

RESEARCH ARTICLE

Accelerating the development of artificial biocrusts using covers for restoration of degraded land in dryland ecosystems

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Abstract

Recent research has revealed the potential for using cyanobacteria inoculation to promote biocrusts on sandy drylands. There is global interest in using this approach to combat land degradation. Nevertheless, in order to use this biotechnology on a large scale, researchers must explore technologies that are simple and efficient to implement. To achieve this aim, we tested the effects of different covers to control dust and stabilize sand surfaces measures—that is, nonwoven fabric, dust-proof net, and sun-shading net—on colonization and development of artificial cyanobacteria crusts on the southeast edge of the Tengger Desert in Northern China. After 80 days, cyanobacteria crusts occurred in all inoculated soils. The best results occurred when using fresh cyanobacteria were covered with two-layer nonwoven fabric and one-layer sun-shading net; this treatment resulted in 50.0% biocrust cover, 2.88 mm biocrust thickness, 19.21 $\mu\text{g cm}^2$ chlorophyll a concentration, 79.05 $\mu\text{g cm}^2$ total carbohydrate content, and 10.00 m s^{-1} threshold friction velocity, which is significantly higher than sand (3.70 m s^{-1}). The results suggest that covering with nets can accelerate development of artificial cyanobacteria crusts, because covers improve micro-environments and remove barriers limiting biocrust colonization. This study describes a potential approach to reconstruct or recover biocrusts, and to restore degraded land in dryland ecosystems.

KEYWORDS

biocrusts, cyanobacteria, degraded land restoration, engineering measures, micro-environments

1 | INTRODUCTION

Climate change and anthropogenic activities have caused desertification to increase, and desertification has become one of the critical social, economic, and environmental issues in much of the world (Reynolds et al., 2007; Schlesinger et al., 1990). Particularly, desertification threatens the productivity of global drylands—a biome that covers about 40% of Earth's land surface and supports 40% of the world population (D'Odorico, Bhattachan, Davis, Ravi, & Runyan, 2013; Reynolds et al., 2007; UNCCD, 2008). Depending on the driver and the geographic setting, desertification can result in increases in bare soil (up to complete denudation of the soil surface),

losses in soil productivity (e.g., loss of nutrients, fine soil grains, and water holding capacity), increases in soil salinity and toxicity, or shifts in vegetation composition (e.g., from perennial to annual species, from palatable to unpalatable grasses, or from grassland to shrubland) (D'Odorico et al., 2013; Reynolds et al., 2007; UNCCD, 2008). Therefore, given the consequences of desertification, it is critical that managers control and rehabilitate degraded lands in dryland ecosystems so that these ecosystems can continue to function and provide ecosystem services that support human populations (Gisladottir & Stocking, 2005; Reynolds et al., 2007). Finding a viable approach to reverse desertification has become an urgent problem for humanity (Feng et al., 2016; Reynolds et al., 2007).

In many low-productivity ecosystems around the world, such as water-limited environments, or early-successional ecosystems, biocrusts form a 'living skin' at the soil surface (Belnap & Lange, 2003). Biocrusts are composed of cyanobacteria, algae, fungi, lichens, and mosses that regulate many functional processes of desert ecosystems (Belnap & Lange, 2003). Recent estimates suggest that these soil surface communities currently cover about 12% of the global terrestrial surface (Rodríguez-Caballero et al., 2018). The contribution of biocrusts to ecosystem function in their habitats makes them an intriguing and promising tool for ecological restoration (Bowker, Reed, Maestre, & Eldridge, 2018; Li, 2012). Artificial cyanobacteria crust cultivation has become one of promising biotechnological strategies for restoring biocrust and soil functionality in arid and semiarid regions (Antoninka et al., 2018; Bowker et al., 2018; Fattahi, Soroush, & Huang, 2020), particularly for increasing soil stability and helping degraded soils resist erosion and increase soil moisture, nutrient content, and biodiversity (Chiquoine, Abella, & Bowker, 2016; Rossi, Li, Liu, & De Philippis, 2017). Artificially induced biocrusts are considered a promising, eco-friendly biotechnological tool to combat desertification (Bowker et al., 2018; Chamizo, Mugnai, Rossi, Certini, & De Philippis, 2018; Zhao, Jia, & Wang, 2019).

In recent years, researchers have studied the feasibility of rapid artificial cultivation and restoration of cyanobacteria crusts, and have obtained important preliminary results. Park, Li, Zhao, Jia, and Hur (2017) showed that manually spraying a combination of fresh cyanobacteria, soil-fixing chemicals, and water once a day for 1 week could facilitate the development of a cyanobacterial crust within 12 months in the Tengger Desert. A study from the western Negev Desert showed that using *Microcoleus vaginatus* with coal fly-ash to enhance soil surface stabilization could improve long-term sustainability of biocrusts (Zaady, Katra, Barkai, Knoll, & Sarig, 2017). However, these technologies are still not widely used (Lan et al., 2014; Li, Hui, & Zhao, 2016). Therefore, to facilitate the wide use of artificial cyanobacteria crust biotechnology in ecological restoration and desertification mitigation projects, technologies must be more efficient and accelerate the formation of cyanobacterial crusts (Perera, Subashchandrabose, Venkateswarlu, Naidu, & Megharaj, 2018; Zhao et al., 2019).

Several studies, such as those in Negev Desert, have demonstrated that biocrust growth is linked to the stability of the sand surface, shading, temperature, and soil surface moisture content (Kidron, 2018; Kidron, Barzilay, & Sachs, 2000). Employing covers—including nonwoven fabric soil covers, dust-proof nets, and sun-shading nets—is a common technique used in China to control dust and stabilize soil and sand surfaces. Soil surfaces covered with dust-proof nets can decrease wind speed 50–70%, can reduce dust emissions by more than 80%, and can decrease light intensity (Xing & Hu, 2004). Furthermore, covering materials and techniques for sand surfaces can be engineered to be efficient and to require low labour costs (Wu, 2010). We hypothesize (a) using covers can enhance artificial biocrust colonization and development; and (b) micro-environment conditions can significantly influence the colonization and development of artificial biocrusts. In the current field-based study, we tested the effects of different soil-covering on colonization

and development of artificial cyanobacteria crusts on the southeast edge of the Tengger Desert in northern China.

2 | MATERIALS AND METHODS

2.1 | Study region

Field experiments were conducted at the Shapotou Desert Research and Experiment State Key Station, located on the southeast edge of the Tengger Desert in Northern China (37°27'36.8"N, 105°00'42.7"E), at an elevation of 1,339 m. The soil substrate is made up of loose and infertile mobile sand: 99.67% sand, 0.01% silt, and 0.22% clay; bulk density is 1.53 g cm³. The mean annual air temperature and wind velocity are 9.6°C and 2.9 m s⁻¹; mean annual rainfall is 186.6 mm and occurs from June to September (Li, He, Duan, Xiao, & Jia, 2007; Li, Jia, Long, & Zerbe, 2005).

