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RESEARCH ARTICLE

Hydroseeding tackifiers and dryland moss restoration potential

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Tackifiers are long-chain carbon compounds used for soil stabilization and hydroseeding and could provide a vehicle for biological soil crust restoration. We examined the sensitivity of two dryland mosses, *Bryum argenteum* and *Syntrichia ruralis,* to three common tackifiers—guar, psyllium, and polyacrylamide (PAM)—at 0.5×, 1.0×, and 2.0× of recommended (×) concentrations for erosion control and revegetation. We measured moss shoot, gemma, and protonema production as well as moss organic matter and bound sand masses as indicators of growth and soil holding ability. We tested sand and tackifier chemistry to investigate potential nutrient and toxicant potential on moss growth. Groups of 10 fragments from field-collected mosses were grown on sand in open petri dishes arranged in a growth chamber in replicated blocks containing each tackifier and concentration combination plus a distilled water control. *Bryum* ($n = 10$) and *Syntrichia* ($n = 9$) growth were measured at the end of 6 and 5 weeks, respectively. Overall model tests yielded statistically significant results ($p < 0.001$) for every variable in each species. When compared to water, guar tended to decrease growth, psyllium tended to increase growth, and PAM's effects were generally neutral to positive. Within tackifier types, increasing concentrations of guar tended to decrease growth, while increasing concentrations of psyllium tended to increase growth. Changes in PAM concentrations had little effect on growth. Increases in guar and psyllium lowered pH and increased P and K. Psyllium and PAM yielded promising results as potential agents of dispersal and adherence of dryland mosses in field restoration.

Key words: biocrust, biological soil crust, dryland mosses, fragment growth, guar, hydroseeding, polyacrylamide, psyllium, soil retention, tackifiers

Implications for Practice

- Hydroseeding technologies could be used to disperse and adhere dryland mosses to exposed soil surfaces.
- Type and concentration of the tackifier used in hydroseeding may dictate moss establishment and restoration success.
- Psyllium and PAM tackifiers promoted or did not interfere with moss growth and warrant further examination in field trials and with other biological soil crust taxa.
- Guar hindered moss growth in growth chamber conditions and caution might be wise if it is used in field trials.
- The mass of sand bound to some mosses varied with combinations of tackifiers suggesting that field trials examine both soil stabilization and biological soil crust growth.

Introduction

Water limitation, soil erosion, invasive species, and an increased probability of fire make ecological restoration of arid and semi-arid ecosystems (drylands) difficult (Bainbridge 2007). Especially in the warmest and driest areas, many attempts to restore native plant communities fail to meet objectives (Hardegree et al. 2011; Knutson et al. 2014). Dryland restoration to date largely focuses on reintroducing vascular plants, with the hope that other missing biotic components will reestablish on their own over time. Many successful practices have a range of soil disturbances from none with herbicides and aerial seeding to severe methods including mechanical techniques (e.g. harrowing, plowing, and ripping soil) and seed drills (e.g. minimum-till drills without disking soil to rangeland drills with disked furrow; Whisenant 1999). Depending on the plant community and the biocrust (e.g. cyanobacteria, lichens, and mosses) species found on the soil surface of a restoration site, it may be advantageous to restore biocrusts since they may resist expansion of invasive species (Condon & Pyke 2018).

Dryland restoration projects that incorporate biocrusts have only recently achieved significant research momentum, but management-scale projects (>50 ha) are rare and only occur

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with cyanobacteria (Bowker 2007; Li et al. 2014). Yet, biocrusts can represent as much as 70% cover of the soil surface (Belnap et al. 2001) and can play important ecological functions (e.g. soil stability, water infiltration, and nutrient cycling) in drylands (Belnap et al. 2001). Mosses have demonstrated their potential for dryland restoration through their desiccation tolerance, ease of propagation, and rapid growth rates (Xu et al. 2008; Antoninka et al. 2016; Condon & Pyke 2016), but an efficient method for delivering and establishing moss propagules is lacking.

Moss physiology makes them ideal for dryland restoration. Mosses absorb water and nutrients through their leaves and can withstand long periods of desiccation (Vanderpoorten & Goffinet 2009). They may propagate sexually via spores or asexually via gametophyte fragmentation or production of gemmae. In addition, dryland mosses may facilitate cyanobacteria and lichen establishment, further enhancing biocrusts in restored communities (Antoninka et al. 2016). Therefore, establishing mosses early in the restoration process could be a valuable first step toward reestablishing a more complete biotic community following disturbance.

Moss fragments are a proven source of restoration propagules, but introduced fragments are prone to secondary dispersal by water and wind. Jute nets have been used to create boundary layers that reduce secondary dispersal and facilitate biocrust establishment (Condon & Pyke 2016; Bowker et al. 2020), but this method is not practical for most management-scale restoration projects. Tackifiers, soil adhesive agents, are commonly used in hydromulching and seeding for landscaping, postfire rehabilitation, and hillslope stabilization (CalTrans 2003; Robichaud et al. 2010) to adhere propagules and mulch to soil surfaces and may be a viable method for distributing and adhering moss fragments to soil at a scale relevant for management-scale restoration projects. A few studies have incorporated tackifier treatments into investigations of biocrust outplanting and cultivation (Park et al. 2017; Chandler et al. 2019), but no study has systematically examined effects of different types and concentrations of tackifiers on moss survival and growth.

In this study, we examined the sensitivity of two cosmopolitan mosses common in drylands (Flowers 1973), *Bryum argenteum* Hedw. (silvery-thread moss) and *Syntrichia ruralis* (Hedw.) F. Web. & D. Mohr (twisted or star moss; hereafter referred to by their generic names), to three common tackifiers (guar, psyllium, and polyacrylamide [PAM]) using a range of concentrations for erosion control and revegetation. We measured the production of protonemata, gemmae, and lateral shoots by adult moss gametophytes as well as moss organic matter mass and substrate mass bound to mosses to address the following questions: (1) To what extent do growth or development of structures (shoots, gemmae and protonemata) in *Bryum* or *Syntrichia* grown with tackifiers differ from those grown without tackifiers (i.e. each tackifier type by concentration combination versus water); (2) Do different concentrations of a given tackifier impact growth or development of structures in *Bryum* or *Syntrichia* (i.e. comparisons among concentrations within each tackifier); (3) Does the type of tackifier impact growth or development of structures in *Bryum* or *Syntrichia* (i.e. comparisons among tackifiers with concentrations pooled); (4) As a post hoc study, could tackifier chemistry be related to growth and development of structures in *Bryum* or *Syntrichia*?

