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REGULAR ARTICLE

Successful field cultivation of moss biocrusts on disturbed soil surfaces in the short term

Chongfeng Bu · Ruxue Li · Chun Wang · Matthew A. Bowker

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Abstract

Aims The artificial cultivation of biocrusts may represent a new low-cost and highly efficient solution to erosion control. However, establishment under varying field environmental conditions is understudied. We tested a variety of methods, arriving at a set of technical recommendations for rapid establishment of moss biocrusts on disturbed slopes, and the industrialization of this process.

Methods In multiple field experiments, aimed at moss biocrust cultivation and establishment, we considered the following factors: nutrient solutions (control and weekly addition); water-retaining agent (control and addition); plant growth regulator (control and biweekly addition); shading (0, 50%, 70% and 90%); dispersal method (broadcast and spray application). In all cases, we initially inoculated soils with 700 g/m^2 of moss biocrust materials. We monitored dynamic changes of the coverage and density of moss biocrusts during the cultivation period, and their biomass at the end.

Results We successfully cultured moss biocrusts in a field setting in as little as two months. Specifically, we found:(1) Regardless of the dispersal method, the nutrient solutions and some degree of shading both increased the coverage, plant density and biomass of moss biocrusts, whereas the water-retaining agent and plant growth regulator had little influence on these parameters. The shading treatments improved the survival rates of moss biocrusts, with the shade rating of 70% exhibiting the best performance. Further, the nutrient solutions had a more positive effect under shaded conditions. (2) The growth of mosses dispersed in the fall exceeded that of mosses dispersed in the summer. (3) Under both dispersal techniques, the maximal coverage of the moss biocrusts exceeded 90%, and the maximal plant density of moss biocrusts reached 120 stems/cm^{2}under broadcast dispersal, and 150 stems/cm^2, under spray dispersal.

Conclusions The rapid restoration of moss biocrusts can best be achieved by spray-dispersal or broadcast-dispersal, while also applying Hoagland solution to supply nutrients and maintaining soil moisture at 15–25%. Fall inoculation appears more likely to lead to better moss establishment, in fact, high moss mortality occurred in summer unless shading was used. We have some evidence, observational in fall, and experimental in summer, that moderate shading favors establishment.

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C. Bu · R. Li · C. Wang
Institute of Soil and Water Conservation, Northwest A & F University, Yangling, Shaanxi 712100, China

C. Bu (✉)
Institute of Soil and Water Conservation, Chinese Academy of Sciences and Ministry of Water Resources, 26 Xinong Road, Yangling, Shaanxi 712100, China
e-mail: buchongfeng@163.com

M. A. Bowker
School of Forestry, Northern Arizona University, 200 East Pine Knoll Drive, PO Box: 15018, Flagstaff, AZ 86011, USA

This technique could feasibly be up scaled and adopted to restore some ecological functions on various types of engineered disturbed surfaces. Over a longer period, the survivorship, succession and sustainability of artificial moss biocrusts should be explored specifically.

Keywords Soil surface damaged by engineering activities · Moss biocrusts · Nutrient solution · Shading rating · Ecological restoration

Introduction

Engineering activities, such as the extraction of energy sources and road construction, have damaged soil surfaces to a great extent throughout the world. These settings may suffer from prolonged ecological degradation, and exhibit various types of dysfunctionality including a propensity for erosion (Sun et al. 2002) and acting as dispersal corridors for non-native plants (Flannery 1994). Revegetation has long been the primary approach for preventing soil erosion, but has some major shortcomings such as high cost, slow growth, poor stress resistance and high rate of failure (Wei 2005). A few previous studies show that the rapid cultivation of an alternative living cover, biological soil crusts (biocrusts: a soil surface community of cryptogams and/or microbes), may offer a new low-cost, high-efficiency approach for soil stabilization, and enhancement of function in damaged soils (Bu et al. 2013a; Antoninka et al. 2015; Bu et al. 2015a, b; Chiquoine et al. 2016; Doherty et al. 2015). In drylands specifically, biocrusts attain variable cover in less disturbed ecosystems, but can commonly dominate the soil surface; reports of 70% cover or greater are common (Li et al. 2015). Biocrusts may be composed of any combination of cyanobacteria, lichens, and mosses among other organisms (Belnap and Gillette 1998). Much progress has been made in artificial culture of cyanobacteria for ecological rehabilitation and related purposes, but recently we are learning that many biocrust mosses can be cultured (Zhao et al. 2016b). In some settings, mosses may be preferable for use in field applications because they function differently than cyanobacteria, and could be used to enhance aesthetic value of degraded land, for example, in the built environment.

As an advanced stage of biocrust succession, moss biocrusts are key carbon-storing organisms in harsh (e.g., dry and barren) environments (Zhao et al. 2016a), and play roles in improving soil fertility (Zhao et al. 2014), increasing soil stability (Patrick 2002), providing erosion resistance (Bu et al. 2015a, b; Yang et al. 2014) and accelerating the recovery of damaged ecosystems (Chiquoine et al. 2016). Importantly for soils disturbed by engineering and construction, moss biocrusts lead to a > 30% decrease in runoff yield and to a > 80% decrease in sediment yield compared to bare soil surfaces (Bu et al. 2015a). However, moss biocrusts may develop very slowly under natural conditions. For example, in China, it takes two years for moss biocrusts to start appearing on bare land in loess regions (Li et al. 2014), and three to four years in sandy deserts (Tian et al. 2006), and the later successional moss components only become prevalent after a decade of growth (Bu 2009). Some regions of the world may develop moss biocrusts much more slowly, after several decades (Weber et al. 2016). During this long recovery process, moss biocrusts development can easily be delayed or thwarted by unfavorable environmental conditions and recurring disturbance. Further, natural development of moss biocrusts is often patchy and heterogeneous, providing incomplete soil protection (Bu et al. 2013b). Possibly, we could control dispersal of mosses to locations in need of treatment, and provide the necessary conditions to rapidly produce a homogenous soil covering of moss biocrusts, as an alternative to simply relying on the natural reestablishment of biocrusts.

