DISSERTATION

INVESTIGATIONS OF *BROMUS TECTORUM*: RESTORATION STRATEGIES AND INTERACTIONS WITH ARBUSCULAR MYCORRHIZAL FUNGI

Submitted by

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Graduate Degree Program in Ecology

In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Spring 2006

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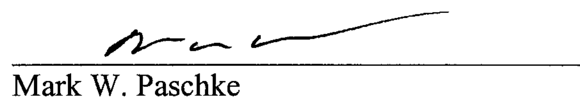
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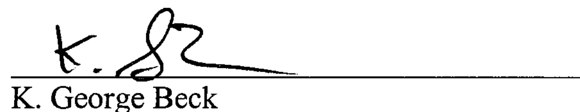
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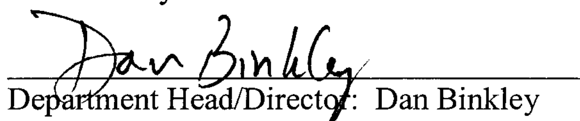
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ABSTRACT OF DISSERTATION

INVESTIGATIONS OF *BROMUS TECTORUM*: RESTORATION STRATEGIES AND INTERACTIONS WITH ARBUSCULAR MYCORRHIZAL FUNGI

Bromus tectorum (L) (cheatgrass, downy brome), one of the most pervasive weeds in the United States, reduces native species diversity and transforms habitats. I conducted four experiments designed to better understand *B. tectorum* ecology and evaluate control and restoration strategies. I measured the responsiveness of six native plants and *B. tectorum* to field and commercial sources of arbuscular mycorrizal fungi (AMF) inoculum. *Bromus tectorum* and early successional plant species were negatively responsive and late successional species were positively responsive to field AMF inoculum, while commercial inoculum was ineffective.

I compared mycorrhizal inoculum potential of field soils from beneath native plants surrounded by *B. tectorum* and the same species surrounded by native vegetation. I found that *B. tectorum* was associated with diminished AMF. Next I tested responses of two native plant species when grown in soils "trained" by *B. tectorum* in a greenhouse. Spore counts, percent root colonized, and final biomass of the native plants were not different amongst *B. tectorum* and native plant trained soils, indicating that *B. tectorum* did not directly affect the AMF community compared with other native plants.

To address decreased AMF found associated with *B. tectorum* soils, I conducted a field experiment that included soil community and sucrose additions with native seed applications to improve native species growth and establishment. Sucrose reduced both

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B. tectorum and other annual plant species abundance and richness. Although soil community addition reduced *B. tectorum*, it did not appear to increase native perennial species.

In a second field experiment, I reduced *B. tectorum* cover to less than 5% with glyphosate and added different seed mixtures based on successional models of tolerance and facilitation. This tested an "ecological bridge" approach in which early successional species replace invasive species and allow succession to proceed. There were no seed treatment differences amongst late successional plant species establishment. The native plant species included may have been marginally effective at forming an "ecological bridge" for other late successional species, but none of the seeding treatments inhibited *B. tectorum* re-invasion.

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PREFACE

The chapters in this dissertation are formatted for submission to scientific journals as follows:

Chapter 1: Rowe, H. I., Brown, C. S., and Claassen, V. P. Comparisons of mycorrhizal responsiveness with field soil and commercial inoculum for six native montane species and *Bromus tectorum*. Restoration Ecology (accepted 2006).

Chapter 2: Rowe, H. I., and Brown, C. S. *Bromus tectorum* associations with arbuscular mycorrhizal fungus community change: causation or correlation? Ecology Letters.

Chapter 3: Rowe, H. I., Brown, C. S., and Paschke, M. W. Testing the influence of soil communities as a restoration strategy in *Bromus tectorum* dominated field sites. Journal of Applied Ecology.

Chapter 4: Rowe, H. I., and Brown, C. S. Testing succession-based seeding for restoration of montane communities invaded by cheatgrass. Restoration Ecology.

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Chapter 1: Comparisons of mycorrhizal responsiveness with field soil and commercial inoculum for six native montane species and *Bromus tectorum*

Abstract

Re-establishing native perennial plants and reducing invasive species are pivotal for many ecological restoration projects. The interactions among plant species, arbuscular mycorrhizal (AM) fungi, and soil P availability may be critical determinants of the success of native and non-native plants in restoration and species invasions. Here we assessed mycorrhizal responsiveness for three late-successional and three early-successional plant species native to Rocky Mountain National Park and for the non-native *Bromus tectorum* L. (downy brome, cheatgrass) using field soil and commercial inoculum. Factorial greenhouse experiments were conducted to compare biomass of plant species with and without field soil and commercial inoculum treatments along a phosphorus (P) gradient, which ranged from ambient field levels to 12% of field levels, using dilutions of native soils. The two field soil inoculum treatments resulted in significant biomass differences for all species studied. Late-successional species responded positively to field inoculum, whereas, early-successional species responded negatively. The two commercial inocula had low colonization rates (14 out of 166

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inoculated plants). The commercial inocula substrates had significant treatment effects on five of the seven species included in the study in the apparent absence of mycorrhizal symbiosis. Soil P levels influenced mycorrhizal responsiveness in only one species, *Aster laevis* L. (smooth blue aster). Our results show that, at least for the species studied here, locally collected field inoculum is the best choice for re-establishment of late successional, native plant species.

Keywords: restoration, field inoculum, commercial inoculum, native species, *Bromus tectorum*, mycorrhiza

Introduction

Ecologists have recognized the importance of arbuscular mycorrhizal fungi (AM fungi) in restoration projects for decades (Reeves et al. 1979; Allen 1984; Miller 1987; Allen 1988; St. John 1998). The relative success of plant species on a disturbed site depends on the availability and identity of AM fungi on the site (Allen 1984; Miller 1987; Allen 1988; Allen 1995). Whether one is seeding or cultivating plants for out planting, knowing the degree of responsiveness to AM fungi can greatly improve establishment success (Gemma et al. 2002).

Researchers have quantified the degree of mycorrhizal responsiveness for over 140 wild grasses and forbs (Tawaraya 2003), native species in Hawaii (Gemma et al. 2002), and tall grass prairie species (Wilson & Hartnett 1998). Comparisons of 95 tallgrass prairie grasses and forb species found that warm season grasses and forbs were more responsive than cool season grasses. Grasses and non-leguminous forbs responded

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positively, whereas, legumes responded negatively to AM fungi (Wilson & Hartnett 1998). Many researchers have noted that late-successional species are generally more responsive to mycorrhizal colonization than early-successional plant species (Allen 1984; Allen & Allen 1984; Reeves 1985; Miller 1987; Allen & Allen 1988; Allen & Allen 1990; Reeves & Redente 1991; Allen 1995; Wilson & Hartnett 1998; Hart et al. 2001; De Deyn et al. 2003; Klironomos 2003). Still, many species have yet to be tested for mycorrhizal responsiveness, including many sub-alpine species.

Because the goal of ecological restoration is often to improve conditions for late-successional species, managers are increasingly aware of a need to ensure a healthy AM fungi community. Mycorrhizal responsiveness is the appropriate measure to estimate how a plant will respond to an increase in AM fungi diversity and abundance (van der Heijden 2002). Relative mycorrhizal responsiveness can be calculated by comparing the growth of a single plant species with and without AM fungi inoculum (Plenchette et al. 1983) or it can be expanded to compare different AM fungi treatments (Klironomos 2002). Mycorrhizal sensitivity, in contrast, has been defined as the variation in responsiveness of plant growth when associated with different AM fungi (van der Heijden 2002).

AM fungi may be a significant factor in success of some invasive plants (Marler et al. 1999; O'Connor et al. 2002; Jin et al. 2004; Carey et al. 2004; Fitter 2005). The mycorrhizal responsiveness of an invader may help predict whether AM fungi facilitate invasion. For example, a low or negative mycorrhizal responsiveness may suggest that a plant will have higher success in a disturbed community with fewer AM fungi propagules and not be as competitive in later seral conditions with a healthy mycorrhizal community

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(sensu Fitter 2005). A plant can have different responsiveness depending on the AM fungi present (van der Heijden et al. 1998; Klironomos 2003) and field soil P levels (Johnson 1993; Johnson 1998). If AM fungi are to be re-introduced for the purposes of restoration, it is imperative to know the responsiveness of the target native species and local invasive plant species on a site.

Although *Bromus tectorum* is a relatively recent invader to our study sites in the sub-alpine regions of Rocky Mountain National Park (RMNP, CO, USA), it occupies approximately 20% of the sagebrush-steppe vegetation zone in the Western U.S., to the point where the establishment of native perennial species is difficult (Knapp 1996). As a winter annual, fall and early spring growth allows *B. tectorum* to gain a competitive advantage over slower growing, native perennial species (Hulbert 1955). *Bromus tectorum* has been shown to have a negative to neutral growth response with AM fungi colonization (Allen 1988). It was found to have a mycorrhizal sensitivity of 4% with fungal species *Glomus etunicatum* at a soil P level of 5-10 mg kg-1 (Wilson & Hartnett 1998). Because of encroachment of *B. tectorum* into the park ecosystem, information regarding effect of restoration treatments on invasive versus native species is expected to improve vegetation management in the park.

For native plant community restoration, the source of AM fungi can be important (see Klironomos 2003; Moora et al. 2004). In some revegetation projects, native inoculum may not be available or commercial inoculum may be perceived to be more effective than native inoculum. Are the strains offered by commercial mycorrhiza dealers the most beneficial or appropriate for native plants? Information regarding the

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mycorrhizal responsiveness of plant species using commercial inoculum will help guide decisions regarding appropriate inoculum source.

The goals of this research were: (1) to compare the mycorrhizal responsiveness of six plant species native to RMNP plus *B. tectorum*, using native and commercial inocula, (2) to assess whether the generality that late-successional species are more dependent on AM fungi than early-successional plant species holds for these seven species, and (3) to test the influence of P availability on mycorrhizal responsiveness for each species.

Methods

Experimental design

We achieved our three goals through testing field soil mycorrhizal responsiveness (Experiment 1) and a commercial inoculum responsiveness (Experiment 2). Separate mycorrhizal inoculation potential trials (Experiment 3) tested the viability of the commercial inoculum used in Experiment 2. The mycorrhizal responsiveness experiments were conducted as two separate trials due to irreconcilable nutrient differences amongst the field soil and commercial inoculum treatments. Each mycorrhizal responsiveness experiment was a complete randomized design with three AM fungi treatments, three P levels, and seven plant species with four replications (252 experimental units).

Soils

Soils were collected from a stockpile of topsoil excavated during a road construction project along Bear Lake Road in RMNP. Soil nutrient levels were compared

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between soils from this stockpile and natural field soils taken from long-term field plots located 1 km away. The stockpiled soil (43 mg kg^{-1} P) had twice the level of phosphorus as the natural field soil (21.7 mg kg^{-1} P) as indicated by weak Bray I extracts for plant available P. Three P levels (P1, P2, and P3) were prepared by diluting the stockpiled soil with sterile, nutrient-poor, coarse sand that contained approximately 1 mg kg^{-1} P.

Plant species collection and seral stage categories

All native seeds were collected within a mile of the inoculum collection sites at elevations of approximately 2,377-2,743 m (7800 – 9000 ft). The *Bromus tectorum* seeds were collected from populations found at 2,000-2750 m (6,562-9,022 ft). Late successional species were characterized as perennial and long-lived and included *Artemisia frigida* Willd. (fringed sage), *Aster laevis*, *Chrysothamnus viscidiflorus* (Hook.) (yellow rabbitbrush), and *Muhlenbergia montana* (Nutt.) Hitchc. (mountain muhly). Early successional species were mostly annual (*B. tectorum,* and *Lappula redowskii* (Hornem.) (flatspine stickseed)), but the short-lived perennial *Elymus elymoides* (Raf.) Swezey (squirreltail) was also included in this category.

Mycorrhizal inoculum

Soil inoculum for the field inoculum experiment was collected from the rhizosphere of a mixed stand of native plants (primarily *Artemisia tridentata* Nutt. (big sagebrush), *Purshia tridentata* (Pursh) DC (antelope bitterbrush), and *M. montana*) in an area with no non-native plants in RMNP at an elevation of 2,540 m (8,339 ft). For the commercial inoculum experiment, two sources were used: a *Glomus intraradices*

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inoculum in a granular form (hereby ComA) and a powdered mixture of seven AM fungi taxa, which included *Glomus mosseae* (20%), *G. intraradices* (20%), *G. fasciculatum* (20%), *G. dussii* (10%), *G. clarum* (10%), *G. deserticola* (10%), and *G. microaggregatum* (10%) (hereby ComB). Application rates were based on manufacturer recommendations (ComA advised four propagules per plant at 20 propagules per gram of inoculum and ComB advised 1 g inoculum per 6.6 plants). Nine of the 84 ComB inoculum pots received 0.58 g of ComB instead of the recommended 0.2 g due to a measurement error.

An additional treatment was added to the field soil inoculum experiment to test for additive effects of commercial inoculum with field soil inoculum. Field inoculum experiment treatments included field soil inoculum only, field soil inoculum+ComAB, and an autoclaved field soil inoculum control. The commercial inoculum experiment treatments were ComA, ComB, and an uninoculated control (no additions).

To evaluate the potential for non-mycorrhizal effects from commercial inoculants, a nutrient analysis was performed on ComA (Modified (DTPA) saturated media extract, A&L Western Agricultural Laboratories, Modesto, CA). We had insufficient volume to perform the analysis for ComB.

Stockpiled soils for use as non-AM fungi potting substrate and soil inoculum controls were autoclaved, allowed to cool overnight, and autoclaved again at 121 °C for 1 hour. We incubated soils with filtrate at room temperature for two weeks to re-introduce soil microbes and stabilize nutrient levels (B. Reeves, 2004, Colorado State University, Fort Collins, CO, personal communication). The filtrate was prepared by passing a

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mixture of 2.20 L of non-autoclaved field inoculum soil and 9 L of deionized water through Whatman # 1 filter paper (Gemma et al. 2002).

Nutrient and AM fungi treatment preparation

Tests for response to P and AM fungi were done in 4.3 x 20.6 cm conetainers (pots containing 164 ml soil volume; Steuwe and Sons, Corvallis, OR, USA). We partially filled conetainers with sterilized sand and autoclaved stockpiled soils to create the three P level treatments for Experiments one and two. For the P1 treatments, which were intended to represent field soil P levels, a 1:1 ratio of sterilized sand to autoclaved stockpiled soil was added to conetainers. To simulate lower P levels, sand was added at a ratio of 1:5 (33 % of field level P1; Treatment P2) and 1:15 (12 % field level P; Treatment P3).

We constructed the AMF treatments by adding either field soil combined with sand (Experiment 1) or commercial inoculum (Experiment 2) to the partially filled conetainers. The upper half of the field soil treatment conetainers were filled with 31 ml of field inoculum mixed with 31 ml sterile sand (field soil treatment), or 31 ml of field inoculum mixed with 31 ml sterile sand plus 0.1515g ComA and 0.2g ComB (field+ComAB treatment), or 31 ml of autoclaved field inoculum mixed with 31 ml sterile sand (field control treatment). The commercial inocula treatments were constructed by adding 0.1515g ComA (ComA treatment), or 0.2g ComB (ComB treatment) or nothing (commercial control treatment) to the mostly filled conetainers. Field inoculum treatments P2 and P3 contained 8.35 mg kg^{-1} and 6.25 mg kg^{-1} bicarbonate extractable P in each respective treatment. Commercial inoculum treatments

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contained approximately 6.7 mg kg^{-1} and 2.5 mg kg^{-1} extractable P for P2 and P3, respectively.

Greenhouse

Artemisia frigida, *As. laevis*, *B. tectorum*, *C. viscidiflorus*, *M. montana* species were seeded and *L. redowskii* and *E. elymoides* were germinated in growth chambers and transplanted. Plants were thinned to one per pot. Racks of conetainers were randomized weekly. Plants were hand watered to field capacity five days each week and were fertilized (0.2 mg/L KNO_3 and 7.25 mg/L NH_4NO_3) to field capacity two days each week, except during the fifth and seventh weeks when plants were watered on five days and fertilized one day. Each species grew until maturity or until a growth effect was evident: *As. laevis*, 111 days; *Ar. frigida*, 121 days; *B. tectorum*, 103 days; *C. viscidiflorus*, 123 days; *E. elymoides*, 115 days; *M. montana*, 121 days; *L. redowskii*, 89 days. When some of the *L. redowskii* and *E. elymoides* transplants did not survive, seeds were planted and reseeded plants were grown seven days longer than transplants in order to provide equivalent growth time.

Calculation of mycorrhizal responsiveness

Mycorrhizal responsiveness for each plant in the field inoculum experiment was calculated using a ratio comparing colonized and uncolonized plants as follows: ln (total dry weight of AM inoculated plant/total dry weight AM control plant). Pairs of colonized and uncolonized plants within a nutrient treatment were randomly matched for this calculation. A positive mycorrhizal responsiveness value indicates a beneficial effect

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of the AM fungi, while a negative value indicates that the AM fungus is acting as a parasite on the plant. By calculating the mycorrhizal responsiveness for each colonized plant, variation of both control and inoculated treatments has been retained and mycorrhizal responsiveness can be compared statistically as a response variable.

We modified the mycorrhizal responsiveness equation for the commercial inoculum experiment to control for possible nutrient elevation in the ComA and ComB treatments. Modified saturated media extract results showed that P, Ca, Na, and B were elevated in ComA compared to the ambient potting media (Table 1.1). To avoid a non-AMF treatment nutrient effect, we compared the colonized individual plants to the mean of the uncolonized plants within treatment, rather than with the control. Thus, mycorrhizal responsiveness for the commercial treatments was calculated using this equation: ln (total dry weight of AM inoculated plant/mean dry weight AM control plant within the same treatment) x 100.

Table 1.1 Analytes that were highly elevated in ComA compared with ambient potting media.

Analyte	Ambient soil levels (mg kg-1)			ComA
	P1	P2	P3	
PO_4-P	21.5	6.7	2.5	32
Calcium	400	441	460	942
Sodium	30	30	32	186
Boron	0.3	0.3	0.3	2.5

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Shoot and root analysis

Roots were fixed in 50% ethanol solution. Roots to be evaluated for AM fungal colonization were removed, weighed, and cleared and stained using a modified vinegar method. Roots were cleared in a 4% KOH solution for 15 minutes, acidified in 1% HCl solution overnight, stained in a hot 5% black Shaeffer ink/95% vinegar solution for three minutes and then destained using a 16:1:1 lactic acid:glycerol:water solution (Kormanik et al. 1980; Koske & Gemma 1989; Vierheilig et al. 1998). AM fungal structures were not quantified in this study because there is often no relationship between percent root colonization and plant growth response (Klironomos 2003). Instead, we verified presence/absence of AM fungi colonization for each plant by placing at least four root segments in four 25 mm rows on a slide and viewing at 100-400x magnification under a compound microscope. If there was any question about the first slide, we assessed a second slide. Shoots and roots not used for AM fungi assessment were dried at 60 °C until they reached a stable weight. Root biomass was the sum of the dry weight of the unused root plus an estimate of the used root weight derived from proportions of fresh to dry root mass (International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi 2005).

Mycorrhizal inoculation potential (Experiment 3)

Mycorrhizal inoculation potential (MIP) for the two commercial inoculants was evaluated as a separate assessment of inoculum viability. Corn seed was grown in 50:50 sand:standard horticultural potting media with commercial inoculum treatment in 100 ml conetainers for 30 days (Moorman & Reeves 1979). Four plants with ComB commercial

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inoculum were grown using the same rate as in the commercial experiment (0.2 g ComB). To assess whether ComA would colonize at higher application rates, ComA was tested at 10 and 100 times the suggested application rate, using three or four replicates, respectively, as available inoculum volumes allowed. There was not enough of the same batch of Com B to test higher application rates. Plants were watered daily to field capacity and pots were randomized once a week.

Roots were cleaned and then cleared and stained (Phillips & Hayman 1970). Percent root colonization was determined by gridline intersect method at 200 X magnification (Giovannetti & Mosse 1980).

