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Title: Hydromulch Tackifier and Sucrose Effects on Microbial Nitrogen and *Bromus tectorum* Biomass

Abstract approved:

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There is growing interest in using nutrient manipulations to control invasive plants such as *Bromus tectorum* L. (cheatgrass). Both labile (sucrose) and recalcitrant (straw and sawdust) carbon sources are added to the soil surface to reduce plant available soil N via soil microbial immobilization. However, the application rates used in previous research can be very high and application techniques are labor intensive. The goal of this research was to determine if established hydromulching technology could be adapted to nutrient-manipulation-based restoration projects to lower costs. Hydromulching is an established technology for treating large areas on a landscape. It requires the use of a tackifier to adhere the treatment to the soil surface. Three tackifiers are available commercially; guar, psyllium, and polyacrylamide (PAM). Tackifiers are long chain carbon compounds that could induce the same soil responses as other recalcitrant carbon compounds. The objectives of this study were

to investigate: 1) whether tackifiers alone or combined with two levels of a labile carbon source (sucrose) decreased cheatgrass biomass; 2) whether the tackifiers differed in their ability to reduce cheatgrass biomass; 3) the degree to which nitrogen immobilization occurs in soil under each tackifier; and each sucrose treatment; and 4) whether treatments affected cheatgrass emergence. Research was conducted in a glasshouse. Cheatgrass biomass was negatively correlated with soil microbial N, although cheatgrass and microbial N responded to different treatments. Cheatgrass biomass was not effected by any of the tackifiers, even though soil microbial N was. None of the treatments had any effect on cheatgrass emergence. If our results are supported in field experiment, hydromulching technology may easily be adapted to nutrient manipulation based restoration projects considerably lowering costs.

Hydromulch Tackifier and Sucrose Effects on Microbial Nitrogen and *Bromus tectorum* Biomass

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Lora B. Perkins, Author

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Hydromulch Tackifier and Sucrose Effects on Microbial Nitrogen and *Bromus tectorum* Biomass

INTRODUCTION

There is a wave of ecological research focusing on biogeochemical cycles and nutrient manipulation as a means of reducing invasive plants (McLendon and Redente 1992; Morghan and Seastedt 1999; Alpert and Maron 2000; Paschke et al. 2000; Torok et al. 2000; Blumenthal et al. 2003; Monaco et al. 2003; Corbin and D'Antonio 2004; Eschen et al. 2006). Most often, a carbon source is added to the soil to reduce invasive plant biomass and, by extrapolation, competitive ability. Treatments often use labile carbon sources such as sucrose (McLendon and Redente 1992; Morghan and Seastedt 1999; Paschke et al. 2000; Blumenthal et al. 2003; Witwicki 2005; Eschen et al. 2006) and more recalcitrant carbon sources such as straw, sawdust or bark (Zink and Allen 1998; Monaco et al. 2003; Corbin and D'Antonio 2004) to reduce plant available N via microbial immobilization. Ruderal nitrophilic plants, which includes many invasive plants, should have a more negative response to the decreased amount of plant available N than late-successional non-nitrophilic species (Paschke et al. 2000).

Cheatgrass (*Bromus tectorum* L.) was introduced in the western U.S.A. in the late 1800's and has expanded to over 10.1 million ha (Pellant et al. 2004). Its persistence in the Great Basin provides ample opportunity to study the control of an invasive ruderal species via nutrient manipulations. The history of attempts to restore native vegetation in this region is nearly as long as the invasion history itself,

beginning in the early 1900's with reseeding and livestock management (Pellant et al. 2004). Many other restoration methods have been attempted, such as reseeding of both non-native (Stewart and Hull 1949) and native plants (Pellant et al. 2004) and herbicide application (Whitson and Koch 1998).

