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Pelletized inoculation of fire mosses in severely burned conifer forests overcomes initial barriers to *Bryum argenteum* establishment but does not increase cover

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ABSTRACT

As wildfires increase in extent and severity, we need new tools to rehabilitate burned landscapes. We tested the effectiveness of adding fire moss tissue, produced in the greenhouse, as a bio-inoculant to severely burned soils in dry mixed conifer forests. We conducted three sequential experiments using knowledge gained from previous experiments to fine-tune fire moss delivery methods. The first two experiments began in July 2017, less than ten days after a wildfire in Arizona, United States. First, we added disaggregated (passed through a 2 mm sieve) cultivated moss tissue to burned soil surfaces, which was immediately collected by ants. In a second experiment, we added two preparations designed to reduce ant collection: moss rolled into pellets using diatomaceous earth and moss ground to a powder. Pelletization increased *Bryum argenteum* cover ($F_{[3,55]} = 12.32$, $p < 0.001$) and the number of distinct moss colonies ($F_{[3,55]} = 11.87$, $p < 0.001$) when compared to untreated control plots, although cover remained low (1%). A third experiment took place four months postfire in New Mexico. Sieved moss, pelletized moss, and pelletized moss at a high (5×) application rate were added to a burned forest. The high-rate pelletized treatment increased *B. argenteum* colony count by 140% compared to controls ($F_{[3,44]} = 2.37$, $p = 0.084$), but did not increase cover ($F_{[3,44]} = 1.19$, $p = 0.325$). At both sites, an extreme drought (Palmer Drought Severity Index < -4) during the winter of 2017–18 likely reduced success. We recommend further refinement and testing of pelletization in non-drought conditions.

1. Introduction

Increases in wildfire extent and severity in the southwestern United States (US) due to climate change and elevated fuel densities create a need to rehabilitate severely burned environments (Keane et al., 2008; Mueller et al., 2020; Singleton et al., 2018). Seeding vascular plants is a common postfire management strategy to quickly increase vegetative cover or reduce invasive species encroachment (Floyd et al., 2006; Peppin et al., 2010). But, seeding often does not result in desired outcomes of increased native plant recovery and reduced soil erosion (Peppin et al., 2010). Fire mosses, which consist of the species *Ceratodon purpureus* (Hedw.) Brid. commonly named “Redshank”, *Funaria hygrometrica* Hedw. commonly named “Cord moss”, and *Bryum argenteum* Hedw. commonly named “Silvergreen moss”, have many traits that could be valuable for postfire rehabilitation (Box 1). Natural fire moss colonization is widespread in the southwestern US after wildfires, but cover remains low (median 3.5%) and does not peak until two years postfire (Grover et al., 2020). Active rehabilitation could increase both the speed and extent of colonization.

While using fire moss in forest rehabilitation seems logical, this is a relatively new field of investigation with two immediate barriers to address. First, an adequate supply of fire moss propagules is needed. Field collection should be limited to reduce additional site disturbance and fire mosses can be grown rapidly in a greenhouse with cover doubling in less than twenty days (Grover et al., 2019). The next barrier is successful inoculation in the field. Ives (2016) found that inoculating field collected fire moss increased cover on burned slash piles from 1 to 9% over controls. However, field inoculation success of greenhouse-grown biocrust mosses in dryland ecosystems of the southwestern US has been limited (Antoninka et al., 2018; Bowker et al., 2019; Young et al., 2019), but this has yet to be tested with fire mosses. We hypothesized that the wetter and more temperate conditions in burned

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conifer forests, compared with drylands, could improve success.

We used the US federal government's Burned Area Emergency Response (BAER) program as a framework to guide our restoration trials. Following a wildfire, the BAER program mandates a rapid assessment of the postfire condition and recommends treatment implementation before the first damaging storm (Rowley, 2018). Treatments are almost exclusively installed on soils that have experienced high burn severity where large erosion responses are more likely (Rowley, 2018; Scott et al., 2009). This limited our experiments to tight timelines for inoculation and specific wildfires but created a more realistic test of inoculation success. Current BAER treatments are implemented via aircraft for speed and safety in the postfire environment (Rowley, 2018); because of this we focused on broadcast dispersal techniques. Our initial objective was to enhance colony counts and moss cover by overcoming propagule limitations through adding greenhouse-grown inoculum (Bowker, 2007).

1.1. Addressing barriers to moss establishment through iterative hypotheses generation

In an initial experiment (Exp. 1), we crushed inoculum through a 2-mm sieve and broadcast it onto the soil surface, a previously successful technique for moss restoration (Condon and Pyke, 2016; Doherty et al., 2019). In Exp. 1, we tested the hypothesis that inoculation of sieved moss would increase cover on recently burned areas.

In a second experiment (Exp. 2) one week after and adjacent to Exp. 1, two treatments were added to the sieving treatment. We hypothesized that pelletization techniques could reduce ant collection that was observed in Exp. 1, especially when coupled with an insect deterrent (Gornish et al., 2019; Madsen et al., 2016). We rolled moss into pellets using diatomaceous earth (Quarles, 1992) to protect propagules and potentially provide a favorable microsite to aid establishment. We also hypothesized that grinding inoculum to a powder could reduce ant collection as they would be less easily targeted by the ants (Slate et al., 2019). Alternatively grinding could reduce growth rates due to smaller, less robust propagules (Grover et al., 2019).