Methods

Moss Species and Source

We collected hundreds of individual small mats (5–10 cm diameter) of dry *Bryum argenteum* and *Syntrichia ruralis* (Fig. 1A) from margins of a park in Bend, Oregon in July 2016 (lat 44°05′36″N, long 121°16′15″W). These margins are Juniper Shrubby Lava Blisters and Juniper Shrubby Pumice Flat Ecological Sites (R010XA023OR and R010XA009OR, Ecosystems Dynamic Interpretive Tool, USDA Natural Resources Conservation Service, https://edit.jornada.nmsu.edu/; accessed 14 May 2019) consisting of *Juniperus occidentalis* Hook. (Western juniper) and occasional *Pinus ponderosa* Lawson & C. Lawson (Ponderosa pine), *Artemisia tridentata* Nutt. ssp. *vaseyana* (Rydb.) Beetle (Mountain big sagebrush), *Purshia tridentata* (Pursh) DC. (Antelope bitterbrush) with an understory of *Festuca idahoensis* Elmer (Idaho fescue), *Pseudoroegneria spicata* (Pursh) Á. Löve (Bluebunch wheatgrass), *Achnatherum thurberianum* (Piper) Barkworth (Thurber's needlegrass), and *Bromus tectorum* L. (Cheatgrass). Soils are shallow to moderately deep sandy loam to loamy sands. Each mat consisted of hundreds of fragments and mats were collected from a series of microhabitats that were assumed to be representative of the various ecotypes and sex ratios at the site (Bowker et al. 2000). The species' identities were verified in the lab while disregarding the fragment's sex and excluding fragments with sporophytes. Specimens were stored (air dried) in brown paper bags at room temperature for 3 to 6 months until used in experiments.

Bryum and *Syntrichia* represent different life history strategies. *Bryum* is an early successional, generalist species found in open areas. It is short in stature (0.5–1.5 cm shoot height) and found in dense cushions. *Syntrichia* is a later successional species with shoots 1–8 cm or more, often arranged in loose cushions. *Syntrichia* is widely accepted as a predominately ectohydric species (i.e. it readily absorbs water from the plant surface), while, in this experiment, *Bryum* demonstrated a more endohydric habit by hydrating more slowly from its surface than *Syntrichia*.

Tackifiers

Three common tackifiers, guar gum (99.89% purity; The Dirty Gardener, Tacoma, Washington), psyllium (M-Binder; Nature's Seed, Lehi, Utah), and PAM (PAM HT; Emerald Seed and Supply, Redmond, Oregon), were applied to moss fragments at three concentrations, 0.5, 1.0, and 2.0 times the manufacturers' recommended concentration for soil stabilization on level sites. The 2.0 times concentration is typical for hillslope applications (CalTrans 2003, 2017) and a 0.5 times concentration was included to examine moss reactions to a broader spectrum of

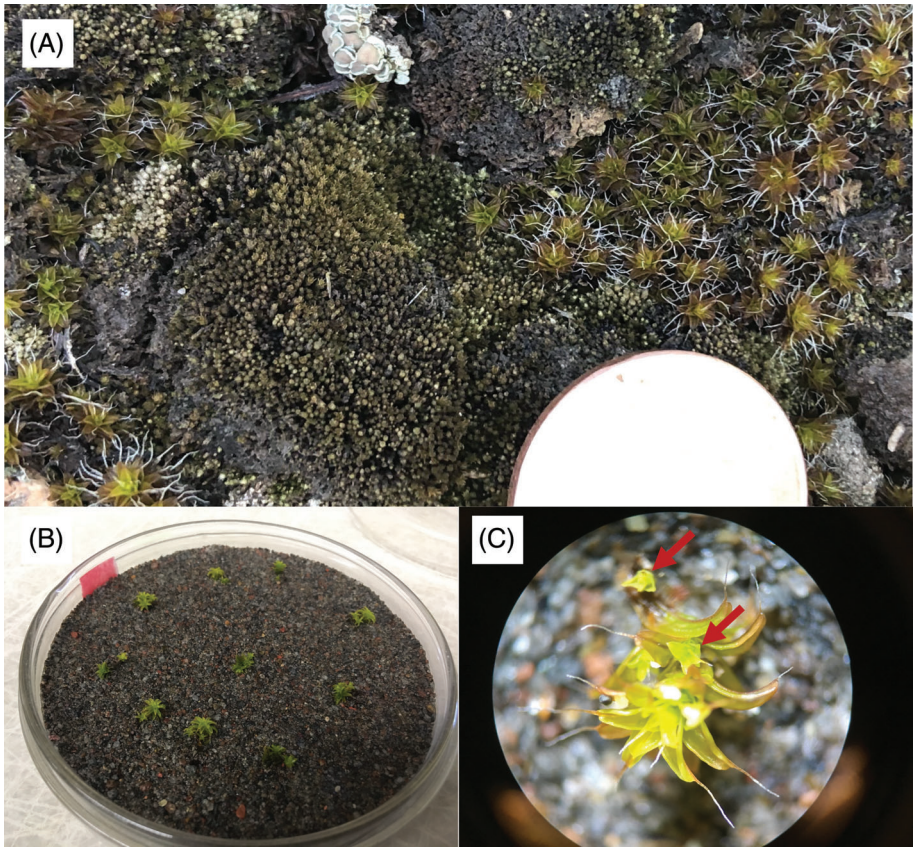


Figure 1. Mats of mosses (A) *Bryum argenteum* (upper left of disk) and *Syntrichia ruralis* (above and right of disk with thread-like appendages on leaf tips) and disk is a U.S. one-cent coin (19.0 mm; Photo by L.A. Condon, June 2019). (B) A replicate petri dish of *Syntrichia* with 10 shoot fragments. (C) A 40× magnification of a *Syntrichia* fragment with two new shoots (red arrows) emerging from leaf axils (B and C; Photos by W.D. Blankenship, October 2016).

concentrations. Guar is an off-white powder and is a polysaccharide derived from seed coats of *Cyamopsis tetragonoloba* (L.) Taubert. Psyllium is a brown powder and is a polysaccharide derived from seed coats of several species of *Plantago* L. PAM is a widely used synthetic polymer available as a crystal line powder. These tackifiers have been used somewhat interchangeably for hydromulching, seeding, and erosion control for decades, but vary in longevity (≤3 months for guar and ≤12 months for psyllium and PAM; CalTrans 2003) and other attributes (CalTrans 2003, 2017; Robichaud et al. 2010). We conducted chemical analyses of each dry tackifier using inductively coupled plasma optical emission spectrometry (Plant Nutrient Analysis, Central Analytical Laboratory, Oregon State University, Corvallis) to understand potential beneficial or detrimental effects each tackifier might have on mosses.

Experimental Design and Sampling

Growth Chamber, Treatments, and Replication. Experiments for each moss species were conducted and analyzed separately. The design included a control (distilled water only) and the three concentrations of each of the three tackifiers for each moss (3 concentrations × 3 tackifiers and a control). Experiments were conducted in a growth chamber (AR-41 L2 Arabidopsis Chamber, Percival Scientific, Perry, IA, U.S.A.) between October 2017 and October 2018. Chamber conditions cycled between 22 light hours (15°C, 70% relative humidity [RH], 75–160 µmol/m^2 s photosynthetic photon flux density [PPFD]) and 2 dark hours (10°C, 85% RH) similar to Jones and Rosentreter (2006). We elected to use this long day length because of the low PPFD in our growth chamber when compared to a typical saturation PPFD of >1,000 µmol/m^2 s (Coe et al. 2014), but opted against using constant light as recommended for achieving maximum moss growth rates (Duckett et al. 2004) because the absence of darkness is completely unnatural.