Cheatgrass has several traits that have both enabled its range expansion and made restoration a formidable challenge. Cheatgrass is a cool season annual grass that gains dominance of a site before native plants are actively growing (Harris 1967). It quickly overwhelms the native seed bank (Humphrey and Schupp 2001), with individual plants capable of producing up to 555 viable seeds (Mack and Pyke 1983). Cheatgrass is nitrophilic with both biomass and density responding positively to increasing soil nitrogen (Monaco et al. 2003; Beckstead and Augspurger 2004; Witwicki 2005). The accumulation of the fine dry litter that is left by cheatgrass can increase wildfire frequency 10-500 times (Hull 1965) to the eventual exclusion of native vegetation (Mack 1981). Once cheatgrass dominates, these areas have experienced a relatively irreversible transition with little probability of autogenic recovery. This altered landscape offers lower native species biodiversity, reduced wildlife habitat and reduced ecosystem services such as soil and nutrient retention (Knapp 1996).

Previous nutrient manipulation experiments have accomplished carbon addition using large doses of sucrose (greater than 1 kg m^{-2}; Corbin and D'Antonio 2004) along with sawdust either broadcast or incorporated into the soil. The quantity of materials and difficulty of application could push these treatments out of the price

range for a practical restoration project. Other large-scale soil applications, such as reseeding after wildfires and erosion control on road-cuts have used hydromulching technology (Paschke et al. 2000; Flanagan et al. 2002; Graber et al. 2006). Hydromulching treatments commonly include a slurry of water, treatment (seeds or mulch) and tackifier. The slurry can be sprayed from helicopters, airplanes or trucks. Tackifiers provide adhesion between the treatment and the soil. Three commonly used tackifiers include guar, psyllium and polyacrylamide (PAM). PAM and guar have been used as soil conditioners and stabilizers in agriculture since the 1950's and have been approved for sensitive uses like food processing and water treatment by the FDA and EPA (Sojka and Lentz 1994). Chemically, all three tackifiers are complex long-chain carbon compounds with only PAM containing any nitrogen (Holliman et al. 2005).

The broad research question of this study is can this established technology be manipulated into a practice for cheatgrass control. The chemical composition of these tackifiers raises the question can these tackifiers be used as recalcitrant carbon sources instead of sawdust or straw? The research objectives were 1) to investigate whether tackifiers alone or combined with sucrose decreased cheatgrass biomass; 2) to investigate whether the three tackifiers differed in their ability to reduce cheatgrass biomass; 3) quantify nitrogen immobilization in the soil under each tackifier and sucrose treatment; and 4) measure any effects of treatments on cheatgrass emergence.

MATERIALS AND METHODS

Experimental design

Cheatgrass seeds and soil were collected south of Vale, on Lincoln Bench (43°54’25’’N,-117°9’27’’W; elevation 927m.) in Malheur County, eastern Oregon. Average annual precipitation is estimated to be 28.5 cm and cheatgrass makes up more than 60% of the vegetative cover (Hempy-Mayer 2004). Seeds were collected in June 2005, cleaned and kept in dry storage in the laboratory at room temperature. Tests established a germination rate of 84%.

The top 10 cm of soil was collected November 2005 and kept covered in large storage tubs in the walk-in cooler until use. While there are no published soil surveys of this area, this depth of soil is characterized as a non-sticky silty clay loam (Hempy-Mayer 2004) with a C:N of 11.1 (Witwicki 2005). To counteract excessive compaction caused by handling and watering in a glasshouse environment, the soil was mixed with coarse sand. Sand was sterilized in an autoclave for 3 hr, allowed to cool, and then mixed with the soil at a ratio of two parts soil to one part sand by volume in a clean cement mixer until visually homogenous. After mixing, the soil was put into sterile 3.5 l plastic pots (approximately 15.5 cm upper diameter and 18 cm depth) and placed in a glasshouse in Corvallis, OR on 10 December 2005. For the duration of the experiment, the glasshouse was kept at 15-18 °C under natural light conditions. Pots were kept approximately at field capacity by daily hand-watering with a hose-end mister using tap water. Treatments were withheld for 30 days to

allow for germination and removal of any seedlings that emerged from the soil seed bank.

After seed bank germination was complete, three cheatgrass seeds were pushed lengthwise into the soil in each pot until the hypocotyl end of the caryopsis was even with the soil surface. Seeds were planted equidistant within a centimeter of the center of the pot. Pots were labeled and located in a randomized block design to help control for any environmental variation in the glasshouse.

