

Research Article

Invaders break assembly rules to beat the natives: how cheatgrass cheats

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Abstract

Understanding how some introduced plants achieve invasive status while most simply become naturalized is a fundamental question in invasion ecology. Traditional approaches comparing native and introduced plants have linked ruderal traits such as annual life history, high fecundity, and rapid growth rates to invasiveness. However, they do not explain why other introduced species bearing similar traits fail to become invasive, possibly because generic comparisons ignore local processes that drive community assembly. Herein, we contrasted native and introduced annuals in the context of local successional processes to elucidate how introduced annual bromes like cheatgrass (*Bromus tectorum*) overtake perennial grasslands in the intermountain western United States. We created disturbed plots and seeded them first with annuals representing natives, naturalized species, or invasive bromes. We then seeded plots with native perennial community dominants to examine how the different annuals influenced succession. Native annuals established transient populations that facilitated perennial establishment compared to unseeded controls, enabling the shift to perennial dominance. Naturalized annuals mirrored the natives, but invasive annuals maintained robust populations at high biomass that inhibited perennial establishment and impeded succession. Mechanistically, invasive annuals reduced soil moisture and elevated plant biomass, litter, and soil N. However, only litter abundance correlated with perennial seedling recruitment across treatments. Overall, litter showed a unimodal relationship wherein lower litter abundance associated with native and naturalized annuals appeared to facilitate perennial seedling establishment while higher litter levels generated by invasives appeared to suppress perennial establishment and inhibit succession. Additional experiments provided little support for the roles of pathogen spill-over or plant-soil feedbacks favoring the introduced bromes. The domination of perennial grasslands by annual bromes may be driven by litter buildup that allows these introduced plants to break local succession rules by acting as both early seral and climax species. Traits like litter accumulation may strongly influence invasion outcomes but are indistinguishable using trait comparisons lacking community context.

Key words: Annual bromes, community assembly, ecosystem engineering, extended phenotype, grass-fire cycle, invasive plants, litter, naturalized plants, pathogen spillover, plant-soil feedback, succession



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Introduction

Some introduced species cause inordinate ecological and economic damage (Mack et al. 2000; Vilà et al. 2011). However, most fail to establish or merely become naturalized with little measurable impact on their new environments (Williamson and Fitter

1996; Pearson et al. 2016). Identifying those unique traits that allow some introduced species to achieve pest status, i.e., become invasive, remains the ultimate objective of invasion ecology (Wallace 1881; Sax and Brown 2000). Traditional approaches to this problem have focused on comparing native and introduced plants to identify traits linked to invasiveness (Van Kleunen et al. 2010; Gallagher et al. 2015; Moravcová et al. 2015). However, these methods compare traits among species without reference to the local community assembly rules that determine the linkage between traits and fitness outcomes (Pearson et al. 2018a). While such studies have broadly linked invasiveness to ruderal strategies such as high fecundity and rapid growth (Pysek and Richardson 2007; Van Kleunen et al. 2010; Jelbert et al. 2015; Moravcová et al. 2015), they do not explain why many introduced plants bearing similar traits simply become naturalized (non-invasive) community members. Comparing native and introduced species in the context of local community assembly processes may help to explain how some introduced species become invasive while others do not (Pearson et al. 2018a), despite sharing superficially similar traits. Moreover, given the strong linkages between ruderal traits, disturbance, and plant invasions (Davis et al. 2000; Pysek and Richardson 2007; Van Kleunen et al. 2010; Jelbert et al. 2015; Moravcová et al. 2015, Pearson et al. 2018b), contrasting native and introduced ruderals in the context of local successional trajectories could prove fruitful.