In recent years, researchers have studied the feasibility of the rapid artificial cultivation and restoration of moss biocrusts, and have obtained some important preliminary knowledge. The growth and development of mosses are primarily affected by soil moisture (Antoninka et al. 2015; Ram and Aaron 2007), light and temperature (Proctor 1972; Yang et al. 2015) and nutrient availability (Jovanovic et al. 2004). Some researchers have applied this knowledge to artificial moss cultivation methods, and have produced 95% coverage of *Didymodon vinealis* (Brid.) Zand. in a phytotron after only 45 days under optimal soil moisture and light conditions and propagule densities (Yang et al. 2015). Other studies have successfully cultured additional species under laboratory or greenhouse conditions in as little as 30 days, also by manipulating these environmental factors (Xu et al. 2008, Doherty et al. 2015, Antoninka et al. 2015), Although rapid production of moss biocrusts in the laboratory is a clearly feasible means to create moss biocrusts inoculum, a challenge to field application is that moss biocrusts that are cultivated under low-stress laboratory conditions may not be able to survive under adverse field

conditions (e.g., intense light, drought and high temperature) when introduced to the field (Zhang 2012). A possible solution worth investigating is to conduct the artificial growth of biocrusts directly in the field. Zhang (2012) found that the plant density of *Bryum argenteum* Hedw. could reach 70 stems/cm^2 after four months of cultivation in the field, by supplying nutrients, shade, mulch, and water. A similar study also found that *B. argenteum* Hedw. coverage could reach 70% after 75 days of cultivation when initially inoculated at a density of 500 g/m^2 and subsequently watered at a rate of 3 L/m^2 every two days (Yang 2016). Although these growth rates are slower than the best results under laboratory conditions, the successful field establishment of mosses is an important advance and should be further studied to adapt the practice to a wider variety of environmental field conditions.

We conducted a set of experiments in an effort to elucidate best practices in the artificial cultivation and establishment of moss biocrusts, for ecological rehabilitation purposes. We used well-developed moss biocrusts in the Loess Plateau as a propagule source. We manipulated four environmental factors including addition of a nutrient solution, a water-retaining agent, a plant growth regulator and shading. We conducted parallel tests using two methods, broadcasting and spraying, to disperse propagules to the field site. We monitored changes in the coverage, plant density and biomass of the moss biocrusts throughout the growth period. We hypothesized that: (1)Environmental stresses such as nutrient limitation, high temperatures and rapid drying limit moss biocrust growth, and eliminating these barriers will speed growth, (2)Clonal growth of mosses can be manipulated with hormones, resulting in faster field establishment, (3)Moss biocrusts created by spray-dispersal can enhance establishment rates and surface stabilization. If moss biocrusts are generated rapidly, their creation may represent an actionable technique to prevent soil erosion, improve hydrological function, establish a living cover on soil surfaces damaged by engineering and potentially rehabilitate natural ecosystems.

Materials and methods

Study site

The experimental station is located in the river valley of the Weishui River in the Yangling Agricultural Hi-tech Industries Demonstration Zone, in Wuquan Township, Shaanxi Province, China (34°–34°20 N, 108°–108°07E). The thermal regime is warm temperate, with an annual mean temperature of 12.9 °C. Easterlies and westerlies are the prevailing winds, and have a maximum wind speed of 21.7 m/s. Precipitation follows a continental monsoonal regime, with an annual average precipitation of 635.1 mm (most falling in the summer and fall) and an average potential evaporation of 1505.3 mm. The soil is a loam, the organic content and total nitrogen of the arable layer is 1.06% and 0.08%, respectively. Biocrusts are widespread, averaging about 35% cover near the experimental station. There is a small meteorological station in the experimental station, and it monitors the surface temperature, ambient temperature, precipitation, and other weather parameters on a 24-h basis. The present study involved the installation of test plots on a 15° slope (Fig. 1).

Moss biocrusts inoculum collection and preparation

Moss biocrust inoculum for the broadcast and spray dispersal processes were collected from a natural slope in the Zhifanggou Small Watershed (which is located at 36°46′99″–36°52′44″N, 109°17′2″–109°18′50″E in the hinterland of the Loess Plateau) in Ansai County, Shaanxi Province. *Populus simonii* Carr., *Caragana korshinskii* Kom. and *Stipa bungeana* Trin. were the primary vegetation species in the sampling area. The moss biocrusts in this sampling area had a coverage of over 80% and an average thickness of 11.45 ± 0.51 mm (n = 9).

Moss biocrusts with a thickness of 10 mm were shoveled into clean plastic bags. Impurities that were discernible to the naked eye (e.g., litter, soil aggregates, and coarse rock fragments) were removed. The sampled moss biocrusts were brought back to the laboratory and allowed to dry in the shade under natural conditions. Afterwards, the moss biocrusts were pulverized using a YB-2000A plant sample pulverizer. The pulverized moss crust samples were then sieved through an 80-mesh sieve to guarantee the uniformity of moss stem and leaf fragments. *D. vinealis* (Brid.) Zand. was the dominant species in the test moss crust samples, but *D. ditrichoides* (Broth.), and *B. ceaspiticium* Hedw. were also present.

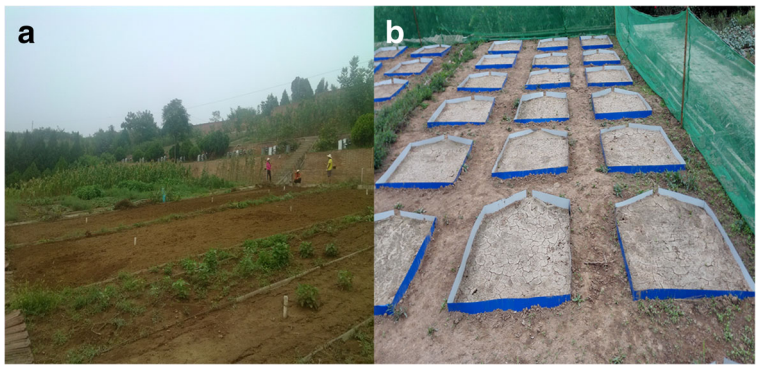

Fig. 1 Photographs of the entire experimental station (**a**) and the test plots (**b**)

Experimental design and process

We conducted one set of experiments (Stage I) between September and November 2015, and another set (Stage II), informed by the first, between May and July 2016. Each test plot had an area of 1 m × 1 m. In both stages, separate but concurrent trials were performed using two dispersal methods: broadcast and spray-dispersal (detailed below). Within each of the concurrent experiments, treatments were assigned to plots randomly. In Stage I, the plots were cleared and smoothed to guarantee the uniformity of the original soil surface before inoculation. We used the same plots for Stage II, and the moss biocrusts were scraped away and discarded from each plot, exposing a new surface with similar conditions to the soil surfaces in Stage I.

