

THESIS

INTERACTIONS BETWEEN *BROMUS TECTORUM* L. (CHEATGRASS) AND NATIVE
RUDERAL SPECIES IN ECOLOGICAL RESTORATION

Submitted by

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ABSTRACT

INTERACTIONS BETWEEN *BROMUS TECTORUM* L. (CHEATGRASS) AND NATIVE RUDERAL SPECIES IN ECOLOGICAL RESTORATION

Bromus tectorum L. is an invasive annual grass that dominates much of the rangeland in western North America. It has shown an impressive ability to invade ecosystems, causing substantial changes in the composition and function of native plant and soil communities in ways that promote further exotic invasion through displacement of native plant species and slowing or halting of succession. *B. tectorum* has several characteristics, including high reproductive rates, affinity for disturbed sites, and the ability to create positive feedback conditions in invaded sites, which make it highly successful at invading new sites, and extremely challenging to remove during revegetation efforts. Traditional control methods including herbicide application, grazing, and burning have largely proven unsuccessful at preventing establishment and spread of *B. tectorum*. Similarly, restoration seed mixes often consist of native perennial grass species, which tend to be slower growing and less robust in disturbed sites, and therefore provide little competition against *B. tectorum* and do not promote the reestablishment of native plant communities. In addition, seed mixes are often planted at a fraction of the rate of annual *B. tectorum* seed production, giving them a distinct disadvantage. Native ruderal species share many traits with *B. tectorum* and could potentially compete with this invader if used at high, competitive seeding rates in restoration efforts, and may alter site characteristics in ways that promote succession of the native plant community.

One key characteristic that may be closely associated with community development is the development and composition of the arbuscular mycorrhizal fungi (AMF) community. AMF are important for resource acquisition by a majority of plant species. They are particularly important for late-seral plant species, which typically exist in low available nutrient conditions. *Bromus tectorum* causes shifts in the mycorrhizal community that could lead to a loss of AMF species richness and abundance in a very short time period, resulting in conditions that are difficult for late-seral species to colonize, due to a lack of access to resources through host-specific plant-AMF relationships. Utilizing native seed mixes composed of species selected for specific functional and competitive traits, and mycorrhizal status, and creating seeding rates designed to increase interspecific competition with *B. tectorum* may provide the missing link for successful restoration of *B. tectorum*-invaded sites.

A study was conducted in northern Colorado to determine whether native ruderal species could suppress *B. tectorum* establishment and persistence in a disturbed site, and how these effects compared to similar effects by sterile wheat. In this study, *B. tectorum* was seeded with and without a high rate native ruderal seed mix and a sterile wheat species (QuickGuard™) used in revegetation efforts for erosion control. *Bromus tectorum* biomass and density data were collected, as well as biomass and density for all seeded native species and sterile wheat. All treatments were seeded in the fall of 2010, and vegetative data collection occurred during the summers of 2011 and 2012. To assess the immediate effects of the establishing plant community on the AMF community, soils were collected from three of the field study treatments after one year of growth: 1) *B. tectorum*, 2) a mixture of native early-seral species, or 3) *B. tectorum* plus native early-seral species. Three mycorrhizal host plant species (*Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths, *Ratibida columnifera* (Nutt.) Woot. & Standl., *Sorghum bicolor* (L.)

Moench ssp. *drummondii* (Nees ex Steud.) de Wet & Harlan) were grown in these soils under greenhouse conditions. Roots were harvested after 30 days and analyzed for AMF colonization.

After one growing season, the native ruderal mix significantly reduced *B. tectorum* biomass in the field. After the second growing season, the effect was no longer detectable in biomass measurements, but was still observed as a reduction in density of *B. tectorum* in the native ruderal plots. The sterile wheat reduced *B. tectorum* biomass after one growing season, but to a lesser extent than the native mix, and had a positive effect on density. In 2012, effects on both biomass and density disappeared. Results from the AMF colonization analysis indicated that the presence of the native species had an impact on AMF richness or abundance within the soils and that *B. tectorum* alters the AMF community in a way that is unique in comparison to weedy native vegetation. Soils from beneath native early-seral plant species had much higher colonization of the host plant species relative to soils from beneath *B. tectorum*. In addition, the native host *R. columnifera* had much higher rates of colonization than the non-native host, *S. bicolor*, indicating that there may be some host-dependent plant-AMF relationships that are more beneficial to the native plant than the non-native plant.

The results of these studies could have important practical field applications for restoring invaded sites, particularly when the goal is to create conditions that promote development of late-seral native plant communities. Utilization of native ruderal species in revegetation mixes could provide a critical missing link for facilitation of late-seral, native plant communities through suppression of *B. tectorum*, as well as rapid facilitation of AMF communities that successfully colonize native late-seral host species. Continued monitoring and assessment of this study site could lend further insight to the long-term dynamics of the native ruderal plant community with *B. tectorum* and development of a late-seral plant community.

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Chapter 1. Tailoring native seed mixes to restore invaded areas: The case of *Bromus tectorum*

1.1 Introduction

Bromus tectorum L. (cheatgrass) is an invasive, annual grass that is native to Eurasia (Hulbert 1955). It germinates in the fall, overwinters as a seedling and grows rapidly in the spring, followed by seed production and senescence early in the summer (Stewart and Hull 1949, Hulbert 1955). *B. tectorum* was introduced to North America in the late 1800s, most likely as a contaminant in grain, although deliberate introduction as a range grass has also been noted (Mack 1981). It has since spread throughout the continent via vectors such as grain seed contamination, bedding straw, and along railways (Mack 1981, Knapp 1996). It has become one of the dominant invasive species throughout the Intermountain West (Hulbert 1955, Mack 1981) occupying approximately 40 million hectares (DiTomaso 2000). *B. tectorum* has been particularly successful at invading overgrazed rangelands (Hulbert 1955, Morrow and Stahlman 1984) and post-fire shrub-steppe habitats (Knapp 1996) especially in disturbance gaps where there is slow regrowth of native species (Hulbert 1955). *B. tectorum* is one of the primary invaders in early- and mid-successional grassland communities (McLendon and Redente 1991, 1992). Once established, *B. tectorum* has demonstrated the capacity to maintain site dominance for extended periods of time, if not indefinitely (Mack 1981).

As land managers struggle to combat this exotic grass, many control methods have been implemented, including herbicide application, grazing, and fire mitigation. These have largely proven unsuccessful at preventing establishment and spread of *B. tectorum*. Reseeding practices are also often inadequate, as they largely consist of species that tend to be slower growing and

less robust in disturbed sites, therefore providing little competition against *B. tectorum*. Consequently, more and more attention is being paid to restoration and reclamation practices and their success. In order to fully understand how to restore *B. tectorum*-invaded ecosystems, we must consider the mechanisms of *B. tectorum* invasion, how it interacts with native ecosystems, and how to select appropriate native species to reduce dominance of *B. tectorum*. In this review, we present a restoration strategy that involves an in-depth understanding of these elements and uses this knowledge to suggest restoration seed mixtures based on species functional and competitive traits. We also suggest seeding rates designed to displace *B. tectorum* and create native-dominated plant communities that are resilient to further disturbances.

1.2 Characteristics for Invasion Success

B. tectorum has many characteristics that contribute to its invasiveness in North American ecosystems. The characteristics defined in the following sections are those that provide substantial influence on the ability of *B. tectorum* to invade new sites and compete with native plants. *B. tectorum* has competitive root system growth patterns, as well as extremely competitive seed production and germination rates that rapidly out-compete other species. In addition, influences to nitrogen cycling and fire regimes create increased opportunities for competition and conditions under which *B. tectorum* is highly successful.

1.2.1 Root System

One trait that provides distinct benefits to *B. tectorum* is its winter annual growth cycle. *B. tectorum* germinates in early fall and actively extends its root system throughout winter and is physiologically active in early spring while most native species are dormant. Acquiring early

season water and nutrients favors *B. tectorum* and often prohibits the establishment of many native species that do not exhibit active root growth until spring (Stewart and Hull 1949, Harris 1967, Booth et al. 2003, Jones et al. 2010).

The majority of the *B. tectorum* root system typically exists in the upper 30 cm of soil (Hulbert 1955). Its presence can deplete resources from this soil layer, limiting resource availability for co-occurring spring annual and perennial seedlings (Harris 1967). Studies have also found that *B. tectorum* has deep roots (>100 cm (Hulbert 1955); >87 cm (Harris 1967)) that compete for water and nutrients with established, deep-rooted perennials. As the growing season advances, *B. tectorum* uses water from deeper in the soil profile to allow for growth and reproduction (Hulbert 1955) after shallow spring moisture and nutrients have been reduced.

1.2.2 Seed Production and Germination

As an annual, *B. tectorum* relies solely on seed production for spread and persistence. Its high seed production and germination rates facilitate invasion through propagule pressure and contribute to post-invasion dominance of the plant community (Stewart and Hull 1949, Hulbert 1955, Mack and Pyke 1983, Humphrey and Schupp 2001). In western North America, *B. tectorum* seed production typically occurs in late spring or early summer and seeds mature between early and late June depending on elevation and moisture (Stewart and Hull 1949). Annual seed production rates can be highly variable and influenced by factors such as individual plant health, site moisture (Stewart and Hull 1949, Hulbert 1955), nutrient availability and intraspecific competition (Stewart and Hull 1949, Hulbert 1955, Mazzola et al. 2011). Estimates of annual seed production in *B. tectorum*-dominated sites range from 5,000 seeds m⁻² (Humphrey and Schupp 2001, Griffith 2010) to over 17,000 seeds m⁻² (Stewart and Hull 1949). Even during

years of extremely low precipitation and overall unfavorable conditions, *B. tectorum* can still produce enough seeds to maintain a presence in the plant community (Stewart and Hull 1949, Hulbert 1955, Mack and Pyke 1983).

In field settings, greater than 98% of viable *B. tectorum* seeds typically germinate in the fall following seed dispersal (Stewart and Hull 1949, Hulbert 1955, Steinbauer and Grigsby 1957, Humphrey and Schupp 2001). Of those that germinate, over 90% become established seedlings (Steinbauer and Grigsby 1957). *B. tectorum* is relatively plastic in its responses to variation in weather; however, its seeds have seasonal controls that generally prevent them from germinating in the middle of summer when moisture is scarce and temperatures are too hot for survival (Hulbert 1955). This process, after-ripening, happens as seeds continue to ripen after falling from the plant based on seasonal changes in temperature and moisture (Allen et al. 1995). The majority of *B. tectorum* seeds produced annually will germinate in the fall and overwinter in the seedling stage. However, in the event that moisture and temperature conditions are not adequate for germination in the fall, germination can be delayed until spring when conditions are more favorable (Stewart and Hull 1949).

