

Can Sainfoin Improve Conditions for Establishment of Native Forbs in Crested Wheatgrass Stands?

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ABSTRACT

Concerns about wildlife habitat quality in western North America has stimulated interest in diversifying *Agropyron cristatum* (crested wheatgrass) stands. Four main obstacles make it difficult to establish native forbs in stands of *A. cristatum*. First, adult *A. cristatum* plants are fierce competitors with native seedlings. Second, *A. cristatum* seedlings emerging from a long-lived seedbank can crowd out native species. Third, *A. cristatum* control may facilitate secondary invaders rather than the desired native species. Fourth, potential soil modification by *A. cristatum* may impede establishment of diverse native plant species. A “bridge species” that is compatible with *A. cristatum* control and improves conditions for native species establishment could facilitate *A. cristatum* stand diversification. We compared native forb growth in soils of former *A. cristatum* stands preconditioned by *A. cristatum*, *Bromus tectorum* (cheatgrass), and *Onobrychis viciifolia* (sainfoin), a glyphosate-tolerant legume. Soils preconditioned by *O. viciifolia* had the greatest P and K availability. Although total plant biomass was similar among treatments, native forbs had greater root colonization by arbuscular mycorrhizal (AM) fungi, less root colonization by non-AM fungal, and lower root-shoot ratios when grown in *O. viciifolia*-conditioned soils, suggesting improved soil microbe and nutrient conditions for native forb establishment. We conclude that *O. viciifolia* may be a useful bridge species for improving soil conditions while allowing for weed control during restoration of *A. cristatum* stands.

Keywords: *Agropyron cristatum*, arbuscular mycorrhizal fungi, *Bromus tectorum*, *Onobrychis viciifolia*, soil feedbacks


Restoration Recap

- We examined a mycorrhizal, glyphosate tolerant legume, *Onobrychis viciifolia* (sainfoin), for the ability to facilitate positive soil feedbacks for native forb establishment in *Agropyron cristatum* (cheatgrass) conditioned soil.
- *Onobrychis viciifolia* increased soil nutrient availability and arbuscular mycorrhizal fungi abundance.
- “Bridge species”, such as *O. viciifolia*, that are compatible with weed control efforts may facilitate diversification of *A. cristatum* stands by improving soil nutrient availability and plant interactions with soil microorganisms, while allowing managers to chemically control and deplete the *A. cristatum* and weedy species seedbank.

Agropyron cristatum (crested wheatgrass) is a perennial caespitose species from Eurasia that has been artificially selected for improved competitive ability, ease of establishment, productivity, and grazing resistance (Rogler and Lorenz 1983, Monsen 2004). *Agropyron cristatum* has been introduced to 13 to 17 million ha in western North America (Heidinga and Wilson 2002) to improve

the condition of degraded rangelands. Once established, *A. cristatum* dominates the seedbank and resists invasion by nonindigenous (Berube and Myers 1982, D’Antonio and Vitousek 1992, Sheley et al. 2008) and native plant species (Marlette and Anderson 1986, Henderson and Naeth 2005, Nafus et al. 2015).

Agropyron cristatum often forms low diversity stands with altered ecosystem function relative to native prairie (Christian and Wilson 1999). *Agropyron cristatum* stands support less bird, mammal, and reptile diversity and abundance than do sites dominated by sagebrush and other native species (Reynolds and Trost 1980, McAdoo et al. 1989, Rottler et al. 2015). Concerns about declining wildlife habitat quality have stimulated interest in diversifying

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A. cristatum stands with native species (Davies et al. 2013). However, *A. cristatum* individuals that survive control measures and recruitment from the seedbank can undermine diversification efforts (Marlette and Anderson 1986, Pyke 1990, Romo et al. 1994, Bakker et al. 2003, Vaness and Wilson 2007). For example, Hulet et al. (2010) used mechanical and chemical treatments to control *A. cristatum* prior to seeding native species. While *A. cristatum* cover initially decreased, all mechanical and herbicide treatment effects disappeared three years after treatment. Moreover, *Bromus tectorum* (cheatgrass) invaded sites after initial *A. cristatum* control and poor native species establishment. Similar results were reported by Fansler and Mangold (2010) who concluded, “suppression of crested wheatgrass may require multiple techniques and multiple years of treatment prior to reintroduction of native plants”. These authors stressed the importance of reducing the weed seedbank before introducing native species.

Plants change soil biological, chemical and structural properties and processes in ways that alter their own growth and the growth of other species (Kulmatiski et al. 2008). Plant-soil interactions are important drivers of plant community composition and ecosystem function (Klironomos 2002, de Kroon et al. 2012). A few studies suggest that *A. cristatum* facilitates changes in soils that may hamper restoration of diverse native plant communities. For example, Jordan et al. (2008) found that *A. cristatum* modified soil in ways that promoted self-facilitation and suppressed native forb growth, presumably due to alteration of soil biota. In a second study, Jordan et al. (2012) found that *A. cristatum* has lower arbuscular mycorrhizae (AM) fungi taxonomic richness and colonization rates than plants in native communities. A study of 100 prairie plant species indicates that forbs have higher AM colonization, and benefit more from AM fungi, than cool season grasses (Wilson and Hartnett 1998). Because AM fungi are important determinants of plant community composition (Middleton and Bever 2012) and soil quality (Rillig and Mummey 2006), the results of Jordan et al. (2008) suggest that reduced AM fungal functionality in *A. cristatum* stands contributes to native forb suppression. Gasch et al. (2015) compared mine reclamation sites in Wyoming seeded with native species or *A. cristatum*. Although sites seeded with *A. cristatum* had similar or higher aboveground biomass, soil microbial biomass was greatly reduced relative to sites seeded with native species. Substrate quality (root exudates and decomposition products) differences between *A. cristatum* and native plant communities (Biondini et al. 1988) may explain these results.

Agropyron cristatum is a strong competitor with *B. tectorum* and is often planted to displace *B. tectorum* and other invasive species. As mentioned above, attempts to diversify *A. cristatum* stands often result in *B. tectorum* invasion and dominance. Like *A. cristatum*, *B. tectorum* may modify the soil environment in ways that inhibit native species growth.