Three tackifier treatments, a guar-based, a psyllium-based and a polymer-based product (Guar gum, R-tack and Rantec RT 9528 Polymer) were obtained from Rantec Corporation, Ranchester, Wyoming. Guar tackifier is powdered *Cyamopsis tetragonalobus* beans. The manufacture's average recommended application rates is 104 kg/ha or 0.19 g/pot for the pot sizes used. The psyllium tackifier is powdered seed husks from either *Plantago ovata* or *Plantago ispaghula*. The manufacture's average recommended application rate is 150 kg/ha or 0.44 g/pot. The polymer tackifier is an anionic polyacrylamide organic polymer (PAM) with the manufacture's average recommended application rate being 7.7 kg/ha or 0.015 g/pot. These tackifier treatments were factorially combined with sucrose in application amounts of 0, 200 kg/ha (84 kg C/ha), and 800 kg/ha (336 kg C/ha) (Table 1) with 15 replicates per treatment. Scaled to the surface area of the pot, the sucrose treatments were 0.345 g/pot and 1.424 g/pot, respectively. Treatments for individual pots were dissolved in 68 ml tap water to mimic the same tackifier-to-water ratio used in the field and poured evenly over the soil surface of seeded pots that were at field capacity.

Table 1. Summary of factorial design of carbon treatments.

Treatment	Sucrose	tackifier
Control	0	0
S1	200 ka/ha	0
S2	800 ka/ha	0
T1	0	Guar
T1S1	200 ka/ha	Guar
T1S2	800 ka/ha	Guar
T2	0	Psyllium
T2S1	200 ka/ha	Psyllium
T2S2	800 ka/ha	Psyllium
T3	0	PAM
T3S1	200 ka/ha	PAM
T3S2	800 ka/ha	PAM

The date of seedling emergence was recorded when the first shoot was visible above the soil surface. Subsequent cheatgrass seedlings as well as other seed bank species that emerged were removed with tweezers and discarded. One cheatgrass plant was allowed to grow per pot.

Leaf number and maximum leaf length were recorded at both 30 and 60 days after planting. At 90 days, leaf number and height were recorded and all above ground biomass was harvested, dried at 60° C for 48 hr and weighed to the nearest mg.

Immediately after the final biomass was harvested, 3 soil cores (4 cm X 4 cm) were taken from each pot, mixed and kept chilled until use in sealed plastic bags. Each sample was put thought a sterile 2mm sieve and divided into 10 g samples each for determining gravimetric water content, available soil N and microbial N. Gravimetric water content was determined by weighing the soil samples before and after drying at 105°C for 48 hours and calculated as ((wet-dry)/dry).

For available soil N determinations, soil samples were extracted with K_2SO_4 within 3 days of collection, placed on a shaker table for 1 hr, filtered, capped and frozen. Samples were prepared for total N analysis using the chloroform extraction method (Horwath and Paul 1994). Soil (10 g) was incubated for three days in chloroform at room temperature and then extracted with K_2SO_4 as explained above. All extracts were digested with potassium persulfate for 50 min in an autoclave at 120°C to transform all nitrogen species to nitrate for analysis using the QuikChem Method 10-107-04-1-L for analysis of Nitrate/Nitrite on a Lachat QuikChem8000 (Lachat Instruments, Milwaukee WI). Microbial N was determined by subtracting the available N from the total N.

The effect of sucrose and tackifier on cheatgrass biomass and microbial N were tested separately using ANOVA (S-plus, 6.1). Parameters included the block location in the glasshouse, the amount of sucrose, presence of tackifier and an interaction between sucrose and tackifier. Microbial N was natural-log transformed to meet the assumptions of the model. Differences between all pairwise comparisons were determined using the Scheffe method.

