Suppression of annual *Bromus tectorum* by perennial *Agropyron cristatum*: roles of soil nitrogen availability and biological soil space

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Abstract. Worldwide, exotic invasive grasses have caused numerous ecosystem perturbations. Rangelands of the western USA have experienced increases in the size and frequency of wildfires largely due to invasion by the annual grass *Bromus tectorum*. Rehabilitation of invaded rangelands is difficult; but long-term success is predicated on establishing healthy and dense perennial grass communities, which suppress *B. tectorum*. This paper reports on two experiments to increase our understanding of soil factors involved in suppression. Water was not limiting in this study. Growth of *B. tectorum* in soil conditioned by and competing with the exotic perennial *Agropyron cristatum* was far less relative to its growth without competition. When competing with *A. cristatum*, replacing a portion of conditioned soil with fresh soil before sowing of *B. tectorum* did not significantly increase its growth. The ability of conditioned soil to suppress *B. tectorum* growth was lost when it was separated from growing *A. cristatum*. Soil that suppressed *B. tectorum* growth was characterized by low mineral nitrogen (N) availability and a high molar ratio of NO₂⁻ in the solution-phase pool of NO₂⁻ + NO₃⁻. Moreover, resin availability of NO₂⁻ + NO₃⁻ explained 66% of the variability in *B. tectorum* above-ground mass, attesting to the importance of *A. cristatum* growth in reducing N availability to *B. tectorum*. Trials in which *B. tectorum* was suppressed the most were characterized by very high shoot/root mass ratios and roots that have less root hair growth relative to non-suppressed counterparts, suggesting co-opting of biological soil space by the perennial grass as another suppressive mechanism. Greater understanding of the role of biological soil space could be used to breed and select plant materials with traits that are more suppressive to invasive annual grasses.

Keywords: Plant–soil relationships; root competition.

Introduction


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In the intermountain region of the western USA, the Eurasian annual grass *Bromus tectorum* is responsible for landscape-level conversion of native *Artemisia* spp. ecosystems to annual grass dominance (Mack 1981; D’Antonio and Vitousek 1992; Billings 1994; Knapp 1996). The major pathway by which *B. tectorum* assumes dominance is by first occupying safesites within the community (often facilitated by disturbance) and expanding from those sites to a critical density and biomass, whereby conditions for large-scale wildfires are promulgated. Following the wildfire, *B. tectorum* readily dominates the site due to lack of competition, its inherently high growth rate, prolific seed production and ability to rapidly utilize post-fire elevated available nutrients (Fig. 1A; Mack 1981; Knapp 1996). Native species recruiting post-wildfire, including perennial grasses, find it difficult to compete against *B. tectorum* from the seedling stage (Francis and Pyke 1996; Arredondo et al. 1998; Brooks 2003; Humphrey and Schupp 2004; Blank 2010).

Fortunately, some plant communities resist invasion by *B. tectorum*; they are able to suppress its growth (James et al. 2008; Blank and Morgan 2012a; Chambers et al. 2014). Common threads to this resistance/suppression are well-established, healthy, and properly-spaced populations of perennial grasses (Fig. 1B–D, Humphrey and Schupp 2004; McGlone et al. 2011). Understanding how soil biochemical attributes affect suppression and how these attributes interact with a perennial grass offers hope of greater success in rehabilitating exotic annual grass-degraded ecosystems (D’Antonio and Thomsen 2004). The nature of suppression is complex and involves biotic and abiotic processes that temporally interact with soil type, the array of plant communities and characteristics of the invasive species (Huenneke et al. 1990; Tilman 1997; Naeem et al. 2000; Gundale et al. 2008). Established perennial plants can simply reduce soil resources to levels below which annual grasses are no longer as competitive (Wedin and Tilman 1990; Claassen and Marler 1998; Prober and Lunt 2009). Suppression of annual grasses may also involve root competition other than nutrient depletion whence perennial roots simply occupy biological soil space and, through chemical signalling, allelopathy included, may forestall competing roots from entering their space (Monk and Gabrielson 1985; McConnaughay and Bazzaz 1992; Schenk 2006).