Succession theory offers the longest-standing framework for explaining plant community assembly (Cowles 1899). Several derivations of succession theory exist (e.g., Cowles 1899; Clements 1916; Gleason 1926; Odum 1969; Connoll and Slatyer 1977) with some arguing for a more deterministic Clementsian model (Clements 1916) and others for a more stochastic Gleasonian (Gleason 1926) perspective. Nonetheless, a common theme is that, following disturbance, plant communities assemble along identifiable stages, known as seres, that culminate in a climax community state reflective of local edaphic and climatic conditions (summarized in Lomolino et al. 2010: 133–134). Within this framework, earlier seres tend to be dominated by pioneering or ruderal species bearing traits such as high fecundity, high vagility, and rapid growth that allow them to quickly exploit the high-resource, low-competition conditions that commonly follow disturbances. Over time, early seral species give way to slower, more competitive or stress tolerant species that may arrive later due to lower fecundity and vagility, but are better adapted to low-resource, high-competition conditions occurring late in succession. The most tolerant and well-adapted of these late seral species comprise a self-perpetuating community referred to as the climax state, which serves as the foundation for classifying communities based on predictable associations with abiotic conditions (e.g., Daubenmire 1966; Pfister and Arno 1980). Yet, how introduced plants may fit into or disrupt these natural successional processes remains understudied (but see Tognetti et al. 2010).

If we apply this succession framework as a benchmark for understanding the rules by which native plant communities assemble (*sensu* Pearson et al. 2018a), we may begin to reveal when and how some introduced plants break the rules to gain advantage over natives in the course of succession. Since many introduced plants exhibit r-selected traits associated with early-seral or ruderal strategies such as high fecundity and rapid growth (Pysek and Richardson 2007; Van Kleunen et al. 2010; Jelbert et al. 2015; Moravcová et al. 2015), we might expect such introduced plants to exploit early successional stages to become abundant immediately following disturbance (e.g., Davis et al. 2000; Jauni et al. 2015; Pearson et al. 2018b). Following the rules of succession described above, these species should give way to lat-

er seral species as seen in native communities (see Lomolino et al. 2010). However, the fact that some introduced annuals come to dominate perennial plant communities where native annuals historically played early seral roles (Mack 1981; Prober et al. 2005; Brooks et al. 2016) suggests that such invaders may somehow break the rules of succession by acting both as early seral species that thrive post disturbance and as climax species that maintain dominance thereafter. Yet, most introduced plants do not overtake native communities (Simberloff 1981; Williamson and Fitter 1996; Ortega and Pearson 2005; Pearson et al. 2016), suggesting that most introduced annuals simply follow the rules of succession. While these patterns are compelling, experimentation is required to understand how some introduced annuals become problematic pests while others simply naturalize.

Mechanistically, introduced plants can impact communities through a variety of processes (see Catford et al. 2009). One process particularly relevant to succession is the ability of invasive plants to alter abiotic and biotic conditions that affect competitive interactions with natives. For example, plant-soil feedbacks (PSF) – defined as species-specific changes in soil properties that influence plant performance (*sensu* van der Putten et al. 2013) – may facilitate succession if early seral species experience stronger negative feedbacks than later seral species (Kardol et al. 2006; Kulmatiski et al. 2008). Hence, invaders could break successional rules by generating positive feedbacks on themselves or escaping negative feedbacks experienced by other community members (Klironomos 2002; Levine et al. 2006). Invasive introduced plants can also benefit by suppressing soil mutualists (Stinson et al. 2006) or by enhancing pathogens (Zhang et al. 2020) if effects are stronger on native competitors. Invaders can also promote seed pathogens that suppress native competitors via spillover effects (Meyer et al. 2007, 2014; Flory and Clay 2013). From the abiotic PSF standpoint, many invasive plants change soil fertility either through altered associations with soil biota involved in nutrient cycling (McLeod et al. 2016) or via changes in litter quantity or quality (Liao et al. 2008), with potential ramifications for succession (Paschke et al. 2000). Hence, invader-mediated shifts in both abiotic and biotic properties represent a broad category of processes by which invasive introduced plants may break successional rules.

Within western North American grasslands, cheatgrass (*Bromus tectorum*) and other annual bromes (*Bromus* spp.) are notorious invaders that have overtaken vast regions of perennial grasslands and shrublands (Mack 1981; Knapp 1996; Ogle et al. 2003; Germino et al. 2016), despite their annual status. Extensive research targeting these species has established that bromes can elevate litter biomass (Knapp 1996; Evans et al. 2001; Lenz et al. 2003; Bansal et al. 2014), alter available soil moisture (Cline et al. 1977; Booth et al. 2003), indirectly suppress native grass emergence through seed pathogen spillover (Beckstead et al. 2010, 2016; Meyer et al. 2014), and directly suppress native seedlings via competition (Humphrey and Schupp 2004; Yelenik and Levine 2010; Parkinson et al. 2013). Annual bromes can also alter N cycling and ammonium and nitrate levels (Evans et al. 2001; Bansal et al. 2014; Stark and Norton 2015; McLeod et al. 2016), and generate PSFs (Perkins and Nowak 2013). These studies provide important insights regarding several mechanisms that might facilitate brome invasion, but to date no study has evaluated these factors within a successional framework to understand how annual bromes might break succession rules to dominate native perennial grasslands.