A shelf of the growth chamber was divided into five replicate blocks to account for positional variation in light and humidity. We randomly assigned treatments to locations within each block. Replicates (10 for *Bryum* and nine for *Syntrichia*) were divided equally between two separate time periods (except for *Syntrichia* with five and four replicates for each period). A replicate (experimental unit) consisted of a 90-mm-diameter by 15-mm-deep sterilized and uncovered petri dish filled with 50 g of dry, autoclaved sand (30 minutes at 1.1 kg/cm^2 and 124°C in autoclave bags; 0.3–0.8-mm and 0.3–0.8-mm diameter grain size for *Bryum* and *Syntrichia*) and 10 moss fragments on the sand. We obtained sand from a garden supply company that

Table 1. Recommended tackifier rates (kg/ha) mixed in water volumes (L/ha) for soil erosion control based on manufacturer-recommended rates and on CalTrans (2003, 2017) and the mixture amounts (g and mL) to achieve the same application rates in the growth chamber study.

	Field Application Rates				*Mixture Amounts Used in Study*			
Concentration	*Water Volume L/ha (US gal/acre)*	*Guar kg/ha (lbs/acre)*	*Psyllium kg/ha (lbs/acre)*	*PAM kg/ha (lbs/acre)*	*Water Volume mL*	*Guar g*	*Psyllium g*	*PAM g*
0.5×	9,462 (1,000)	28.38 (25)	56.75 (50)	2.25 (2.0)	500	1.5	3.0	0.125
1.0×	9,462 (1,000)	56.75 (50)	113.50 (100)	4.50 (4.0)	500	3.0	6.0	0.250
2.0×	9,462 (1,000)	113.50 (100)	227.00 (200)	9.00 (8.0)	500	6.0	12.0	0.500

was typical of commercially available sands for greenhouse and home use. Sands with different sized grains were used for each moss because *Bryum* fragments were easily buried in the *Syntrichia* sand. An additional set of replicates per treatment ran for 6 weeks without mosses to determine the mean contribution of combined tackifier and sand organic matter to total mass of organic matter in each dish (five replications with the sand for *Bryum* and only two replications with the sand for *Syntrichia* because of an insufficient supply of sand).

Moss fragments were extracted from dry, field-collected moss mats. We separated individual moss shoots (gametophytes) from mats under a dissecting microscope (5–40×) and sectioned shoots with fine forceps and a scalpel into individual fragments of equal lengths from the terminal, 1.5 mm for *Bryum* and 6.5 mm for *Syntrichia*, because maximum generative ability is suspected to reside in the green, terminal ends of mosses (Barker et al. 2005). Terminal fragments contained no visually apparent developing lateral buds or gametophyte shoots, sporangia, protonemata, gemmae, or rhizoids.

Tackifier Application. We mixed tackifiers with distilled water using a consensus of recommended rates (Table 1) obtained from governmental agencies (CalTrans 2003, 2017), local hydroseeding retailers, and tackifier manufacturers. All batches were prepared and maintained in a uniform mixture with a stirring plate during the set-up of the experiment. Additionally, PAM batches were agitated with a hand blender to break up their dry crystals in water and ensure even exposure of moss fragments to the tackifier. Each dish of sand was sprayed with distilled water until nearly saturated and the surface of the sand momentarily glistened. Moss fragments were collectively hydrated by spraying them with distilled water.

Ten hydrated moss fragments (subsamples within a replicate dish) were dipped into the assigned tackifier-concentration mixture with forceps and placed on top of the wet sand in a circle about 20 mm from the dish's outer edge (Fig. 1B). Between about 6 and 10 mL of the remaining tackifier-concentration mixture was poured over fragments so each fragment received between 0.6 and 1 mL. We used forceps to return any fragments buried in sand to the surface. The high viscosity of mixtures made it difficult to achieve exact application rates noted in Table 1, but since we mixed each concentration according the amounts in Table 1, our rates approximate the recommended field application rates.

Dishes prepared without mosses to determine organic matter mass contributions of tackifiers and sand were prepared as described above, except the 10 mL of tackifier-concentration mixture for each replicate was poured evenly over the center of the dish instead of in a ring at the edge of the dish to ensure that sampling at the end of the experiment would only collect sand exposed to tackifier.

Watering Frequency. Treatments received distilled water multiple times per day beginning the day after dishes were placed in the growth chamber. *Bryum* and *Syntrichia* dishes were watered two and four times daily to maintain constantly hydrated moss fragments. Dishes without moss (used in the sand and tackifier organic matter mass adjustment) were watered twice daily. Watering was done manually with a spray bottle until sand was just below field capacity (droplets of water stood on the surface of the sand but infiltrated into the sand). When fungal incursions were observed in dishes, the sand and fungi in affected areas were removed with a microspatula. Fungi did not appear to be associated with a particular treatment. In a few rare occurrences when fungi appeared to impact fragments, the fragment was also removed, and their absence was accounted for in the subsampling data analysis.

Data Collection

Data were collected at the end of each study period (5 weeks for *Bryum* and 6 weeks for *Syntrichia*). Each fragment was viewed from above with a dissecting microscope at up to 40× magnification and with a probe. Both species can produce rhizoids which are nonphotosynthetic, filamentous appendages that anchor mosses to soils and can help absorb water (Schofield 1981; Glime 2017). They can also produce protonemata, which are a juvenile stage of thread-like strands of cells (often green) that extend from the central axis and expand the footprint of the moss gametophyte. Gemmae are moss asexual propagules that can become new gametophytes.

Fragments were examined to ensure they did not have preexisting lateral shoots, gemmae, protonemata, or rhizoids before they were used in the experiment. For both species, we determined number of shoots, bound sand mass, and moss organic matter mass. In addition, we determined gemma, and protonema presence for *Bryum* and total shoot length for *Syntrichia*.

Total Shoot Length and Number of Shoots. The length of each shoot on every *Syntrichia* fragment was measured in millimeters and summed for a dish total (Fig. 1C). The minute stature of *Bryum* precluded it from being quantified this way.

The number of shoots was counted for every fragment and summed by dish for both species.

Gemma and Protonema Presence in *Bryum*. When gemmae were observed extending from a fragment, they were considered present for that fragment. Protonemata were considered meaningfully present if there were more than five extending from a fragment and into the sand. Presence observations were counted separately per fragment and summed within each dish for a maximum count of 10 for each variable.

Bound Sand Mass and Moss Organic Matter Mass. After collecting the above-mentioned data, fragments were grasped individually with fine forceps and pulled gently from their dish (perpendicular to the surface) with attached sand. They were pooled by dish into a small, furnace-dried (150°C), preweighed foil packet (*Bryum*) or vial (*Syntrichia*). Any rhizoids or protonemata remaining in dishes were removed along with their attached sand and added to pooled samples.

Packets and vials were oven dried at 65°C for 48 hours, allowed to cool in a desiccator, and weighed to determine dry mass of their contents (*Bryum*, M2P, Sartorius AG balance; *Syntrichia* R300S Sartorius AG balance, Goettingen, Germany). Packets and vials were then transferred to a furnace and maintained at 500°C for 10 hours before being cooled in a desiccator and weighed to determine their change in mass following combustion of organic materials. The remaining contents were sand and some quantity of ash unadjusted for organic matter from sands and tackifiers.

We obtained moss organic matter estimates by removing the sand and tackifier organic matter contribution from the total organic matter estimate (moss + tackifier + sand) via multiplying a sand and tackifier organic matter adjustment ratio to the total mass of sand, tackifier, and moss before burning to gain an estimate of the proportion that each tackifier and sand combination made to total organic matter contribution. This product was subtracted from the total organic matter estimate to yield moss organic matter content.