![Figure 1.](https://academic.oup.com/aobpla/article-abstract/doi/10.1093/aobpla/plv006/199314)
In a previous study, we explored the mechanistic underpinnings of perennial grass suppression of *B. tectorum* (Blank and Morgan 2012b). The data suggest that perennial grass roots reduced soil nitrogen (N) and phosphorous (P) availability and occupied biological soil space, thereby reducing *B. tectorum* growth. This paper reports on additional experiments to more definitively elucidate soil factors involved in suppression of *B. tectorum*. The perennial grass used was *Agropyron cristatum*, a native to Russia and central Asia. This grass is often used to rehabilitate degraded rangelands in the western USA, and well-established stands effectively suppress *B. tectorum* (Evans and Young 1978; Wicks 1997). Two hypotheses were tested: (i) soil conditioning brought about by established *A. cristatum* will reduce availability of soil mineral N and P to levels low enough to significantly reduce growth of *B. tectorum*, and (ii) occupation of biological soil space by roots of *A. cristatum* will cause roots of *B. tectorum* to alter their architecture, morphology and activity resulting in reduced growth, i.e. suppression.

**Methods**

Two experiments were conducted in a greenhouse at Reno, NV, USA (39° 32′ 17.20″ N; 119° 48′ 22.89″ W). Prior to each experiment, soil substrate was freshly collected from a *Krascheninnikovia lanata* (winterfat) site, invaded by *B. tectorum* for about 12 years, ~80 km northwest of Reno, NV, USA (40° 7′ 59.43″ N; 120° 6′ 56.18″ W). This soil, conditioned by *B. tectorum*, has elevated soil N availability relative to nearby soil conditioned by native vegetation (Blank and Morgan 2013). Surface soils (0–25 cm, corresponding to the A horizon) were composited from an area of ~10 m². Soils, loamy sand in texture, were sieved to <4 mm to remove coarse fragments and medium-to-large roots and homogenized by hand mixing on a greenhouse bench. Four replicates of this soil were analysed for various attributes (see below). This original soil—referred to as fresh soil—is taken from a soil classified as a coarse-loamy, mixed, superactive, calcarous, mesic Typic Torriorthent.

Experiment 1 quantified the suppression of *B. tectorum* (cheatgrass) by established *A. cristatum* (crested wheatgrass). Twelve replicate clear plastic rhizotrons, 5 × 30 × 100 cm depth, were filled with equal volumes of soil. The outsides of the rhizotrons were covered with insulation that could be removed from the back to observe rooting patterns. Prior to seed planting, rhizotrons were paired in adjoining plastic containers to maintain a slight angle so that roots would readily intercept the clear rhizotron backing for observation, and deionized water was added to reach field capacity—~6% by weight for the soils used—over the entire rhizotron. Two seeds of *A. cristatum* were sown in the rhizotrons 6 cm from each edge to leave an 18 cm space in between for later planting of *B. tectorum*, and allowed to establish for 68 days, the conditioning phase. We define conditioning as the plant species-dependent engendering specific traits such as carbon flow, root exudation, nutrient uptake, root occupancy of soil space, alteration of the soil microbial community etc. that might affect competitive interactions. During establishment, *A. cristatum* was supplemented with 500–1000 mL of deionized water per week depending on depletion in the rhizotron as gauged by visual inspection when opaque backs were removed. After establishment, four treatments were randomly imposed to three replicate rhizotrons. In one treatment, *B. tectorum* was sown directly between *A. cristatum* in the conditioned soil as a test for maximal suppression. For the next treatment, 500 g of soil were removed from between the established *A. cristatum* plants, replaced with 500 g of fresh soil and *B. tectorum* sown in the new soil. The purpose of this treatment was to test how fresh soil mitigated against suppression. In another treatment, 500 g of conditioned soil were removed from between the established *A. cristatum* plants; then a nylon mesh (2-mm opening) was placed in the excavated area, 500 g of fresh soil were placed over the mesh and *B. tectorum* sown. Our purpose was to examine how reduced root movement into the fresh soil from *A. cristatum*, but still allowing diffusion of gases, solutes and microbes from the adjacent conditioned soil, affects suppression. For the last treatment, 500 g of soil were removed from between the established *A. cristatum* plants; then a plastic barrier was placed in the hole and filled with 500 g of fresh soil, and sown to *B. tectorum*. This treatment tested *B. tectorum* suppression upon total blocking of *A. cristatum* roots, which would affect biological soil space and exposure to potential pathogenic organisms and allelochemicals in soil conditioned by *A. cristatum*. Deionized water was immediately applied to soil above the newly sown seeds of *B. tectorum*. A small sub-sample of the homogenized 500 g conditioned soil was analysed for several soil attributes (see below). We also grew *B. tectorum* without competition in small containers filled with 500 g of either fresh soil or conditioned soil (from the soil excavated from rhizotrons). During growth of *B. tectorum*, rhizotrons and containers were watered twice weekly; water was not limiting to *B. tectorum* in this study. Supplemental lighting, using four high-pressure sodium lamps each producing 124 000 lumens at 2100 K temperature, was used to assure at least 12 h of daylight. After 70 days of growth, *B. tectorum* was clipped at the soil surface, dried for 48 h at 70 °C and weight recorded. Soil within the rooting zone of *B. tectorum* was excavated and roots reserved. Subsamples of roots from each treatment and replicates were
washed, immediately observed with a light microscope and photographs taken. These subsamples were then added to the original sample, dried for 48 h at 70 °C and weight recorded. After harvest, soil within the rooting zone of *B. tectorum* of each treatment was homogenized and analysed for solution-phase anions (Cl⁻, NO₂⁻, NO₃⁻, SO₄²⁻ and ortho-P) using immiscible displacement (Mubarak and Olsen 1976) with quantification by ion chromatography (Dionex® AS11-HC column with gradient elution) and mineral N, defined as NH₄⁺ + NO₂⁻ + NO₃⁻, by 1.5 M KCl extraction (Keeney and Nelson 1982).