Statistics

In order to establish whether species' biomass were significantly different between mycorrhizal and non-mycorrhizal treatments and, thus, significantly mycorrhizal responsive, a two way analysis of variance (ANOVA, SAS version 8.0 SAS Institute, Inc., SAS OnlineDoc®, Cary, NC, 1999) was performed on each species using biomass means as the response variable and nutrients and AMF as the independent variables. Analysis of variance was then used to compare the response variable mycorrhizal responsiveness amongst species with species, nutrients and AMF as independent variables for each of the commercial and field inoculum experiments. Tukey's HSD (honestly significant difference) test was used for post hoc means comparisons. Diagnostic plots of studentized residuals versus predicted values were used to check assumptions of homogeneity of variance and to identify outliers. No transformations were required.

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Results

Mycorrhizal colonization

In the commercial inoculum experiment, 14 out of 166 (8.4%) plants in the ComA or ComB treatments had AM fungi colonization. A further two of the 81 commercial control plants were colonized, due either to the incorrect treatment application or to contamination in the greenhouse (Table 1.2). Because none of the field inoculum control treatment pots became colonized, the former is the more likely cause. In the MIP tests of the commercial inoculum, ComB did not colonize any of the corn plants at the levels used in the commercial inoculum experiment. ComA did not colonize any of the four corn plants at the 10 times rate, but colonization for the three corn plants grown at the 100 times rate was 3, 93, and 92%.

Table 1.2 Percentage of plants colonized by AMF in each treatment.

Species	Field treatment	n	Field+ ComAB	n	Field control	N	ComA	n	ComB	n	Commercial control	n
Artemisia frigida	100%	12	100%	12	0%	12	18%	11	8%	12	0%	10
Aster laevis	100%	12	100%	12	0%	12	8%	12	17%	12	0%	12
Bromus tectorum	100%	11	100%	12	0%	12	0%	12	8%	12	0%	12
Chrsysothamnus viscidiflorus	100%	11	100%	10	0%	12	0%	11	0%	12	9%	11
Elymus elymoides	100%	12	100%	12	0%	12	8%	12	8%	12	0%	12
Lappula occidentalis	100%	12	92%	12	0%	12	17%	12	8%	12	0%	12
Muhlenbergia montana	100%	12	82%	11	0%	12	8%	12	8%	12	8%	12

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In the field inoculum experiment, between 90 and 100% of the plants in each treatment were colonized by AM fungi (Table 1.2). None of the plants in the uninoculated field control treatments were colonized.

Individual species biomass comparisons

All plants in the field inoculum experiment showed significant growth differences between the inoculated and control treatments or else showed a significant interaction effect (Table 1.3). Biomass for late successional species increased with colonization except in *Muhlenbergia montana*, in which aboveground biomass increased while belowground decreased, for an overall non-significant change in biomass. *Aster laevis* showed a significant P by AM fungi interaction; biomass of AM fungi treated *As. laevis* remained fairly even across P availability levels, but biomass of uncolonized *As. laevis* declined dramatically with decreasing P availability (Table 1.3).

Biomass of late successional plants did not increase with the addition of ComAB to the field inoculum compared to the field inoculum only (Table 1.3). Biomass of early successional species in the field+ComAB treatment tended to be smaller than uninoculated control plants but larger than plants with field inoculum only (Table 1.3).

There were not enough colonized plants in the commercial inoculum experiment to assess mycorrhizal responsiveness. However, there were within species differences in plant biomass among treatments whether or not the 16 colonized plants were included in the analysis. Thus, the differences in biomass among treatments cannot be attributed to AM fungi colonization. Results excluding all colonized plants are reported in Figs. 1.1 & 1.2. Biomass differences amongst treatments were found for five of the seven species

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Table 1.3 Field soil inoculum experiment (Experiment 1) biomass. Data are expressed as means ± SE. Values with different letters are significantly different at the $p \leq 0.05$ level using Tukey's honestly significant difference test for each species within a row. The interaction between P level and AM fungi inocula was significant for *As. laevis* ($F_{[4,26]} = 7.27, p = 0.0005$). Unless otherwise indicated, biomass values were averaged across nutrient levels for each species.

				Treatments		
Plant species	Biomass	n	P level	Field soil	Field+ComAB	Control
Late succession species						
Ar. frigida	total	36		0.20±0.01^a	0.23±0.02^a	0.15±0.01^b
As. laevis	total	11	P1	0.30±0.03	0.24±0.02	0.41±0.05
	total	12	P2	0.25±0.02	0.27±0.04	0.34±0.04
	total	12	P3	0.25±0.01	0.26±0.05	0.12±0.02
C. viscidiflorus	total	33		0.16±0.01^a	0.16±0.01^a	0.11±0.01^b
M. montana	belowground	36		0.16±0.02^b	0.16±0.02^b	0.24±0.02^a
	aboveground	36		0.30±0.01^a	0.30±0.02^a	0.25±0.01^b
	total	36		0.46±0.03	0.47±0.03	0.49±0.02
Early succession species						
B. tectorum	total	35		0.33±0.03^b	0.34±0.02^b	0.44±0.02^a
E. elymoides	total	35		0.30±0.01^c	0.36±0.02^b	0.45±0.02^a
L. redowskii	total	35		0.19±0.02^b	0.26±0.02^{ab}	0.29±0.03^a

assessed (Figs. 1.1 & 1.2). Significant P by AM fungi interactions were found for *C. viscidiflorus* belowground biomass, and *E. elymoides*, and *L. redowskii* aboveground biomass (Figs. 1.1 & 1.2).

Mycorrhizal responsiveness among species

In the field inoculum experiment, when comparing mycorrhizal responsiveness across species, species and nutrients had a significant interaction effect (Table 1.4, Fig. 1.3). Mycorrhizal responsiveness of *As. laevis* rose sharply as soil P levels declined (Fig. 1.3). If *As. laevis* was removed from the analysis, this interaction was no longer significant ($F_{[10,98]} = 1.48, p = 0.16$). Late-successional plants tested in the study all

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responded positively and early-successional plants all responded negatively to the mycorrhizal treatments when averaged over P levels (Fig. 1.4), with the exception of late

Figure 1.1 Mean belowground biomass for uncolonized plants in commercial inoculum treatments (Experiment 2) for seven species. Error bars represent one standard error of the mean. Asterisks denote significant P level by AM fungi treatment interactions within species. Treatments with different letters are significantly different within species based on Tukey's honestly significant difference test at $p < 0.05$.

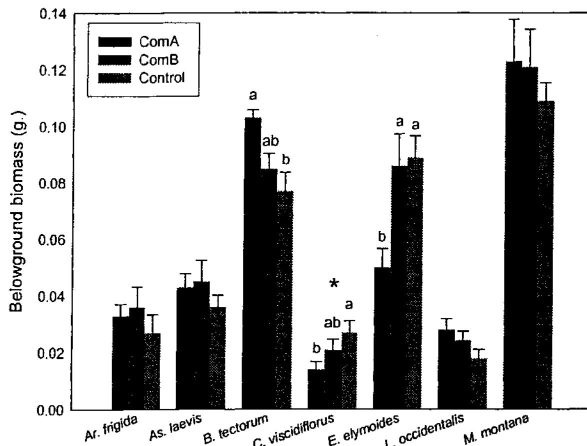

Figure 1.2 Mean aboveground biomass for uncolonized plants in commercial inoculum treatments (Experiment 2) for seven species. Error bars represent one standard error of the mean. Asterisks denote significant P level by AM fungi treatment interactions within species. Treatments with different letters are significantly different within species based on Tukey's honestly significant difference test at $p < 0.05$.

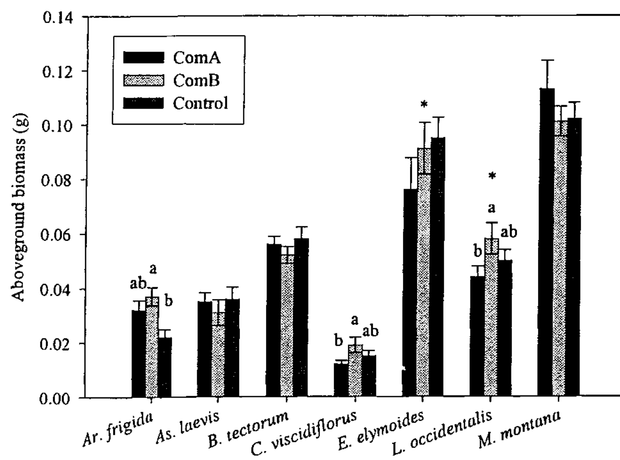

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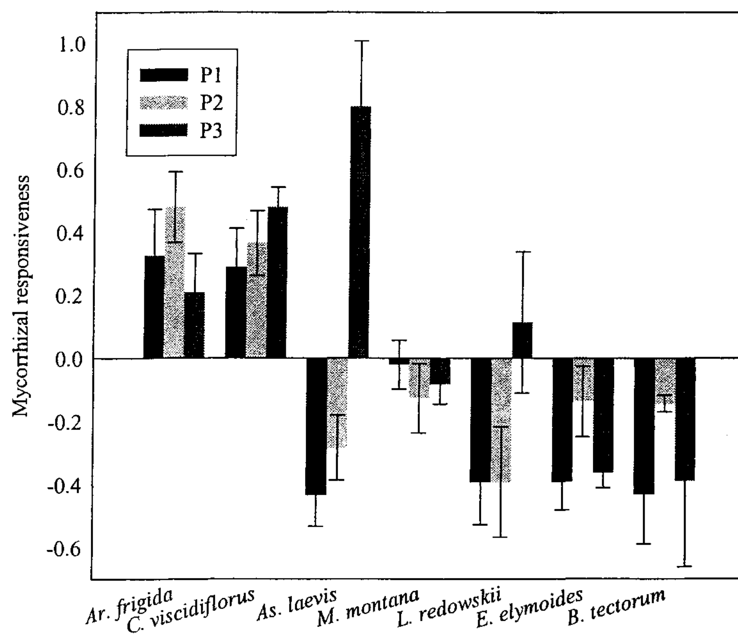
Table 1.4. Analysis of variance (ANOVA) results for mycorrhizal responsiveness to field inoculum (Experiment 1).

Source of variation	df	Sum of squares	F value
Species	6	11.16	12.22***
Nutrients	2	1.53	5.03**
Species * P level	12	7.68	4.21***
AM fungi	1	0.4	2.63
Species * AM fungi	6	0.57	0.62
AM fungi * P level	2	0.12	0.39
Species * AM fungi * P level	12	0.68	0.37
Error	115	17.51	.

$^{**}p = 0.01$; $^{***}p = 0.001$

Figure 1.3 Mycorrhizal responsiveness of seven species to field inoculum treatments (Experiment 1) (averaged) at each P level to show species by P interaction. Error bars represent standard error of the mean.



successional *M. montana*. Root biomass of this species significantly decreased while shoot biomass significantly increased, providing a non-significant, neutral overall growth response to AM fungi treatments (Fig. 1.4).

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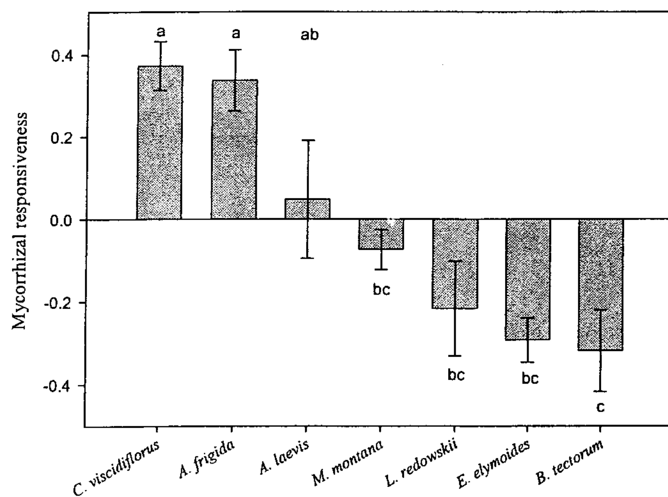
Figure 1.4 Mycorrhizal responsiveness to field inoculum treatments (Experiment 1) for seven species averaged over P levels with field inoculum. Error bars represent one standard error of the mean. Mycorrhizal responsiveness of species with different letters is significantly different based on Tukey's honestly significant difference test at $p < 0.05$.



When mycorrhizal responsiveness of the 14 individual colonized plants in the commercial inocula treatments were compared, ComA and ComB inoculated plants responded significantly differently (ComA -0.28 ± 0.10, ComB 0.30 ± 0.13, $F_{(1,5)} = 15.19$, $p = 0.01$, significant with Tukey's HSD at $\alpha = 0.05$). Plants colonized with ComA all grew less than the uncolonized plants in the same treatments, whereas, all but two species responded positively to ComB (Table 1.5).

Table 1.5 Mean mycorrhizal responsiveness to commercial inocula (Experiment 2) by species.

Species	n	Mean	SE
ComA inoculation			
Ar. frigida	2	-0.54	0.22
As. laevis	1	-0.26	.
B. tectorum	0	.	.
E. elymoides	1	-0.40	.
L. redowskii	2	-0.08	0.14
M. montana	1	-0.09	.
ComB inoculation			
Ar. frigida	1	0.49	.

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Species	n	Mean	SE
As. laevis	2	0.49	0.12
B. tectorum	1	-0.03	.
E. elymoides	1	-0.26	.
L. redowskii	1	0.18	.
M. montana	1	0.73	.

Discussion

Of the plant species used in the study, all late-successional plant species responded positively and all early-successional plant species responded negatively to field inoculum. This result supports the trend found by others (Allen 1984; Allen & Allen 1984; Reeves 1985; Miller 1987; Allen & Allen 1988; Allen & Allen 1990; Reeves & Redente 1991; Allen 1995; Wilson & Hartnett 1998; Hart et al. 2001; De Deyn et al. 2003; Klironomos 2003), including a recent field study in which adding field collected inoculum with native seed enhanced species richness and abundance of native plants while suppressing exotic and ruderal plant abundance (Korb et al. 2004). Because *Bromus tectorum* responded negatively to field inoculum, the application of AM fungi as a restoration strategy in the field is not expected to benefit this invasive plant.

Commercial inoculum was largely ineffective when used at recommended rates in this study. In the field inoculum experiment, there were no significant differences between field soil and field+ComAB treatments for late successional species, showing that no additive growth effect occurred with commercial inoculum. Early successional plant species tended to grow more with field+ComAB than with field soil inoculation alone. However, it is unclear, based on the lack of colonization in the commercial inoculum experiment and the nutrient analyses of the commercial inoculum, whether this benefit resulted from the commercial inoculum substrate or from AM fungi colonization.

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The MIP tests confirmed the low colonization rates seen on plant roots treated with commercial inoculum. Analyses that excluded AM fungi colonized plants indicate that the commercial substrates themselves affected plant growth. Though the manufacturers list no fertilizers on the commercial inoculum labels, the nutrient analysis showed elevated levels of P, Ca, Na and B for ComA. While nutrient effects may explain the significant plant growth treatment differences, the reader should keep in mind that a) only 0.151 g of ComA or 0.2 g of ComB were added to each container and b) nutrient analyses to assess nutrient differences in plant tissues were not performed. No definite conclusions can be made to explain the underlying causes for the differences in commercial inoculum treatments other than to note that they occurred. These results may raise serious questions for practitioners unable to assess root colonization; they will not be able to distinguish between plant biomass changes caused by nutrient changes and those caused by AM fungi colonization.

When possible nutrient effects were controlled for, the mixture of *Glomus spp.* (ComB) produced more beneficial associations with the inoculated plants than *Glomus intraradices* (ComA) alone (Table 1.5). Plants colonized by *Glomus intraradices* (ComA) all responded negatively compared with the uncolonized plants, whereas mycorrhizal responsiveness with ComB tended to be more similar to field inoculum (n = 14, Table 1.5). Others have also found that *G. intraradices* suppresses biomass in species rich macrocosms, has a neutral effect in less species rich macrocosms (Klironomos et al. 2000), and has a short term growth effect that disappears after five years in semiarid systems (Requena et al. 2001). Although different sources of field inoculum were not tested in this study, a recent study helps elucidate the necessity of collecting inoculum

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from a nearby "desirable" reference site. The study showed that the origin of field inoculum can determine competitive outcomes amongst plant species and alter the degree of benefit conferred by AM fungi to plant species (Moora et al. 2004).

Surprisingly, soil P levels had little effect on AM fungi growth responses in the species studied here. Critical levels for sufficient plant available P for wildlands plants on granitic soils are not known, but the P3 treatment at 2.7 mg kg-1 P would generally be considered low enough to limit plant growth (Olsen and Sommers 1982). Under these conditions, additional P assumed to be made available by mycorrhizal hyphae would be expected to allow increases in plant biomass. In the mutualism - parasitism continuum of plant-AM fungi interactions, however, AM fungi can become parasitic when environmental conditions make costs of symbiosis greater than benefits (Johnson et al. 1997). Across the three levels of P availability in this study, the field inoculum had either a mutualistic relationship with plant hosts (*Ar. frigida, C. viscidiflorus, M. montana*) or parasitic relationship with plant hosts (*B. tectorum, E. elymoides, L. redowskii*). Only for *As. laevis* did a change in environmental conditions cause a significant reversal of costs and benefits. The field inoculum acted as a strong symbiont with *As. laevis* at the low P levels (benefits of AM fungi colonization exceeded costs), but acted as a parasite at field P levels (costs exceeded benefits) (Fig. 1.3, Table 1.3).

In summary, field soil was a highly effective inoculum. It positively increased growth of the late-successional native species and negatively affected growth of the early-successional native and invasive species studied. The commercial inocula tested were largely ineffective for achieving AM fungi colonization in the target species. Results from this study suggest that field inocula, rather than commercial inocula, should

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be added with perennial plant seeds in field sites in which AM fungi presence is suspected to be low. Phosphorus deficiency had a limited effect on mycorrhizal responsiveness. Although care must be taken in transferring lessons from the greenhouse to the field, restoration applications that involve seeding native perennial species should ensure adequate AM fungi presence. Further, managers may be reassured that we found adding field inoculum did not stimulate growth for *B. tectorum* or other annual plant species.

Implications for Practice
❑ Locally collected field inoculum appeared to be more effective than commercial inocula for establishing late successional plant species. ❑ Native species tended to respond better to commercial inocula that contain higher AM fungal species diversity compared with commercial single species inoculum. ❑ AM fungal inoculation should not increase the spread of *Bromus tectorum* (cheatgrass). ❑ For two of the tested perennial native species, AM fungi improved establishment and growth regardless of the soil P levels. In one (*As. laevis*) mycorrhizal response was more positive at low soil P levels.

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Chapter 2: *Bromus tectorum* associations with arbuscular mycorrhizal fungus community change: causation or correlation?

ABSTRACT

Non-native plant species may alter arbuscular mycorrhizal fungi (AMF) communities in ways that benefit themselves in competitive relationships. Studies show that *Bromus tectorum* L. invasion is associated with shifts in AMF community composition and decreased AMF richness. Using two experiments, we examined whether *B. tectorum* directly changed AMF communities or whether *B. tectorum* invasion was correlated with areas low in AMF. In one experiment we compared mycorrhizal inoculation potential of native plant soils when surrounded by either *B. tectorum* or native vegetation. Mycorrhizal inoculation potential was lower in the *B. tectorum* dominated areas for both species. In a second experiment we examined how *B. tectorum* and three native plant species would condition two sources of soil. *B. tectorum* did not affect the AMF or final plant biomass differently than other species studied. These results suggest that *B. tectorum* does not directly affect the mycorrhizal community in the short term.

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INTRODUCTION

It has been suggested that arbuscular mycorrhizal fungi (AMF) can aid plant invasions by altering competitive relations with native plant species. For example, invasive *Centaurea maculosa* auct. Non Lam. had greater biomass when grown with a native bunchgrass (*Festuca idahoensis* Elmer) and AMF treatment than without AMF (Marler *et al.* 1999). *Centaurea maculosa* can derive direct benefits from AMF as well as indirect benefits such as carbon transfer from *F. idahoensis* to *C. maculosa* (Carey *et al.* 2004). Another study on Chongming Island, China showed that the AMF colonization increased linearly with time since invasion by the exotic host plant, *Solidago canadensis* L. The authors concluded that the AMF symbiosis helped the invasion of this *Solidago* species. (Jin *et al.* 2004). A recent review article summarized results showing that five species of exotic/invasive plants made significant changes to the AMF community, however, the authors caution that it is difficult to link structural changes in belowground communities with changes in plant function (Wolfe & Klironomos 2005). By looking at the proportion of naturalized and native species in Britain that occur in families with distinct mycorrhizal status, Fitter (2005) suggests that the ability to form mycorrhizal symbiosis may increase the chance of a plant becoming naturalized. Conversely, introduction and range expansion patterns of Cyperaceae and Brassicaceae, two non-mycorrhizal families, suggests that non-mycorrhizal plants may have difficulty invading undisturbed communities (Fitter 2005). Restoration studies have shown that the presence of AMF alters the plant community trajectory to favor later successional, less weedy species. In plant communities with high weed colonization and low mycorrhizal

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activity, annual non-AMF weeds persisted up to 10 years. However with low weed and high AMF activity, shrubs dominated (Allen 1988). Generally, whether AMF presence helps or hinders invasions may be partially explained by the mycorrhizal dependency of the native and non-native plant species in the community.