In a third experiment (Exp. 3), we carried forward the most successful ant collection deterrent from Exp. 2 and tested the hypothesis that final cover is positively affected by the number of propagules added. Additionally, we hypothesized that the postfire conditions in Exp. 1 and 2 could be limiting moss growth, to explore this we moved Exp. 3 to a recently burned area with different postfire conditions. We retained the sieved moss treatment in Exp. 3 to maintain continuity between all three experiments and examine if the initial barriers to moss colonization were more widespread.

2. Materials and methods

2.1. Study sites

We selected study sites where a fire occurred the summer of the inoculation year, had adequate coverage of high soil burn severity, and had previously been a mixed conifer forest to target a specific climate envelope (Grover et al., 2020). Exp. 1 and 2 were installed on the Boundary Fire, which was 33.5 km northwest of Flagstaff, Arizona (Table 1). The Boundary Fire was fully contained on 03 July 2017 and burned 7200 ha primarily in heavy downed fuel within the perimeter of a previous burn, the 2000 Pumpkin Fire. Exp. 1 plots contained only dead and down fuel before the Boundary Fire (35.42524588° N, −111.88336642° W, elev 2469 m). Exp. 2 plots contained dead and down fuel and a 15-year-old *Pinus Ponderosa* plantation pre-fire and high soil burn severity was more widespread than in Exp. 1 (35.42848517° N, −111.8827725° W, elev 2451 m, Fig. 1a). Prior to the Pumpkin fire both sites were dominated by *Pinus ponderosa* with some *Populus tremuloides* present. Soils in Exp. 1 and 2 are Ustolls derived from a Rhyolitic parent material with a silt loam surface texture (0-1 cm) (Kettler et al., 2001; Ramcharan et al., 2018). Average precipitation and temperature from 1981 to 2010 are 671 mm and 7 °C respectively, with cold wet periods occurring from Nov-Mar and monsoonal moisture occurring from July-Aug (Daly et al., 2008).

We installed Exp. 3 on the Cajete Fire in the Jemez Mountains 13.5 km northeast of Jemez Springs, New Mexico (Table 1). The Cajete Fire was fully contained on 24 June 2017 and burned 570 ha in previously unburned dry mixed conifer forest dominated by *P. ponderosa* with some *P. tremuloides* and *Pseudotsuga menziesii* (Table 1). Exp 3 was installed in a patch of continuous high soil burn severity (35.818068° N, −106.554721° W, elev 2607 m, Fig. 1c). Soils are Ustolls derived from a Rhyolitic parent material with a loam surface texture (0–1 cm) (Kettler et al., 2001; Ramcharan et al., 2018). Average precipitation and temperature from 1981 to 2010 are 625 mm and 5.9 °C respectively, with low precipitation from Sep-June and monsoonal moisture occurring from July-Aug (Daly et al., 2008).

2.2. Moss collection and processing

When collecting moss, we focused on finding local populations in similar ecosystem types to the study sites to increase local adaptation and reduce the possibility of introducing nonnative vascular plants. For Exp. 1 and 2, we sourced *F. hygrometrica* from the 2016 Camillo Fire, southeast of Flagstaff, Arizona, November 2016 (34.863940° N, −111.419616° W, 2318 m). We sourced *B. argenteum* and *C. purpureus* from the 2014 Slide Fire (35.025599° N, −111.792511° W, 2118 m). For Exp. 3, we sourced *B. argenteum* and *C. purpureus* from the 2013 Thompson Ridge Fire but could not find a local source of *F. hygrometrica*

Box 1
Fire Moss traits that could make them a valuable tool for postfire rehabilitation.

1. Natural colonization provides soil stability, increased soil nutrients, and microbial activity, but may or may not influence infiltration and runoff on steep slopes (García-Carmona et al., 2020; Grover et al., 2020; Silva et al., 2019).
2. It has been suggested that fire moss colonization could enhance reestablishment of vascular plants after wildfire (Muñoz-Rojas et al., 2021). Currently, this hypothesis is being tested experimentally in the southwestern US.
3. As opposed to vascular plants, fire mosses are desiccation tolerant, meaning their vegetative structures can dry out completely without dying and remain viable for years (Robinson et al., 2000; Werner et al., 1991). This does not preclude their need for precipitation to establish and grow.
4. Fire mosses can reproduce vegetatively through gametophyte fragments, or specialized tissues in the case of *B. argenteum* (Frey and Kürschner, 2011). They are also prolific spore producers creating a potential variety of propagule production and delivery techniques (Rosentreter, 2019).
5. Fire mosses have global distributions, making them a potentially valuable rehabilitation material in burned areas worldwide (Longton, 1981; McDaniel and Shaw, 2005).

Table 1
Descriptive information for each experiment. Temp and Precip averages from 1981 to 2010. Soil burn severity and the number of days between complete fire containment and the beginning of each experiment (Days after fire) are from BAER burned area reports. Study size (ha) was calculated by drawing a polygon around all plot locations. Reps is number of replications for each treatment.