1.2.3 Nitrogen Use

As with many early-seral species, *B. tectorum* is highly productive in nitrogen-rich soils (Hulbert 1955, Kay and Evans 1965) and efficient utilization of available nitrogen often leads to an increase in plant vigor, above-ground biomass (Ball et al. 1996), seed production (Hulbert 1955, Anderson 1991) and site dominance (Kay and Evans 1965, McLendon and Redente 1992). After uptake, nitrogen becomes tied up in the plant until senescence when decomposition returns the nitrogen back to the soil (McLendon and Redente 1992, Holly et al. 2009). *B. tectorum* has

been associated with increases in total nitrogen both immediately following invasion (Belnap et al. 2005) and as time since invasion increases (Blank 2008). *B. tectorum* litter has higher C:N ratios than the native species it generally replaces, which can stimulate increased bacteria and microbe growth (Blank 2008). These alterations to the microbial community promote increased rates of litter breakdown and nitrogen mineralization, providing an ongoing source of nitrogen for seedlings and mature plants (Holly et al. 2009).

Seeming conflicts exist regarding the potential for *B. tectorum* to impact the nitrogen cycle within invaded areas. Some studies indicate increased nitrogen availability in *B. tectorum* invaded areas, both short term (Belnap et al. 2005) and long term (Blank 2008), while others indicate that nitrogen availability is lower in invaded areas due to higher C:N ratios in the plant litter (Evans et al. 2001, Rimer and Evans 2006). However, these seeming contradictions may be mediated by further results indicating that plant available nitrogen is leached into deeper soils rather than retained at the soil surface (Sperry et al. 2006). This study found that forms of available nitrogen from decomposition of litter at the surface were leached into deeper soils where they were taken up by *B. tectorum* roots and incorporated back into litter (Sperry et al. 2006). The surface soils studies indicating a loss of nitrogen in *B. tectorum* sites utilized soils where leached nitrogen would not be captured, while deeper soil studies would incorporate this leached nitrogen (Sperry et al. 2006). Indeed, nitrogen availability was found in another study to be greatest deeper within the soil (60-100 cm) than at the surface in invaded areas (Blank 2008). Such a nitrogen cycle can contribute to a positive feedback loop that can work in conjunction with increased decomposition rates to promote continued site dominance by nitrophilic *B. tectorum*.

1.2.4 Fire Regime

Following maturation, senescence of *B. tectorum* leaves a large amount of residual dry, fine litter (Klemmedson and Smith 1964, Young and Evans 1978, Knapp 1996, Brown et al. 2008). Persistent standing dead material burns easily and quickly (Stewart and Hull 1949, D'Antonio and Vitousek 1992) and can promote the movement of wildfires into previously unburned areas by increasing connectivity (Hunter 1991, Knick and Rotenberry 1997). *B. tectorum* litter alters the local fire regime in both intensity and frequency due to its flammability (Stewart and Hull 1949, D'Antonio and Vitousek 1992, Knapp 1996, Knick and Rotenberry 1997). Historic fire regimes in rangelands of the western US are estimated to have had fire return intervals between 60- and 110-years (Whisenant 1990). Following invasion by *B. tectorum*, fire return intervals have been shortened to 3-5 years (D'Antonio and Vitousek 1992). These alterations to the fire regime have detrimental effects on native fire-adapted species (Young and Evans 1978, Melgoza et al. 1990, Knick and Rotenberry 1997), which demonstrate a certain level of resilience to natural fire disturbances of their communities (Brown et al. 2008). Persistent short fire return intervals caused by dominance of *B. tectorum* prevent most mature perennial species from reestablishing and can lead to local extirpation of these species (Humphrey and Schupp 2001). Following a fire, *B. tectorum* can quickly move into gaps, leading to competition with native annuals and perennials that do reestablish (Melgoza et al. 1990, Knapp 1996) and contributing to a further loss of species diversity from a wide variety of plant functional types (Young and Evans 1978).

1.3 Interactions with Native Ecosystems

1.3.1 Seed Bank

Soil seed banks play an important role in plant community resistance to invasion, particularly after disturbances. Communities that have persistent seed banks are better able to recover after disturbance, even if the disturbance has completely removed aboveground vegetation (Bossuyt and Hermy 2001). In shrub-steppe habitats, some of the dominant native perennials and shrubs such as *Artemisia tridentata* Nutt. (big sagebrush) and *Chrysothamnus viscidiflorus* (Hook.) Nutt. (yellow rabbitbrush) have extremely limited seed banks (Young and Evans 1978). Perennial grasses typically have short-lived seeds (Bekker et al. 1998) and, if eliminated from the plant community, reestablishment from the seed bank can be slow (Bossuyt and Hermy 2001). These seed bank limitations can negatively impact the recovery potential of the plant community following disturbance (Humphrey and Schupp 2001).

With increasing disturbance severity and damage to resprouting species, recovery becomes increasingly reliant on germination of residual seeds (Klimkowska et al. 2010) and dispersal from outside sources. Frequent disturbance favors early-seral species that establish quickly from seed (Henderson et al. 1988) rather than by vegetative reproduction. However, ecosystems that have gone for over 50 years without disturbance are unlikely to have an extensive ruderal seed bank because even long-lived seeds of ruderal species are unlikely to remain viable for more than 50 years (Bossuyt and Hermy 2001). Consequently, in areas where natural disturbances have been altered substantially from historic regimes, seed banks are likely limited and reduced in their capacity for recovery following disturbance.

Reestablishment of perennial species following disturbance is often challenging in ecosystems where invaders such as *B. tectorum* have colonized, likely because of alterations to

seed bank composition and reduced recovery potential of as a result of invasion. Communities dominated by *B. tectorum* tend to have sparse native seed banks (Humphrey and Schupp 2001), and persistent *B. tectorum* components. When coupled with a diminished seed bank, higher seed mortality due to increases in fire frequency from invasion (Stewart and Hull 1949, D'Antonio and Vitousek 1992, Knapp 1996, Knick and Rotenberry 1997, Brooks and Pyke 2001) could further lead to a depleted native seed bank and a minimized ability for system recovery after disturbance.

1.3.2 Microorganisms

Arbuscular mycorrhizal fungi (AMF) are very important members of the soil community that impact succession and disturbance recovery through parasitic, mutualistic and decomposer mechanisms (Callaway et al. 2003). AMF promote the growth of many plant species, especially late-seral, dominant species (Janos 1980, Hartnett and Wilson 1999, Rowe et al. 2007). Benefits incurred by plants via AMF-root associations include: increased nutrient capture (Hart et al. 2003, Batten et al. 2006, Van Der Heijden and Horton 2009), disease protection, and access to water (Hartnett and Wilson 2002). AMF tend to be more abundant in poor soils (Hart et al. 2003) and promote obligate mycotrophic perennial species, giving them a competitive advantage (Hartnett and Wilson 1999), while native perennial plants also support greater diversity of fungi (Belnap and Phillips 2001).

While AMF benefits local, native plant species, some exotic species are also able to use the local AMF community to their advantage (Marler et al. 1999, Klironomos 2002, Callaway et al. 2004, Van Der Putten et al. 2007, Wurst et al. 2011). In invaded areas, exotic plants can be released from the pressure of pathogenic soil organisms of their home soils while receiving

benefits from local AMF (Klironomos 2002, Callaway et al. 2004, Reinhart and Callaway 2006, Batten et al. 2008). Exotic plant invasions can also cause changes in nutrient availability for native plants by decreasing the presence and diversity of AMF in the microbial community (Batten et al. 2006, Batten et al. 2008).

B. tectorum can promote a positive feedback loop for its own success through negative pressure on native species caused by modifications to the AMF community (Batten et al. 2008, Jordan et al. 2008). *B. tectorum* is a facultative mycotroph (Knapp 1996) and offers low AMF colonization potential relative to known native mycorrhizal species (Busby 2011).

B. tectorum has been shown to modify soil microbial communities over a small number of growth cycles by decreasing AMF while increasing saprophytic fungi and bacteria (Hawkes et al. 2006, Jordan et al. 2008), while decreasing the diversity and abundance of fungi (Belnap et al. 2005). The impact of *B. tectorum* on the abundance and composition of AMF within a plant community can involve an overall degradation of the community and reduction of those AMF that are most beneficial to the native plant community (Busby et al. 2011), decreasing the capacity for nutrient transfer from the soil to native plant species (Hawkes et al. 2006), and limiting the establishment and survival of mycorrhizal native species – particularly late-seral species.

1.4 Selection of Native Species for Restoration Seed Mixes

Although *B. tectorum* has been widely studied for over half a century, there is still a general lack of information regarding how to successfully restore invaded areas. Typical approaches, including herbicide application (Whitson and Koch 1998), mowing and grazing management (DiTomaso 2000), and fire mitigation are largely unsuccessful for a variety of reasons including

prohibitive costs, high *B. tectorum* propagule pressure, herbicide application challenges (Klemmedson and Smith 1964, Whitson and Koch 1998, Pellant et al. 1999) and the plasticity of *B.tectorum* responses to disturbance and changing conditions (Stewart and Hull 1949, Mack and Pyke 1983, Leffler et al. 2011). By combining existing knowledge of this species' mechanisms of invasion with current restoration methods, novel approaches may lead to strategies that offer improved restoration success through utilization of interspecific competition and increased seeding rates of native species (Table 1.1).

In areas where *B. tectorum* has become established, reestablishment of native species through natural succession is very limited (Hulbert 1955). This is typically due to the factors discussed previously including competition for moisture (Stewart and Hull 1949, Harris 1967, Melgoza et al. 1990), high seed production rates (Stewart and Hull 1949, Hulbert 1955, Mack and Pyke 1983, Humphrey and Schupp 2001), alterations to nutrient cycling (Kay and Evans 1965, McLendon and Redente 1992) and changes in the microbial community (Hawkes et al. 2006, Jordan et al. 2008). Disturbed early-seral sites, such as those invaded by *B. tectorum*, are frequently the focus of restoration and are characterized by high nutrient availability (McLendon and Redente 1991) including large amounts of plant-available nitrogen.

Table 1.1. Matching restoration strategies to *Bromus tectorum* characteristics. To combat *B. tectorum*, a well-rounded approach considering the mechanisms by which it moves into ecosystems and gains advantage must be encouraged and implemented. Competition with *B. tectorum* may be improved by using a restoration mix of species that share functional or resource-use traits. A diverse mix is likely to be most successful in competing for resources, both for short term site establishment and long term site dominance. Specific traits to consider are high nitrogen utilization, rapid growth, early germination and maturity, and high seed production. In addition, including nitrophilic and mycorrhizal species could help create conditions that facilitate natural succession toward late-seral conditions and species. Using a seeding rate comparable to annual *B. tectorum* seed production could also increase interspecific competition for resources, especially during seedling establishment.