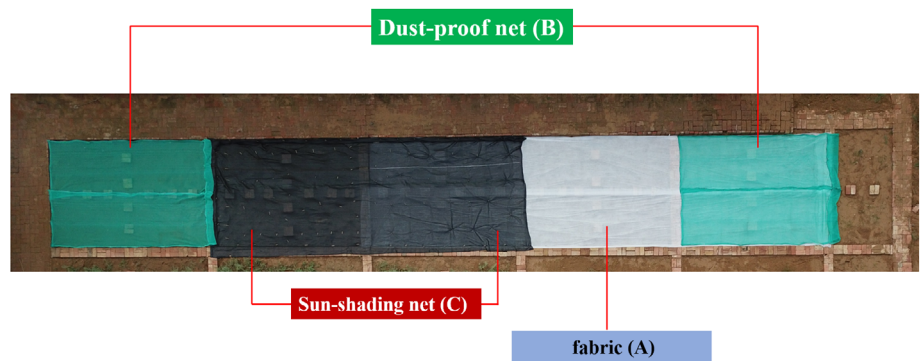
2.2 | Field experiment design

The study site was established after a mobile dune was flattened. We established experimental plots, each with 3.0 m² (1.5 m × 2.0 m) area. Soil surface plant litter and stones were removed from each experimental plot.

In early August 2018, we established the following 7 treatments with 4 replicates each and each plot was arranged in a randomized manner: (a) uncovered fresh cyanobacteria (CK); (b) fresh cyanobacteria covered with two-layer nonwoven fabric (NWF); (c) fresh cyanobacteria covered with dust-proof net (DPN); (d) fresh cyanobacteria covered with sun-shading net (SSN); (e) fresh cyanobacteria covered with two-layer nonwoven fabric first and then covered with dust-proof net (NWF + DPN); (f) fresh cyanobacteria covered with two-layer nonwoven fabric first and then covered with sun-shading net (NWF + SSN); and (g) natural biocrust as a reference treatment (N-BSC). Application of inoculum of cyanobacteria, the nonwoven fabric, dust-proof net and sun-shading net were spread over the soil (the covers on touch with the treated surface). We applied fresh cyanobacteria at 200 g fresh weight m⁻² (about 10 g dry weight, or 11.13 mg m² chlorophyll a concentration, about 0.2 mm thickness) by spraying each treatment over the plot surfaces once.

The nonwoven fabric was white, 1.60-m wide, and weighed 30 g m² (Hualuo Sealing Material Co. Ltd., Hebei Province, China). The nonwoven fabric was often broken by sun and wind within short periods of time, thus we covered each plot with two-layer nonwoven fabric; if one layer was broken, the other layer remained. We replaced broken fabric in the field as soon as possible, and replaced all two-layer nonwoven fabric every 15 days. The dust-proof net was green, polyester fiber, and 1.50-m wide, with 800 mesh per 100 cm² (Oushun Chemical Fibre Rope Net Co. Ltd., Shandong Province, China). The sun-shading net was black, high-density polyethylene, and 5.0-m wide, with 6 needle density (Jinjunma Sun-Shading Net Co. Ltd., Shandong Province, China). For images of the nonwoven

FIGURE 1 Images of the nonwoven fabric (a), dust-proof net (b), and sun-shading net (c) used in current study [Colour figure can be viewed at wileyonlinelibrary.com]



fabric, dust-proof net and sun-shading net, see Figure 1. The N-BSC reference treatment was a 6-year-old developed natural biocrust, 300–500 m from the incubated biocrusts plots. We did not add supplemental water to any of the plots. Rainfall during the study was only source of water (for the daily amount of rainfall during the experiment, please see Table S1).

2.3 | Mass cultivation of cyanobacteria

We collected samples of cyanobacteria crust from crustal locations along the southeast edge of the Tengger Desert. We used the methods described in Park, Li, Jia, and Hur (2014) to isolate and culture the cyanobacteria samples. Because species differ in their preferences and functional traits, and to help us identify which species were most successful at our sites (Antoninka et al., 2018; Bowker & Antoninka, 2016), we used five mixed cyanobacterial species in our artificial crust cultivation: *Anabaena* sp., *Nostoc* sp., *Phormidium* sp., *Scytonema* sp. and *Tolypothrix* sp. Cyanobacteria were cultured en masse in an open cement container (1.0 × 1.0 × 15.0 m), which allowed them to directly exploit sunlight (Rossi et al., 2017). The container was filled with BG-11 liquid medium to a depth of 0.50 m for 10 days in late July to early August 2018, following methods described in Zhao et al. (2019). To provide reasonable light intensity and temperature, culturing took place in a greenhouse with the top covered by a shade net. Given that cyanobacteria can accumulate on the floor of the container, we used pumps to agitate cyanobacteria during the cultivation period, bringing cyanobacteria deposited on the floor of the container to the water surface (Li et al., 2016; Rossi et al., 2017). In order to provide relatively even agitation for different parts of container, we installed two pumps in the middle and head of the container. For an image of our facilities for large volume cultivation of cyanobacterial inoculants, please see Figure S1. The temperature of the liquid medium was 20–25°C under artificial radiation 12 hr per day; light intensity was 600–800 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$; air was supplemented by immersible pump (QDX15-10-0.75L2, Shimgo Pump Industry Group Co., Ltd., Zhejiang Province, China). We harvested cyanobacterial cells during the exponential growth phase (Park et al., 2017) using a nylon filter net with a pore diameter of 25 μm ; the growth curve of mixed

cyanobacterial species cultivated in a cement container is described in our previous study (Li et al., 2016). We used cyanobacteria cultured in the laboratory as seed for mass cultivation in the field at Shapotou Station.

2.4 | Field measurements

We conducted our fieldwork from early August to end of October 2018. We used point sampling frames to measure biocrust coverage after 30, 60, and 80 days of development in the field (Li, He, Zerbe, Li, & Liu, 2010), and measured biocrust thicknesses at the same time using a Vernier caliper.

Artificial biocrust and control samples were collected at 80 days of development in the field. We used a stainless-steel circular box, with inner diameter 1.76 cm (2.43 cm^2) and 0.50 cm depth, for regular sampling of biocrusts and the treated soils. To account for spatial heterogeneity, we randomly collected 18 cores total for each treatment. Next, 3 cores were randomly selected to mix together to form a composite biocrust sample. Using this method, we obtained 6 mixed samples for each treatment, 3 samples for measuring the chlorophyll a concentration and 3 samples for measuring total carbohydrate contents.