This tackifier and sand organic matter ratio was determined by applying the same tackifier concentrations to the same sand types used in the moss growth study (*Bryum* sand $n = 5$; *Syntrichia* sand $n = 2$ because of the limited amount of the original *Syntrichia* sand). We used an inverted 10-mL graduated pipette to collect eight equal-sized columns of sand and tackifier (surface to bottom of the dish) from each dish and pooled these samples by dish into foil packets. Using the same furnace procedures described above, a ratio of total organic matter of tackifier and sand to the total mass of tackifier and sand before burning away organics was created and used as the moss organic matter adjustment.

Having only two replicates of *Syntrichia* sand led to uncertainty in generating average organic matter adjustment ratios because of high variability in this sand's organic matter. To manage this shortcoming, we first plotted means and individual replicate points for the *Bryum* sand's ratios to obtain the expected shape of the ratio response to increases in each tackifier's concentrations. We did the same for the *Syntrichia* ratios. These initial sets of curves were similar in shape and were separated by the expected difference in organic matter that came in the two sand types with a few exceptions. The lack of replicates and high variability in *Syntrichia* sand created some anomalies in these means for the three concentrations of PAM and for the Psyllium1.0× concentration which had extremely low or high adjustments. We elected to remove these anomalous samples and to use a linear regression of the remaining ratio points against their respective tackifier concentrations and to equate the zero concentration points to the ratios of the water controls for the *Syntrichia* sand. The organic matter adjustment ratios for *Syntrichia* sand was the average of the two replicates for all tackifiers and concentrations except for Psyllium1.0× and the three concentrations for PAM where we used the regression's estimated ratio (Fig. S1, Supporting Information).

Sand and Tackifier Chemistry. Since we detected differences in moss species growth with the different tackifiers, we conducted post hoc chemical analyses of raw tackifiers and of sand type by tackifier type and concentration to elucidate potential nutrient or toxicant effects these combinations might have on moss growth. We used the original type of sand used to test *Bryum* growth. Since we had a limited amount of the original sand for *Syntrichia*, we tested the original sand without tackifiers as a control (original sand). We obtained a similar sand based on texture and color that came from a different delivery to our greenhouse for testing tackifier types and concentrations plus an additional control (new sand control) to compare to our original sand. Sands were autoclaved as before, then we mixed 3 replicates ($n = 3$) of 20 mL of each tackifier type and concentration with 100 g of each sand type. All controls had 20 mL of distilled water without tackifiers mixed with the sand. Soil pH was measured using 1:1 ratio of water to sand plus tackifier, while cation exchange capacity was calculated from cation extraction. Nitrate nitrogen was extracted using KCl techniques and measured using a Lachet. Other chemicals were extracted using Mehlich 3 and quantified using ICP-OES as above (Producer's Soil Nutrient Analysis, Central Analytical Laboratory, Oregon State University, Corvallis).

Data Analysis

All analyses were done with R version 3.4.3 (R Core Team 2017) using the subsample means for each variable. Shoot length, number of shoots, and masses of moss organic matter and bound sand were analyzed with a linear mixed model (LMM), using block as a random effect and the nine tackifier-concentration combinations and the water control as a fixed effect with the nlme package (version 3.1.131; Pinheiro et al. 2017). A single, combined variable (tackifier type and concentration) was used instead of two factors and their interaction because the control group did not have varying concentrations.

Model assumptions of constant variance and normality of errors were checked graphically with residual plots. When residuals were right-skewed with nonconstant variance, a natural logarithmic transformation of data was attempted. When the

assumption of constant variance was not met, variances could vary by tackifier type or treatment. When a natural logarithmic transformation was required, comparisons among treatment groups were back-transformed and reported as ratios of medians.

Proportions of gemma and protonema presence were analyzed using a binomial generalized linear mixed model (GLMM) with a logit link with the lme4 package (Bates et al. 2015), using the same fixed and random variables as the LMM. The GLMM was checked for overdispersion. If overdispersion was present, an observation-level random effect was added to the model. Checks of model fit were done using simulated residuals with the DHARMa package (version 0.2.0; Hartig 2018). Comparisons from the GLMMs are reported as odds ratios. Estimated values and 95% confidence limits of each variable are reported for every tackifier-concentration combination of *Bryum* and *Syntrichia*.

Tests for differences in each variable among treatment means, medians, or odds were done with *F* tests (LMM) and drop-in-deviance chi-square tests (GLMM). The final model for each variable was used to compare the effect of each tackifier-concentration combination with that of water (all vs. control), the effect of concentration within each tackifier type, and the mean effect of each tackifier (the mean value of a variable for all concentrations of a tackifier compared across tackifiers). All comparisons were done using package emmeans (version 1.2.4; Lenth 2018).

Tackifier and sand combined with tackifier chemicals were graphed as means (±95% confidence interval [CI] using Student's *t* score) and were compared for overlapping CI and means. No official test of covariance with moss growth was conducted since these measures were obtained post hoc to the moss growth and the sand medium source for *Syntrichia* sand was known to differ between these two observations. We used these data to provide insight into potential correlations between moss growth and tackifier or sand chemistry.

Results

Number of shoots, bound sand mass, and moss organic matter mass for *Bryum argenteum* ($F[9, 81] \geq 4.9$, $p < 0.001$) and the same variables plus total shoot length for *Syntrichia ruralis* ($F[9, 72] \geq 11.1$, $p < 0.001$) differed among treatments for all three proposed questions on moss growth. The odds of gemma presence per fragment and protonema presence per fragment for *Bryum* (drop-in-deviance $\chi^2[9] \geq 63.6$, $p < 0.001$) differed among treatments for all three questions about moss growth within tackifiers. See Supporting Information Tables S1 and S2 (Supporting Information) for all estimates of differences in central tendency, confidence limits, and probabilities of tackifier and concentration comparisons related to the three questions.

Growth Response Between Tackifier Type — Concentration Versus Water

Bryum fragments grown in psyllium had greater growth than fragments grown in water regardless of the concentration except for protonema. For *Syntrichia,* only the 2× concentration of psyllium had greater growth (Figs. 2 & 3). One exception to this occurred in *Syntrichia* sand mass where fragments grown in higher concentrations of psyllium held less sand mass than fragments grown in water (Fig. 3C). In contrast, growth of fragments in water exceeded those grown in Guar1.0× or Guar2.0× for organic matter mass, protonema, and gemma of *Bryum* and for shoot length, number of shoots, and organic matter mass of *Syntrichia* (Figs. 2C–E, 3A, 3B, & 3D). *Bryum* fragments grown in PAM had more bound sand mass (Fig. 2B) and higher gemma presence (Fig. 2E) than fragments grown in water, while *Syntrichia* fragments grown in PAM1.0× and PAM2.0× had less moss organic matter than fragments grown in water (Fig. 3D). Otherwise, fragments grown in PAM did not vary appreciably from fragments grown in water.

Growth Response With Varying Concentrations Within Each Tackifier

Tackifier concentration, regardless of tackifier type, did not affect the number of shoots or moss organic mass for *Bryum* (Figs. 2A & 2C; Q2—Table S1). Regardless of species, fragment growth in the lowest concentration of guar exceeded growth in the higher concentrations on all growth variables except for number of shoots on *Bryum* (Figs. 2 & 3; Q2—Tables S1 & S2). In contrast, the highest concentration of psyllium enhanced *Syntrichia* growth for all variables over growth in the lowest concentration, except for sand mass where fragments grown in the lowest concentration exceeded those grown in higher concentrations (Fig. 3; Q2—Table S2). Different concentrations of PAM did not lead to appreciable differences in fragment growth, except in *Bryum* protonema presence where growth in the lowest concentration was greater than the highest (Fig. 2D).