<i>Bromus tectorum</i> Characteristics	Current General Restoration Strategies	Alternate Restoration Strategies for <i>Bromus tectorum</i> Invaded Areas	Suggested example native species
Seed production rates: 5,000 – 17,000 PLS/m ²	Recommended seeding rates: 516 – 1,032 PLS/m ²	Increase seeding rates to be more comparable to <i>B. tectorum</i> seed production rates. Use species with high seed production rates	<i>Elymus elymoides</i> , <i>Helianthus annuus</i> , <i>Ambrosia artemisiifolia</i> , <i>Chenopodium fremontii</i> , <i>Elymus multisetus</i>
Fall germination, winter root growth and early aboveground growth in spring	Seed mix dominated by spring germinating grass species	Use seed mix containing diverse mix of native species including fall germinating and fast-growing, annual grass and forb species	<i>E. elymoides</i> , <i>E. multisetus</i> , <i>Vulpia octoflora</i> , <i>Poa, secunda</i> , <i>Linum lewisii</i> , <i>C. fremontii</i> , <i>Amaranthus retroflexus</i>
Rapid growth and high productivity in sites with high nutrient availability	Seed mix dominated by perennial grass species that are slow-growing and poor competitors in high nutrient sites		
Reduces presence of AMF in soil community while increasing the presence of bacteria and saprophytes, creating self-promoting conditions	Seed mix dominated by later-seral perennial species that are largely dependent upon AMF for successful establishment and persistence	Include early-seral mycorrhizal species in seed mix to promote AMF community development and facilitate the establishment of later-seral, mycorrhizal species	<i>H. annuus</i> , <i>Sphareralcea coccinea</i> , <i>L. lewisii</i>

While ruderal species respond quickly to fluxes of plant-available nitrogen with increased biomass, many native perennials do not, or are delayed in their response (Bilbrough and Caldwell 1997). Ruderal species typically maintain dominance of the plant community until they deplete the amount of available nitrogen to the point where they no longer have an advantage, which results in a successional transition toward mid- and late-seral species (McLendon and Redente 1992, Paschke et al. 2000). Many late-seral species have mycorrhizal associations and can therefore access nutrients from nutrient-depleted soils that many ruderal species cannot, giving them a greater advantage in late-seral conditions (McLendon and Redente 1992). In addition, disturbed sites are frequently low in AMF presence, and many early successional species do not typically associate with AMF, making these species excellent candidates for use in disturbed sites (Brown et al. 2008).

Many species typically utilized in restoration seed mixes are mid- and late-seral perennial grasses. Given the nutrient abundance of most disturbed sites, these species are often at a competitive disadvantage against invading ruderal species such as *B. tectorum* (Hulbert 1955, Kay and Evans 1965, Anderson 1991, McLendon and Redente 1992, Ball et al. 1996). By tailoring seed mixes to include native ruderal, nitrophilic species, the establishment of invasive *B. tectorum* could be reduced (Herron 2010), shifting the advantage toward native species (Rowe and Brown 2008) (Figure 1.1). In addition, because the soil community is a major driver of plant community succession via shifts in microbial composition (Van Der Putten et al. 2007), seed mixes containing mycorrhizal ruderal species such as *Helianthus annuus* L. (common sunflower) (Rinaudo et al. 2010) and *Sphaeralcea coccinea* (Nutt.) Rydb. (scarlet globemallow) (Reeves et al. 1979) could lead to the promotion of AMF species necessary to increase establishment of native later-seral perennial species (Van Der Heijden and Horton 2009, Busby et al. 2011).

Studies focused on reestablishment of native communities in invaded areas have indicated relationships between functional groups and traits of successful revegetation species and those of the invasive species they compete most effectively with (Dukes 2002, Booth et al. 2003, Fargione et al. 2003, Bakker and Wilson 2004, Pokorny et al. 2005, Hooper and Dukes 2010). A focus on functional trait overlap between restoration species and invading species could prove useful for limiting exotic establishment and promoting successional development of native plant communities (Brown 2004) and as has been indicated through research. For example, Pokorny et al. (2005) found that native forb species were the most effective competitors against *Centaurea maculosa* auct. non Lam. (spotted knapweed), an invasive forb, due mainly to similarities in nutrient acquisition and ecological role. *Centaurea solstitialis* L. (yellow starthistle), a warm-season forb species, was most inhibited by a native forb *Hemizonia congesta* DC. ssp. *luzulifolia* (DC.) Bab. & H.M. Hall (hayfield tarweed), with similar phenology (Dukes 2002). A study by Fargione et al. (2003) showed that invasive species from four different functional groups were most strongly inhibited by resident native species from corresponding functional groups.

Competition against *B. tectorum* invasion may be improved by seeding with a mixture of species that share certain traits and resource use patterns (Funk et al. 2008). In particular, when dealing with an invading annual grass, important traits to focus on include short life cycle, dispersal ability, high germination rate, early flowering, and rapid emergence (Pywell et al. 2003, Jones et al. 2010) as well as amount and seasonality of soil water use, and nitrogen use (Brown et al. 2008). *Vulpia octoflora* Walter Rydb. (six-weeks fescue), which is a native, winter annual grass, could offer direct competition with *B. tectorum* for moisture and nutrients due to its overlap in seasonal germination, growth, and its historic role as a native early-seral species in the

United States (Mack 1981). Established *Elymus elymoides* (Raf.) Swezey (squirreltail), a native C3 grass, was shown in a study by Booth et al. (2003) to suppress the establishment of *B. tectorum* (also a C3 grass) while at the same time allowing for the establishment of *A. tridentate*, indicating the potential for limiting *B. tectorum* invasion. In addition, *E. elymoides* and *Poa secunda* J. Presl (sandberg bluegrass) share traits with *B. tectorum* such as short-lived life cycles, early maturity, and high rates of reproduction (Jones et al. 2010), which could contribute to continued site dominance of the natives and limit establishment and invasion by *B. tectorum*.

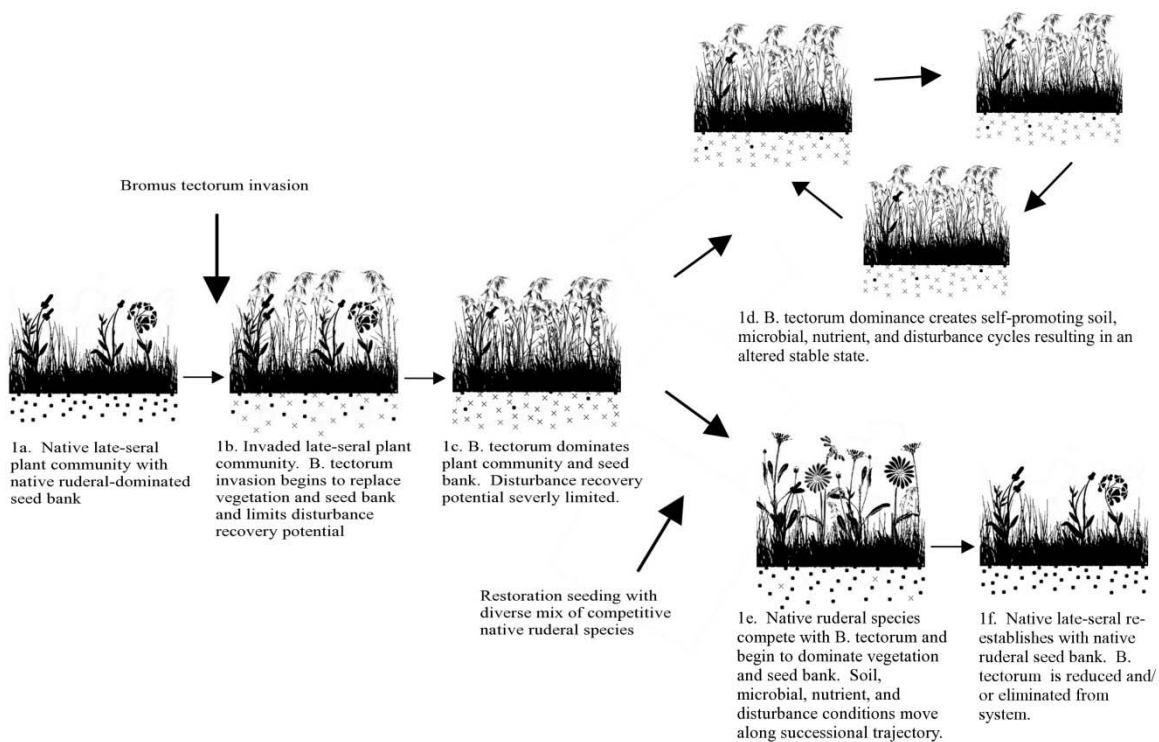


Figure 1.1. *Bromus tectorum* invasions and dominance reduction through competitive native seeding. As *B. tectorum* abundance increases in the plant community following invasion, native soil communities (circles) are replaced by soil communities associated with *B. tectorum* (x's). Eventually, these changing conditions create a positive feedback loop that supports *B. tectorum* persistence at the site, while limiting growth and success of native species. Restoration seed mixes and methods may be tailored to be more competitive against this invasive species through the use of native fast-growing, highly reproductive species that provide resource competition with *B. tectorum*. These native ruderal species may also contribute to a return to successional development of late-seral plant communities, and avoidance of positive feedback conditions of *B. tectorum* dominance.

Other species may have characteristics that demonstrate some level of competition with *B. tectorum*, yet not fall into the ‘functional group’ category. For example, *H. annuus* L. and *Ambrosia artemisiifolia* L. (annual ragweed) are fast-growing, annual forbs that have been shown in a greenhouse experiment to reduce the biomass of *B. tectorum* and increase the presence of native perennial species in direct competition (Perry et al. 2009). Some species may provide more direct competition with *B. tectorum* during germination and establishment, while others may provide great competition once already established. This diversity of modes of competition could increase the scope of limitation to *B. tectorum* growth and invasion.