The biomass of cultivated biocrust was determined by measuring the chlorophyll a concentration. We estimated chlorophyll a concentrations from samples collected after 80 days of development in the field. Briefly, 20 ml of ethanol (99.9%) was added to 2.43 cm^2 soil in a 50-ml cap tube, then allowed to stand for 5 min in a 80°C water bath, after which the mixture was allowed to cool for 30 min and centrifuged at 4000 rpm for 5 min. Light absorption of the extracted solutions was then measured at wavelengths of 665 nm (A665) using a spectrophotometer (UV-1700 PharmaSpec, Japan), see Li et al. (2016). Generally, chlorophyll a concentrations are estimated according to Equation (1) (Ritchie, 2006). However, we estimated chlorophyll a concentration per unit area; thus we changed the denominator 'sample weight (g) × cell path length (cm)' to 'sample area (cm^{-2})', and changed the unit of chlorophyll a concentration to $\mu\text{g cm}^{-2}$ instead of $\mu\text{g g}$ (Li et al., 2016). We calculated chlorophyll a concentrations according to the Equation (2):

$$\text{Chlorophyll a concentration } (\mu\text{g g}) = \frac{11.9035A665 \times \text{ethanol (ml)}}{\text{sample weight (g)} \times \text{cell path length (cm)}} \quad (1)$$

$$\text{Chlorophyll a concentration } (\mu\text{g cm}^2) = \frac{11.9035A665 \times \text{ethanol (ml)}}{\text{sample area (cm}^2\text{)}} \quad (2)$$

We determined total carbohydrate contents according to the phenol sulfuric acid method. Briefly, an area (2.43 cm² in current study) of the biocrust sample was placed into a test tube, then added 1 ml of distilled water and 5% phenol solution. Next, we added 5 ml of concentrated sulfuric acid, and let it stand 1 hr at room temperature. The tubes were then centrifuged at 4600 g for 10 min to remove the suspended sediments. Light absorption of the extracted solutions was then measured at wavelengths of 485 nm using a spectrophotometer (UV-1700 PharmaSpec, Japan) (Safařík & Šantrůčková, 1992). A linear regression curve was obtained using glucose (Sigma-Aldrich, St. Louis, MO) as a standard polysaccharide material (Park et al., 2017). The total carbohydrate content was calculated using a regression equation. All treatments were tested in triplicate.

The threshold friction velocity (TFV) was determined using a portable wind tunnel. The tunnel is open-circuit, with a 15.0 × 15.0-cm cross section, a 2.4-m length of transparent polycarbonate and a 3:1 contraction section with a honeycomb flow straightener. Wind speed was estimated by a pitot tube (∅ 0.03 m) inside the tunnel attached to a MP120 manometer (Kimo, Montpon Ménestérol, France). Wind tunnels were placed on each experiment plot and the pitot tube was set at 7.5 cm above the soil surface, as described in Park et al. (2017). Wind speed was then gradually increased until the threshold friction velocity of each cyanobacterial crust occurred; the threshold friction velocity was maintained for 3 min. The threshold friction velocity was defined as the value at which particles or small fragments were initially detached and moved forward from the soil surface (Li et al., 2010; Li, He, et al., 2010).

Soil gravimetric moisture content (SMC) of the surface soil (depth 0–2.0 cm) was measured by oven-drying method. Soil surface temperature (SSTem) and air relative humidity (ARHum) at 0.10 m were measured with DT616CT temperature and humidity data loggers (Hong Kong CEM company, Hong Kong), with an accuracy of ±0.2°C in temperature and ±2% in humidity, as described in Zhao, Li, Zhang, Hu, and Huang (2015). All measurements were made on clear days in mid-August, September, and October.

To calculate shading rate (SR), we measured light intensity under uncovered (UNC) and covered (C) conditions with TES1339 illuminometer (Taishi Electronic Industry co., LTD, Taiwan), with a measuring range from 0.00018 to 18,000 μmol m⁻² s⁻¹, and a measurement resolution of 0.00018 μmol m⁻² s⁻¹. The measurements were made on clear days in mid-August, September, and October at 14:00 local time. Shading rate was calculated according to the formula:

$$\text{SR}(\%) = \text{C}/\text{UNC} \times 100 \quad (3)$$

For our survey of herbaceous plants, we established 1.0 × 1.0 m² survey quadrats on each plot, and measured the coverage, density, and height of herbaceous plants at the end of September (see Figure S2 for descriptions of herb characteristics).

2.5 | Data analyses

We used one-way ANOVA to test for differences in the biocrust coverage, thickness, chlorophyll a, and total carbohydrate content among different treatments. A post hoc test was conducted using Duncan when equal variance occurred and Tamhane's T2 when equal variance did not occur. The relationships between incubated biocrust properties (coverage, thickness, chlorophyll a, and total carbohydrate content) and micro-environment factors (soil moisture content, soil surface temperature, air relative humidity, and shading rate) were fitted by linear function. In addition, we conducted stepwise linear regression analysis with incubated biocrust coverage, thickness, chlorophyll a and total carbohydrate content as dependent variables, and with micro-environment factors and herb characteristics as independent variables. The best model to predict dependent variables was selected based on the determination coefficient (*R*²) and Akaike information criterion (AIC), which is a penalized likelihood criterion; the best statistical model minimized the value of AIC (Burnham & Anderson, 2002). The data used in linear and stepwise linear regression analysis did not include natural growing biocrusts. The data obtained were used as input data for a statistical analyses. Prior to these analyses, data were tested for assumptions of normality and homogeneity of variances and then sine- or log-transformed when necessary. All analyses were performed using SPSS 16.0 statistical software (SPSS Inc., Chicago, IL).

3 | RESULTS

3.1 | Coverage and thickness of incubated biocrust

The incubated biocrusts successfully colonized the sand surface in all treatments. The coverage of biocrusts rapidly increased during the first 30 days after spraying, particularly in the NWF + SSN treatment (Figures 2 and 4A). Coverage remained relatively stable in all treatments over the next 50 days of the experiment; variation in cover ranged no more than 5% for any treatment (Figures 2 and 4A). After 80 days of development all treatments had significantly more biocrust coverage than did control plots (CK), which had no cyanobacterial crust. Biocrust coverage was most extensive in the NWF + SSN treatment (*p* < .05). After 80 days, the biocrust coverages were 17.5 for NWF, 9.0% for DPN, 15.0% for SSN, 20.8% for NWF + DPN and 50.0% for NWF + SSN. The biocrust coverage of all treatments was lower than that of naturally occurring biocrusts (N-BSC) 81.2% (*p* < .05; Figures 2 and 3A).