Exp.	Temp (°C)	Precip (mm)	Soil parent material, suborder, texture	Pre-fire condition	Soil burn severity	Days after fire	Study size (ha)	Moss species added	Treatments	Reps
1	7	671	Rhyolite, ustoll, silt loam	Heavy dead/down fuel no overstory cover	Moderate-High	2	0.3	*B. argenteum, C. purpureus, F. hygrometrica*	Control, Sieve	15
2	7	671	Rhyolite, ustoll, silt loam	Heavy dead/down fuel, 15-year-old *Pinus ponderosa* plantation	High	9	0.2	*B. argenteum, C. purpureus, F. hygrometrica*	Control, Sieve, Grind, Pellet	15
3	5.9	625	Rhyolite, ustoll, loam	Dry mixed conifer forest	High	134	0.2	*B. argenteum, C. purpureus*	Control, Sieve Pellet, Pellet 5×	12

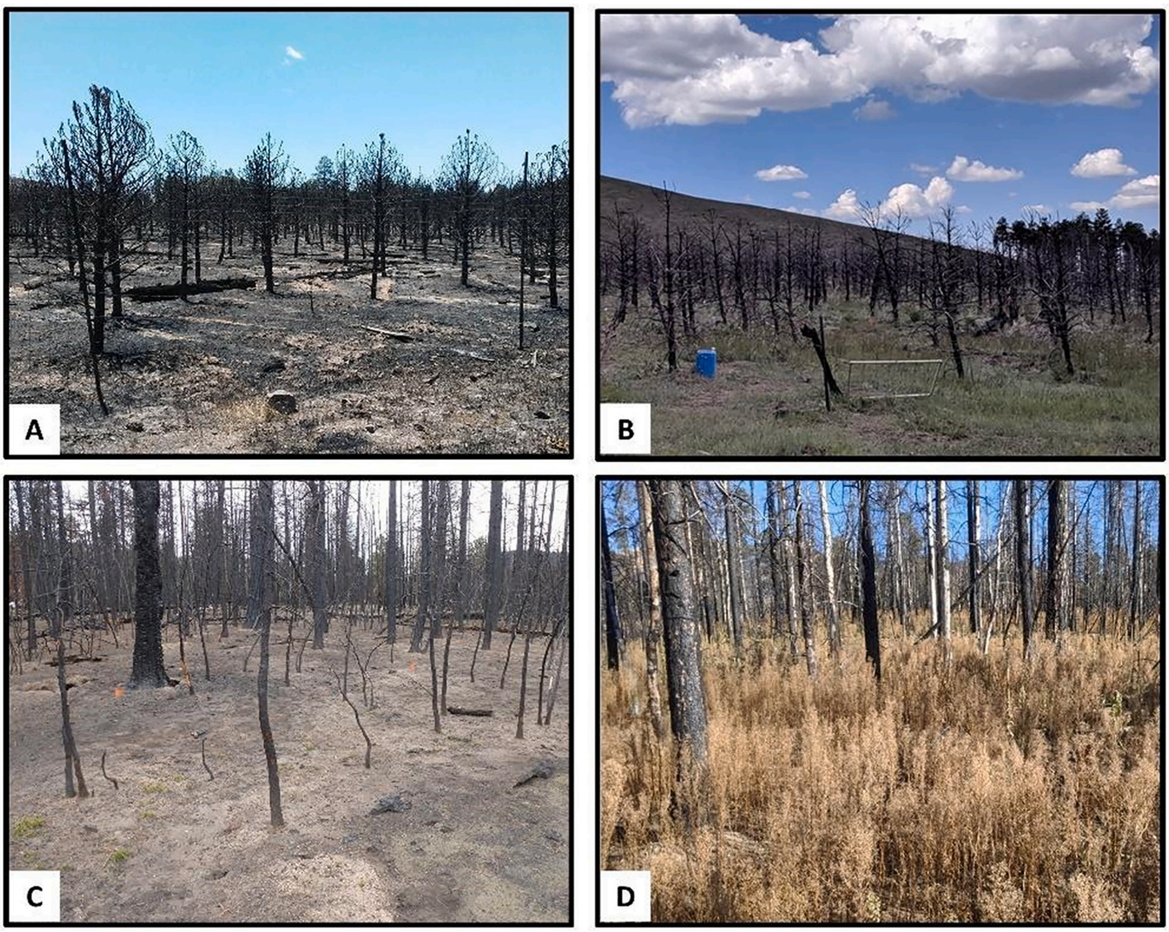

Fig. 1. (A) Exp. 2 directly after fire, prior to inoculation, (B) two years after inoculation, (C) Exp. 3 four months after fire, prior to inoculation, and (D) two years after inoculation.

(35.893059° N, −106.577049° W, 2711 m). In all experiments, mosses were dried over a period of 10 or more days, with a light shade covering to prevent damage due to rapid desiccation (Proctor et al., 2007). We removed excess soil, crushed mosses through a 2 mm sieve to break up colonies, and stored them in paper bags in the dark at room temperature (23 °C) until cultivation.