Additional competition may be provided through the use of invader-adapted ecotypes of competitive native species. Over time, native species growing in concert with invasive species can become more tolerant of the invaders (Mealor and Hild 2006) through natural selective processes associated with invader-caused changes in the environment (Mealor et al. 2004). One study found that *Sporobolus airoides* (Torr.) Torr. (alkali sacaton) growing in areas invaded by *Acroptilon repens* (L.) DC. (Russian knapweed) had increased tillering and vegetative growth over *S. airoides* from uninvaded areas, which are changes that can increase persistence against disturbances (Bergum et al. 2012). *Elymus multisetus* M.E. Jones (big squirreltail) collected from *B. tectorum* populations initiated growth earlier in the growing season than those from non-invaded sites, which is an early season establishment advantage (Leger 2008). The *E. multisetus* from the invaded areas were also much more competitive with *B. tectorum* than the same species from uninvaded sites (Leger 2008). Other similar studies on native grasses in forb-invaded systems have shown similar results, indicating that, over time, native species can adapt to the presence of invaders and become more resistant to negative effects (Mealor et al. 2004, Callaway et al. 2005, Mealor and Hild 2006). In addition, Callaway et al. (2005) found that the invasive *C.*

maculosa had reduced biomass production when grown with native grasses taken from invaded areas relative to grasses taken from uninvaded areas. Using species ecotypes that have evolved in the presence of *B. tectorum* could prove to be advantageous when choosing native species for a native seed mix, given the genetic limitations of cultivated revegetation species (Leger 2008).

In addition to creating a seed mix tailored toward *B. tectorum* competition based on function, applying the mix at a competitive seeding rate is likely to be important. Typically, commercial and consumer seeding rates used in restoration practices are low relative to annual seed production of *B. tectorum*. For example, the Natural Resources Conservation Service (NRCS) recommends broadcast seeding rates between 512 and 1,032 pure live seeds (PLS) m⁻² depending on seed size (John et al. 2008) while *B. tectorum* produces seed at a rate of 5,000-17,000 seed m⁻² (Stewart and Hull 1949, Humphrey and Schupp 2001, Griffith 2010). When coupled with potentially diminished native seed banks, high seed production rates of *B. tectorum* could prohibit disturbed ecosystems from recovery through successional processes. Studies have found evidence that seeding rates of native species that are at or higher than invader seeding rates promotes the establishment of the native species (Mazzola et al. 2011) and that increased seeding rates of native species have disproportionately higher native species establishment than lower seeding rates (Sheley et al. 2006). Using a seeding rate that is comparable to annual *B. tectorum* seed production could offer increased competition for resources via increased native species establishment, especially during the initial stages of seedling establishment and ecosystem development.

Using existing knowledge of *B. tectorum* invasion biology in combination with demonstrated and potential competitors, there is great opportunity to develop restoration practices that limit success of this invader in disturbed ecosystems. Creating a diverse seed mix that is heavy in

native ruderal species, particularly those that are nitrophilic and promote AMF development could promote succession and facilitate the establishment of later-seral species. In addition, using a seeding rate that is comparable to *B. tectorum* seed production rates could further encourage the competitive effects of seeded native species. These approaches to reseeding could be used in combination with current control methods such as herbicide application or mowing, for an integrated approach to *B. tectorum* control and ecosystem restoration (Sheley et al. 2006). To truly combat this aggressive invader, a well-rounded approach considering the mechanisms by which *B. tectorum* moves into ecosystems and gains advantage must be encouraged and implemented.

1.5 Further Application

Many exotic species are similar to *B. tectorum* in that they readily invade disturbed areas with limited competition from native species. The restoration approach presented in this paper is based on knowledge of invader characteristics and designing a seed mix to include functional traits expected to be most effective competing with exotic species. As demonstrated in this review, we suggest that collecting substantial information about the target exotic species is critical for successful restoration seed mix development. For many invasive species, scientific studies are lacking, but for extensively studied invaders like *B. tectorum*, a substantial amount of scientific knowledge is available in the literature. Characteristics such as nutrient and water use efficiency, seed production and germination rates, potential effects on invaded areas, and characteristics that are unique or rare may all be important to understanding the target species' competitive advantages. From this, restoration species can be selected for either overall functional trait overlap, or for competition in specific characteristics. We suggest that a diverse

mix seeded at a competitive rate may be the most advantageous for promoting interspecific competition with the target exotic species and promoting succession toward native late-seral communities. It is also important to consider functional traits and functional diversity as separate and different from species diversity as differences in species do not necessarily imply or guarantee differences in community function (Lavorel et al. 1998, Díaz and Cabido 2001, Dukes 2001, Aubin et al. 2009).

While we present an approach to restoration in this review, we acknowledge the need for further research on this topic, both with regard to *B. tectorum* specifically, as well as invasive plant species in general. We suggest that research looking into tailored restoration seed mixes as a component of an integrated weed management approach for species management would lend significant insight to the methods and ideas presented within this review and further successes in restoration of disturbed lands.

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Chapter 2. The exotic invader *Bromus tectorum* is reduced through seeding of competitive native ruderal species

2.1 Introduction

Bromus tectorum L. (cheatgrass) is an annual grass that is native to Eurasia and has proven to be an aggressive invader in the United States. It was first introduced in the late 1800s and has become one of the most abundant exotic species in the western U.S. (Hulbert 1955, Mack 1981). *B. tectorum* is highly successful at invading rangelands through gaps created by disturbance events (Hulbert 1955, Morrow and Stahlman 1984, Knapp 1996). Its phenological characteristics make it a particularly challenging species to displace in restoration efforts. *B. tectorum* is a winter annual that germinates in the fall and overwinters as a seedling, allowing enhanced access to water and nutrients in the spring (Stewart and Hull 1949, Harris 1967). High rates of seed production (5,000 (Humphrey and Schupp 2001, Griffith 2010) – 17,000 PLS/m² (Stewart and Hull 1949, Humphrey and Schupp 2001, Griffith 2010)) and germination rates greater than 98% (Stewart and Hull 1949, Steinbauer and Grigsby 1957, Humphrey and Schupp 2001) further promote the success of this species and lend insight to some of the difficulties encountered when trying to restore invaded areas.

As *B. tectorum* invades new sites, alterations to resource availability (Melgoza et al. 1990, McLendon and Redente 1992, Walker and Smith 1997) and the microbial community (Hawkes et al. 2006, Jordan et al. 2008) discourage the growth and succession of native communities (Hawkes et al. 2006, Busby 2011). *B. tectorum* can efficiently utilize available nitrogen, often an abundant resource in disturbed sites, to increase its biomass (Ball et al. 1996), fecundity

(Hulbert 1955, Anderson 1991), and site dominance (Kay and Evans 1965, McLendon and Redente 1992). Perennial species, on the other hand, tend to be less responsive to high nitrogen availability, and are therefore at a competitive disadvantage in high-nitrogen sites (McLendon and Redente 1992).

Invasion by *B. tectorum* has been associated with limited presence of perennial species in soil seed banks (Humphrey and Schupp 2001). Native perennial species tend to have seeds that are short-lived in the seed bank (Bekker et al. 1998), while ruderal species tend towards long-term seed viability, leading to late-successional, perennial-dominated plant communities with ruderal-dominated seed banks (Henderson et al. 1988, Tekle and Bekele 2000, Bossuyt and Hermy 2001). When disturbance regimes are reduced or altered in an ecosystem, the native ruderal seed bank may become diminished due to lack of restocking. This, combined with invasion by *B. tectorum* (Stewart and Hull 1949, Steinbauer and Grigsby 1957, Humphrey and Schupp 2001) could greatly limit the recovery potential of a disturbed native plant community. Restoration of disturbed plant communities through reseeded efforts may be necessary for community recovery in these cases.

While there are many approaches for *B. tectorum* removal including grazing management, herbicide application, and fire management, these are not typically successful at long-term *B. tectorum* management (Klemmedson and Smith 1964, Whitson and Koch 1998, Pellant et al. 1999). Restoration seeding can be incorporated to management practices in an effort to increase success of Seed mixes currently used to restore *B. tectorum* invaded areas typically consist of perennial, late-seral grasses. These grasses may be at a competitive disadvantage against invading ruderal species in high nutrient, disturbed sites, as they do not respond to nutrient fluxes with rapid plant growth and production in the rapid manner that ruderal species do (McLendon

and Redente 1992). Also, seed mixes are often broadcast seeded at rates between 500 and 1,000 PLS/m² (John et al. 2008). When compared with annual *B. tectorum* seed production rates of 5,000 to 17,000 PLS/m², the standard seeding rate may be too low to provide adequate competition with *B. tectorum*. Using a higher seeding rate of native plants may allow for increased competition with *B. tectorum*, as well as help to restock the diminished native seed bank.

In this study, we seeded a native ruderal-dominated mix at a high rate with and without *B. tectorum* to assess the ability of the native mix to suppress *B. tectorum*. We also seeded a sterile wheat hybrid (QuickGuard™) that is often used for erosion control, to compare its effects on *B. tectorum* establishment with those of the native mix. We predicted that (H1) seeding a native ruderal species mix would suppress *B. tectorum* establishment; (H2) seeding a native ruderal species mix would suppress *B. tectorum* better than sterile wheat.

2.2 Methods

2.2.1 Study site

The study site is located on a 130-ha research property owned by Colorado State University and located north of Fort Collins, Colorado (Lat. 40° N, Long. -105° W). The site (elevation 1,584 m, 1-3% slopes) occurs on Stoneham loam soils (NRCS 2012). Existing vegetation was dominated by *Agropyron cristatum* L. Gaertn. (crested wheatgrass) and *Ericameria nauseosus* (Pall. Ex Pursh) G.L. Nesom & Baird (rubber rabbitbrush). In addition to these dominant species, the site contained scattered patches of *Cirsium arvense* (L.) Scop. (Canada thistle), *B. tectorum*, and *Bromus arvensis* (L.) (field brome).

In September, 2010, all *E. nauseosus* shrubs and their root crowns were pulled from the treatment area. The entire study site was mowed to stubble height of 5 cm. In October, 2010, a tractor-driven ripper consisting of rolling coulters and ripper shanks was run through the site twice (lengthwise and crosswise). The site was then rototilled to a depth of 12-15 cm twice using a tractor-powered tiller, with one week between tilling treatments. During both tilling events, the site was first tilled lengthwise and then crosswise. Large rocks were removed from the study plots by hand.

2.2.2 Study setup

The 45 x 25-m study site consisted of 50, 3x3 m plots surrounded on each side by a one-meter buffer. In November, 2010, plots were seeded randomly with one of six different treatments (Table 2.1): *B. tectorum* (BROMUS), native ruderal mix (NATIVE) (Table 2.2), sterile wheat (WHEAT), native ruderal mix plus *B. tectorum* (NATBRO), sterile wheat plus *B. tectorum* (WHTBRO) and an unseeded control (UNSEED). Two of the treatments (NATBRO, WHTBRO) received two seed “mixes” in additive quantities. The native ruderal seed mix and the sterile wheat treatments have five replicates each. The other four treatments were replicated 10 times each (these plots were doubled in replication for use as part of a long-term study in which half of these plots will be drill seeded with native perennial species).