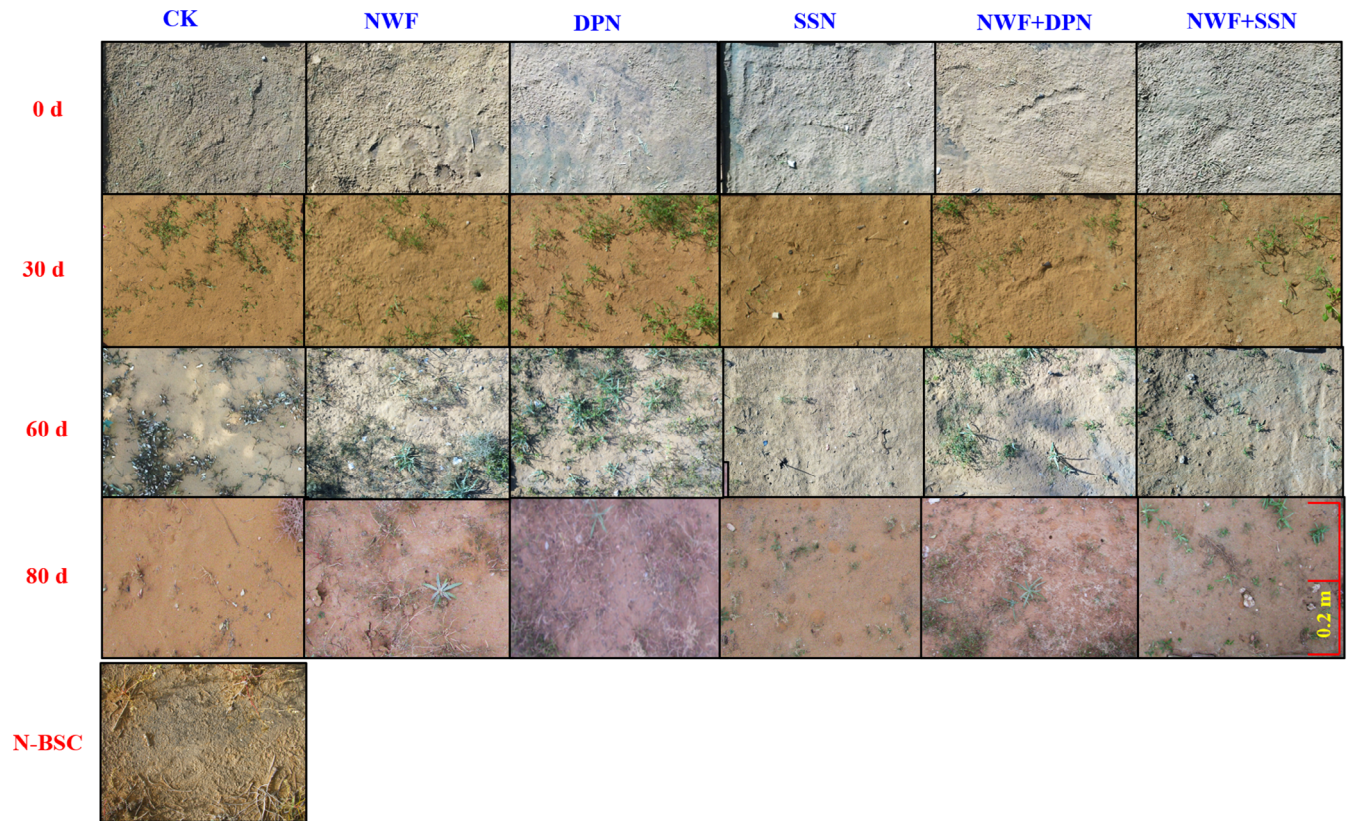


FIGURE 2 Images of incubated biocrusts in the field 0, 30, 60, and 80 days after inoculation and natural biocrust. Treatments: fresh cyanobacteria (CK); fresh cyanobacteria covered with two-layer nonwoven fabric (NWF); fresh cyanobacteria covered with dust-proof net (DPN); fresh cyanobacteria covered with sun-shading net (SSN); fresh cyanobacteria covered with two-layer nonwoven fabric and dust-proof net (NWF + DPN); fresh cyanobacteria covered with two-layer nonwoven fabric and sun-shading net (NWF + SSN); natural biocrust (N-BSC) [Colour figure can be viewed at wileyonlinelibrary.com]

The thicknesses of the incubated biocrusts rapidly increased during the first 30 days of development, particularly in NWF + SSN treatment. After 30, 60, and 80 days, the incubated biocrusts in the NWF + SSN treatment were thicker (2.71, 2.85, and 2.88 mm, respectively) than the incubated biocrusts in the other treatments, but still thinner than N-BSC, which was 3.60 mm ($p < .05$; Figures 3 and 4B).

3.2 | Chlorophyll a and total carbohydrate of incubated biocrust

After 80 days, the chlorophyll a concentration of the incubated biocrusts were no different across all treatments (13.05–15.21 $\mu\text{g cm}^{-2}$), but they were roughly half of the biocrust chlorophyll a in N-BSC (22.45 $\mu\text{g cm}^{-2}$) ($p < .05$, Figure 4C).

After 80 days of development, total carbohydrate content of incubated biocrusts in the NWF + SSN treatment (79.05 $\mu\text{g cm}^{-2}$) were higher than the biocrusts in the DPN and SSN treatments (54.15 and 61.48 $\mu\text{g cm}^{-2}$, respectively), but less than half of total carbohydrate content in N-BSC (162.43 $\mu\text{g cm}^{-2}$) ($p < .05$, Figure 4D).

3.3 | Threshold friction velocity of incubated biocrust

The soil fragments of cyanobacterial crusts developed over 80 days were initially detached when wind speeds reached between 8.50–10.00 m s^{-1} (statistically indistinguishable from N-BSC) in all treatments other than the CK control soil, which detached at much lower wind speeds (3.70 m s^{-1}) (Figure 5).