To quantify an allometric relationship between leaf number, maximum leaf length and biomass, an additional set of 45 cheatgrass plants were grown in 3 different soil types (coarse sand, soil collected from Lincoln Bench and a planting mix available at the glasshouse). The growing conditions were the same as described above. A third of the plants were used at 30 days, 60 days and 90 days after planting. Each plant was measured for leaf number and maximum leaf length and then the above ground biomass was harvested and dried at 60° C for 48 hrs. Data were analyzed with both ANOVA and a mixed effects linear regression (S-plus 6.1). Separate linear regression models were derived for 30 and 60-days to predict biomass using leaf number and maximum leaf length. These models were then applied to the large data set to help elucidate if there was a change in cheatgrass biomass though time due to the carbon treatments.

RESULTS

Both tackifier and sucrose treatments had significant effects on microbial N ($p < 0.01$) but no significant interaction existed between the two treatments ($p=0.262$). Only the high level of sucrose (800 kg/ha) and the psyllium tackifier increased microbial N significantly over the control (fig.1).

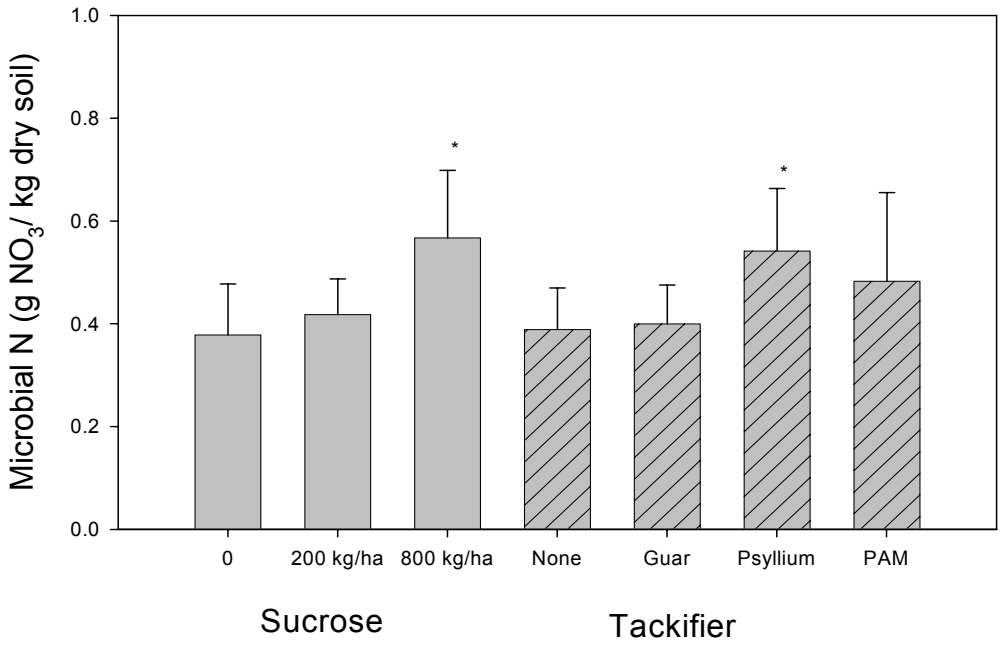

fig.1. . Microbial N under sucrose and tackifier treatments. Bars represent 95% CI and * indicates a significant difference from the control.

Both sucrose levels lowered cheatgrass aboveground dry biomass by a mean of 0.38 g/plant (95% C.I. 0.203-0.572) (fig. 2). However, none of the other variables (block, tackifier and sucrose X tackifier) had a significant effect on cheatgrass biomass (min. p-value 0.244).