Growth Responses Among Tackifiers

The mean growth of fragments grown in psyllium exceeded those grown in guar in all variables for the two species ($p < 0.001$; Q3—Tables S1 & S2). Mean growth of fragments in PAM also exceeded those grown in guar in all cases ($p < 0.017$), except for *Bryum* number of shoots ($p = 0.089$) and *Syntrichia* moss organic matter mass ($p = 0.428$) where there were no appreciable differences between these tackifiers.

Except for *Bryum* protonema presence (where there was not an appreciable difference), the mean growth of *Bryum* fragments in psyllium exceeded growth of *Bryum* fragments in PAM in all cases ($p < 0.007$; Q3—Table S1). The mean number of shoots and moss organic matter mass of *Syntrichia* fragments grown in psyllium exceeded their growth in PAM ($p < 0.038$), but the opposite was true for sand mass ($p = 0.019$; Q3—Table S2).

Sand and Tackifier Chemistry

Tackifiers are dominated by C, N, P, and K, but differed in levels of each (Fig. 4). PAM differed from the other two in its high levels of K, lack of P, and had the lowest levels of

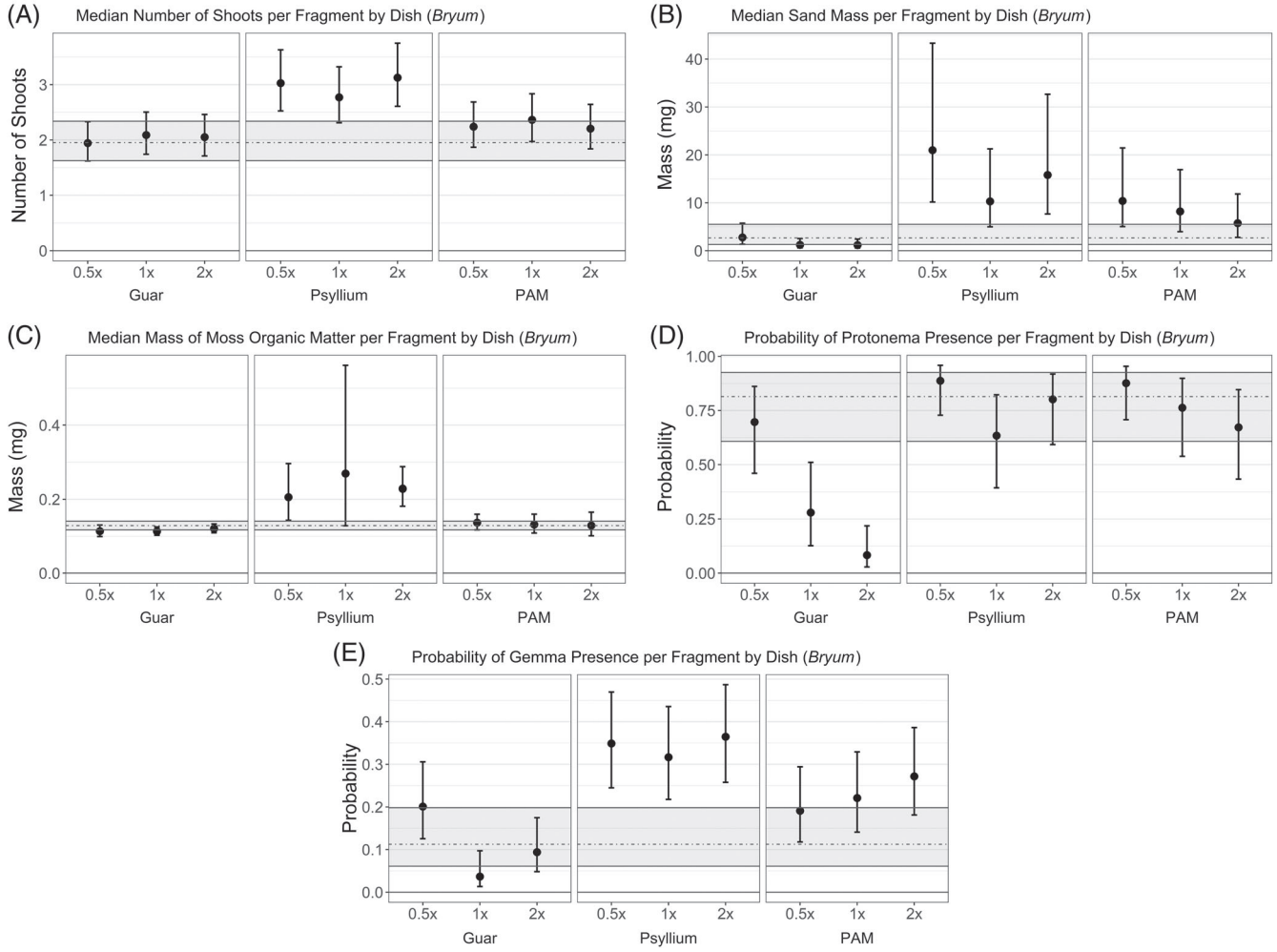


Figure 2. *Bryum* medians for each growth variable (A–E; odds for protonema presence and gemma presence) relative to tackifier (guar, psyllium, or PAM) and concentration (0.5×, 1.0×, and 2.0×) and to water (control). Horizontal dotted line depicts estimated value for the control and gray shading represents its ±95% CI. Error bars are ±95% CI.

other chemicals. When tackifiers were mixed with water to their recommended concentrations (Table 1), all chemical levels in psyllium and guar dominated over PAM except for K where psyllium dominated, but guar and PAM were similar due to their mass ratios of 12:1.

The different sands used for each moss species were chemically similar in pH (7.3), and most common major nutrients like C (0.01–0.3%), N (undetectable) and K (50–70 µg/g) (horizontal line on Figs. 5 & 6). Differences, when they occurred, were always higher in the *Syntrichia* sand (P 95 vs. 10 µg/g horizontal line Fig. 6), Zn (2.9 vs. 0.4 µg/g) and Fe (95 vs. 27 µg/g). Although we had limited amounts of the original lot of *Syntrichia* sand that was used in the moss growth experiments, the new lot of *Syntrichia* sand was within 95% CI of the original sand for all chemicals (Figs. 5 & 6). Relationships between tackifier concentration and chemistry were most consistent with psyllium. Both P and K increased from the lower to the higher concentration on both sand types, whereas pH had an opposite response, making the sands slightly more acid (Figs. 5 & 6). Guar had a similar effect to psyllium with pH decreasing as concentration increased. Chemical contents did not increase with PAM concentration across tested chemicals. None of the potential toxic elements (Zn, Fe, Cu, and S) were found to change with changing concentrations of any tackifier.

Discussion

This is the first study to our knowledge to examine moss growth when exposed to differing tackifier types and concentrations. Tackifiers varied in their effect on moss growth in this study. When compared to water, guar generally decreased growth, psyllium generally increased growth, and PAM was generally neutral for growth of *Bryum argenteum* and *Syntrichia ruralis* fragments. Within tackifier types, increasing concentrations of guar tended to decrease moss growth, while increasing concentrations of psyllium tended to increase growth and changes in PAM concentrations had little impact on moss growth.