2.3. Cultivation

We combined moss species in equal proportions for greenhouse cultivation using the methods outlined in the bulking experiment of Grover et al. (2019). Briefly, moss was dispersed on an organic gardening soil, kept moist continuously and shaded using a row cover (temperature 17 °C, relative humidity 41%). When moss cover exceeded 80%, usually within 60 days, we dried moss and organic substrate slowly under shade cloth in the greenhouse, then crushed it through a 2-mm sieve. Moss was not separated from the substrate to maintain an external source of favorable nutrients during inoculation (Grover et al., 2019). Inoculum was placed into a bucket and stored at room temperature (23 °C) for no more than three months until field experiments commenced.

2.4. Plot selection and installation

We targeted patches of high soil burn severity during plot installation for all experiments to reduce heterogeneity of experimental units (Parsons et al., 2010). Plots consisted of 1 × 1 m quadrats placed at least 2 m apart marked with permanent iron stakes. We selected low slope angles (<10%) to reduce loss of bio-inoculants to erosion from our small plots. Exp. 1 began on 5 July 2017, Exp. 2 on 12 July 2017, and Exp. 3 on 4 Nov 2017 (Table 1).

2.5. Experimental design

The design of each experiment was modified using knowledge gained from the previous experiments to overcome initial barriers to moss establishment and to test successful techniques on different wildfires. In Exp. 1 we replicated treatment levels of untreated control and sieved moss 15 times each and randomly assigned treatments to plots ($n = 30$). During application, we scooped moss inoculum out of a bucket using a 250 ml cup and shook it evenly onto each plot. This was equal to roughly 25% cover on each plot. We stirred moss to prevent settling, prior to and periodically throughout the inoculation process.

In Exp. 2 we replicated four treatment levels of control, sieved moss, moss pellets, and ground moss 15 times each and randomly assigned them to plots ($n = 60$). For each replicate of each treatment, we extracted 250 ml of sieved moss inoculum from the bucket in the same manner as Exp. 1. The sieve treatment matched Exp. 1 exactly. Moss pellet replicates were made individually. We mixed the inoculum with diatomaceous earth (1:1 by volume) in a 20-l bucket. Using a hand pump sprayer, we periodically added a fine jet of water to the mixture while rotating the bucket from the handle. This had the effect of rolling the mixture into unequally sized pellets ranging from 1 to 15 mm in diameter (Fig. 2b), which we then dried slowly in the greenhouse. We stored replicates in individual paper bags and spread them evenly onto plots during inoculation. For the final treatment we ground moss to a fine powder using a mill (Thomas® Wiley Mill, Swedesburo, NJ), stored them separately, then spread them evenly on the plots.

In Exp. 3 we applied four treatment levels of control, sieved moss, pellets at the same volume as Exp. 2, and moss pellets at five times the volume of Exp. 2, referred to in the subsequent text as pellet 5×. Each treatment was replicated 12 times and randomly assigned to each plot ($n = 48$).

2.6. Monitoring

Directly after installing each experiment, HG performed a 30-min timed search looking for ant collection of inoculum, starting with the plots that had been set up first. We did not monitor cover directly after inoculation because treatment manipulations made comparisons impossible. We monitored cover every fall and spring when mosses were dry, this occurred once for Exp. 1 in fall 2017, five times for Exp. 2 and

Fig. 2. (A) ants (*Myrmica* sp.) collecting sieved inoculum on Exp. 1. (B) Moss just after pelletization, (C) pellets one week after inoculation on Exp. 2, and (D) pellets one year after inoculation on Exp. 2.

concluded in fall 2019, and three times for Exp. 3 and concluded in spring 2019. We divided plots into 4 quadrants and HG visually estimated cover to the nearest 0.1% on each quadrant for all visits. Visual estimation is particularly accurate when the class of interest is at relatively low cover, as was often the case with our mosses (Meese and Tomich, 1992). A reference sheet illustrating cover amounts was used for consistency. Basal cover classes were: moss to species, moss protonema, bare soil, rock, litter, and vascular plant. Canopy cover classes were: forb, graminoid, shrub, and tree.

On Exp. 2 and 3 we counted the number of distinct moss colonies in each plot quadrant as a measure of colonization events (Doherty et al., 2019). A colony was defined as a spatially discrete patch of moss that we interpreted as having radiated from a central colonization event of a single propagule. Physical separation between patches could be no less than 2 mm. If moss colonies were found in a quadrant where the visual estimates had recorded no moss, we changed the cover value to one half of the lowest cover value (0.05%). This allowed us to differentiate low cover from true absences more accurately. Quadrant level data was aggregated to the plot scale before analysis by taking the mean of all quadrants for cover and the sum for colony count.

In the final monitoring timepoint of Exp. 3, fall 2019, dense annual vascular plant growth and seed dispersal obscured our plots (Fig. 1d), preventing accurate visual estimates of both moss cover and colonies. To deal with this, we instead estimated moss cover with a point intercept method using a 40 × 40 cm gridded frame and reading 50 points per plot and did not attempt to count colonies.

