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Producing moss-colonized burlap fabric in a fog chamber for restoration of biocrust

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ABSTRACT

We developed a system that waters biocrust moss with fog on a burlap substrate, tested its production capacity, and evaluated field establishment of the moss-colonized fabrics it produced. First, we studied effects of application rate, watering period, and pulverization on biomass increase of *Syntrichia ruralis*, a globally distributed moss. We observed increases at both high and low application rates, though pulverization impeded growth. In a subsequent experiment studying growth more frequently we observed this species increase by 1.5% in the first 30 days and 17% over 81 days. This confirmed our expectation of a non-linear relationship between time and yield when cultivating tissues that have incurred stress prior to cultivation from the field or during storage. In both experiments moss was loosely attached to burlap following cultivation. We field tested moss-colonized burlap produced in a multi-level fog system, installing it face-up or down, and compared cover change with the same material detached from burlap and with wild moss. We observed losses in all treatments after five months, perhaps due to inactivity and consequent displacement by wind during an anomalous summer drought. However, face-down treatments retained the most cover and regenerated to 60% (initial levels) by the following spring. These levels were four-fold higher than fog chamber materials without burlap association. Fog materials with no burlap association established 8% more cover than wild moss, suggesting that fog materials are as field-ready as wild materials. Thus, moss-colonized burlap is effective at rapidly establishing biocrust in the field, even during drought.

1. Introduction

Researchers and practitioners are focused on increasing the production capacity and diversity of plant materials to conduct restoration in various systems (Peppin et al., 2010; Copeland et al., 2018). While vascular plants are typically employed for this task, surface-dwelling soil organisms known as biocrusts are also an integral component of many systems, and new technologies for rapidly cultivating and deploying biocrust organisms present an opportunity for inclusion of this group in restoration treatments (Bowker et al., 2017; Zhao et al., 2016). Biocrusts are desirable in this role because they are drought tolerant, excel at soil stabilization, and fix carbon and other nutrients in vascular plant interspaces (Belnap and Büdel, 2016; Sancho et al., 2016; Stark, 2017). As with plant materials in general, best practices for sustainable production and field establishment of biocrusts are active areas of inquiry.

Human activities have reduced biocrust abundance globally, though recent innovations in biocrust materials production may aid recovery efforts (Belnap and Eldridge, 2001; Bowker, 2007; Zaady et al., 2016). Recovery timelines in disturbed areas vary by proximity to propagule sources, climate, and taxonomic group, though adding propagules can hasten recovery in some areas (Condon and Pyke, 2016; Weber et al., 2016; Zhao et al., 2016; Warren et al., 2019; Condon et al., 2020; Doherty et al., 2020; Slate et al., 2020). Various cultivation systems and substrates are suitable for rapid production of microbial and moss biocrust materials for use in active restoration (Chen et al., 2006; Xu et al., 2008; Doherty et al., 2015; Antoninka et al., 2016; Giraldo-Silva et al. 2020; Grover et al., 2020). The relative benefits of these systems haven't been formally assessed across the breadth of biocrust groups, though growth rates, supported taxa, space-use efficiency, and subsequent field-survivorship are important considerations.

Establishing biocrusts, specifically cultivated materials, in the field has proven challenging in many systems. Physical disturbances such as wind and intense rain may displace loosely broadcast materials (Young

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et al., 2019). The mechanisms for stress tolerance are plastic in some taxa, and biocrusts may lose stress tolerance when hydrated for prolonged periods during cultivation and die upon out-planting (Stark, 2017; Antoninka et al., 2018; Bowker et al., 2020; Giraldo-Silva et al., 2020). Timing treatments to avoid extreme annual weather patterns and to leverage gentler periods of cool-season precipitation may improve establishment outcomes, though the importance of timing may be site dependent (Condon and Pyke, 2016; Bu et al., 2018; Young et al., 2019). Additionally, amelioration strategies such as soil imprinting, shading, and installation of erosion control fabrics improve establishment (Condon and Pyke, 2016; Bu et al., 2018; Antoninka et al., 2018; Bowker et al., 2020; Doherty et al., 2020; Slate et al., 2020). These recent modest gains in field establishment highlight how much work remains before cultivated biocrust can be confidently used in large-scale restoration treatments.

Syntrichia ruralis (Hedw.) F. Weber & D. Mohr is a widespread biocrust moss that provides desirable ecosystem services and is amenable to cultivation (Doherty et al., 2018). *S. ruralis* has fewer dispersal adaptations than ruderal species, but is among the tallest biocrust mosses, a factor which gives it comparatively high carbon sequestration and water interception potential per unit area (Mishler and Oliver, 1991; Proctor et al., 1998; Rosentreter et al., 2007; Frey and Kürschner, 2011; Stark et al., 2017; Doherty et al., 2018). *S. ruralis* is also highly desiccation tolerant and can survive drying to water content levels far lower than most vascular plants (Stark, 2017). All moss species can absorb liquid water directly through leaves and can also hydrate via water vapor alone under conditions of high relative humidity (Pan et al., 2016; Stark, 2017; Slate et al., 2018). The capacity of moss to collect water in various forms is interesting and unexplored in the context of biocrust cultivation research.