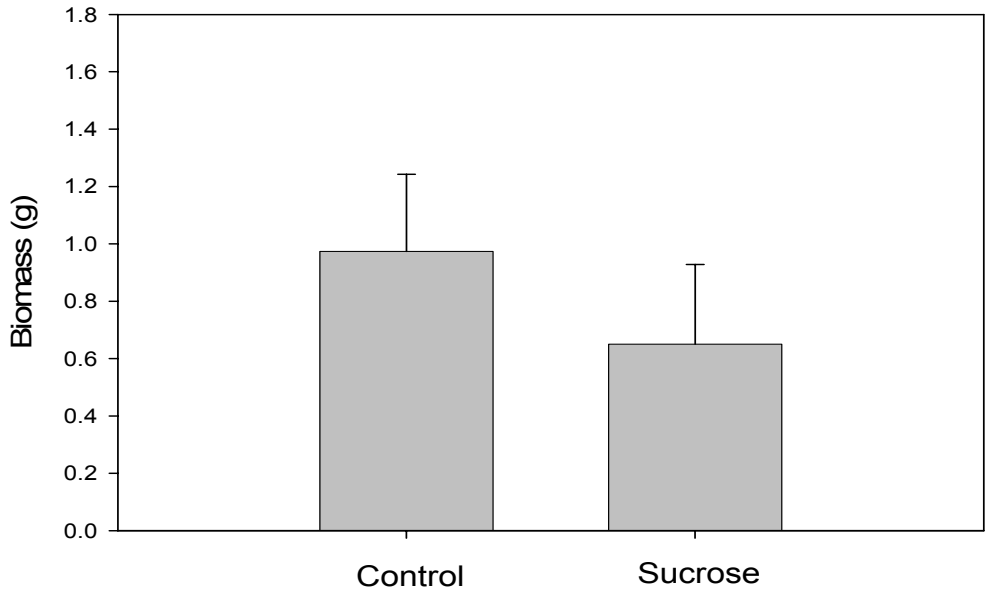

fig. 2. Grams of cheatgrass dry biomass per plant with and without (control) sugar (mean of both sugar levels) applied to soil surface. Bars indicate 95% CI.

A linear mixed effects model regression of cheatgrass aboveground biomass on microbial N revealed a negative correlation ($p=0.004$). As soil microbial N increased by 1 mg/ kg soil across our treatments, the mean cheatgrass biomass decreased by 0.3816 g. (95% C.I. 0.1233- 0.6399) (fig. 3).

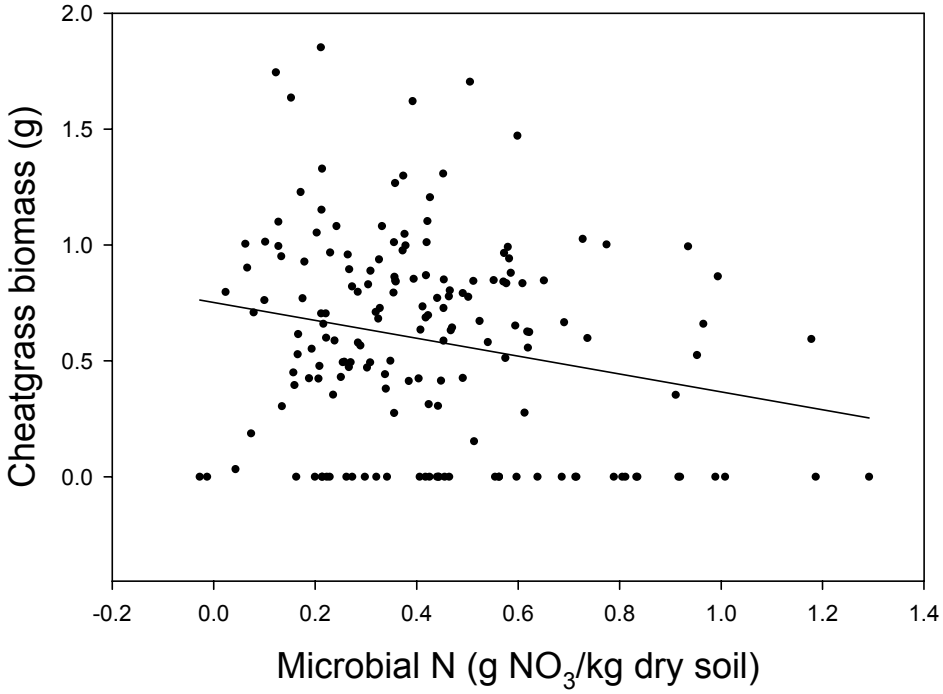

fig. 3. Regression of cheatgrass biomass (g) with soil microbial N (mg/kg soil).

