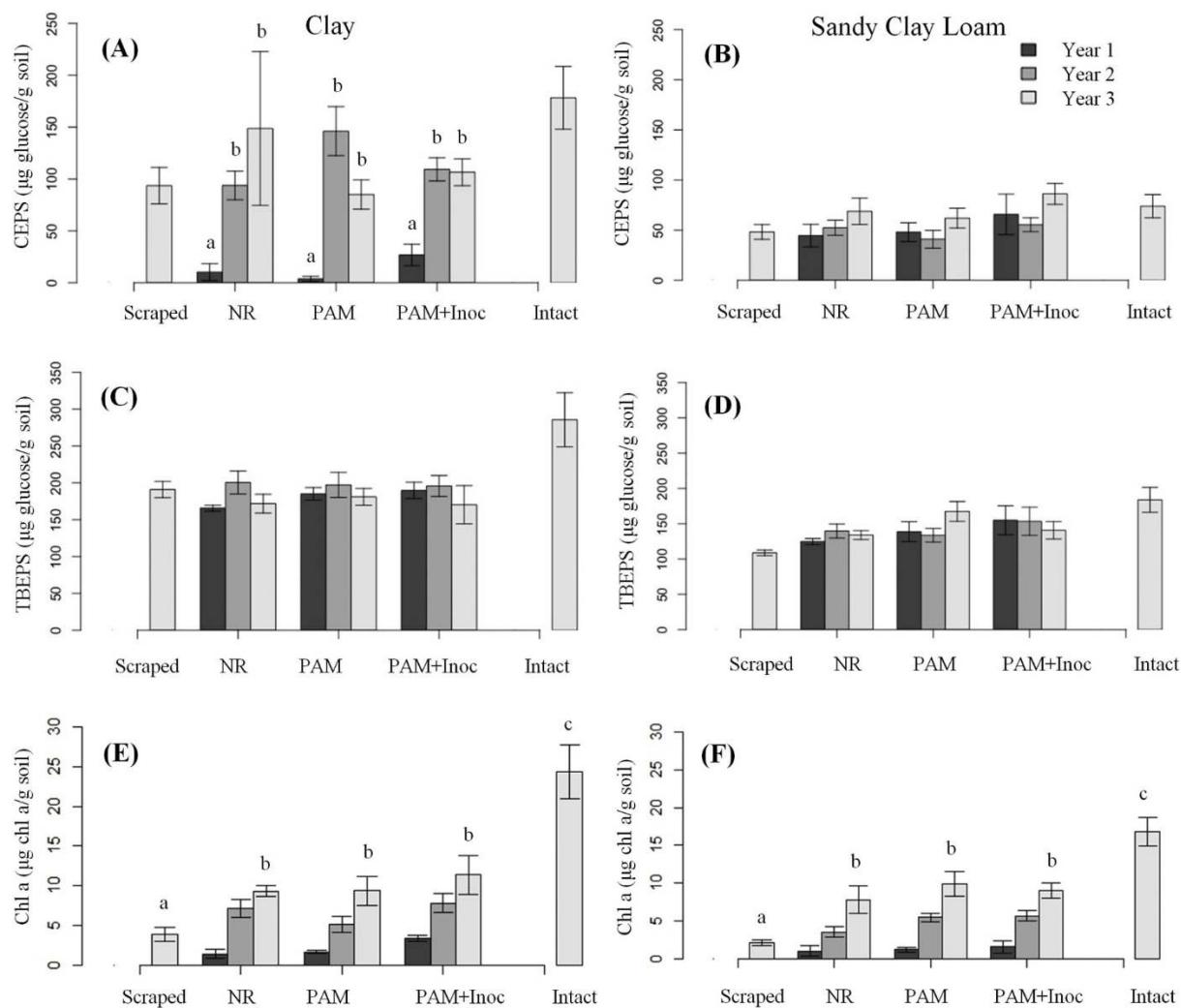


Fig. 6. Results from artificial soil stabilization experiment using PAM on clay and sandy clay loam soils. Bars show mean \pm 1 SD. Letters denote significant differences ($p < 0.05$) between treatments in Year 3. (A) CEPS responses on clay soil; (B) CEPS responses on sandy clay loam soil; (C) TBEPS responses on clay soil; (D) CEPS responses on sandy clay loam soil; (E) Chl a responses on clay soil; (F) Chl a responses on sandy clay loam soil. NR: natural recovery. Scrapped: scraped control. Intact: intact control. PAM: polyacrylamide. Scrapped and intact controls were established in Year 3.

