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SHORT REPORT

Acquiring high-quality and sufficient propagules/fragments for cyanobacteria crust inoculation and restoration of degraded soils in a sandy desert

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Abstract

Sowing naturally developing biocrust fragments is a fast and effective approach to cultivate biocrusts for the restoration of soil function. However, producing large amounts of biocrust fragments/propagules is difficult for ecological restoration, especially at large scales. In this study, we use fresh cyanobacteria to cultivate fragments and then broadcast these to culture artificial biocrusts under field conditions. Our results show that the cultivated biocrust coverage, thickness, chlorophyll a and total carbohydrate concentrations continuously increase after broadcasting. The cyanobacterial communities in incubated biocrusts were dominated by *Phormidium*, *Chroococcidiopsaceae*, *Crinalium* and *Tychonema*. Our study shows that fragments can allow for successful incubation of artificial biocrusts in short periods. We suggest a two-step nursery and sowing procedure (i.e., first acquire fragments and then broadcast them) to incubate artificial biocrusts at large scale.

KEYWORDS
arid land, artificial biocrusts, cyanobacterial communities, land degradation, sandy desert

1 | INTRODUCTION

Artificial biocrust inoculation (i.e., the cultivation of soil with cyanobacteria/algae or moss) is emerging as biotechnology for restoring disturbed or destroyed biocrust and degenerated land in drylands around the World. Artificial biocrusts can increase soil surface stability, soil water and nutrient content, and the diversity of plants and soil microbes (Antoninka et al., 2018; Zhao, Wang, et al., 2021). Biocrust incubation is considered a promising, eco-friendly biotechnology strategy to combat desertification (Chamizo et al., 2018).

Field-harvested biocrusts are ideal materials for artificial biocrust cultivation. They contain almost the entire range of species present in biocrusts and can therefore enhance ecosystem function (Tucker et al., 2020). Sowing naturally developing biocrust fragments is a fast and effective approach to cultivate biocrusts. However, harvesting intact biocrusts on-site—for example, only 10% coverage collection—can lead to new disturbances (Antoninka et al., 2018) and land degradation (Antoninka et al., 2020). Therefore, the direct use of field-collected biocrusts is not always satisfactory because it puts too much pressure on naturally occurring biocrusts. Hence, it is critical to develop alternative methods to produce large amounts of biocrust fragments for large-scale ecological restoration.

Researchers and managers consider cyanobacterial crusts, which are dominated by cyanobacteria, to be one important solution for the restoration of degraded soil. Cyanobacteria play a key role in maintaining soil stability, and also improve soil fertility (Antoninka et al., 2020). Therefore, understanding the characteristics of cyanobacterial community composition of artificial biocrust is a precondition to exploring incubated biocrusts, particularly in field conditions.

2 | MATERIALS AND METHODS

We conducted this work on the southeast edge of the Tengger Desert (Shapotou Desert Research and Experiment State Key Station, Chinese Academy of Sciences), northern China (37°27′36.8″N,