Stage I We performed a full-factorial manipulation of three factors, namely the nutrient solutions (Hoagland), the water-retaining agent (Polyacrylamide with molecular weight of 3 million), the plant growth regulator (IBA: 0.1 mg/L indole 3 butyric acid). Two different levels were designed for each factor, a control and an addition. There were a total of eight different treatments. Each of the 8 treatment combinations treatment was replicated 3 times, 24 plots each in the broadcast and spray-seeding trials.

In the treatments receiving the water-retaining agent, we removed a 1 cm layer of topsoil from the plot surface two days before dispersal of moss inoculum. The soil was homogeneously mixed with polyacrilimide in a 0.02% proportion to dry soil, then returned to the plot surface.

During the broadcast-dispersal process, moss biocrust fragments (around 2 mm thickness) were disaggregated and homogeneously broadcast over the plot surface at a rate of 700 g/m^2 (based on dry mass of moss tissue, and determined in our previous work, Yang 2015) using broadcast-dispersal that we designed (supplemental data. 1).

To implement the spray dispersal process, we developed and used a machine composed of storage barrel, stirrer, pump and sprayer (supplemental data. 2). Wood fiber (100 g/m^2), binder (3 g/m^2) and water (4.2 L/m^2), were combined with moss inoculum, to create a slurry which was sprayed on the plots using the machine.

During the cultivation period, Hoagland nutrients were applied to plots as 2.1 L/m^2 weekly using an electric sprayer. The IBA was applied as 2.1 L/m^2 in a similar fashion biweekly. In the case of the spray-dispersal trials, the initial application of Hoagland solution and IBA was mixed into the slurry.

Soil moisture content in the 0–5 cm layer was monitored intermittently using a TRIME-PICO32 time-domain reflectometer, and used to indicate when irrigation was needed. We maintained soil moisture between 15 and 25%. In addition to the natural precipitation, supplemental irrigation amounting to around 70 L per plot was supplied for both broadcast and spray dispersal treatments.

Stage II Because in Stage I we observed obviously superior growth of moss biocrusts in the shadows created by the flashing surrounding the plots, a targeted shading design was conducted in Stage II. During this experiment, Hoagland nutrient (control and addition) and four shading levels (0%, 50%, 70% and 90%) were considered, totaling 8 treatments in a full-factorial design, each with 3 replicates. Parallel spray and broadcast dispersal trials were implemented using the same techniques as above. Shading with 50%, 70% and 90% was

fixed by a wooden frame of 120 cm × 120 cm × 20 cm over the corresponding plot. We measured the light intensity, surface temperature and air humidity in each test plot simultaneously using a portable illuminometer (AR823, China) and a split-type hygrothermograph (AR847, China). Top soil moisture content was monitored and maintained in the same way as in Stage I. During stage II, around 80 L water per plot was applied to both broadcast and spray dispersal trials, in addition to the natural precipitation received.

Monitoring and maintenance

Starting at day 30 of cultivation, we measured growth indices of each moss crust every 15 days until there was no significant change in the coverage of the moss biocrusts, at which time the cultivation was considered complete. Crust coverage was measured using the gridded quadrat method (mesh size: 2.5 cm × 2.5 cm) (Li et al. 2010). We determined moss stem density by counting and averaging the number of moss stems in a total of five grid 2.5 cm × 2.5 cm, positioned at equidistant points on the diagonals of each test plot. Chlorophyll-*a* (chl-*a*) was used to indicate the biomass of moss biocrusts in the plots (Li et al. 2005). To estimate chlorophyll a concentrations at the plot level, we sampled 2.01 cm^2 cores at 3 points positioned randomly along the diagonal direction, once at the conclusion of the experiment. The specific steps are as follows: 5 ml of 95% ethanol, a few quartz sand grains and $CaCO_3$ were added to the moss samples and ground for 5 min to homogenize. Afterward, we transferred the extraction to a volumetric flask of 25 ml and metered volume using 95% ethanol; then, the absorbance at 649 nm (A649) and 665 nm (A665) was measured separately by UV spectrophotometer (UV-2450, China). The equation, chlorophyll *a* = 13.95 × A665 nm-6.88 × A649 nm × V × N / S (V: volume of extraction; N: dilution ratio; S: sampling area), was used to calculate the chlorophyll *a* contents (Bao and Leng 2005).

Data analysis

In stage I experiments, we analyzed cover and density data using a full-factorial repeated measures MANOVA. Chlorophyll *a* was measured only once, and was analyzed using a three factor ANOVA. Broadcast- and spray dispersal trials were tested separately, and are not compared statistically. In stage II, unshaded treatments experienced 100% mortality, this we could not analyze the entire model without introducing a major violation of the heterogeneity of variance assumption. Instead, we analyzed the remaining treatments as a full factorial model of 3 levels of shading, and 2 levels of nutrient addition. Otherwise, a similar combination of repeated measures MANOVA and 2-way ANOVA was applied. We applied post-hoc Tukey Kramer HSD tests to determine which factor levels were most distinct form one another. All statistics were performed in JMP Pro 12.2 (2015 SAS Inst.).

Results

Stage I experiments

Dynamic changes in cover

In stage I experiments, biocrust coverage increased relatively rapidly in the first 45 days of cultivation, after which the coverage increased at a decreasing rate. Spray-dispersed plots had nearly attained their maximal coverage by the 45th day, whereas broadcast-dispersal plots required about 60 days. In both the broadcast-dispersal and spray-dispersal experiments, the strongest effect by far was that of time ($F = 1079.3$, $P < 0.0001$; $F = 2750.8$, $P < 0.0001$; Fig. 2).The most influential main effect was a negative influence of polyacrilimide addition ($F = 87.1$, $P < 0.0001$; $F = 1333.8$, $P < 0.0001$). A positive effect of nutrient addition was also detected ($F = 34.1$, $P < 0.0001$; $F = 22.9$, $P = 0.0002$).There was a weaker negative effect of the IBA, and several additional interactive effects (Table 1). At the end of the fall cultivation period, the moss biocrust cover exceeded 85% in both dispersal methods.

