AN ABSTRACT OF THE THESIS OF

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Abstract approved:

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There is growing interest in using biocrusts (assemblages of cyanobacteria, lichens, mosses, and other taxa in various proportions covering the upper few millimeters of the soil surface) to assist in restoring ecosystem function and native plant communities in dryland ecosystems. Biocrusts can be transplanted and established using jute or thatch, but these methods are difficult to expand for restoration at a landscape scale. Tackifiers are organic or synthetic long-chain carbon compounds used for soil stabilization and hydroseeding and could provide a more scalable option for biocrust restoration. We examined the sensitivity of two dryland mosses, *Bryum argenteum* and *Syntrichia ruralis*, to three common tackifiers - guar, psyllium, and polyacrylamide (PAM) - at 0.5x, 1x, and 2x of recommended concentrations for erosion control and revegetation. We measured moss shoot, gemma, and protonema production as well as moss organic matter and bound substrate masses as indicators of growth. Groups of ten fragments from field-collected mosses were grown on sand in petri dishes arranged in a growth chamber in replicated blocks containing each tackifier-concentration treatment. Ten replications of *Bryum* and nine replications of *Syntrichia* were measured at the end of six and five weeks, respectively. The growth responses of fragments in each tackifier-concentration combination were compared

with those of a control treatment (fragments grown on sand with distilled water) as well as by concentration within tackifier type and by tackifier type. Overall model tests yielded statistically significant results ($p<0.001$) for every variable in both 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 moss growth, while increasing concentrations of psyllium tended to increase growth. Varying concentrations of PAM had little effect on growth. Further research should examine impacts of this suite of tackifiers on moss growth and biocrust establishment in the field.

Tackifier Type and Concentration have Varying Impacts on Growth of Dryland Mosses

by
Wyatt Dillon Blankenship

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Wyatt Dillon Blankenship, Author

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CONTRIBUTION OF AUTHORS

Drs. David Pyke and Lea Condon assisted with project conception, study design, data interpretation and editing of the manuscript. Ariel Muldoon assisted with study design and statistical analysis.

TABLE OF CONTENTS

LIST OF FIGURES

Figure Page

LIST OF TABLES

Table Page

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 (Knutson et al. 2014). Dryland restoration to date largely focuses on reintroducing vascular plants, with the hope that other missing biotic components will re-establish on their own over time. Dryland restoration projects have rarely incorporated biocrusts, which include cyanobacteria, lichens, and mosses that often fill interspaces between vascular plants in the upper few millimeters of the soil surface and are present to some degree in most terrestrial environments (Bowker 2007). Biocrusts can represent over half of all surface cover and may promote soil stability and water infiltration and increase nutrient cycling in drylands (Belnap et al. 2001). Mosses, in particular, have demonstrated their potential for dryland restoration through their desiccation tolerance, ease of propagation, and rapid growth rates (Xu et al. 2008; Condon & Pyke 2016; Antoninka et al. 2016).

The physiology of mosses 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 are also phenotypically plastic and can propagate vegetatively. 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 towards re-establishing 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 runoff and wind. In previous efforts, jute net has been used to create a boundary layer to reduce secondary dispersal and facilitate establishment (Condon & Pyke 2016), but this method is not practical for large-scale restoration projects. Tackifiers are used in hydromulching and seeding for post-fire rehabilitation and hillslope stabilization

(Robichaud et al. 2010) and may be a viable method for distributing and adhering moss fragments to the soil at a scale relevant for landscape restoration. A few studies have incorporated a tackifier treatment into investigations of biocrust outplanting and cultivation (Park et al. 2017a; Park et al. 2017b; Chandler et al. 2018), 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, *Bryum argenteum* Hedw. and *Syntrichia ruralis* (Hedw.) F. Web. & D. Mohr (hereafter referred to by the genus name), to three common tackifiers (guar, psyllium, and polyacrylamide) using a range of concentrations applied for erosion control and revegetation. We measured moss shoot, gemma, and protonema production as well as moss organic matter mass and substrate mass bound to mosses to address the following questions:

1. To what extent do structures (shoots, gemmae and protonemata) in *Syntrichia* and *Bryum* grown with tackifiers differ from structures in *Syntrichia* and *Bryum* grown without tackifiers? (All tackifier types and concentration combinations *vs*. water)
2. Do different concentrations of a given tackifier impact growth or development of structures (shoots, gemmae and protonemata) in *Syntrichia* or *Bryum*? (Each concentration compared within each tackifier)
3. Does the type of tackifier used impact the growth or development of structures (shoots, gemmae and protonemata) in *Syntrichia* or *Bryum*? (Mean of structure response pooled across concentrations within a tackifier compared among tackifiers)

METHODS

Moss Species and Source

Small mats of dry *Bryum argenteum* and *Syntrichia ruralis* (syn: *Tortula ruralis*) were collected from a public park in Bend, Oregon in July 2016. Each mat consisted of hundreds of fragments

of the targeted species. The species' identities were verified in the lab. Specimens were stored in brown paper bags at room temperature until they were used in experiments.

Bryum and *Syntrichia* represent different life history strategies. *Bryum* is an early successional, generalist species found in open areas. It is an acrocarp of short stature (0.5 – 1.5 cm stem height) found in dense cushions. *Syntrichia* is a later successional species with stems greater than 1 cm, often arranged in loose cushions. *Syntrichia* is widely accepted as a predominately ectohydric species (meaning it readily absorbs water from the plant surface), while *Bryum* can demonstrate a more endohydric habit from our observations (more slowly taking up water from its surface and conducting water via a central strand). Both species have cosmopolitan distributions and are common in the semi-arid sagebrush steppe of North America (Flowers 1973) .