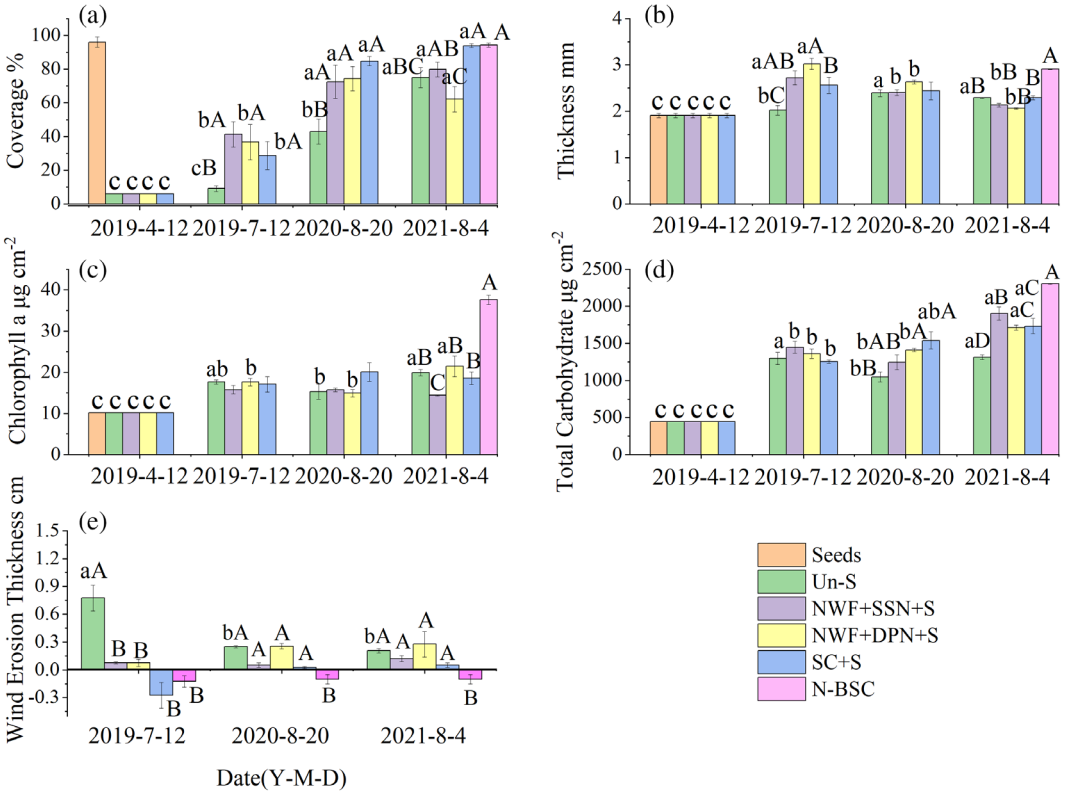

FIGURE 1 Measurements of artificial biocrusts in restoration field plots. (a) Coverage, (b) thickness (c), chlorophyll a concentration, (d) Total carbohydrate and (e) wind erosion thickness measurements of artificial biocrusts development in July 2019, august 2020 and august 2021, respectively. Treatments: Uncovered- fragments (un-S); broadcast fragments and cover with two-layer non-woven fabric first and then cover with sun-shading net (NWF + SSN + S); broadcast fragments and cover with two-layer non-woven fabric first and then cover with dust-proof net (NWF + DPN + S); straw checkerboard (1.0 m × 1.0 m) first and then broadcast fragments (SC + S); and natural biocrust as a reference treatment (N-BSC). Bars reflect mean ± standard error. Small and capital letters reflect statistically differences among same treatment measurement in different time and among treatments in same time ($p < 0.05$) [Colour figure can be viewed at wileyonlinelibrary.com]

105°00′42.7″E; elevation 1339 m). The soil type is aeolian sandy soil. The average annual precipitation, air temperature and wind speed are 186.6 mm, 9.6°C and 2.9 m s^{-1}, respectively.

In early April 2019, the following 5 treatments were established with 3 replicates each and each restoration plot (2.0 m × 4.0 m) was arranged in a randomized manner: (1) uncovered fragments (Un-S); (2) broadcasting fragments and covering with non-woven fabric (two-layers) and sun-shading net (NWF + SSN + S); (3) broadcasting fragments and covering with non-woven fabric (two-layers) and dust-proof net (NWF + DPN + S); (4) straw checkerboard (1.0 m × 1.0 m) and broadcasting fragments (SC + S); and (5) reference treatment (natural biocrust, N-BSC). Here, we define "fragments" as biocrusts cultivated using artificial methods and then harvested for artificial biocrust incubation. The fragments used for artificial biocrust were cultivated in a 3.0 m^2 (1.5 m × 2.0 m) area with 3 replicates using fresh cyanobacteria and covered with net; for a detailed description see our previous study (Zhao, Wang, et al., 2021). The fragments were cultivated during the 9 months following August 2018. We collected the fragments 1 day before sowing in experimental plots. The fragments more than 95% coverage in seeded field plot, 1.91 mm thickness, 10.13 μg cm^{-2} chlorophyll a and 440.54 μg cm^{-2} total carbohydrate concentrations, Figure 1) we broadcast 6% cover on each restoration plot once. The reference treatment was a natural biocrust (N-BSC), about 1 km from the experimental plots. For images and information describing each treatment, see Figure S1. During the study period, precipitation was the only source of water, and no supplemental water added to experimental plots.

We estimated biocrust coverage using a vertical picture of each sample (20 cm × 20 cm) taken with a Canon Eos 70D digital camera. We visually estimated the cover of cyanobacteria. Biocrusts thicknesses was measured using a vernier caliper. Chlorophyll a and total carbohydrate concentrations were used ethanol method and the phenol sulfuric acid method, respectively (Ritchie, 2006; Safařík & Šantrůčková, 1992; Zhao, Wang, et al., 2021). Four steel pin (length × diameter = 0.5 m × 0.02 m) were randomly placed into the soil within each plot to measure wind erosion thickness. We made all measurements in July 2019, August 2020 and 2021, respectively. The biocrust layers were collected at the end of August 2020 and used to analyze cyanobacterial community composition and diversity (Wang et al., 2020). We compared cyanobacterial communities between fragments/natural biocrust and artificial biocrust treatments using an index of similarity which was used to evaluate similarity degree