Dynamic changes in plant density

Plant density increased steadily until the end of the experiments, in contrast to cover which maximized after 45 or 60d. Thus, time was the most influential factor affecting density ($F = 3093.6$, $P < 0.0001$; $F = 4471.4$, $P < 0.0001$; Fig. 3). Nutrient addition resulted in as much as 40% greater density ($F = 600.0$, $P < 0.0001$; $F = 707.9$, $P < 0.0001$), and interestingly exerted an increasingly strong effect as the experiment progressed ($F = 87.7$, $P < 0.0001$; $F = 114.6$, $P < 0.0001$). Polyacrilimide addition resulted in as much as 25% less

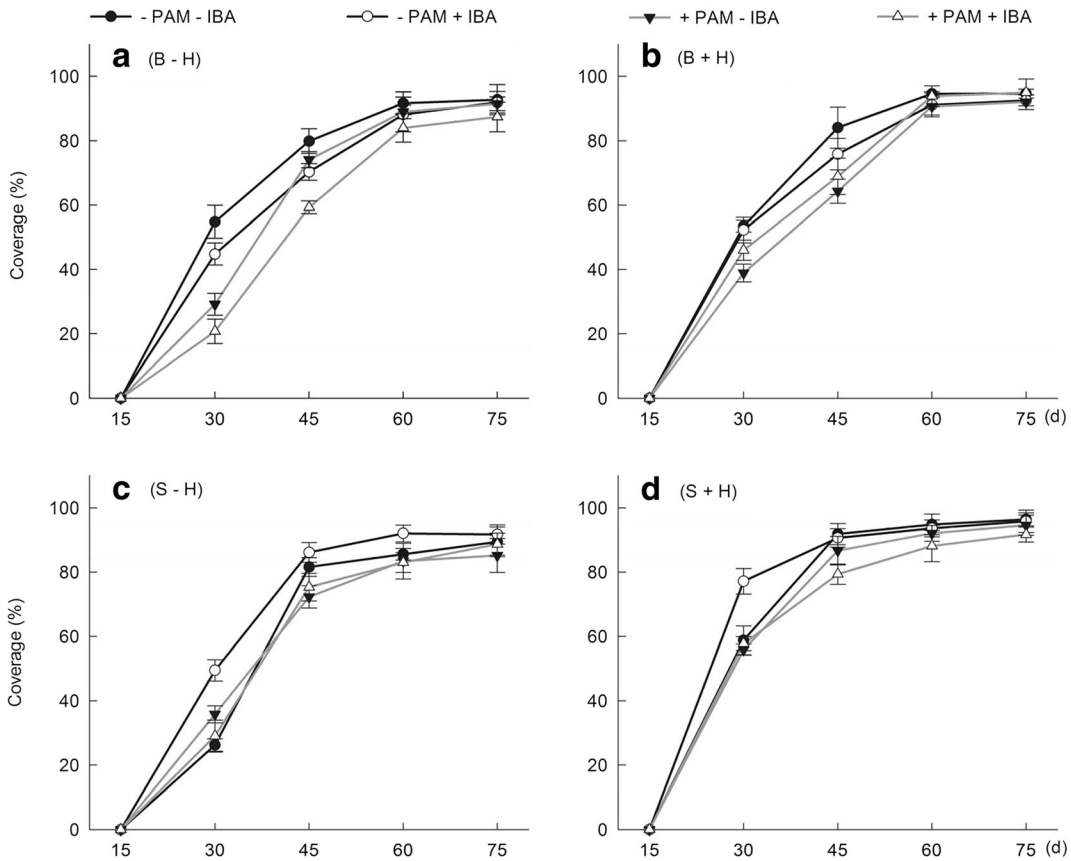

Fig. 2 Changes in the coverage of broadcast- dispersed and spray-dispersed moss biocrusts, which were subjected to different treatments in stage I experiments, over time. (−) refers to the control and (+) refers to the treatment, PAM and IBA refers to polyacrilimide and indole 3 butyric acid, H refers to Hoagland, B and S refers to broadcast dispersal and spray dispersal. (the same meaning as Figs. 3, 4, 5, 6, and 7)

density ($F = 138.0$, $P < 0.0001$; $F = 87.63$, $P < 0.0001$). No main effect of IBA was detected ($F = 1.6$, $P = 0.23$; $F = 1.5$, $P = 0.24$). Several interactive effects, albeit weaker than those described above, were also detected (Table 1). The final density of the optimal treatment was 120 stems/cm^2 under broadcast dispersal, and 150 stems/cm^2 under spray dispersal.

Chlorophyll a

In the broadcast-dispersal experiment, the only notable effect on chlorophyll *a* concentrations was a positive effect of polyacrilimide addition ($F = 12.3$, $P = 0.009$). We obtained dramatically different results in the spray-dispersal experiment. Nutrient addition approximately doubled chlorophyll *a* on average ($F = 144.6$, $P < 0.0001$; Fig. 4). The nutrient effect interacted with other factors such that magnitude of the nutrient effect was dampened when polyacrilimide was added ($F = 13.5$, $P = 0.002$), IBA was added ($F = 10.7$, $P = 0.006$), or both were added ($F = 6.3$, $P = 0.02$). An inhibitory effect of the plant growth regulator was detected ($F = 17.5$, $P = 0.0007$). In addition, the maximal chlorophyll *a* content was only 8.54 μg/cm^2 under broadcast-dispersal but 19.62 μg/cm^2 under spray-dispersal at the end of the experiments.

Stage II experiments

In stage II, mosses failed to grow without some form of shade. Cover, density and chlorophyll *a* were all below detection limits by the initiation of monitoring at day 30. In the results below, we compare the effect of different levels of shading with the acknowledgement that the complete effect of shading, inclusive of mortality or survival, is not captured there.

Table 1 The results of full-factorial repeated measures MANOVA and the three factor ANOVA in stage I experiments