Tackifiers

Treatments consisted of three common tackifiers – guar gum (99.89% purity from The Dirty Gardener, Tacoma, WA), psyllium (M-Binder®), and polyacrylamide (PAM HT®) - applied in three concentrations - 0.5x, 1x, and 2x - of their manufacturers' recommendation (x) for soil stabilization on level sites. Concentrations greater than 1x are recommended for hillslope applications and a 0.5x concentration was included in this experiment to examine moss reactions to a broader spectrum of treatments. Guar is sold as an off-white powder and is a polysaccharide derived from seed coats of the guar plant, *Cyamopsis tetragonoloba* L. Psyllium comes as a brown powder and is a polysaccharide derived from seed coats of several species in the plant genus *Plantago*. Polyacrylamide (PAM) is a widely used synthetic polymer which is sold in the form of superabsorbent crystals. The three tackifiers have been used somewhat interchangeably for hydromulching, seeding, and erosion control operations for decades, but have been shown to vary in longevity and other attributes once applied (CalTrans 2003; McLaughlin & Brown 2006; Sojka et al. 2007; Robichaud et al. 2010; CalTrans 2017; Polizzi 2018). All are mixed in water for application.

Experiment Set-up

Fragments and Dishes

Each moss species was tested separately. A replicate (experimental unit) consisted of a dish with multiple moss fragments as described below. *Bryum* had ten replicates per treatment grown on sand (0.3-0.8 mm diameter) for six weeks. *Syntrichia* had nine replicates per treatment grown on sand (0.3-2 mm diameter) for five weeks. An additional seven replicates per treatment ran for six weeks without mosses to determine the organic matter contributions of tackifier and organic matter in the sand to each dish's total mass of organic matter (five replications with the sand used in *Bryum* dishes, but only two replications with the sand used in *Syntrichia* dishes because of an insufficient supply of the latter). The experimental design included a control (distilled water only) treatment and the three concentrations of each of the three tackifiers for each moss (3 concentrations x 3 tackifiers and a control = 10 treatment levels per species). Experiments ran between October 2017 and October 2018 and were divided into two runs of five replicates (four replicates for one run of *Syntrichia*) because of size constraints of the growth chamber.

Moss fragments were extracted from dry, field-collected moss mats. Individual moss shoots were separated from mats under a dissecting microscope (5x to 40x magnification) and sectioned with fine forceps and a scalpel into individual fragments of equal size. Fragments were collected from the terminal 1.5 mm of *Bryum* and 6.5 mm of *Syntrichia* because maximum vitality resides in the green, terminal ends of mosses (Barker et al. 2005).

Both species can produce rhizoids which are non-photosynthetic, 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 asexual propagules produced by the moss that can become new gametophytes. Fragments were examined to ensure they did not have pre-existing lateral shoots, gemmae, protonemata, or rhizoids before they were used in the experiment.

Each replicate consisted of a 90-mm diameter x 15-mm deep petri dish bottom washed with soapy water and bleach and filled with 50 g of dry, autoclaved sand (30 mins at 1.1 kg/cm² and 124°C in autoclave bags). A shelf of the growth chamber was divided into five replicate blocks to account for positional variation in light and humidity. Each treatment was randomly assigned to a location within a block.

Application of Tackifiers

Tackifiers were mixed according to their recommended concentration for hydroseeding per 1,000 gallons of water/acre. Where manufacturer's recommendations were unavailable, we used a consensus of recommendations obtained from governmental agencies, hydroseeding retailers, and manufacturers of comparable products. We converted units to grams per liter and made small batches of 0.5x, 1x, and 2x of the recommended concentrations in 500 mL of distilled water (Table 1).

Table 1: The mass of dry tackifier used to make each tackifier-concentration mixture and the field application recommendations for 1x concentrations (recommended concentration for erosion control on flat ground).

		TACKIFIER TYPE		
	Concentration	**Guar**	**Psyllium**	**PAM**
In 1,000gal Water	1x	*50lbs*	*100lbs*	*4lbs*
In 500mL Water	**0.5x**	1.5g	3g	0.125g
	1x	3g	6g	0.25g
	2x	6g	12g	0.5g

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 glistened. Moss fragments were collectively hydrated by spraying them with distilled water immediately after saturating the sand. 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. Approximately 10 mL of the same tackifier-concentration mixture was carefully poured over the fragments in the dish so each fragment received about 1 mL. Any fragments buried in sand by this process were returned to the sand's surface using forceps.

Dishes prepared without mosses for the run to determine organic matter mass contributions of tackifier and organic matter in the sand were prepared the same 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.

Growth Chamber Settings and Watering Frequency

Dishes without lids were placed into a growth chamber (Percival® AR-41L2). The growth chamber was set to a daily cycle of 22 light hours at 15°C / 70% RH and 2 dark hours at 10°C / 85% RH similar to Jones and Rosentreter (2006). The photon flux in the growth chamber ranged from 75 to 160 μmol/m^2s.

Treatments received distilled water multiple times per day beginning the day after dishes were placed in the growth chamber. *Bryum* dishes were watered twice per day and *Syntrichia* dishes were watered four times per day based on observations of what was required to maintain constantly hydrated moss fragments. Dishes without moss for the sand and tackifier organic matter mass adjustment were watered twice per day. Watering was done manually with a spray bottle until sand was just below field capacity (droplets of water began to stand at the surface of the sand, but infiltrated into the sand). Where fungal incursions were observed in dishes, the sand and fungi in affected areas were removed with a microspatula. This was a rare occurrence in each run. In the few cases where fragments were impacted by fungi, they were removed from their dishes and their absence was accounted for in the data analysis.