Although nutrient levels vary in *B. tectorum* invasions depending on environmental context (Rimer and Evans 2006, Stark and Norton 2015), there is general agreement that *B. tectorum* invasion lowers AM fungi abundance and alters AM fungi community composition (Al-Qawari 2002, Hawkes et al. 2006, Busby et al. 2012, Lekberg et al. 2013). Because it is a winter annual, *B. tectorum* alters the temporal dynamics of AM fungi activity (Busby et al. 2012), potentially selecting for ruderal species that can rapidly infect roots.

Bromus tectorum produces large amounts of relatively short-lived seed (Humphrey and Schupp 2001). Like *A. cristatum*, reducing the *B. tectorum* seed bank is critical for native species establishment. We’ve found that sufficient *A. cristatum* seeds can remain viable after two years to recapture field sites (personal observation). Removing seed heads by mowing or grazing can reduce *A. cristatum* seed production (Benson 2011). However, herbicide-based methods are needed to control mature *A. cristatum* plants and seedlings emerging from the seedbank prior to seeding native plant communities. Following to delete the seedbank removes the primary soil carbon source, plants, causing soil quality to decline (Bronick and Lal 2005), which can limit seedling recruitment and vigor (Awadhwai and Thierstein 1985). From the perspective of wildlife management, fallowed areas provide no interim benefits to wildlife.

Bridge species are used in restoration to reduce erosion and compete with weeds. A bridge species that is compatible with *A. cristatum* and annual grass seedling control strategies could expedite native species reestablishment. Bridge species must be able to establish rapidly and facilitate, or not inhibit, native species establishment. Bridge species have been examined for weed suppression and to facilitate plant community succession (i.e., Perry et al. 2009, Milchunas et al. 2011, Leger et al. 2014). Although a rich body of work examines soil amendments to alleviate negative soil feedbacks (reviewed by Ohsowski et al. 2012), the potential for bridge species to alleviate negative soil feedbacks and improve soil conditions for native species establishment has received relatively little attention in the restoration community (Eviner and Hawkes 2008).

Onobrychis viciifolia (sainfoin) has characteristics that suggest it is a good bridge species for *A. cristatum* stand diversification. Although *O. viciifolia* is not native to North America it is not considered weedy or invasive (USDA, NRCS). *Onobrychis viciifolia* is a legume that hosts nitrogen-fixing bacteria, potentially increasing nitrogen availability that may limit plant growth in former *A. cristatum* stands (Lesica and Deluca 1996; Christian and Wilson 1999). Leguminous species have a high capacity to mobilize soil P through root exudates and can increase P nutrition (Pypers et al. 2007). *Onobrychis viciifolia* is mycotrophic and may increase the abundance and diversity of AM fungal communities important to native forb and warm season grass abundance and diversity (Wilson and Hartnett

1998, Carbonero et al. 2011). *Onobrychis viciifolia* may improve soil nutrient and microbial conditions before native species are planted. It may also help land managers meet wildlife habitat improvement goals during and after *A. cristatum* stand diversification. *Onobrychis viciifolia* has excellent forage value for ungulates and upland game birds (USDA, NRCS) and is a rich source of pollen and nectar for pollinator species (Carbonero et al. 2011).

Onobrychis viciifolia tolerates the low amounts of glyphosate herbicide commonly used to control grass seedlings (Peel et al. 2012). We have used glyphosate to control invasive seedlings on restoration sites planted with *O. viciifolia*. Although glyphosate treatments can stunt *O. viciifolia* growth, seedlings survive under field conditions after treatment with 0.27 kg glyphosate ha⁻¹. First year *O. viciifolia* plants thrive after treatment with 0.54 kg glyphosate ha⁻¹. Glyphosate can therefore be used within *O. viciifolia* plantings to control invasive species seedlings and reduce the seedbank prior to seeding native species.

Here, we examine the potential for *O. viciifolia* to alter soils and facilitate native forb establishment. We hypothesized that conditioning soils with *O. viciifolia* would increase N and P availability relative to *B. tectorum* and *A. cristatum* conditioned soils. AM fungi abundance is important to forb abundance and diversity. We hypothesized that soils conditioned with *O. viciifolia* support increased AM fungi abundance, forb growth, and plant resource allocation to shoots. To investigate the role of microbial communities in promoting soil feedbacks on plant growth, we treated soils to remove AM fungi. We hypothesized that exotic grasses would exhibit a positive growth response, and native forbs a negative response, to soil sterilization treatment.

Methods

We conducted a greenhouse experiment to examine the potential for *O. viciifolia* to facilitate positive soil feedbacks on native seedling establishment in *A. cristatum* and *B. tectorum* conditioned soils. The experiment was conducted in two phases: a soil preconditioning phase and a bioassay phase (Figure 1). During the preconditioning phase, *A. cristatum*, *B. tectorum*, and *O. viciifolia* were grown for three generations to condition sterilized and non-sterilized soils. During the bioassay phase, species used to condition the soils and three native forb species were planted into each preconditioned soil.

We collected surface soil (25-cm depth; loamy-skeletal, mixed, frigid Typic Haploxerolls in the Bigarm gravelly loam series) inside two stands of *A. cristatum* older than 20 years in the Bitterroot Valley of Western Montana, USA. At each site, we used a shovel to excavate soils from interspaces between *A. cristatum* plants in a 10-m² area. We removed coarse materials and homogenized the soil by sieving (4-mm mesh). Half of the soil was heat-treated

to remove AM fungi (sterilization treatment). One-gallon buckets were filled with soil, moistened, covered with foil and heated until the soil was uniformly 85°C, a temperature that kills AM fungi propagules (Thompson 1990). We mixed soils with sand (25% vol/vol) to facilitate root harvest. The sand was rinsed three times with water to remove salts and other contaminants that could influence plant and microbial growth. Soil mixtures were added to 600-cm³ pots. Each soil preconditioning species was planted in 60 pots containing untreated field soil and 60 pots containing heat-treated field soil.