Experiment 2 explored the role of soil nutrient availability of N and P in the suppression process and tested to a greater extent if and how conditioned soil affects suppression. Twelve rhizotrons were filled with freshly collected soil and planted to *A. cristatum* (see Experiment 1). Soil was conditioned by *A. cristatum* for 64 days with lighting and watering as described for Experiment 1. Four treatments were imposed following conditioning by *A. cristatum*. In four randomly chosen rhizotrons, *B. tectorum* was sown directly between established *A. cristatum* to test for maximal suppression (Treatment 1). The remaining eight rhizotrons had their backs removed and soil was separated by depths (0–30 cm (Treatment 2), 30–60 cm (Treatment 3) and 60–90 cm (Treatment 4)), and homogenized along with any roots present. For each soil depth separate, 2500 g was placed in containers and *B. tectorum* immediately sown. These treatments tested the suppressive ability of conditioned soil, by depth, without live plants of *A. cristatum*, but with different amounts of now inactive roots present depending on soil depth, greatest in the 0–30 cm depth increment and least in the 60–90 cm depth increment. In similar-sized containers, *B. tectorum* was sown in fresh soil to serve as unsuppressed controls (six replicates). To gauge the influence of *B. tectorum* growth on post-harvest soil attributes, five replicates of unplanted controls in fresh soil were prepared in similar-sized containers. For all experimental units, one anion and cation exchange resin capsule (Unibest®) was placed at 15 cm directly beneath where *B. tectorum* was sown to gauge nutrient availability. After 64 days of growth, above-ground and root biomass of *B. tectorum* were harvested, dried and weighed. Resin capsules were removed, washed extensively with deionized water, dried and treated with 40 mL of 1 N HCl and shaken for 30 min. Resin availability of NH₄⁺, NO₂⁻ + NO₃⁻ and ortho-P were quantified using a Lachat® autoanalyser. Soil in the rooting zone of *B. tectorum* was homogenized and analysed for mineral N and soil-solution anions as stated in Experiment 1. Availability of micronutrients was determined using the DTPA method (Lindsay and Norvell 1978).

Nitrogen mineralization potential was quantified using a moist 30-day incubation procedure (Bundy and Meisinger 1994). Total soil C and N were quantified using a LECO Truspec® analyser.

The data structure for Experiment 1 includes eight treatments with replication for a total of 38 experimental units. Experiment 2 had 10 treatments with replication for a total of 43 experimental units. For each experiment, a separate ANOVA was performed and means separated using Tukey’s honest significant difference test. A backward selective regression was used to identify variables possibly related to above-ground *B. tectorum* biomass. The procedure was applied separately to Experiments 1 and 2 and to the combined data set.

**Results**

**Experiment 1**

Competition against established *A. cristatum*, in either conditioned or fresh soil, significantly reduced above-ground biomass of *B. tectorum*, relative to its growth in fresh soil without competition (Fig. 2). Growth of *B. tectorum* improved using fresh soil above a mesh, but not significantly so, relative to its growth competing with *A. cristatum* in either fresh or conditioned soil. Above-ground biomass of *B. tectorum* was far greater when fresh soil was placed in a plastic barrier between established *A. cristatum*. When competing with *A. cristatum*, *B. tectorum* was marked by very high shoot/root mass ratios relative to its ratios when not in competition (Fig. 2). The most suppressed plants of *B. tectorum* were characterized by minimal root branching and some consisted of one very long root. In the most suppressed *B. tectorum* trials, roots had fewer and shorter root hairs based upon microscopic inspection.