Interactions between the invasive grass *Bromus tectorum* L. (downy brome, cheatgrass) and AMF may be among the reasons it has come to be the most ubiquitous weed in steppe vegetation in western North America (Goodwin 1992). The success of *B. tectorum* as an invading species has been largely attributed to its winter annual lifecycle (Hulbert 1955), competitive advantage in disturbed, high nutrient environments (Kay & Evans 1965; McLendon & Redente 1991; Dakheel *et al.* 1993), and the accumulation of early dry fine fuels that contribute to increased fire cycles that reduce native species abundance and increase *B. tectorum* dominance (Klemmedson 1964; Humphrey & Schupp 2001). Evidence suggests that AMF associations may be another factor in the ability of *B. tectorum* to invade and dominate plant communities. Invasion of *B. tectorum* into native grasslands has corresponded with changes in the AMF composition and lower AMF richness in native grasses (Hawkes *et al.* in press), altered soil food web structure, and a shift from mutualistic soil fungal composition to more parasitic and saprophitic forms (Belnap & Philips 2001). Another study showed low mycorrhizal inoculation potential in areas with *B. tectorum* dominance (Al-Qarawi 2002). None of these studies have investigated whether *B. tectorum* directly influences AMF communities. Decreases in AMF richness or changes in AMF species composition can result in profound changes in the plant community and ecosystem function (Allen *et al.* 1995; van der Heijden *et al.* 1998; van der Heijden *et al.* 1998; Eom *et al.* 2000;

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Klironomos *et al.* 2000; Stampe & Daehler 2003; van der Heijden *et al.* 2003). Thus, it is important to determine whether *B. tectorum* actually causes changes in the AMF community or whether *B. tectorum* simply establishes better in areas with a disturbed AMF community. The findings by Hawkes and others (2005) in an undisturbed landscape suggest causation. AMF taxa respond differently to host plant species (van der Heijden *et al.* 1998), and because *B. tectorum* has been found to be non-mycorrhizal in monoculture (Goodwin 1992), as well as facultative and negatively responsive to AMF (Allen 1988; Rowe *et al.* in press), *B. tectorum* may not provide as much benefit to the local AMF taxa as native plants do. As a consequence, *B. tectorum* may not support an AMF community as diverse or abundant as supported by the native plant community or it may select for AMF species that are less beneficial to the local native plant species (positive feedback Bever *et al.* 2002; Hawkes *et al.* in press). However, until the case for causation has been demonstrated, we must consider the possibility that *B. tectorum* spread is correlated with lower mycorrhizal activity. As an early successional plant species, *B. tectorum* is commonly found on disturbed sites (Klemmedson 1964; Mack 1981; Pierson & Mack 1990). Disturbed sites have been found to have lower potential for mycorrhizal inoculation of plant roots than undisturbed communities (Moorman & Reeves 1979).

If causation is demonstrated, *B. tectorum* alteration of AMF communities could be a significant contributing factor to *B. tectorum* invasion success. Lower levels of AMF spores on a site can prevent establishment of obligate mycorrhizal plants, which are often late-successional species (Allen *et al.* 1995). Klironomos (2002) demonstrated that plants grow better in AMF isolated from their own soil compared with AMF from soils of

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different host plants. An invader that changes the AMF community may diminish the productivity or diversity of a native plant community (van der Heijden *et al.* 1998; van der Heijden *et al.* 1998; Hartnett & Wilson 1999; Klironomos *et al.* 2000; Hartnett & Wilson 2002).

In this study we evaluated *B. tectorum* interactions with the AMF community in two experiments. The objective of the first study was to establish whether *B. tectorum* is associated with a modified AMF community in the field. The objective of the second study was to investigate whether *B. tectorum* differentially affects the AMF community of two native plant species.

MATERIALS AND METHODS

Experiment 1:

Aggregate soil samples were taken from: *Muhlenbergia montana* (Nutt.) Hitchc. surrounded by undisturbed native vegetation (Mm), *Chrysothamnus viscidiflorus* (Hook.) surrounded by undisturbed native vegetation (Cv), *M. montana* surrounded by *B. tectorum* (MmB), *C. viscidiflorus* surrounded by *B. tectorum* (CvB), and from a *B. tectorum* monoculture (B) at 2,377 m (7,800 ft) on a south-facing slope in Rocky Mountain National Park (RMNP) dominated by *Pascopyrum smithii* (Rydberg) Love, *Carex microptera* Mackenzie, *Artemisia frigida* Wildenow, *C. viscidiflorus*. Each aggregate sample contained soil from at least three separate plants. In an effort to minimize site environmental heterogeneity, all samples were taken within a 10-meter radius. We collected the soils into plastic ziplock bags in the latter part of the growing season, September 2004, and stored the soil at 5° C until the experiment began in mid-

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October 2004. The two target species were chosen to represent positive (*C. viscidiflorus*) and neutral (*M. montana*) responses to AMF as shown by previous work (Rowe *et al.* in press).

Equal portions of the five aggregate soil samples were mixed together to prepare the non-AMF control inoculum (C). Coarse sand (used as potting material, Crystal Landscape Supplies, Inc., Fort Collins, CO) and the control inoculum were autoclaved, allowed to cool over night, and autoclaved again at 121 °C for 1 hour. We incubated soils with filtrate at room temperature for 2 weeks to re-introduce soil microbes and stabilize nutrient levels (B. Reeves, 2004, Colorado State University, Fort Collins, CO, personal communication). The filtrate was prepared by passing a mixture of 2.2 L of non-autoclaved control inoculum soil and 9 L of deionized water through Whatman # 1 filter paper (Gemma *et al.* 2002).

We filled 30 16.5 cm plastic pots with sand (six inoculum treatments [Cv, Mm, CvB, MmB, B, C], five replications), and a center column of 40 ml of either inoculum or control inoculum. We used corn plants to bioassay mycorrhizal inoculation potential (MIP) because they are known to host many AMF species (Moorman & Reeves 1979). Corn seed was surface sterilized by soaking in 70% ethyl alcohol for 10 minutes. Plants were thinned to one per pot, watered daily to field capacity, and grown for 30 days (Moorman & Reeves 1979). We re-randomized pot positions on the bench weekly. Although non-surviving plants were repeatedly re-seeded, three pots from three different treatments did not produce corn plants.

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Corn roots were cleaned, stored in 50% ethyl alcohol, cleared (Phillips & Hayman 1970), and stained with Shaeffer black ink (Vierheilig *et al.* 1998). Percent root colonization was determined by gridline transect method (Giovannetti & Mosse 1980).

Experiment 1 was a completely randomized design with five replicates of each of the six treatments. Variances and outliers were analyzed using studentized residual plots; no transformations were required. MIP data were analyzed as a one-way analysis of variance with percent colonization as the response variable (ANOVA, SAS Institute, Inc., version 9.1, Cary, NC, 2002-2003). Differences in treatment means were compared using Tukey's test.

Experiment 2:

A soil conditioning experiment was designed to test whether *B. tectorum* directly affects resident native species by modifying the AMF community. The approach is depicted in Fig. 1 (Bever *et al.* 2002). *Artemisia frigida* and *C. viscidiflorus* were chosen as the soil inocula source/final plant species because their high mycorrhizal responsiveness to native soil inoculum (Rowe *et al.* in press) may make them more sensitive to soil conditioning. Four trainer plant species were chosen as intermediary species to provide a gradient of mycorrhizal responsiveness. The two highly responsive species acted as trainer plants as well as soil source and final plants to allow for a comparison between conditioning the soil with the same species to the effect of conditioning that soil with the other trainer species (Fig. 2.1). *Elymus elymoides* (Raf.) Swezey was chosen as a trainer species because of its similarity to *B. tectorum* in life history and response to AMF. *Elymus elymoides* is a short lived perennial with a

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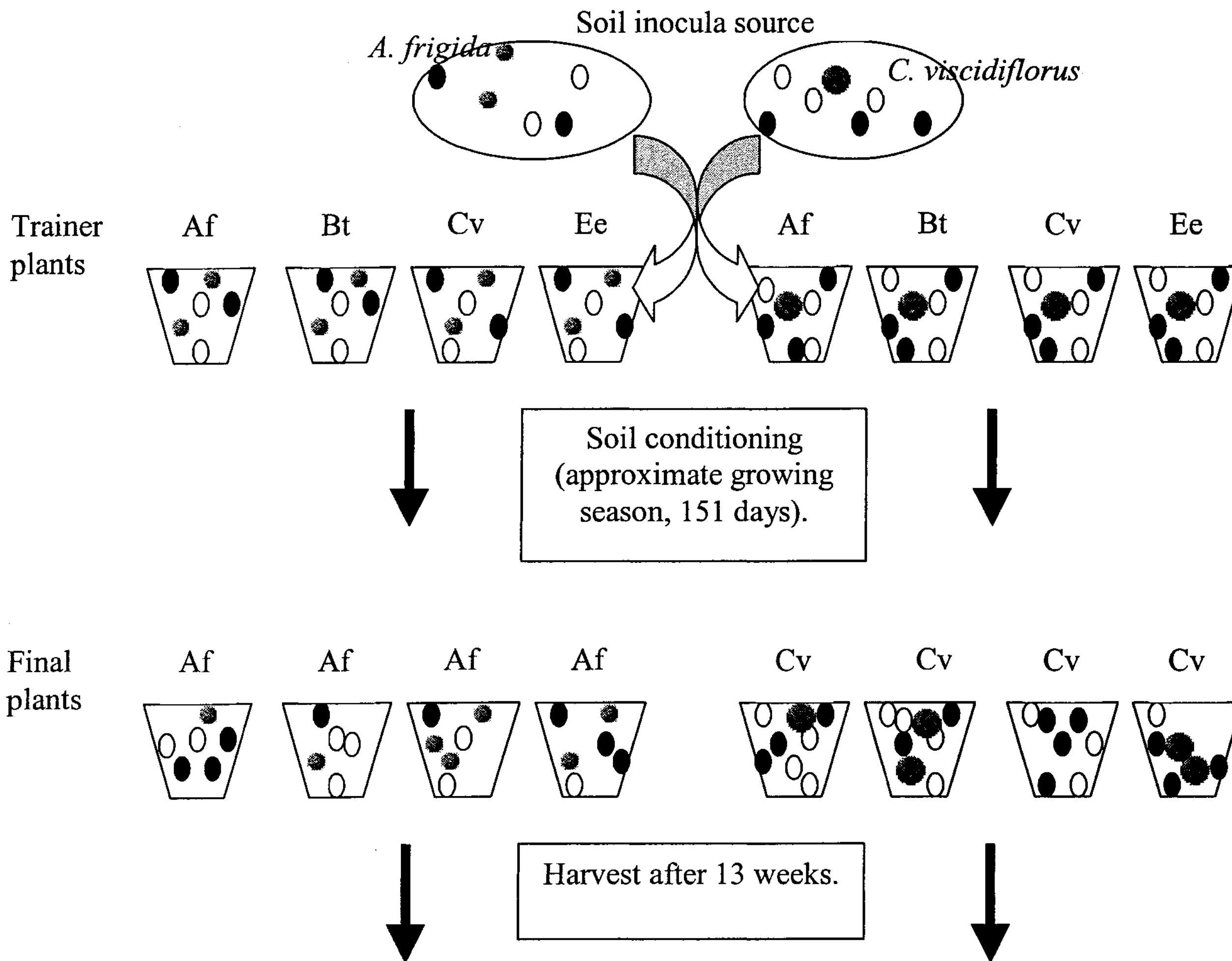

Figure 2.1 Experimental design for soil conditioning experiment. Ovals and dots represent spores from different AMF species. Soil was collected from the roots of *Artemisia frigida* and *Chrysothamnus viscidiflorus* and used to inoculate replicate"trainer plants" (*A. frigida, Bromus tectorum, C. viscidiflorus*, and *Elymus elymoides*). The same soils were sterilized and washed with filtrate and added to complementary pots for each trainer plant. The composition of the AMF community may differentiate during the "soil conditioning" period of five months. The final plants, *A. frigida* and *C. viscidiflorus* were planted in the final phase in their original, conditioned soils and harvested after three months. The final plants should act as indicators as to how much the trainer plants changed the AMF community (after Bever *et al.* 2002).

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negative mycorrhizal responsiveness of –0.29, *B. tectorum* has a dependency of –0.32 with the same native RMNP soil inoculum (Rowe *et al.* in press).

Seeds for each species were collected at RMNP at approximately 2,438 m (8,000 ft.). Soils were collected from RMNP from a stockpile of topsoil. The stockpiled soil (43 ppm P[1]) had twice the level of phosphorus as natural field soil at RMNP (21.7 ppm P[1]). To achieve field soil levels of P, course sand was added as a 1:1 ratio. Two aggregate soil inocula were collected, one from the rhizospheres of *A. frigida* plants and one from the rhizospheres of *C. viscidiflorus* plants in RMNP.

Stockpiled soils and half of the inoculum soils to be used for controls were autoclaved and incubated with soil filtrate as in Experiment 1. Sixty-four pots were filled with 1.55 g of 1:1 sand:soil with 50 ml of either *A. frigida* or *C. viscidiflorus* soil inoculum or autoclaved control soils in the center column. We planted seeds of *B. tectorum, E. elymoides, A. frigida*, or *C. viscidiflorus* into these pots and watered twice daily with drip irrigation for 10 days, and then watered daily to field capacity for another 14 days to promote germination and establishment. After 2 weeks, watering was reduced to three times weekly for 5 minutes to allow soil to dry between watering cycles. At 74 and 81 days soil surfaces of all pots were scraped to kill off moss or algae growth, even if no moss was formed. The scraper was sterilized in between pots. Once seedlings were sufficiently strong to withstand water pressure (25 days), they were watered an additional time each week to field capacity with ammonium nitrate (0.0145g/L) and potassium nitrate (0.04g/L). We moved pots to different locations on the bench weekly using a randomized design with precautions taken to sterilize irrigation emitters before replacing them in pots.

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[1] Using the weak Bray method.

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After 151 days, all trainer plants were harvested at soil level and final plants *A. frigida* and *C. viscidiflorus* were seeded into pots containing their respective original soil inoculum (Fig. 2.1). We seeded directly into existing pots rather than using inoculum in new pots to retain as many existing AMF reproductive structures, including the external hyphal mycelium, roots, and spores as possible. We watered final plants twice daily for 31 days, then three times a week for 5 minutes. At 4 weeks, watering to field capacity with the above nitrogen fertilizer was introduced once a week, for a total of nine times before harvest at 13 weeks.

After the initial soil conditioning, when trainer plants were harvested, a single 2.5 cm diameter core sample (50 ml soil) was taken from each pot directly adjacent to the plant shoot and stored at 4 °C. Roots were removed from soils and fixed in a 50% alcohol solution. Roots were cleared in a 4% KOH solution for 15 minutes, acidified in 1% HCl solution overnight, stained in a hot 5% Shaeffer black ink/95% vinegar solution for 3 minutes and then destained using a 16:1:1 lactic acid:glycerol:water solution (Kormanik *et al.* 1980; Koske & Gemma 1989; Vierheilig *et al.* 1998). We verified presence/absence of AMF colonization for each plant by placing salvageable root segments in 25 mm rows on a slide and viewing at 100-400x magnification. Most samples (80%) had enough root segments for four rows, 9% had three rows, 8 % had two rows, one sample had one row (1.5%), and for one sample no roots could be salvaged (1.5%) (*C. viscidiflorus* trainer plant in its own soil). All plant roots in inoculated pots were colonized, and no AMF colonization was found in the controls. No parasitic fungi were seen in any of the roots.

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Spores were extracted from 50 ml of each soil core sample using the wet sieve and sucrose gradient method (Daniels & Skipper 1982). Spores were counted based on low-density direct counts (see http://invam.caf.wvu.edu/methods/spores/enumeration.htm accessed 7/2005). Larger spores were separated with a 250 micron sieve and viewed at 7x magnification. All spores were removed and placed on slides with 50:50 PVLG (polyvinyl lacto-glycerol):Meltzer's solution. Only spores that released lipids when cover slip was pressed were included in the final count. The remaining smaller spore suspension was diluted to 25 ml, vortexed, and 1 ml was transferred to each of four watch glasses. Watch glasses were viewed at 40 x magnification. Spores were removed and counted in the same manner as the larger spores. The total spore count per sample was calculated as the large spore count plus the average number of spores per watch glass multiplied by the number of ml in the initial solution (25 ml). Because spores are primarily reproductive bodies, spore counts can be used as a proxy to measure population growth (Bever 2002).

Final *A. frigida* and *C. viscidiflorus* plant roots were cleaned, stored, and stained as described above for the trainer roots. Percent colonization of hyphae, vesicles, and arbuscules was assessed by viewing 100 intersections per sample using the magnified intersection method (McGonigle *et al.* 1990). This method allows for analysis of relative AMF function; for example, a greater number of arbuscules can indicate greater benefit to the plant because they are sites of nutrient supply to the plant (Johnson *et al.* 2003). Similarly, intraradical hyphae spread may indicate potential nutrient uptake (McGonigle *et al.* 1990), and vesicle density may indicate greater fungal fitness (Smith & Read 1997; Johnson *et al.* 2003). Percent hyphae and percent AMF (total) were highly correlated (R

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= 0.99, $p < 0.0001$), so percent AMF is not presented. Root samples from final plants in all inoculated pots were colonized, and none of the controls had any signs of AMF. Though trace amounts of parasitic fungi were found in roots of three final control plants in three different treatments, none of these final plants represented the lowest shoot biomass in their respective treatment groups, thus it is assumed that the parasites had a negligible effect on the experiment.

Presence of trainer plant roots in the pots complicated the final plant root harvest. Every effort was made to carefully separate the trainer roots from the final roots, then estimates were made of how much inextricable trainer root remained in the final root before drying. Shoots and roots were dried to constant mass at 55-60 °C and weighed. Established calculations from International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM) website were used to estimate dry biomass for the root sections taken for AMF assessment (Rowe *et al.* in press). Estimates of remaining trainer roots were subtracted from the root totals to calculate a modified final root weight. This modification helped reduce some variation, but the variation was still high. Final shoot biomass correlated well with the final root biomass ($R = 0.67$, $p < 0.0001$) and inclusion of the root data within analysis of final biomass did not alter the trends of the shoot data. Thus, only shoot biomass data are presented.

Mycorrhizal responsiveness (MR) for each plant was calculated using a ratio comparing colonized and uncolonized plants as follows: Ln (total dry weight of AMF colonized plant/total dry weight uncolonized control plant). Pairs of colonized and uncolonized plants within a treatment were randomly matched for this calculation. By calculating the MR for each colonized plant, all variation of control and inoculated

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treatments has been retained and the MR can be compared statistically as a response variable. For both final and trainer plants this ratio allows for comparison of AMF effects across plant species. For the final plant analysis, this ratio also controls for any possible non-mycorrhizal nutrient effects that the trainer plant might have had on the final plants.

A positive MR indicates a beneficial effect of the AMF while a negative MR indicates that the AMF are acting as parasites on the plant; the plant does not receive adequate benefit to compensate for the cost of supporting the fungi.

Experiment 2 was a completely randomized design with 4 replicates x 2 soil inoculum types (*A. frigida* and *C. viscidiflorus*) x 2 AMF types (autoclaved and not autoclaved) x 4 intermediate trainer plant species (see Fig. 2.1). Variances and outliers were analyzed using studentized residual plots. Spore counts and final *C. viscidiflorus* shoot biomass were square root transformed. Outliers were detected for the mycorrhizal responsiveness (MR) of trainer plants and trainer biomass. Unless otherwise noted in the text, removal of the outliers did not change the significance and results are reported with the outliers retained.