Time of emergence was not impacted by tackifiers ($p = 0.94$), sucrose ($p = 0.32$), or sucrose X tackifier interaction ($p = 0.33$). Mean emergence occurred 7.6 days after planting (95 % CI ± 0.2 days).

Allometric model to predict aboveground biomass using height and leaf number were derived using data from the extra set of cheatgrass for 30-day and 60-day harvests separately (R^2= .944 at 30-day and 0.936 for 60-day). These models were then applied to the original set of cheatgrass to derive a surrogate value for biomass at 30-days and 60-days. Using the output from these models as a proxy for biomass, only sucrose had a significant effect ($p=0.004$ for 30-day and $p=0.001$ at 60-days) and

no response to tackifier treatments ($p=0.07$ for 30-day and $p=0.12$ for 60-day) was found. Subtle changes might not have been picked up in the model that would have been evident if 30 day and 60 day biomass was actually measured.

DISCUSSION

Increased soil C is often related to increased N immobilization (Barrett and Burke 2000). Labile forms of carbon, such as sucrose, will be utilized quickly and are thought to produce only a short-term effect (Torok et al. 2000; Blumenthal et al. 2003). In an attempt to extend this effect, more recalcitrant forms of carbon (e.g., sawdust, straw, wood chips) are often added to the soil (Whitford et al. 1989; Torok et al. 2000; Zink and Allen 1998). Tackifiers are readily available materials that may provide a source of recalcitrant carbon while also providing soil stabilization.

The reductions in biomass we observed associated with sucrose application are similar to those seen in other studies (Morghan and Seastedt 1999; Alpert and Maron 2000; Blumenthal et al. 2003; Eschen et al. 2006) and are concurrent with other studies looking specifically at cheatgrass (Monaco et al. 2003; Beckstead and Auspurger 2004; Witwicki 2005). Unexpectantly, the highest level of sucrose did not result in further reductions in aboveground biomass than those caused by the lower level, although others have seen inverse relationships with increasing concentrations of sucrose (Blumenthal et al. 2003). The amount of carbon needed might depend on initial soil fertility (Blumenthal et al. 2003) and the fertility of the soils we used was low even before being diluted with sand. If our finding that even a relatively low sucrose addition caused a substantial decrease in cheatgrass biomass is also observed in field studies, the cost of labile carbon additions in restoration projects might be dramatically reduced.

Both sucrose and the psyllium tackifier increased soil microbial N. Although the microbial N values observed with psyllium addition were similar to those seen at the highest sucrose dose after 90 days of growth, microbial N values under the psyllium treatment may have risen relatively late in the growth cycle of cheatgrass. This could allow cheatgrass the opportunity to access available N before psyllium induced immobilization by the soil microbes. It is possible psyllium was the only tackifier that increased microbial N because the other tackifiers were not microbially available or had effects on the soil microbial community that did not result in an increase in nitrogen immobilization. The exact structure of the psyllium mucilage is still under debate. It seems to be a complex ring and branch polysaccharide system of arabinose and xylose with a very high molecular weight (Fischer et al. 2004). Guar tackifier is a galactomannan polymer consisting of galactose and mannose monomers with a medium molecular weight (Graber et al. 2006). Guar tackifier's efficacy as a soil conditioner is reported to be shorter lived in the field than PAM (Wallace 1986). PAM, most often used in agricultural settings to promote soil aggregate stability, is a long polymer of acrylamide monomers with a medium to high molecular weight (Graber et al. 2006). In a lab setting, PAM has promoted growth in *Pseudomonas* spp. and some sulfur reducing bacteria (Barvenik 1994) but in the field is reported to be resistant to microbial degradation (Seybold 1994). The lack of effect of PAM on our soil microbial N in this study may result from its resistance to microbial degradation. Being ground seed husks, psyllium could be the most physically similar to other

recalcitrant carbon compounds such as straw and bark that cause similar increases in soil microbial N (Whitford et al. 1989; Zink and Allen 1998).