We tested a system that waters biocrust moss with fog on a burlap fabric substrate. Our aims were to investigate new methods of moss production and investigate materials that could improve field establishment of this group. We conducted three experiments to: 1) identify treatments that improve biomass yield of *S. ruralis*, 2) reveal *S. ruralis* growth patterns over time in a field-to-greenhouse production context, and 3) evaluate establishment potential of *S. ruralis*-colonized burlap (moss burlap, hereafter) produced by fog watering. In experiment 1 we manipulated application rate, pulverized tissues or left them intact, and observed the outcome of these treatments over time. Because *S. ruralis* is a comparatively tall moss species and may accumulate appreciable vertical biomass, we expected growth to continue regardless of application rate (Doherty et al., 2018). We expected pulverizing to have a suppressive effect on growth, but we were interested in this treatment because it was described in prior work (Shaw, 1986) and allowed for a more uniform application on burlap. In experiment 2 we frequently observed growth of *S. ruralis*, expecting slow initial rates as mosses recovered from a dry state, followed by a period of rapid growth, and then a deceleration in growth as stems matured (Coe et al., 2012; Stark, 2017; Stark et al., 2017). Finally, in experiment 3 we installed moss burlap in the field after 10 weeks of fog watering. We expected that moss burlap would provide anchoring and amelioration properties to improve establishment relative to loosely broadcast materials. Additionally, we expected that cultivated moss would perform poorly compared to wild materials due to loss of stress tolerance while in the fog chamber.

2. Methods

2.1. Sourcing and processing moss for experimentation and bulk cultivation

We collected aboveground tissues of *S. ruralis* from MPG Ranch in the Bitterroot Valley, Montana, USA in September 2017 (−114.017° W, 46.668° N, 1047 m). Following collection, we slowly dried tissues to minimize stress, and stored them at 27 °C in the dark until processing. We did not monitor humidity levels of our storage facilities prior to our experiments, but the building is climate controlled and more recent measurements indicate they were stable at 40%. Because we made a single initial collection of moss, storage times varied by experiment and are indicated in section 2.3.

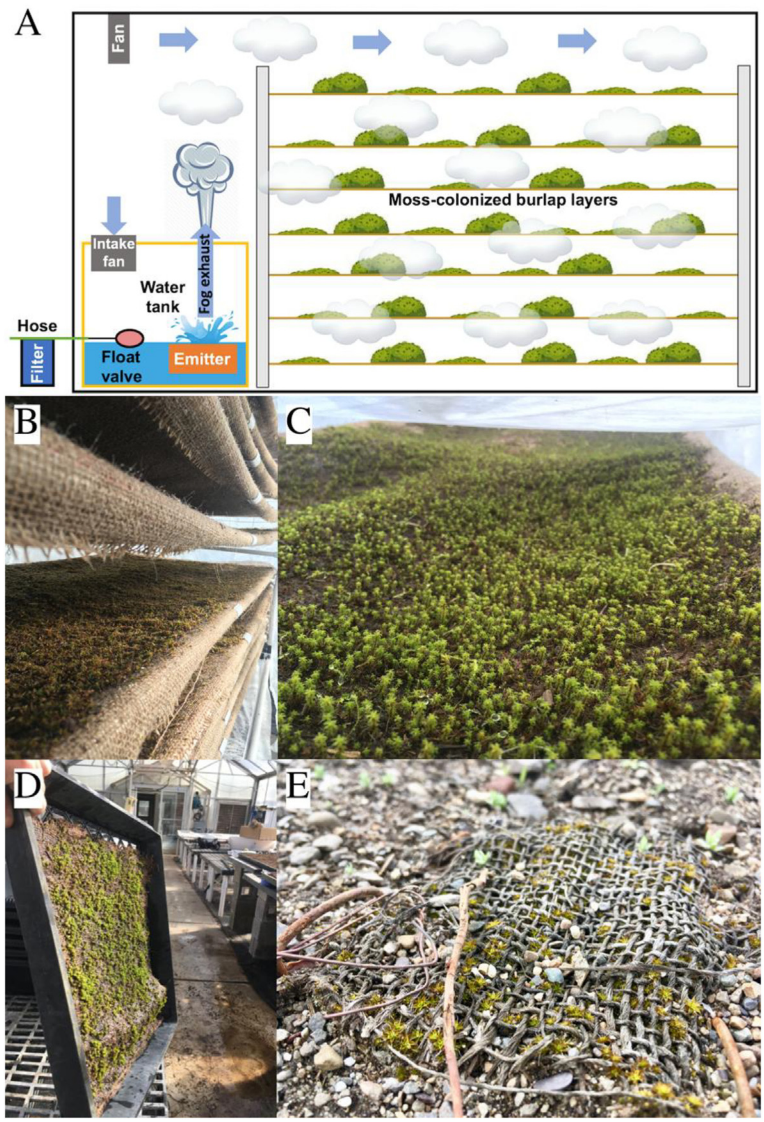
For processing in all experiments, we first disaggregated dry moss cushions by passing them through a 4 mm sieve, collecting moss stems (gametophytes) on a 2 mm sieve. For moss pulverization treatments in experiment 1, we processed tissues in a Hario Skerton brand espresso mill after sieving; resultant particles ranged from 250 to 400 μm in size. We conducted additional cleaning of a subset *S. ruralis* tissues for experiment 2 to remove soil and organic residue. These materials were removed because their decomposition might obscure mass gain data. We first rinsed tissues and then immersed them in water. When immersed in water, the bulk of hydrated moss stems sink while other organic litter floats. We immersed moss stems, skimmed off litter, and retrieved materials for three successive iterations. After each iteration, we removed any observed woody debris.