Soil conditioning species were grown until *B. tectorum* flowered (2 to 3 months). Shoots were clipped after each generation and placed on the soil surface. Pots were dried in a greenhouse for three weeks between generations to desiccate and kill the plants. After each reseeded, regrowth from established plants was clipped as soon as detected. At the end of the third soil preconditioning generation, soil samples were collected for analysis (see below). During the bioassay phase, each soil conditioning species and three native forbs were grown separately in each conditioned soil (N = 10).

Average greenhouse temperatures ranged from 10–30°C. Supplemental lighting was provided for 6 hours each day between December and March using 400-watt high-pressure sodium lamps. Pots were repositioned each week to minimize positional effects.

Plant Materials

Onobrychis viciifolia seed (Shoshone cultivar) was purchased from the Bighorn Sainfoin Seed Co. (Powell, WY). *Dalea purpurea* (Purple prairie clover; Bismark cultivar), *Ratibida columnifera* (upright prairie coneflower), and *Rudbeckia hirta* (black-eyed Susan) were purchased from Granite Seed Company (Lehi, UT). *Bromus tectorum* and *A. cristatum* seeds were collected from MPG Ranch (restorationmap.MPGRanch.com) in the Bitterroot Valley of western Montana (46°40'50.81" N, 114°1'37.22" W).

Plant Analyses

We collected biomass of all species at the end of the bioassay phase (Figure 1). Plant roots were carefully washed to remove soil particles. Roots and shoots of each plant were dried (48 h, 85°C) and weighed to determine above and belowground biomass. The root-shoot ratio of each plant was calculated by dividing its root weight by its shoot weight.

Soil Analyses

Subsamples of sterilized and non-sterilized soils conditioned by *B. tectorum*, *A. cristatum*, and *O. viciifolia* were analyzed to evaluate differences in chemical properties. Soil pH was measured electrometrically in a 1:1 (soil/H₂O, v/v) solution. NO₃-N was extracted with calcium phosphate, and PO₄-P extracted with Mehlich III

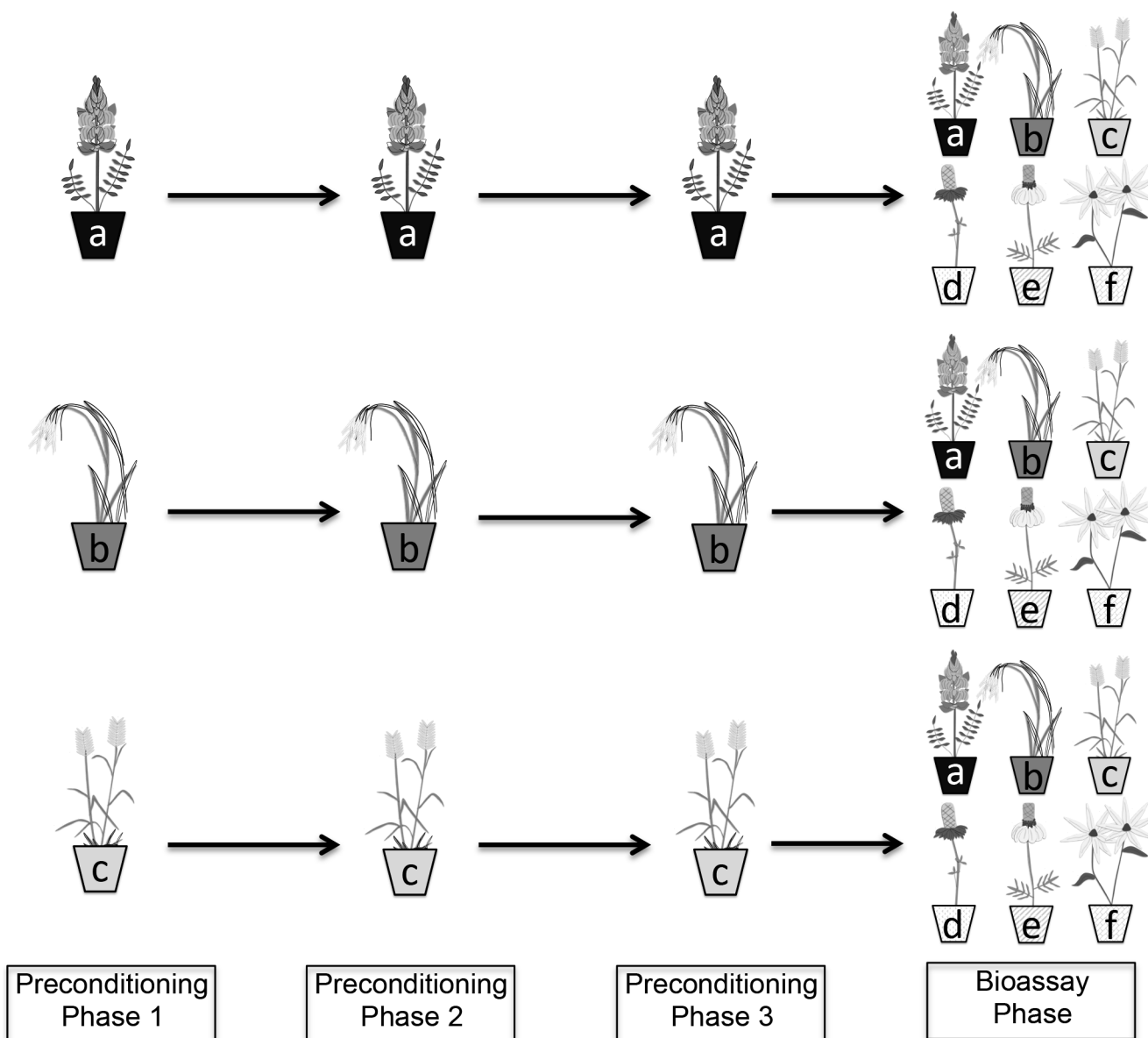


Figure 1. Experimental design. We grew each soil preconditioning species: a) *Onobrychis viciifolia* (sainfoin), b) *Bromus tectorum* (cheatgrass), and c) *Agropyron cristatum* (crested wheatgrass) in 60 pots containing untreated field soil and 60 pots containing heat-treated field soil for three generations. During the bioassay phase, we grew each preconditioning species and d) *Dalea purpurea* (purple prairie clover; Bismark cultivar), e) *Ratibida columnifera* (upright prairie coneflower), and f) *Rudbeckia hirta* (black-eyed Susan) in 10 pots of each untreated and heat-treated preconditioned soil.

extracting solution (Mehlich 1984), before analysis using a Lachat QUIKCHEM 8000 flow injection analyzer (Lachat Instruments, Loveland, CO). Potassium was extracted in NH_4OAc and analyzed by inductively coupled plasma optical emission spectrometry using a iCAP 6500-ICP-OES analyzer (Thermo Scientific Inc.).