Aboveground trainer biomass means within each trainer species were analyzed using a two-way ANOVA with soil source and presence of AMF as factors (ANOVA, SAS Institute, Inc., version 9.1, Cary, NC, 2002-2003) to confirm the significance of the trainer plant MR. Aboveground final plant biomass within soil source/final plant was analyzed using a t-test to test for differences between colonized and uncolonized plants to confirm the significance of the final plant MR. Mycorrhizal responsiveness for trainer and final plants, spore counts from after the soil training, and percent hyphae, arbuscules,

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and vesicles of the final plants were analyzed in a two-way ANOVA with soil type/final plant species and trainer plant species as the independent variables. Differences in treatment means were compared using Tukey's HSD. Pearson correlation coefficients were used to compare correlations between appropriate data sets.

RESULTS

Experiment 1

The inoculation potential of soils under *C. viscidiflorus* and *M. montana* was lower when the plants were surrounded by *B. tectorum* than when surrounded by native vegetation. Soil MIP of *B. tectorum* and *M. montana* with *B. tectorum* were no different from the autoclaved control soils with no AMF colonization (Fig. 2.2). The MIP of *C.*

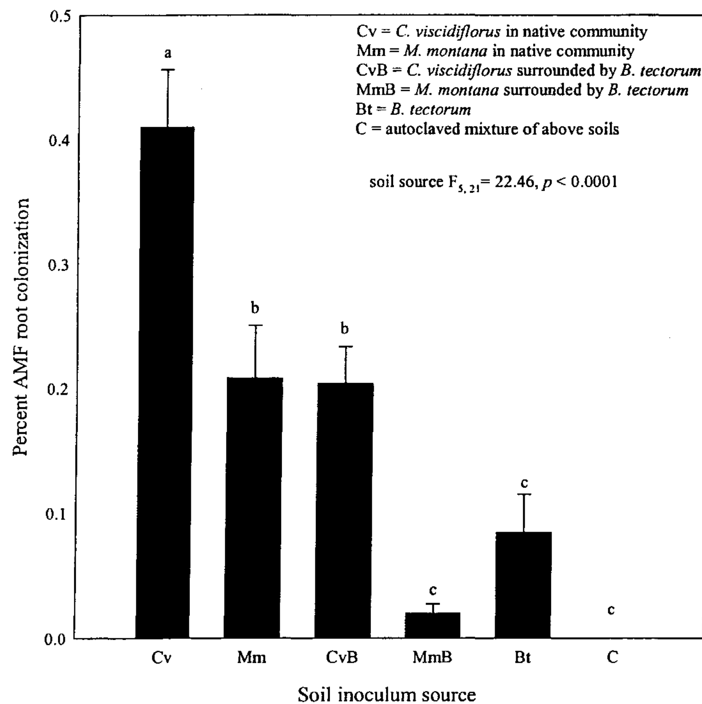

Figure 2.2 Mycorrhizal inoculation potential for *C. viscidiflorus* and *M. montana* growing with *B. tectorum* and native vegetation, *B. tectorum* alone and the autoclaved control soil. Different letters denote differences at α= 0.05 using Tukey's HSD test. Error bars represent one standard error of the mean.

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viscidiflorus soil invaded by *B. tectorum* was similar to that of *M. montana* soil, a less mycorrhizal dependent species.

Experiment 2

Trainer plants

Contrary to expectations, mean trainer plant biomass was lower in AMF inoculated pots compared with the sterile control within each trainer species, with the exception of *C. viscidiflorus* (Fig. 2.3). *Chrysothamnus viscidiflorus* responded

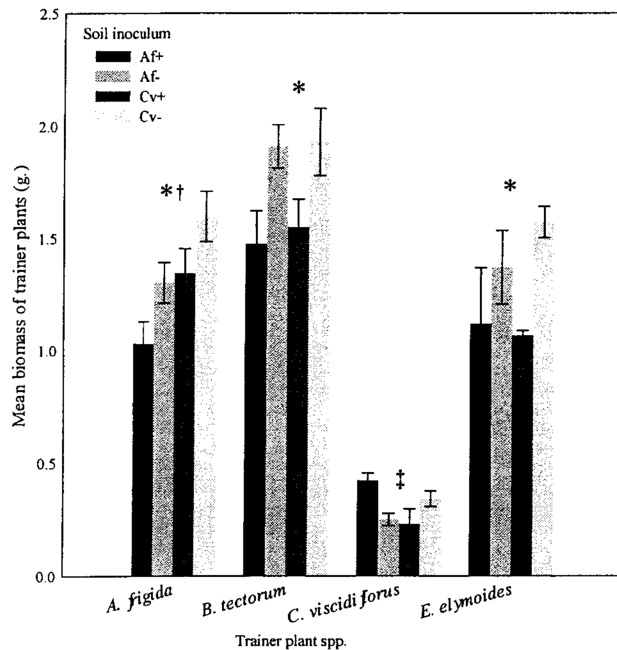

Figure 2.3 Mean biomass differences between inoculated and control treatments within trainer plant species. Trainer plant biomass was not compared amongst species due to inherent species biomass differences. Error bars represent one standard error of the mean. Soil inocula are abbreviated in the figure: Af+ is soil inocula from *A. frigida* plants, Af- is the sterilized *A. frigida* soil, Cv+ is soil inoculum from *C. viscidiflorus* plants, Cv- is the sterilized *C. viscidiflorus* soil.
* Biomass difference between inoculated and uninoculated plants ($p < 0.05$). † Biomass difference between *A. frigida* and *C. viscidiflorus* soil sources ($p < 0.05$). ‡ Significant interaction effect between soil source and AMF treatments ($p < 0.05$).

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positively to AMF inoculation from *A. frigida* soils and negatively to AMF inoculation from *C. viscidiflorus* soils (Fig. 2.3) (negative feedback Bever *et al.* 2002). Besides the interaction effect, *A. frigida* was the only trainer plant to show significant differences between soil inoculum sources. Its biomass was higher in *C. viscidiflorus* soils than in *A. frigida* soils, averaged over AMF inoculation (Fig. 2.3). Analyzing MR across species revealed the same *C. viscidiflorus* interaction effect described above (Fig. 2.4). If we

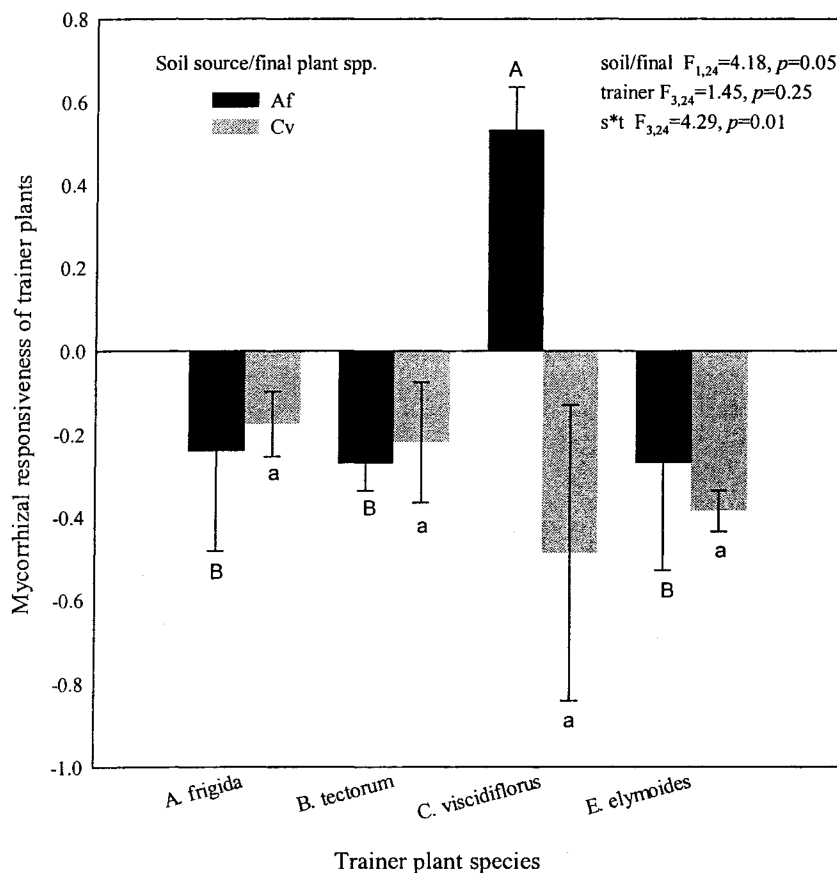

Figure 2.4 Mycorrhizal responsiveness of trainer plants for each soil inoculation source type. Upper case letters denote differences between trainer species within *A. frigida* final plants; lower case letters denote differences between trainer species within *C. viscidiflorus* final plants using Tukey's HSD (α= 0.05). Error bars represent one standard error of the mean.

remove *C. viscidiflorus* from the analysis, no significant differences remain amongst MR of trainer species. When trainer plant MR was analyzed separately by soil source, *C.*

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viscidiflorus was more responsive in *A. frigida* soils and less responsive in its own soils than the other trainer species (Fig. 2.4). Due to an outlier in the *C. viscidiflorus* soil inoculated *C. viscidiflorus* trainer plants, the difference between *C. viscidiflorus* trainer plants and other trainer plants in *C. viscidiflorus* soils is only significant if the outlier is removed.

Arbuscular mycorrhizal fungi

Mean number of spores found in pots at the end of the soil conditioning phase was higher in the *A. frigida* trained soils than in the soils of any of the other trainer species (Fig. 2.5d). Spore numbers were also higher in pots inoculated with soil from *C. viscidiflorus* compared with *A. frigida*, when averaged across trainer plant species (Fig. 2.5d).

Proportion of final plant roots colonized by vesicles, hyphae, and arbuscules differed amongst soil source/final plant species (Figs. 2.5 a-c). When averaged across trainer plant species, *A. frigida* soil source/final plant had more vesicles, whereas *C. viscidiflorus* soil

source/final plant had more hyphae and arbuscules (Figs. 5a, b and c, respectively). Trainer plant *C. viscidiflorus* diminished the proportion of hyphae and arbuscules in the final plants compared to the other three trainer plant species, when averaged across soil source/final plant spp. (Figs. 2.5 b & c). Trainer plants had an interaction effect in which the proportion of arbuscules in the final plant depended on the initial soil source/final plant species; this effect was pronounced for *B. tectorum* and *E. elymoides* (Fig. 2.5c).

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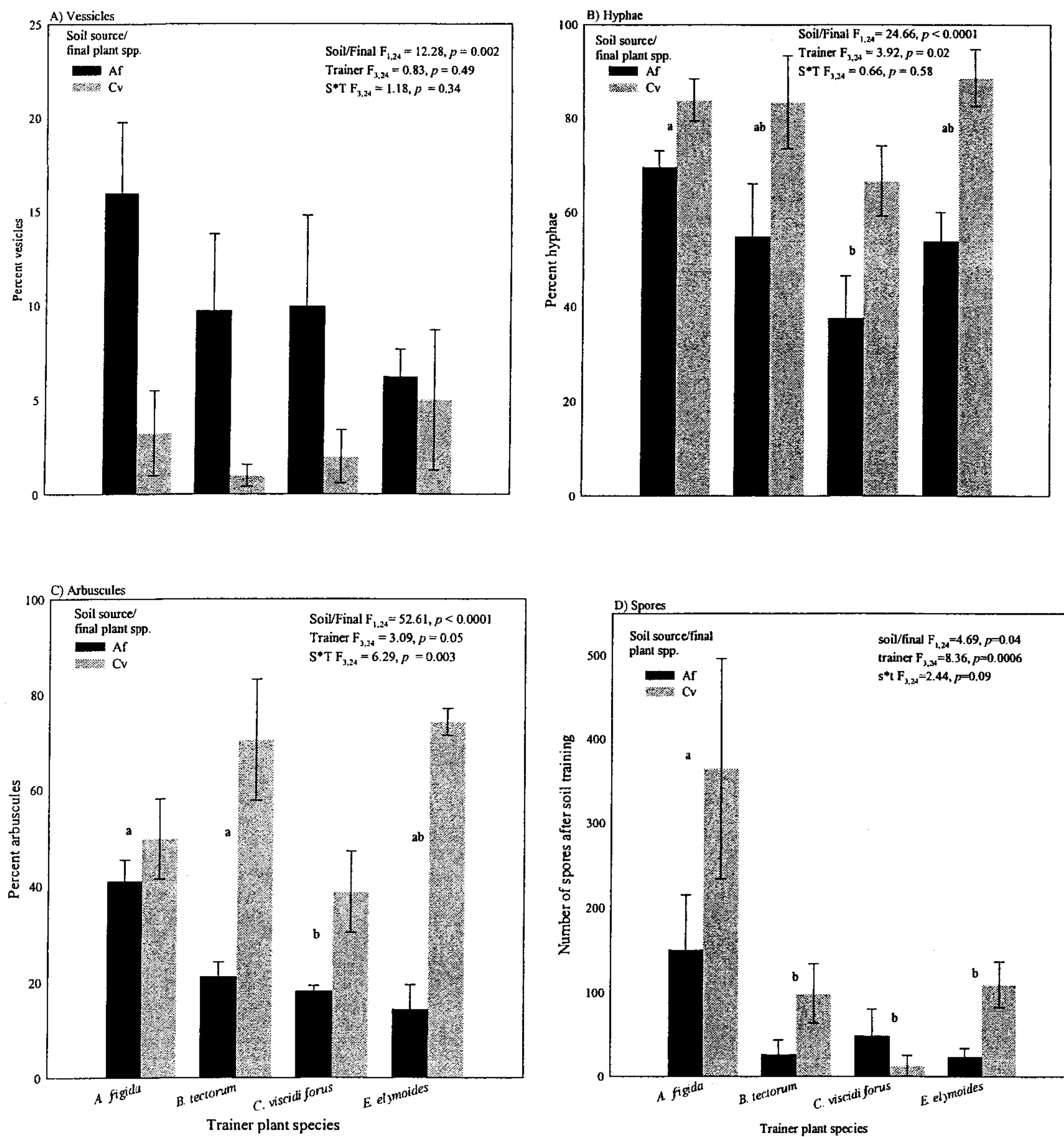

Figure 2.5 Percent (a) vesicle, (b) hyphae, and (c) arbuscule colonization of final plant roots of *A. frigida* and *C. viscidiflorus* and (d) mean number of spores produced during soil conditioning by trainer plants. Different letters indicate differences between trainer plant treatments averaged across soil source/final plant species using Tukey's HSD ($\alpha = 0.05$ for hyphae and spores, $\alpha = 0.01$ for arbuscles). Error bars represent one standard error of the mean.

The mean number of spores after the soil conditioning was correlated with the proportion of the final plant roots colonized with hyphae and arbuscules (Pearson's correlation coefficient $r = 0.43, p = 0.01$, $r = 0.37, p = 0.04$, respectively). Percentages of

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root colonized by hyphae and arbuscules in the final roots were also correlated (r = 0.79, $p < 0.0001$).

Final plants

Final plant species were both highly responsive to AMF. The mean MR value for *C. viscidiflorus* was 1.26 ± 0.17 and 1.04 ± 0.22 for *A. frigida*. T-tests confirmed that final colonized and uncolonized plants were different (positively responsive) within final plant species ($p < 0.001$).

No overall treatment differences were detected. Within soil source/final plant species, *C. viscidiflorus* grew more in its own soil when *B. tectorum* had conditioned the soil than when it had conditioned its own soil, but no differences were detected for *A. frigida* soil source/final plant species (Fig. 2.6). Trainer plant MR was not correlated with final plant MR (r = 0.13, $p = 0.47$), spore counts (r = 0.05, $p = 0.78$), percent hyphae (r = -0.24, $p = 0.19$), percent arbuscules (r = -0.30, $p = 0.10$), or percent vesicles (r = 0.28, $p = 0.13$).

DISCUSSION

Results from the first experiment support previous research (Al-Qarawi 2002) showing a link between low mycorrhizal inoculation potential and *B. tectorum* populations. It was the objective of the second experiment to distinguish whether the lowered mycorrhizal inoculation potential was caused directly by *B. tectorum* or if the effect was simply correlated with another factor, such as disturbance. If *B. tectorum* caused low inoculation potential, we should have seen a negative effect on the

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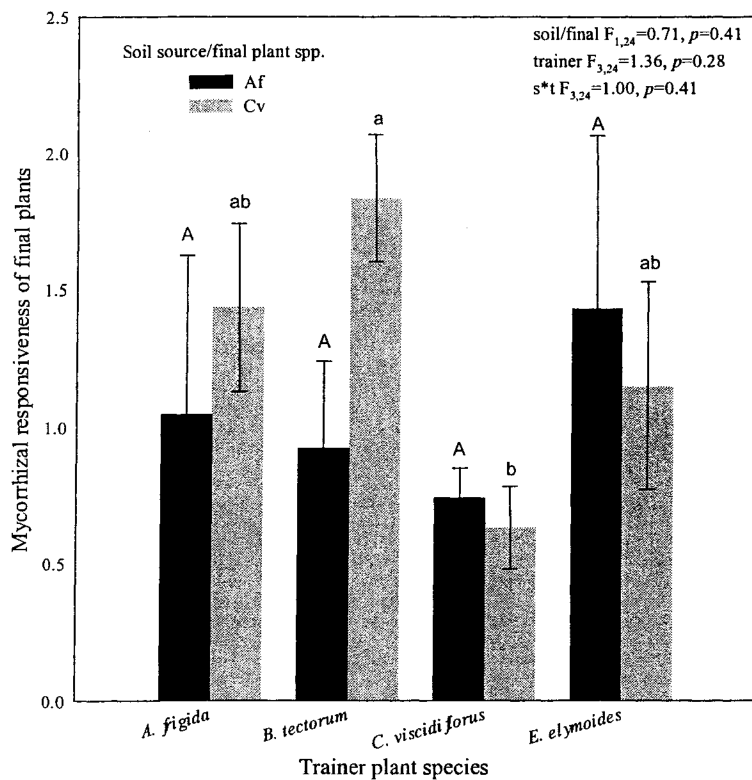

Figure 2.6 Mycorrhizal responsiveness of final plants as influenced by trainer plant species and soil source. Upper case letters denote differences between trainer species within *A. frigida* final plants; lower case letters denote differences between trainer species within *C. viscidiflorus* final plants using Tukey's HSD (α= 0.05). Error bars represent one standard error of the mean.

mycorrhizal community or final plant growth in the samples conditioned by *B. tectorum* compared with the other three trainer species. The second experiment gave no indication that *B. tectorum* affected the mycorrhizal community differently than the other trainer plants. Final plants were not adversely affected by soils conditioned by *B. tectorum.* In fact, *C. viscidiflorus* final plants responded more to the *B. tectorum* conditioned AMF community than soil conditioned by plants of its own species (Fig. 2.6). From this evidence we would conclude that *B. tectorum* does not directly alter the AMF community, however we consider some other issues below.

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One may question whether the unexpected negative responsiveness of all the trainer plants (except *C. viscidiflorus* in *A. frigida* soil inoculum) adversely affected the experiment. We had anticipated a steeper gradient of mycorrhizal responsiveness from highly positive to negative (Rowe *et al.* in press) and expected that this gradient would differentially affect the soil AMF community and final plant growth. However, because trainer mycorrhizal responsiveness was not correlated with final plant responsiveness, spore counts, or percent colonization of the hyphae, vesicles, or arbuscules, it appears that responsiveness was not a strong determinant of the AMF community or final plant responsiveness.

The results presented here differ from the recent work done by Hawkes and others (2005). They found that the presence of *B. tectorum* corresponded with a change in AMF community composition and a decrease in AMF species richness in two native grass species in an undisturbed area. These differences may be reconciled in a number of ways: 1) different variables were measured; 2) *B. tectorum* may affect grass and forb AMF communities differently; 3) AMF communities may have reacted differentially to trainer plant clipping according to annual or perennial life history; 4) *B. tectorum* and AMF community changes may be correlated with changes in soil N. First, we did not examine the genetic composition of the AMF community. In our experiment, *B. tectorum* may have changed the AMF community composition without differentially affecting the final plant biomass of the two final plant species studied here, but this is unlikely given that distinct AMF species and AMF communities have been shown to benefit plant species differently (Bever *et al.* 1996; Bever 2002; Klironomos 2003). Alternatively, each of the trainer plants, non-native and native, may have altered the soil

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AMF community in different ways with the same net growth effect in the two final plant species.