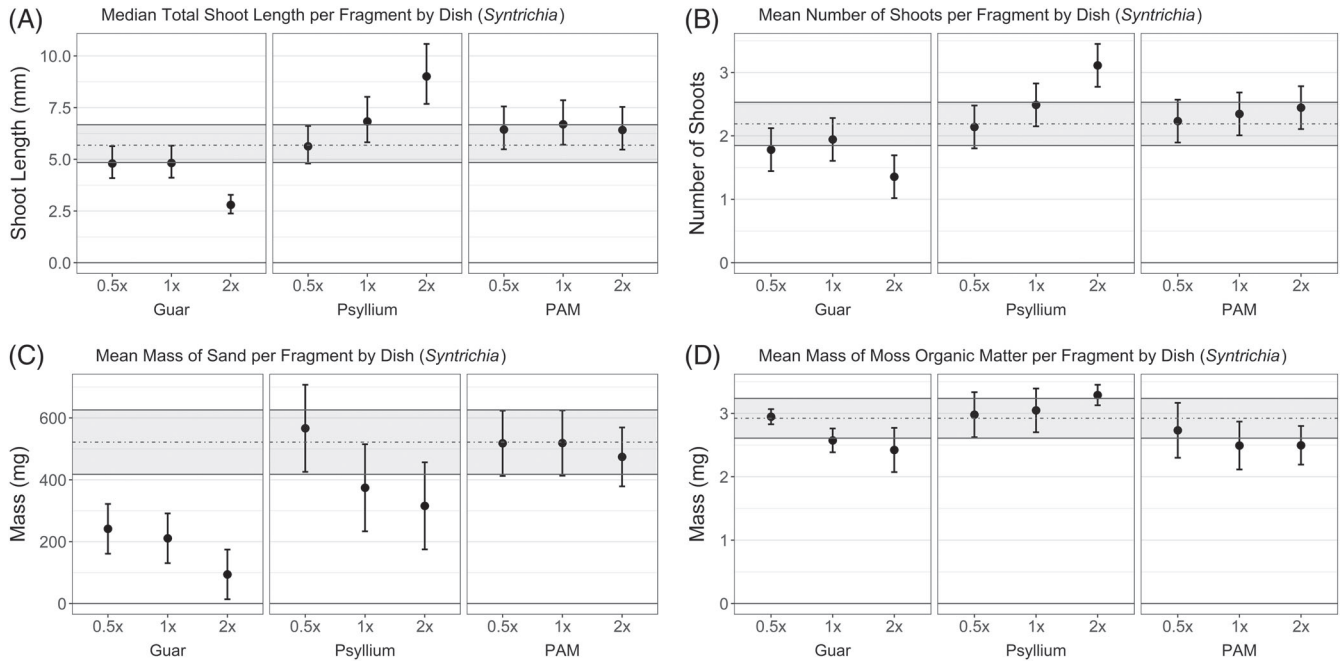


Figure 3. *Syntrichia* estimates of means for each growth variable (A–D; medians for total shoot length) relative to tackifier (guar, psyllium or PAM) and concentration (0.5×, 1.0×, and 2.0×) and to water (control). Horizontal dotted line depicts estimated value for the control treatment and gray shading represents its ±95% CI. Error bars are ±95% CI.

Growth chamber studies are excellent for limiting environmental variation so that we can manipulate other factors to examine their impact on growth, but they also have caveats to understand. For example, we kept moss fragments continuously hydrated and used a 22:2 hours light:dark photoperiod due to our chamber's low PPFD. Dryland mosses grown in their environments typically experience daily fluctuations of moisture and sunlight, resulting in repeated cycles of hydration and desiccation which we did not examine. Moreover, ecotypes have been shown to exist in these moss species (Condon & Pyke 2016) and might lead to additional variation in results.

Changes in moss growth observed in this experiment may be a dose response to the amount or composition of tackifier or both. Though all three tackifiers have been used in hydroseeding of vascular plants, it is possible that their varying physical or chemical properties (CalTrans 2003, 2017) interact with the unique characteristics of bryophytes. Our chemical tests of tackifiers, alone and with sands, provide some potential explanation for growth differences we detected with the differing tackifiers and their concentrations. Both guar and psyllium tended to acidify these sands slightly with increasing concentrations. Guar at low concentrations was more alkaline trending toward neutral at higher concentrations. *Bryum* tends to favor acidic soils in Australia (Eldridge & Tozer 1997) and may provide a partial explanation for poor moss growth with guar.

Chemicals known to be toxic to mosses when in compounds with other chemicals (Zn, Cu, Fe, and S; Oregon State University: http://bryophytes.science.oregonstate.edu/page18.htm [accessed 1 May 2019]) either were undetectable or did not change with increasing levels of tackifiers. Nutrient limitations may be a potential cause for growth differences. Other growth chamber experiments with mosses have typically incorporated nutrients, generally in the form of a standardized solution of nitrogen, phosphorus, and potassium (Duckett et al. 2004; Jones & Rosentreter 2006; Xu et al. 2008). Our decision to not include nutrients may have inadvertently created nutrient-limited environments, where slight differences in tackifier composition and concentration may have provided gradients of increasing nutrient abundance corresponding with increased growth as we saw with psyllium. No tackifier provided measurable levels of N, but psyllium did increase both P and K over levels found in the sand and increased with increasing concentrations.

PAM is resistant to microbial degradation (Seybold 1994), which may explain its tendency of yielding neutral growth responses. We did detect high levels of K in PAM, but these did not consistently increase with increasing concentrations (exception was in the *Bryum* sand). Finally, previous experiments with the same moss species yielded increased growth when associated with jute net (Condon & Pyke 2016) and biological soil crusts in general have benefited from jute nets (Bowker et al. 2020), which likely create a microhabitat more conducive for moss growth, buffering fragments from extreme fluctuations of temperature and moisture in the field. The seed husks of psyllium might have acted like jute net for promoting moss growth. More detailed physiological studies may be required to ascertain direct causes.

Other biological soil crusts studies have tested growth with only low concentrations of PAM. Like our results, cyanobacterial crust growth was neutral to positive when associated with PAM in dryland restoration studies (Park et al. 2017;

