

Research Article

Suppression of *Bromus tectorum* L. by Established Perennial Grasses: Potential Mechanisms—Part One

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Bromus tectorum L. (cheatgrass) is an Eurasian annual grass that has invaded ecosystems throughout the Intermountain west of the United States. Our purpose was to examine mechanisms by which established perennial grasses suppress the growth of *B. tectorum*. Using rhizotrons, the experiment was conducted over 5 growth cycles: (1) *B. tectorum* planted between perennial grasses; (2) perennials clipped and *B. tectorum* planted; (3) perennials clipped and *B. tectorum* planted into soil mixed with activated carbon; (4) perennials clipped, *B. tectorum* planted, and top-dressed with fertilizer, and; (5) perennial grasses killed and *B. tectorum* planted. Water was not limiting in this study. Response variables measured at the end of each growth cycle included above-ground mass and tissue nutrient concentrations. Relative to controls (*B. tectorum* without competition), established perennial grasses significantly hindered the growth of *B. tectorum*. Overall, biomass of *B. tectorum*, grown between established perennials, increased considerably after fertilizer addition and dramatically upon death of the perennials. Potential mechanisms involved in the suppression of *B. tectorum* include reduced nitrogen (possibly phosphorus) availability and coopting of biological soil space by perennial roots. Our data cannot confirm or reject allelopathic suppression. Understanding the mechanisms involved with suppression may lead to novel control strategies against *B. tectorum*.

1. Introduction

The Eurasian annual grass *B. tectorum* L. (cheatgrass, downy brome) has come to dominate many ecosystems in the Intermountain Region of the Western United States [1]. Pathways by which *B. tectorum* facilitate its expansion are myriad and include phenotypic plasticity to new host environments [2], it increases the rates and sizes of wildfires, which fosters more invasion [3], rapid above- and below-ground growth rates [4, 5], prolific seed production [6], landscape disturbance [7–9], and elevated atmospheric CO₂ [10, 11].

Given the invasion success of *B. tectorum*, one might conclude that it is competitive. In the seedling stage, *B. tectorum* is quite competitive against native and introduced perennial grasses [12–14]. Particular ecosystems, however, are resistant to invasion by *B. tectorum* [15]. A common thread in ecosystem resistance to *B. tectorum* invasion is healthy, well-established, perennial grass communities such as the natives *Pseudoroegneria spicata* (bluebunch wheatgrass), *Elymus elymoides* (bottlebrush squirreltail), *Poa secunda* (bluegrass),

and *Festuca idahoensis* (Idaho fescue), and the introduced Eurasian *Agropyron cristatum* (crested wheatgrass) [16–18]. Established perennial grasses that grow early in the spring and maintain growth during the winter have been shown to suppress annual weeds [19]. By what mechanisms do these perennial grass communities resist *B. tectorum* invasion? Very meager literature exists on specific mechanisms of plant suppression. In the framework of ecological resistance theory [20], what factors in a host environment populated by healthy perennial grasses might constrain the invasiveness of *B. tectorum*?

The biotic and abiotic factors that influence community resistance can work in synergism making it difficult to decipher-specific mechanisms. In the case of *B. tectorum*, empirical evidence implicates soil resource availability, particularly nitrogen and phosphorus, as an important candidate mechanism [16, 17, 21, 22]. Addition of nitrogen fertilizer greatly increases the growth and competitive stature of *B. tectorum* [23, 24]. Conversely, immobilization of mineral soil nitrogen via the addition of a labile carbon

source can greatly reduce populations of *B. tectorum* [25, 26], although factors other than nitrogen immobilization may be partially responsible [27]. We ask the question: do healthy perennial grass communities, through a network of established perennial roots, control soil nutrient availability at a low level such that *B. tectorum* is less successful [28]?