2.2. Design of fog watering system

Our watering system consisted of fog emitters that produced a continuous flow of sub-micron water droplets circulating across one or more layers of burlap within a fabric chamber (1 W × 2.5 L × 1.5H m; Box 1A). We immersed two House of Hydro brand, 5-disc fog emitters in a closed 60 L tank of carbon filtered water. The tank was regularly refilled with an automated float valve. The emitters were set on alternating, one-minute cycles to prevent heat-related damage or malfunction. We installed a fan intake and exhaust hole in the airspace at the top of the water tank so fog would be forcibly ejected from the tank and circulate within the chamber. The chamber walls were made of Row Cover Ultimate brand fabric, which allowed for 30% light transmission. The walls were not sealed but were sufficient to concentrate fog within the chamber. We estimated fog chamber conditions to have a mean relative humidity of 93%, ranging from 88 to 98%, a mean temperature of 19.4 °C, ranging from 13.7 to 25.8 °C, and mean photosynthetically active radiation at midday of 138 μmol m^{-2} s^{-1}, ranging from 124 to 152 μmol m^{-2} s^{-1}. We used Vigoro brand burlap for our cultivation substrate. Burlap is a ∼ 2 mm grid form of jute fabric woven from the cellulosic plant fibers of the genus *Corchorus* (Rabbani, 1965). For experiments 1 and 2, we suspended burlap units on plastic netting 5 cm from the bottom of the chamber, so that fog would circulate both above and below the burlap. For bulk moss cultivation in experiment 3, we stacked ten layers of burlap sheets vertically, with 5 cm of separation between each layer to allow for fog circulation. During cultivation we rotated layers up one level each week to reduce the effects of differential light levels. We moved the top layer to the bottom during each cycle.

2.3. A note on experiment order and the relevance of storage time

The following experiments are presented in an order that first focuses on ex situ cultivation outcomes and concludes with a field trial testing the performance of moss-colonized burlap. This order is different than the sequence in which the experiments occurred. We made an initial collection of wild moss in September 2017 (details found in Section 2.1) and used these materials in all of our subsequent cultivation experiments. Experiment 1 began October 2017 (storage time one month), the cultivation step of experiment 3 began January 2018 (storage time 16 months), and experiment 2 began January 2019 (storage time three months). This information may be relevant, particularly to interpretation of our results in experiment 2, because recent work has shown that prolonged storage can impact the regenerative abilities of some moss species (Guo et al., 2020). Further information may be found in section 4.1 of the discussion.



Box 1. We present a system (A) for watering biocrust mosses with sub-micron water droplets (fog) on a burlap substrate. This system may incorporate one or more layers (B) and produces a moss-colonized burlap fabric (C). When watered in a fog chamber some species will attach to the burlap substrate (D). Attachment aids in establishment when deployed in the field (E). Burlap may buffer some environmental stressors and dispersing moss on a colonized fabric may maintain the moss colony structures that improve water retention.

2.4. Experiment 1: assessing relative effects of treatments on moss biomass yield

We studied the relative effects of three factors on moss biomass yield in our fog cultivation system: 1) application rate (26.7 g/m^2 or 53.3 g/m^2), 2) pulverization (ground or intact stems) and 3) duration of cultivation (one or two months). Application rates corresponded to approximately 50% cover in the low rate and 100% cover in the high rate treatments. This was a full factorial design with 5 replicates of each treatment combination ($n = 40$). We cut 7.5 × 7.5 cm squares of burlap and took the initial mass of each. We then wetted the squares by immersion in water to improve adhesion and evenly broadcast dry inoculum onto the squares, applying 0.15 g for the low rate and 0.3 g for the high rate. We randomized and placed the units in the fog chamber suspended on a single layer of plastic netting. We conducted two harvests, collecting half of the units at 30 days and the remainder at 60 days. We recorded dry mass of moss burlap units after the final harvest and subtracted the initial mass of burlap from these values for analysis. In addition, we added 10 units of uninoculated burlap controls to the chamber during the experiment, harvesting five at 30 days and five at 60 days, to assess if decomposition of burlap occurred in the fog chamber. The experiment ran from October to December 2017.

2.5. Experiment 2: patterns of S. ruralis biomass yield through time

We cultivated *S. ruralis* in the fog chamber and observed biomass yield through time at sub-weekly intervals. First, we inoculated 0.5 g of dry intact moss stems, cleaned by the washing and immersion process described earlier, onto 72 wetted burlap squares measuring 10 × 10 cm. This resulted in an application rate of 50 g/m^2. The tissue preparation and application rate were informed by experiment 1, though we made slight changes to burlap size and rate to balance the considerations of space constraints, sample size, edge effects, and tissue availability for future work. We placed units in the fog chamber in the same manner as Experiment 1. Starting at time zero, we harvested units every ~3.5 days, taking three units at each timepoint for a total of 24 harvests. After each harvest the units were slowly dried for ~3.5 days and stored in the dark at 23 °C until the end of the experiment. After the final harvest, we detached moss stems from each burlap unit, and then took mass measurements of burlap and moss separately. We took the mass of burlap to investigate if decomposition of this material occurred in the fog chamber. This experiment ran for 81 days from February until May 2019.