Factors	Broadcast-dispersal						Spray-dispersal					
	Cover		Density		Chlorophyll *a*		Cover		Density		Chlorophyll *a*	
	F =	*P* =	*F* =	*P* =	*F* =	*P* =	*F* =	*P* =	*F* =	*P* =	*F* =	*P* =
N	34.1	<0.0001	600.0	<0.0001	0.2	0.68	22.9	0.0002	707.9	<0.0001	144.6	<0.0001
P	87.1	<0.0001	138	<0.0001	9	0.009	133.8	<0.0001	87.6	<0.0001	2.6	0.13
IBA	13.1	0.002	1.6	0.23	4	0.06	20.9	0.0003	1.5	0.24	17.5	0.0007
N x P	5.5	0.03	15.7	0.001	0.1	0.80	1.2	0.29	2.1	0.17	13.5	0.002
N x IBA	16.1	0.001	3.7	0.07	1.7	0.21	19.6	0.0004	3.4	0.08	10.7	0.005
P x IBA	3.8	0.07	8	0.01	0.3	0.58	4.6	0.05	31.0	<0.0001	2.6	0.13
N x P x IBA	7.6	0.01	27.5	<0.0001	0.3	0.57	7.8	0.01	22.7	0.0002	6.3	0.02
T	1079.3	<0.0001	3093.6	<0.0001	–	–	2750.8	<0.0001	4471.4	<0.0001	–	–
T x N	9.0	0.001	87.7	<0.0001	–	–	22.2	<0.0001	114.6	<0.0001	–	–
T x P	29.1	<0.0001	20.2	<0.0001	–	–	61.8	<0.0001	33.4	<0.0001	–	–
T x IBA	3.3	0.05	1.4	0.27	–	–	5.5	0.01	3.0	0.07	–	–
T x N x P	9.7	0.001	9	0.001	–	–	13.5	0.0002	8.7	0.002	–	–
T x N x IBA	2.3	0.12	7.8	0.003	–	–	6.5	0.006	3.7	0.04	–	–
T x P x IBA	0.7	0.58	9.8	0.001	–	–	1.9	0.17	15.0	0.0001	–	–
T x N x P x IBA	0.7	0.58	3.2	0.06	–	–	1.7	0.21	24.9	<0.0001	–	–

N, Nutrient addition; *P*, Polyacrilimide; *T*, Time

Changes in environmental factors under shaded conditions

The air humidity, illumination, temperature and soil moisture of shaded and unshaded test plots are shown in supplemental Fig. S3. Shading led to higher air humidity (F = 2.9, $P = 0.046$) and soil moisture content (F = 26.618, $P < 0.001$), lower light intensity (F = 78.4, $P < 0.0001$), but did not strongly alter temperature ($F = 0.048$, $P = 0.986$). When the ambient light intensity reached the highest level of the day (at 14:00), the light intensities under the sunshades with shade ratings of 50%, 70% and 90% were 60%, 77% and 85% lower than the ambient light intensity, respectively.

Dynamic changes in cover

In both trials, cover of mosses increased to a gradual plateau as time progressed ($F = 879.9$, $P < 0.0001$; $F = 266.9$, $P < 0.0001$; Fig. 5). Nutrient addition strongly promoted moss biocrust cover by 40–50% compared to controls ($F = 149.8$, $P < 0.0001$; $F = 106.4$, $P < 0.0001$). We also detected an effect of shading, which appeared to be driven by higher cover under 70% shade compared to 50% or 90% shade Additional, weaker interactive effects of experimental factors are listed in Table 2. Overall, regardless of the dispersal method, moss coverage was maximized by the simultaneous application of nutrients, and provision of 70% shade. By day 75, these treatments had achieved about 70% (spray-dispersal) to 80% (broadcast-dispersal) coverage.

Dynamic changes in density

In the broadcast-dispersal experiment, we observed a steady increase of density through time ($F = 266.9$, $P < 0.0001$). Nutrients enhanced density at all time points by at least 40% ($F = 48.7$, $P < 0.0001$); this nutrient effect was more pronounced in later time points than the first. Nutrient addition and shade interacted such that the combinations of 70% shade and nutrient addition resulted in the greatest moss density by far ($F = 14.8$, $P = 0.006$; Fig. 6). Results from the spray-dispersal trail were

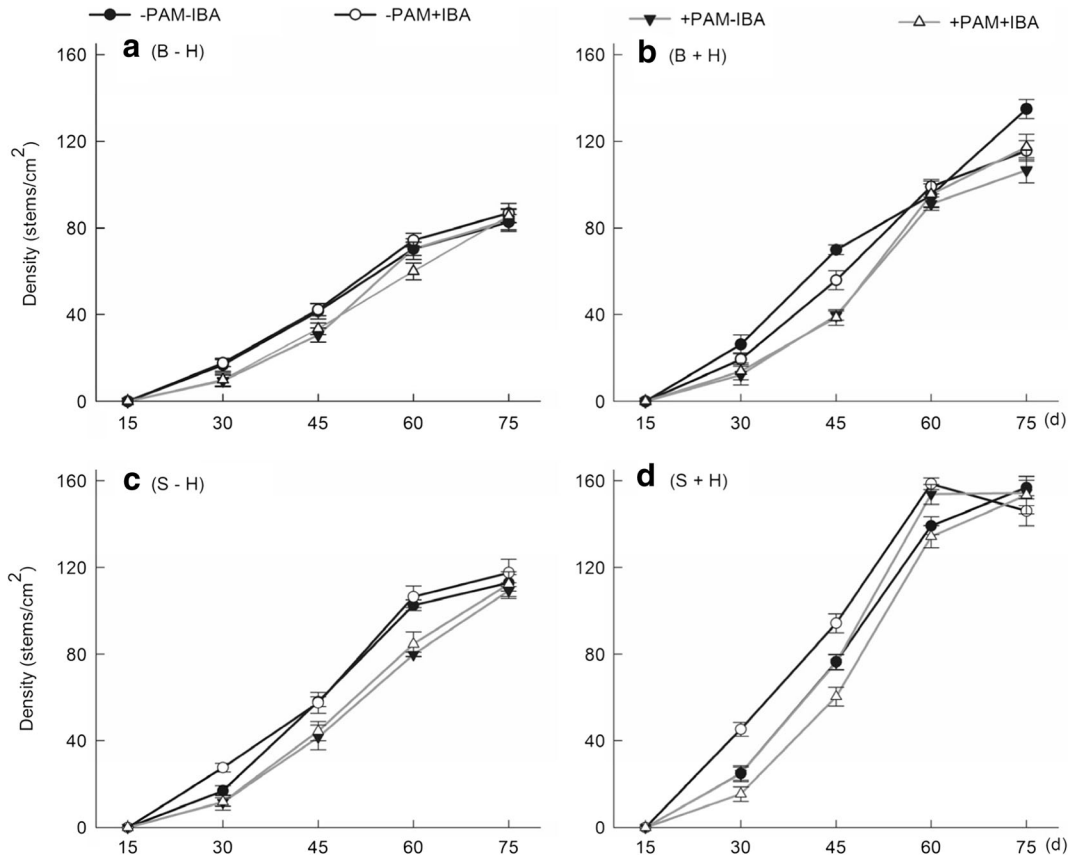

Fig. 3 Changes in the stem density of broadcast- dispersed and spray-dispersed moss biocrusts, which were subjected to different treatments in stage I experiments, over time

mostly similar, except that the interactive effect of shade and nutrient addition was even stronger (F = 89.5, P < 0.0001). Additional interactive effects from both experiments are presented in Table 2.