Resistance to plant invasion via root competition can manifest itself in mechanisms other than decreasing nutrient availability [29]. The concept of biological soil space has relevance here. One aspect of biological soil space is that it provides an open niche that plant roots can exploit and indeed is necessary for plants to establish [30]. Another aspect is that the physical space, itself, may also be a soil resource [31]. The mechanistic underpinnings of how physical space influences plant growth are speculative, but it is suggested that flexibility to deploy roots to fragmented areas of physical space may confer greater ability to access soil nutrients [32]. If physical soil space is a resource, then a plant root that occupies that soil space must reduce the utility of that space to a competing root. Indeed the chemical and biological environment near-established perennial roots may negatively affect the roots of potential plant invaders. Some invasive species exude allelopathies to gain a competitive advantage in their new host environments [33, 34]. Conversely, some native plants resist invasion by producing allelopathies [35, 36]. We ask another question: do healthy perennial grasses resist *B. tectorum* invasion by coopting biological soil space or by possibly exuding allelopathies?

This study was designed to test if and by what mechanisms established perennial grasses suppress *B. tectorum*. Our approach was to use multiple growth cycles to evaluate different aspects of suppression including declining soil nutrient resources, allelopathy, root competition, supplemental soil nutrients, and finally death of the perennial grasses. The working hypotheses posit that (1) established perennial grasses will suppress the growth of *B. tectorum*; (2) established perennial grasses will control soil nutrients, particularly nitrogen, to levels low enough such that growth of *B. tectorum* is reduced; (3) established perennial grass roots will occupy biological soil space and/or exude allelopathies thus causing reduction in the growth potential of *B. tectorum*.

2. Materials and Methods

The experiment was conducted in a greenhouse at Reno, NV, USA (39°32'17.20''N; 119°48'22.89''W). The soil substrate was freshly collected from a *Krascheninnikovia lanata* (winterfat) site, invaded by *B. tectorum* L. for about 10 years, about 80 km northwest of Reno, NV, USA. Surface soil (0–25 cm, corresponding to the A horizon) was composited from an area of about 10 m². The soil, loamy sand in texture, was sieved to remove coarse fragments and medium-to-large roots and homogenized by hand mixing on a greenhouse bench. Replicate clear plastic rhizotrons, 5 × 30 × 100 cm depth, were filled with equal volume of the 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 the soil was saturated with deionized water. The competitive effect of three established native perennial grasses against *B. tectorum* was tested. *Elymus wawawaiensis* (Snake River wheatgrass) is a long-lived cool season grass common to the Intermountain Region of the Western USA and grows from 40 to 120 cm tall. *Achnatherum hymenoides* (Indian ricegrass) is adapted to sand-textured soils, is drought-tolerant, and ranges from 5 to 75 cm tall. *Leymus triticoides* (creeping wild rye) is a rhizomatous grass common to moist soils and can reach over 1 m in height.

Further experimental protocols are summarized in Table 1. Two seeds of each grass were sown in the rhizotrons 6 cm from each edge to leave an 18 cm space between for planting of *B. tectorum*. During initial establishment, perennial grasses were supplemented with 500 to 1000 mL of deionized water per week. The experiment was continued for five separate *B. tectorum* growth cycles (Table 1). Additionally, for each growth cycle four replicate rhizotrons were sown to just *B. tectorum* to serve as control and thereby gauge the influence of established perennial grasses on *B. tectorum* growth through time. Supplemental lighting, using 4 high pressure sodium lamps each producing 124,000 lumens at 2,100°K temperature, was used to assure at least 12 hours of daylight. For each harvest, above-ground tissue was dried for 48 hrs at 70°C, weight recorded and reserved for nutrient analyses. Following each harvest, *B. tectorum* roots were left undisturbed except in preparation for the 3rd growth cycle (Table 1). Through the experiment, deionized water was added to the soil surface twice weekly amounting to between 300 to 1000 mL depending on visual indication of soil moisture content as viewed through the plastic rhizotron backs. Additionally, small amounts of water were added as needed in the immediate vicinity of the competed *B. tectorum* to assure water availability—water was not limiting in this study. Following final harvest, using a 2.5 cm diameter-coring device, soil was collected at depths between 0–30 and 30–60 cm directly beneath perennial grasses and directly beneath *B. tectorum*, both with or without competition. For these soil samples and original soil before planting: (1) phosphorus in the soil solution was extracted using immiscible displacement [37] and quantified by ion chromatography; (2) the mineral nitrogen pool was extracted with KCl [38] and NO₃⁻-N and NH₄⁺-N quantified by flow-injection methodology (Lachat); (3) the bicarbonate-extractable phosphorus pool was quantified using the Olsen method [39] with quantification using molybdenum-blue colorimetry; (4) total nitrogen and carbon were quantified by Dumas combustion using a Leco analyzer. Total nitrogen in plant tissue was quantified by Dumas combustion using a LECO analyzer. Phosphorus in plant tissue was determined using a dry ash procedure with solubilization in 1 N HCl [40] followed by quantification using vanadomolybdate chemistry. Standards were NIST-(National Institute of Standards and Technology) certified, and NIST-certified reference plant tissue was used as a check.