Root Colonization

We used microscopy to analyze AM and non-AM fungi root colonization. Dried roots were re-hydrated and immersed in 10% potassium hydroxide solution for 3 days. Roots were then rinsed with deionized water and

immersed in 3% hydrochloric acid for 24 hours before being stained with Trypan blue and mounted on slides. We assessed mycorrhizal colonization at 200 \times magnification by the gridline intersect method (McGonigle et al. 1990) at 50 randomly selected locations covering the entire slide, scoring any AM and non-AM fungal structures as positive for colonization (hyphae, vesicles, arbuscules). AM fungi were differentiated from other root colonizing fungi, such as Ascomycete and Basidiomycete, based on morphological characteristics (Rillig et al. 1999).

Statistical Analyses

We used two-way ANOVAs to evaluate the effects of soil sterilization (heat-treated or not-heat-treated), soil preconditioning species (*O. viciifolia*, *B. tectorum*, or *A. cristatum*) and interactions between soil treatment and conditioning species on soil nutrient availability and bioassay plant biomass. The effect of soil-preconditioning species on AM and non-AM fungi root colonization of *O. viciifolia*, *B. tectorum* and *A. cristatum* was evaluated using one-way ANOVAs. We evaluated native forb root-shoot ratio, AM fungi root colonization, and non-AM fungi root colonization using two-way ANOVAs with native species and soil preconditioning species as fixed factors. AM fungi and non-AM fungi root colonization data were log-transformed prior to analysis to meet ANOVA normality and homogeneity of variance assumptions. All statistical analyses were performed using SPSS (version 20).

Results

Soil Analyses

Our results partially support our hypothesis that soil conditioning with *O. viciifolia* increases nutrient availability relative to soil conditioning with *A. cristatum* or *B. tectorum*. Available P was significantly influenced by the interaction of soil treatment and conditioning species ($F_{2,39} = 4.3$, $p = 0.02$; Figure 2A). *Bromus tectorum*-conditioned soils had significantly less P compared to *O. viciifolia* and *A. cristatum*-conditioned soils (Figure 2A). Soil treatment and soil treatment interactions with conditioning species significantly influenced pH ($F_{2,39} = 9.7$, $p < 0.01$; Figure 2B).

Sterilization to kill AM fungi reduced the pH of *B. tectorum* and *A. cristatum* conditioned soils and increased the pH in *O. viciifolia*-conditioned soils ($F_{2,39} = 9.7$; $p < 0.01$; Figure 2B). Potassium concentration was significantly influenced by soil treatment ($F_{1,39} = 10.4$, $p < 0.01$) and conditioning species ($F_{2,39} = 6.5$, $p < 0.01$), but not their interaction (Figure 2C). *Onobrychis viciifolia* conditioned soils had higher K content than both *B. tectorum* and *A. cristatum* conditioned soils. Potassium content was lower in heat-treated soil for all three soil conditioning treatments (Figure 2C). The mean nitrate content was highly variable and not significantly different among soil treatments or cultivator species (Figure 2D).

Biomass Measurements

In support of our hypothesis that forbs would exhibit a negative growth response to soil sterilization, soil sterilization reduced the total biomass of *O. viciifolia* ($F_{1,50} = 24.9$, $p < 0.01$), *D. purpurea* ($F_{1,46} = 8.8$, $p < 0.01$), *R. columnifera* ($F_{1,43} = 17.0$, $p < 0.01$), and *R. hirta* ($F_{1,53} = 15.8$, $p < 0.01$; Figure 3). However, *O. viciifolia* and the three native forb species showed no significant biomass response to soil conditioner species. Conversely, soil conditioner species

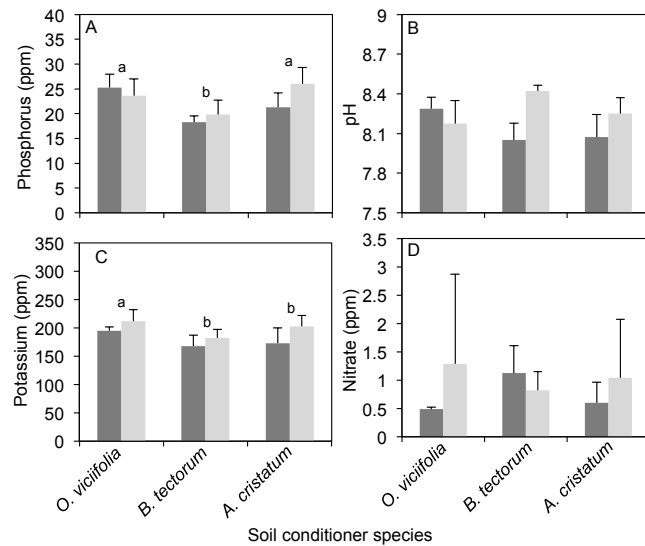


Figure 2. Soil nutrient concentrations after soil preconditioning treatments. Results of two-way ANOVA to evaluate the importance of soil sterilization treatment (ST), soil pre-conditioning species (CS), and interactions between ST and CS. The dark bars indicate the sterilized soil treatment and the light bars the non-sterilized soil treatment. Different letters above bars indicate significant differences ($p < 0.05$) between CS treatments. Error bars indicate standard deviation.

significantly influenced the total biomass of *B. tectorum* ($F_{2,53} = 19.9$, $p < 0.01$) and *A. cristatum* ($F_{2,47} = 10.1$, $p < 0.01$), but neither species was affected by the soil sterilization treatment (Figures 3B and 3C). Both species had greater total biomass in soils conditioned by *O. viciifolia* compared with soils conditioned by *B. tectorum* and *A. cristatum* (Figures 3B and 3C). The total biomass of *B. tectorum* was significantly lower in *B. tectorum*-conditioned soils (Figure 3B). Interactions between soil treatment and soil conditioner species influenced *A. cristatum* total biomass ($F_{2,47} = 5.5$, $p < 0.01$) (Figure 3C). *Bromus tectorum* and *A. cristatum* produced the greatest total biomass in sterilized soils conditioned by *O. viciifolia* and the least biomass in sterilized soils conditioned by *B. tectorum* (Figures 3B and 3C).