We explored the above ideas within Intermountain Grasslands of the western United States that are susceptible to takeover by introduced annual brome grasses

(Mack 1981). These native grasslands are dominated by perennial bunchgrasses and forbs in their climax state (Mueggler and Stewart 1980). In this system, natural disturbances sufficient to displace community dominants are followed by flushes of native annuals that rapidly establish and flourish but ultimately give way to the dominant perennial plant community. Within this context, we established a field experiment wherein we created disturbances and initiated succession by seeding plots with either 1) native annuals, 2) naturalized introduced annuals, or 3) invasive introduced annual bromes. We then seeded six species of dominant native perennial grasses and forbs (climax species) into each plot to examine how succession progressed. Despite the fact that all our annuals were ruderal taxa with high fecundity and rapid growth rates, we predicted that 1) native annuals would succumb to perennials, allowing succession to proceed as seen in natural grasslands, 2) naturalized annuals would behave like the natives, similarly allowing succession to proceed, and 3) invasive annuals would suppress the native perennials and maintain dominance by inhibiting succession. To understand how the different ruderal groups influenced successional trajectories, we evaluated their effects on standing biomass, litter, and soil properties. Finally, we complemented the succession experiment with secondary experiments evaluating PSFs and pathogen spillover.

Materials and methods

Field experiment

We established our main experiment within a fenced space at Diettert Gardens on the University of Montana campus, Missoula, MT, USA in 2014 (46.841981, -113.992030). The site reflects abiotic conditions found in the adjacent intermountain bluebunch wheatgrass habitat (Mueggler and Stewart 1980) and was historically dominated by this grassland community. The native plants selected for the experiment represent perennial community dominants and common early seral annuals based on Mueggler and Stewart (1980) and extensive vegetation surveys conducted across these systems (Pearson et al. 2016). For the invasive introduced (hereafter “invasive”) species, we chose cheatgrass (*Bromus tectorum*) and Japanese brome (*B. japonicus*) because these species are highly invasive annuals capable of dominating perennial grasslands of western North America (Mack 1981; Knapp 1996; Ogle et al. 2003; Germino et al. 2016). For the naturalized introduced (hereafter “naturalized”) species, we selected four common annuals that thrive following disturbances but fail to dominate intermountain grasslands (mean cover $\leq 3\%$ as reported by Pearson et al. 2016).

We prepared the site by watering for two weeks to initiate seed germination and purge the seedbank in August 2015. We then applied Roundup® to kill actively growing plants in September. In October, after the herbicide had degraded, we delineated 100 0.7×0.7 m (0.5-m^2) plots with 0.8-m spacing (separated by weed cloth to maintain plot integrity) in a rectangular array and simulated disturbance in each plot by digging the soil to a depth of ~ 15 cm and removing plant biomass. We then sowed each plot with 1000 seeds of one of nine focal annual species, with ten replicate plots per species randomly assigned to represent the following treatments: 1) natives (*Collinsia parviflora*, *Clarkia pulchella*, or *Plantago patagonica*), 2) naturalized taxa (*Alyssum alyssoides*, *Arenaria serpyllifolia*, *Filago arvensis*, or *Veronica verna*), and 3) invasives (*Bromus japonicus* or *B. tectorum*). The remaining 10

plots were left unseeded to represent controls. In September 2016, after one growing season, we seeded all plots with three species of native perennial grasses (*Festuca idahoensis*, *Koeleria macrantha*, and *Pseudoroegneria spicata*) and three species of native perennial forbs (*Achillea millefolium*, *Balsamorhiza sagittata*, and *Lupinus sericeus*), all representing climax species that dominate undisturbed grasslands in this system (seeding rates were 100, 150, and 200 seeds per plot for large, medium, and small-seeded species, respectively, to reflect natural variation in fecundity [sensu Maron et al. 2012]; Suppl. material 1: table S1). All seeding and sampling targeted the center 0.5 × 0.5 m of each plot, allowing a 0.1 m wide buffer zone on all sides. Non-study species were weeded to < 10% cover in all plots each year, and focal invasives were completely weeded from treatments where not sown to prevent them from overtaking other treatments. Other focal annuals were allowed to colonize plots where they were not sown because it was not possible to remove them before they went to seed without disturbing perennial seedlings.