The data structure is 3 perennial grass species in competition with *B. tectorum* replicated 4 times for a total of 12 rhizotrons plus 4 replicates of *B. tectorum* grown without

TABLE 1: Summary and time line of experimental protocols.

Action	Time (days)	Purpose
Establish perennial grasses	60	Create suppressive conditions
1st growth cycle: single seed of <i>B. tectorum</i> sown between established perennial grasses (in competition) and alone (without competition)	89	Quantify the degree of suppression by three perennial grasses in competition with <i>B. tectorum</i> relative to <i>B. tectorum</i> grown without competition
1st growth cycle harvest: perennials clipped at 15 cm above soil surface and <i>B. tectorum</i> clipped at soil surface. Dry tissue weights measured	1	
2nd growth cycle: repeat 1st cycle planting protocols	78	Clipping perennial grasses simulates the effects of herbivory on suppression. Removal of perennial above-ground mass will test the effect of declining soil resources on suppression
2nd growth cycle harvest: Repeat 1st cycle harvest protocols	1	
3rd growth cycle: excavate 400 g soil from beneath clipped <i>B. tectorum</i> , mix with 4 g activated carbon, and return to rhizotrons, then repeat planting protocols	81	Test if activated carbon addition affects suppression via amelioration of allelopathy. Disturbance will create root-free soil space and increase biological soil space, at least initially
3rd growth cycle harvest: repeat 1st cycle harvest protocols	1	
4th growth cycle: repeat planting protocols then, after emergence of <i>B. tectorum</i> , top-dress each rhizotron soil with a complete fertilizer solution (Rapid Gro) containing 82 mM N, 6.7 mM P, and 9.0 mM K and micronutrients in a 500 mL solution added in 100 mL increments over 5 weeks	84	Test if suppression is partly due to lack of nutrients
4th growth cycle harvest: repeat 1st cycle harvest protocols	1	
5th growth cycle: perennial grasses cut below growing nodes to kill plant	83	Test how perennial grass death affects suppression
5th growth cycle harvest: <i>B. tectorum</i> clipped at soil surface. Soil samples taken	1	

competition. Given changing greenhouse conditions over the five growth cycles, it is not justified to compare between growth cycles for individual attributes. Therefore, a separate ANOVA was run for each growth cycle to compare between above-ground biomasses and tissue nutrient concentrations, with Tukey's Honest Significant difference as a post hoc test. As an index of changing plant biomass over the five growth cycles, for each growth cycle, a ratio of the average of above-ground biomass between *B. tectorum* without competition to that of competed *B. tectorum* above-ground biomass was computed. ANOVA was used to analyze soil data after the 5th harvest with categorical variables PLANT (beneath perennial grasses, beneath competed *B. tectorum*, and beneath *B. tectorum* without competition) and DEPTH (0–30 and 30–60 cm). Tukey's was also used as a post hoc test. Before ANOVAs were run, data were normalized as necessary using square root or log transformations.