Secondly, it is possible that *B. tectorum* influence on the AMF community may change depending upon the life form and identity of the neighbor plants. At a California study site, Hawkes and others (2005) found that in the presence of non-native grasses *Avena barbata* Pott ex Link and *Bromus hordeaceus* L., AMF richness increased in a native forb while it decreased in native grasses. In Utah, they studied *B. tectorum* interactions with native grasses, whereas we studied the effects of *B. tectorum* on forbs. We don't know whether AMF communities respond differently to *B. tectorum* soil conditioning depending on life form identity.

As a third explanation, clipping trainer plants prior to senescence may have had stronger suppressive effects upon the AMF community associated with the perennial plants compared to those associated with *B. tectorum*, causing their communities to appear similar despite possible negative associations with *B. tectorum*. Prior to senescence, AMF will often transfer resources to spores, relatively long-lived reproductive structures. By cutting the trainer plants prior to senescence, the AMF may not have received the signal to sporulate. This effect may be more pronounced in annual than in perennial plants, because annual plants senesce earlier. In other words, at the time we cut the trainer plants, the AMF community associated with the annual plant *B. tectorum* may have begun to sporulate, whereas, that of the perennials may not have. This difference might help explain the absence of differences between *B. tectorum* and perennial conditioned soils (Jim Bever, Indiana University, personal communication, March 2, 2006). We have no way of knowing whether the *B. tectorum* trainer plants

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sporulated more than the perennials prior to clipping; the *B. tectorum* trainer plants did not produce seed or show aboveground signs of senescence at the time of clipping. It is also unknown whether the clipping had an adverse effect on the AMF root structures and external hyphae. While it is true that hyphae and root fragments are not as robust as spores, they have been shown to be effective inoculum even when isolated from their host plant (Smith & Read 1997).

Finally, although AMF community changes could not be attributed to disturbance, Evans and others (2001), who studied the same field site as Hawkes and others (2005), found that high C:N and lignin:N ratios in *B. tectorum* litter lowered available N at invaded sites. Thus changes in soil N levels due to *B. tectorum* litter could have produced AMF community changes because soil N availability has been shown to affect AMF community identity (Johnson *et al.* 2003). Our experiment was not designed to detect indirect effects and the N fertilizer treatments in our experiment would have prevented any N ameliorated differentiation.

Evidence from Experiment 2 suggests that *B. tectorum* may not directly influence mycorrhizal communities in the short term. It is possible litter deposition may indirectly change AMF communities by altering the C:N and lignin:N soil ratios (Evans *et al.* 2001). In some cases, *B. tectorum* may simply proliferate in areas already low in AMF propagules, perhaps as a result of disturbance. Disturbance leads to decreased AMF diversity (Helgason *et al.* 1998) and reduced AMF infectivity and spore density (Loree & Williams 1984; Doerr *et al.* 1984; Biondini *et al.* 1985; Waaland & Allen 1987) . *B. tectorum* has been shown to favor disturbed areas with high nutrient availability and low interspecific competition (Kay & Evans 1965; McLendon & Redente 1991; Dakheel *et*

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al. 1993) and has been repeatedly shown to grow as well or better in the absence of AMF (Allen 1988; Rowe *et al.* in press). Although the case for a correlation between *B. tectorum* and altered AMF communities is building, researchers should be cautious to conclude that *B. tectorum* is directly responsible for those changes in all cases.

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Chapter 3: Testing the influence of soil communities as a restoration strategy in *Bromus tectorum* dominated field sites

Summary

1. Cheatgrass has invaded approximately 40,000,000 hectares of rangelands in the United States, reducing native species diversity and transforming habitats. Cheatgrass continues to expand its influence and can now be found in montane ecosystems that are important habitats for wildlife and livestock. In addition to well understood mechanisms by which cheatgrass gains competitive advantage, recent studies show cheatgrass is associated with changes in arbuscular mycorrhizal fungi (AMF) communities.
2. We test the idea that cheatgrass is associated with depleted mycorrhizal communities by implementing relevant restoration strategies, soil community and sucrose additions with seeding. Sucrose additions are a proven tool for initiating conditions that accelerate successional change from annual- to perennial-dominated communities.
3. Because most perennial plants respond to AMF with increased growth compared to no AMF, if the AMF community is depleted, the addition of AMF with sucrose should have an additive effect on perennial establishment and growth.

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4. Sucrose amendments had the expected effect of reducing annual plant cover, increasing perennial seedling establishment, and minimally increasing perennial forb cover. Cheatgrass was inhibited by both sucrose and soil community amendments. However, we detected no response of native perennial species to the soil community additions.
5. **'Synthesis and applications'** Despite increased awareness of the need to provide adequate AMF inocula to restoration sites, managers should be aware that sites in which native perennial plants persist may already have sufficient AMF in the soil community. Although a reduced level of AMF has been found in the presence of cheatgrass, it seems that, at least where native species coexist with cheatgrass, the existing soil community can be adequate for successful recruitment of perennial species.

Keywords: arbuscular mycorrhizal fungi, soil community, cheatgrass, inoculum, restoration ecology, seeding, sucrose

Introduction

It is estimated that 40,000,000 ha of rangelands in the United States, once dominated by perennial bunchgrasses and shrubs, are now infested with cheatgrass (*Bromus tectorum* L.) (DiTomaso 2000), a cool-season annual grass introduced from Eurasia (Klemmedson 1964). Approximately 20% of the sagebrush-steppe vegetation zone in the Western U.S. is dominated by cheatgrass to the point where the establishment of native perennial species is very difficult (Knapp 1996). It is estimated that cheatgrass and other rangeland weeds together result in economic losses of $2 billion annually in the U.S. (DiTomaso 2000).

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Cheatgrass is the most ubiquitous weed in steppe vegetation in Western North America (Mack 1981).

The success of *B. tectorum* as an invading species has been largely attributed to its winter annual lifecycle (Hulbert 1955), competitive advantage in disturbed, high nutrient environments (Kay & Evans 1965; McLendon & Redente 1992; Dakheel, Radosevich, & Barbour 1993), and the accumulation of early dry fine fuels that contribute to increased fire cycles that reduce native species abundance and increase *B. tectorum* dominance (Klemmedson 1964; Knapp 1996; Humphrey & Schupp 2001). Nitrogen has been shown to improve the ability of cheatgrass to compete (Kay & Evans 1965; Dakheel, Radosevich, & Barbour 1993; Rasmussen 1995), inhibit establishment of native late-successional plant species (Cherfas 1991), and slow the succession of a site from weedy annuals to native herbaceous perennials (McLendon & Redente 1992; Paschke, McLendon, & Redente 2000). Conversely, decreased N availability has been correlated with the replacement of early-successional species by mid-successional species in a variety of systems (Wedin & Tilman 1990; Tilman & Wedin 1991; McLendon & Redente 1991; McLendon & Redente 1992; Klein et al. 1996; Paschke, McLendon, & Redente 2000; Evans et al. 2001; Blumenthal, Jordan, & Russelle 2003).

Sucrose amendments, which lower soil mineral N availability, have been successful in reducing cheatgrass biomass and community dominance (McLendon & Redente 1994; Paschke, McLendon, & Redente 2000). In an effort to elucidate differing responses in other studies using carbon additions as a restoration tool, Blumenthal, et al. (2003) found three conditions necessary for carbon additions to facilitate native species: 1) initial conditions must favor weeds over native species, 2) weeds must be more nitrophilic relative to native

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species, and 3) the carbon additions must decrease available N for long enough to alter the initial competitive dynamics between annuals and native perennials (Blumenthal, Jordan, & Russelle 2003). The application of sucrose at a larger scale is prohibitively expensive, though perhaps it could be used successfully in combination with other restoration techniques (Paschke, McLendon, & Redente 2000; Blumenthal, Jordan, & Russelle 2003).

Studies show an association between cheatgrass establishment and reduced AMF (Al-Qarawi 2002; Rowe 2006), altered AMF composition, and lower AMF richness in native grasses (Hawkes et al. in press). Decreases in AMF richness or changes in AMF species composition can result in profound changes in the plant community and ecosystem function (Allen et al. 1995; van der Heijden et al. 1998; van der Heijden et al. 1998; Eom, Hartnett, & Wilson 2000; Klironomos, McCune, & Neville 2000; Stampe & Daehler 2003; van der Heijden, Wiemken, & Sanders 2003). Evidence suggests that these changes in the AMF community may not be directly caused by cheatgrass, but by associated factors such as mineral N or disturbance (Rowe 2006). Regardless of the cause, a depleted AMF community may inhibit growth and natural regeneration of perennial species (Allen 1988; Allen 1995). One model proposes that as relative AMF responsiveness of the subordinate species in a plant community rises, the number of plant species increases in the presence of AMF (Urcelay & Diaz 2003). In cheatgrass dominated stands, the dominant species is negatively responsive to AMF and the desired subordinate species, perennials, tend to be more positively responsive (Rowe 2006). Thus, following the model, if the soils in the cheatgrass dominated communities are low in AMF, re-inoculating the site should increase the desired perennials, resulting in increased plant diversity.

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In a field setting, we tested a combination of sucrose addition and soil community inoculation to evaluate their combined efficacy for restoring cheatgrass dominated stands to native perennial communities. The objectives were to: 1) Use sucrose to decrease cheatgrass and annual plant abundance and create conditions to promote establishment and growth of native perennial species; 2) Test whether adding soil community inoculum can increase establishment of native plant species that are highly mycorrhizal responsive in cheatgrass dominated sites; 3) Test whether soil community inoculation in combination with sucrose has a greater effect in shifting the composition of a community towards a late-successional community compared with sucrose treatments alone. If soil community addition treatments increase species richness in the plots, enhance perennial establishment from seed, and increase growth of existing perennial species, we can confirm that the beneficial mutualisms in the soil communities were suppressed or pathogens were enhanced. If no positive growth effect is found, we can conclude that the soil community was sufficient even in the presence of cheatgrass.

Materials and methods

Description

The experimental plots were located between 2,377 and 2,438 m (7800 – 8000 ft) on a south-facing slope in Rocky Mountain National Park (RMNP). *Helianthus pumilus* Nutall [low], *Eriogonum umbelatum* Torrey, *Chrysothamnus viscidiflorus* (Hook.), *Purshia tridentata* (Pursh) de Candolle, *Muhlenbergia montana* (Nutt.) Hitchc., *Hesperostipa comata* (Trinius & Ruprecht) Barkworth [maned] comprise much of the natural vegetation with scattered *Pinus ponderosa* Douglas subsp. *scopularum* (Watson) Weber. Cheatgrass

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invasion on this site is patchy, ranging from no cheatgrass in some areas, to high dominance (70-80 % cover) in others.

Test plots

The experiment was a two by two factorial design with (1) added soil community inoculation (SC+) or ambient soil with no addition (C) and (2) sucrose amendments (N-) or ambient mineral N (N). Blocking was used to control for environmental gradients; there were 48 1.5 m^2 plots and 12 blocks with one replicate of each treatment pair in each block. All treatment plots had cheatgrass cover between 55 and 70%. Soil type and rockiness on plots were as similar as possible within blocks. Treatments were randomly assigned to plots with the restriction that C plots were not closer than 1 m distance apart or directly down slope of SC+ plots to prevent contamination.

Seeding

All plots were broadcast seeded with the same mixture of annual and perennial species collected within approximately one mile of the field site in the same drainage basin at elevations between 2,377 and 2,743 m (7800 – 9000 ft). Perennials included: *Artemisia frigida* Willd. and *C. Viscidiflorus, Aster laevis* L, and *E. umbellatum*, *M. montana*, and short-lived *Elymus elymoides* (Raf.) Swezey. Annual species included *Lappula redowskii* (Hornem.), *Chenopodium leptophyllum* (Nuttall) Watson, and *Polygonum douglasii* Greene. Seeding rates of a nearby RMNP restoration project (646 seeds/m^2) were followed, so that annual species and *E. elymoides* were applied at 80.75 filled seeds m^2 and the remaining species at 64.6 filled seeds/m^2. Germination and dormancy (tetrazolium) tests (Redente,

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Ogle, & Hargis 1982; Fulbright, Redente, & Hargis 1982; Association of Official Seed Analysts (AOSA) 2003) were conducted on 4 replicate trays of 50 collected seeds. All species contained viable seed (Table 3.1).

Table 3.1. Seed viability results from growth chamber germination and tetrazolium tests for seeded species. Percent viability is the sum of percent germination and percent dormancy.

	Percent viability of filled seed	s.e.
Artemisia frigida	91.68	0.02
Aster laevis	93.68	0.01
Chenopodium leptophyllum	32*	-
Chrysothamnus viscidiflorus	39.59	0.03
Elymus elymoides	92.96	0.03
Eriogonum umbellatum	90.15	0.03
Lappula occidentalis	76.25	0.03
Muhlenbergia montana	88.63	0.03
Polygonum douglasii	70.82	0.04

**Che. leptophyllum* did not germinate, this result is from TZ tests taken on one sample. The same batch of seeds germinated well in greenhouse conditions and in the field.

Soil community inoculum

Soil inoculum was collected from a mixed stand of native plants (primarily *Artemisia tridentata* Nutt., *P. tridentata*, *A. frigida*, *C. viscidiflorus* and *M. montana*) in an area without weeds in RMNP at an elevation of 2,540 m (8,339 ft) in October 2003 and again in September 2004. We used whole soil to introduce soil organisms from a reference community in an effort to replicate easily accessible restoration practices. To collect soil, we loosened soil from the rhizosphere of each plant and collected 250 – 500 ml soil; this process was haphazardly repeated across 0.5 hectares to collect a representative sample of all the species in the community. We collected 40 L in 2003 and 16 L in 2004. Soil was mixed and

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coarsely sieved to remove rocks and other debris larger than 1 cm. Roots caught in the filter were cut to provide more even inoculum distribution and added back into the soil. Gloves were worn when working with soil to prevent contamination. Both batches of collected soil successfully inoculated plants with AMF in separate greenhouse experiments (Rowe 2006; Rowe, Brown, & Claassen in press).

We incorporated seed and 0.237 L soil community inoculum by hand-raking each 1.5 m^2 plot in October 2003 and re-inoculated in 2004 with the new soil collection without raking. Two sources of commercial inoculum were included in the 2003 application, but due to poor colonization rates in the greenhouse it was not reapplied in 2004 (Rowe, Brown, & Claassen in press). The commercial inocula consisted of a granular *Glomus intraradices* and a powdered mixture of seven AM fungi taxa, which included Glomus *mosseae* (20%), *G. intraradices* (20%), *G. fasciculatum* (20%), *G. dussii* (10%), *G. clarum* (10%), *G. deserticola* (10%), and *G. microaggregatum* (10%). Application rates were based on manufacturer recommendations (113 g/1.5 m^2 *G. intraradices* and 15 g/1.5 m^2 of the mixed AMF inocula).

Sucrose addition

We applied sucrose nine times each season (2003-04, 2004-05) to immobilize mineral N and other nutrients in the soil. Three monthly applications were made in the fall and bi-monthly applications were initiated when the snow melted in the spring until cheatgrass senescence in June. Application rates were 6,500 kg/ha sucrose in 2003-4 and reduced slightly to 5,900 kg/ha the following season. Three to four ion exchange resin bags were placed in each study plot to document the relative differences in soil mineral N availability during the growing season (October 2003– July 2004, October 2004– August 2005)

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(Paschke, McLendon, & Redente 2000). Mineral N was extracted from the resins following methods in Paschke and others (2000) with the exception that the resin bags were not air dried overnight. Samples were analyzed on an Alp Chem Flow Solution IV (O.I. Analytical, College Station, TX).

Monitoring treatment effects

Pre-treatment baseline estimates of plant cover were taken in August 2003. A single individual visually estimated plant cover with the aid of reference cards the size of 1, 5, and 10% plot area and also counted individual seedlings by species in each plot in July and again in August 2004 and 2005. Only the center 0.5 m^2 of each plot was surveyed to minimize edge effects. After the final field measurements in August 2005, annual aboveground primary production biomass was collected, dried at 36 °C for 72 hours, and weighed. Monthly temperature and precipitation for the duration of the study were similar to the 30-year monthly averages, with the following notable deviations: February 2003 and 2004 were cooler, July 2003 and 2005 were hotter, and March 2003, April, June, and July 2004 were wetter. However, climate data for the years of this study are not fully comparable to 30 year averages because the Estes Park weather station was moved in 2001 (see Appendix A).

Cover and richness of existing species in the community were analyzed by functional group (Tables 3.2 &3.3) (Lauenroth, Dodd, & Sims 1978; Paschke, McLendon, & Redente 2000). Classification into groups was determined by descriptions on USDA Plants database (http://plants.usda.gov/index.html). If a plant species was described as annual, biennial, and perennial, annual and biennial, or biennial and perennial it was categorized as biennial (Table 3.2). Cheatgrass was treated as a separate category.

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Table 3.2 Cover estimates of all plant species by functional group. Cover data from all plots and observations from 2003-2005 were used to calculate species mean and standard error of the mean.

Functional group	Percent cover	
species	mean	s.e.
Bromus tectorum	58.797	1.206
Annual forbs	**17.777**	**1.090**
Native annual forbs	**1.260**	**0.294**
Chenopodium leptophyllum	0.946	0.207
Polygonum douglasii	0.314	0.160
Non-native annual forbs	**16.517**	**1.059**
Alyssum spp.	10.261	0.772
Amaranthus reflexus	0.652	0.155
Chamaesyce maculata	0.441	0.127
Lepidium densiflorum	5.120	0.747
Salsola iberica	0.016	0.010
Taraxacum officinale	0.028	0.022
Biennials	**2.284**	**0.562**
Native biennial forbs	**1.248**	**0.410**
Bahia dissecta	0.440	0.133
Cerastium fontanum	0.001	0.001
Erigeron flagellaris	0.454	0.334
Lappula redowskii	0.347	0.135
Machaeranthera biglovii	0.007	0.005
Non-native biennial forbs	**1.036**	**0.390**
Tragopon dubius	0.319	0.147
Verbascum thapsus	0.717	0.364
Perennial forbs	**6.685**	**0.783**
Artemisia frigida	0.246	0.068
Artemisia ludoviciana	1.676	0.367
Aster laevis	0.005	0.003
Astragalus flexuosus	0.820	0.261
Eriogonum alatum	0.003	0.002
Eriogonum umbellatum	0.468	0.089
Euphorbia brachyceras	0.008	0.005
Grindelia squarrosa	0.022	0.022
Helianthus pumilus	2.405	0.600
Heterotheca villosa	0.044	0.021
Lithospermum incism	0.015	0.010
Oenothera coroni	0.182	0.081
Oxybaphus hirsutus	0.575	0.163
Oxytropis lambertii	0.004	0.003
Penstemon glaber	0.186	0.064
Phacelia heterophylla	0.022	0.014
Potentilla pensylvanica	0.003	0.003

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Functional group	Percent cover	
species	mean	s.e.
Perennial grasses	**10.796**	**0.926**
Bouteloua gracilis	2.255	0.368
Carex microptera	1.230	0.258
Elymus elymoides	1.512	0.242
Elymus trachycalum	0.408	0.159
Hesperostipa comata	1.045	0.252
Muhlenbergia montana	1.572	0.477
Pascopyron smithii	2.014	0.407
Sporobolus cryptandrus	0.759	0.220
Perennial shrubs	**3.202**	**0.603**
Chrysothamnus viscidiflorus	0.892	0.133
Mahonia repens	0.006	0.005
Opuntia polycantha	0.011	0.005
Purshia tridentata	2.293	0.592

*For species authorities see: Weber, William A. and Wittmann, Ronald C. (2001) *Colorado Flora, Eastern Slope*, Third edn. University Press of Colorado, Boulder.

Analysis

Studentized residual plots were used to evaluate the distributional properties of the data. We transformed the data to best meet assumptions of normality and homogeneity of variance and to minimize outlier effects. Nitrogen data from resin bags were log transformed. Seedling counts and cover data of all functional groups were rank transformed. Richness data were square root transformed only for native annual and biennial forbs, non-native biennial forbs, and perennial shrubs. Cheatgrass cover, total, native biennial forb, and perennial forb and grass richness were normal and did not require transformations.
Mineral N was analyzed for years 2004 and 2005 with soil community and N as fixed effects and block as a random effect using the Procedure Mixed (ANOVA, SAS Institute, Inc., version 9.1, Cary, NC, 2002-2003). We analyzed the total number of seedlings with soil community and N as fixed effects and block as a random effect using Procedure Mixed (ANOVA, SAS Institute, Inc., version 9.1, Cary, NC, 2002-2003). Species that germinated in more than three plots were then analyzed separately with soil community and N as fixed

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Table 3.3 ANOVA table for percent cover for each functional group, total species richness, and correlations between biomass and cover estimates. DF columns include degrees of freedom numerator followed by degrees of freedom denominator.