Nutrient attributes quantified for Experiment 1 differed significantly among treatments (Table 1). Soil mineral N content was greatest in the fresh soil (0.450 mmol kg⁻¹) and did not decline significantly after conditioning by *A. cristatum* (0.332 mmol kg⁻¹). Following harvest of *B. tectorum*, soil in its rooting zone of all experimental units had significantly less mineral N than the fresh soil; notable was the far lower mineral N remaining after plant growth in its rooting zone when competed with *A. cristatum*. The molar proportion of NO₂⁻ in the solution-phase NO₂⁻ + NO₃⁻ pool varied widely among treatments. Notable are the very high values in trials where *A. cristatum* competed against *B. tectorum*, with exception of the plastic barrier treatment. Solution-phase ortho-P was less variable among treatments than mineral N, and plant growth facilitated elevated P values relative to fresh soil. Using a backwards regression variable-selection
procedure, root mass explained 88% of above-ground biomass; but no measured soil nutrient attributes significantly predicted above-ground biomass of *B. tectorum*.

**Experiment 2**

Similar to Experiment 1, established *A. cristatum* suppressed the growth of *B. tectorum* relative to its

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**Table 1.** Selected soil attributes for Experiment 1.1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mineral N (mmol kg⁻¹)</th>
<th>Mole NO₂⁻ (%)</th>
<th>Ortho-P (μmol L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freshly collected field soil</td>
<td>0.450⁶</td>
<td>49BC</td>
<td>20.6⁸</td>
</tr>
<tr>
<td>Conditioned soil prior to sowing <em>B. tectorum</em>²</td>
<td>0.332⁴AB</td>
<td>16C</td>
<td>30.8⁴A</td>
</tr>
<tr>
<td>Conditioned soil post-harvest <em>B. tectorum</em>³</td>
<td>0.058⁴C</td>
<td>81AB</td>
<td>20.1¹AB</td>
</tr>
<tr>
<td>Fresh soil post-harvest <em>B. tectorum</em>⁴</td>
<td>0.048⁴C</td>
<td>72AB</td>
<td>29.1⁴AB</td>
</tr>
<tr>
<td>Fresh soil above mesh post-harvest <em>B. tectorum</em>⁴</td>
<td>0.059⁴C</td>
<td>95A</td>
<td>27.3³AB</td>
</tr>
<tr>
<td>Fresh soil above plastic barrier post-harvest <em>B. tectorum</em>⁵</td>
<td>0.064⁴C</td>
<td>46BC</td>
<td>29.5³AB</td>
</tr>
<tr>
<td>Fresh soil post-harvest <em>B. tectorum</em> without competition⁵</td>
<td>0.228⁴BC</td>
<td>10⁴C</td>
<td>34.6⁴A</td>
</tr>
<tr>
<td>Conditioned soil post-harvest <em>B. tectorum</em> without competition⁵</td>
<td>0.154⁴C</td>
<td>7⁴C</td>
<td>34.9⁴A</td>
</tr>
<tr>
<td>ANOVA</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

¹For each column, means with different superscripted letters are significantly different at the ≤0.05 level; mineral N is total NH₄⁺ + NO₂⁻ + NO₃⁻ extractable by KCl; mole NO₂⁻ is the molar proportion of NO₂⁻ in the solution-phase pool of NO₂⁻ + NO₃⁻. Attributes unaffected by treatment included solution-phase NO₂⁻, NO₃⁻ and SO₄²⁻.

²Soil from a homogenized subsample taken between *A. cristatum* that established for 60 days in rhizotrons.

³Soil from a homogenized subsample taken from the rooting zone of *B. tectorum* in competition with *A. cristatum* in rhizotrons.

⁴Soil from homogenized subsamples of the fresh soil and the fresh soil placed above the mesh or plastic barrier in rhizotrons.

⁵Soil from a homogenized subsample of entire container.