3. Results

3.1. Soil and Plant Growth. Throughout the Intermountain West of the United States, healthy, established perennial grass communities suppress annual weeds including *B. tectorum* (Figure 1). We investigated mechanisms involved in the suppression of *B. tectorum* in the greenhouse (Figure 1). The soil used in this experiment is low in total carbon and nitrogen, typical of an arid land soil (Table 2). This low carbon/nitrogen ratio indicates the potential to mineralize organic-bound nitrogen. Levels of phosphorus in the soil-solution pool and bicarbonate-extractable pool, as well as

TABLE 2: Selected attributes of the soil before planting.

Attribute	Mean
N (%)	0.037 (0.002)
C (%)	0.251 (0.017)
C/N	6.84 (0.56)
Soil solution P ($\mu\text{mol L}^{-1}$)	8.95 (0.53)
Bicarbonate P (mmol kg^{-1})	0.25 (0.01)
Mineral N (mmol kg^{-1})	0.25 (0.01)
pH	7.25 (0.05)

Data are average of 4 subsamples taken from homogenized soil with standard errors in parentheses.

mineral nitrogen, indicated that the soil is relatively infertile. Using this soil, established perennial grasses significantly suppressed *B. tectorum* growth, but the magnitude depended on growth cycle and the competing perennial species (Tables 3 and 4). In absolute terms, biomass of *B. tectorum* in competition increased with each growth cycle. Clipped biomass of the perennial grasses and *B. tectorum* without competition generally declined with growth cycle, but all increased biomass after the 4th growth cycle (fertilizer addition). All the tested perennial species suppressed *B. tectorum*, but for most growth cycles, suppression was least between *A. hymenoides*. Greatest suppression of *B. tectorum* occurred after the 1st growth cycle, where its above-ground biomass averaged 3480 times less than *B. tectorum* grown without competition. The above-ground biomass ratio of *B.*

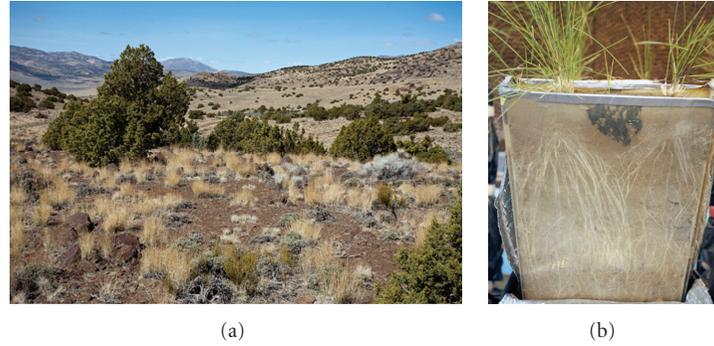


FIGURE 1: (a) was taken in the Virginia Range of northern Nevada, USA. These high elevation communities are dominated by the low sagebrush species *Artemisia arbuscula* due to the clay-textured soils. The trees are *Juniperus occidentalis* that are greatly expanding their range. The grass in the foreground is the native perennial bunchgrass *Psuedoreogneria spicata*, which effectively suppresses growth of *Bromus tectorum*. The vegetation in the background is dominated by *B. tectorum*, which we suspect is due to loss of *P. spicata*. (b) shows a rhizotron used in the greenhouse experiment with the back removed to observe rooting patterns. Photograph was taken near the completion of the 3rd growth cycle (addition of activated carbon) and shows the perennial grass *A. hymenoides* with a small plant of *B. tectorum* between.

TABLE 3: Mean above-ground biomass (g) by plant species and growth cycle.