Response variable	Source of variation														Correlation of cover estimates to biomass	
	Soil		Sucrose		Time		Soil*Time		N*Time		N*Soil		Soil*N*Time			
	DF	F value	DF	F value	DF	F value	DF	F value	DF	F value	DF	F value	DF	F value	R	*P*
B. tectorum	1, 32	3.99*	1, 32	6.55*	4, 170	14.98***	4, 170	0.37	4, 170	2.22	1, 32	0.14			0.68	<.0001
Annual forbs	1, 202	0.15	1, 202	25.22***	4, 202	17.04***	4, 202	0.46	4, 202	3.6**	1, 202	0.9			0.94	<.0001
Native annual forbs	1,202	0.59	1,202	11.99***	4, 202	21.62***	4, 202	1.19	4, 202	5.92***	1,202	0.04			1.00	<.0001
Non-native annual forbs	1, 202	0.82	1, 202	17.28***	4, 202	14.18***	4, 202	0.23	4, 202	3.8**	1, 202	1.99			0.95	<.0001
Biennial forbs	1, 32	0	1, 32	1.06	4,202	5.49***	4,202	0.75	4,202	0.65	1, 32	2.83			0.91	<.0001
Native biennial forbs	1,202	0	1,202	0.04	4,202	5.14***	4,202	1.03	4,202	0.64	1,202	5.97*			0.90	<.0001
Non-native biennial forbs	1,202	0.39	1,202	1.14	4,202	2.6*	4,202	0.29	4,202	1.19	1,202	0.01			0.87	<.0001
Perennial grasses	1, 198	0	1, 198	0.11	4, 198	44.27***	4, 198	0.57	4, 198	1.07	1, 202	0.01	4, 198	2.39*	0.84	<.0001
Perennial forbs	1, 202	0.1	1, 202	3.17	4, 202	25.36***	4, 202	1.55	4, 202	0.88	1, 202	1.57			0.82	<.0001
Perennial shrubs	1, 202	9.76*	1, 202	0.51	4, 202	4.28**	4, 202	0.88	4, 202	1.16	1, 202	0.1			0.97	<.0001
Total species richness	1, 32	0.37	1, 32	1.36	4, 170	84.8***	4, 170	0.55	4, 170	0.5	1, 32	0.35				

* significant at P= 0.05 level, ** significant at P= 0.01 level, *** significant at P= 0.001 level

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effects and block as a random effect. *As. laevis,* and *P. douglasii* seeds germinated in three or fewer plots.

A repeated measures design with soil community and N as fixed effects and blocks as a random effect was used to evaluate cheatgrass, annual forb (total, native, and non-native), biennial forb (total, native, and non-native), perennial grass, forb, and shrub cover and richness differences between treatment plots over time using Procedure Mixed (ANOVA, SAS Institute, Inc., version 9.1, Cary, NC, 2002-2003). Autoregressive, lag error structure was used with the repeated measures to account for higher correlation in time points closer together than those farther apart. For simplicity, we present only analyses for total richness and richness for functional groups with treatment differences other than those found in the overall community. Pre-treatment cover estimate comparisons showed that prior differences occurred on soil community and sucrose treatment plots for perennial shrub cover, but for no other functional groups (ANOVA, SAS Institute, Inc., version 9.1, Cary, NC, 2002-2003). Biomass data were highly correlated with cover estimated immediately before cutting (August 2005) and support using the three seasons of cover to estimate vegetation changes over time as a surrogate measure of aboveground biomass (Table 3.3).

Due to technical difficulties, cover and richness data for August 2005 were missing for six plots (all treatments from one block and non-soil community treatments from another block). In addition, one non-soil community sucrose plot was sprayed with herbicide and was excluded from the experiment. Biomass samples for one control plot were lost.

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Results

Sucrose applications lowered mineral N in sucrose treatment plots relative to the non-treated control plots (2004 N- 6.44±1.56 ppm, N 24.80±3.74, $F_{[1,32]} = 32.26$, $P < 0.0001$; 2005 sucrose 12.06±3.06, control 28.37±3.01, $F_{[1,31]} = 28.05$, $P < 0.0001$). When averaged together, seedlings had no overall treatment differences or interactions. Species responded differentially to sucrose; some of the perennial species tended to establish better in the N- plots, while *C. leptophyllum* established better with ambient levels of mineral N (N plots) (Fig. 3.1a). Increased establishment of *C. leptophyllum* in N plots the first year after seeding created a spike in native annual forb species richness that helped explain a sucrose by time interaction ($F_{[4,170]} = 5.34$, $P = 0.0004$). Although seedling responses to soil community treatments were not statistically significant

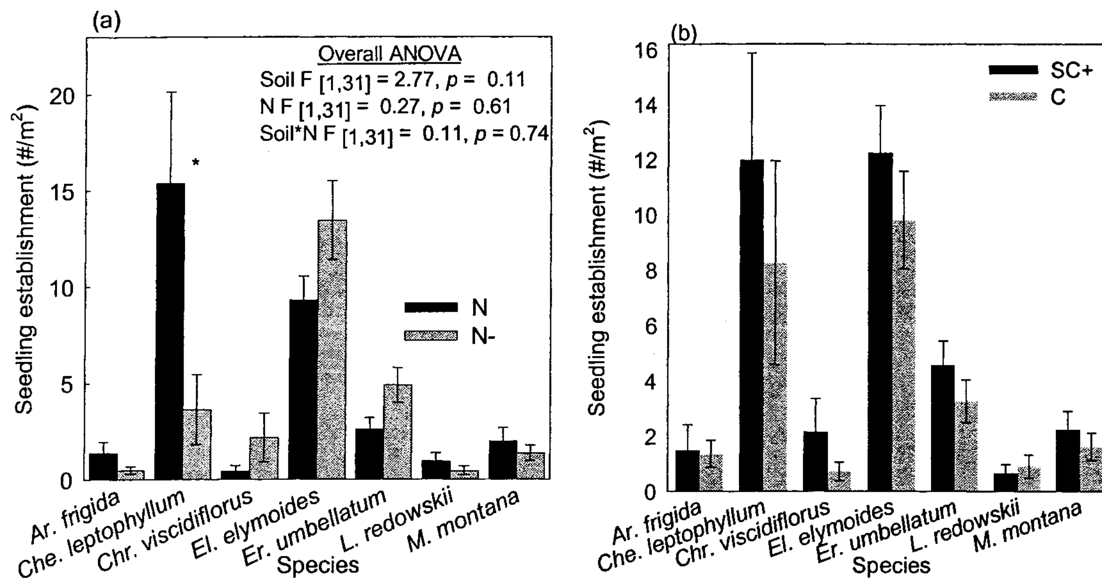

Figure 3.1 Within species comparisons of seedling establishment in (a) N treatments and (b) soil community treatments. Bars represent one standard error of the mean. Asterisk highlights significant difference within *Che. leptophyllum* between sucrose treatments at the $P = 0.01$ level. *As. laevis* and *P. douglasii* were not included due to low representation in the plots. Treatments are abbreviated as follows: sucrose addition (N-), no sucrose addition control (N), soil community addition (SC+), and no soil addition control (C). In the ANOVA table, N denotes the sucrose treatment response variable and Soil denotes the soil community treatment response variable.

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averaged over species or within species, the trend indicates that most species respond favorably to the soil community treatment (Fig. 3.1b).

Annual forb cover decreased over time in the N- plots and increased in N plots (Fig. 3.2). Perennial forb cover had an N treatment effect at the $\alpha = 0.10$ level (N 4.56±0.69%, N- 8.91±1.4%). Cheatgrass cover decreased in N- compared with N plots (Fig. 3.3a) and in SC+ plots compared with ambient soil community (Fig. 3.3b). Native biennial forb cover and richness response to soil community additions depended upon the level of mineral N (Fig. 3.4). A three-way interaction for perennial grass cover (Table 3.3) can be explained by a pronounced seasonal change in the grasses in control plots compared with treated plots.

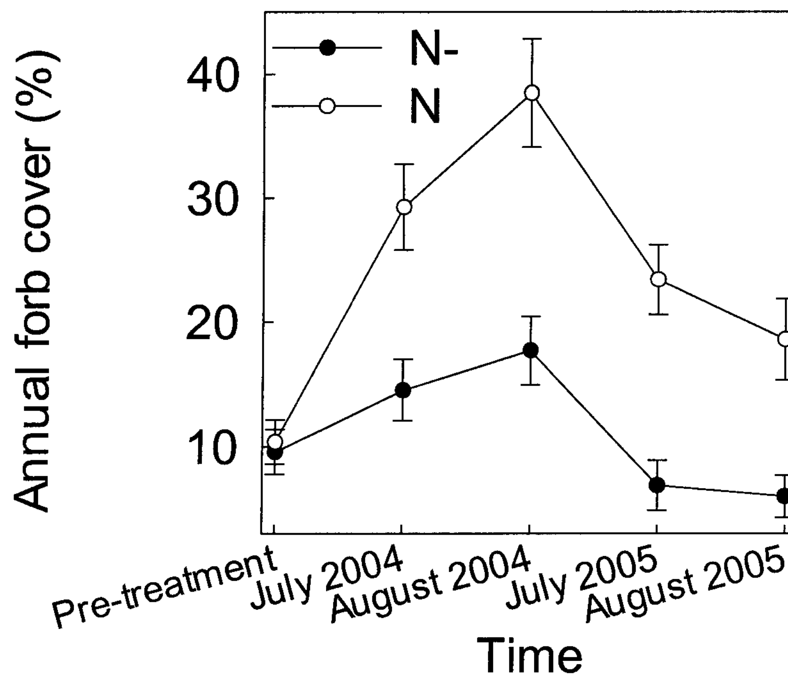

Figure 3.2 Annual forb cover for each sampling time with and without sucrose additions. Bars represent one standard error of the mean. Treatments are abbreviated as follows: sucrose addition (N-), and no sucrose addition control (N).

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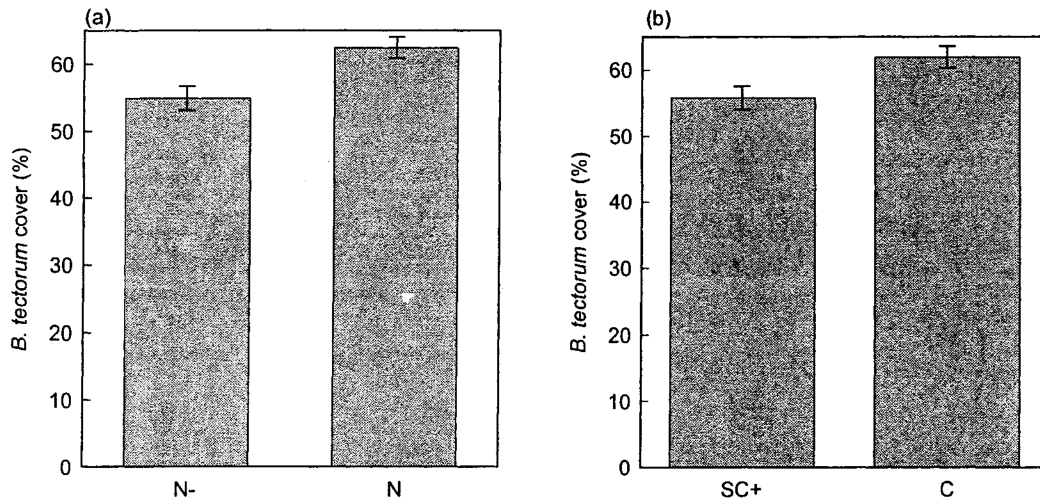

Figure 3.3 *B. tectorum* response to N averaged across soil community and time (a) and *B. tectorum* response to soil community averaged across time and N (b). Bars represent one standard error of the mean. Treatments are abbreviated as follows: sucrose addition (N-), no sucrose addition control (N), soil community addition (SC+), and no soil addition control (C).

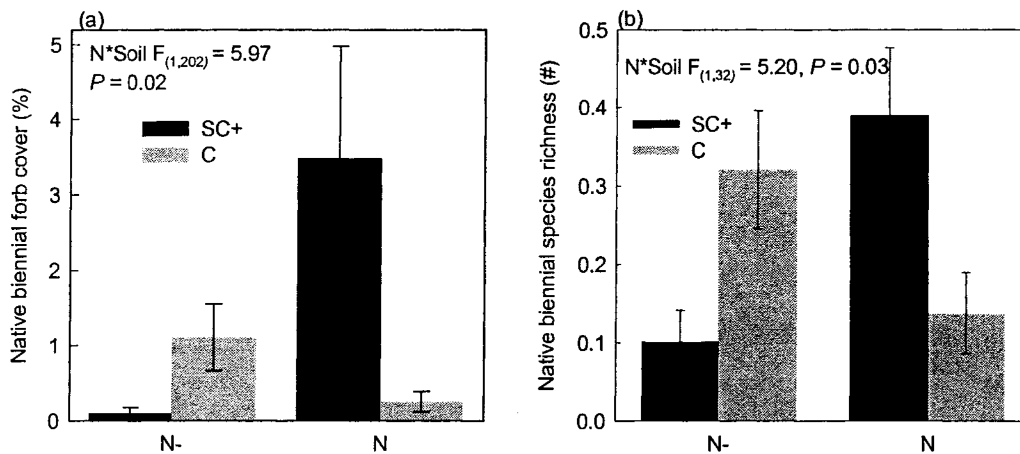

Figure 3.4 Interaction response of native biennial forbs to soil community and sucrose for cover estimates (a) and species richness (b). Bars represent one standard error of the mean. Treatments and are abbreviated as follows: sucrose addition (N-), no sucrose addition control (N), soil community addition (SC+), and no soil addition control (C). In the ANOVA output, N denotes sucrose treatment response variable and Soil denotes soil community treatment response variable.

Total species richness increased over time, probably in part due to improved researcher botanical skills (Table 3.3). N and soil community treatment differences in

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perennial shrub cover did not change beyond the initial pre-treatment baseline conditions (no treatment by time interactions, Table 3.3).

Discussion

Soil community additions reduced cheatgrass cover but had no effect on perennials in this study. With AMF inoculation, we expect a negative response from cheatgrass and positive responses from perennials. In a previous greenhouse study, cheatgrass and other annual species responded negatively and perennials generally responded positively to the same batch of field inoculum used in this study and potting media with soil P levels similar to this field site (Rowe, Brown, & Claassen in press). In a separate study on our field site, we confirmed low AMF associated with native plants surrounded with cheatgrass compared to the same plant species surrounded by other native plants (Rowe 2006). Although the presence of AMF inoculum was confirmed for the soil community inoculum, this treatment probably also included other parasitic and mutualistic soil organisms. Therefore, it is impossible to definitively attribute any treatment effects to one soil organism.

It stands to reason that responses to soil community inoculum would be more detectable in dominant plant species relative to rarer plant species in a plot. This could explain why cheatgrass has the strongest response to the soil community treatments and why other sub-dominant perennial cover and seedlings showed no treatment effect. Alternatively, ambient AMF in the plots may have been sufficient for perennial establishment and growth, despite our expectations that AMF would be low given the cheatgrass dominance on the site. AMF can provide a spectrum of benefit to plants

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ranging from parasitism to mutualism depending on AMF species identity (Johnson, Graham, & Smith 1997; van der Heijden et al. 1998; Klironomos, McCune, & Neville 2000; Klironomos 2003; van der Heijden, Wiemken, & Sanders 2003). It is possible that species of AMF introduced to our site provided no added benefit for perennial plants and had a parasitic effect on the cheatgrass. Alternatively, the soil community inoculum may have contained parasitic fungi or other pathogens that affected the cheatgrass, but had little effect on the native species. The latter interaction seems unlikely because soil pathogens have been found to affect native species more than non-native invasive species (Klironomos 2002).

Sucrose treatments decreased cheatgrass and annual forb cover, as seen in previous studies (McLendon & Redente 1992; Paschke, McLendon, & Redente 2000; Blumenthal, Jordan, & Russelle 2003, but see Corbin & D'Antonio 2004). Our study met at least two of the three conditions required for carbon mediated change from annual to perennial dominated communities: the plots were all dominated by weeds and the weeds were more nitrophilic than the desired perennial species (Blumenthal, Jordan, & Russelle 2003). With respect to the third condition, it is possible that the carbon additions decreased available mineral N for long enough to alter the initial competitive dynamics between annuals and perennials. Reduced soil mineral N and decreased cheatgrass and annual forb cover and biomass show that sucrose amendments decreased plant available N. In addition, perennial forb cover increased (weakly) and three seeded perennial species tended to establish better in the sucrose amended plots. However, perennial shrubs and grasses did not show a growth response in the two years of this experiment. Perennials have five main competitive advantages over annuals in conditions of low soil

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mineral N availability. Perennials (1) have high root:shoot ratios, which allow greater accessibility to soil nutrients, (2) have lower mineral N requirements because they do not have to re-grow all their tissue each year, (3) can reabsorb and reallocate portions of N from senescing tissue prior to loss, (4) have greater C:N ratios due to greater amounts of structural material, and (5) their mutualistic relationships with the decomposer subsystem provide them with increased access to soil mineral N (McLendon & Redente 1992; McLendon & Redente 1994). Thus, sucrose treatments may have initiated conditions of N immobilization required for compositional changes leading to increased perennial dominance in these plots (McLendon & Redente 1992; McLendon & Redente 1994) and community change will continue along this trajectory.

The interaction between soil community and mineral N for native biennial forbs cannot be fully explained. If the soil community treatments were primarily AMF, we would expect an interaction of soil community and mineral N that was the reverse of what occurred in this study. Elevated levels of mineral N have been associated with less beneficial AMF associations (Johnson et al. 2003), thus in plots with higher mineral N (control plots) we expect AMF to have a less beneficial effect. In lower nutrient conditions, we anticipated greater benefit from the AMF (Johnson 1993). The reverse occurred in this experiment for native biennial forbs. Soil organisms other than AMF may be responsible for this effect.(Johnson, Graham, & Smith 1997; van der Heijden et al. 1998; Klironomos, McCune, & Neville 2000; Klironomos 2003)

Overall, mineral N and soil community treatments greatly affected the abundances of cheatgrass and native plant species in this field experiment. We found that: 1) Decreased soil mineral N reduced cheatgrass and other annual plant abundances

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and increased establishment of some perennial seedlings, 2) Adding soil community inoculum did not increase perennial establishment on the site in general, but it increased some native biennial forbs, depending on soil mineral N, and 3) Mycorrhizal inoculation in combination with sucrose did not appear to have a greater effect in shifting the composition of the community towards late-successional species compared to sucrose treatments alone, but soil community additions decreased cheatgrass abundance in the field. While sucrose amendments seem to have begun shifting the community dominance from cheatgrass and annual forbs to perennials, it appears that soil community additions did not contribute directly to the restoration of perennial species. Although previous studies found that AMF is depleted in cheatgrass dominated communities (Al-Qarawi 2002; Hawkes et al. in press), the results presented here cast doubt as to whether this reduction actually affects native plant communities (Rowe 2006). The inhibition of cheatgrass with soil community additions appears promising, but because responses of plant functional groups cannot be adequately explained, this application may require further investigation.

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Chapter 4: Testing succession-based seeding for restoration of montane communities invaded by cheatgrass

Abstract

It is estimated that 40,000,000 ha of rangelands in the United States, once perennial grass and shrublands, are now dominated with cheatgrass (*Bromus tectorum* L.). We must develop effective methods for the control of cheatgrass and restoration of invaded sites to conserve biological diversity and ecosystem functioning. Here we apply seeding treatments based on facilitation and tolerance models of succession to evaluate the success of using native seeds as an "ecological bridge". The succession models explain different interactions among early and late successional species that ultimately allow late successional species to dominate, as is our goal for restoration. The "ecological bridge" describes the approach of using hardy early successional plant species to compete against the invasive plant and allow native perennial communities to establish. In this field study, we evaluated the ecological bridge approach according to whether treatments favored perennial plant species and reduced cheatgrass re-establishment after herbicide control. We compared the appropriateness of the models

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for choosing seeding mixtures through the performance of late successional species in six seeding treatments. Cheatgrass was reduced to five percent the first year with glyphosate. In our experiment, there was weak evidence that facilitation within the seed mixtures improved late successional seedling establishment when late and early successional seeds were planted together compared with controls. Seed rain contributed significantly to seedling recruitment. The ecological bridge approach was partially supported because treatments did not inhibit late successional seedling establishment and cover. However, none of the seeding treatments reduced cheatgrass re-establishment.