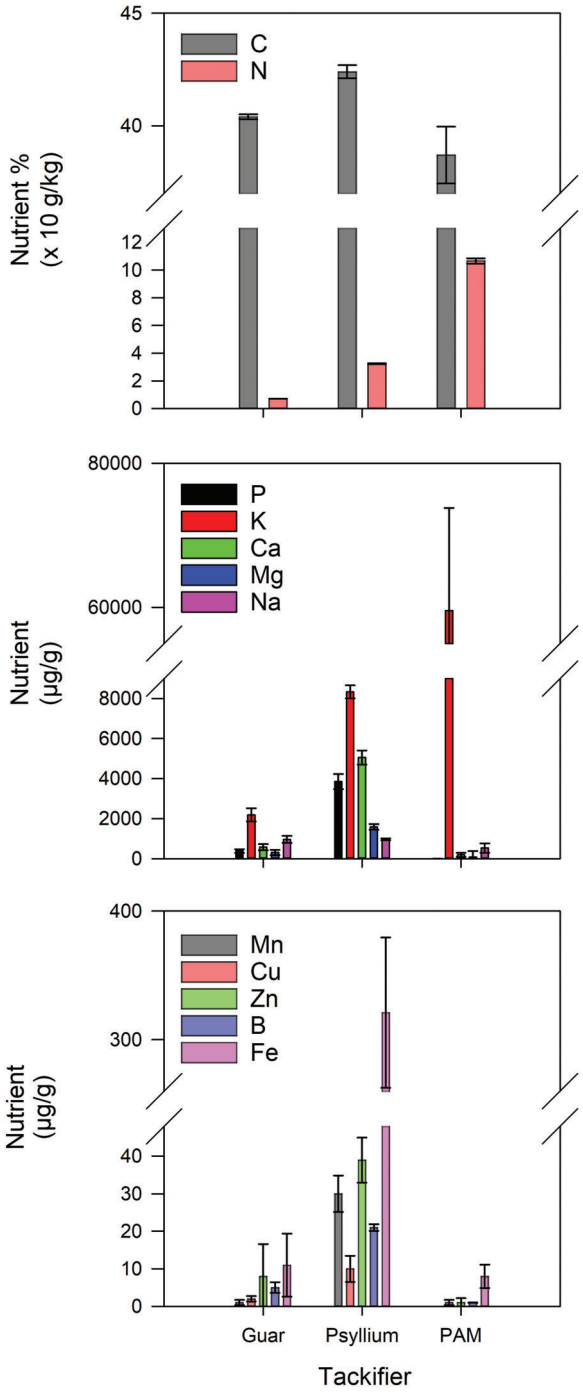


Figure 4. Chemical analysis for equal masses of each of three tackifiers, guar, psyllium, and PAM. Error bars are ±95% CI.

Chandler et al. 2019). Neutral growth responses of biocrusts to tackifiers may still have a positive effect on restoration success if the tackifier is adhering biocrust propagules to the soil, preventing secondary fragment dispersal and encouraging establishment and growth, while also enhancing soil aggregate stability (Eldridge 2001). This is especially important for soil stabilization in locations prone to wind or water erosion, such as recently burned areas (Robichaud et al. 2010). Once established, mosses may extend rhizoids and protonemata, as we saw in *Bryum* and *Syntrichia,* and bind with additional soil, which we saw with psyllium, there by defending against raindrop–soil impacts as they extend moss mats.

Soil stabilization is a common benefit of biocrusts (Belnap et al. 2001) and is exemplified in our measurements of sand mass where control treatments of tiny moss fragments became bound to quantities of sand that were tens to hundreds of times greater than their own mass. Cyanobacteria are commonly viewed as another soil stabilizing agent that often is a precursor to mosses in some environments. Tackifiers in this respect may also act as a soil stabilizing agent, thus mimicking cyanobacteria and stabilizing soil for moss establishment. In our attempt to quantify moss and tackifier adherence to soils, we examined both the moss organic matter mass and the mass of sand particles that adhered to the moss fragments and found these parameters also varied among tackifiers and their concentrations. Most mass responses mirrored the observed aboveground growth responses to psyllium and PAM, except for *Syntrichia* sand mass which, when grown in higher psyllium concentrations, yielded fragments bound with less sand than fragments in the water treatment. This negative effect on *Syntrichia* was substantial enough that sand mass bound to fragments in PAM treatments exceeded the sand mass bound to fragments grown in psyllium treatments. The moss organic matter mass for *Syntrichia* fragments grown in psyllium had an opposite trajectory with concentration, which could reflect differences in the efficacy of tackifier types for binding moss fragments to substrate or variable impacts of tackifiers on other belowground attributes of mosses which we did not measure. This may be due to its greater molecular weight providing greater capacity to hold coarse soil textures than lighter molecular weight tackifiers like guar and psyllium (Graber et al. 2006).

Tackifier molecular weights also contribute to water adsorption and infiltration (Graber et al. 2006) in field applications and may assist with moss hydration and seed germination. Since our watering regime maintained near field capacity conditions and the growth chamber maintained high humidity (>80%), we do not believe water was a limiting resource in our study. For future field studies, however, water availability will likely be important and the type of tackifier, its concentration, and the texture of the soil may influence not just moss growth, but germination of vascular plant seeds. Seeds may include those intentionally seeded as part of a hydroseeding mixture and residual seeds in the seed bank. The seed bank may include invasive plants that may also benefit from the application of a tackifier. This aspect also warrants further consideration in field studies.

Our study aimed to inform future field studies on the restoration of mosses and other biocrusts. As jute (Condon & Pyke 2016; Bowker et al. 2020) and straw checkerboard (Li et al. 2003; Li et al. 2014) have facilitated biocrust establishment in field studies, it is timely to test tackifiers as a delivery and establishment agent for moss and other biocrust species. We envision that moss fragments might not come solely from terminal ends, as we used, but that whole dry moss fragments could be shredded or crushed from mats and then mixed with tackifiers,