Data Collection

Data were collected at the end of each experiment. Each fragment was viewed from above with a dissecting microscope at up to 40x magnification and examined with a probe. Number of shoots, gemma presence, protonema presence, bound sand mass, and moss organic matter mass were determined for *Bryum*. Total shoot length, number of shoots, bound sand mass, and moss organic matter mass were determined for *Syntrichia*.

Total Shoot Length and Number of Shoots

The length of each *Syntrichia* shoot on every fragment was measured in millimeters and summed for a dish total. 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

The presence of gemmae was considered a measurement of fecundity. Where gemmae were observed extending from a fragment, they were considered present for that fragment. The presence of abundant protonemata was considered a measure of expansion and potential soil stabilization. Protonemata were considered meaningfully present if there were more than five protonemata 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 ten for each variable.

Bound Sand Mass and Moss Organic Matter Mass

After collecting the above-mentioned data, fragments were grasped individually with fine forceps, pulled gently from their dish (perpendicular to the surface) with attached sand, and pooled by dish into a small, furnace-dried (150°C), pre-weighed foil packet or vial, depending on the species. Any rhizoids or protonemata remaining in dishes were removed along with the sand

bound to them and added to the pooled samples. Fragments from *Bryum* dishes were combined in 45 mm x 55 mm rectangular pieces of aluminum foil folded into packets. Fragments from *Syntrichia* dishes were combined in 20 mL glass vials with 40 mm x 40 mm square pieces of aluminum foil folded over them as lids. Glass vials were used for *Syntrichia* fragments to accommodate their larger size and greater volume of accompanying material. *Bryum* fragments were maintained in foil packets because it allowed their mass to be determined with a more precise scale than could be used for the heavier glass vials.

Packets and vials were oven dried at 65° C for 48 hours, allowed to cool in a desiccator, and weighed to determine the dry mass of their contents. *Bryum* packets were weighed (Sartorius® M2P scale) to the nearest microgram. *Syntrichia* vials were weighed (Sartorius® R300S) to the nearest 0.1 milligram. 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. The change in mass (from combustion) represented the organic contribution of the tackifier, the original moss fragment and any product of fragment growth, as well as organic matter that came in the sand.

After accounting for packet or vial mass, the total bound sand mass and total organic matter mass for each replicate were determined where:

$$Total\ bound\ sand\ mass = mass\ of\ combusted\ sample$$

$$Total\ organic\ matter\ mass = mass\ of\ dry\ sample - mass\ of\ combusted\ sample$$

To determine the proportions of tackifier and sand organic matter in total organic matter mass, we used an inverted 10 mL graduated pipette to collect eight equal-sized substrate samples from each dish in the dishes with only sand and tackifier and without moss. Samples were collected from the sand at the center of each dish where a tackifier mixture was poured, included the entire column of sand from the substrate surface to the bottom of the dish, and were pooled by dish into

foil packets. Using the same mass determination steps described for dishes with moss fragments, a ratio of total bound sand mass to total organic matter mass was created for dishes that did not have moss fragments. Ratios were averaged by treatment for both sand types to yield an average organic matter mass adjustment ratio for each tackifier-concentration combination. This ratio can be written as:

$$Organic\ matter\ mass\ adjustment\ ratio = \frac{Total\ organic\ matter\ mass_{without\ moss}}{Total\ sand\ mass_{without\ moss}}$$

Having only two replicates of *Syntrichia* sand led to uncertainty in the process of generating average mass adjustment ratios because of outliers. To manage this shortcoming, we plotted separate curves for each sand type (Fig 1). The two curves were remarkably similar in shape, but were separated by the difference in organic matter mass that came in the various sand types. Where the *Syntrichia* curve departed markedly from the shape of the *Bryum* curve, that average ratio was removed and a new point was generated from a line fitted to the remaining average ratios of that tackifier type, using the average ratio of the water treatment as a 0x concentration.

Finally, the organic matter mass adjustment ratios calculated from dishes without moss fragments were used with the measurements of *Bryum* and *Syntrichia* total organic matter and bound sand masses from dishes with moss fragments to determine moss organic matter mass for the samples from each dish with moss fragments, which can be written as:

$$Total\ moss\ organic\ matter\ (OM)\ mass = Total\ OM\ mass - (OM\ \text{mass}\ adjustment\ ratio\ x\ Total\ bound\ sand\ mass)$$

Though fragment masses were not measured during the experiment set-up, it was assumed that standardized fragment lengths yielded approximately equal initial fragment masses across dishes within a species. Thus, differences in total fragment mass (moss organic matter mass) at the end of the experiment should reflect differences in growth amount across dishes and treatments.

Differences in moss organic matter mass capture above-ground and below-ground growth and other changes in mass that are not otherwise easily quantified.