Plant	Growth cycle				
	1st	2nd	3rd	4th	5th
<i>B. tectorum</i> (without competition)	11.11 ^A	1.79 ^D	0.56 ^B	7.56 ^A	6.52 ^A
<i>L. triticoides</i>	4.24 ^C	2.92 ^C	0.91 ^{AB}	5.06 ^A	
<i>B. tectorum</i> (in competition between <i>L. triticoides</i>)	0.006 ^D	0.012 ^E	0.016 ^B	0.191 ^B	2.47 ^B
<i>E. wawawaiensis</i>	3.27 ^C	5.70 ^A	1.56 ^A	7.16 ^A	
<i>B. tectorum</i> (in competition between <i>E. wawawaiensis</i>)	0.004 ^D	0.004 ^E	0.010 ^B	0.137 ^B	1.86 ^B
<i>A. hymenoides</i>	8.31 ^B	4.18 ^B	0.81 ^{AB}	6.37 ^A	
<i>B. tectorum</i> (in competition between <i>A. hymenoides</i>)	0.038 ^D	0.045	0.108 ^B	0.422 ^B	2.92 ^B
ANOVA	$P < 0.0001$	$P < 0.0001$	$P = 0.0002$	$P < 0.0001$	$P = 0.0058$

Separate ANOVAs used for each growth cycle. For each column, different superscripted letters denote significant differences.

TABLE 4: Above-ground biomass ratios of *B. tectorum* grown without competition (WC) to *B. tectorum* grown in competition (IC), by growth cycle.

Ratio	Growth cycle				
	1st	2nd	3rd	4th	5th
WC/IC	3480	242	42	65	3

tectorum without competition to *B. tectorum* in competition decreased with each growth cycle, but the greatest relative decrease occurred after the perennial plants were killed (5th growth cycle).

3.2. Tissue Nutrient Concentrations and Uptake of Nitrogen and Phosphorus. Tissue nitrogen concentrations varied among the tested plant species and growth cycles (Table 5). For the first two growth cycles, tissue nitrogen in *B. tectorum* without competition was significantly greater than tissue nitrogen of perennial grasses and far greater than that of *B. tectorum* in competition. All perennial grasses and *B. tectorum* in competition had much greater tissue nitrogen after the 3rd growth cycle; indeed approaching that of *B. tectorum* without competition. Noteworthy are the high tissue

nitrogen levels for *A. hymenoides*, known to fix nitrogen in its rhizosphere [41], after the 3rd cycle. Following fertilizer top-dressing (4th cycle), *B. tectorum* grown with and without competition appeared to utilize added nitrogen judging from the large increase in tissue nitrogen. Likewise, it seems that *L. triticoides* utilized the fertilizer nitrogen; however, *E. wawawaiensis* and *A. hymenoides* did not.

In almost all instances, *B. tectorum* had significantly greater tissue phosphorus concentrations than the perennial grasses (Table 5). *Bromus tectorum*, both in and without competition, had statistically similar tissue phosphorus concentrations after the 3rd and 4th growth cycles; however, after the 5th growth cycle (perennials killed), the formerly competed *B. tectorum* had statistically greater tissue phosphorus concentrations. For the perennial grasses, tissue phosphorus increased from the 1st to the 3rd growth cycles (*A. hymenoides* declined in the 2nd), and then declined in the 4th growth cycle.

Over the first four growth cycles, *B. tectorum* grown without competition had far greater uptake of nitrogen and phosphorus than the perennial grasses (Figure 2). It was not until the 5th growth cycle (perennials killed) that *B. tectorum* in competition uptook sizeable levels of nitrogen or phosphorus.

TABLE 5: Mean above-ground tissue nitrogen and phosphorus concentrations by plant species and growth cycle.

Plant	Growth cycle				
	1st	2nd	3rd	4th	5th
Tissue nitrogen (%)					
<i>B. tectorum</i> (without competition)	2.07 ^A	2.27 ^A	2.27 ^A	3.68 ^{AB}	3.35
<i>B. tectorum</i> (in competition)	1.26 ^{AB}	1.50 ^B	2.55 ^{A-C}	4.20 ^A	3.86
<i>L. triticoides</i>	1.02 ^B	1.13 ^{BC}	2.56 ^{BC}	3.58 ^{AB}	
<i>E. wawawaiensis</i>	0.97 ^B	0.97 ^{BC}	2.13 ^C	2.09 ^C	
<i>A. hymenoides</i>	1.46 ^B	0.78 ^C	3.34 ^A	3.00 ^{BC}	
ANOVA	$P = 0.0002$	$P < 0.0001$	$P = 0.0054$	$P = 0.0004$	$P = 0.0994$
Tissue phosphorus (mmol kg ⁻¹)					
<i>B. tectorum</i> (without competition)	91.0 ^A	110.7 ^A	122.8 ^{AB}	112.7 ^{AB}	93.3 ^B
<i>B. tectorum</i> (in competition)	nd	nd	131.6 ^A	134.6 ^A	129.3 ^A
<i>L. triticoides</i>	24.2 ^{BC}	32.9 ^B	94.5 ^C	84.5 ^{BC}	
<i>E. wawawaiensis</i>	18.2 ^C	27.8 ^{BC}	67.7 ^D	58.2 ^C	
<i>A. hymenoides</i>	41.2 ^B	19.6 ^C	99.8 ^{BC}	58.7 ^C	
ANOVA	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P = 0.0476$