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**Figure 2.** Above-ground biomass and shoot/root mass ratios of *B. tectorum* plants following harvest of Experiments 1 and 2. For each panel, ANOVA results are provided and bars with non-overlapping letters are significantly different at the ≤0.05 level.
non-competed growth in fresh or conditioned soil (Fig. 1). Suppressed B. tectorum, akin to Experiment 1, had very high shoot to root mass ratios. Growth of B. tectorum was not nearly as suppressed when grown in conditioned soil taken from three depths in rhizotrons planted to A. cristatum (Fig. 1). Moreover, shoot to root mass ratios of B. tectorum grown non-competed in this conditioned soil were similar to those of non-competed B. tectorum grown in fresh soil (Experiment 1).

Following harvest of B. tectorum, mineral N and resin availability of NO₂⁻ + NO₃⁻ were greatest in unplanted controls (Table 2). Relative to all other treatments, mineral N was by far lowest (0.026 mmol kg⁻¹) in the 0–30 cm depth increment when competed with A. cristatum in the rhizotrons. After conditioning of soils by A. cristatum growth, mineral N was not significantly reduced relative to fresh soil. Moreover, following the harvest of B. tectorum not competing with A. cristatum, soil mineral N was not significantly reduced relative to fresh soil. Resin availability of NO₂⁻ + NO₃⁻ mirrored mineral N data with the unplanted controls having the greatest resin availability and the competed rhizotron values (placed at 15 cm) the least. The molar proportion of NO₂⁻ in the solution-phase NO₂⁻ + NO₃⁻ pool was by far greatest in the conditioned soil sown to B. tectorum treatment. In general, plant growth, be it A. cristatum or B. tectorum, facilitated an increase in soil-solution ortho-P relative to fresh soil. Soil-solution ortho-P values were quite similar among the treatments with plant growth and only solution ortho-P of the fresh soil was significantly less (Table 2). There were no significant differences among the samples measured in resin availability of P (Table 2). Micronutrient availability of Zn did not vary much among treatments with the only significant difference between the fresh soil and the non-competed conditioned soil from the 30–60 cm depth increment. Manganese availability differed considerably among treatments with the highest values occurring in the rhizotron soils at depths of 30–60 and 60–90 cm and the lowest values in the fresh soil, the unplanted control soils and the non-competed soils. Using a backwards regression variable-selection procedure, applied to only data set 2, a combination of root biomass, resin-available NO₂⁻ + NO₃⁻, and solution-phase NO₃⁻ explained 94% of above-ground biomass variability. With combined data sets, root biomass and solution-phase NO₃⁻ explained 87% of the variability in B. tectorum above-ground biomass (Fig. 2).

Discussion
We partially accept hypothesis 1 that established A. cristatum will reduce availability of soil mineral N and P to levels low enough to suppress growth of B. tectorum. In regard to availability of soil P, there simply is no evidence from our data that established A. cristatum has reduced its availability sufficiently to suppress B. tectorum (Tables 1 and 2). We do accept the hypothesis that established A. cristatum has reduced the availability of soil N and thereby suppressed B. tectorum. Firstly, in both experiments, B. tectorum competing against established A. cristatum was significantly suppressed relative to its growth un-competed (Fig. 2). Secondly, following harvest of B. tectorum, mineral N was far less in soils with established A. cristatum relative to soil in non-competed trials (Tables 1 and 2). Thirdly, for Experiment 2, 66% of the variability in above-ground biomass of B. tectorum is explained by resin availability of NO₂⁻ + NO₃⁻ (Fig. 3). Finally, solution-phase NO₃⁻ was a significant variable in predicting above-ground mass of B. tectorum in the combined data set (Fig. 3).

It is not surprising that lowered soil N availability, due to established A. cristatum, would suppress the growth of B. tectorum. Many annual grasses, including B. tectorum, are nitrophiles and their growth is stimulated by additions of mineral N (Hueneke et al. 1990; Brooks 2003; Vasquez et al. 2008). Conversely, growth of annual grasses are often suppressed when mineral N is lowered by manipulating solution culture (Muller and Garnier 1990) or by addition of labile C sources that immobilize soil N (McLendon and Redente 1992; Young et al. 1998; Blank and Young 2009).