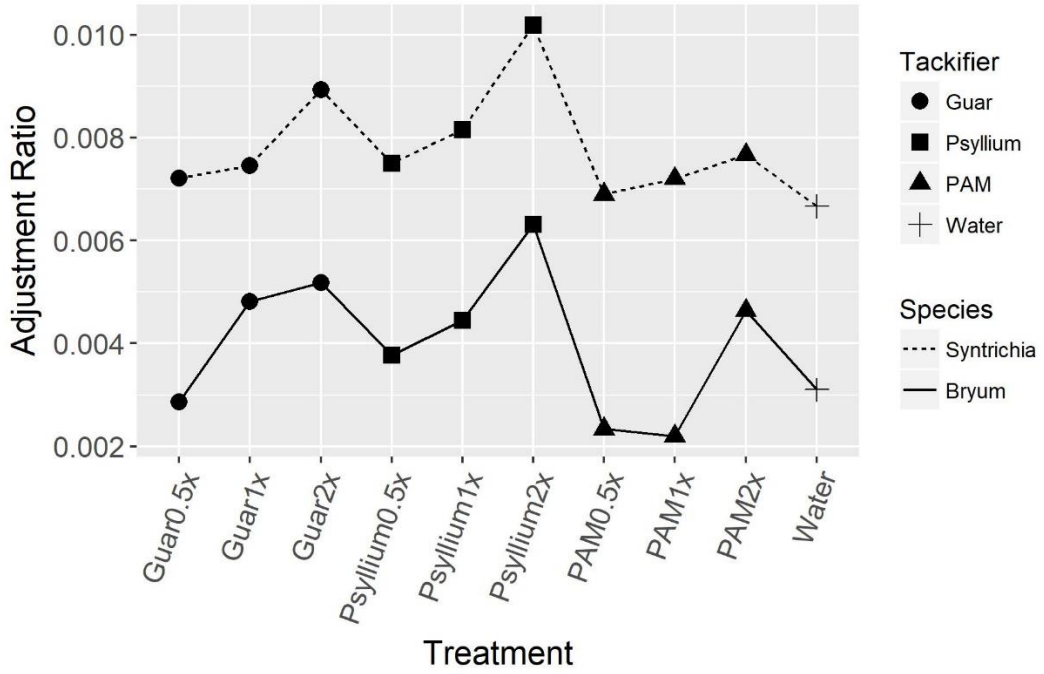

Figure 1: Plot of average adjustment ratios (connected by lines) used to calculate the moss organic matter mass of fragments from Bryum *and* Syntrichia *dishes.* A*djustment ratios are an average of ratios of organic matter mass to sand mass in dishes without moss fragments with five replications for each* Bryum *treatment and two replications for each* Syntrichia *treatment. Outliers were removed from the PAM0.5x and PAM2x treatments for* Syntrichia *based on the values from the* Bryum *curve. Ratios for the Psyllium0.5x and PAM1x treatments for* Syntrichia *were calculated using a best fit line through the remaining points of the respective tackifier type and the average adjustment ratio of the water treatment as a 0x concentration using the least squares method.*

Data Analysis

Dish level data for each variable was divided by the number of fragments in each dish to account for the loss of some fragments in the course of a run. All analyses were done with R version 3.4.3 (R Core Team 2017). 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 a treatment variable representing the nine tackifier-concentration combinations (and

the water control) as the fixed effect with the nlme package (version 3.1.131, Pinheiro et al. 2017). A single, combined variable 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 non-constant variance, a natural logarithmic transformation of data was attempted. When the assumption of constant variance was not met, variances were allowed to vary by tackifier type or treatment. Where a natural logarithmic transformation was required to fit the model, comparisons among treatment groups were back-transformed and reported as ratios of medians.

Counted proportions of gemma presence 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 intervals 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 (as appropriate) were done with F tests (LMM) and drop-in-deviance χ^2 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).

RESULTS

Number of shoots, bound sand mass, moss organic matter mass, and total shoot length differed among treatments based on linear mixed models (Table 2). Generalized linear mixed models testing the odds of gemma presence per fragment (drop-in-deviance χ^2 test $\chi^2_9 = 73.3$, $p < 0.001$) and protonema presence per fragment (drop-in-deviance χ^2 test $\chi^2_9 = 63.6$ $p < 0.001$) also yielded statistically significant differences.

Table 2: Results from overall F-tests (degrees of freedom {df}, F-statistic, and p-value) for models of each variable by species.

	Variable	Between df	Within df	F-statistic	p-value
Bryum	Number of Shoots	9	81	4.9	<0.001
	Bound Sand Mass	9	81	12.8	<0.001
	Moss Organic Matter Mass	9	81	7.5	<0.001
Syntrichia	Total Shoot Length	9	72	20.0	<0.001
	Number of Shoots	9	72	11.1	<0.001
	Bound Sand Mass	9	34	27.4	<0.001
	Moss Organic Mass	9	34	12.6	<0.001

Fragments grown in psyllium exceeded fragments grown in water in seven of nine responses between *Bryum* and *Syntrichia* and seemed to produce the largest values at higher concentrations (Q1 - Tables 3 & 4, 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). Fragments grown in Guar1x or Guar2x were exceeded by fragments grown in water in eight of nine responses between the two species. *Bryum* fragments grown in PAM had more bound sand mass (Fig 2b) and higher gemma presence (Fig 2e) than fragments grown in water, but *Syntrichia* fragments grown in PAM1x and PAM2x had less moss organic matter than fragments grown in water (Fig 3d). Otherwise, fragments growth in PAM did not vary appreciably from fragments grown in water.