3.4 | Micro-environment characteristics in different treatments

During the study period, the average soil moisture content was highest in NWF + SSN treatment (2.34%), and was highest in the DPN treatment in August (3.69%) and in the NWF + SSN treatment in September (3.01%, $p < .05$; Figure 6A). The soil surface temperature was lowest in the NWF + SSN treatment (27.33 and 22.67°C, $p < .05$) in September and October (Figure 6B). In August, the air relative humidity was higher in CK, DPN, SSN, and NWF + SSN treatments (52.33, 53.33, 52.97, and 54.57%, respectively) than in NWF and NWF + DPN treatments (48.96 and

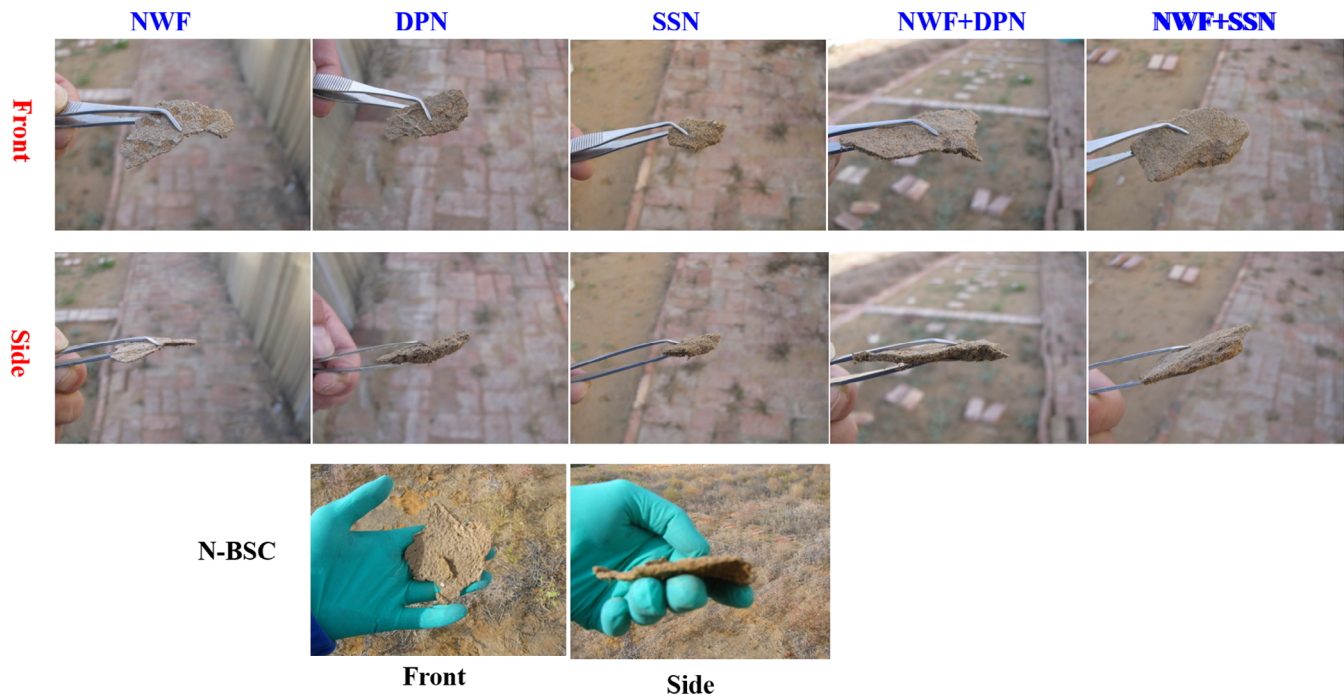


FIGURE 3 Images of the front and side of incubated biocrusts in the field 80 days after inoculation and natural biocrust. Treatments: fresh cyanobacteria covered with two-layer nonwoven fabric (NWF); fresh cyanobacteria covered with dust-proof net (DPN); fresh cyanobacteria covered with sun-shading net (SSN); fresh cyanobacteria covered with two-layer nonwoven fabric and dust-proof net (NWF + DPN); fresh cyanobacteria covered with two-layer nonwoven fabric and sun-shading net (NWF + SSN); natural biocrust (N-BSC) [Colour figure can be viewed at wileyonlinelibrary.com]

47.53%, respectively; $p < .05$; Figure 6C). The shading rate in the NWF + SSN treatment (85.47%) was higher than other treatments ($p < .05$; Figure 6D).

3.5 | Relationships between coverage, thickness, chlorophyll a and total carbohydrate content of incubated biocrust, micro-environment factors, and herb characteristics

Soil surface temperature (SSTem) and shading rate (SR) were significantly related to coverage of incubated biocrust ($R^2 = 0.771$ and 0.680 , $p = .021$ and $.044$, respectively; Figure 7A and B); air relative humidity (ARHum) was marginally negatively related to coverage of incubated biocrust ($R^2 = 0.562$, $p = .086$; Figure 7C). Soil moisture content (SMC), SR and threshold friction velocity (TFV) were positively related to thickness of the incubated biocrust ($R^2 = 0.724$, 0.544 , and 0.881 ; $p = .032$, $.094$, and $.006$, respectively; Figure 7D, E, and F). TFV was positively related to chlorophyll a of the incubated biocrust ($R^2 = 0.950$, $p < .001$; Figure 7G). The SR and TFV were positively related to total carbohydrate content of the incubated biocrust ($R^2 = 0.595$ and 0.927 , $p = .070$ and $.002$, respectively; Figure 7H and I). Herb coverage (HC), abundance (HA), and height (HH) had no significant relationships to coverage and thickness of incubated biocrusts (Table S2).

3.6 | Stepwise regression analysis of the effects of micro-environment factors and herb characteristics on development of incubated biocrust

Stepwise regression analyses suggested that the R^2 and AIC values for the model for coverage increased when both SSTem and TFV were included as explanatory variables (SSTem alone: $R^2 = 0.772$, AIC = -498.62 ; SSTem and TFV: $R^2 = 0.948$ with AIC = -523.40). The model for thickness using just TFV as an explanatory variable explained 88.1% of the variation in thickness (as reflected by R^2) with an AIC value of -614.96 ; adding SMC as an explanatory variable increased the model's explanatory power to 98.2% (as reflected by R^2) with an AIC value of -646.67 . The model for chlorophyll a using just TFV as an explanatory variable explained 92.3% of the variation in chlorophyll a (as reflected by R^2) with an AIC value of -564.84 . The model for total carbohydrate content using just TFV as an explanatory variable explained 92.8% of the variation in total carbohydrate (as reflected by R^2) with an AIC value of -501.64 ; adding ARHum as an explanatory variable increased the model's explanatory power to 99.8% (as reflected by R^2) with an AIC value of -563.21 ; and adding SMC as an explanatory variable further increased the model's explanatory power to 100.0% (as reflected by R^2) with an AIC value of -611.89 . The inclusion of SR, HC, HA, and HH did not increase the explanatory power of the models (Table 1 and Table S3).