2.7. Quantification of drought

To help interpret how post-inoculation weather affected inoculation success, we extracted drought severity values for Exp. 2 and 3. We used Google Earth Engine to extract Palmer Drought Severity Indices (PDSI) at a spatial resolution of 4 km and a temporal resolution of 10 days as generated by Abatzoglou et al. (2014). PDSI accounts for both precipitation and potential evapotranspiration and is comparable within regions such as the southwestern US (Dai, 2011). PDSI is often reported from less than −5 (Exceptional Drought) to greater than 5 (Exceptionally Moist) but more extreme values are possible.

2.8. Soil sample analysis

To test for edaphic differences between study sites at the time of inoculation, we took a composite soil sample with greater than 10 subsamples throughout Exp. 2 and 3 at a depth of 0–1 cm. We sieved samples to 2 mm and analyzed them for pH, total carbon and nitrogen, available phosphate, and a suite of available cations and trace metals, following the Forest Inventory Analysis Protocol (Amacher et al., 2003). We determined pH using a glass electrode immersed in a saturated paste of 10 mg soil mixed with a 0.01 M $CaCl_2$ solution and allowed to stand for 30 min. To test for total carbon and nitrogen content, we ground and analyzed samples using a 4010 Elemental Combustion System (Costech Analytical Technologies Inc. Valencia, CA). We extracted phosphate using the Olsen (pH > 6) and Bray (pH ≤ 6) methods and analyzed using a Lachat Instruments QuikChem 8500 series Flow Injection Analyzer (Lachat Instruments, Loveland, CO). We extracted water soluble cations and metals using a 1 M NH_4Cl solution. We used an argon gas carrier and analyzed samples on a Thermo Scientific™ iCAP™ 7000 Series ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometry) or mass spectrometry (ICP-MS), using Qtegra™ ISDS™ Software. Cations and metals consisted of calcium, magnesium, potassium, sodium, sulfur, aluminum, and manganese.

2.9. Statistical analysis

All statistical analyses for this manuscript were conducted in R version 3.6.1 (R Core Team, 2019). To analyze Exp. 1, we used a linear model with the fixed effect of treatment and control and a response of moss cover. Due to non-normality and unequal variance in model residuals we used stratified bootstrapping to obtain 95% confidence intervals for mean moss cover. We used the boot package (Canty, 2002) and 10,000 replicates to perform residual resampling and stratified by treatment levels.

To analyze Exp. 2 and 3 we conducted ANOVAs on linear models with fixed effects of treatment, time since inoculation (time), and their interaction. Time was modeled as a categorical variable because its relationship with moss growth was non-linear. A random effect of plot was included to account for repeated measurements on the same plots. Response variables of moss cover and moss colony count were modeled separately for each experiment for a total of four models. To satisfy model assumptions of equal variance and normality we performed a cube root transformation on all response variables before conducting our analysis. Analyses were completed using the lmerTest package creating a type three ANOVA table with a Satterthwaite's degrees of freedom calculation (Kuznetsova et al., 2017). In Exp. 2, one replicate in the grind treatment was a strong outlier with greater than 30 times the average moss cover in spring 2019. Based on visual evidence, we concluded this was due to convergent overland flow depositing moss from other plots onto this plot and excluded it from cover and colony analyses. In Exp. 3, a high number of zero cover values and colony counts were found during the first monitoring visit in spring 2018 due to a lack of precipitation and pellets obscuring moss within them. We excluded this monitoring visit from statistical analysis because we were unable to accurately measure the response variables.

3. Results

3.1. Experiment 1

After inoculating Exp. 1 we immediately saw ants from the genus *Myrmica* collect moss fragments and remove them from the treated plots (Fig. 2a). By the time we had finished setting up the experiment (~1 h), we returned to the plots that we treated first and found no moss propagules or ants. In the fall 2017 monitoring we found that treated plot cover (mean = 0.11%, 95% CI 0.013–0.33%) did not differ from control cover (mean = 0.01%, 95% CI = 0.00–0.08%). No further monitoring was conducted.

3.2. Soil nutrients for Exp. 2 and 3

Soil nutrient levels at inoculation differed between Exp. 2 and 3. Soil carbon and nitrogen were roughly twice as high on Exp. 2; however, Exp. 3 generally had higher cations and trace metals (Table 2). We interpreted these differences as being driven by the different fire return intervals between sites. Exp. 2 had one high soil burn severity fire in 2000 and again prior to our experiment, whereas Exp. 3 had only experienced the fire prior to our experiment.

3.3. Experiment 2

After installing Exp. 2 we immediately saw ants collect moss fragments and remove them from the plots that received sieved moss, we did not see ant collection on grind or pellet treated plots. In this experiment, pelletization was the most successful treatment (Fig. 2b & c), increasing *B. argenteum* cover ($F_{[3,55]} = 12.32$, $p < 0.001$) and colony count ($F_{[3,55]} = 11.87$, $p < 0.001$) compared to the control (Fig. 3a & b). Mean cover on pelletized plots remained low (1.0 ± 0.4%) throughout the experiment and fluctuated over time ($F_{[4220]} = 6.90$, $p < 0.001$). The worst performing treatment was sieved moss with a maximum mean cover of 0.2 ± 0.1% by fall 2019. Background colonization of *B. argenteum* on control plots was lower still, reaching a mean of 0.1 ± 0.05% cover by fall 2019. During the first three monitoring visits we found no colonization of *C. purpureus* on any treatment, but by spring 2019 we observed

Table 2
Soil properties for Exp. 2 and 3 at inoculation July 2017 and November 2017, respectively.