There were no effects of within-tackifier concentration on number of shoots or moss organic mass for *Bryum* (Q2 - Table 3, Figs 2a,c). Fragments grown in higher concentrations of guar were exceeded by fragments grown in the lowest concentration of guar on all other variables for

the two species (Figs 2 and 3). For *Syntrichia,* the highest concentration of psyllium resulted in fragments with higher values for every response compared to fragments grown in Psyllium0.5x and Psyllium1x, except for sand mass where fragments grown in the lowest concentration exceeded those grown in higher concentrations (Q2 – Table 4, Fig 3). Different concentrations of PAM did not lead to appreciable differences within response variables, except in *Bryum* protonema presence (Fig 2d).

The mean value of fragments grown in psyllium exceeded the mean value of fragments grown in guar in all nine responses between the two species (Q3 - Tables 3 & 4). The mean value of fragments grown in PAM also exceeded the mean value of fragments grown in guar in all cases, except for *Bryum* number of shoots and *Syntrichia* moss organic matter mass where there were no appreciable differences.

Except for *Bryum* protonema presence (where there was not an appreciable difference), the mean value of *Bryum* fragments grown in psyllium exceeded the mean value of *Bryum* fragments grown in PAM in all cases. The mean value of *Syntrichia* fragments grown in psyllium exceeded the mean value of *Syntrichia* fragments grown in PAM in number of shoots and moss organic matter mass, but the opposite was true for sand mass.

Table 3: Summary of results for Bryum *comparisons of medians (or odds) per fragment for variables where p<0.05. Estimates of ratios of medians (ratios of odds for gemma presence and protonema presence), lower and upper confidence limits (LCL, UCL), degrees of freedom (df), the values of the test statistic (z for gemma presence and protonema presence, t otherwise), and p-values are reported. Comparisons are of (Q1) all concentrations* vs. *water, (Q2) all concentrations* vs. *each other within each tackifier type, and (Q3) the mean of all concentration treatments of each tackifier compared across tackifiers.*

	Response Variable	Comparison	Est.	LCL	UCL	df	test stat	p-value
Q1	Number of Shoots	Psyllium0.5x > Water	1.551	1.238	1.944	81	3.8	<0.001
		Psyllium1x > Water	1.420	1.133	1.779	81	3.1	0.003
		Psyllium2x > Water	1.602	1.279	2.008	81	4.2	<0.001
	Moss Organic Matter Mass (mg)	Water > Guar1x	1.136	1.034	1.248	81	2.7	0.009
		Psyllium0.5x > Water	1.599	1.160	2.205	81	2.9	0.005
		Psyllium1x > Water	2.090	1.091	4.002	81	2.3	0.027
		Psyllium2x > Water	1.774	1.444	2.180	81	5.5	<0.001
	Sand Mass (mg)	Water > Guar2x	2.273	1.012	5.102	81	2.0	0.047
		Psyllium0.5x > Water	7.885	3.514	17.694	81	5.1	<0.001
		Psyllium1x > Water	3.870	1.725	8.684	81	3.3	0.001
		Psyllium2x > Water	5.944	2.649	13.337	81	4.4	<0.001
		PAM0.5x > Water	3.904	1.740	8.760	81	3.4	0.001
		PAM1x > Water	3.078	1.372	6.908	81	2.8	0.007
	Gemma Presence†	Water > Guar1x	3.328	1.032	10.733	-	2.0	0.044
		Psyllium0.5x > Water	4.234	2.020	8.877	-	3.8	<0.001
		Psyllium1x > Water	3.660	1.738	7.711	-	3.4	<0.001
		Psyllium2x > Water	4.535	2.163	9.507	-	4.0	<0.001
		PAM1x > Water	2.241	1.037	4.841	-	2.1	0.040
		PAM2x > Water	2.947	1.387	6.230	-	2.8	0.005
	Protonema Presence†	Water > Guar1x	11.257	3.355	16.007	-	3.9	<0.001
		Water > Guar2x	48.309	12.678	183.813	-	5.7	<0.001
Q2	Number of Shoots	NONE	-	-	-	-	-	-
	Moss Organic Matter Mass (mg)	NONE	-	-	-	-	-	-
	Sand Mass (mg)	Guar0.5x > Guar1x	2.264	1.009	5.081	81	2.0	0.048
		Guar0.5x > Guar2x	2.367	1.055	5.312	81	2.1	0.037
	Gemma Presence†	Guar0.5x > Guar1x	6.609	2.170	20.128	-	3.3	<0.001
		Guar0.5x > Guar2x	2.427	1.069	5.506	-	2.1	0.034
	Protonema Presence†	Guar0.5x > Guar1x	5.913	1.845	18.957	-	3.0	0.003
		Guar0.5x > Guar2x	25.357	6.984	92.058	-	4.9	<0.001
		Guar1x > Guar2x	4.288	1.206	15.248	-	2.2	0.025
		Psyllium0.5x > Psyllium1x	4.545	1.328	15.550	-	2.4	0.016
		PAM0.5x > PAM2x	3.451	1.012	11.774	-	2.0	0.048
Q3	Number of Shoots	Psyllium > Guar	1.465	1.286	1.669	81	5.8	<0.001
		Psyllium > PAM	1.309	1.149	1.492	81	4.1	<0.001
	Moss Organic Matter Mass (mg)	Psyllium > Guar	2.011	1.560	2.591	81	5.5	<0.001
		Psyllium > PAM	1.758	1.346	2.296	81	4.2	<0.001
		PAM > Guar	1.144	1.025	1.276	81	2.4	0.017
	Sand Mass (mg)	Psyllium > Guar	9.499	5.957	15.147	81	9.6	<0.001
		Psyllium > PAM	1.913	1.200	3.051	81	2.8	0.007
		PAM > Guar	4.965	3.114	7.918	81	6.8	<0.001
	Gemma Presence†	Psyllium > Guar	5.241	3.188	8.616	-	6.5	<0.001
		Psyllium > PAM	1.788	1.239	2.579	-	3.1	0.002
		PAM > Guar	2.932	1.760	4.884	-	4.1	<0.001
	Protonema Presence†	Psyllium > Guar	8.792	4.244	18.211	-	5.9	<0.001
		PAM > Guar	8.322	4.023	17.217	-	5.7	<0.001