Separate ANOVAs used for each growth cycle. For each column, different superscripted letters denote significant differences. nd: not determined due to lack of sufficient plant tissue for analyses.

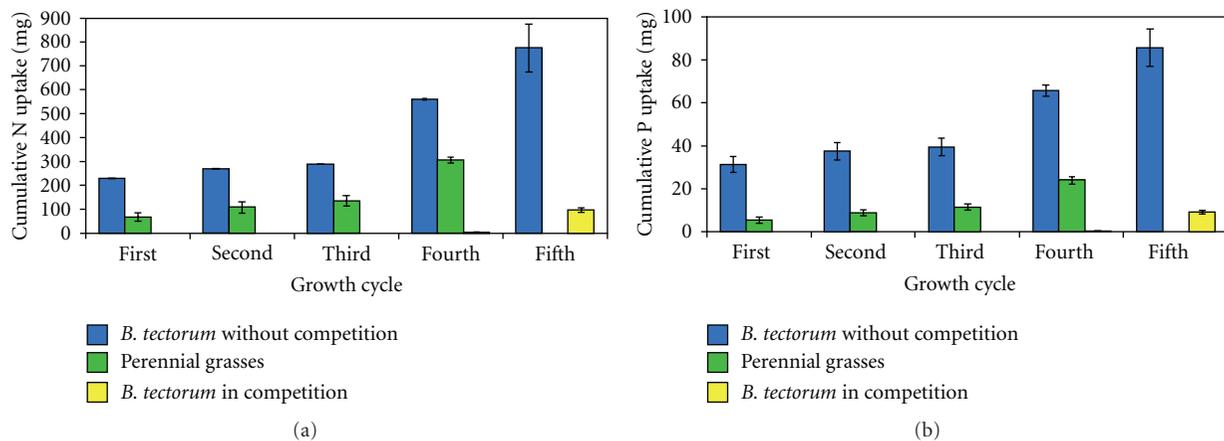


FIGURE 2: Graphs showing cumulative plant uptake of nitrogen and phosphorus by growth cycle.

3.3. Soil Nutrients Following Final Harvest. Following the 5th growth cycle, soil nutrient pools were quantified (Table 6). Mineral nitrogen pools exceeded the initial soil values (Table 1) at all depths and microsites, which is likely due to fertilizer additions during the 4th growth cycle. Mineral nitrogen in the soil beneath competed *B. tectorum* is statistically similar to that beneath *B. tectorum* grown without competition. The lowest mineral nitrogen pool occurred beneath the surface 30 cm of perennial grasses. The molar proportion of $\text{NH}_4^+\text{-N}$ in the mineral nitrogen pool was statistically greater in the surface 30 cm beneath competed *B. tectorum* and the perennial grasses. The soil solution P pool was statistically similar among microsites. Values of phosphorus for the surface 30 cm are similar to initial values of the soil (Table 1), but phosphorus values for the 30–60 cm depth increment were statistically lower.