In the first growing season after seeding perennials, we documented recruitment by counting seedlings in each plot twice during the main germination window, May–June 2017. We carefully removed perennial seedlings after the final count and then reseeded the plots with the same species in September 2017, following the prior year's methods. This approach allowed us to quantify perennial recruitment over two growing seasons. In spring 2018, we counted perennial seedlings as done previously, but this time we left plants to grow. To represent recruitment per plot and year in analyses, we took the maximum count across the two surveys for each sown perennial species and summed these across species. Late in the growing season of 2019, we harvested all plots for aboveground biomass. Biomass of live focal annuals, sown perennials and litter (dead material from past years) was separated and dried for 48 hours at 62 °C before weighing.

To track populations of individual focal annuals in the years preceding harvest, 2016–2018, we visually estimated cover of both seeded and colonizing species in each plot (to the nearest 1% if < 10%, and nearest 5% if > 10%) aided by a frame marked in 1% increments. For examination of factors influencing perennial recruitment, we considered total focal annual cover summed across all functionally similar species per plot. For native, naturalized, and control treatments, this metric included focal native and naturalized annuals directly sown in the plots and those colonizing from other plots, as justified by comparison of perennial recruitment among plots established with these taxa (Suppl. material 1: fig. S1). Similarly, we treated the invasives *B. japonicus* and *B. tectorum* as a species complex given that they intermixed to some degree and were difficult to distinguish until late season (i.e., after cover surveys). Biomass harvested in 2019 showed that plots were dominated by the original sown *Bromus* in 70% of n = 20 cases, and perennial recruitment did not differ between plots sown with the two species (Suppl. material 1: fig. S1). We followed these same pooling conventions for consideration of focal annual biomass. We also measured litter cover in each year prior to harvest, 2016–2018. Cover estimation was repeated in 2019 prior to harvest for comparison to biomass.

To understand how focal annuals might affect abiotic conditions, we measured soil properties in each study plot in 2018. We extracted four soil cores (2.5 cm diameter × 10 cm deep) from each plot in mid-May. Soil cores were pooled by plot and sieved through a 2 mm sieve before lab analyses. We used a subset of each fresh soil sample to quantify available N (NO_3^- and NH_4^+) via KCl extraction (Hart et al. 1994) followed by colorimetric analyses on a Synergy 2 Microplate Reader (BioTek,

Winooski, VT, USA) according to Weatherburn (1967) and Doane and Horwath (2003). The remainder of each soil sample was air dried and sent to Ward Laboratories (Kearney, NE, USA) for measurements of SOM (LOI%), pH (water), P_{Merlich} , K, S, Zn, Fe, Mn, Cu, Ca, Mg, Na, and CEC. We sampled soil moisture (volumetric water content) at 4 cm depth weekly from May–June using a handheld probe (Field Scout® TDR-100; Spectrum Technologies, Inc., Plainfield, Illinois, USA) at two locations near the center of each plot and used the mean value per plot in analyses.