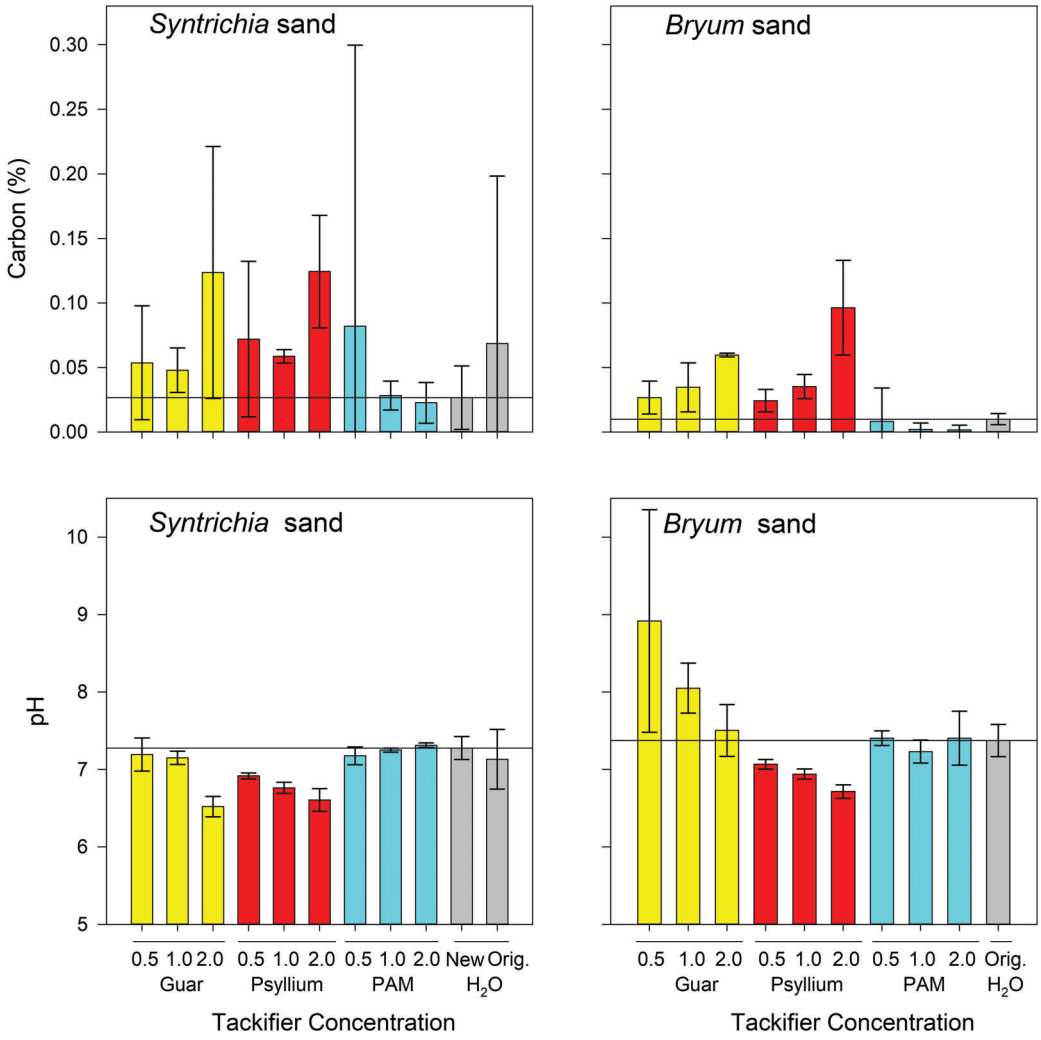


Figure 5. Percent C and pH for sand type (*Syntrichia* and *Bryum*) and tackifiers (guar, psyllium, and PAM) at applied concentrations (0.5×, 1.0×, and 2.0×, where × is the recommended concentration for soil erosion control on flat land). Horizontal line equals water control level. Error bars are ±95% CI.

vascular plant seeds, and mulch. This mixture could be applied with current hydroseeding methods thus providing a mechanism for biocrust restoration that is both more efficient than jute nets, is commercially available with application technologies, and can be extended to larger spatial scales.

Of the three tackifiers that we tested, psyllium and PAM were the most consistent in being neutral or positive to moss growth, had the least negative effects, and would likely be the best types to use in field studies. Hydroseeding methods often include straw and we believe this may aid to create microrelief and an additional carbon source for soil microbes involved in decomposition and slow release of nutrients which may reduce annual grasses such as cheatgrass (Paschke et al. 2000). We believe future investigation may wish to examine a range of concentrations in field studies, since soil textures and other environmental factors may interact with tackifier types and concentrations affecting moss growth.

We used fragment tips as the source material, but this is not likely to be practical as projects begin to scale up to small plots (m^2) and management level restorations (ha). Future studies examining grinding or shredding moss for restoration source material may be useful. *Bryum* and *Syntrichia* responded similarly to our tackifier treatments, despite their differences in stature and life history. *Ceratadon purpureus* and *Syntrichia caninervis* are additional dryland mosses with potential for restoration and may be good candidates for future studies with tackifiers (Jones & Rosentreter 2006; Antoninka et al. 2016).

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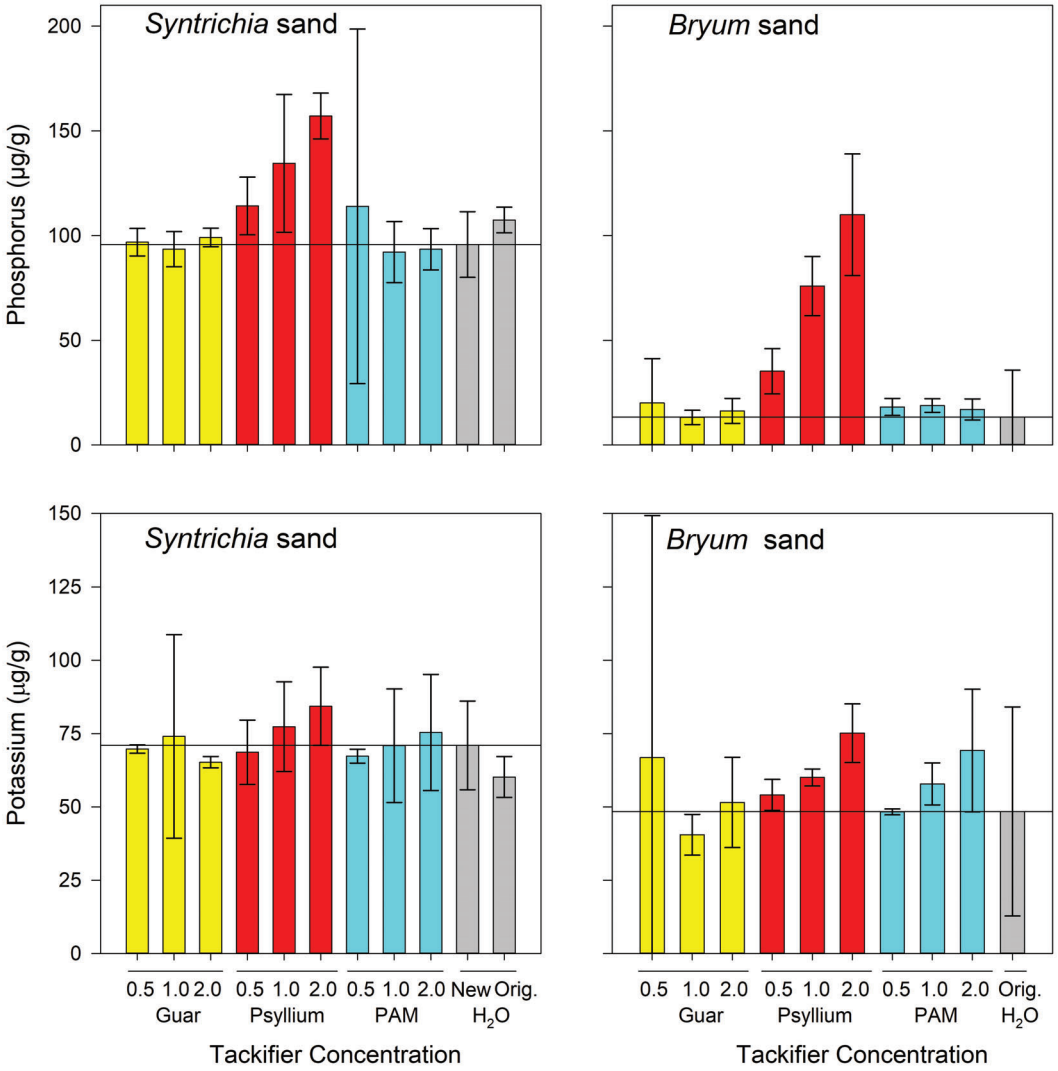


Figure 6. Phosphorus and potassium for sand type (*Syntrichia* and *Bryum*) and tackifiers (guar, psyllium, and PAM) at applied concentrations (0.5×, 1.0×, and 2.0×, where × is the recommended concentration for soil erosion control on flat land). Horizontal line equals water control level. Error bars are ±95% CI.

for critical review of an earlier version of the paper. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government or Oregon State University. Data and metadata are available through https//:doi.org/10.5066/F7D50KWR.

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Supporting Information

The following information may be found in the online version of this article:

Table S1. Comparisons of *Bryum argenteum* median estimated differences between treatment pairs.

Table S2. Comparisons of *Syntrichia ruralis* median estimated differences between treatment pairs.

Figure S1. Plot of average adjustment ratios used to calculate the moss organic matter mass of fragments from *Bryum argenteum* and *Syntrichia ruralis* dishes.

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