2.6. Experiment 3: field establishment of moss burlap

We produced moss-colonized burlap in a multi-level fog system, tested its field establishment potential as a function of delivery mode, and compared outcomes with wild materials of the same population collected immediately prior to the field test. The source *S. ruralis* materials for cultivation we collected from the field as described in section 2.1. We cut ten pieces of burlap that measured 2 × 1 m and inoculated 200 g of sieve-disaggregated *S. ruralis* stems onto each. We increased application rates relative to prior experiments to maximize stem density for water retention in the field and because we expected some loss during transport from the fog chamber to our field site. We installed the ten layers in a vertical stack, described above, within the fog chamber (Box 1A). We watered these moss tissues for ten weeks from January to April 2018. At the end of this period we slowly dried the products, and stored them in the dark at 23 °C.

We then transported moss burlap to an herbivore exclosure in the Bitterroot Valley, Montana, USA (−114.034° W, 46.679° N, 998 m) where we conducted field establishment trials. The exclosure was located 1.7 km from the site of initial tissue harvest and ~ 50 m lower in elevation. This site had been tilled and covered in ground cloth for five years prior to our experiment. The site was flat, free of initial vegetation, and weeded of vascular plants regularly during the growing season.

We cut 30 squares measuring 7.5 × 7.5 cm from a single sheet of moss burlap, targeting regions that exceeded 50% cover. We detached stems and measured volumes from ten of these units, finding that they corresponded to 21.5 ± 2.3 (SE) mL. We used this mean volume to determine addition rate for our wild materials, which were collected from the same location as our cultivated materials two days prior to installing our experiment. Finally, we installed ten replicates of four treatments ($n = 40$): burlap-attached cultivated moss with stems facing up, cultivated moss attached facing down, cultivated moss detached from burlap and broadcast directly onto soil without burlap, and wild moss broadcast onto soil without burlap. We placed four erosion control staples in the corners of each the of 7.5 × 7.5 cm plots. Staples were installed over burlap in treatments with these materials. The same observer monitored units three times over the period of a year in May 2018 (time zero), September 2018, and May 2019. During each observation we installed temporary 7.5 × 7.5 cm monitoring frames over the plots, aligning them with the plot corners delimited by staples. Within each frame we determined percent cover of moss tissue by ocular estimation, similar to the methods described in Daubenmire (1959). We assigned our observations to percentage classes of 0, 1, 2, 5, or increments of 5 for greater cover classes.

2.7. Data analysis

In both greenhouse cultivation experiments we converted biomass measurements to yield as a function of unit area (g/m^2) to facilitate comparison of space-use efficiency in future studies. In experiment 1 we evaluated effects of application rate and pulverization over time with an effect size approach. We calculated Hedges' G and associated bootstrapped 95% confidence intervals, contrasting one (control) and two months of cultivation within a treatment combination (Hedges and Olkin, 1985; Kelly, 2005). In this contrast framework positive values of the lower confidence limit indicated statistically significant growth. If a confidence interval intersected zero, growth was not significant. To test for the effects of decomposition of burlap in this experiment we conducted a paired *t*-test comparing the ending mass of burlap controls at one and two months.

To model the functional form of biomass yield over time in experiment 2, we evaluated three regression models which we fit to the mean yield at each time point. We fit a linear model, second order polynomial, and the logistic function, then calculated adjusted R-squared and AIC for each and drew inference from the best performing model. To assess the effects of burlap decomposition over time we fit a linear model, predicting mean burlap mass at each timepoint as a function of time.

For comparison of treatments studying moss burlap field establishment in experiment 3 we calculated effect sizes and associated 95% confidence intervals (bootstrapped) at the final timepoint (May 2019). In order of treatments followed by controls we contrasted: 1) fog-watered moss detached from burlap with wild mosses, 2) fog-watered face-up with fog-watered detached, and 3) fog-watered face-down with fog-watered detached to test the hypotheses stated earlier. In this framework a treatment effect was positive and significant relative to the control only if the lower confidence limit was positive.

We conducted all analyses in the R programming language (R Core Team, 2019). We fit linear and polynomial models, conducted *t*-tests, and calculated AIC with the stats package. We fit a logistic function using a dose response curve provided in the drc package. We calculated effect sizes and bootstrapped confidence intervals with the code provided in the appendices of Kelly (2005).

3. Results

3.1. Experiment 1: assessing relative effects of treatments on moss biomass yield

We observed increases in biomass between one and two months only in intact (unpulverized) treatments, though only intact high rate treatments exceeded the initial amounts added (Fig. 1). The effect size of cultivation time on yield was large and positive (> 0.8; Cohen, 1988) at both low (1.84[1.29, 2.46]; effect size followed by confidence limits in brackets) and high (2.35[1.56, 3.27]) application rates. These effects corresponded to a mean increase of 7.2 g/m^2 in low rate intact treatments and 11.1 g/m^2 mean increase in high rate treatments between one and two months. We did not observe significant yield increases in pulverized treatments at low (−0.99[−1.5, 0.22]) or high (0.04[−1.09,1.51] application rates.