Table 2.1. Seeding treatments and rates utilized to assess the effects of a native ruderal seed mix and sterile wheat species on the establishment and persistence of *Bromus tectorum*. Treatments that received multiple seed mixes were additive, with the total amount of seed applied for each treatment indicated in the “Total” column. The native ruderal mix was incorporated via rototilling into the top 10 cm of soil, resulting in an effective seeding rate of 2,000 PLS/m² based on even distribution throughout the 10 cm and potential germination of the seeds remaining in the top centimeter (10%).

Treatment	<i>B. tectorum</i>	Native mix	Sterile wheat	Total
	PLS/m ²			
Native Ruderal mix (NATIVE)	0	20,000	0	20,000
Sterile wheat (WHEAT)	0	0	2,000	2,000
<i>Bromus tectorum</i> (BROMUS)	7,650	0	0	7,650
Unseeded (UNSEED)	0	0	0	0
Native Ruderal plus <i>B. tectorum</i> (NATBRO)	7,650	20,000	0	27,650
Sterile wheat plus <i>B. tectorum</i> (WHTBRO)	7,650	0	2,000	9,650

Table 2.2. Native ruderal seed mix species. Plants listed below were included in the native ruderal seed mix (NATIVE treatment) at the indicated rates. Growth habit indicates forbs (F) and grasses (G) while type indicates annual (A), biennial (B) and perennial (P) life spans. Species seeding rates (PLS/m²) were adjusted based on estimated and desired contribution to community composition, and to total a seed mix rate of 20,000 PLS/m².

Type	Growth Habit	Latin name	Common Name	PLS/m ²
A	F	<i>Amaranthus retroflexus</i> L.	Redroot amaranth	2800
A/P	G	<i>Aristida purpurea</i> Nutt.	Purple three-awn	1400
A	F	<i>Chenopodium album</i> L.	Lambsquarters	2500
A	F	<i>Cleome serrulata</i> Pursh	Rocky Mountain beeplant	2400
P	F	<i>Gaillardia aristata</i> Pursh	Blanketflower	1700
A	F	<i>Helianthus annuus</i> L.	Common sunflower	2000
A	G	<i>Vulpia octoflora</i> (Walter) Rydb.	Six-weeks fescue	2400
B/P	F	<i>Erysimum capitatum</i> (Douglas ex Hook.) Greene	Sanddune wallflower	2400
B/P	F	<i>Sphaeralcea coccinea</i> (Nutt.) Rydb.	Scarlet globemallow	2400
Total				20,000

The native ruderal seed mix (20,000 PLS/m²) was broadcast by species and rototilled with a rear-tine garden tiller into the top 10 cm of soil. Because the study site had been dominated by a non-native bunchgrass for several decades and the native seed bank was likely limited, we used this seeding method as a means to reestablish a native seed bank for use in a long-term study, which will not be addressed in this paper. After tilling, we estimate that the effective seeding rate for the native ruderal seed mix was the seed located in the top centimeter of soil – approximately 10% (2,000 PLS/m²) of the seed mix. Based on our observed germination data of 1,000 – 1,200 PLS/m², which is consistent with an expected rate of 50-60% germination of grasses and forbs.

The sterile wheat and *B. tectorum* were broadcast following the incorporation of the native ruderal mix into the soil. Sterile wheat was seeded at the same rate as the effective seeding rate for the native mix (2,000 PLS/m²). *B. tectorum* was seeded at a rate of 7,650 PLS/m², which was determined from both the range of estimates for annual seed production and the amount of seed available onsite for collection. All plots were raked after seeding to increase seed-to-soil contact and were rolled with a water-filled roller to firm the seedbed.

2.2.3 Study Maintenance

Both 2011 and 2012 were dry years relative to standard average precipitation for this area. Average precipitation for this site during the early growing season (March through May) is 15.06 cm. For this period in 2011, the site received 5.94 cm, while in 2012 only 3.58 cm of precipitation fell. To overcome the potential for limited establishment and to allow for the testing of our hypotheses, supplemental water was applied in 0.5 cm increments between April and June 2011 and 2012 to increase soil moisture while still staying below average precipitation

conditions. In 2011, initial establishment was of greater concern, given barren soils and no litter to retain moisture, so greater moisture quantities were added in the spring of 2011. In 2012, existing plant litter from the previous growth likely assisted in retaining moisture and limiting rapid evaporation, therefore water addition was a slightly lesser concern. In 2011, total spring moisture (precipitation plus supplemental) for the site was 9.75 cm; in 2012, total spring moisture was 5.88 cm. No soil moisture data was collected in either year, so the exact influence of moisture additions cannot be addressed.

2.2.4 Data collection

Vegetation in each study plot was sampled on 27 and 28 of June, 2011 and 2012. Four 0.25 x 0.75 m frames were destructively sampled in each of the 50 plots. Density counts of seeded species were conducted in each sampling frame. Biomass was collected by species in each frame by clipping plants at ground level and retaining all plant material from the current growing season. Biomass and density counts were pooled for the four frames in each plot. Biomass was dried to constant mass at 65°C. Dried samples were weighed to determine total aboveground production per plot for each species.

2.2.5 Statistical analysis

The effect of native ruderal mix and sterile wheat establishment on *B. tectorum*, as well as the effect of *B. tectorum* establishment on the native ruderal mix and wheat was analyzed using one-way analysis of variance in the mixed procedure in SAS 9.2 (SAS Institute, Cary, NC, USA) for each treatment. Biomass and density data were log transformed to adjust for normality and a Kenward-Rogers adjustment was used, when necessary, to account for heterogeneity of

variances. Biomass and density of *B. tectorum* were analyzed for differences between all treatments containing *B. tectorum* (BROMUS, NATBRO, WHTBRO). Pairwise comparisons were made using the Tukey's adjustment for *B.tectorum* analyses. Biomass and density of the native ruderal species and sterile wheat were analyzed between NATIVE and NATBRO plots, and between WHEAT and WHTBRO treatments, respectively. To better understand dynamics and effects through time, all analyses were conducted for each year separately.

2.3 Results

All seeded treatments resulted in establishment of the seeded species. Native ruderal species were present in both years, though composition varied between years (Table 2.3). Plots not seeded with *Bromus tectorum* (UNSEED, NATIVE, WHEAT), did not have notable establishment of *B. tectorum* in this study (data not shown). *Chenopodium album* (L.) established from the seedbank in addition to from seed, and was found in low numbers in all treatments in both years (Table 3). Sterile wheat was only present in WHEAT and WHTBRO plots, and only in the first growing season.

Table 2.3. Native seed mix biomass and density results. Seeded native mix species biomass and density data were recorded in all six treatments (*Bromus tectorum* (BROMUS), native ruderal seed mix (NATIVE), sterile wheat (WHEAT), native mix plus *B. tectorum* (NATBRO), sterile wheat plus *B. tectorum* (WHTBRO), and unseeded (UNSEED)) for both growing seasons. Biomass and density were both recorded in an effort to better understand the dynamics through which the seeded native species, *B. tectorum*, and sterile wheat were interacting. Means and standard errors (SE) were calculated for each species' biomass (g/m^2) and density (\#/m^2). Means were calculated across data from all plots (NATIVE, WHEAT: $n = 5$; BROMUS, NATBRO, WHTBRO, UNSEED: $n = 10$).

2.3a. Biomass (g/m^2)	Treatment	Year	<i>A. retroflexus</i>		<i>C. album</i>		<i>C. serrulata</i>		<i>E. capitatum</i>		<i>G. aristata</i>		<i>H. annuus</i>		<i>S. coccinea</i>		<i>V. octoflora</i>	
			Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
	BROMUS	2011	0.00	0.00	0.12	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.55	0.28	0.00	0.00	0.00	0.00
	NATBRO	2011	0.06	0.04	6.92	1.58	0.40	0.08	0.00	0.00	1.09	0.43	131.90	8.50	0.00	0.00	0.00	0.00
	NATIVE	2011	4.18	0.02	1.30	0.59	0.02	0.16	1.75	0.01	119.69	0.29	30.67	11.93	0.00	0.00	0.00	0.00
	WHTBRO	2011	0.00	0.00	0.20	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.40	0.29	0.00	0.00	0.00	0.00
	WHEAT	2011	0.00	0.00	4.32	2.46	0.00	0.00	0.00	0.00	0.02	0.02	2.67	2.67	0.00	0.00	0.02	0.02
	UNSEED	2011	0.00	0.00	0.25	0.24	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.03	0.03	0.00	0.00
	BROMUS	2012	0.00	0.00	0.49	0.19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	NATBRO	2012	0.00	0.00	0.20	0.09	0.33	0.24	0.01	0.01	0.03	0.03	4.80	1.89	0.00	0.00	0.00	0.00
	NATIVE	2012	0.00	0.00	0.32	0.27	0.03	0.03	0.00	0.00	0.16	0.11	7.48	6.86	0.00	0.00	0.00	0.00
	WHTBRO	2012	0.00	0.00	0.67	0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00
	WHEAT	2012	0.00	0.00	0.89	0.76	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.00
	UNSEED	2012	0.00	0.00	0.24	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.09	0.00	0.00

2.3b. Density (#/m ²)	Treatment	Year	<i>A. retroflexus</i>		<i>C. album</i>		<i>C. serrulata</i>		<i>E. capitatum</i>		<i>G. aristata</i>		<i>H. annuus</i>		<i>S. coccinea</i>		<i>V. octoflora</i>	
			Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
	BROMUS	2011	0	0.13	1	0.36	0	0.00	0	0.00	0	0.00	1	0.22	0	0.00	0	0.00
	NATBRO	2011	1	0.70	70	6.66	23	4.40	0	0.00	54	11.31	981	146.56	0	0.00	0	0.13
	NATIVE	2011	3	2.59	69	7.55	27	7.61	0	0.27	118	7.94	822	117.29	0	0.00	0	0.00
	WHTBRO	2011	0	0.00	1	0.50	0	0.00	0	0.00	0	0.13	1	0.31	0	0.00	0	0.13
	WHEAT	2011	0	0.00	1	0.36	0	0.00	0	0.27	0	0.27	1	0.53	0	0.00	0	0.27
	UNSEED	2011	0	0.00	0	0.18	0	0.00	0	0.00	0	0.13	0	0.00	0	0.40	0	0.00
	BROMUS	2012	0	0.00	17	6.44	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
	NATBRO	2012	0	0.00	5	1.84	14	8.06	0	0.10	0	0.10	24	7.90	0	0.00	0	0.00
	NATIVE	2012	0	0.00	5	3.11	0	0.20	0	0.00	3	2.13	16	9.22	0	0.00	0	0.00
	WHTBRO	2012	0	0.00	20	25.75	0	0.00	0	0.00	0	0.00	0	0.10	0	0.00	0	0.00
	WHEAT	2012	0	0.00	31	3.88	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
	UNSEED	2012	0	0.00	6	3.59	0	0.00	0	0.00	0	0.00	0	0.00	1	0.70	0	0.00