Why did the microbial N show a response to only the higher level of sucrose and to psyllium treatments while both low and high levels of sucrose addition, but no tackifier, reduced cheatgrass biomass? Most investigations with nutrient manipulations for invasive plant control have the underlying assumption that microbes are better competitors than plants for soil nitrogen (McLendon and Redente 1992; Morghan and Seastedt 1999; Alpert and Maron 2000; Paschke et al. 2000; Blumenthal et al. 2003; Monaco et al. 2003; Corbin and D'Antonio 2004). In arid ecosystems after water, soil microbial activity is carbon-limited and responds to additions of carbon with cellular division and community growth, thus immobilizing nitrogen (Whitford et al. 1989). However, in both arid and mesic systems, soil microbes are not always the better competitor than plants for nitrogen (Kaye and Hart 1997; Wang and Bakken 1997; Hodge et al. 2000; and Schimel and Bennett 2004). There are many other issues such as the magnitude, composition and efficiency of the soil microbial community as well as successional changes and trophic interactions that are possibly stimulated by the addition of carbon (Bardgett et al. 2005; Wolf and Wagner 2005) that can influence the outcome of the competition for N by microbes vs. plants. Microbial activity levels rather than simple population size might also be highly important for decomposition and mineralization (Whitford et al. 1989). Significant cheatgrass decrease has been shown after sucrose addition without any detectable difference in the soil microbial N (Witwicki 2005). This lends support to the idea that

our measure of soil microbial N might be too rough a measure to give us any more than a vague glimpse at what is really happening. Molecular methods such as PLFA (phospholipid fatty acid analysis) (Bottomley 2005) and T-RFLP (terminal restriction fragment length polymorphism) analysis (Kuske et al. 2002; Wawrik et al. 2005) could provide insight into soil microbial dynamics and help explain the underlying mechanism.

PAM can increase percent emergence of crop plants (Cook and Nelson 1986) and range grasses *Panicum anlidotale* and *Bouteloua curtipendula* but it had no effect on emergence of *Bothricochloa ischaemum* and *Sporobolus airsoides* (Rubio et al. 1992). We found no effect of any treatment on time to emergence.

Despite the uncertainty surrounding how exactly nutrient manipulations decrease cheatgrass, there is mounting evidence that manipulations of nutrient cycling through carbon application negatively affect cheatgrass biomass (Monaco et al. 2003; Beckstead and Augspruger 2004; Witwicki 2005). Cheatgrass biomass is highly correlated with its seed production (Hulburt 1955) and cheatgrass seeds have low year-to-year reserve in the seed bank (Pyke 1994). While native plant responses to carbon additions have been mixed, controlling cheatgrass production for a short time could open up space for native plant establishment.

Refinement of both carbon dose and application procedures to a realistic approach is needed if it is going to be incorporated into a restoration strategy. Hydromulch technology is readily available for treating large areas and can easily be adapted for nutrient manipulation treatments. Of the hydromulch tackifiers available, psyllium

might be the best choice for microbial stimulation in place of or in addition to other recalcitrant carbon sources. Sucrose requirements might be tied to original site fertility (Blumenthal et al. 2003) and further dose responses should be established in the field.

CONCLUSION

Nutrient manipulations especially carbon applications are emerging as a promising restoration tool for controlling ruderal nitrophilic invasive plants including cheatgrass. Adjustments of treatments and refinements of application techniques from the experimental setting must be made if these techniques are going to be used in a practical setting. Treatment cost is also an issue. The high levels of carbon and labor intensive application procedures that have been applied in experimental settings can be cost prohibitive. Our results show a substantial effect with lower sucrose levels than in previous experiments, if supported in field studies, is one step in helping lower treatment costs. Psyllium tackifier shows some promise of helping to increase soil microbial N and could further reduce treatment costs as a substitute for sawdust or straw as a more recalcitrant carbon source. Hydromulching is an established technology that can easily treat large land areas and is easily modified for nutrient manipulation.

Recommendations for future research include field studies for dose response and investigation of possible extended effects of the tackifiers that were not seen in the 90 days of this study. It is also possible that abiotic factors like UV rays and increased soil moisture fluctuations found in the field could influence tackifier effects on both cheatgrass biomass and microbial N.

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