3.2. Experiment 2: observing patterns of S. ruralis biomass yield through time

We found that a second order polynomial model performed best when predicting biomass yield through time (adjusted-R^2 = 0.77, AIC = 89.22; Fig. 2). Our model showed that moss biomass increased 1.5% in the first 30 days, 7% between 30 and 60 days, and projected a 12% increase from 60 to 90 days. Overall, the model predicted a 17% increase from time zero until the end of this 81-day experiment. In both this experiment and the prior one, initial moss stems senesced and gave rise to new green stems (Fig. 3). This result confirmed our expectation of an initial lag phase followed by biomass increase, though rates of growth were increasing at the end of the experiment and we did not observe a point at which growth attenuated.

3.3. Experiment 3: field establishment of moss burlap

We found that fog-watered mosses attached to burlap had significantly higher establishment than fog-watered mosses detached from burlap and loosely broadcast onto soil without burlap installation (Fig. 4). When moss burlap was installed face-up we observed a positive and moderate effect (effect size lower confidence limit > 0.5; 1.25[0.59, 1.86]) on establishment relative to loose fog-watered materials. When installed face-down we observed a positive and large effect (effect size lower confidence limit > 0.8; 2.29[1.30, 3.86]) relative to loose fog-watered materials. These effects corresponded to a positive

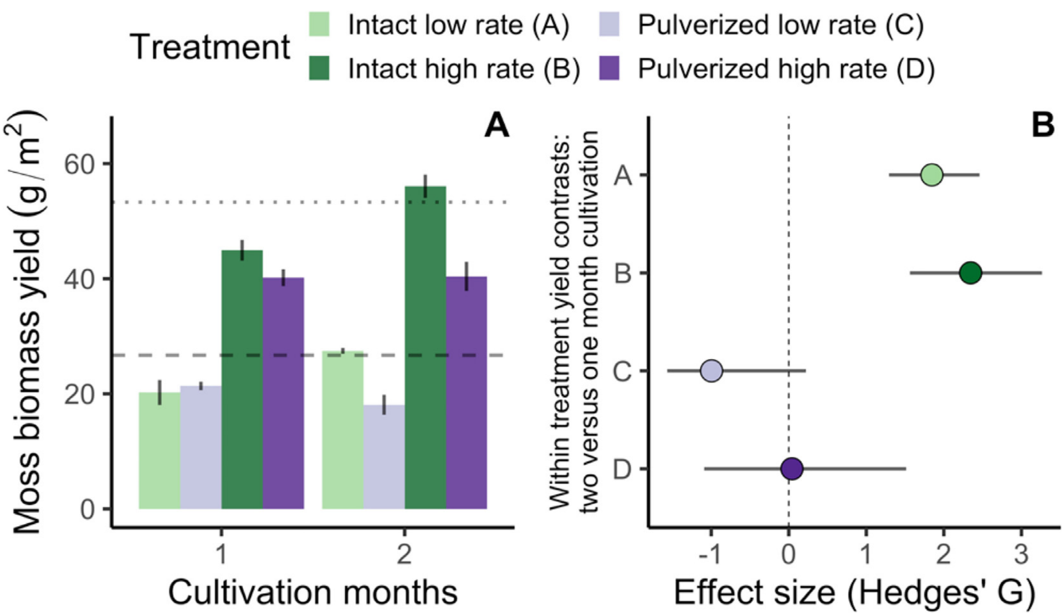


Fig. 1. We measured biomass of *S. ruralis* watered in a fog chamber after one and two months of cultivation (x-axis; A). We added moss tissues at high (darker shaded bars) or low application rates (lighter shaded bars) and stems were left intact (green bars) or were pulverized (purple bars). Dashed horizontal lines indicate initial low application rates and dotted horizontal lines indicate initial high application rates. Vertical black lines indicate SE +/− for each group. In panel B we show test results for differences in biomass between one and two months presented as bootstrap estimates of the standardized mean difference, Hedges' G (x-axis), for all treatment combinations (y-axis). Dots indicate the bootstrapped mean of effect size and horizontal lines indicate the bounds of the 95% confidence interval for that estimate. Positive values of effect size indicate growth occurred between one and two months of cultivation for a given treatment combination. An effect is only significant if the confidence interval does not intersect zero (dashed vertical line). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

mean percent cover difference of 24% in face-up treatments and 47% in face-down treatments relative to loose fog-watered materials at the end of the experiment. We also found that loosely broadcast fog-watered mosses installed without burlap had slightly higher establishment (effect size lower confidence limit > 0.2; 1.2[0.21, 1.96]) relative to wild mosses of the same population that received no fog watering and were applied in the same manner. This effect corresponded to an 8% positive mean difference in final cover. Cover loss and regeneration occurred throughout the experiment, though ending cover relative to initial levels was only higher in the face-down treatment (+2%). Losses in cover coincided with an anomalous drought in the summer following installation of the experiment (Daly and Bryan, 2013; Fig. S1). We also found evidence of moss rhizoids from burlap-attached units aggregating soil particles at the conclusion of the experiment (Fig. S2).