Exp.	pH	C (%)	N (%)	P (ppm)	Ca (ppm)	Mg (ppm)	K (ppm)	Na (ppm)	S (ppm)	Al (ppm)	Mn (ppm)
2	7.15	5.22	0.36	93	3190	332.7	719.9	83.7	59.6	12.6	31.4
3	6.72	2.56	0.19	91	5098	654.3	1034	290.1	50	56.5	118.3

C = Carbon, N = Nitrogen, P = Phosphate, Mg = Magnesium, K = Potassium, S = Sulfur, Al = Aluminum, Mn = Manganese.

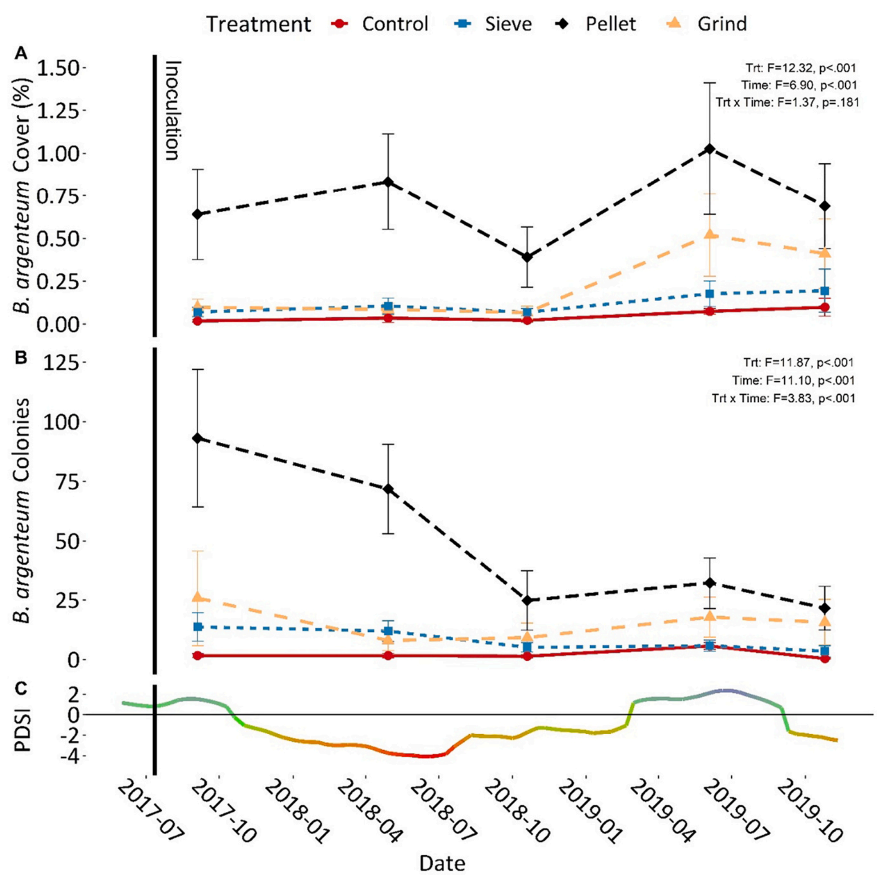

Fig. 3. Exp. 2, vertical lines indicate date of inoculation. (A) *B. argenteum* cover and (B) colony count at monitoring visits. (C) PDSI throughout the experiment; trending negative values indicate increasing drought while trending positive values indicate more moist conditions. Error bars represent ±SE.

trace amounts on pellet and grind treatments. We found no *F. hygrometrica* on any treated plot.

B. argenteum colony counts at the first monitoring visit were highest on pelletized plots with a mean of 93/m^2 (Fig. 3b). The number of colonies on pelletized plots declined between fall 2017 and fall 2018 which is supported by a strong main effect of time ($F_{[4220]} = 11.10$, $p < 0.001$) and the interaction between treatment and time ($F_{[12,220]} = 3.83$, $p < 0.001$). This decline corresponds with an extreme drought that occurred over the winter of 2017/2018 with PDSI values below −4 (Fig. 3c). Pelletized colony counts leveled off between fall 2018 and 2019 as the drought subsided averaging roughly 30/m^2.

3.4. Experiment 3

After installing Exp. 3 we did not see ant collection on any treatment during our timed search. We returned to the experiment the following morning and mosses were still present on all treatments. There was no effect of treatment on cover ($F_{[3,44]} = 1.19$, $p = 0.325$, Fig. 4a), however there was a weak effect of treatment on colony count ($F_{[3,44]} = 2.36$, $p = 0.084$, Fig. 4b). Pelletization at 5× volume achieved the highest cover of 10.5 ± 2.5% and a mean colony count of 412 ± 124 by June 2019. The sieved and pelletized treatments had similar cover values throughout the experiment and performed only slightly better than the controls (Fig. 4a). Natural recruitment of *B. argenteum* on control plots was high compared to Exp. 1 and 2, reaching 6.8 ± 2.3% cover in June 2019. Moss cover at the first monitoring visit, 6 months post-inoculation, was extremely low with a mean of less than 0.02% across all treatments. However, pellets remained with an average cover of 42.2 ± 2% and 8.7 ± 0.5% for pellet 5× and pellet treatments respectively. This was most likely due to the exceptional drought that occurred over the winter of 2017/2018 with PDSI values below −5 (Fig. 4c). We detected *C. purpureus* on one pelletized and one sieve plot at the final monitoring visit. We did not find *F. hygrometrica* at any time.

