United States Department of Agriculture

January 2022

FINAL STUDY REPORT
Aberdeen Plant Materials Center
Aberdeen, Idaho

Preliminary greenhouse study to evaluate the effects of native soil amendment on seedling growth of early- and late-seral natives compared to cheatgrass (*Bromus tectorum*), an exotic annual

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ABSTRACT

Cheatgrass (*Bromus tectorum*), an exotic early seral annual, has been shown to alter soil microbial communities and nutrient cycling, making restoration of the native plant community difficult. This greenhouse study was conducted to evaluate the effects of Biologically Enhanced Agricultural Management (BEAM) compost on the growth of cheatgrass and six native species [annual sunflower (*Helianthus annuus*), curlycup gumweed (*Grindelia squarrosa*), lobeleaf groundsel (*Packera multilobata*), sand dropseed (*Sporobolus cryptandrus*), bluebunch wheatgrass (*Pseudoroegneria spicata*) and western wheatgrass (*Pascopyrum smithii*)] in a Kecko-Clems-Vining association fine sandy loam collected from a cheatgrass-dominated site near American Falls, ID. A BEAM compost pile was inoculated during construction with a small amount of soil from undisturbed native plant communities, and the finished compost was applied as a seed treatment. For each species, treated seed was compared with an untreated control. After 12 weeks of growth, shoot length, shoot dry weight, and root dry weight were measured. The multilobed groundsel, an early seral native, and western wheatgrass, a late seral native, showed growth responses to BEAM treatment. Treated multilobed groundsel had significantly greater mean shoot length (6.8 cm) than the untreated control (5.8 cm). The mean shoot length of treated western wheatgrass plants was significantly less than that of untreated plants, 55.8 cm and 61.8 cm, respectively. The mean root weight of treated western wheatgrass plants was significantly greater than that of the untreated plants, 2.35 g vs. 1.79 g, while shoot weight was not significantly different between the two treatments. The remaining species, including cheatgrass, were not significantly affected by BEAM treatment. Seed treatment with BEAM compost may in some cases prime the soil biota to enhance the growth of native species.

INTRODUCTION

Cheatgrass (*Bromus tectorum*), an exotic annual, has invaded and replaced millions of acres of rangeland in the Intermountain West leading to significant degradation including loss of species diversity and decreases in productivity (Knapp, 1996). Once cheatgrass becomes dominant at a site, it is very difficult to restore the native plant community.

One obstacle that may be limiting ecological restoration is the common practice of attempting restoration to the pre-disturbance "climax" plant community rather than utilizing natural plant succession. Most restoration efforts have focused on re-inserting late-seral native species directly into the invaded area (Jones & Johnson, 1998; Ogle et al., 2014); however restoration results have

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been discouraging (Kulpa & Leger, 2013; Svejcar et al., 2017). Recent studies have indicated however, that some early seral native species have a higher competitive ability against invasive species than late seral (Leger et al., 2014; Uselman et al., 2015; Tilley et al., 2020). Early seral native species are adapted to the same disturbance conditions that favor annual weeds, and they provide a priming effect for later-seral species. It is possible that part of this priming effect is the result of early-seral species' ability to rebuild soil microbial communities, and therefore soil functionality, after disturbance (Harris, 2009; van der Putten et al., 2013).

Cheatgrass is thought to increase its competitive advantage by negatively impacting the soil microbial community and N-cycling processes (Reitstetter & Rittenhouse, 2017; Busby et al., 2011; Kieffer Stube, 2012). Carey et al. (2017) found that active microbial biomass was lower in soils invaded by exotic annual grasses compared to native plant communities. Additionally, Busby et al. (2011) found a lack of mycorrhizal fungi in cheatgrass invaded soils. Paschke et al. (2000) studied old crop fields that had been invaded by cheatgrass. They hypothesized that the redevelopment of microbially-mediated N cycling after disturbance is an important factor in system redevelopment. They used sucrose to promote growth of the soil microbial community, which then immobilized N. The sucrose plots shifted plant community composition toward an increase in perennial species. Inoculating seed with beneficial microbes from native soil may prime the re-establishment of a native soil microbial community and improve restoration outcomes.