The invasive annuals *B. tectorum* and *B. japonicus* host the seed pathogen *Pyrenophora semeniperda* which can spillover onto and suppress germination of native grasses (Beckstead et al. 2010, 2016; Meyer et al. 2014). To evaluate whether this pathogen and/or other generalist seed pathogens like *Fusarium* (Meyer et al. 2014, 2016) played a role in our experimental results, we carefully tracked fates of marked seeds of *P. spicata* in a subset of field plots. This effort focused on *P. spicata* 1) to render the experiment manageable, 2) because this species is known to be susceptible to these pathogens, and 3) it is the dominant perennial grass in our system. To track the fate of *P. spicata* seeds, we glued individual seeds to the base of a wooden toothpick with Elmer's School Glue® and placed it in the ground so that the seed was buried in the soil (after Meyer et al. 2014). This was done for 20 seeds per plot in all *B. tectorum* and *B. japonicus* plots ($n = 10$ each), all control plots ($n = 10$), and half of the plots seeded with each native and naturalized species, respectively ($n = 35$). Seeds were planted in the center of each plot in a 5×4 grid with 10 cm spacing in early October 2018. In November 2018, we scored fall emergence and in early May 2019 we scored survival of fall seedlings and emergence of spring seedlings. We could not follow survival of marked seedlings beyond this point because the sample size of those remaining was limited for invasive plots (80% of plots had < 3 seedlings; range 0–5 seedlings). We recovered marked seeds with no evidence of emergence in field surveys and examined them under a dissecting scope for signs of pathogen attack including typical “black fingers of death” structures associated with *Pyrenophora* and infection cushions associated with *Fusarium* (Meyer et al. 2016).

Greenhouse PSF experiments

We initiated two PSF experiments in the greenhouse. The first experiment was designed to evaluate feedbacks of individual focal annuals on themselves and the second was designed to assay feedbacks from the focal invasive, *B. tectorum*, on the native perennials sown in our field experiment. This second experiment focused on *B. tectorum* to simplify logistics. Because we were interested in both abiotic and biotic PSFs, we followed the approach outlined in Castle et al. (2016) where soil was first trained by relevant species in “round 1” of the experiment. Shoot biomass was then harvested, and soil was sieved (10 mm) to remove most roots, placed back in the pot, and seeded with species for “round 2.”

In round 1 of the first experiment, each focal annual species sown in the field experiment was grown for three months (Nov–Jan) in 650 ml pots filled to approximately 500 ml with sieved (3 mm) soil collected to 10 cm deep from the experimental site and mixed with heat-treated sand and Turface (2:1:1, v:v:v). In round 2 of this experiment, seeds of the same species were planted in conspecific-trained soil (8 replicates per species in each round of the experiment for $n = 72$ pots per round). In both rounds, we grew three plants per plot after weeding out extra plants from the initial seeding effort and watered with tap water as needed. At the midpoint of

each round, all pots received 20 mL of a 0.5 g/L 20-2-20 (N-P-K, Peters Professional fertilizer, JR Peters, Inc., Allentown, PA, USA) solution to address nutrient limitation indicated by yellowing of leaves. At the end of each round, we harvested shoots, dried them for 48 hours at 62 °C, and weighed them. PSFs in this experiment were calculated by comparing biomass between rounds 1 and 2. To address possible differences in environmental conditions between rounds, we also grew each species in an inert medium (1:1 mix of Black Gold® Seedling Mix and Miracle Gro® Seed Starting Potting Mix) during each round ($n = 3$ replicates/species). In the second experiment, we first trained soils in round 1 by growing either *B. tectorum* or non-*Bromus* annuals (each of seven focal species) in pots for three months using the protocol described for the first experiment. In round 2, each of the 6 perennial species sown in our field experiment was grown either in the *B. tectorum*-trained soils (8 replicates/perennial species for 48 total pots) or in soils trained by non-*Bromus* annuals (7 replicates/perennial species comprised of 1 pot per non-*Bromus* species for 42 total pots). A single perennial plant was grown per pot after weeding out extra plants from the initial seeding effort, and plants were watered as needed with tap water. After two months, we harvested shoot biomass as described above. To evaluate PSF in this experiment, we compared biomass of perennials grown in soil trained by *B. tectorum* vs. by non-*Bromus* annuals. In both experiments, pots were not root-bound at the end of either round, and the few plants that died were excluded from analyses ($n = 4$ and $n = 8$ plants from each experiment, respectively).