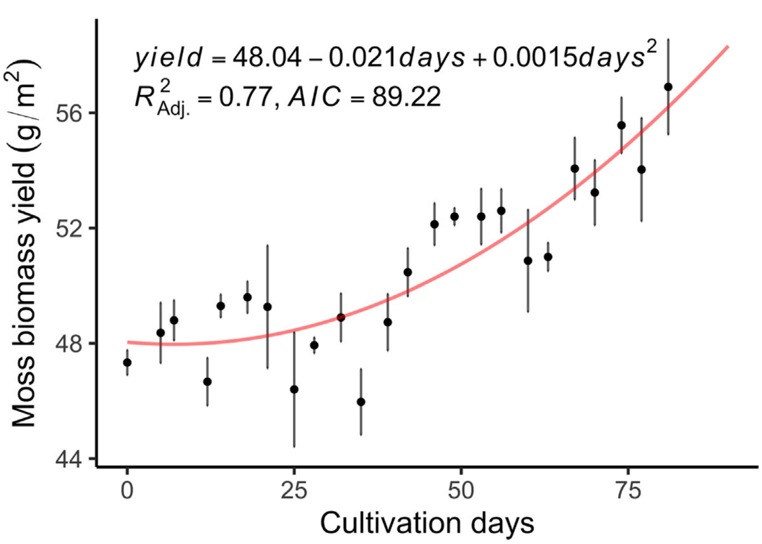


Fig. 2. Results from a greenhouse cultivation experiment of the moss *S. ruralis*. Units were cultivated in a fog chamber for 81 days, and harvested at regular intervals, three at a time. Dots represent the mean biomass yield of the three units at each time point, vertical lines indicate the SE+/−. We modeled the mean biomass increase with a second order polynomial, represented by the red line. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.4. *Test of decomposition of burlap in the fog chamber*

We found no difference in final mass between one- and two-month burlap controls from Experiment 1 ($t = 0.78_{[4]}$, $p = 0.48$). Furthermore, we found no relationship between burlap mass and time in Experiment 2 (adjusted-$R^2 = -0.01$). Thus, we did not encounter evidence of burlap decomposition in either experiment.

4. Discussion

4.1. *Interpretation of fog cultivation growth patterns*

S. ruralis biomass increased over time in both greenhouse cultivation experiments. In experiment 1, *S. ruralis* increased at both low and

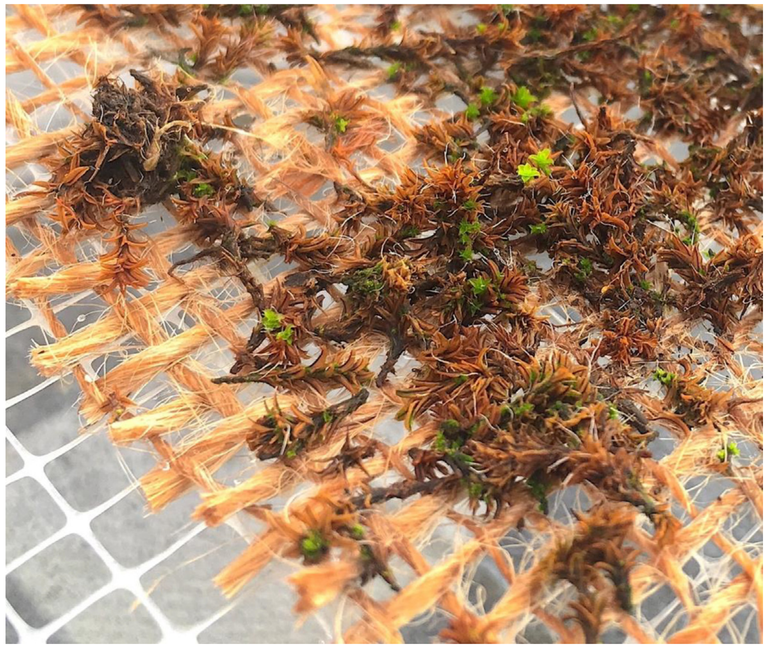


Fig. 3. New *S. ruralis* stems emerging from senesced parent stems after 25 days of fog cultivation on burlap. This phenomenon can explain a delay in net biomass increase when cultivating field collected moss.