all of which can be significant barriers to biocrust establishment (Bowker et al., 2008). In greenhouse efforts, shading has had varied effects on biocrust growth, depending on how much light intensity and temperature are shifted from their optima (Bu et al., 2014). Other studies attempting to grow biocrust inoculum in the greenhouse also found that water addition and light reduction had positive effects on biocrust growth (Velasco Ayuso et al., 2016), and that 70% shading improved the survival rates of moss biocrusts (Bu et al., 2017). Thus, we can infer that shade likely assisted biocrust growth by increasing soil moisture and reducing UV stress. The finer textured clay soil likely retained more moisture than the SCL soil, which could have contributed to the stronger effect of shade on biocrust growth in the clay soils. Moisture availability has been shown to stimulate EPS production, possibly creating positive feedbacks due to EPS high absorption capacities (Austin et al., 2004; Kidron and Benenson, 2013; Rossi et al., 2017). Even with these advantages, physically shading large areas of land for biocrust rehabilitation would be both costly and time consuming. Utilizing natural shading such as that provided by vegetation, or prioritizing biocrust rehabilitation on sites that experience more insolation may be most effective.

Artificial soil stabilization created by polyacrylamide (PAM) early in

the establishment process did not influence EPS production or chl a amounts after three years in this experiment. Unlike our results, in a previous study, PAM reapplied in 6 month intervals was observed to reduce chl a quantum yield and efficiency on sandy soils after two years (Davidson et al., 2002). In yet other studies, the use of artificial soil stabilizers effectively stabilized moving sand dunes and helped to retain water (Li et al., 2006; Zhao et al., 2016). Our neutral observations of PAM additions may be because on finer textured soils like those in our experiment, the inherent soil aggregate stability properties may outweigh PAM's stability effects.

There were no strong biocrust rehabilitation treatment effects from either inoculum addition or physical manipulations. Chl a, an index of photosynthetic biomass, increased two-to three-fold over the three year period across all plots regardless of treatment applied. CEPS amounts did not differ by treatment and were similar to those of intact controls after three years. Unlike chl a, CEPS did not always increase incrementally by year and could have been more strongly influenced by soil type, time since disturbance, and other factors (Austin et al., 2004; Rossi et al., 2017; Wingender et al., 1999). In addition, CEPS have high turnover rates (Chen et al., 2014), which could have also contributed to their variability. Furthermore, small scale disturbances in small test