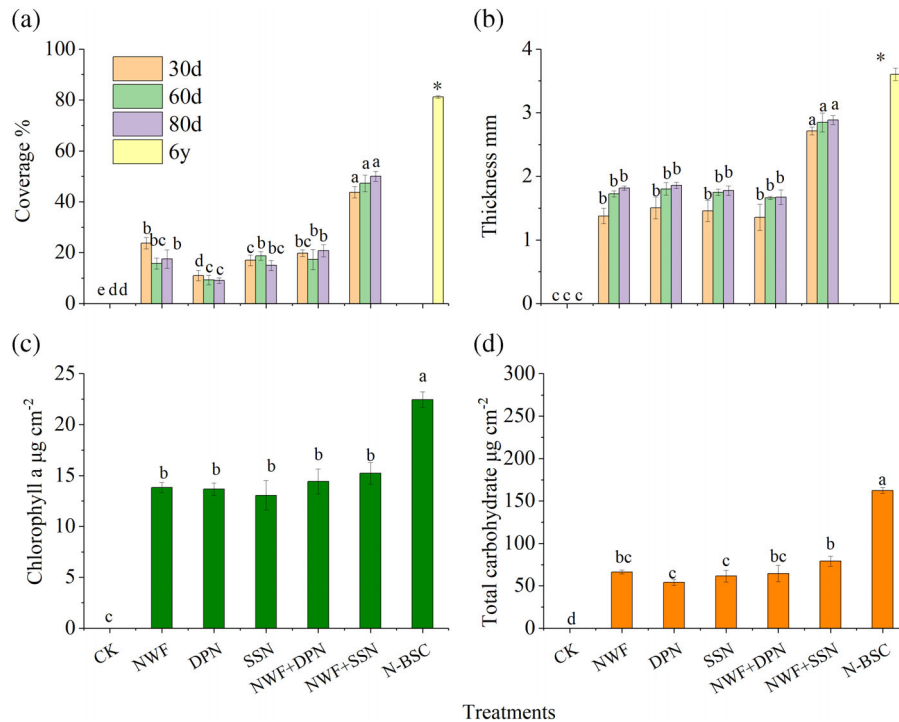


FIGURE 4 Measurements of biocrusts in field plots. (a) Coverage and (b) thickness measurements given after 30, 60, and 80 days of development in the field for experimental treatments and after 6 years for the naturally occurring biocrust control. (c) Chlorophyll a concentration and (d) Total carbohydrate measurements given after 80 days of development. Treatments: fresh cyanobacteria (CK); fresh cyanobacteria covered with two-layer nonwoven fabric (NWF); fresh cyanobacteria covered with dust-proof net (DPN); fresh cyanobacteria covered with sun-shading net (SSN); fresh cyanobacteria covered with two-layer nonwoven fabric and dust-proof net (NWF + DPN); fresh cyanobacteria covered with two-layer nonwoven fabric and sun-shading net (NWF + SSN) and developed 6-year natural biocrust (N-BSC). Bars reflect mean \pm SE. Letters reflect statistically differences between treatments ($p < .05$) [Colour figure can be viewed at wileyonlinelibrary.com]

4 | DISCUSSION

4.1 | Assessing quality of artificial biocrust cultivated by covered net methods

To assess incubated biocrusts quality, studies have measured biocrust coverage, thickness, chlorophyll a content, and total carbohydrate content (Chiquoine et al., 2016; Li et al., 2016; Williams et al., 2018). According to Eldridge and Leys (2003), a crust cover of approximately 20% is required to maintain sediment transport below an erosion control target of $5 \text{ g m}^{-1} \text{ s}^{-1}$ for a 65 km hr wind at 10 m height. In our study, artificial biocrust coverages after 80 days of development reached that 20% threshold in two treatments: NWF + DPN (21%) and NWF + SSN (50%). The thicknesses of the incubated biocrusts in the NWF + SSN treatment (2.88 mm) were higher than the incubated biocrusts in the other treatments. Furthermore, after 80 days of development, coverage and thickness of inoculated crust in the NWF + SSN treatment continued to increase, suggesting that the NWF + SSN treatment provided more stable and suitable conditions for biocrust colonization and development. Thus, the NWF + SSN method to incubate artificial biocrusts appears to be the best method that we tested, as measured by biocrust coverage and thickness.

Several studies have indicated the excretion of total carbohydrate as a key physiological process affecting the outcome of the inoculation process (Mugnai et al., 2017). Most importantly, soil aggregate stability and resistance to wind erosion are closely related to total carbohydrate secreted by cyanobacteria and the moisture content of the topsoil, because carbohydrates tightly bind sand particles (Li et al., 2016; Mugnai et al., 2017). We found the highest total carbohydrate values in the NWF + SSN treatment after 80 days development.

In our study area, there are 11 days on average each year with wind speeds higher than 17.30 m s . There are typically 49 days each year with wind speeds higher than 5.00 m s (Jia, Li, Liu, Gao, & Zhang, 2012). Therefore, wind erosion during the early stage of cyanobacterial crust incubation will occur in soils with a threshold friction velocity lower than 5.00 m s . In our study, the threshold friction velocity reached more than 8.50 m s in all treatments except CK. The NWF + SSN treatment reached 10.00 m s , suggesting that all fabric and shading treatments, especially the NWF + SSN treatment, were strong enough to resist erosion by wind in our study region.

Chlorophyll a is another key index to accessing biocrust (Williams et al., 2018). Our results showed that after 80 days of culture, the chlorophyll a of the incubated biocrusts were between $13.05\text{--}15.21 \mu\text{g cm}$; chlorophyll a in the NWF + SSN treatment reached more than 65% of the chlorophyll a present in the N-BSC. Previous studies have shown

relatively modest levels of chlorophyll a in inoculated biocrusts relative to our study. For example, Park et al. (2017) found levels of cyanobacterial biomass in inoculated biocrusts of 3.1–2.7 $\mu\text{g g}$ after 12 months field incubation. In another study, Antoninka et al. (2018)

found chlorophyll a levels of cyanobacteria of no more than 3.0 $\mu\text{g g}$ after 12 months in field conditions.

After measuring multiple indicators of effectiveness, our study indicates that fresh cyanobacteria covered with two-layer nonwoven fabric and one-layer sun-shading net (NWF + SSN) is an effective method for future artificial biocrust cultivation in arid and semi-arid regions.