The Johnson-Su bioreactor creates Biologically Enhanced Agricultural Management (BEAM) compost that contains a microbially diverse and fungal-dominant soil microbiome (Johnson & DeSimio, 2017; CSU, 2021). The resulting compost can be applied to the soil or used as a seed treatment. BEAM compost is not intended to be a bulk amendment that supplies organic matter and nutrients. Rather, it is more accurately described as a source of "probiotics" for the soil. BEAM compost is already being adopted by farmers in Idaho as a way to improve soil health in cropping systems. The BEAM method is simple, inexpensive, and accessible and thus could be easily adopted by land managers and restoration practitioners. However, little is known regarding the applicability of BEAM compost to restoring native plant communities.

To better understand the effects of BEAM compost on native species under controlled conditions, IDPMC initiated a greenhouse trial comparing inoculated versus non-inoculated seed of 7 species. This greenhouse study is a preliminary step to evaluating the seed treatment at a field scale. The objective of this study is to evaluate whether supplementing the soil microbial community of cheatgrass-invaded soils with BEAM compost (inoculated with soil from an intact late-seral plant community) will improve the growth of early- and late-seral native plants in a cheatgrass-invaded soil, and if this inoculation also affects cheatgrass growth.

MATERIALS AND METHODS

We constructed a Johnson-Su bioreactor using the method described by Johnson & DeSimio (2017). It consists of an upright cylindrical frame made of wire re-mesh lined with landscape cloth. Our bioreactor is 106 cm (42 in) high and 91 cm (36 in) in diameter. The Johnson-Su bioreactor is a static (non-turning) composting system; therefore, aeration is critical. The bioreactor is placed on a wooden pallet to allow air circulation from underneath. Before filling the frame with feed material, six septic system drainfield pipes were placed vertically within the frame, evenly spaced to ensure that air could circulate within the filled bioreactor. After the fill material settled, the pipes were removed, leaving open columns of air within the compost. The fill was kept damp with a small amount of water applied daily. To reduce moisture loss, the top of the frame was kept covered with landscape cloth.

Feed material for the compost included local herbicide-free yard waste (lawn clippings and deciduous leaves), pasture clippings (grass, alfalfa and forbs), and llama manure. We were not able to include worms in our bioreactor, although they are one of the standard components of the BEAM composting system. However, we inoculated the feed material during the filling process with approximately 2.2 kg (5 lb) of native soil from two undisturbed late seral plant communities. We let the bioreactor run its composting process for the recommended year before sampling, testing, and using the finished compost.

Species evaluated are shown in Table 1. We evaluated one exotic early seral grass (cheatgrass), one early seral native grass (sand dropseed), 3 early seral native forbs (annual sunflower, curlycup gumweed, and lobeleaf groundsel), and 2 late seral native grasses (bluebunch wheatgrass and western wheatgrass). Seeds of annual sunflower and curlycup gumweed were cold stratified in damp sand at 37 °F for 60 days beforehand to promote rapid germination once planted in the greenhouse (Kramer & Foxx, 2016; Luna, 2008). Each species was subjected to 2 treatments: 1) seed treatment with BEAM compost and 2) untreated control.

Table 1. Species evaluated in the greenhouse study.

Species	Common Name	Family	
Bromus tectorum	cheatgrass	Poaceae	exotic early seral[1/]
Helianthus annuus	annual sunflower	Asteraceae	early seral[2/]
Grindelia squarrosa	curlycup gumweed	Asteraceae	early seral[3/]
Sporobolus cryptandrus	sand dropseed	Poaceae	early seral[4/]
Packera multilobata	lobeleaf groundsel	Asteraceae	early seral[5/]
Pseudoroegneria spicata	bluebunch wheatgrass	Poaceae	late seral[6/] [7/]
Pascopyrum smithii	western wheatgrass	Poaceae	late seral[6/] [7/]

[1/]Knapp, 1996; [2/]Perry et al., 2009; Herron et al, 2013; ; [3/]Tilley & Pickett, 2019; [4/]Welsh, 2003; [5/]Ott et al., 2003; Hoelzle et al., 2012; [6/]Ott et al, 2019; [7/]Tilley et al., 2021.