†comparisons are reported as ratios of odds, tested with the z-statistic

Table 4: Summary of results for Syntrichia *comparisons of means (or medians) per fragment for variables where p<0.05. Estimates of differences of means (medians for shoot length), lower and upper confidence limits (LCL, UCL), degrees of freedom (df), the value of the t-statistic, and the p-value are reported. Comparisons are of (Q1) all concentrations* vs. *water, (Q2) all concentrations* vs. *each other within each tackifier type, and (Q3) the mean of all concentration treatments of each tackifier compared across tackifiers.*

	Response Variable	Comparison	Est.	LCL	UCL	df	t stat	p-value
Q1	Total Shoot Length (mm)††	Water > Guar2x	2.031	1.671	2.469	72	7.2	<0.001
		Psyllium2x > Water	1.586	1.304	1.928	72	4.7	<0.001
	Number of Shoots	Water > Guar0.5x	0.406	0.013	0.800	72	2.1	0.043
		Water > Guar2x	0.833	0.440	1.230	72	4.2	<0.001
		Psyllium2x > Water	0.922	0.529	1.316	72	4.7	<0.001
	Moss Organic Matter Mass (mg)	Water > Guar1x	0.349	0.085	0.613	34	2.7	0.011
		Water > Guar2x	0.500	0.159	0.840	34	3.0	0.005
		Psyllium2x > Water	0.367	0.112	0.621	34	2.9	0.006
		Water > PAM1x	0.430	0.073	0.787	34	2.4	0.020
		Water > PAM2x	0.426	0.109	0.743	34	2.7	0.010
	Sand Mass (mg)	Water > Guar0.5x	280.180	191.789	368.571	34	6.4	<0.001
		Water > Guar1x	310.810	222.419	399.201	34	7.1	<0.001
		Water > Guar2x	427.558	339.167	515.949	34	9.8	<0.001
		Water > Psyllium1x	147.410	25.095	269.725	34	2.4	0.020
		Water > Psyllium2x	206.064	83.749	328.379	34	3.4	0.002
Q2	Total Shoot Length (mm)††	Guar0.5x > Guar2x	1.716	1.412	2.087	72	5.5	<0.001
		Guar1x > Guar2x	1.724	1.418	2.096	72	5.6	<0.001
		Psyllium2x > Psyllium0.5x	1.600	1.316	1.945	72	4.8	<0.001
		Psyllium 2x > Psyllium1x	1.319	1.085	1.603	72	2.8	0.006
	Number of Shoots	Guar0.5x > Guar2x	0.427	0.034	0.821	72	2.2	0.034
		Guar1x > Guar2x	0.589	0.195	0.983	72	3.0	0.004
		Psyllium2x > Psyllium0.5x	0.970	0.577	1.364	72	4.9	<0.001
		Psyllium2x > Psyllium1x	0.622	0.229	1.016	72	3.2	0.002
	Moss Organic Matter Mass (mg)	Guar0.5x > Guar1x	0.374	0.216	0.532	34	4.8	<0.001
		Guar0.5x > Guar2x	0.524	0.257	0.791	34	4.0	<0.001
		Psyllium2x > Psyllium0.5x	0.310	0.027	0.593	34	2.2	0.033
	Sand Mass	Guar0.5x > Guar2x	147.378	73.371	221.385	34	4.0	<0.001
		Guar1x > Guar2x	116.748	42.741	190.755	34	3.2	0.003
		Psyllium0.5x > Psyllium1x	192.124	51.508	332.740	34	2.8	0.009
		Psyllium0.5x > Psyllium2x	250.778	110.162	391.394	34	3.6	<0.001
Q3	Total Shoot Length (mm)††	Psyllium > Guar	1.749	1.563	1.958	72	9.9	<0.001
		PAM > Guar	1.622	1.449	1.816	72	8.6	<0.001
	Number of Shoots	Psyllium > Guar	0.886	0.659	1.113	72	7.8	<0.001
		Psyllium > PAM	0.232	0.004	0.459	72	2.0	0.046
		PAM > Guar	0.654	0.427	0.882	72	5.7	<0.001
	Moss Organic Matter Mass (mg)	Psyllium > Guar	0.457	0.297	0.618	34	5.8	<0.001
		Psyllium > PAM	0.531	0.329	0.733	34	5.3	<0.001
	Sand Mass (mg)	Psyllium > Guar	236.596	171.725	301.468	34	7.4	<0.001
		PAM > Psyllium	84.811	14.549	155.073	34	2.5	0.019
		PAM > Guar	321.407	270.869	371.945	34	12.9	<0.001