Besides availability of N, other aspects of the soil N cycle may be involved, at least tangentially, in suppression of B. tectorum. Soil conditioned by A. cristatum and competing with B. tectorum had very high molar proportions of NO₃⁻ in the solution-phase NO₂⁻ + NO₃⁻ pool (Tables 1 and 2). Moreover, that soils only conditioned by A. cristatum prior to sowing B. tectorum had far lower molar NO₃⁻ levels suggests that the grasses interact with the soil differently when combined than they do individually. Perennial grasses differentially affect soil N cycling (Wedin and Tilman 1990; Vinton and Burke 1995), but we are unaware of any literature that tested the combined effect of a perennial grass and an annual grass on the soil N cycle. Roots of grasses can inhibit nitrite-oxidizers (Munro 1966); but why then did the greatest molar content occur only upon growth of B. tectorum. Bromus tectorum has high affinity to uptake N in the NO₃⁻–N form relative the NH₄⁺–N form (MacKown et al. 2009), but we are unaware of any data on its ability to uptake the NO₂⁻–N form. If B. tectorum does not have a high affinity to uptake the NO₂⁻–N form, then perennial grasses that inhibit nitrate-oxidizers would likely elevated their suppressive ability.
Table 2. Selected soil attributes for Experiment 2.1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mineral N (mmol kg$^{-1}$)</th>
<th>Resin N (μmol)</th>
<th>Mole NO$_2$ (%)</th>
<th>Solution P (μmol L$^{-1}$)</th>
<th>Resin P (μmol)</th>
<th>DTPA Zn (μmol kg$^{-1}$)</th>
<th>DTPA Mn (μmol kg$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freshly collected field soil</td>
<td>0.430$^{BC}$</td>
<td>nd</td>
<td>5.2$^{BC}$</td>
<td>17.8$^{C}$</td>
<td>nd</td>
<td>5.91$^{AB}$</td>
<td>53.1$^{A}$</td>
</tr>
<tr>
<td>Conditioned soil, 0–30 cm, prior to placing in containers and sowing B. tectorum$^2$</td>
<td>0.160$^{CD}$</td>
<td>nd</td>
<td>0.3$^{C}$</td>
<td>35.2$^{AB}$</td>
<td>nd</td>
<td>4.76$^{AB}$</td>
<td>12.4$^{D}$</td>
</tr>
<tr>
<td>Conditioned soil, 30–60 cm, prior to placing in containers and sowing B. tectorum$^2$</td>
<td>0.260$^{CD}$</td>
<td>nd</td>
<td>0.8$^{C}$</td>
<td>30.7$^{B}$</td>
<td>nd</td>
<td>3.94$^{B}$</td>
<td>16.1$^{CD}$</td>
</tr>
<tr>
<td>Conditioned soil, 60–90 cm, prior to placing in containers and sowing B. tectorum$^2$</td>
<td>0.250$^{CD}$</td>
<td>nd</td>
<td>0.5$^{C}$</td>
<td>32.9$^{AB}$</td>
<td>nd</td>
<td>3.59$^{B}$</td>
<td>16.4$^{CD}$</td>
</tr>
<tr>
<td>Conditioned soil, post-harvest B. tectorum$^3$</td>
<td>0.026$^{D}$</td>
<td>0.6$^{C}$</td>
<td>50.3$^{A}$</td>
<td>36.6$^{A}$</td>
<td>1.18</td>
<td>4.54$^{AB}$</td>
<td>25.2$^{B}$</td>
</tr>
<tr>
<td>Container soil following B. tectorum harvest growing in fresh soil$^4$</td>
<td>0.550$^{B}$</td>
<td>35.0$^{A}$</td>
<td>0.6$^{C}$</td>
<td>36.2$^{A}$</td>
<td>0.92</td>
<td>6.38$^{A}$</td>
<td>20.3$^{BC}$</td>
</tr>
<tr>
<td>Container soil following B. tectorum harvest growing in conditioned soil, 0–30 cm$^6$</td>
<td>0.315$^{C}$</td>
<td>10.5$^{BC}$</td>
<td>11.1$^{BC}$</td>
<td>35.1$^{AB}$</td>
<td>1.21</td>
<td>4.81$^{AB}$</td>
<td>17.0$^{CD}$</td>
</tr>
<tr>
<td>Container soil following B. tectorum harvest growing in conditioned soil, 30–60 cm$^6$</td>
<td>0.364$^{BC}$</td>
<td>18.7$^{B}$</td>
<td>9.6$^{BC}$</td>
<td>37.7$^{A}$</td>
<td>1.21</td>
<td>4.46$^{B}$</td>
<td>18.3$^{B-D}$</td>
</tr>
<tr>
<td>Container soil following B. tectorum harvest growing non-competed in conditioned soil, 60–90 cm$^6$</td>
<td>0.414$^{BC}$</td>
<td>11.6$^{BC}$</td>
<td>11.6$^{BC}$</td>
<td>34.8$^{AB}$</td>
<td>1.26</td>
<td>4.53$^{AB}$</td>
<td>19.8$^{BC}$</td>
</tr>
<tr>
<td>Fresh soil unplanted control$^4$</td>
<td>1.280$^{A}$</td>
<td>45.6$^{A}$</td>
<td>20.6$^{B}$</td>
<td>34.5$^{AB}$</td>
<td>0.75</td>
<td>5.56$^{AB}$</td>
<td>21.1$^{BC}$</td>
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<td>0.6754</td>
<td>0.0023</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