We collected soil for use in the greenhouse study from a cheatgrass-dominated site near American Falls in Power County, Idaho. The site is mapped as the Kecko-Clems-Vining association; all three soil components have a fine sandy loam surface (USDA NRCS, 2021). We collected soil from the A horizon [top 15 cm (6 in)] on 23 June 2021, after cheatgrass senescence. Soil was screened with 2.5 mm round hole mesh to remove residue and larger weed seeds. The screened soil was used to fill 500 cm^3 (4 in) pots into which the treatments were planted. Samples of this soil were submitted to Ward Laboratories (Kearney, NE) for a basic agronomic analysis (NPK, pH, organic matter %). Additionally, soil microbial community composition and activity were analyzed with phospholipid fatty acid (PLFA) and soil respiration tests. A sample of the BEAM compost was also submitted for PLFA analysis. Analysis results are shown in Appendix A.

The BEAM compost seed treatment was accomplished in the following way: The BEAM compost pile was cored several times with a soil sampling probe. The cores were mixed with non-chlorinated municipal water then strained through a kitchen sieve. The resulting slurry was used to coat the treated seeds using the slurry method of coating legume seed with inoculant (Johnson & DeSimio, 2017; Wolf et al., 2021). The tiny seeds of sand dropseed were treated by dipping a fingertip in the slurry, touching the fingertip to seeds to pick up several, touching slurry/seeds to the soil in the pot, then sprinkling a very thin layer of additional soil on top of the slurry/seeds. Seeds were planted at the appropriate depth immediately after BEAM treatment was applied following recommendations in Ogle et al. (2014).

Seeded pots were randomized on a greenhouse table with automatic watering. Pots were initially watered twice a day for 20 min. After one week, the schedule was cut back to one daily 20 min watering for the remainder of the study. Seedlings were thinned to one plant per pot and any germinating weeds were removed. No fertilizer was applied. The small amount of BEAM compost used in seed treatment supplied a negligible amount of nutrients to the growing plants.

Annual sunflower and cheatgrass plants grew rapidly in the small pots and had begun to senesce by week 10. We evaluated those 2 species at week 10 to avoid tissue loss from senescence. All other species were allowed to grow for 12 weeks. At the end of each species' growth period, we measured the length of the longest shoot in each pot, including any inflorescences. The plants were removed from the pots and shoots were separated from the roots. The root masses were washed with running water through a No. C 1/12 in round screen (Seedburo Equipment Company, Chicago). Both shoots and roots were oven dried for 48 hours at 60 °C in a drying oven and then weighed immediately after removal.

The experiment was arranged in a completely randomized design with 12 replications. Each component (root weight, shoot weight, shoot length) of each species was analyzed separately with Statistix 10 software (Tallahassee, FL). Two Sample t-tests ($P < 0.05$) were used for all means comparisons except in cases where the data did not meet test assumptions of normal distribution or homogeneity of variances. Sunflower root weight and shoot length, lobeleaf groundsel root weight and shoot length, and sand dropseed shoot weight and shoot length did not meet the Two Sample t-test assumptions and were analyzed with the nonparametric Wilcoxon Rank Sum test. The Wilcoxon Rank Sum test calculates and tests the significance of differences between mean ranks. Significant differences in mean rank were only detected in shoot length of multilobed groundsel; these mean ranks are reported as treatment means in Table 2.

RESULTS AND DISCUSSION

Results are shown in Table 2. Lobeleaf groundsel mean shoot length was significantly greater for the BEAM treatment (6.8 cm) than for the untreated control (5.8 cm). However, there were no significant differences in root or shoot weight between the two treatments.

The mean shoot length of treated western wheatgrass plants was significantly less than that of untreated plants, 55.8 cm and 61.8 cm, respectively. However, the mean root weight of treated western wheatgrass plants was significantly greater than that of the untreated plants, 2.35 g vs. 1.79 g. Shoot weight was not significantly different between the two treatments. It appears that the BEAM treatment encouraged root growth over shoot growth in western wheatgrass.

For cheatgrass, sand dropseed, curlycup gumweed, common sunflower, and bluebunch wheatgrass, treatment with BEAM compost resulted in no significant difference in shoot length, root weight, or shoot weight compared to the untreated control.