2.3.1 *Bromus tectorum* establishment

In 2011, *B. tectorum* biomass was significantly lower in NATBRO treatments (35.69 g/m²) relative to BROMUS treatments (95.65 g/m², $p < 0.001$); however, in 2012 *B. tectorum* biomass was not different between treatments (NATBRO: 3.46 g/m², BROMUS: 3.54 g/m², $p = 0.850$) (Figure 2.1a). Density counts (Figure 2.1b) exhibited the opposite relationship where *B. tectorum* counts were not significantly different between NATBRO (229 plants/m²) and BROMUS (247 plants/m²) in 2011 ($p = 0.823$); however, were significantly different in 2012 (NATBRO: 52 plants/m², BROMUS: 200 plants/m², $p = 0.015$). Biomass of *B. tectorum* was significantly higher in WHTBRO treatments (65.50 g/m²) than NATBRO ($p = 0.004$) in 2011 and significantly lower than BROMUS ($p = 0.045$) in the same year. In 2012, *B. tectorum* biomass in WHTBRO plots (4.87 g/m²) was not significantly different from other treatments (NATBRO: $p = 0.804$, BROMUS: $p = 0.996$) (Figure 2.1a). Density of *B. tectorum* in 2011 was significantly higher in WHTBRO (352 plants/m²) than NATBRO plots ($p = 0.017$), though not significantly different from BROMUS plots ($p = 0.0656$) (Figure 2.1b). In 2012, density counts in WHTBRO (228 plants/m²) were not significantly different from BROMUS plots ($p = 0.925$), yet were significantly higher than NATBRO plots ($p = 0.035$).

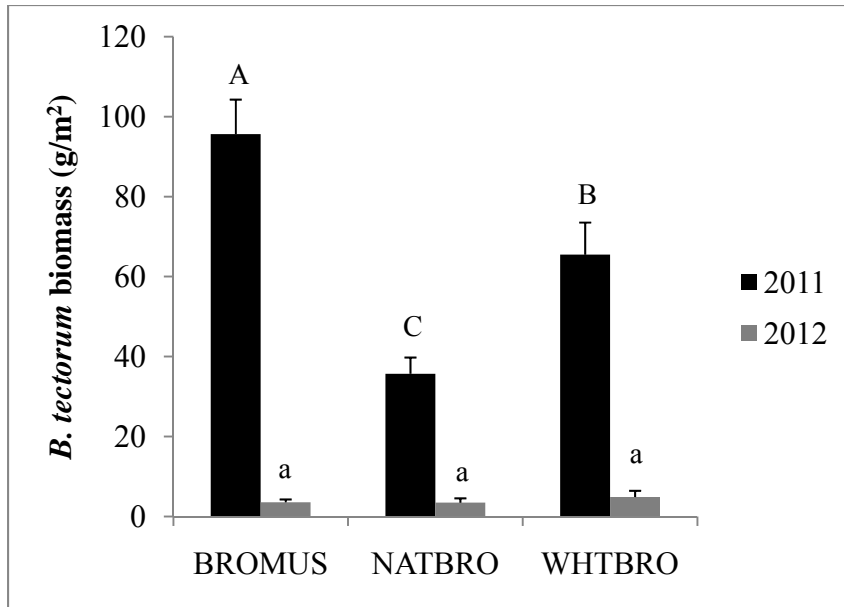


Fig. 2.1a

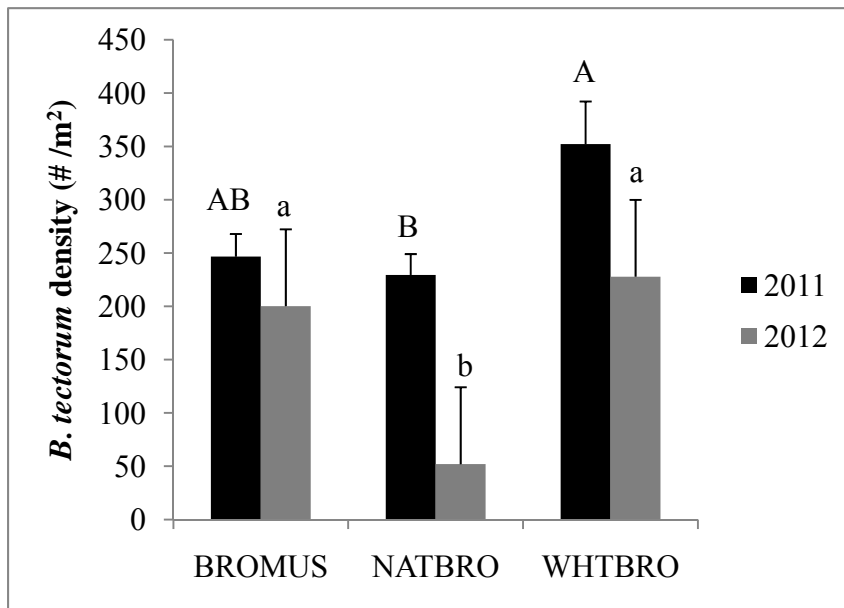


Fig. 2.1b

Figure 2.1. *Bromus tectorum* biomass (Fig. 2.1a) and density (Fig. 2.1b) comparison between *B. tectorum*-seeded treatments (means of original data presented). *Bromus tectorum* was seeded alone (BROMUS), with a native ruderal species mix (NATBRO), and with a sterile wheat (WHTBRO) to assess the potential utility of the two seeding treatments for suppressing *B. tectorum*. One-way analysis of variance of log-transformed means of *B. tectorum* biomass showed that native ruderal species and sterile wheat reduced *B. tectorum* biomass during the initial growing season after seeding (2011) (Fig. 2.1a). These effects diminish in the second growing season. Similar analyses of log-transformed density data showed no effect of native species on *B. tectorum* density in the first growing season, but a reduction in *B. tectorum* density in the second growing season (Fig. 2.1b). Sterile wheat increased *B. tectorum* density during the first growing season, but had no effect in the second growing season. For each year, bars with different letters differ significantly at $\alpha = 0.05$ following Tukey's adjustment.

2.3.2 *Bromus tectorum* effects on other species

Biomass and density values of native ruderal species were not significantly different between NATIVE and NATBRO treatments in either year. Sterile wheat biomass was significantly lower in WHTBRO plots (3.92 g/m²) relative to WHEAT plots (15.95 g/m²) in 2011 (p = 0.004). Density between WHTBRO (8 plants/m²) and WHEAT (7 plants/m²) was not significantly different (p = 0.766). No sterile wheat plants were found in 2012.

2.4 Discussion

Seeding a native ruderal mix resulted in a reduction of *B. tectorum* biomass after one growing season and reduced *B. tectorum* density in the second growing season. Density was counted in order to provide additional insight as to how *B. tectorum* was being affected by the various treatments. After one growing season, *B. tectorum* density was not significantly impacted by the presence of native species. However, given the impact of natives on *B. tectorum* biomass, it seems that germination of *B. tectorum* was similar between the two treatments, but that the plants growing amongst the native ruderal mix were smaller individuals, leading to the significant difference in biomass between the treatments. It is likely that this reduction in plant vigor was due to resource competition with the native ruderal species. The lack of a similar reduction in the native species in the presence of *B. tectorum* indicates that native ruderal species may be good competitors with *B. tectorum*, as has been suggested by others (Booth et al. 2003, Perry et al. 2009, Herron et al. in press).

Sterile wheat seeded at the same effective rate as the native ruderal mix did not lead to equivalent suppression of *B. tectorum* establishment. Sterile wheat did suppress *B. tectorum*, but not to the same level as the native seed mix. Density of *B. tectorum* was lower in WHTBRO

plots relative to BROMUS plots, indicating a lower germination or establishment rate of *B. tectorum* in that treatment. However, because both biomass and density were reduced in the presence of sterile wheat, we do not have enough information to infer anything about the size of individual plants between the treatments. Unlike with the native ruderal mix, the sterile wheat was negatively impacted in terms of both density and biomass by the presence of *B. tectorum*, suggesting that it is perhaps not as competitive with *B. tectorum* as the native mix.

By 2012, the initial suppression of *B. tectorum* biomass by the native ruderal mix had declined. However, there was still an effect on *B. tectorum* density (NATBRO: 52 vs. BROMUS: 200 plants/m²). Given the equivalence in biomass but difference in density between treatments, it appears that *B. tectorum* growing with the natives species are larger individuals than those in the BROMUS treatments. This could be a result of 2011 *B. tectorum* producing more seed in the BROMUS plots, or fewer recruits in the NATBRO plots.

The 2010 sterile wheat seeding did not negatively impact *B. tectorum* in 2012. There are some indications that biomass and density of *B. tectorum* may actually be increasing in WHTBRO treatments. This is a very interesting result, and not necessarily expected, as sterile wheat is frequently used in restoration seed mixes to limit establishment of undesirable species. However, skeptics of sterile species use in revegetation have suggested that they may actually create conditions that allow exotics to establish more successfully in the second year following seeding via creation of an “ecological vacuum” (Keeley 2006). Essentially, the successful establishment of the sterile species is thought to prevent competitive native species from establishing, thus creating open sites ideal for invasive species in the second year. The results presented in this study may support this theory, though long-term data will be required for substantiation.

2.5 Conclusion

Based on the results of this study, seeding native ruderal species at a high rate may suppress and alter the establishment and growth of *Bromus tectorum* in field settings. Our results indicate that biomass and density of *B. tectorum* were both affected by the presence of the native ruderal seed mix, though not in the same year. Long-term data could shed more light on the continued effects of this treatment on the development of a *B. tectorum* population and the plant community at the site.

A sterile wheat cover crop showed promise for reducing *B. tectorum* in the initial growing season. However, biomass and density data from the second growing season indicate the potential for increases after the initial competitive effects disappeared. Additional research focusing on longer-term effects of sterile wheat should be conducted to ensure that use of this seeding approach does not contradict its intended goals in the long-term.

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Chapter 3. Soils from native ruderal plant communities promote higher arbuscular mycorrhizal colonization than cheatgrass (*Bromus tectorum*) soils

3.1 Introduction

Bromus tectorum L. (cheatgrass) is a common invasive grass in western North America that readily establishes in lands that have been disturbed through events such as overgrazing (Hulbert 1955, Morrow and Stahlman 1984) and fires (Knapp 1996). *B. tectorum* has many characteristics that facilitate its success in invaded ecosystems including efficient competition for resources (Stewart and Hull 1949, Harris 1967, Melgoza et al. 1990), high rates of seed production and seedling establishment (Stewart and Hull 1949, Hulbert 1955, Mack and Pyke 1983, Humphrey and Schupp 2001), and self-promoting changes in nutrient cycling, fire regimes (Young and Evans 1978, Melgoza et al. 1990, Knapp 1996, Knick and Rotenberry 1997) and microbial community composition (Hawkes et al. 2006, Jordan et al. 2008, Busby et al. 2012). Many of these changes can lead to slowing or a total halt of natural succession in the disturbed area (Hulbert 1955, McLendon and Redente 1992, Paschke et al. 2000).