We hypothesized that soil conditioning would influence how plants allocated biomass. *Onobrychis viciifolia* root-shoot ratio was significantly influenced by conditioning species ($F_{2,50} = 4.0$, $p = 0.03$). *Onobrychis viciifolia* allocated significantly more biomass to shoot tissue when grown in *O. viciifolia* conditioned soil, and more biomass to root tissue when grown in *B. tectorum* or *A. cristatum* conditioned soils (Figure 4A). *Bromus tectorum* root-shoot ratio was also influenced by conditioning species ($F_{2,53} = 4.2$, $p = 0.02$). *Bromus tectorum* allocated significantly more biomass to shoots when grown in *B. tectorum*-conditioned soil (Figure 4B). *Agropyron cristatum* and *D. purpurea* showed no significant differences in tissue allocation between treatments (Figure 4C and 4D). Conditioning species influenced

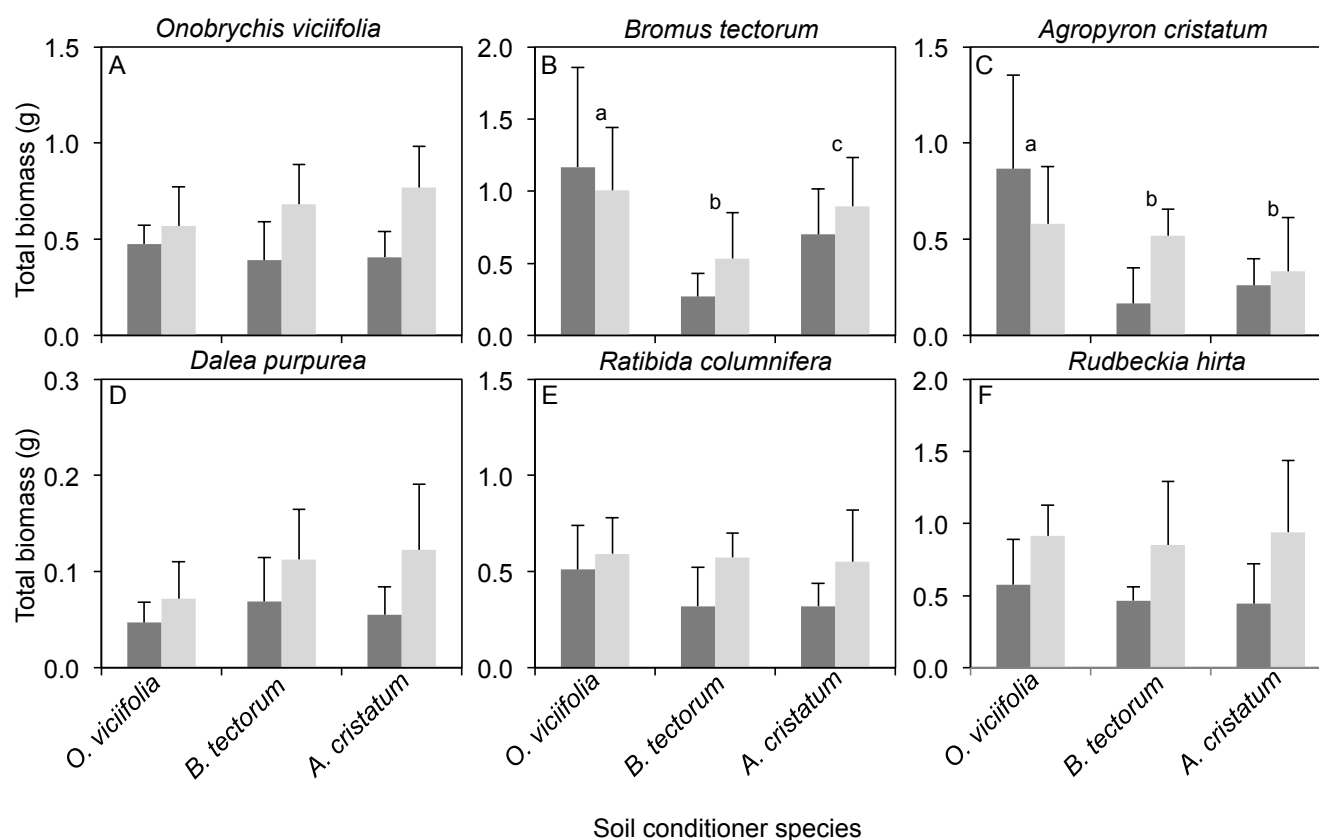


Figure 3. Average total biomass of bioassay plants. Results of two-way ANOVA to evaluate the importance of soil sterilization treatment (ST), soil pre-conditioning species (CS) and interactions between ST and CS. Different letters above bars indicate significant differences ($p < 0.05$) between CS treatments. The dark bars indicate the sterilized soil treatment and the light bars the non-sterilized soil treatment. Error bars indicate standard deviation.

the root-shoot ratio of *R. columnifera* ($F_{2,43} = 5.8, p < 0.01$) and *R. hirta* ($F_{1,53} = 5.8, p < 0.01$). Both species had the lowest root-shoot biomass ratio in *O. viciifolia*-conditioned soils and the highest in *A. cristatum*-conditioned soils (Figure 4E, 4F). Overall, native forb root-shoot ratio in non-sterilized soils was lowest in *O. viciifolia*-conditioned soils and highest in *A. cristatum*-conditioned soils ($F_{2,154} = 3.93; p = 0.024$).