4.2 | Effect of micro-environment on the colonization and development of artificial biocrust

Micro-environment factors can significantly influence biocrust colonization and development (Lan et al., 2014; Li, 2012; Park et al., 2017). In our study, cover nets changed light intensity and soil surface temperature, increased soil moisture content and air humidity, and stabilized the sand soil surface. Regression models suggest that the positive effects of the treatments were due to increased shading and soil moisture content, and reduced soil surface temperatures. Additionally, our study occurred during the wet season, which may have further enhanced soil moisture. Similar results were observed in artificial biocrusts in the Qubqi Desert, where shady slopes exposed to less direct solar radiation had more water availability, which hastened the establishment of biocrusts and facilitated their succession (Lan et al., 2014). Another study also showed that shade can promote biocrust growth by facilitating longer durations of wetness and promoting biocrust coverage, density and chlorophyll a in the Negev desert (Kidron, 2016, 2018) and in Loess Plateau (Bu, Li, Wang, & Bowker, 2018; Ma et al., 2012). Also, research in the Negev Desert found that biocrust chlorophyll a was negatively associated with average substrate temperatures and higher air humidity

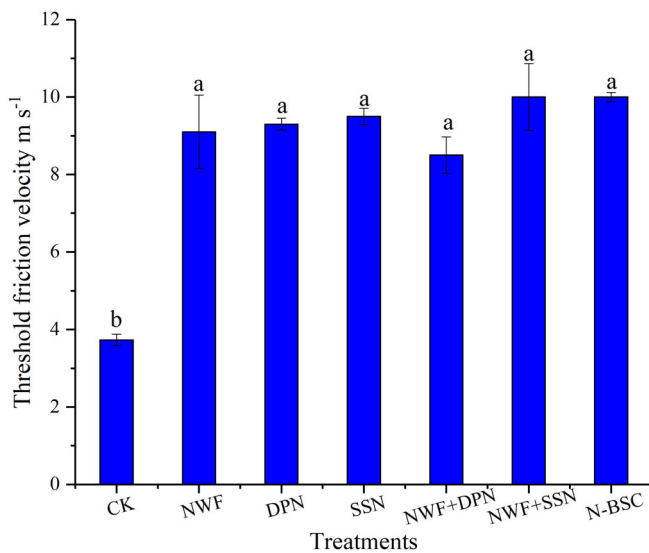


FIGURE 5 The threshold friction velocity of incubated crust after inoculation 80 days in fresh cyanobacteria (CK); fresh cyanobacteria covered with two layer nonwoven fabric (NWF); fresh cyanobacteria covered with dust-proof net (DPN); fresh cyanobacteria covered with sun-shading net (SSN); fresh cyanobacteria covered with two-layer nonwoven fabric and dust-proof net (NWF + DPN); fresh cyanobacteria covered with two layer nonwoven fabric and sun-shading net (NWF + SSN) and developed 6-year natural biocrust (N-BSC). Letters reflect statistically differences between treatments ($p < .05$) [Colour figure can be viewed at wileyonlinelibrary.com]

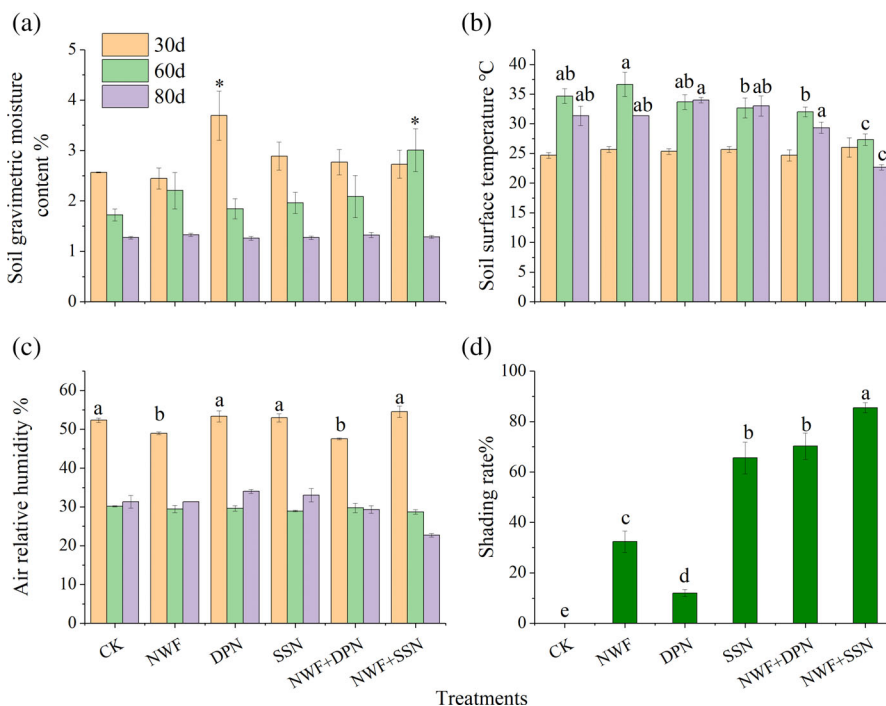


FIGURE 6 Micro-environment, soil moisture content, soil surface temperature, air relative humidity, and shading rate fresh cyanobacteria (CK); fresh cyanobacteria covered with two layer nonwoven fabric (NWF); fresh cyanobacteria covered with dust-proof net (DPN); fresh cyanobacteria covered with sun-shading net (SSN); fresh cyanobacteria covered with two-layer nonwoven fabric and dust-proof net (NWF + DPN); fresh cyanobacteria covered with two-layer nonwoven fabric and sun-shading net (NWF + SSN). Different letters and denote mean statistical significance between treatments ($p < .05$) [Colour figure can be viewed at wileyonlinelibrary.com]