Soil communities have a profound effect on the progression of natural succession in plant communities through plant-soil interactions that range from negative to neutral to positive (Callaway et al. 2003). Arbuscular mycorrhizal fungi (AMF) are a critical component of the soil community that have beneficial effects on many plant species (Janos 1980, Hartnett and Wilson 1999) through increased access to nutrients (Hart et al. 2003, Batten et al. 2006, Van Der Heijden and Horton 2009), disease protection, and increased access to water (Hartnett and Wilson 2002). During succession, sites tend to transition from high nutrient availability to low-nutrient availability. Early-seral species utilize readily available nutrients from the system (McLendon and Redente 1991) until they become limiting and later successional plants adapted to low-

nutrient soils replace the early-seral species (Reynolds et al. 2003). Several studies have suggested a close association between native plants, AMF, and successional development such that AMF colonization increases as a plant community transitions from early- to late-seral conditions (Doerr et al. 1984, Rowe et al. 2007) due to increased access to nutrients gained by plants associated with AMF (McLendon and Redente 1992, Hartnett and Wilson 1999).

Exotic species cause many changes to ecosystem nutrient cycling via alterations to the soil microbial community following invasion (Batten et al. 2006). Ecosystem invasion by *B. tectorum* has been associated with significant changes in the abundance of bacteria and saprophytic fungi (Hawkes et al. 2006, Jordan et al. 2008) and decreases in the abundance and species richness of AMF (Hawkes et al. 2006, Busby et al. 2012). Due to its difference in phenology from most native plant species, the presence or dominance of *B. tectorum* (a poor mycorrhizal host) in a plant community may provide an overwinter AMF host that promotes different species of AMF than are typically associated with native plant communities (Busby et al. 2012). Over time, if AMF species requiring plant hosts for survival are without the proper hosts, they may die off and further limit establishment of mycotrophic plant species (Doerr et al. 1984, Busby et al. 2012). Reductions of AMF to very low levels can decrease the chances of a mycotrophic seedling becoming colonized, thereby reducing its chances of successful establishment and persistence (Doerr et al. 1984, Gange et al. 1993). Changes to the soil community can occur in one growing season (Batten et al. 2008) and may limit the success of the native community while promoting continued success of *B. tectorum* (Batten et al. 2008, Jordan et al. 2008). While Rowe and Brown (2008) found that *B. tectorum* did not lead to differences in native plant establishment, the soils used in their study had been previously invaded by *B. tectorum*, therefore delayed and residual effects of *B. tectorum* on soil community could not be

extrapolated. Instead these soils may demonstrate that the removal of *B. tectorum* via herbicide application may not cause immediate changes in the soil community that benefit native species.

In 2010, a field study was initiated in northern Colorado, USA to assess the competitive effects of native early-seral plant species that share fundamental characteristics with *B. tectorum*. A native seed mix consisting of predominantly early-seral species (Table 3.1) was seeded at a high rate in replicated test plots with or without *B. tectorum* to determine if this type of seeding approach could be successful in reducing *B. tectorum* establishment. The present study utilized soil from these field plots in a greenhouse study to determine if, after one year of field growth, there was a difference in AMF colonization between the soils collected from three field treatments containing either *B. tectorum* (BROMUS), native early-seral species (NATIVE), or both (NATBRO). Many early-seral plants are often considered to be facultative AMF hosts and offer the potential to influence the AMF community in many ways (Busby et al. 2011), including the promotion of AMF species required by late-seral species. Native late-seral species tend to respond well to soils that have been conditioned by a mix of native species (Vogelsang and Bever 2009, Jordan et al. 2011). We predicted that (H1) field soils collected from plots seeded with the NATIVE treatment would result in greater AMF root colonization than soils from plots seeded with BROMUS and (H2) root colonization of plants grown in NATBRO soils would be intermediate between NATIVE and BROMUS treatments.

Table 3.1. Native early-seral seeding mix and resulting native community relationships. A native early-seral mix was seeded with and without *Bromus tectorum* in a field study to assess the ability of the native mix to suppress *B. tectorum* establishment and persistence. The native early-seral mix was composed of a diverse assemblage of species with different growth habits (forb or grass), growth duration (A: annual, B: biennial, P: perennial), mycorrhizal status (NM: non-mycorrhizal, AM: arbuscular mycorrhizal, U: unknown), and resource use patterns, which were selected for their potential to compete with *B. tectorum*. Each species composed, at most, 14% of the seed mix. The total seeding rate of the mix was 20,000 PLS/m². In the first growth season, the majority of the seeded native species comprised less than 1% of the plant community, both by biomass and density. *Helianthus annuus* dominated the native plant community in both biomass and density.

Genus	Species	Family	Mycorrhizal status	Growth Duration	% seed mix	PLS/m ²	% of native plant community (biomass)	% native plant community (density)
<i>Amaranthus</i>	<i>retroflexus</i> L.	Amaranthaceae	NM	A	14	2800	<1	<1
<i>Aristida</i>	<i>purpurea</i> Nutt.	Poaceae	AM	A/P	7	1400	0	0
<i>Chenopodium</i>	<i>album</i> L.	Chenopodiaceae	NM	A	12.5	2500	3-5	6-7
<i>Cleome</i>	<i>serrulata</i> Pursh	Capparaceae	NM	A	12	2400	<1	2-3
<i>Gaillardia</i>	<i>aristata</i> Pursh	Asteraceae	U	P	8.5	1700	.5-1.5	4-11
<i>Helianthus</i>	<i>annuus</i> L.	Asteraceae	AM	A	10	2000	93-95	79-86
<i>Vulpia</i>	<i>octoflora</i> (Walter) Rydb.	Poaceae	U	A	12	2400	<1	<1
<i>Erysimum</i>	<i>capitatum</i> (Douglas ex Hook.) Greene	Brassicaceae	U	B/P	12	2400	<1	<1
<i>Sphaeralcea</i>	<i>coccinea</i> (Nutt.) Rydb.	Malvaceae	AM	B/P	12	2400	0	0
Total						20,000		

3.2 Methods

3.2.1 Field Study

Soils were collected from a field study (Stube 2012) north of Fort Collins, Colorado (40N 42'56", 105W 6' 4", elevation 1584 m, 1-3 % slopes) on a 130-hectare property owned by Colorado State University. Soils are dominated by Stoneham loam of mixed alluvium and/or eolian deposit parent material (fine-loamy, mixed, mesic Aridic Haplustalfs) (NRCS 2012). Dominant vegetation at the site prior to establishment of the field study consisted of *Agropyron cristatum* L. Gaertn. (crested wheatgrass) and *Ericameria nauseosus* (Pall. ex Pursh) G.L. Nesom & Baird (rubber rabbitbrush). The field study consisted of 50 – 3x3 m plots randomly assigned with one of six seeding treatments (Stube 2012), three of which were used in the present study: *Bromus tectorum* (BROMUS), a mixture of native early-seral species (NATIVE), and *B. tectorum* seeded with the native early-seral mix (NATBRO). The NATIVE treatment was replicated five times, while BROMUS and NATBRO treatments were replicated 10 times. The plots were seeded in October of 2010. The native early-seral seed mix was broadcast first by species, at a total seeding rate of 20,000 pure live seed (PLS) m⁻². After tilling to a depth of 10 cm to repopulate a native seed bank, we estimate the effective seeding rate in the top centimeter of soil for the native early-seral mix to be approximately 2,000 PLS/m². *B. tectorum* was broadcast following the native early-seral mix at a rate of 7,650 PLS/m², a number that falls within its estimated seed production rate. After seeding, all plots were raked by hand to improve seed to soil contact and firmed with a water-filled roller. The first year of plant establishment and growth occurred during the spring and summer of 2011. Results from the first growing season showed that *B. tectorum* was significantly suppressed in plant growth when seeded with the native early-seral mix (NATBRO) relative to BROMUS treatments. Results also indicated

that the native early-seral mix was not significantly suppressed by *B. tectorum* in the NATBRO treatment relative to NATIVE treatment. In the native early-seral treatments, *Helianthus annuus* L. was the dominant species in the first growing season, accounting for approximately 95% of native seeded species by biomass and approximately 80% of the native species by density in both NATIVE and NATBRO plots.

3.2.2 Greenhouse Study

In October of 2011, following the first growing season of the field study, soil samples were collected from three seeding treatments: BROMUS, NATBRO, and NATIVE. Five plots were randomly sampled from each seeding treatment (15 plots total). Five soil cores were sampled from each of the 15 plots (one from the center and one from the middle of each edge) and combined into one sample per plot totaling approximately 1000 ml of soil. Samples were sieved to remove rocks; root clumps were broken down to evenly distribute root fragments throughout the soil sample.

Three host plant species were selected in order to assay soils for mycorrhizae: two species were native mycorrhizal species *Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths (blue grama), and *Ratibida columnifera* (Nutt.) Woot. & Standl. (upright prairie coneflower), and one was a non-native promiscuous AMF host *Sorghum bicolor* (L.) Moench ssp. *drummondii* (sudangrass). Seeds were surface sterilized using 70% ethanol and planted in 150 ml cones containing a 1:1 mixture of autoclaved sand and field soil. Five cones per host plant were planted for each soil sample (plot). Cones were placed in random order on a misting bench in the greenhouse under consistent temperatures of 20 C° (night) and 24° C (day). They received natural and supplemental light to total 16 hours of light each day and were misted every 30

minutes for 30 seconds for the duration of growth. Cones were re-randomized on the misting bench weekly. Upon germination, seedlings were thinned to one plant per cone. Seedlings were harvested after 30 days of growth and roots were removed, washed and stored in 70% ethanol.

All collected root samples for each cone were cut into 2.5 cm pieces and processed for AMF colonization calculation. Root samples were cleared using 2.5% KOH for 30 minutes at 90° C, rinsed, and acidified in 1% HCl for two hours (Koske and Gemma 1989). Samples were stained with 0.05% trypan blue in acid glycerol for 30 minutes at 90° C and destained in acid glycerol for 30 minutes at 90° C (Koske and Gemma 1989). Root samples were mounted in glycerol on microscope slides and observed under 400X magnification. Colonization of roots by hyphae, arbuscules, and vesicles was counted using 100 root intersections per slide (McGonigle et al. 1990).