In contrast to Exp. 2, the number of colonies for all treatments in Exp. 3 increased through time (Fig. 4b). As the drought began to subside there was an increase in colony counts in the fall 2018 monitoring (Fig. 4b & c). Colony counts plateaued from fall 2018 to spring 2019, but there was a relatively large increase in cover during that time period (Fig. 4a).

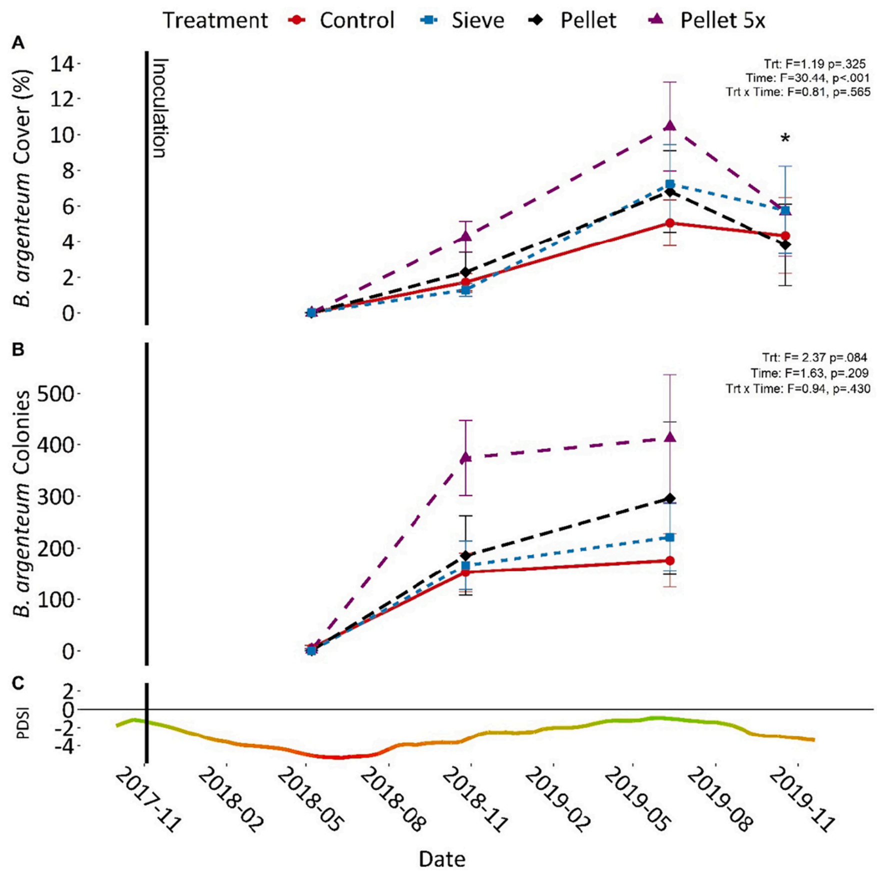

Fig. 4. Exp. 3, vertical line indicates date of inoculation. (A) *B. argenteum* cover and (B) colony count at monitoring visits. *Cover methodology changed, and no colony count data was taken in final monitoring visit due to extensive vascular plant colonization. (C) PDSI throughout the experiment; trending negative values indicate increasing drought while trending positive values indicate more moist conditions. Error bars represent ±SE.

4. Discussion

4.1. Ant collection is a novel barrier to biocrust rehabilitation

To our knowledge, ant collection of propagules has not been identified as a barrier to biocrust rehabilitation success prior to this study (Bowker, 2007). A possible explanation for the collection behavior we observed was for the construction of large structures of earth, moss, and dead vegetation called solaria that *Myrmica* spp. build in the spring for brood rearing (Radchenko and Elmes, 2010). Perhaps ant collection was a localized occurrence driven by the moss only inoculum mix, postfire environment, time of year, or ecosystem type, but it may also be a more widespread barrier to biocrust restoration. Biocrust inoculum collection by ants has also been documented anecdotally in a degraded *Juniperus monosperma* woodland ~22 km from Exp. 3 (K. Young personal communication, Young et al., 2019). Future research in collaboration with entomologists should quantify insect collection and account for or mitigate it when necessary.