††comparisons are reported as ratios of medians

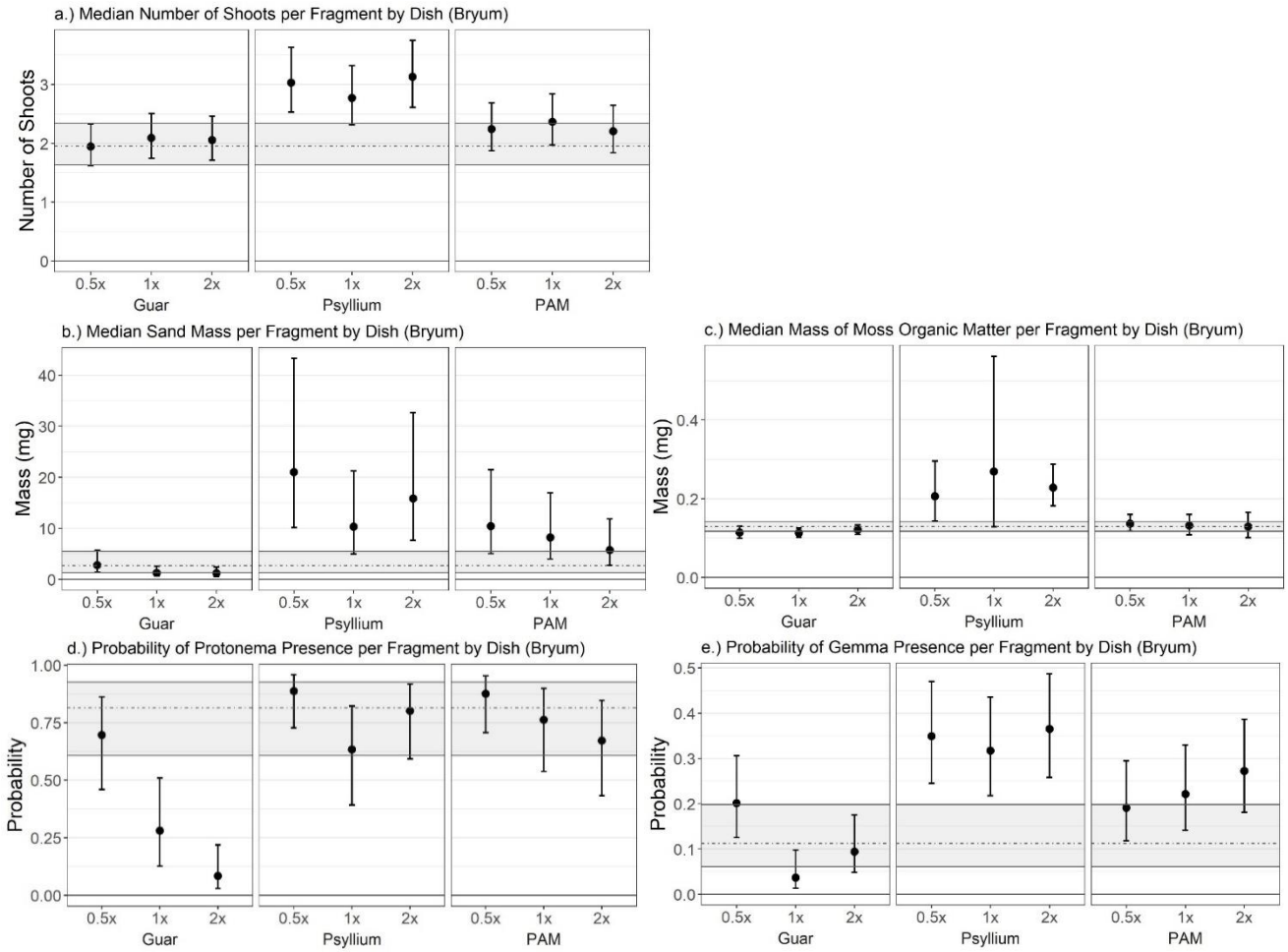

Figure 2: Graphical summary of Bryum *estimates of medians of treatments for each variable (a-e) (odds for protonema presence and gemma presence). Horizontal dotted line depicts estimated value for the water (control) treatment and gray shading represents its 95% confidence interval. Error bars are the 95% confidence interval for each variable.*