Regardless of dispersal method, the combination of nutrient addition and 70% shade was clearly superior to other treatment combinations and the final density were 47 stems/cm^2 under broadcast-dispersal and 57 stems/cm^2 under spray-dispersal (Fig. 6).

Chlorophyll a

In the broadcast-dispersal experiment, nutrient addition approximately doubled chlorophyll *a* vales (F = 179.9,

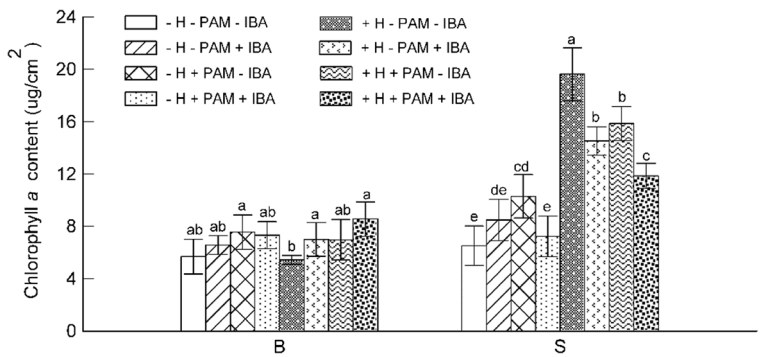

Fig. 4 Final Chl-*a* content of moss biocrusts that were subjected to different treatments in stage I experiments

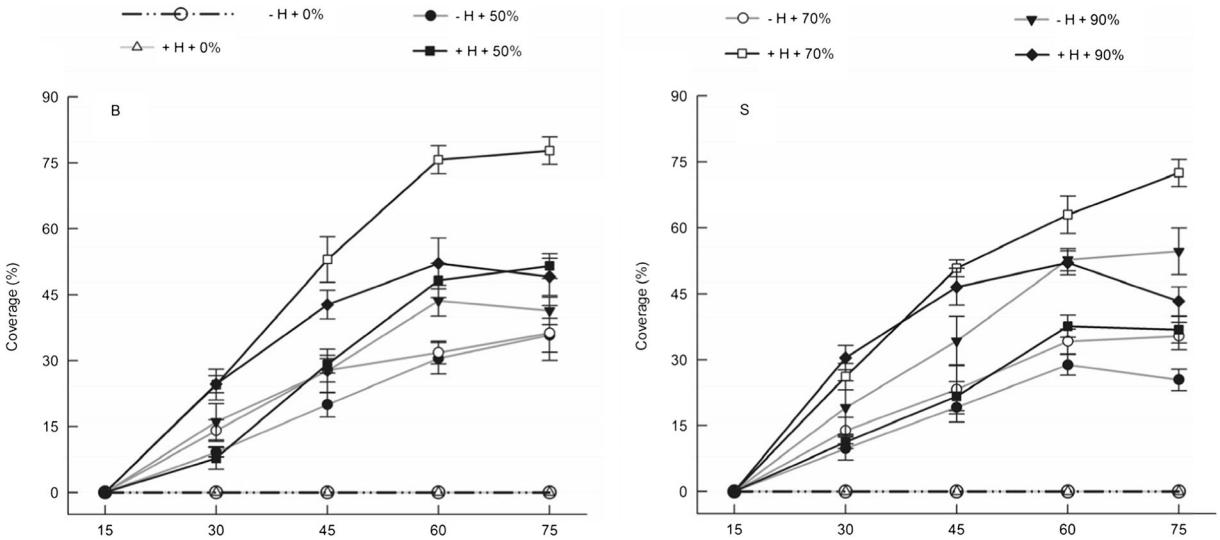

Fig. 5 Changes in the coverage of broadcast- dispersed and spray-dispersed moss biocrusts, which were subjected to different treatments in stage II experiments, over time. Because of the death of the moss biocrusts that were subjected to unshaded treatments, these data are unavailable

$P < 0.0001$). The effects of shading were primarily interactive; the combination of nutrient addition and 70% shade produced the greatest chlorophyll *a* values. In the spray-dispersal trial, very similar results were obtained (Table 2, Fig. 7), except that a main effect of shading was obtained such that 70% shading increased chlorophyll *a* both when nutrients were added and when they were not ($F = 17.7$, $P = 0.0003$). In the best performing treatments (+ nutrients, 70% shade), spray-dispersal resulted in very similar chlorophyll *a* values (Fig. 7).

Discussion

Successful resource augmentation: nutrient addition and shade provision

Most terrestrial ecosystems exhibit limitations of soil resources such as nutrients and water. The present study found that the field application of Hoagland's nutrient solution could promote the rapid development of moss biocrust coverage, density and biomass, regardless of dispersal techniques. These findings corroborate

Table 2 The results of full-factorial repeated measures MANOVA and the 2-way ANOVA in stage II experiments

Factors	Broadcast-dispersal						Spray-dispersal					
	Cover		Density		Chlorophyll *a*		Cover		Density		Chlorophyll *a*	
	F =	*P* =	*F* =	*P* =	*F* =	*P* =	*F* =	*P* =	*F* =	*P* =	*F* =	*P* =
N	149.8	<0.0001	48.7	<0.0001	108	< 0.0001	106.4	<0.0001	187	<0.0001	81.4	<0.0001
S	32.9	<0.0001	11.2	0.0018	2.6	0.11	93.5	<0.0001	56.1	<0.0001	17.7	0.0003
N x S	23.7	<0.0001	14.8	0.0006	13.4	0.0009	41.7	<0.0001	89.5	<0.0001	26.2	<0.0001
T	879.9	<0.0001	266.9	<0.0001	–	–	266.9	<0.0001	454.2	<0.0001	–	–
T x N	67.9	<0.0001	27.0	<0.0001	–	–	3.9	0.04	27.8	<0.0001	–	–
T x S	7.2	0.0003	11.3	<0.0001	–	–	7	0.0004	9.7	<0.0001	–	–
T x N x S	8.6	0.0001	7.6	0.0002	–	–	16.3	<0.0001	19.1	<0.0001	–	–