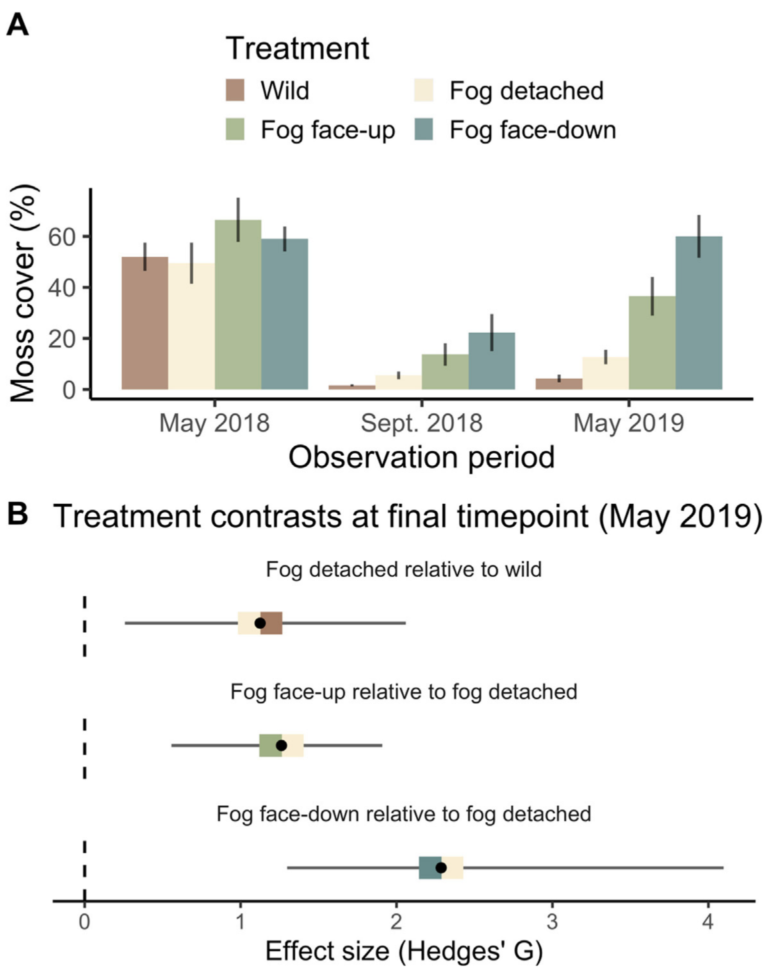


Fig. 4. We installed *S. ruralis* watered in a fog system in the field and observed changes in percent cover over time, drawing comparison with wild transplanted materials from the same population (panel A, brown bars). Moss burlap was installed face-up (green bars), face-down (blue bars), and moss was detached from burlap and broadcast directly onto the soil (beige bars). Black lines indicate SE +/− for each group. In panel B we show the results of three treatment contrasts we tested at the final timepoint. We calculated bootstrap estimates of the standardized mean difference, Hedges' G (x-axis), listed in the order of treatment relative to controls, e.g., fog detached (treatment) relative to wild (control). Dots indicate the bootstrapped mean of effect size and horizontal lines indicate the limits of the 95% confidence interval for that estimate. Colored boxes behind dots depict the treatment group (left box) and control group (right box) considered in the contrasts and match bar colors in panel A. Positive effect sizes indicate higher establishment of the treatment relative to the control, and an effect is only significant if the confidence interval does not intersect zero (dashed vertical line). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

high application rates when moss stems were left intact, but not when pulverized. We observed the greatest increase in grams per square meter at higher application rates (~100% cover), suggesting, as in prior work (Doherty et al., 2018), that vertical growth in *S. ruralis* is not impeded at high stem densities. Testing a gradient of application rates could reveal an intermediate application rate that further optimizes yield in terms of biomass increase per unit area. While past work has shown that pulverized mosses can regenerate in a greenhouse context (Shaw, 1986), we did not observe growth in *S. ruralis* following pulverization in our system, though perhaps fragmenting tissues more coarsely could reveal some benefit to this approach. In our second experiment we found that the relationship between biomass increase and time was not linear, as shown by a 1.5% increase in the first 30 days compared to a 17% increase over the entire 81-day experiment.

Several factors may have contributed to the lag phase observed in the second experiment. Even healthy tissues of *Syntrichia* spp. must undergo repair and reactivate metabolism when reviving from a dry state, and four or more hours are needed for positive carbon balances, though cell repair continues for days and may limit growth during this period (Bewley, 1995; Coe et al., 2012; Reed et al., 2012). Rapid drying and rapid wetting are also damaging, and we took care to mitigate rapid drying, though we were unaware of the damaging effects of rapid wetting, which may have occurred during sample washing (Reed et al., 2012; Slate et al., 2018). Storage conditions and duration can negatively impact moss health, particularly for *S. ruralis* when relative humidity exceeds 50%, though our storage conditions did not pass this threshold (Proctor, 2001; Proctor, 2003; Stark, 2017). Guo et al. (2020) conducted a storage trial studying three moss species (none *S. ruralis*) and found stems lost between 13 and 96% regenerative capacity after a 197-day period. Thus, it is possible that the tissues tested in experiment 2, which had been stored for 16 months, also lost some capacity for new growth. Finally, there may have been plant litter residues present in the inoculant, which could have decomposed in the fog chamber and influenced mass reading, though we expect these effects to be minimal given our washing protocols. Repair was required for all factors that caused cell damage, some tissues decomposed, and the sum of these effects delayed biomass increase by nearly a month.

Growth increased following the initial lag phase, and we were unable to identify a peak or deceleration of increase. The majority of greenhouse cultivation studies of this moss and other *Syntrichia* spp. evaluated growth over time on soil media and in the context of percent cover only, which makes comparison challenging (Antoninka et al., 2016; Bowker and Antoninka, 2016; Bowker et al., 2017; Doherty et al., 2018). It is a reasonable assumption that percent cover increase correlates with biomass increase. The longest observed period of cover increase was five months, though growth slowed between four and five months in this study, perhaps because of competition with other species or changes in greenhouse conditions (Bowker and Antoninka, 2016). Antoninka et al. (2016) found cover peaked, decreased, and recovered, perhaps due to ephemeral adverse greenhouse conditions. In a natural setting, *S. ruralis* stems elongate, branch, and resume growth in new branches based upon seasonal environmental cues, and replication of such cues may be required to maintain long-term growth (Mishler and Oliver, 1991). Additional growth over longer periods may be possible in a fog system, and long-term relationships between yield and time needs further study to optimize cultivation protocols.

4.2. The utility of fog systems

Some benefits of a fog system may include space savings of stacked cultivation and production of moss burlap, though the system is not without drawbacks. There are unique costs associated with burlap substrate and the fog emitters, which require 150 watts of power per unit to operate. Additionally, nutrients may be limiting in this soilless system, as dust and soil are the native inputs of macro and micro nutrients critical to growth (Bowker et al., 2016). The mosses grown in our fog experiments had minimal soil residues, which may have been sufficient for short-term growth, or they may have relied on reserves assimilated prior to cultivation. Thus, regular nutrient inputs may be required to accelerate growth for cultivation over longer periods, and there are media appropriate for this purpose (Xu et al., 2008; Antoninka et al., 2016).