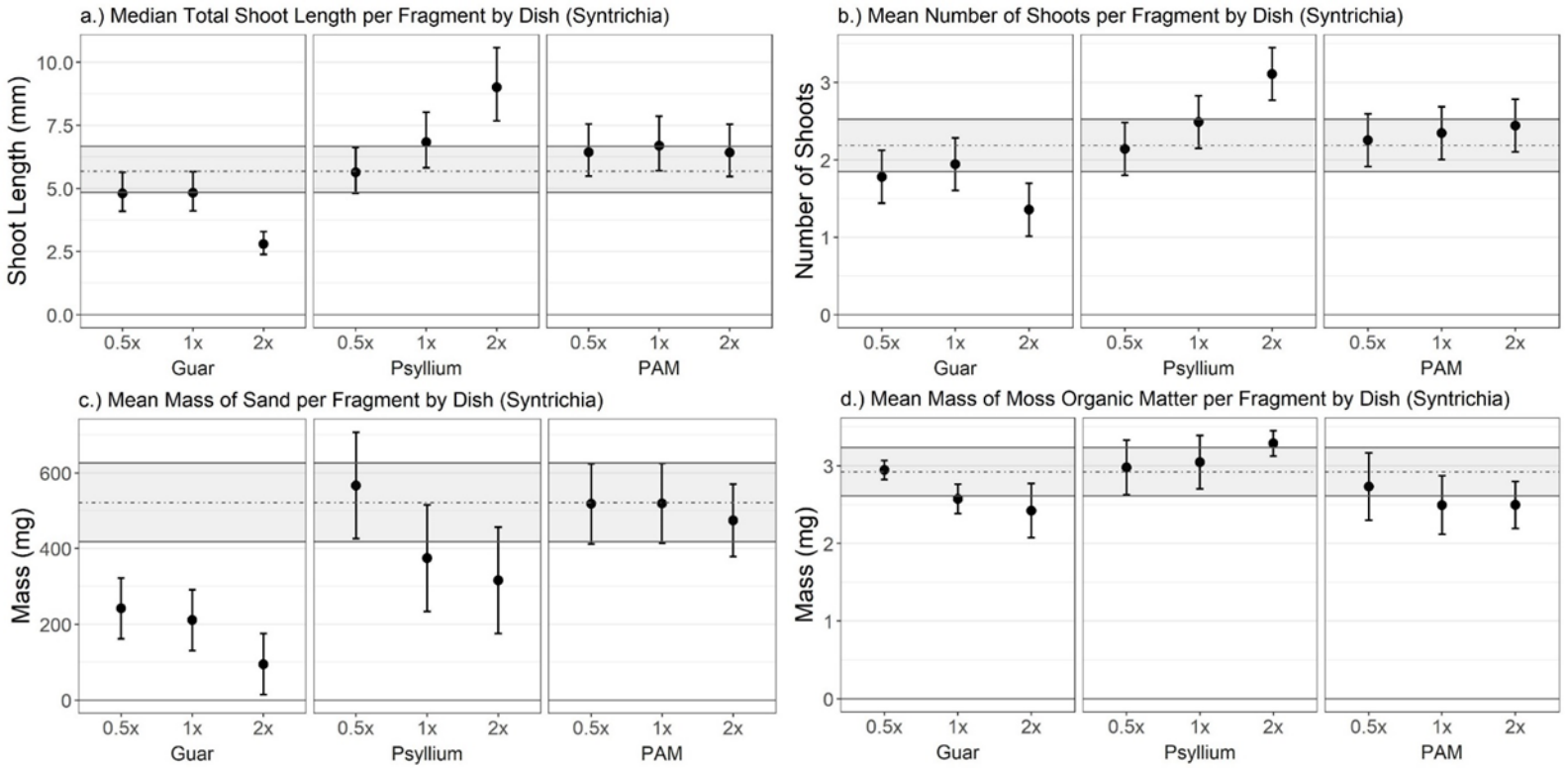

Figure 3: Graphical summary of Syntrichia *estimates of means of treatments for each variable (a-d) (medians for total shoot length). Horizontal dotted line depicts estimated value for the water (control) treatment and gray shading represents its 95% confidence interval. Error bars are the 95% confidence interval for each variable.*

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* and *Syntrichia* fragments. Within tackifier types, increasing concentrations of guar tended to decrease moss growth, while increasing concentrations of psyllium tended to increase growth, and PAM concentrations had little impact on moss growth.

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; CalTrans 2017) interact with the unique characteristics of vegetative propagation in bryophytes. For instance, a tackifier containing trace amounts of copper or zinc, which are detrimental to mosses, from processing methods, storage, or transportation could reduce fragment growth when compared to fragments grown without tackifier. Additionally, though water was not limiting, changes to osmotic potential (not measured) caused by a tackifier's physical properties could impact the availability of water to mosses and could have caused some of the differences in growth we observed.

Further, other growth chamber experiments with mosses have typically incorporated additional nutrients, generally in the form of a standardized solution of nitrogen, phosphorous, and potassium (Jones & Rosentreter 2006; Xu et al. 2008; Duckett et al. 2014). Our decision to not include nutrients may have created nutrient-limited environments, where slight differences in tackifier composition and concentration may have inadvertently provided a gradient of increasing nutrient abundance corresponding with increased growth as we saw with psyllium. Psyllium is a somewhat labile carbon source and has been noted to be broken down by microbes (Perkins 2006) which could make other nutrients available for moss growth, especially as husks from the seed coats remained bound to moss fragments in some capacity for the duration of the experiment. PAM is resistant to microbial degradation (Seybold 1994), which may explain its

tendency of yielding neutral growth responses. Finally, previous experiments with the same moss species yielded increased growth when associated with jute net, which likely created a microhabitat more conducive for moss growth, buffering fragments from extreme fluctuations of temperature and moisture (Condon & Pyke 2016). The aforementioned seed husks of psyllium might have acted similar to jute net for promoting moss growth.

Like our results, cyanobacterial crust growth was neutral to positive when associated with PAM in restoration studies in natural drylands (Park et al. 2017b; Chandler et al. 2018). Neutral growth responses of biocrusts to tackifiers may still have a positive effect on restoration success if the tackifier is adhering the biocrust propagule to the soil and preventing secondary fragment dispersal, while also enhancing soil aggregate stability. 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, the moss may extend rhizoids and protonemata, as we saw in *Bryum* and *Syntrichia,* and bind with soil while extending the moss mat.

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. 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 were similar to our above ground 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 below ground attributes of mosses which we did not measure.

Our study is aimed at informing restoration, highlighting the above- and below- ground attributes of mosses that could be practical in the field. As jute (Condon & Pyke 2016) and straw checkerboard (Li et al. 2003; Li et al. 2014) have facilitated biocrust establishment in field studies, it is timely to test moss growth in tackifiers, which could provide a mechanism for biocrust restoration that is both more efficient and can be extended to larger spatial scales. *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 could be good candidates for future studies with tackifiers (Jones & Rosentreter 2006, Antoninka et al. 2016). Future research should be mindful of tackifier type and tackifier concentration in biocrust restoration and consider the suitability of different tackifier applications to various site conditions.

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