Table 2. Mean values for shoot and root weight (oven dry) and shoot length for BEAM compost-treated vs. untreated control for various species grown from seed for 12 weeks in the greenhouse at the Aberdeen PMC, Idaho.

Species	BEAM-Treated	Control	Significance[1]
Bromus tectorum[2]			
Shoot (g)	1.33	1.26	NS
Root (g)	5.68	8.64	NS
Length (cm)	14.2	12.7	NS
Sporobolus cryptandrus			
Shoot (g)	1.64	1.39	NS
Root (g)	0.96	1.22	NS
Length (cm)	54.7	56.5	NS
Grindelia squarrosa			
Shoot (g)	1.30	1.24	NS
Root (g)	3.96	4.09	NS
Length (cm)	8.0	8.3	NS
Helianthus annuus[2]			
Shoot (g)	5.07	4.91	NS
Root (g)	3.16	3.37	NS
Length (cm)	95.3	96.3	NS
Packera multilobata			
Shoot (g)	0.80	0.61	NS
Root (g)	1.80	1.35	NS
Length (cm)	6.8	5.8	*
Pseudoroegneria spicata			
Shoot (g)	0.82	0.79	NS
Root (g)	2.21	2.04	NS
Length (cm)	28.3	30.1	NS
Pascopyrum smithii			
Shoot (g)	1.81	1.66	NS
Root (g)	2.35	1.79	*
Length (cm)	55.8	61.8	*

[1] Significance (*) is indicated by $P < 0.05$.

[2] Grown for 10 weeks rather than 12 weeks because plants were starting to senesce in the small pots.

We hypothesized that inoculation with probiotic treatment derived from native soil would promote the growth of native species, particularly late seral natives. However, this was only the case with western wheatgrass and lobeleaf groundsel. It may be that early seral species are adapted to disturbed soils with weak microbial communities, and do not benefit from the soil microbial community present in late seral communities. It is also possible that 12 weeks is simply insufficient time for the microbial community in BEAM compost to colonize the rhizosphere to the extent that plant growth is affected by the treatment, regardless of sere.

CONCLUSION

For 5 out of the 7 species evaluated in this study, seed treatment with BEAM compost had no effect on shoot length, shoot weight, or root weight. Cheatgrass, an exotic early seral annual, was one of the species not affected by BEAM treatment. Lobeleaf groundsel, the only early seral native species to show a significant BEAM treatment effect, had significantly greater shoot length but not shoot or root weight. Western wheatgrass was the only late seral species to show a significant response to BEAM treatment, with greater root weight and smaller shoot length.

It is possible that 12 weeks is insufficient time for the microbial community in BEAM compost to colonize the rhizosphere to the extent that plant growth is affected. Further studies that evaluate the effects of BEAM compost on plant growth should be longer term field studies that last at least one growing season and contain a wider range of species. Additional measurements of soil health at the end of the study would confirm any changes to soil microbiology.

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APPENDIX A

PLFA test results for cheatgrass-dominated soil and BEAM compost (Ward Laboratories, Kearney, NE).

	Cheatgrass-Dominated Soil		BEAM Compost	
	Biomass, PLFA ng/g	% Total Biomass	Biomass, PLFA ng/g	% Total Biomass
Functional Group				
Total Bacteria	237	24.1	1221	40.1
Gram +	104	10.6	772	25.4
Actinomycetes	35	3.6	200	6.6
Gram -	133	13.6	449	14.7
Rhizobia	0	0	0	0
Total Fungi	73	7.5	451	14.8
Arbuscular Mycorrhizal	13	1.3	192	6.3
Saprophytes	60	6.2	258	8.5
Protozoa	0	0	0	0
Undifferentiated	672	68.4	1375	45.1
Total Living Microbial Biomass	982		3047	

Soil test results for cheatgrass-dominated soil, top 6 in of profile sampled on 23 June 2021 after cheatgrass senescence (Ward Laboratories, Kearney, NE).

pH	7.2
OM % (LOI)	1.7
NO_3-N (ppm)	3.7
NH_4-N (ppm)	6.7
P (ppm)	66
K (ppm)	333
Soil respiration CO_2-C (ppm C)	53.6

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