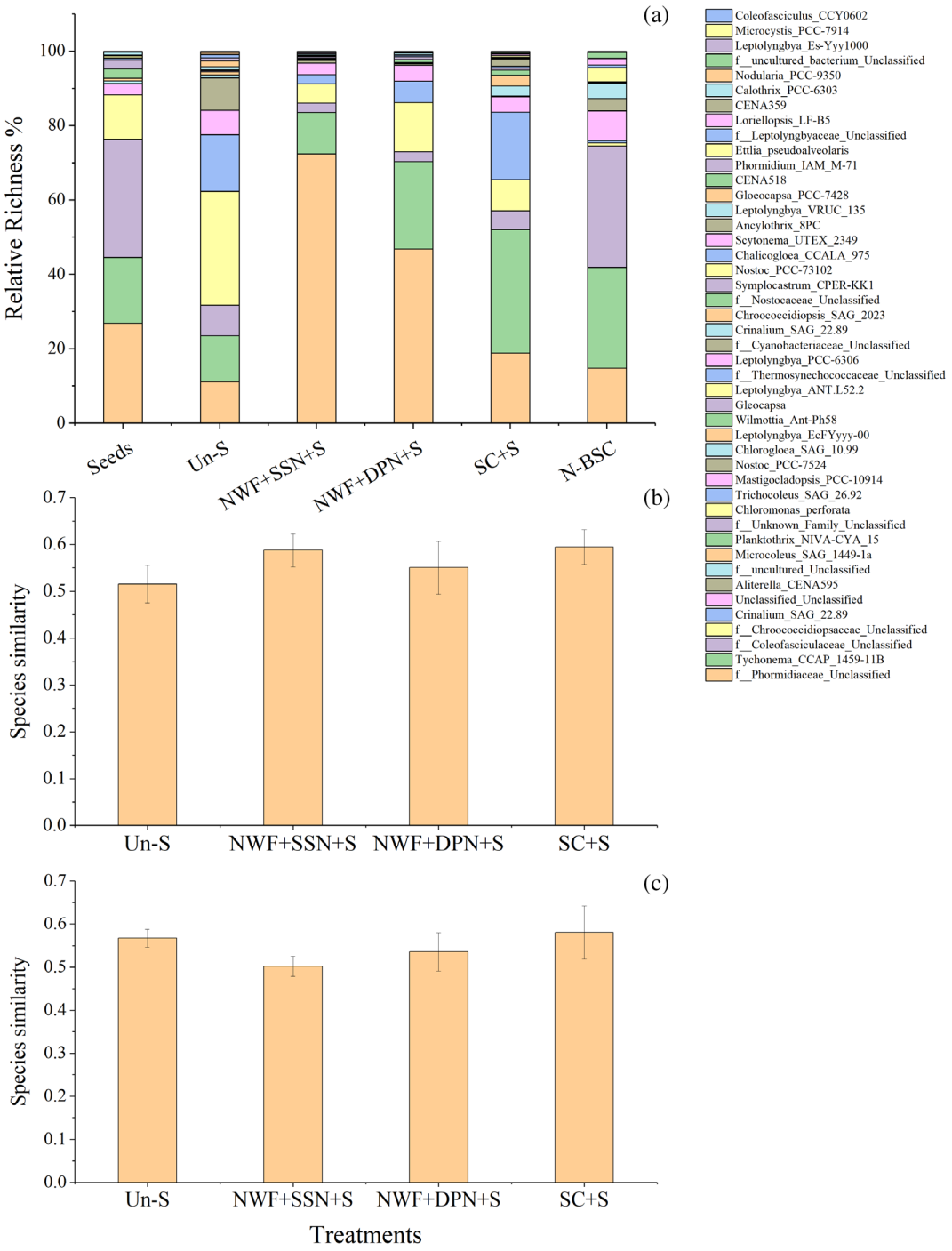

FIGURE 2 Cyanobacterial community composition at the genus level (a) based on 97% similarity and species similarity index between fragments/propagules and incubated biocrusts treatments (b) and between N-BSC and incubated biocrusts treatments (c). Treatments: Uncovered fragments (un-S); broadcast fragments and cover with two-layer non-woven fabric first and then cover with sun-shading net (NWF + SSN + S); broadcastfragments and cover with two-layer non-woven fabric first and then cover with dust-proof net (NWF + DPN + S); straw checkerboard (1.0 m × 1.0 m) first and then broadcast fragments (SC + S); and natural biocrust as a reference treatment (N-BSC) [Colour figure can be viewed at wileyonlinelibrary.com]

between communities or experimental plots. Diversity indices of cyanobacterial operational taxonomic units (OTUs) at the genus level were calculated with "vegan" packages in R version 3.5.1. At the end of July 2021, the 0–2 cm depth soil were collected and used to analyze soil properties and enzyme activities (Guan, 1986); and measured the coverage, height, species number and density of herbaceous plants, each plots are 20 × 20 cm with 9 replicates. All analyses were performed using SPSS 16.0 statistical software (SPSS Inc., Chicago, IL, USA). One-way ANOVA was used to assess differences in variables of incubated biocrusts among the treatments.