N, Nutrient addition; *S*, Shading; *T*, Time

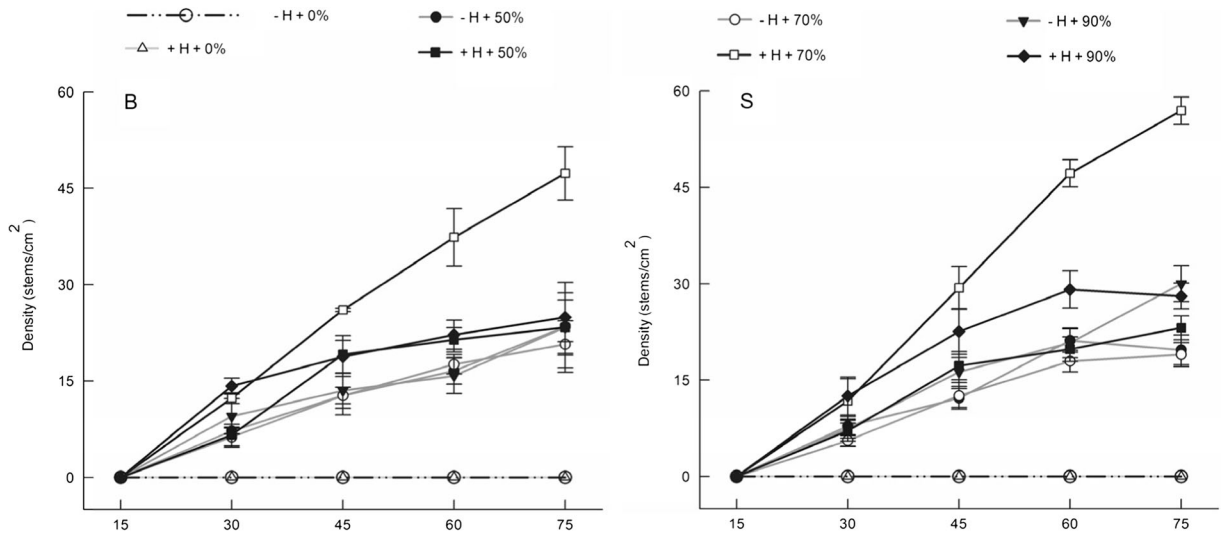

Fig. 6 Changes in the plant density of broadcast- dispersed and spray-dispersed moss biocrusts, which were subjected to different treatments in stage II experiments, over time Because of the death of the moss biocrusts that were subjected to unshaded treatments, these data are unavailable

previous results in the laboratory which identified Hoagland's solution as the most effective among Knop's, Murashige and Skoog's, Benecke's, and Part solutions (Yang 2015). Likely, some of these other solutions would also have promoted growth as has been demonstrated elsewhere (Antoninka et al. 2015, Xu et al. 2008). Bowker et al. (2005) found that the distribution and development of moss biocrusts are positively correlated with the Mn, Mg, K and Zn contents of the soil, in addition to N, Hoagland's NS also contains macronutrients such as K, P and Ca, and some micronutrients. These elements are not only the components of important compounds in plant cells, but also play active roles in the physiological metabolic activities of plants (e.g., they promote the activation of enzymes and increase light-use efficiency and photosynthesis) (Gao et al. 2003), which supported our conclusion.. Shading represents a passive means to reduce stress by reducing temperature, and augment resource availability by increasing soil moisture retention. Reduced light is not a problem for most mosses because they are tolerant of, or even prefer, low light conditions compared to vascular plants (Alpert and Oechel 1987). The clear result of the study was that in Stage II, which occurred during summer, because air humidity and soil moisture were elevated relative to fall to some extent, all shaded treatments developed better moss biocrusts than unshaded ones. Consistent with prior work (Ma et al. 2012), these conditions induced by shade clearly promoted moss biocrust growth, and the 70% shade cloth most effectively promoted *D. vinealis* cover, density and biomass at least under summer conditions. A plausible

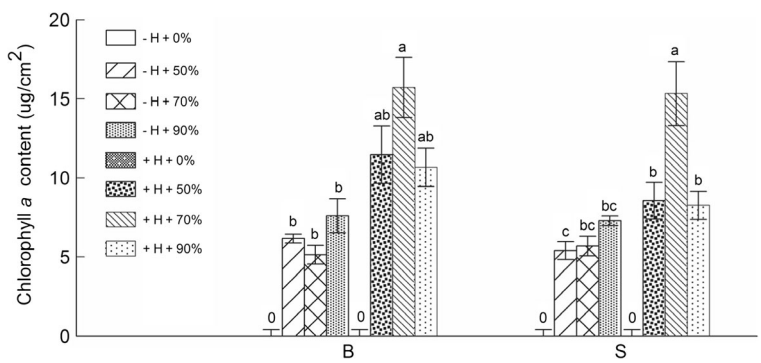

Fig. 7 Final Chl-*a* content of moss biocrusts that were subjected to different treatments in stage II experiments Because of the death of the moss biocrusts that were subjected to unshaded treatments, these data are unavailable

explanation may be related directly to the light environment, and the response of moss protonemata. Protonemata are essential to vegetative reproduction and may differentiate into various forms of tissue. Vashistha and Chopra (1987) found that protonematal growth is stimulated at 3500–4500 lx. Unshaded and 50% shade treatments exceeded 4500 lx. This behavior may occur because bryophytes generally conduct photosynthesis and other metabolic activities under low or medium light intensity conditions, and excessively low or high light intensity will disrupt the synthesis of chlorophyll in moss crusts (Wu et al. 2001). In direct contrast, Xu et al. (2008) found that the light intensity has no significant impact on the growth of *Syntrichia caninervis* Mitt. Because of the biological and physiological differences between different bryophyte species, as well as the long-term adaptation of different bryophyte species to their environments (Liu et al. 2005), the light intensity requirements may vary significantly between different bryophyte species. Thus, our specific results may not generalize to all biocrust mosses. Additional experimentation will be helpful here to identify whether 70% shade cloth is optimal in fall as well as summer, or whether another shade rating might be preferable.