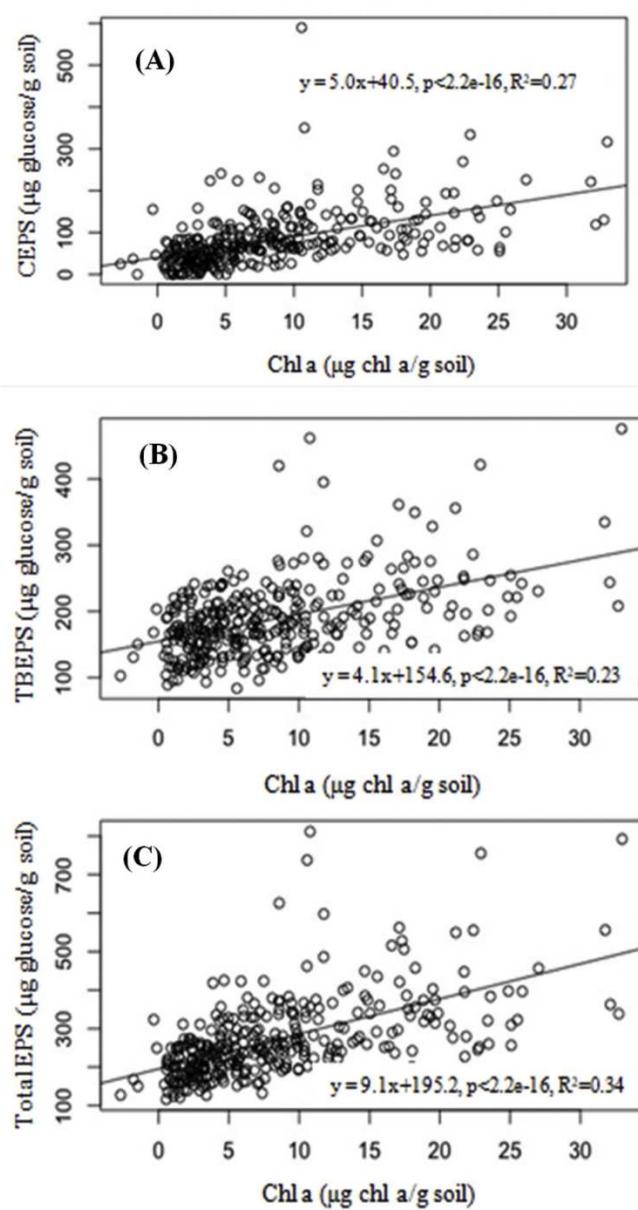


Fig. 7. Correlations between chl a amounts and (A) CEPS; (B) TBEPS; and (C) total EPS.

plots may have had faster recovery of biocrust metrics compared to larger scale disturbances due to dispersal factors (Dojani et al., 2011) and edge effects.

In contrast to CEPSs, TBEPS were more stable throughout time and similar across soil types. This may have been due to early successional biocrust species such as *Microcoleus* spp. establishing quickly and producing large amounts of TBEPS in the first year of recovery (Campbell, 1979), and from other bacteria, microfungi, and microalgal species contained in the inoculum (Velasco Ayuso et al., 2016; Supplementary Fig. S1). Additionally, there could have been remnant TBEPS left behind following the original scraping. We scraped the top 2 cm of the soil surface which removed the majority of the active biocrust; however, EPS in the soil matrix have been observed as deep as 10 cm in undisturbed biocrusts (Belnap and Gardner, 1993). A similar study found that about 20–30% of CEPS and TBEPS in active biocrusts were present 20 cm below the biocrust surface, even though active enzyme activity was reduced to minimal levels at this depth (Chen et al., 2014).

EPS and chl a were more abundant on the clay soil type, indicating faster recovery on the finer textured soil. As demonstrated in other

studies, early successional filamentous cyanobacteria such as *Microcoleus* spp. colonize quickly on finer textured soils due to the ability of cyanobacterial trichomes to bridge smaller spaces between soil particles (Belnap and Gardner, 1993; Rozenstein et al., 2014; Zheng et al., 2011). In this study, rapid biocrust natural recovery rates were likely due to the high soil silt and clay content. In contrast, the majority of past biocrust restoration and recovery studies have been conducted on sandier soils (Colica et al., 2014; Mager et al., 2011; Zhao et al., 2016). Our results from finer textured soils suggest that biocrust rehabilitation through inoculum addition and physical treatment implementation might be prioritized on sandier soils, which have slower natural recovery (Rozenstein et al., 2014) and are more likely to require artificial soil stabilization. Alternatively, if sandy, mobile soils are too difficult to rehabilitate (Li et al., 2004) in the timeline needed, restoration prioritization may instead be focused on finer soil types that are more responsive to these efforts.

Soil aggregate stability demonstrated a rapid increase, taking only two years to recover to the highest level, although chl a amounts were still increasing after two years. This illustrates an interesting contrast between functional recovery versus that of biocrust community composition. Although the functionality of stability and other metrics we tested appeared to have recovered in the three year timeframe, biocrust community composition lagged and was still dominated by early successional cyanobacteria.

Total EPS were correlated with soil aggregate stability, with similar results to those observed in Rossi et al. (2012). However, EPS had little to no effect on overall soil stability including shear strength and soil surface resistance. This inconsistency may have arisen because inherent soil stability from high clay content (42% and 28% for clay and SCL, respectively) may have obscured direct effects of EPS on soil stability. Finer soil textures are more stable than coarser texture soils due to their higher surface area to volume ratio, increasing their ability to bind to minerals and organic matter (NRCS, 1996). However, soil aggregate stability was better explained by EPS on SCL, which contains larger particle sizes and may benefit more from EPS soil aggregation than clay soils.

5. Conclusions

The results of this study can be used to direct future biocrust restoration efforts to regain ecosystem functions in dryland ecosystems. The combination of shade and inoculum is an effective treatment on fine-textured soils, but site-specific treatments must be considered on different soil types. Soil texture must be characterized and taken into account when deciding on biocrust restoration treatments to address rehabilitation barriers present at a site, in conjunction with climatic conditions and other factors (Dojani et al., 2011; Hawkes and Flechtnner, 2002). Though more research is needed to address scaling up restoration techniques to the landscape level, we provide insight into factors that should be considered in order to tailor biocrust restoration efforts to target desired ecosystem functions.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jaridenv.2018.10.007>.

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