Statistical analyses

For our field experiment, we used generalized linear models (GLMs) in SAS (PROC GLIMMIX, SAS Institute 2013) to compare responses (plant/litter abundance metrics, perennial seedling recruitment, marked *P. spicata* seed/seedling fates, and soil properties) among annual plant treatments, i.e., plots seeded with native, naturalized or invasive annuals, and unseeded controls. For responses measured in multiple years, we ran a separate model for each year. The annual plant treatment was included as a fixed factor in all cases. We did not include focal species identity in models as a random factor given that 1) species within each treatment category functioned similarly (Suppl. material 1: fig. S1) and were allowed to intermix (see above), and 2) we did not intend to extrapolate results to all potential species in each treatment category (as would apply if species was included as a random factor) but rather contrast the behavior of annual bromes to a reasonable representation of native and naturalized annuals, respectively. To further evaluate significant treatment effects ($P < 0.05$), we tested for post-hoc differences using multiple comparisons wherein the P -value was adjusted for the number of comparisons via the Bonferroni method. Response variables were fit to the most appropriate distribution, as assessed by examining scatterplots of residuals against predicted values (negative binomial for perennial seedling counts; lognormal distribution for biomass and soil P, K, S, Cu, and CEC; beta for proportion of marked *P. spicata* seeds emerging and seedlings surviving, respectively; and normal for remaining variables). We present least squares means and SEs back-transformed from the scale used in analysis as relevant. For analysis of pathogen presence on unemerged *P. spicata* seeds, we simplified data to the plot level and tested for differences in attack frequency using Fisher's Exact Test, as more complex analyses were limited by the lack of detections.

We also evaluated the relationship between recruitment of native perennials and conditions in plots to explore potential mechanisms governing succession in our field experiment. To do so, we used GLMs with perennial seedling counts from 2018 (the cohort linked to sampling of soil properties and to final biomass measures in the subsequent year) as the response fit with a negative binomial distribution. Based on observed differences among treatments, we opted to construct separate models for 1) plots established with “non-invasive” annuals (native or naturalized treatments) or eventually colonized by these species (controls), and 2) invasive annual plots. This allowed us to take a simple approach to examining whether mechanisms of perennial establishment might differ for communities dominated by either non-invasive or invasive annuals. Model covariates represented measured biotic and abiotic conditions from 2018, and all were considered in the same multivariate model to isolate the independent influence of each covariate (i.e., when variation attributable to other covariates was accounted for). These covariates were total focal annual cover, litter cover, soil moisture, and soil NH_4^+ and NO_3^- (remaining soil properties showed minimal differences among treatments; see Results). We also included an additional measure of litter abundance, litter biomass, in models because litter properties differed between non-invasive/control and invasive annual plots. For plots dominated by non-invasive species, litter cover captured within-year variation in litter abundance (e.g., litter cover and biomass measured in 2019 were significantly correlated: $r = 0.12$, $P = 0.002$), which was largely two-dimensional. However, in invasive plots, old plant material accumulated in three dimensions and litter cover did not suffice to capture variation in litter quantity among plots (e.g., litter cover and biomass measured in 2019 were not significantly correlated: $r < 0.1$, $P = 0.18$; see Results). Therefore, in addition to including litter cover in models (as measured in 2018 to align with seedling counts), we also included litter biomass (only measured in 2019). We screened for potential multicollinearity issues by testing whether model covariates were highly correlated in bivariate space ($r > 0.9$; Tabachnick and Fidell 2007), but these correlations were modest ($r < 0.6$). While P -values tested for significant covariate relationships, we also wanted a means of comparing the relative contribution of each covariate to overall variation in recruitment (akin to an r^2 value). For this purpose, we dropped covariates one at a time from the full model to determine the effect on AICc (Akaike’s Information Criterion corrected for small sample sizes), which provides a measure of expected predictive power weighted by the number of model parameters (Bolker et al. 2009).

For the greenhouse experiment designed to compare PSFs of individual focal annuals among native, naturalized and invasive taxa, we treated shoot biomass (mean of three plants per pot) as the response in a generalized linear mixed model in SAS (PROC GLIMMIX, SAS Institute 2013). Fixed factors were round (round 1: growth in untrained soil vs round 2: growth in conspecific-trained soil), annual plant type, and their interaction, and pot was included as a random factor. To test whether environmental conditions differed between rounds for focal annuals grown in an inert medium, we ran a GLM with shoot biomass (mean of three plants per pot and species) as the response and round as a fixed factor. For the experiment designed to test for PSF from the focal invasive, *B. tectorum*, on native perennials, we used a GLM with shoot biomass as the response. Fixed factors in this model were soil type (soil trained by *B. tectorum* vs. by non-*Bromus* annuals), species (each of six perennials from our field experiment), and the soil type x species interaction. For all three analyses of greenhouse biomass responses, we specified a normal distribution based on satisfaction of criteria described above.