1For each column, means with different superscripted letters are significantly different at the < 0.05 level; mineral N is total NH$_4^+$ + NO$_2$ + NO$_3$ extractable by KCl; resin N includes NO$_2$ + NO$_3$; mole NO$_2$ is the molar proportion of NO$_2$ in the solution-phase pool of NO$_2$ + NO$_3$; nd, not determined. Attributes unaffected by treatment included 30-day aerobic incubated NH$_4^+$ and NO$_3$; net N mineralization potentials, total C and N, and DTPA extractable Fe and Cu.

2Soils taken after 64 days conditioning by A. cristatum from rhizotrons and homogenized by depth.

3Soils collected from within rooting zone of B. tectorum that were conditioned by A. cristatum for 64 days.

4Soils from homogenized sample of entire container.
We accept hypothesis 2 that occupation of biological soil space by established roots of *A. cristatum* will suppress growth of *B. tectorum*. Compelling aspects of our data include high shoot to root ratios of *B. tectorum* when competing with established *A. cristatum* (Fig. 2), response of *B. tectorum* to varying soil treatments (Fig. 2), and distinct elongated root architectures with far fewer root hairs in the most suppressed trials. The concept of biological soil space implies that physical space is a resource in itself, beyond that of access to nutrients and water (McConnaughay and Bazzaz 1991, 1992). In this construct, occupation of physical space by roots of established *A. cristatum* will constrain root growth of *B. tectorum*. The mechanistic underpinnings of suppression via biological soil space may involve root signalling or root toxicity (Schenk 2006). It is possible that the elevated shoot to root ratios in *B. tectorum* competing against established *A. cristatum* in this study is likely less due to reduced availability of N than interactions with pre-existing roots of *A. cristatum*. Low soil N availability should stimulate rather than decrease root growth (Hill et al. 2006). If reduced biological soil space due to established *A. cristatum* roots is partly responsible for suppression of *B. tectorum*; then replacement of conditioned soil between established *A. cristatum* plants should have increased biological space for *B. tectorum* root growth and also increased nutrient availability resulting in less suppression—yet suppression still occurred. One possibility is that roots of *A. cristatum* may have proliferated in the fresh soil and simply occupied soil space faster than roots of the newly sown *B. tectorum*. Visual inspection upon harvesting *B. tectorum* did reveal the presence of *A. cristatum* roots. Moreover, very low post-harvest mineral N levels in the fresh soil (Table 1) lend support to the re-occupation of biological soil space by *A. cristatum* as such small plants of *B. tectorum* simply could not have depleted that much mineral N. We also expected a mesh would limit new root encroachment by *A. cristatum* and the fresh soil placed above the mesh would have much un-occupied biological soil space for roots of *B. tectorum* to proliferate; yet, *B. tectorum* planted in this soil was still suppressed and had high shoot to root ratios. Indeed, as the mesh was removed from the rhizotrons following harvest of *B. tectorum*, visible inspection indicated very few roots of *A. cristatum* had penetrated the mesh. Nonetheless, the fresh soil added above the mesh had very low mineral N content after harvest, in fact the lowest among all the treatments. Clearly, enough *A. cristatum* roots had penetrated the mesh to reduce mineral N content and thereby partially suppressed *B. tectorum* via lowered N availability. In all individual trials of *B. tectorum* competing with established *A. cristatum*, no matter the treatment, post-harvest *B. tectorum* had high shoot to root ratios (Fig. 2) and decreased root hair formation. Recent research has demonstrated that root competition is far more complex than simple resource depletion (see review by Schenk 2006). In this new construct of root to root interactions, it is possible that newly establishing roots of *B. tectorum* sense the presence of *A. cristatum* roots and do not grow appreciably into the fresh soil provided. Alternatively, established roots of *A. cristatum* may exude toxic substances that affect *B. tectorum* root architecture; unfortunately our experimental protocols are not able to rigorously test this conjecture.