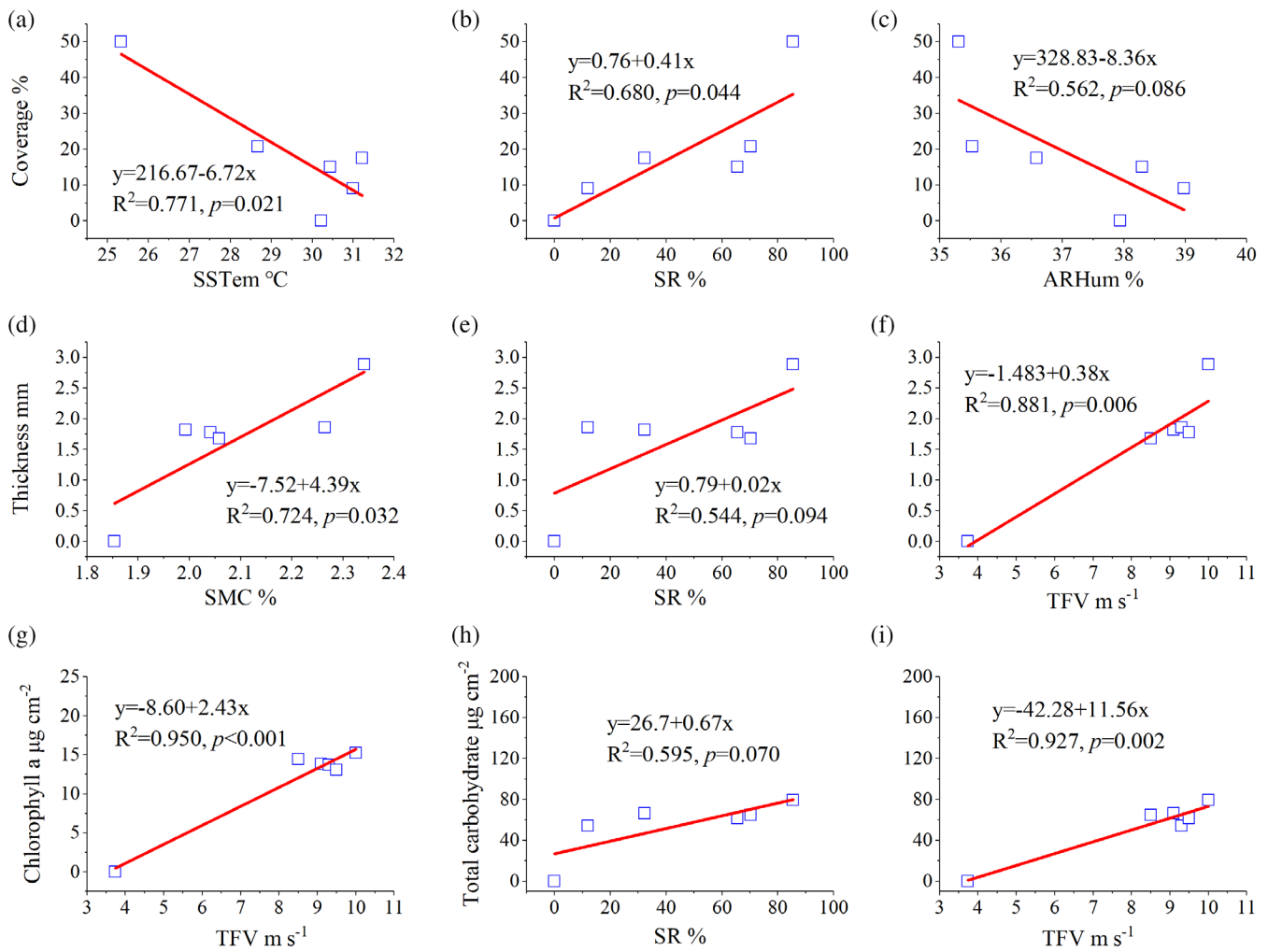


FIGURE 7 Observed (square) and fitted linear regression (solid line) relationships between coverage (a, b, and c), thickness (d, e, and f), biomass (g) and exopolysaccharides (EPS; h and i) of incubated crust and micro-environments factors. We did not include natural growing biocrust in this analysis. ARHum, Air relative humidity; SMC, Soil gravimetric moisture content; SR, Shading rate; SSTem, Soil surface temperature; TFV, Threshold friction velocity [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Stepwise regression analysis on effect of micro-environment factors on colonization and coverage, thickness, chlorophyll a and total carbohydrate content of incubated biocrust, the date were not include natural growing biocrust in this analysis

Dependent variables	Model	Constant	SMC %	SSTem °C	ARHum %	TFV m s ⁻¹	R ²	F	p	AIC
Coverage %	1	216.681		-6.716			0.772	13.514	.021	-498.620
	2	165.216		-5.872		3.185	0.948	27.575	.012	-523.400
Thickness mm	1	-1.479				0.376	0.881	29.631	.006	-614.958
	2	2.846		-0.137		0.343	0.982	80.994	.002	-646.668
Chlorophyll a µg cm ²	1	-8.597				2.429	0.923	47.939	.002	-564.842
Total carbohydrate content µg cm ⁻²	1	-42.226				11.549	0.928	51.475	.002	-501.643
	2	148.962			-4.973	10.755	0.998	709.453	.000	-563.209
		166.978	-10.223		-5.014	11.336	1.000	5,276.0	.000	-611.892

Note: Bold types mean significance at $p < .05$ and $.100$ level.

Abbreviations: ARHum, Air relative humanity; SMC, Soil gravimetric moisture content; SSTem, Soil surface temperature; TFV, Threshold friction velocity.

(Li, 2012). In short, shading by cover nets reduces stress by reducing temperature, and augments resource availability by increasing soil moisture retention and air humidity.

4.3 | Current artificial technologies to future reverse desertification

We find that applying nonwoven fabric and sun-shading net on sand surface can greatly improve micro-environment conditions for biocrust development by, for example, decreasing light intensity and soil surface temperature, and increasing soil surface stability and soil moisture content. The results are comparable or better to biocrust restoration in other locations. For example, we achieved artificial biocrust coverage of 50.0% after 80 days of development. The study from the Qubqi Desert found 60% biocrust coverage 8 years after inoculation (Lan et al., 2014). For biomass, we achieved chlorophyll a values at 60% of naturally growing references after 80 days of cultivation. In the Tengger Desert, the level of cyanobacterial crust chlorophyll a in the inoculated crust reached more than 66% of the naturally growing reference biocrusts (Park et al., 2017). Additionally, our best treatment (NWF + SSN) achieved a threshold friction velocity of 10.00 m s^{-1} after 80 days of cultivation, higher than artificial cyanobacteria crusts cultivated over 12 months by Park et al. (2017), which initially detached at wind speeds of 8.80 m s^{-1} , and also by Zaady et al. (2017), which achieved a threshold friction velocity of no more than 8.00 m s^{-1} using coal fly-ash and bio-inoculant as substrate. In short, covering with nets, particularly NWF + SSN treatment, can accelerate the development of artificial cyanobacteria crusts.

5 | CONCLUSIONS

We successfully cultivated artificial cyanobacteria crusts using fresh cyanobacteria covered with two-layer nonwoven fabric and one-layer sun-shading net for 80 days, resulting in 50.0% biocrust cover, 2.88 mm thickness, $19.21 \mu\text{g cm}^{-2}$ chlorophyll a concentration, $79.05 \mu\text{g cm}^{-2}$ total carbohydrate content, and 10.00 m s^{-1} threshold friction velocity. Reductions in wind erosion and increases in water duration caused by covered nets were crucial for the establishment, coverage and chlorophyll a of the crusts; the nets extended water duration, substantially impeded surface erosion, and reduced soil surface temperature and light intensity. Results suggest that covering with nets can accelerate the development of artificial cyanobacteria crusts by improving micro-environments and removing barriers limiting biocrusts colonization. This study describes a potential approach to biocrust reconstruction or recover and to ecological restoration of degraded dryland ecosystems. Our approach provides a foundation for future improvements and may facilitate wide use of artificial cyanobacterial crust cultivation. For wide use, more research and large scale experiments are required.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Yang Zhao and Zhi Shan Zhang designed research, analyzed data and wrote the paper; Nan Wang analyzed data and edited the paper; Rong Liang Jia and Yan Xia Pan acquired and analyzed data.

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