Fungal Endophyte Colonization

AM fungi root colonization averaged 3.7% (s.d. = 3.4) in sterilized soils and 49.2% (s.d. = 20.7) in soils that were not sterilized. Soil sterilization reduced AM fungi (> 90% reduction) more than non-AM fungi (60% reduction). Sterilized soil pots in which AM fungi root colonization was above 5% were considered contaminated and excluded from further analyses.

We hypothesized that soils conditioned with *O. viciifolia* would support increased AM fungi abundance relative to *A. cristatum* and *B. tectorum*. Although *O. viciifolia* and *A. cristatum* trended towards greater AM fungi colonization when grown in soils conditioned by *O. viciifolia*, AM fungi colonization didn't differ significantly between treatments for the soil conditioning species (*O. viciifolia*, *B. tectorum* and *A. cristatum*) during the bioassay phase

(Figure 5A, 5B and 5C). AM fungi colonization of native forbs was greater in *O. viciifolia* preconditioned soils than in *B. tectorum* or *A. cristatum* preconditioned soils ($F_{2,48} = 4.8, p = 0.013$; Figure 6).

Soil conditioning significantly influenced non-AM fungi root colonization of *B. tectorum* ($F_{2,27} = 7.3, p < 0.01$) and *A. cristatum* ($F_{2,27} = 8.6, p < 0.01$). *Bromus tectorum* and *A. cristatum* had greater non-AM fungi root colonization when grown in *B. tectorum* and *A. cristatum* conditioned soils (Figures 5E and 5F). *Onobrychis viciifolia* non-AM fungi root colonization was highly variable in *B. tectorum* and *A. cristatum* conditioned soils and not significantly different between soil conditioning treatments (Figure 5D). Native forb, non-AM fungi root colonization was influenced by bioassay species ($F_{2,48} = 4.9, p = 0.012$) and soil conditioning species ($F_{2,48} = 10.2, p < 0.01$). Native forb, non-AM fungi colonization was lowest for plants grown in *O. viciifolia* preconditioned soils (Figure 6).

Discussion

Agropyron cristatum stands can have decreased soil nutrient availability relative to native plant communities (Lesica and Deluca 1996, Christian and Wilson 1999). We hypothesized that soils conditioned by *O. viciifolia* would have

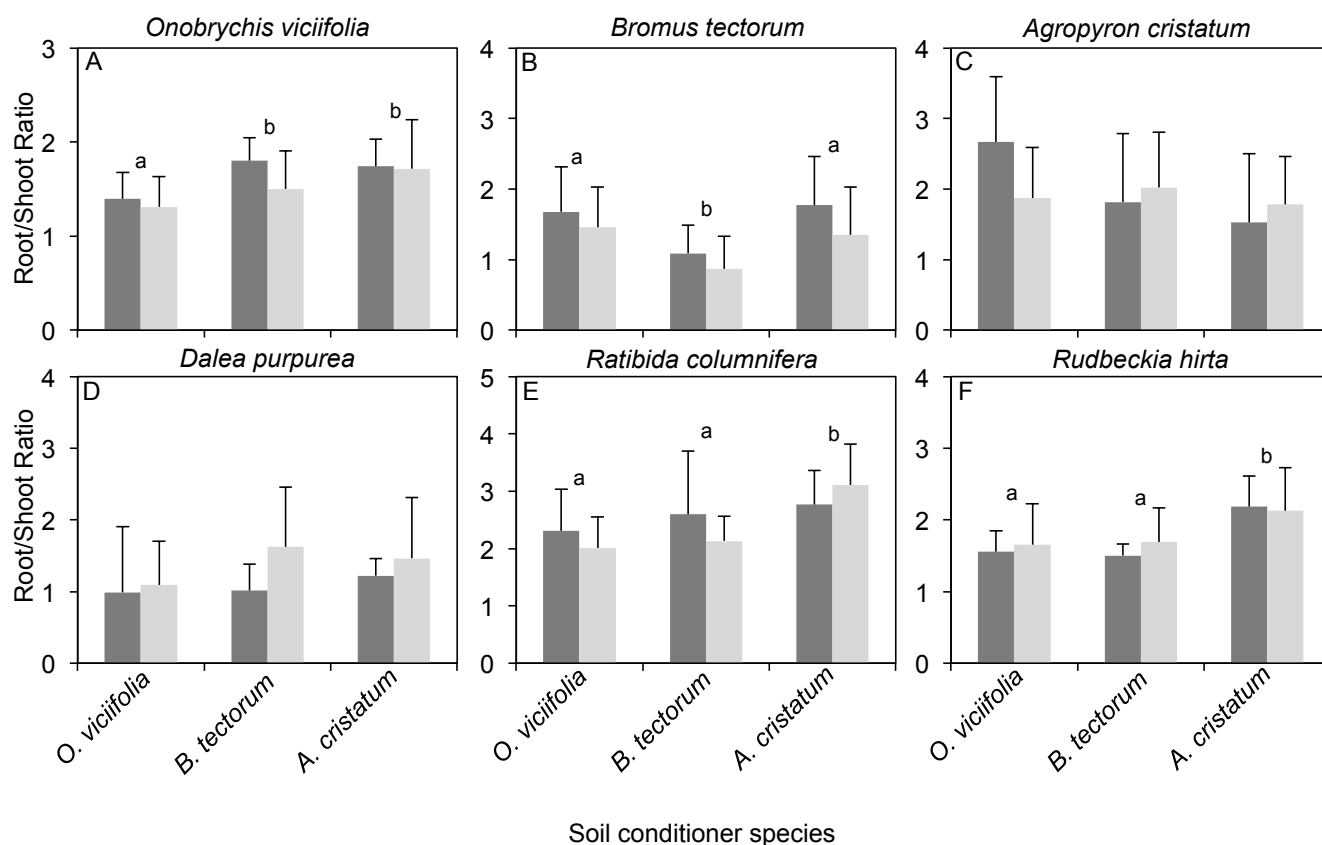


Figure 4. The average root-shoot ratio of bioassay plants. Results of two-way analysis to evaluate the importance of soil sterilization treatment (ST), soil pre-conditioning species (CS), and interactions between ST and CS. Different letters above bars indicate significant differences ($p < 0.05$) between CS treatments. The dark bars indicate the sterilized soil treatment and the light bars the non-sterilized soil treatment. Error bars indicate standard deviation.