Figure 3. Graphs showing variables strongly related to above-ground biomass as determined by backward selection regression. Top graph, combined Experiments 1 and 2, relates root biomass with above-ground biomass. Middle graph relates resin availability of NO$_2^−$ + NO$_3^−$ with above-ground biomass; resin data were only collected for Experiment 2. Bottom graph, combined Experiments 1 and 2, relates predicted above-ground biomass using the combination of root biomass and solution-phase NO$_3^−$. 

We accept hypothesis 2 that occupation of biological soil space by established roots of *A. cristatum* will suppress growth of *B. tectorum*. Compelling aspects of our data include high shoot to root ratios of *B. tectorum* when competing with established *A. cristatum* (Fig. 2), response of *B. tectorum* to varying soil treatments (Fig. 2), and distinct elongated root architectures with far fewer root hairs in the most suppressed trials. The concept of biological soil space implies that physical space is a resource in itself, beyond that of access to nutrients and water (McConnaughay and Bazzaz 1991, 1992). In this construct, occupation of physical space by roots of established *A. cristatum* will constrain root growth of *B. tectorum*. The mechanistic underpinnings of
and sown to B. tectorum produced far more above-ground biomass than it did when competing with A. cristatum. Our expectation was that the conditioned soil separated from A. cristatum would retain its ability to suppress B. tectorum because the soil would have depleted N availability, at least initially. Moreover, we expected conditioned soil from the 0–30 cm depth increment would have greater filling of biological soil space with established roots of A. cristatum and therefore be more suppressive to B. tectorum than conditioned soil from lower depths. In fact, conditioned soil removed from rhizotrons did not suppress B. tectorum and shoot to root mass ratios were not elevated as in competed trials. Lack of suppression in this situation may be explained by the following. Firstly, the now dead roots of A. cristatum have mineralized and contributed N to enhance B. tectorum growth. The relatively high mineral N and resin available N levels post-harvest for these trials lend credence to this possibility. Secondly, the lack of an established root system of A. cristatum due to homogenization prior to sowing B. tectorum in containers may free up biological soil space for B. tectorum resulting in lower root to root signalling and exudation of toxins (Schenk 2006).

We expected that B. tectorum growing in the fresh soil above the plastic barrier in the rhizotrons would have above-ground biomass similar to its growth, non-competed, in fresh soil. Why then did the plastic barrier facilitate even greater growth of B. tectorum? Speculating, given the equal watering regimes used in all experimental units, the plastic barrier could have reduced water flow beyond the rooting zone of B. tectorum and essentially provided greater water availability.

Conclusions
The non-native perennial grass A. cristatum, when established, suppresses the growth of the exotic annual grass, B. tectorum. Reduced soil N availability and co-opting of soil space by perennial grass roots are potential soil factors involved in suppression. If only it were as easy to establish perennial grasses on B. tectorum-invaded rangelands as it is in the greenhouse, rehabilitation of B. tectorum degraded rangelands would be easier and far less expensive. The use of non-native plant materials to facilitate rehabilitation of exotic annual grass-invaded rangelands is controversial (D’Antonio and Meyerson 2002). Some researchers, however, make the case that particular non-natives possess attributes that allow for stronger and more effective rehabilitation (Asay et al. 2001; Ewel and Putz 2004). The reality that A. cristatum suppresses B. tectorum so effectively offers opportunities to use this species and other non-native competitive grasses as a successional bridge to encourage subsequent native plant recruitment (Cox and Anderson 2004; Brown et al. 2008; Davies et al. 2013). Perennial grasses differ markedly in their ability to suppress annual grasses (Borman et al. 1990, 1991). A portion of the suppressive ability of A. cristatum is via utilization of soil N resources such that it is less available to B. tectorum; however, the annual is also an effective competitor for soil N (Monaco et al. 2003). If perennial grasses do not strongly couple root uptake of N with the timing of its availability in soil, pulses of availability can occur leading to less suppression. We believe greater understanding of aspects of suppression via biological soil space can be a fruitful area of research.

What specific properties do established perennial grasses engender to biological soil space to resist subsequent growth of alien annual grasses? Is allelopathy involved? Is alteration of the soil microbial community involved? Understanding specific mechanisms could direct plant breeding strategies to develop perennial grasses more suppressive to exotic annual grasses.

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Contributions by the Authors
R.R.B. designed the experiment, analysed the data and was the principle writer. T.M. designed and built rhizotrons. T.M. and F.A. collected soils, monitored the experiments and conducted soil analyses.

Conflicts of Interest Statement
None declared.

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Literature Cited