3 | RESULTS AND DISCUSSION

During the first 3 months, the incubated biocrust coverage rapidly increased after broadcasting fragments, especially in the NWF + SSN + S and NWF + DPN + S treatments. Coverage increased in all incubated treatments over the next 2 years of the experiment. In the third year, the biocrust coverage of NWF + SSN + S and SC + S treatments was not significantly different than N-BSC treatment (Figure 1a and Figure S2). The incubated biocrust thicknesses rapidly grew during the first 3 months of development; however the thickness increased very little across all treatments over the next 2 years of the experiment. After 3 years, all incubated biocrust treatments were still thinner than the reference treatment ($p < 0.05$; Figure 1b,c). Chlorophyll a and total carbohydrate concentrations of incubated biocrusts continuously increased after broadcasting fragments; the highest values occurred in the third year. However, the incubated biocrusts in all treatments had lower chlorophyll a concentrations than N-BSC ($p < 0.05$; Figure 1d). Wind erosion thickness decreased in all treatments over time, showing that sand surface stability increased with biocrust colonization (Figure 1e). Wind erosion can significantly impact on the biocrust development, especially during early stages, in areas with little coverage (Kidron et al., 2017). Therefore, maintaining sand surface stability during the early stages of biocrust incubation was very important. These results suggest that fragments/propagules can be used to successfully incubate artificial biocrusts over short durations in field conditions, particularly with stable sand surface conditions.

The cyanobacterial communities in fragments were dominated by *Phormidium*, *Coleofasciculaceae* and *Tychonema*. The Un-S treatment was dominated by *Chroococcidiopsaceae*, followed by *Crinalium* and *Tychonema*. The NWF + SSN + S NWF + DPN + S and SC + S treatments were dominated by *Phormidium* and *Coleofasciculaceae*. The N-BSC treatment was dominated by *Coleofasciculaceae*, *Tychonema* and *Phormidium*. The species similarity index values between fragments and incubated biocrust treatments and between N-BSC and incubated biocrust treatments were no more than 0.60 (Figure 2). Our results indicated that the dominant cyanobacterial species of biocrusts can be rapidly restored or reconstructed using artificial cultivation methods.

Incubated biocrusts from fragments established very rapidly, attaining more than 70% cover in only 1 year and producing sufficient quantity. However, natural biocrusts had higher cyanobacterial species richness, biomass, total carbohydrate concentrations and level of development, as well as higher soil nutrition contents, soil enzyme activities, coverage and height of herbaceous plants (Figures S3–S5). This was because incubated biocrusts did not develop the level of diversity and function of natural biocrusts within the timeframe of the experiment. More importantly, the cyanobacterial community composition differed between natural biocrusts versus incubated biocrusts. However, the complete restoration of cyanobacterial diversity and ecological function of biocrusts is a long-term process. The restoration process is significantly affected by environmental factors, such as precipitation, wind speeds, light intensity and soil physical and chemical properties (Wang et al., 2020; Zhao, Xu, & Wang, 2021). The primary goal of adding biocrust inoculum is to reestablish ecosystem function in damaged systems, and the speed at which functional recovery occurs can be critical (Antoninka et al., 2018). Cultivated seeds and natural biocrust fragments/propagules have different advantages in biocrust and ecological function recovery. Thus, the use of both artificially cultivated and naturally available fragments to maintain the diversity and stability of the species and ecological functions deserve further study.

4 | CONCLUSIONS

In conclusion, our three-year study has shown that seeding with fragments/propagules can allow for the successful incubation of artificial biocrusts in short periods of time and can help keep sand surfaces stable. Hence, for artificial biocrust incubation in field conditions, we suggest a two-step procedure in which researchers or managers first incubate biocrusts (in nurseries, for acquiring fragments), and then sow cultivated biocrust fragments to repopulate target restoration areas. We suggested that fragments can be widely used to restore damaged biocrusts or degraded soils at large scales. Our study also suggests that the character of cyanobacterial community's composition, diversity and ecological functions in incubated biocrusts should be continuously monitored during the incubation period.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

No data are available.

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SUPPORTING INFORMATION

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