increased nutrient availability relative to soils conditioned by *A. cristatum* or *B. tectorum*. *Onobrychis viciifolia* facilitated small but significant soil nutrient increases. Phosphorus availability increased in soils preconditioned by *O. viciifolia* relative to *B. tectorum*. Potassium availability was greater in *O. viciifolia* conditioned soils compared with *B. tectorum* and *A. cristatum* conditioned soils. We expected increased nitrogen availability in *O. viciifolia* conditioned soils. Although we observed nodulation of *O. viciifolia* roots, indicating the presence of nitrogen-fixing bacteria, nitrate concentrations in *O. viciifolia* conditioned soils were highly variable and not significantly greater than those of *A. cristatum* or *B. tectorum* preconditioned soils.

Our hypothesis that native forb biomass would be greater for plants grown in *O. viciifolia*-conditioned soils was not supported by the data. Native forb total biomass did not differ between treatments. However, as hypothesized, forbs growing in *O. viciifolia*-conditioned soils allocated fewer resources to roots relative to shoots. Plants respond to their environment by altering resource allocation to optimize resource use. When N and P availability are low, increased root growth allows plants better access to limiting soil resources. When N or P availability increases, more resources are allocated to shoots to provide greater photosynthetic area (Marschner et al. 1996, Andrews et al.

1999, Hermans et al. 2006, Poorter et al. 2012). Increased resource allocation to shoots in *O. viciifolia*-conditioned soils suggests a more favorable nutrient status for forb growth. Whether or not this translates into greater survivorship under field conditions is uncertain. Increased resource allocation to roots by young plants could lead to increased biomass and survival over the long term under field conditions, where plants experience drought and herbivory.

Similar to increased nutrient availability, AM fungi root colonization, by increasing the host plants ability to acquire P and other nutrients (Smith et al. 2011), can decrease the root-shoot ratio (Veresoglou et al. 2012). Our results support the hypothesis that soil conditioning by *O. viciifolia* increases AM fungi abundance. Native forb species had greater AM fungi root colonization when grown in *O. viciifolia*-conditioned soils. However, our results provide little evidence that differences in AM fungi abundance influenced plant biomass. Although soil steaming was effective for killing AM fungi, it influenced soil nutrients, pH, and non-AM fungal communities, all of which influence plant growth, complicating estimation of AM fungi influences on plant growth characteristics. However, differences in AM fungal abundance may have contributed to differences in the way plants growing in untreated soils

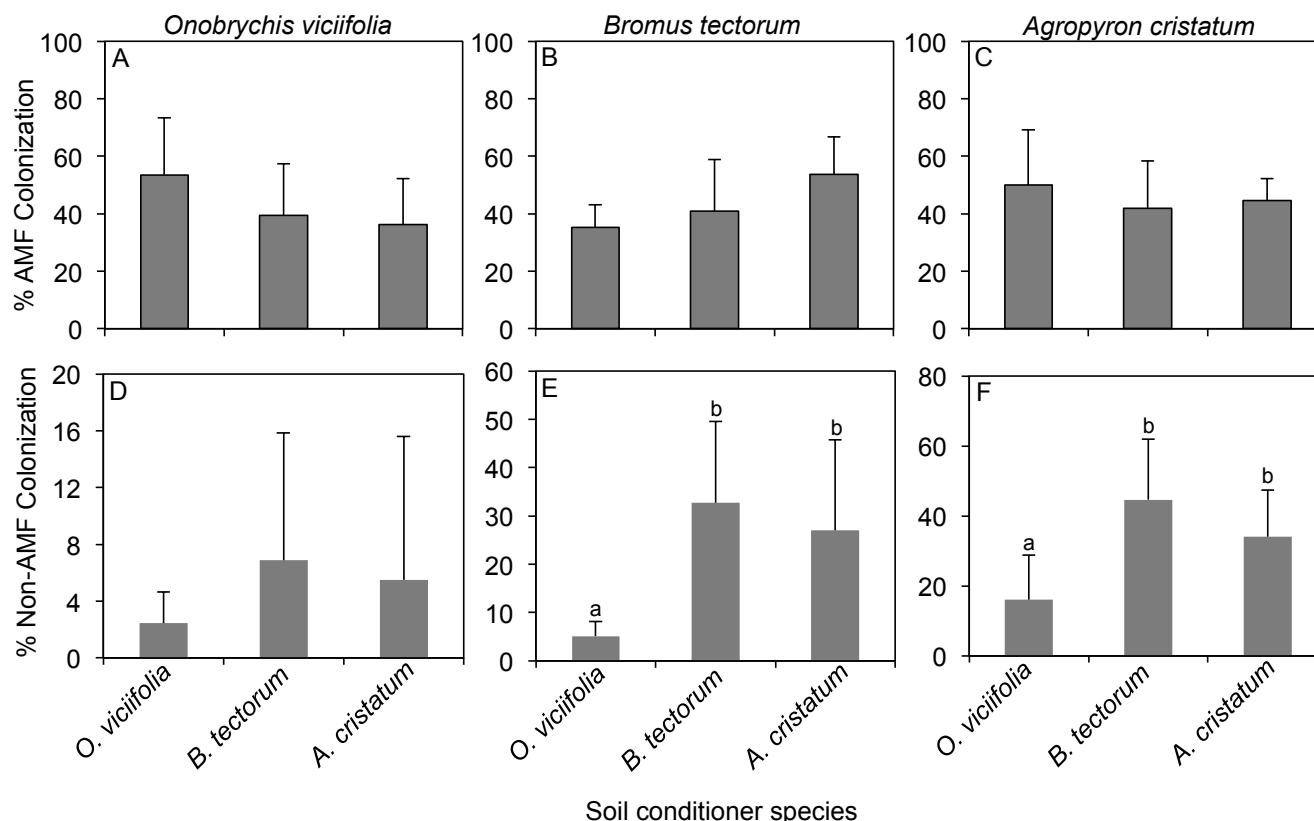


Figure 5. Average percent AM fungi and non-AM fungi root colonization of *O. viciifolia*, *B. tectorum* and *A. cristatum*. Treatments with different letters differ significantly ($p < 0.05$).

allocated resources. AM fungi are known to increase water use efficiency and may help alleviate drought stress (Boyer et al. 2014), potentially increasing forb growth and survival under field conditions.