4.2. Pelletization increased moss colonization and could provide other benefits

Pelletization has three potential benefits compared to the sieved and ground moss treatment: protection from insect collection, protection from wind and water erosion, and aiding in inoculum delivery. These along with the increased colonization in Exp. 3 and the increased colonization and cover in Exp. 2 make it a potentially promising restoration technology. Pelletization has had mixed results in vascular seed restoration trials but is often successful at preventing seed collection by insects (Gornish et al., 2019). Diatomaceous earth is an insect deterrent (Quarles, 1992), but perhaps physically encasing moss fragments was sufficient to deter collection. Exp. 2 inoculation occurred at the onset of monsoon season; one week later, Exp. 2 had received 26 mm of rain, the diatomaceous earth had disintegrated, and pellets had melted into the soil surface (Fig. 2c). This suggests that the physical attachment of moss to the soil surface also reduces ant collection. Refinement of pelletization techniques such as using psyllium husk powder as a binding agent could improve soil attachment (Blankenship et al., 2020; Fick et al., 2019). Pellets also increased propagule density two-fold and colony size up to seven-fold compared to sieved mosses, reducing the risk of loss from wind and water erosion. Finally, pelletization is an effective method for maintaining even application of very small seeds when using aerial broadcast techniques, an equally important consideration for fire moss application (Madsen et al., 2016).

In Exp. 2, the grind treatment did attain higher moss cover and colony counts than the control, but before the drought, pelletization had greater than three times the colonization rate of the grind treatment. Perhaps this was because ground moss fragments were less viable or were more easily eroded off plots. This and other considerations addressed previously in this section lead us to suggest pelletization over grinding as a treatment for further study.

4.3. Drought likely reduced inoculation success

Pelletization and initial monsoonal moisture allowed *B. argenteum* to successfully colonize in Exp. 2, but many of the colonies did not survive or proliferate and cover did not increase. The long hydration periods and cooler temperatures provided by winter precipitation is especially important for moss growth, making the first winter after inoculation critical for establishment and success (Bu et al., 2018; Condon and Pyke, 2016; Fick et al., 2019). The extreme drought that occurred during the winter of 2017–2018 was likely the main contributor to low cover in Exp 2.

Stronger treatment differences in Exp. 2 than in Exp. 3 likely resulted from increased natural colonization, more severe drought, and the timing of inoculation in Exp. 3. We suspect that the higher natural colonization of *B. argenteum* in Exp. 3 than 2 is due to different postfire edaphic conditions (section 3.1). Perhaps this was driven by the reburn in Exp. 2 given that parent material, climate, and potential vegetation type were similar between Exp. 2 and 3 (section 2.1). The winter 2017/2018 drought in Exp. 3 was deeper and never completely subsided when compared to Exp. 2. Additionally, mosses in Exp. 3 had to withstand the drought within pellets, whereas in Exp. 2 mosses were added before an active 2017 monsoon season allowing them to partially establish. In Exp. 3 the pellet treatment did not perform significantly better than the control or sieved treatment and the much higher effort pellet 5× treatment performed marginally better suggesting that survivorship was low under exceptional drought conditions.

4.4. Moss species traits may affect establishment success

B. argenteum was the only species to establish, possibly due to the asexual reproductive structures it creates. *B. argenteum* was also the dominant natural colonizer suggesting that our study sites may have been outside the ecological niche of *F. hygrometrica* or *C. purpureus,* especially in drought conditions. We believe the specialized asexual reproductive structures found on *B. argenteum* such as bulbils, rhizoidal tubers, and protonemal gemmae helped it establish from vegetative inoculum (Frey and Kürschner, 2011; Rosentreter, 2019). Rosentreter (2019) suggests using *F. hygrometrica* and *C. purpureus* spores for rehabilitation, which we did not attempt. It is possible to induce sporophyte growth in fire mosses (Grover et al., 2019), but alternative techniques would be needed to collect spores and inoculate burned soils.

5. Conclusions

In this series of studies, we explored the potential to rehabilitate recently severely burned soils in two dry mixed conifer forest ecosystems using a blend of moss species as a bioinoculant. Generally, the addition of greenhouse-produced mosses to the field promoteed its colonization of burned soil. However, such evidence was not strong. A larger number of colonies established when we inoculated. This suggests that further testing and refinement is warranted. The finding that moss colonies did not coalesce into more cover suggests that our current methods are insufficient overcome all establishment barriers. We documented that collection of added moss inoculum by ants may present a barrier to the success of moss bio-inoculation efforts. One of our approaches to countering this barrier – pelletization of mosses in diatomaceous earth – did boost the number of colonies established, whereas grinding mosses into a powder did not. Another probable barrier to success was that our experiments were conducted during an extreme drought. We found that even the addition of a high rate of moss inoculum was insufficient to elicit a clear increase in moss cover under these conditions. Successfully establishing mosses through bio-inoculation, if possible, may require us to simultaneously address multiple barriers to establishment including insects, drought, and others. Repetition of similar trials with and without experimental irrigation or rainfall reduction could directly confirm and quantify the influence of drought conditions. Accounting for the fact that one type of pellet created a positive effect, a broader investigation of pellet types could be beneficial: the pellets should be designed to protect mosses, to increase erosion resistance, and to extend viability during drought.

Authors contribution

HG designed, conducted the experiment, conducted the analysis, wrote the manuscript, MB, PF, CS secured funding, advised in the experimental design and analysis, edited the manuscript, AA secured funding, edited the manuscript.

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

Supplementary material: ANOVA tables of *B. argenteum* cover and colony linear models for Exp. 2 and 3 may be found online at https://doi.org/10.1016/j.ecoleng.2021.106513.

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