We tested biomass increases only in a single-layer implementation of the fog system, and further work is necessary to establish if growth rates are comparable when implemented as a multi-level system. If appreciable moss growth is possible in a multi-level fog chamber it would offer a unique avenue to increase yield in facilities with space limitations.

4.3. Moss burlap as a restoration material

After a year in the field we found that mosses watered in a fog system established more cover when attached to burlap compared to detached mosses watered by the same system. We suspect that these results may be due to a combination of habitat amelioration and anchoring provided by burlap fabric. While some mosses do have the capacity to tolerate drying for prolonged periods, they cannot develop anchoring structures while in a dry state, and loose dry propagules are vulnerable to wind displacement and other disturbances (Stark, 2017; Young et al., 2019). Given the exceptionally dry summer following moss addition, we suspect our losses are largely attributable to displacement from wind, though burlap attachment mitigated this effect. Additionally, face-down materials were more shaded and protected from the wind than face-up treatments, and these mitigations likely helped moss regenerate to initial high levels of cover. Factors that maintain higher humidity levels for longer and buffer against rapid changes in water content were beneficial in other field trials (Coe et al., 2014; Condon and Pyke, 2016; Antoninka et al., 2018; Doherty et al., 2020; Slate et al., 2020). Thus, loosely broadcast mosses of any origin would likely benefit from installation under a layer of burlap. However, the colony structure in *Syntrichia* spp. is critical for water storage and extends activity periods (Sand-Jensen and Hammer, 2012; Wu et al., 2014). Loosely disaggregated stems blanketed in burlap would not benefit from the water holding capacity leant by adjacent and parallel stems, which may be maintained on moss burlap, though the benefits of colony effects should be a focus of further study.

Direct sun and wind exposure are probable drivers of reduced establishment in face-up relative to face-down treatments. In spite of this, ending cover in face-up treatments was more than twice that of unattached materials originating from the fog chamber, which highlights the benefits of burlap anchoring. While this difference is still smaller than that of face-down relative to unattached, face-up treatments may differ in function from face-down treatments and may still be of interest to practitioners. For example, mosses build soils by entrapping dust between stems (Danin and Gaynor, 1991), and a burlap cover may impede or enhance this function and others. For this reason, additional work is needed to determine if there are functional differences resulting from installation method.

Though there is some question as to whether hardening biocrust materials to field conditions is beneficial for establishment (Bowker et al., 2020), we show that hardening protocols are unnecessary to achieve high levels of *S. ruralis* cover in the grassland systems of western Montana, USA. Fog-watered materials installed without burlap had small but significantly higher rates of establishment than wild mosses of the same population, which had been pre-exposed to field conditions and were expected to be in a hardened state. This may suggest that hardening by prior exposure to field stressors is not a pathway to improve success of at least some moss species in the field. Alternatively, small reductions in water content are sufficient to induce hardening in some mosses (Stark, 2017) and minor fluctuations in cultivation conditions that cause partial drying may prepare *S. ruralis* and others for field stress.

The feasibility of restoration projects with moss burlap may be determined by the size and condition of a target site. Installation methods in experiment 3 are similar to those of erosion control fabrics or weed cloth held in place by staples, which are estimated to consume 100 personnel hours per hectare to install (Clarke personal communication). We advocate for treatments that include both vascular plants and biocrust, but a mixed strategy where moss burlap is stapled in place will still prove practical only for small sites of high value. Rather than stapling, a more scalable approach may involve broadcasting weighted fragments of moss burlap into plant interspaces. Furthermore, biocrusts are adapted to soil surface air currents and transfer to other contexts will shorten their activity periods and cause stress from rapid drying (Coe et al., 2014). Thus, installing moss burlap sheets over densely vegetated areas or otherwise elevating them above the soil surface is not advised. Finally, though many moss species, including *S. ruralis*, are broadly distributed across North America and globally, they harbor microbes which could prove to be non-native (Rosentreter et al., 2007; Doherty et al., 2018). Thus, provenance choices should favor more local sources when available.

4.4. Conclusions

While our present work offers an alternative cultivation system that generates field-ready biocrust restoration materials, key challenges remain in the pursuit of sustainable biocrust restoration technologies. Patterns of moss growth during long-term culture are understudied (Stark personal communication), though we must establish practices for maintaining sources that do not rely on continued harvest of field materials. Additionally, treatments that augment storage tolerance, such as equilibrating tissues to a low but undamaging water content, should be explored further to effectively stockpile restoration relevant quantities (Proctor, 2001; Proctor, 2003; Stark, 2017; Stark et al., 2017; Guo et al., 2020). At least some cells of *S. ruralis* can withstand prolonged dormancy, which were culturable after 20 years storage in an herbarium and 400 years underneath a recently receded glacier (La Farge et al., 2013; Stark et al., 2017). Given its broad range, ecological importance, amenability to cultivation, and resultant field success, we advocate for continued development of *S. ruralis* as a restoration material and encourage exploration of fog cultivation in other taxa.

Authors contribution

All authors contributed to the conception and design of the experiment. KD, HG, AA executed the experiment and monitoring. KD conducted statistical analyses and data visualizations. All authors contributed to writing and editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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