Plant functional groups differ in AM fungi colonization rates and growth response to AM fungi. C3 grasses, such as *A. cristatum* and *B. tectorum*, are thought to be the poorest AM fungi hosts and forbs and C4 grasses the best (Bunn et al. 2015). Late-successional plant species are generally more responsive to AM fungi than are early-successional species (Middleton and Bever 2012, Koziol and Bever 2015). Low AM fungal abundance and diversity in *A. cristatum* stands may slow succession toward highly valued but difficult to establish, late-successional species. Soil disturbances (i.e., harrowing, seedbed preparation, seeding) and elimination of AM fungi host plants decreases AM fungal abundance, potentially favoring early-successional species and weeds (Koziol and Bever 2015). AM fungi community composition, as well as abundance, determines AM fungi community function (Johnson et al. 1997). *Agropyron cristatum* stands have low AM fungi-host diversity and relatively uniform edaphic conditions that may act as habitat filters, decreasing AM fungal diversity (Hiiesalu et al. 2014). Although analysis of AM fungal community composition is beyond the scope of this study, how bridge species alter AM fungal species abundance relationships (i.e., increase low abundance species relative to grass-enriched species)

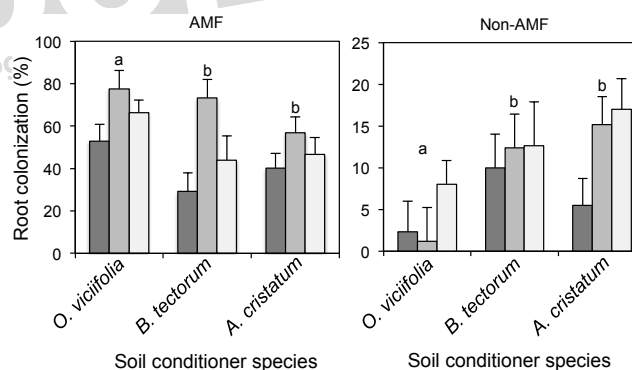


Figure 6. Average percent AM and non-AM fungi root colonization of native forbs. Dark bars indicate *R. hirta*, intermediate bars indicate *D. purpurea*, and light bars *R. columnifera*. Treatments with different letters differ significantly ($p < 0.05$).

may be as important to plant interactions as changes to AM fungi community abundance.

Vascular plants host a great variety of fungi besides AM fungi. Similar to AM fungi, non-AM fungal root endophytes can be mutualistic or parasitic (Schulz and Boyle 2005). Unlike AM fungi, non-AM fungi can also be pathogens. *Agropyron cristatum* and *B. tectorum* had significantly more non-AM fungi root colonization, but less plant biomass, when grown in soils preconditioned

by *A. cristatum* or *B. tectorum*. It is well established that species-specific pathogens can build up in low diversity plant communities. For example, pathogen enrichment necessitates agricultural crop rotations to maintain yields (van der Putten et al. 2013). It is also clear that closely related plant species, or species within the same functional group (i.e., grasses or forbs), are more likely to share the same pathogens (Petermann et al. 2008).

Direct competition is assumed to be responsible for *A. cristatum* stand resistance to *B. tectorum* invasion. Our results suggest the possibility that negative soil feedbacks created by *A. cristatum* may impede grass establishment and contribute to its invasion resistance. Negative soil feedbacks may also decrease self-recruitment. *Agropyron cristatum* establishment between persistent seeded rows can be low even in 50 year old stands (Anderson and Marlette 1986).

Although both exotic grass species examined exhibited enhanced growth in *O. viciifolia*-conditioned soils, *O. viciifolia*'s intrinsic glyphosate tolerance provides managers with a way to control grass seedlings and reduce the weed seedbank before native species are planted. Although we didn't evaluate the effects of soil preconditioning on native grass species growth, *O. viciifolia* may reduce negative soil feedbacks on native, as well as exotic, grasses. Additional research is needed to examine the potential for *O. viciifolia* to reduce negative feedbacks on native grass species establishment.

Non-AM fungal colonization was highest for native forbs grown in *A. cristatum* or *B. tectorum*-conditioned soils, indicating that non-AM fungal species enriched during soil conditioning by grasses infected all plant functional groups examined to different extents. Increased AM fungi abundance in *O. viciifolia*-conditioned soils may have an antagonistic effect on non-AM root colonizing fungal species. Wehner et al. (2011) found that non-AM fungal root colonization decreased with increased AM fungi root colonization. Rillig et al. (2014) demonstrated that AM and non-AM fungal root endophytes interact to influence plant community composition, suggesting that these relationships can be manipulated to improve restoration outcomes.

Onobrychis viciifolia grew equally well in soils preconditioned by itself, *A. cristatum*, and *B. tectorum*. This suggests that it is a good pioneer species able to thrive in soils conditioned by either *A. cristatum* or *B. tectorum*. Although *O. viciifolia* soil preconditioning did not alter native forb total biomass under greenhouse conditions, alteration of nutrients and fungal communities would be expected to influence plant community dynamics in the field. In addition to altering soil conditions, established plants can ameliorate harsh environmental conditions for seedling establishment (Padilla and Pugnaire 2006). Future research will examine how *O. viciifolia* and other potential bridge species influence microclimate conditions, soil microbial

communities and native species community assembly under field conditions.

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