Keywords: succession, facilitation, tolerance, inhibition, cheatgrass, *B. tectorum*, seeding techniques, restoration

Introduction

Approximately 20% of the sagebrush-steppe vegetation zone in the Western U.S. is dominated by cheatgrass to the point where the establishment of native perennial species is nearly impossible (Knapp 1996). It is estimated that cheatgrass and other rangeland weeds together result in economic losses of $2 billion annually in the U.S. (DiTomaso 2000). Cheatgrass is the most ubiquitous weed in steppe vegetation in Western North America (Mack 1981).

The success of cheatgrass as an invading species in the Western U.S. is largely attributed to its winter annual lifecycle (Hulbert 1955). Fall-germinated seedlings of cheatgrass spend winter in a semi-dormant state and grow rapidly in the spring as conditions become favorable (Stewart & Hull 1949; Hulbert 1955; Mitich 1999). This early growth allows cheatgrass to gain a competitive advantage over slower growing native perennial species as cheatgrass completes its lifecycle by early summer (Hulbert

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1955). The resulting accumulation of fine fuels from senescent cheatgrass often results in fires that further reduce native species abundance, and increase cheatgrass dominance (Knapp 1996).

A relatively new term, "ecological bridge", describing the use of early successional species as an intermediate step to ameliorate site conditions for late successional species, has been recently applied to the restoration of weed dominated landscapes (Hardy & Palazzo 2002). If bridging to desired late successional species is to be successful, facilitation or tolerance (Connell & Slatyer 1977) must be the predominant model at work. Connell and Slatyer (1977) described three models of succession. In all models, disturbance opens a space and colonizers with early successional traits establish on the site. The models differ in how late successional species become established. With facilitation, early successional species create conditions favorable for establishment of late successional species. In both the tolerance and inhibition models, late successional species do not require amelioration of the site by other species. In the tolerance model, late successional species establish later than early species because of their life history traits, but will eventually dominate due to their superior competitive abilities. The early species on this site will neither increase nor decrease the rates of recruitment or growth of other species. The inhibition model describes conditions by which the initial colonizers inhibit establishment of subsequent species on the site. The initial colonizers must die or be damaged in order for other species to establish (Connell & Slatyer 1977). By basing seed mixtures on the facilitation and tolerance models of succession, we can find out which model best allows late successional species to establish, one requirement of the "ecological bridge".

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The goal of the research was to test the "ecological bridge" approach for reducing cheatgrass and restoring native, late successional perennials in cheatgrass invaded montane communities. Specifically, we tested which succession based seed mixture a) resulted in the highest establishment of late successional species and b) best resisted cheatgrass.

Methods

Description

An experiment was conducted to evaluate the effects of succession-based seed mixtures on the establishment of late successional species and their ability to deter cheatgrass invasion. Early successional species would be included in a facilitation based seed mixture to aid the establishment of late successional species. With tolerance as the model, early successional species would not be included because it is expected that they do not aid late successional species establishment. In our study, the two seed mixtures representing facilitation were an early and late successional (ELS1) species mixture planted simultaneously in the same year and a mixture planted sequentially, the early successional mixture in the first year and the late successional mixture in the second year (ELS2). The tolerance mixtures were a mixture of late successional (LS1) species planted the first year alone and a mixture of late successional species planted the second year into plots not seeded the first year (LS2). These seed mixtures were planted into an existing plant community comprised of native and non-native plant species. The dominant weed, cheatgrass, was reduced with herbicide the first year as described below.

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The experiment was located at an elevation of about 2,378 m on a south-facing slope in Rocky Mountain National Park (RMNP). *Helianthus pumilus* Nutt. (little sunflower), *Eriogonum umbelatum* Torr. (sulfur-flower buckwheat), *Chrysothamnus viscidiflorus* (Hook.) (yellow rabbitbrush), *Purshia tridentata* (Pursh) de Candolle (antelope bitterbrush), *Muhlenbergia montana montana* (Nutt.) Hitchc. (mountain muhly) and *Hesperostipa comata* (Trin. & Rupr.) Barkworth (needle and thread) comprised much of the natural vegetation with scattered *Pinus ponderosa* Douglas subsp. *scopularum* (Watson) Weber. Cheatgrass invasion on this site was patchy, ranging from no cheatgrass in some areas, to high dominance (70-80 % cover) in others.

Experimental design

A randomized complete block design was used to control for environmental gradients; there were 72 1.5 m^2 plots and 12 blocks with one replicate of each of the 6 treatments (late successional species, one year (LS1), early and late successional species, one year (ELS1), early successional species, one year (ES1), non-seeded control (C), no seed first year, late successional species second year (LS2), early successional species first year, late successional species second year (ELS2) in each block. We selected block locations to minimize differences in soil type, cheatgrass and soil cover among plots within blocks. All treatment plots had cheatgrass cover between 55 and 70%. Soil type and rockiness on plots were as similar as possible within blocks.

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Cheatgrass control

In order to create an opening for seedlings to establish, cheatgrass was removed to ≤ 5% cover in April 2004 with glyphosate (Roundup®), applied at a rate of 0.55 kg/ha with a CO_2 pressurized backpack sprayer (Whitson & Koch 1998). Native plants were covered to protect them from spray.

Seed mixes

We collected seeds within 2 km of the field site in the same drainage basin at elevations of approximately 2,377-2,743 m (7800 – 9000 ft) and broadcast seeded with rake incorporation following seeding rates of a nearby RMNP restoration project (646 seeds m^{-2}). For species in the LS1 and ES1 mixtures, the total seeding density (646 filled seed m^{-2}) was evenly divided among species. The late-early (ELS1) treatment received half the seed density of the ES1 and of LS1 treatments to equal the same total seed density as the LS1 and ES1 treatments. Keeping seeding densities constant in the first year treatments allowed us to compare seeding treatment effects on cheatgrass re-establishment. The two-year seeding treatments (ELS2 and LS2) followed an additive design, such that the seeding density was the same for each year and application. In the first year ELS2 was seeded with the ES1 mixture at the 646 filled seeds m^{-2} rate and the LS2 was not seeded. In the second year both treatments were seeded with the LS1 mixture at the same 646 filled seed m^{-2} rate. Same seeding rates in year two enabled us to compare establishment of late successional species seeded at the same densities in the same year with and without the presence of seeded early successional species.

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We characterized late successional seed mix species as perennial and long-lived; our late successional mixture included two shrubs (*Artemisia frigida* Willd. [fringed sage] and *Prunus virginiana* L [chokecherry]), two forbs (*Aster laevis* L [smooth blue aster], and *E. umbellatum*), and two grasses (*M. montana* and *H. comata*). *Prunus virginiana* was grown from seed in the greenhouse and transplanted in the field, but herbivores claimed most of these, thus, this species was omitted from the analyses. Early successional seed mix species were shorter lived perennials and annuals and included no shrubs, five forbs (*Lappula redowskii* (Hornem.) [flatspine stickseed], *H. pumilus*, *Chenopodium leptophyllum* (Moq.) Nutt. [narrowleaf goosefoot], *Polygonum douglasii* Greene [Douglas' knotweed] and *Heterotheca villosa* (Pursh) Shinners [hairy false goldenaster] and one grass *Elymus elymoides* (Raf.) Swezey [squirreltail]). Germination and dormancy (tetrazolium) tests (Redente *et al.* 1982; Fulbright *et al.* 1982; Association of Official Seed Analysts (AOSA) 2003) were conducted on 4 replicate trays of 50 collected seeds. All species contained viable seed (Table 4.1).

Monitoring treatment effects

Pre-treatment baseline estimates of plant cover were taken in August 2003. A single individual visually estimated plant cover with the aid of reference cards the size of 1, 5, and 10% plot area and also counted individual seedlings by species in each plot in July and again in August 2004 and 2005. Only the center 0.5 m^2 of each plot was surveyed to minimize edge effects. After the final field measurements in August 2005, annual aboveground primary production was collected, dried at 36 °C for 72 hours, and weighed. Monthly temperature and precipitation for the duration of the study were

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Table 4.1. Germination results for seeded species. Percent viability includes germination and dormancy tests for filled seed.

	Percent viability of filled seed	s.e.
Early spp.		
Chenopodium leptophyllum	32*	-
Elymus elymoides	92.96	0.03
Helianthus pumilus	35.13	0.20
Heterotheca villosa	83.31	0.06
Lappula redowskii	76.25	0.03
Polygonum douglasii	70.82	0.04
Late spp.		
Artemisia frigida	91.68	0.02
Aster laevis	93.68	0.01
Chrysothamnus viscidiflorus	39.59	0.03
Eriogonum umbellatum	90.15	0.03
Hesperostipa comata	74.00	0.02
Muhlenbergia montana	88.63	0.03

**Che. .leptophyllum* did not germinate, this result is from TZ tests taken on one sample. The same batch of seeds germinated well in greenhouse conditions and in the field.

similar to the 30-year monthly averages, with the following notable deviations: February 2003 and 2004 were cooler, July 2003 and 2005 were hotter, and March 2003, April, June, and July 2004 were wetter. However, climate data for the years of this study are not fully comparable to 30 year averages because the Estes Park weather station was moved in 2001 (see Appendix A).

Cover and richness of existing species in the community were analyzed by functional group (Table 4.2) (Lauenroth *et al.* 1978; Paschke *et al.* 2000). Classification into groups was determined by descriptions on USDA Plants database (http://plants.usda.gov/index.html). If a plant species was described as annual, biennial, and perennial, annual and biennial, or biennial and perennial it was categorized as biennial (Table 4.2). Cheatgrass was treated as a separate category.

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Table 4.2. Cover estimates of all plant species by functional group. Cover data from all plots and observations from 2003-2005 were used to calculate species mean and standard error of the mean.

Functional group	Percent cover	
species	**mean**	**s.e.**
Bromus tectorum	39.572	1.399
Annual forbs	**22.285**	**0.918**
Native annual forbs	**0.572**	**0.097**
Chenopodium leptophyllum	0.451	0.084
Epilobium brachycarpum	0.002	0.001
Polygonum douglasii	0.119	0.052
Non-native annual forbs	**21.713**	**0.919**
Alyssum spp.	13.471	0.753
Amaranthus reflexus	1.558	0.265
Chamaesyce maculata	0.543	0.120
Lepidium densiflorum	6.140	0.507
Salsola iberica	0.001	0.001
Taraxacum officinale	0.012	0.011
Biennials	**1.399**	**0.197**
Native biennial forbs	**0.896**	**0.162**
Bahia dissecta	0.354	0.095
Erigeron flagellaris	0.049	0.049
Lappula redowskii	0.300	0.061
Machaeranthera biglovii	0.193	0.085
Non-native biennial forbs	**0.504**	**0.116**
Camelina microcarpa	0.013	0.009
Non-native biennial forbs	**0.504**	**0.116**
Sisimbrium altissimum	0.057	0.045
Tragopon dubius	0.214	0.064
Verbascum thapsus	0.219	0.079
Perennial forbs	**6.191**	**0.557**
Artemisia frigida	0.495	0.130
Artemisia ludoviciana	1.445	0.260
Aster laevis	0.000	0.000
Astragalus flexuosus	0.062	0.026
Cirsium undulatum	0.047	0.029
Eriogonum alatum	0.003	0.002
Eriogonum umbellatum	0.431	0.085
Helianthus pumilus	2.411	0.425
Heterotheca villosa	0.049	0.015
Lithospermum incism	0.014	0.012
Oenothera caespitosa spp macroglottis	0.014	0.008
Oenothera coroni	0.391	0.098
Oxybaphus hirsutus	0.346	0.108
Oxytropis lambertii	0.009	0.009
Penstemon glaber	0.156	0.085

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Functional group	Percent cover	
species	mean	s.e.
Phacelia heterophylla	0.001	0.001
Potentilla fissa	0.003	0.003
Potentilla pensylvanica	0.007	0.006
Sollidago spp.	0.310	0.157
Perennial grasses	**9.750**	**0.784**
Bouteloua gracilis	1.643	0.198
Carex microptera	1.723	0.337
Elymus elymoides	0.458	0.075
Elymus lanceolatus	0.003	0.003
Elymus trachycalum	0.094	0.044
Hesperostipa comata	1.321	0.263
Koeleria macrantha	0.007	0.007
Muhlenbergia montana	2.835	0.434
Pascopyron smithii	0.766	0.139
Sporobolus cryptandrus	0.902	0.198
Perennial shrubs	**2.533**	**0.360**
Ceanothus fendleri	0.026	0.014
Chrysothamnus viscidiflorus	1.113	0.180
Mahonia repens	0.334	0.153
Opuntia polycantha	0.254	0.096
Prunus virginiana (transplant)	0.015	0.006
Purshia tridentata	0.761	0.163
Ribes cereum	0.007	0.006
Pinus ponderosa	0.005	0.003

For species authorities see Weber, W.A. and R.C. Wittmann. 2001. Colorado Flora, Eastern Slope. Third edition. University Press of Colorado, Boulder.

Analysis

Studentized residual plots were used to evaluate the distributional properties of the data. We transformed the data to best meet assumptions of normality and homogeneity of variance and to minimize outlier effects. Cover estimate and number of seedling data were rank transformed. Richness data were arcsine square-root transformed. Cheatgrass cover data did not require transformation.

We analyzed the early successional and late successional seedling establishment separately with treatment as a fixed effect and block as a random effect using Procedure Mixed (ANOVA, SAS Institute, Inc., version 9.1, Cary, NC, 2002-2003). Analysis of

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early successional seedling establishment included all treatments, because early successional species were all seeded in the same year. We compared the establishment of late successional species separately for the one-year and two-year treatments because, at the last sampling date, the late successional species had only one year to establish in the two-year treatments (LS2, ELS2), whereas the one-year treatments (ELS1, LS1) had two years to establish. The one-year and the two-year treatments shared the control (C) treatment.

Early and late successional seedling establishment was also analyzed within species with treatment as a fixed effect and block as a random effect. F-protected differences of least squared means were used to separate means with $\alpha = 0.05$. *Aster laevis* seedlings and *P. virginiana* transplants were found in fewer than three plots each and were excluded from these analyses.

A repeated measures design with treatments as fixed effects and blocks as a random effect was used to evaluate cheatgrass, annual forb (total, native, and non-native), biennial forb (total, native, and non-native), perennial grass, forb, and shrub cover and richness differences among treatment plots over time using Procedure Mixed (ANOVA, SAS Institute, Inc., version 9.1, Cary, NC, 2002-2003). Autoregressive, lag error structure was used with the repeated measures to account for higher correlation in time points closer together than those farther apart. Annual natives and non-natives and biennial natives and non-natives were analyzed separately, but were presented individually only if the final results differed from the analyses of the combined variables. Pre-treatment cover estimate comparisons showed that prior treatment differences occurred for perennial forb cover and species richness, but for no other functional groups

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(ANOVA, SAS Institute, Inc., version 9.1, Cary, NC, 2002-2003). The one-year and two-year treatments were analyzed separately and together. The only difference between the two approaches was that, when analyzed separately, there were no differences in biennial forb cover and richness, but when comparing all treatments together, biennial forb cover and richness had treatment differences amongst the one-year and two-year treatments. For simplicity, we present the combined analysis. Biomass data were highly correlated with cover estimated immediately before cutting (August 2005) and support using the three seasons of cover to estimate changes in vegetation production over time (Table 4.3).

Table 4.3. ANOVA table for percent cover and species richness analyses, and correlations between biomass and cover estimates. DF columns include numerator degrees of freedom followed by denominator degrees of freedom. Treatments include all six single year and multi-year treatments.

Response variable	Source of variation						Correlation of cover estimates to biomass	
	Treatment		Time		Treatment*Time			
	DF	F value	DF	F value	DF	F value	R	*P*
Cover								
B. tectorum	5, 55	1.05	4, 255	530.78***	20, 255	1.15	0.72	<0.0001
Annual forbs	5, 55	1.41	4, 255	40.49***	20, 255	0.64	0.74	<0.0001
Biennial forbs								
native	5, 55	2.41*	4, 255	12.63***	20, 255	1.25	0.94	<0.0001
Non-native	5, 55	2.2	4, 255	3.53**	20, 255	1.17	0.95	<0.0001
Perennial grasses	5, 55	0.25	4, 255	51.86***	20, 255	1.2	0.80	<0.0001
Perennial forbs	5, 55	2.96*	4, 255	22.81***	20, 255	0.58	0.90	<0.0001
Perennial shrubs	5, 55	0.37	4, 255	10.42***	20, 255	0.89	0.95	<0.0001
Richness								
Annual forbs	5, 55	0.92	4, 255	26.46***	20, 255	0.53		
Biennial forbs								
native	5, 55	2.92*	4, 255	12.34***	20, 255	1.26		
Non-native	5, 55	2.11	4, 255	3.32**	20, 255	1.17		
Perennial grasses	5, 55	0.63	4, 255	39.58***	20, 255	2.14**		
Perennial forbs	5, 55	3.64**	4, 255	21.21***	20, 255	0.93		
Perennial shrubs	5, 55	0.52	4, 255	9.32***	20, 255	0.8		

* significant at P= 0.05 level, ** significant at P= 0.01 level, *** significant at P= 0.001 level

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Due to technical difficulties, cover and richness data for August 2005 were missing for nine plots (all treatments except ELS2 from one block and all single year treatments from another block).

Results

Seeding treatment differences

Late successional species seedling establishment in the one-year treatments differed by treatment (Fig. 4.1a). On average, more late successional seedlings established in the

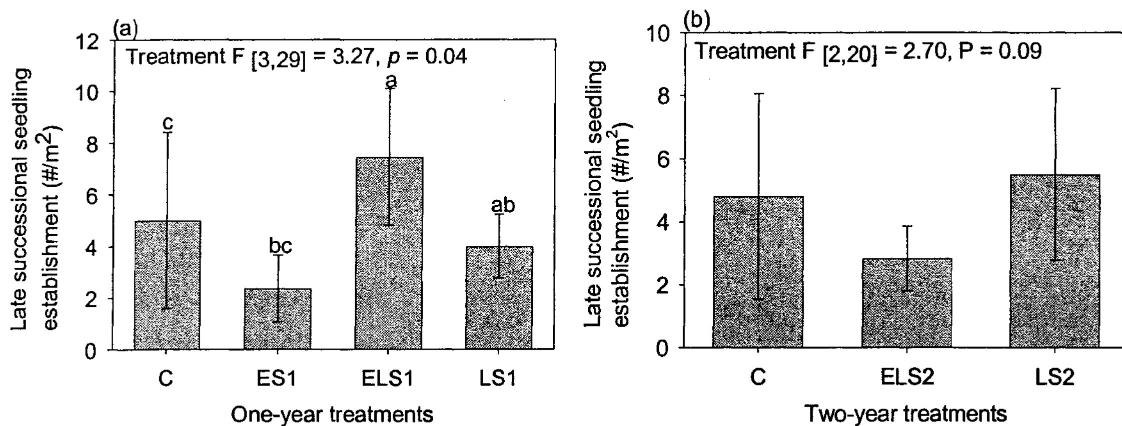

Figure 4.1 Establishment of late successional seeded species by treatment averaged over species for (a) one-year treatments and (b) two-year treatments. Species included in the analysis are: *Ar. frigida, Chr..viscidiflorus, Er. umbellatum, Hes. comata, M. montana.* Error bars represent one standard error of the mean. Different letters indicate treatment differences within species using differences of least squared means (α = 0.05). Seed mixture treatments are abbreviated as follows: no seeded control (C), early successional species seeded year one (ES1), early and late successional species seeded year one (ELS1), late successional species seeded year one (LS1), early successional species planted year one, late successional species planted year two (ELS2), no seed planted year one, late successional species planted year two (LS2).

facilitation treatment (ELS1) compared with treatments without late successional species (C and ES1, Fig. 4.1a). The tolerance treatment (LS1) was not different from the

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facilitation treatment (ELS1) or from the early successional treatment (ES1, Fig. 4.1a). There were no differences amongst the two-year treatments (Fig. 4.1b).