4. Discussion

Established perennial grasses significantly suppressed the growth of *B. tectorum*, thus supporting our first hypothesis. The magnitude of suppression was remarkable in the 1st and 2nd growth cycles; *B. tectorum* never produced more than 3 leaves after 70 days of growth. By comparison, *B. tectorum* grown without competition produced hundreds of leaves during the same periods. Water availability can be a key determinant in the competitive ability of *B. tectorum* [42]; however, in this study, growth suppression is not due to water limitation as plants were frequently supplemented with water. What then are the mechanistic underpinnings for suppression of *B. tectorum*? Resistance to invasion is exceedingly complex, an interaction of various biotic and abiotic factors in the host soil environment [43].

TABLE 6: Selected soil nutrient measurements following the 5th harvest.

Depth (cm)	<i>B. tectorum</i> without competition	<i>B. tectorum</i> in competition	Perennial grasses	ANOVA
				Plant $P = 0.8345$
				Depth $P = 0.0025$
				Inter. $P = 0.0631$
				Plant $P = 0.0087$
				Depth $P < 0.0001$
				Inter. $P = 0.0430$
				Plant $P = 0.3256$
				Depth $P < 0.0001$
				Inter. $P = 0.6458$

In our study, growth of *B. tectorum* in competition increased minimally from the 1st to 2nd growth cycles, slightly more after the 3rd growth cycle (activated carbon addition), sizably after the 4th growth cycle (fertilizer addition), and greatly after the 5th growth cycle (perennial death). When one compares the ratio between *B. tectorum* grown without competition and plants in competition over the five growth cycles, a different picture emerges. From the 1st through 3rd growth cycles, this ratio declined precipitously. Fertilizer addition (4th cycle) resulted in greater relative growth of controls. It is only after the death of perennials (5th growth cycle) that competed *B. tectorum* vastly increased its growth relative the controls. These data suggest that a combination of reduced nitrogen availability and coopting of biological soil space may be responsible for suppression of *B. tectorum* by established perennial grasses. Our data do not allow acceptance or rejection of allelopathy as a mechanism in suppression.

The first mechanism our data supports is that established perennial grasses reduced the availability of nitrogen (possibly phosphorus) in the soil of establishing *B. tectorum* plants, thus supporting our second hypothesis. Prober and Lunt [35] reported that the native perennial grass *Themeda australis* reduced soil NO_3^- -N and thereby decreased invasion by nitrophilic annuals. Evidence for a similar mechanism operating in this study included lower tissue nitrogen concentrations for the 1st and 2nd growth cycles for *B. tectorum* in competition, relative to *B. tectorum* grown without competition (Table 5) and the increase in absolute growth and tissue nitrogen concentrations of *B. tectorum* in competition after fertilizer addition. *Bromus tectorum* is a nitophile, and its growth and competitive ability is increased as nitrogen availability is elevated [21, 44, 45] and lowered resource availability has been suggested as to why some plant communities are resistant to *B. tectorum* invasion [15]. It is by no means clear, however, that lowered soil nitrogen availability can by itself completely assure that *B. tectorum* cannot invade particular sites [43]. Due to lack of sufficient sample we could not quantify phosphorus in *B. tectorum* in competition for the 1st and 2nd growth cycles. It is possible that like nitrogen, phosphorus was less available. Gundale et al. [23] determined that soil beneath perennial bunchgrasses

was phosphorus-limited and negatively affected the growth of *B. tectorum*.