Unsuccessful stress alleviation: polyacrilimides

As a mini-reservoir of water in soil, polyacrilimides can increase the effective soil moisture content in the plant root zone as well as retain soil moisture (Li and Huang 2001) and improve soil properties (Sojka et al. 2007). Therefore, they are a commonly used auxiliary material for vegetation restoration in arid regions and generally play positive effects (Liu et al. 2014; Yang et al. 2012). However, in our study, polyacrlimide generally exerted negative effects on cover and density of mosses. In the case of broadcast-dispersed plots of stage I, polyacrilimide appeared to promote chlorophyll a concentration in the soil substantially. Since neither moss cover nor density showed this result, we suspect that perhaps the polyacrilimide promoted cyanobacterial colonization. Cyanobacteria are another source of chlorophyll a, and are not undesirable, but promotion of cyanobacterial biocrusts was neither a goal nor focus of this study. We propose two non-exclusive hypotheses for why the polyacrilimides did not benefit mosses. First, the average soil moisture in the 0–5 cm layer of the test plots ranged from 15% to 25% throughout the experiment, which is enough to meet the requirements for the normal growth and development of moss biocrusts. In contrast, Yang (2016) found that polyacrilimides significantly promote moss biocrusts growth specifically under low soil moisture content. Thus water may never have been sufficiently limiting for polyacrilimides to confer benefit. Second, water absorbed in polyacrilimide does not automatically release into soil but can be passively absorbed by plant roots; however, the rhizoids of mosses do not function in water transport and may not represent and effective water absorption channel from the polyacrilimides (Kallio and Karenlampi 1975).

Finally, the water-retaining capacity and the effects of the polyacrilimide may also vary with the polyacrilimide type, soil temperature, salinity, mixing ratio, application method, irrigation water volume and irrigation method (Ran et al. 2015). At present, the application of polyacrilimide during the rapid cultivation of moss biocrusts is still at the exploratory stage, and does not appear to be needed for rapid establishment of moss biocrusts. Relevant issues regarding effects of polyacrilimide types, dosages and application techniques still require further clarification.

Inconsistent effects of a plant growth regulator

A plant growth regulator may positively induce moss growth and may also inhibit it. The present study showed that IBA had few effects on the growth of the mosses in the field, and when there was an effect it was negative. A previous laboratory experimental study (Yang 2015) showed that 0.1 mg/L IBA promoted the coverage, plant density and biomass of *D. vinealis* crusts. The reasons for this discrepancy may be because the field experiments involve a large number of factors with considerable variation. In the present study, the applied IBA may have undergone partial photolysis. Further, the IBA was applied at a relatively small dosage. Because of the relatively high soil moisture content, the IBA may have been diluted. This finding does not indicate that there are no benefits to application of plant growth hormones. There are a variety of these substances and studies regarding their effects on mosses are sparse. Effects of some plant growth regulators have been shown to exert a dosage-dependent effect (Liu 1998). Investigating applications of additional plant growth regulator types in varying dosages to multiple moss species is one of the important research gaps to address to improve the rapid cultivation of moss biocrusts.

Timing matters more than the means of dispersal

Broadcast- and spray-dispersal both achieved establishment of moss biocrusts. More often than not, the spray-seeding method resulted either in better biocrust development, or faster development, but this was not always true. Observed beneficial effects may be partly explained by the wood fiber used in the spray-seeding method, which had shading and water-retaining effects, which may have reduced the rate at which moisture evaporated, providing a more favorable environment for the growth of moss biocrusts. In stage II, the sunshades, which also had shading and water-retaining effects, may have concealed the effects of the wood fiber. Spray-dispersal also had a soil-aggregating effect which may have benefited moss biocrust development, especially when not protected from rain splash by shade cloth. From an economic standpoint, the spray-dispersal method was more efficient; spraying an area of 100 m2 in only 10 min. The spray-dispersal method is suitable for a number of adverse site conditions such as steep slopes.

The overall growth of moss biocrusts at stage I outperformed that at stage II, possibly due to different climate conditions. Stage I of the test was conducted between September and November of 2015. During this period, the average ambient temperature ranged from 15 °C to 25 °C, which was ideal for the growth of the moss crusts (Bu et al. 2011; Zhang 2012). In addition, there was ample rainfall during this period, which was distributed evenly. The cumulative precipitation reached 234.6 mm (approximately 46% of total rainfall). As a result, the soil moisture content at a depth of 0–5 cm was generally maintained between 15% and 25%. In contrast, stage II of our study was conducted between May and July of 2016. During this period, while the total precipitation reached 224.8 mm (approximately 44% of total rainfall) and the time of soil wetness duration at 0–5 cm layer was relatively short, the average surface temperature exceeded 35 °C, which had a negative impact on the growth and development of moss biocrusts and also resulted in an increase in evaporative water loss. In particular, the relative surface humidity was only 40% after 12:00 each day. These factors resulted in the relatively poor overall growth of moss biocrusts at stage II. Therefore, selecting a suitable dispersal time is very important because it dictates the suitability of soil moisture, temperature and light regimes (Ram and Aaron 2007).

Are artificial moss biocrusts self-sustaining?

Our field trials attempt to rapidly establish biocrusts by temporarily reducing stress and removing limiting factors. Since these activities cannot be economically sustained indefinitely, especially for larger-scale applications, it is important that we are able to produce a self-sustaining biocrust cover. After our Stage II experiment, the artificial moss biocrusts did not show any degeneration after the removal of the shade and water/nutrition addition, though they were susceptible to weed colonization in summer 2017. Our previous findings (Bu et al. 2017) suggest possible mechanisms that could be exploited to enhance survivorship, induced by non-lethal exposure of mosses to moderate dehydration-rehydration stress or brief exposure to high temperature. These exposures induce stress resistance that could prepare mosses for a high stress environment. Possibly, simply cultivating biocrusts in outdoor conditions, as opposed to laboratory conditions, provides the necessary non-lethal stress exposure to induce stress resistance, resulting in persistence of our moss biocrusts beyond the duration of the experiment. An important next step in further developing and upscaling this technology will be explicit long-term tests of artificial moss survivorship, and methods for enhancing long-term survivorship.

Conclusions

Synthesizing our results from all 4 trials, we recommend moss inoculation in fall rather than summer. Spray-dispersal with wood fiber (100 g/m^2) and a binder (3 g/m^2) may accelerate establishment or final density of moss biocrust economically, but broadcast-dispersal is also a viable strategy. Application of Hoagland's nutrient solution weekly can speed moss biocrust development. Shading to some degree benefits moss biocrust development. A 70% shading coefficient was the best in summer, but the optimum shading coefficient in fall is not known. Finally, supplemental watering to keep high soil water content is useful during the growth period, but may be terminated after a high coverage of mosses is achieved. This technique appears to be amenable to upscaling, and may be applied to enhance ecosystem functioning on engineered slopes and in natural ecosystems.

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