When analyzing the species separately, *Er. umbellatum* was the only late successional species with significant treatment differences (Figs. 4.2 a & b). *Eriogonum umbellatum* established better when seeded the second year into unseeded plots (LS2) than ELS2 and C (Fig. 4.2b). Although seed rain from outside the plots may have contributed to *Er. umbellatum* establishment, there were no pre-treatment differences in *Er. umbellatum* cover ($F_{[5,55]} = 0.43$, $p = 0.82$).

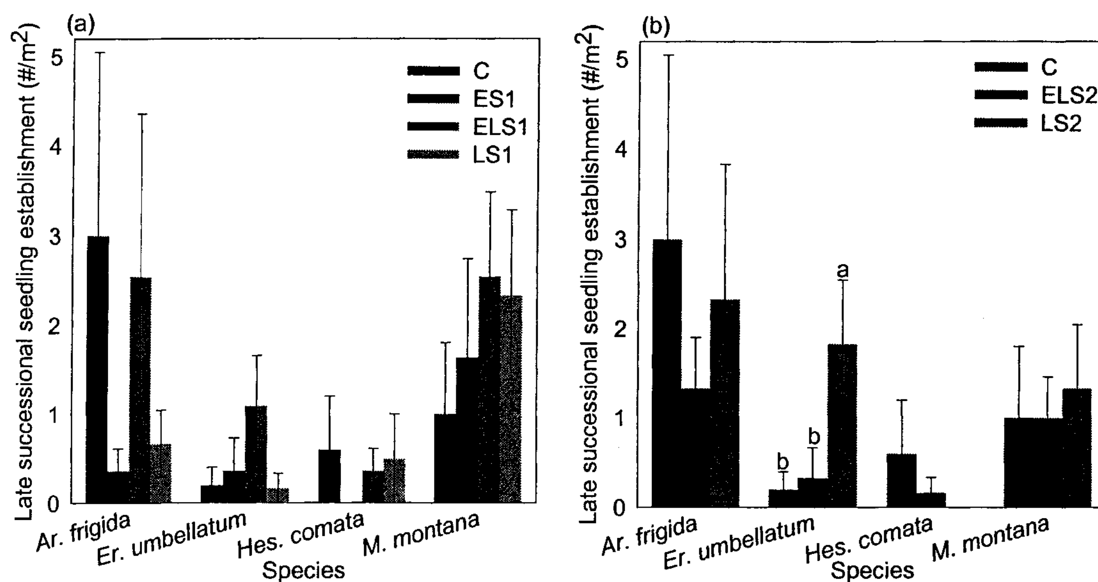

Figure 4.2 Within species comparisons of late successional species establishment in the one-year (a) and two-year (b) treatments. Error bars represent one standard error of the mean. Different letters indicate treatment differences within species using differences of least squared means ($\alpha = 0.05$). Seed mixture treatments are abbreviated as follows: no seeded control (C), early successional species seeded year one (ES1), early and late successional species seeded year one (ELS1), late successional species seeded year one (LS1), early successional species planted year one, late successional species planted year two (ELS2), no seed planted year one, late successional species planted year two (LS2).

Overall, early successional species seedling establishment was higher with the seeding treatments (ELS2, ES1, ELS1) than the non-seeded treatments (C, LS1, LS2; F

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$_{[5,51]} = 10.15, p < 0.001)$. *Elymus elymoides, Het. villosa,* and *L. redowskii* established best in treatments in which they were planted (ES1, ELS1, ELS2, Fig. 4.3). In contrast, *C. leptophyllum* establishment was greatest in plots in which it was not seeded (C and LS1, Fig. 4.3).

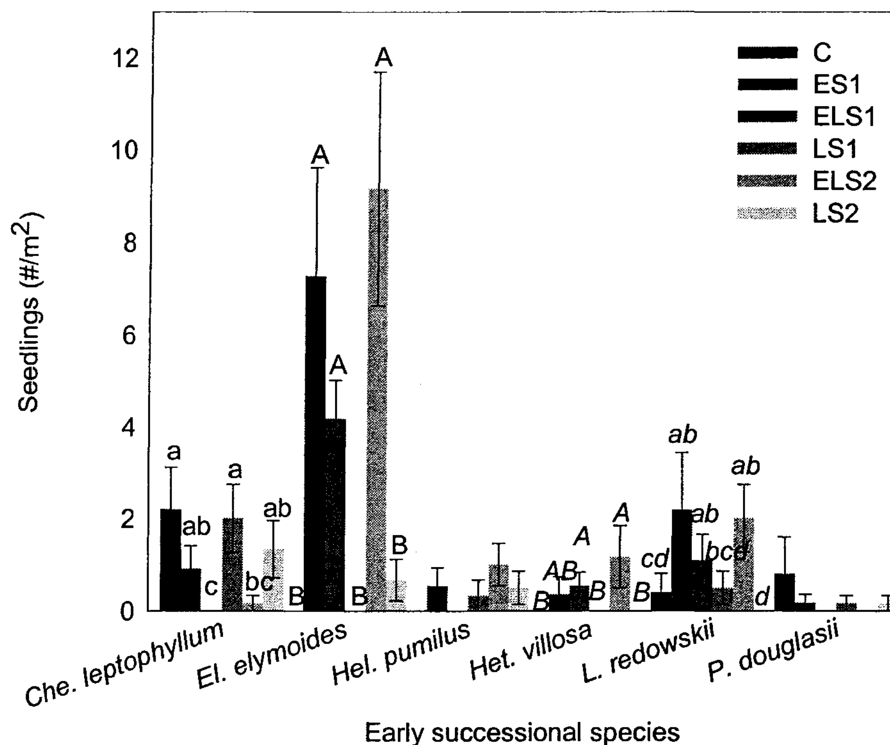

Figure 4.3 Within species comparisons of early successional species establishment amongst treatments. Error bars represent one standard error of the mean. Different letters indicate treatment differences within species using differences of least squared means ($\alpha = 0.05$). Seed mixture treatments are abbreviated as follows: no seeded control (C), early successional species seeded year one (ES1), early and late successional species seeded year one (ELS1), late successional species seeded year one (LS1), early successional species planted year one, late successional species planted year two (ELS2), no seed planted year one, late successional species planted year two (LS2).

Existing plant community

Percent cover of all functional groups increased over time (Table 4.3, Fig. 4.4). *Bromus tectorum* decreased in response to the glyphosate treatment, but returned to pre-treatment levels the following year (Fig. 4.4). Perennial grass richness over time

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depended upon treatment (Table 4.3, Fig. 4.5). ELS1 treatments, which included late successional grass species *M. montana* and *Hes. comata* as well as early successional,

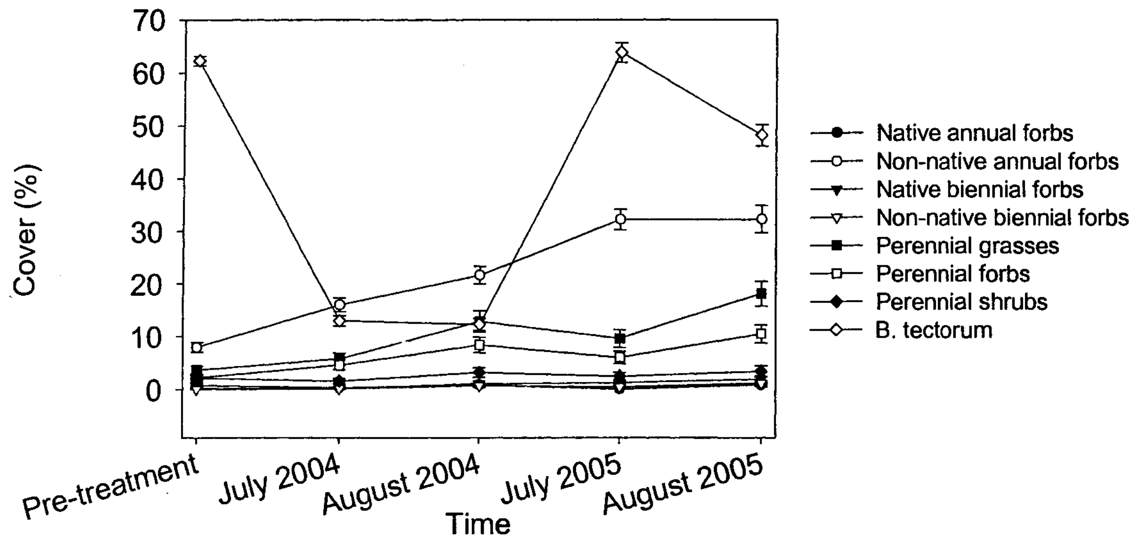

Figure 4.4 Mean plant cover for each sampling date by functional group. Bars represent one standard error of the mean.

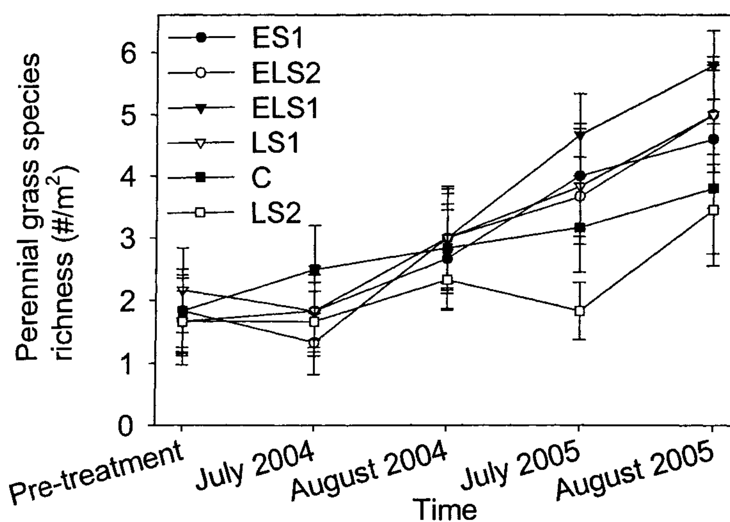

Figure 4.5 Perennial grass species richness change in estimated cover over time. Bars represent one standard error of the mean. Seed mixture treatments are abbreviated as follows: no seeded control (C), early successional species seeded year one (ES1), early and late successional species seeded year one (ELS1), late successional species seeded year one (LS1), early successional species planted year one, late successional species planted year two (ELS2), no seed planted year one, late successional species planted year two (LS2).

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short-lived, perennial *El. elymoides*, gave the greatest increase in perennial grass richness over time (Fig. 4.5). The C and the LS2 treatments had lower richness of perennial grasses over time (Fig. 4.5).

Existing plant cover and richness of biennial native species increased in some of the treatment mixtures (ELS1 and ELS2) compared with treatments that did not include early successional species (LS2, Fig. 4.6). These differences can be partially attributed to the inclusion of biennial *L. redowskii* in the early successional seed mixtures.

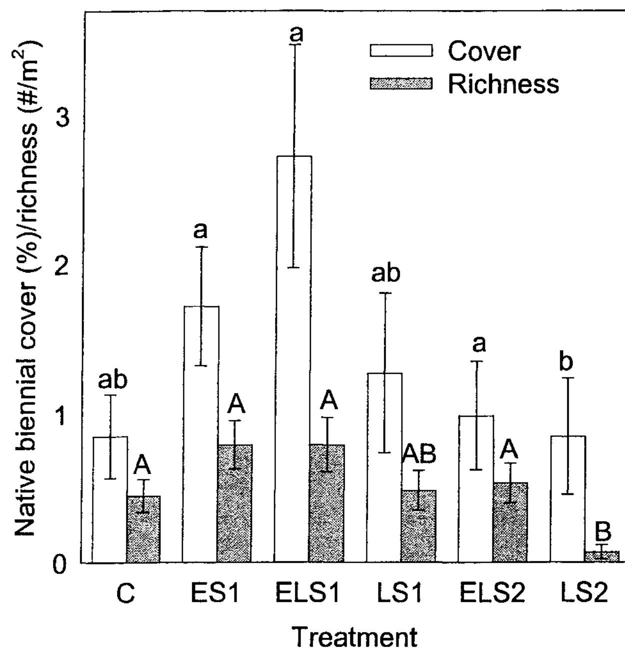

Figure 4.6 Biennial forb cover and richness treatment differences. Bars represent one standard error of the mean. Lower case letters represent significant treatment differences using least square means ($\alpha = 0.05$) for cover estimates; upper case represent same for richness. Seed mixture treatments are abbreviated as follows: no seeded control (C), early successional species seeded year one (ES1), early and late successional species seeded year one (ELS1), late successional species seeded year one (LS1), early successional species planted year one, late successional species planted year two (ELS2), no seed planted year one, late successional species planted year two (LS2).

Treatment differences in perennial forb cover and richness did not change beyond the initial pre-treatment baseline conditions (no significant treatment by time interactions,

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Table 4.3). Initial perennial forb cover was significantly lower in LS1 plots than in any of the other plots ($F_{[5,55]} = 2.96$, $p = 0.02$); mean cover of perennial forbs on pre-treatment plots was: LS2 4.86 ± 1.90%, C 1.96 ± 0.70%, ELS2 2.25 ± 0.94%, ELS1 1.65 ± 0.70%, and ES1 1.25 ± 0.68% and LS1 1.17 ± 1.00%.

Discussion

Succession based seeding treatments did not result in the desired "ecological bridge" towards a community with diminished cheatgrass and increased perennial cover in the time frame of this experiment. Although we had successful late successional species recruitment from our seeding, establishment of late successional species did not clearly show which model of succession should guide seeding mixture composition. In the one-year treatments, the success of late successional seedling establishment with the facilitation (ELS1) treatment gives marginal support to using facilitation based seed mixtures, because this was the only treatment in which late successional species establishment was better than both treatments without late successional species (ES1 and C). Although the two-year treatments were not statistically different at the final sampling date, the two-year treatments had only one year to germinate and establish compared with two years in the one-year treatments. Thus, it is possible treatment differences may become more pronounced with time.

None of the seeding treatments suppressed cheatgrass or increased perennial plant cover. Cheatgrass recovered from the glyphosate treatments by the second year and non-native annual forbs also increased over the course of the experiment. In this study, we seeded native early successional species instead of introduced non-invasive species

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previously used in an "ecological bridge" approach. Hardy and Palazzo (2002) had success in using Vavilov Siberian wheatgrass as an ecological bridge in a cheatgrass dominated system. Vavilov significantly reduced cheatgrass and, when seeded with Vavilov, bluebunch wheatgrass was also able to establish (Hardy & Palazzo 2002). Since National Park regulations do not allow the introduction of non-native varieties, it was hoped that early successional native species could act as a bridge in our field site.

As in the study by Hardy and Palazzo (2002), early successional species that were intended to serve as an "ecological bridge" established successfully. Of the four early successional species in our study that had significant treatment effects, three of these species successfully established from seeds in the treatments (*El. elymoides, Het. villosa, L. redowskii*). Seed rain was just as effective for seedling recruitment as seeding treatments for the other early successional species, *P. douglasii, CHE.leptophyllum,* and *Hel. pumilis*. However, in the time frame of this experiment none of these treatments established a community that could resist cheatgrass. It is possible that the success of native species as an "ecological bridge" requires a longer time period.

Perennial forb and grass cover did not differ by seed mixture treatment. However, perennial cover increased over time. These changes may have resulted from natural seed rain, environmental conditions, or the temporary release from cheatgrass competition or some combination of the three factors. There was a treatment effect in perennial grass species richness over time in the one-year facilitation mixture (ELS1) compared with other treatments, but this effect is an artifact of unequal number of perennial grass species in the mixtures. There were three species of perennial grasses in

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ELS1 as compared with two in the late species mixture and one in the early successional species mixture.

Native biennial cover and richness increased in treatments with native biennial *L. redowskii* (ES1, ELS1, ELS2) compared with one control (LS2). However, these treatments were not significantly different from other treatments without early successional species (C, LS1). Seed rain probably caused this mixed result. Other treatment differences appear to be complicated by ambient seed rain; establishment of late successional species in three treatments with late successional species (LS1 in the one-year, LS2 and ELS2 in the two-year experiments) were not different from treatments in which they were not seeded (ES1 and C in the one-year, C in the two-year experiments). Due to the Park Service requirement that we use native species, all of the species included in the late successional seed mixes were already present on the field site, except for *As. laevis*, which did not establish on the plots. Thus, it was impossible to distinguish seedlings established by our treatments from those established through natural recruitment processes.

Although most models of community structure are based on competition and neglect facilitation, a large body of evidence shows that facilitation can be important in determining plant distributions, productivity, diversity and reproduction (Callaway 1995; Callaway & Pugnaire 1999; Gomez-Aparicio *et al.* 2004). Although some work shows that facilitation occurs along a stress-gradient (Lortie & Callaway 2006), two meta-data analyses have shown that facilitation may be important in other environments as well (Gomez-Aparicio *et al.* 2004; Maestre *et al.* 2005; Maestre *et al.* 2006). Some of the mechanisms for facilitation include resource modifications of light and temperature, soil

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moisture, nutrients, and oxygenation, substrate modification, protection from herbivores, pollination, root grafts, mycorrhizae, and soil microbes (Callaway 1995; Callaway & Pugnaire 1999). It may be possible that early successional seedlings, with their faster growth, alleviated some herbivore pressure from the late successional seedlings, but we did not collect herbivory data.

Amongst the one-year treatments, the facilitation based seed mixtures seemed to be a good approach for late successional seedling establishment. Early successional species establishment did not inhibit the establishment of late successional species. As a test for the "ecological bridge" concept, our results show that although late successional seedlings can establish in these seeding mixtures, the native species did not successfully suppress or resist cheatgrass. It is possible that the experiment needed more time to allow the native species to establish and compete against the cheatgrass. However, with such a vigorous competitor as cheatgrass, it is likely that plant species with rapid growth rates might be required to form an effective "ecological bridge".

Acknowledgements

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Appendix A: Estes Park mean monthly temperature and precipitation for 2003-2005 with comparisons to thirty-year averages.

	Mean temperature (°F)		Mean precipitation (inches)	
2003	Temperature	Deviation from 1971-2000 average	Precipitation	Deviation from 1971-2000 average
JAN	34.2	5.8	0.08	-0.24
FEB	23.6	-6.5	1.32	0.85
MAR	32.5	-2.1	5.44	4.54
APR	41.3	0.9	0.8	-0.62
MAY	49	0.5	1.65	-0.46
JUN	55.6	-1.8	1.25	-0.18
JUL	68.9	6.2	2.44	0.28
AUG	64.3	3.4	2.31	0.33
SEP	51.2	-3	0.7	-0.53
OCT	48.6	3.5	0.28	-0.65
NOV	31.2	-3.7	0.97	0.34
DEC	30	1.2	0.46	0.09
YEAR	44.20	0.37	1.48	0.31
2004				
JAN	27	-1.4	0.82	0.5
FEB	23.8	-6.3	0.43	-0.04
MAR	37.7	3.1	0.59	-0.31
APR	39.6	-0.8	3.37	1.95
MAY	50.3	1.8	2.16	0.05
JUN	54.9	-2.5	4.85	3.42
JUL	61.1	-1.6	5.35	3.19
AUG	58.2	-2.7	2.74	0.76
SEP	53.7	-0.5	1.66	0.43
OCT	43.8	-1.3	0.76	-0.17
NOV	31.9	-3	1.66	1.03
DEC	29.9	1.1	0.13	-0.24
YEAR	42.66	-1.18	2.04	0.88
2005				
JAN	30.9	2.5	1.3	0.98
FEB	26.8	-3.3	0.57	0.1
MAR	32.6	-2	1.16	0.26
APR	39.7	-0.7	2.29	0.87
MAY	47.6	-0.9	2.29	0.18
JUN	57.5	0.1	2	0.57
JUL	66.7	4	1.45	-0.71

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	Mean temperature (°F)		Mean precipitation (inches)	
2003	Temperature	Deviation from 1971-2000 average	Precipitation	Deviation from 1971-2000 average
AUG	60.7	-0.2	2.44	0.46
SEP	56.3	2.1	0.31	-0.92
OCT	45.9	0.8	1.45	0.52

Data from Western Regional Climate Center, http://www.wrcc.dri.edu/

* Data after year 2001 are not fully comparable to 30 year averages because the Estes Park weather station was moved in 2001 from 40°22'46"x105°29'09" at 7,480' elevation to 40°22'08"x105°30'39" at 7,785' elevation.

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