An aspect of mineral nitrogen, other than its availability, may influence the suppression of *B. tectorum*. Surface soil beneath the perennial grasses and competed *B. tectorum* has a significantly greater proportion of mineral N in the NH_4^+ -N form compared to *B. tectorum* grown without competition (Table 6). Empirical evidence has shown that *B. tectorum* has greater uptake kinetics for nitrogen in the NO_3^- -N form rather than the NH_4^+ -N form [46]. Indeed, *B. tectorum*-occupied soils have elevated nitrification rates, relative to native ecosystems, such that a greater proportion of mineral nitrogen is in the NO_3^- -N form [42]. In a well-established and healthy stand of the perennial grass *Agropyron cristatum* in northern Nevada, which resists invasion by *B. tectorum*, the molar proportion of NH_4^+ -N in the mineral N fraction often exceeds 90% [47]. We conjecture that a portion of perennial grass suppression of *B. tectorum* growth in the present study may be due to inhibited nitrification, which reduces the availability of NO_3^- -N. In a west African shrub savannah, grasses have been shown to inhibit soil nitrification [48]. Data from our experimental treatments, at least initially, argue against nitrification inhibition as a major factor in the suppression of *B. tectorum*. Allelochemicals are often implicated in nitrification inhibition [49]; yet, the activated carbon treatment (3rd growth cycle), which can ameliorate allelopathic activity [50], caused very little increase in the growth of *B. tectorum* in competition. The veracity of our inferences that activated carbon ameliorates allelopathy must be questioned based on recent research [51, 52]. A more persuasive argument that nitrification inhibition may not have a major role in suppression is that application of large quantities of NO_3^- -N (4th growth cycle) did significantly increase *B. tectorum* above-ground biomass grown in competition relative to above-ground biomass of *B. tectorum* grown without competition.

Death of competing perennial grasses (5th growth cycle) greatly released the growth potential of *B. tectorum*. What aspects of the death of a competing plant might so affect the growth of *B. tectorum*? Established perennials may reduce light resources to *B. tectorum*; however, the spacing afforded in this rhizotron experiment and perennial grass clipping

argues against any light limitation. Water resources could be so depleted by the established perennial grasses such that newly added seeds of *B. tectorum* could not attain maximum growth. Again, as stated earlier, the competing *B. tectorum* plants in this study were given adequate water. Death of plant roots can provide nutrients to new plants [53] and thereby facilitate the growth of *B. tectorum*. We doubt, however, that this process could so increase the growth of *B. tectorum* for two reasons. Firstly, *B. tectorum* grown in competition did not greatly respond to fertilizer top-dressing relative to its increase at perennial plant death. Secondly, the cumulative total plant uptake of nitrogen and phosphorus over the first four growth cycles was far greater for *B. tectorum* growth without competition than the perennial grasses (Figure 1). This fact suggests that, even after four growth cycles, soil beneath perennial grasses had nutrient availability that was adequate to support very high growth of *B. tectorum* grown in competition.

We believe that our data supports a part of hypothesis three, that established perennial grass roots occupy biological soil space and/or exuded allelopathies, thus causing reduction in the growth potential of *B. tectorum*. A recent review essay has presented a compelling case that root competition is far more complex than just resource depletion [28]. By occupying biological space, intact perennial roots can alter the architecture and activity of an invading root [31]. Plants can sense neighboring roots via chemical signaling (allelochemicals) and adjust growth patterns accordingly [49]. We believe that a portion of the growth reduction seen in competed *B. tectorum* may be due to its sensing of soil occupied by perennial roots. From the seedling stage, roots of *B. tectorum* seem unaffected by perennial grass neighbors, indeed they intermingle and likely coopt space and nutrients [14]. Clearly, established perennial grass roots are able to suppress root growth of *B. tectorum* as indicated by its lack of rooting in the rhizotrons. By what specific mechanism(s) might occupation of biological space affect root suppression? Unfortunately, specific mechanisms of how biological soil space influences plant growth remain elusive [31]. A reasonable inference based on our research is that established perennial roots simply interfere with the expansion of roots of *B. tectorum*. Due to the limitations of activated carbon in allelopathic research [51, 52], we cannot state with confidence that the perennial grasses exuded allelopathies, which suppressed *B. tectorum*. Published literature, however, reports that allelopathies, exuded from native flora, assist in the resistance to invasive plants [35].

5. Conclusions

Established perennial grasses greatly suppress the growth of *B. tectorum*. Our research hints at causative mechanisms including reduced nutrient availability and coopting of biological soil space; unfortunately, the definitive mechanisms have not been identified. Understanding the suppressive nature of established perennial grasses on growth of *B. tectorum* may lead to greater success in rehabilitation of native plant communities invaded by *B. tectorum*. Future studies

should attempt to design appropriate experiments to tease out the role of biological soil space in suppression.

Disclosure

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