3.2.3 Statistical analysis

The effect of seeding treatment on root colonization was analyzed using one-way analysis of variance in the mixed procedure in SAS 9.2 (SAS Institute, Cary, NC, USA) for each species. Data were arcsine square root transformed to adjust for normality and a Kenward-Rogers adjustment was used, when necessary, to account for heterogeneity of variances. Hyphal, arbuscular, and total colonization differences between field soil treatments were analyzed for each host species; due to low detection rates leading to zero-inflation in the datasets, vesicular colonization was not analyzed. However, it is captured in the total colonization data. Pairwise comparisons were made using a Tukey adjustment for all analyses.

3.3 Results

Results are presented for *R. columnifera* and *S. bicolor* only as *B. gracilis* suffered large replicate losses during growth in the greenhouse. In addition, the majority of surviving *B. gracilis* individuals had insufficient root growth to complete the analysis. For *R. columnifera* and *S. bicolor*, data are presented using percent colonization for hyphal, arbuscular, and total colonization.

3.3.1 *Ratibida columnifera*

Hyphal colonization was lowest in *R. columnifera* plants grown in BROMUS soils (6.35%), which had significantly lower colonization than *R. columnifera* plants grown in either the NATBRO (10.64%, $p = 0.02$) or the NATIVE (12.84%, $p < 0.001$) soils (Figure 3.1a). There was no significant difference between colonization in NATBRO and NATIVE soils ($p = 0.56$). Arbuscular colonization was lowest in BROMUS soils (8.55%) and was significantly lower than NATBRO (16.56%, $p < 0.01$) and NATIVE (18.48%, $p < 0.001$) soils while NATBRO and NATIVE soils were not significantly different ($p = 0.73$) (Figure 3.1a). Total colonization followed the same pattern with BROMUS colonization (14.78%) significantly lower than NATBRO (27.03%, $p < 0.01$) and NATIVE (30.96%, $p < 0.001$) soils, and NATBRO and NATIVE soils were not significantly different ($p = 0.65$) (Figure 3.1a).

3.3.2 *Sorghum bicolor*

Hyphal colonization of *S. bicolor* was lowest in BROMUS soils (2.34%) and was significantly different than colonization in NATIVE soils (4.85%, $p < 0.01$) (Figure 3.1b). Hyphal colonization in NATBRO (3.73%) soils was not different from either BROMUS ($p =$

0.10) or NATIVE soils ($p = 0.60$) soils (Figure 3.1b). Arbuscular colonization followed the same pattern as BROMUS (0.35%) soils had significantly lower colonization than NATIVE soils (1.09%, $p = 0.03$), but NATBRO (0.78%) soils showed no significant differences from BROMUS ($p = 0.17$) or NATIVE ($p = 0.71$) soils. Total colonization was lowest in BROMUS (2.68%) soils and was significantly lower than in NATIVE (5.89%, $p < 0.01$) soils. Colonization in NATBRO (4.50%) soils was not different from either BROMUS ($p = 0.09$) or NATIVE ($p = 0.62$) soils in total colonization.

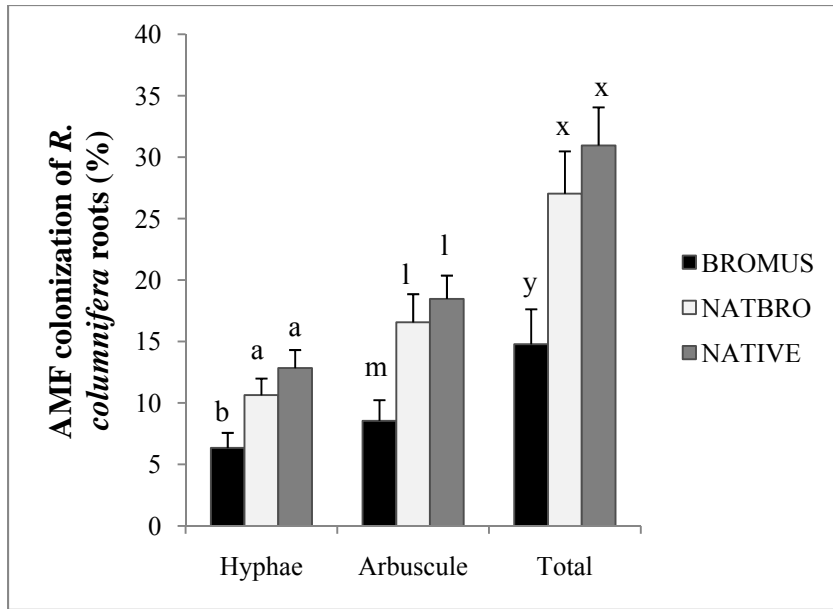


Fig. 3.1a

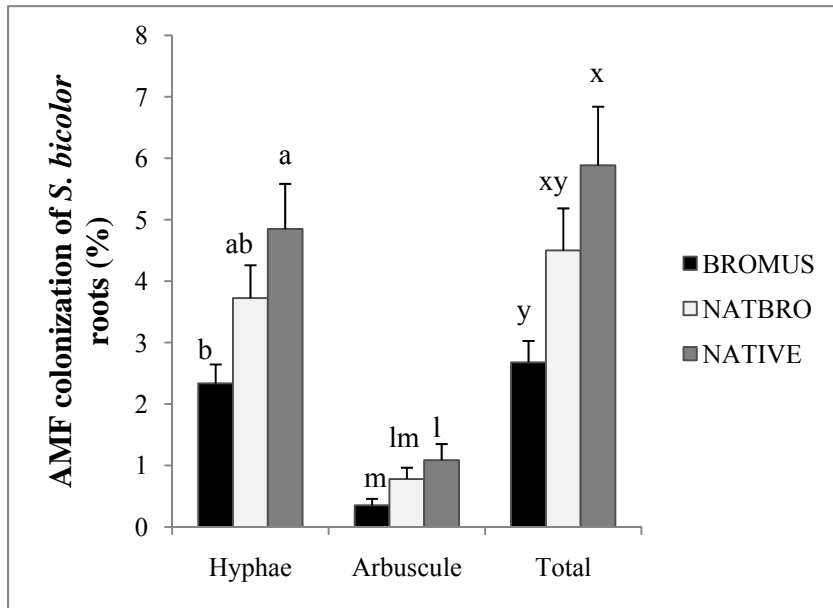


Fig. 3.1b

Figure 3.1. Colonization of host plant species by arbuscular mycorrhizal fungi (AMF) after 30 days of growth. Percent colonization by AMF was analyzed in a native mycorrhizal host (*Ratibidia columnifera*) (Fig. 3.1a.) and a promiscuous non-native mycorrhizal host (*Sorghum bicolor* ssp. *Drummondii*) (Fig. 3.1b.) in soils collected from three field treatments: 1) *Bromus tectorum* soil (BROMUS) 2), native early-seral mix soil (NATIVE), and 3) native early-seral mix and *B. tectorum* soil (NATBRO). Analyses were conducted on hyphal, arbuscular and total colonization between treatments, by species under 400X magnification. For each AMF structural grouping, bars with different letters differ significantly at $\alpha = 0.05$, using Tukey's adjustment.

3.4 Discussion

Results of this study support previous studies that indicate *B. tectorum* may significantly affect mycorrhizal communities of invaded areas (Al-Qarawi 2002, Hawkes et al. 2006, Jordan et al. 2008, Busby et al. 2012) and show that these effects can begin to emerge early in the invasion process. The increased colonization of hyphae and arbuscules in soils seeded with native early-seral plants relative to soils seeded with *B. tectorum* supports our first hypothesis and suggests that native early-seral species may promote a higher diversity or abundance of AMF species that provide greater colonization potential, particularly for native host species.

Colonization of the native host *R. columnifera* by mycorrhizae was lowest in plots seeded with *B. tectorum* relative to those seeded either with native early-seral species or native early-seral species plus *B. tectorum*, suggesting that the presence of these native early-seral species in the plant community is more important than the absence of *B. tectorum*. In the non-native plant host *S. bicolor*, hyphal and total colonization were significantly lower in soils seeded with *B. tectorum* than those seeded with the native early-seral seed mix. However, colonization in soils seeded with *B. tectorum* plus the native early-seral mix was not significantly different from either soils seeded with only *B. tectorum* or those seeded with only the native early-seral seed mix. This may suggest that, for *S. bicolor*, the presence of *B. tectorum* has a significant impact on the mycorrhizae that colonize it.

The observed differences in colonization between *R. columnifera* and *S. bicolor* were surprising, as it was expected that *S. bicolor* would colonize in the presence of all available AMF species due to its known status as a promiscuous colonizer. Evidence for host-dependent plant-AMF relationships in plant communities (Bever et al. 1996, Bever et al. 2001) could explain some of this unexpected variation in response between the two host species. In the NATIVE and

NATBRO field treatments, *Helianthus annuus* L. was the most successful early-seral species (Table 1). Given this species' status as a mycorrhizal species, it is possible that its dominance of the plant community created conditions promoting AMF species that were more beneficial to native late-seral *R. columnifera* than to non-native *S. bicolor*. Vogelsang and Bever (2009) similarly found that native host plants exhibited higher colonization rates from native soils than did non-native host plants. Growth rates of AMF species have been shown to be highly dependent upon the host plant species (Bever et al. 2001). The results of this study could indicate that the *R. columnifera* showed a positive host-specific relationship with the AMF species in the soils relative to *S. bicolor*. Studies of host-dependence indicate that the species of AMF present may, in fact, be more important to the plant community than AMF diversity and richness (Bever et al. 2001, Vogelsang et al. 2006).

Based on the results of this study, it seems that establishing a native early-seral plant community could prove useful for promoting an AMF community able to colonize, and likely, promote late-successional species. This could prove critical for successional development to a late-seral plant community (Doerr et al. 1984, Rowe et al. 2007). In addition, for native mycorrhizal host species such as *R. columnifera*, it seems that focusing on establishing native early-seral species is more important, and perhaps a higher priority for resources, than completely eliminating invaders like *B. tectorum* from the plant community. Further studies carrying out this sort of analysis for repeated years following establishment of native early-seral species could aid in determining long-term effects of initial colonizing species. Also, AMF species composition changes could allow more specific targeting as to how changes in AMF communities are occurring.

3.5 Conclusions

Based on the results of this study, seeding disturbed sites with native early-seral species could be an important step in restoration, particularly for promoting AMF communities that can colonize native species and facilitate succession toward native, late-seral communities. More specifically, native early-seral mycorrhizal species may play a critical role in the facilitation of AMF species that readily colonize native late-seral host plant species.

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