Results

Early seral community composition

Focal annuals established rapidly and formed monocultures in plots where sown in the first year after seeding, 2016 (Fig. 1a, Suppl. material 1: table S2). In this year, mean cover per seeded focal species was substantial ($\geq 40\%$) in all seeded treatments, though more than twice as high for invasive compared to native or naturalized annuals ($P < 0.001$). In 2017, seeded annual species retained their dominance where sown, with similar cover among seeded treatments (Fig. 1b,

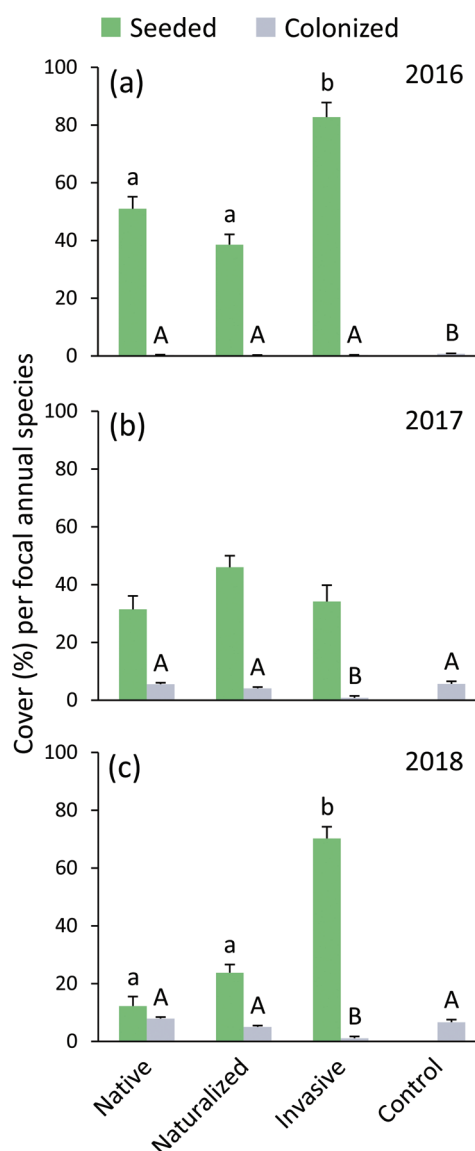


Figure 1. Abundance of seeded and colonizing focal annual species by treatment. Given is mean (+ 1 SE) cover per focal species seeded into experimental plots to represent native, naturalized, or invasive annuals, 2016–2018. Plots were disturbed and purged of plants prior to seeding in fall 2015, and control plots were not seeded. Sown native and naturalized annuals were allowed to colonize plots where unseeded, and mean (+ 1 SE) cover per species across this set of colonizing taxa is given for comparison to seeded species cover. Within-year patterns for each variable were evaluated with post-hoc comparisons when the treatment effect was significant ($P < 0.05$), and means that do not share letters (seeded species: lower case, colonizing species: upper case) are significantly different.

Suppl. material 1: table S2). Focal native and naturalized annuals spread into other plots, except the invasive annual plots, where colonization was negligible (80% lower than other treatments; $P < 0.001$). In 2018, seeded focal annuals remained a prominent component in all treatments where sown, but only invasive annuals retained monoculture status (Fig. 1c, Suppl. material 1: table S2). By this time, mean cover per seeded annual had diminished to modest levels for native and naturalized species but was more than three times greater for invasives ($P < 0.001$).

Perennial establishment and succession

Invasive annuals suppressed recruitment of sown native perennials in both 2017 ($F_{3,96} = 15.3$, $P < 0.001$) and 2018 ($F_{3,96} = 24.1$, $P < 0.001$; Fig. 2a). In 2017, perennial seedling counts averaged 50% lower in the invasive annual treatment relative to remaining treatments, with no differences among the latter. Despite much higher overall recruitment levels in 2018, the pattern was similar, with 42% fewer seedlings in the invasive treatment compared to native and naturalized treatments, which again did not differ from each other. However, seedling counts in 2018 were also depressed in unseeded controls relative to treatments established with native and naturalized annuals, indicating that these taxa facilitated native perennial recruitment.

This pattern of recruitment translated to marked differences in community composition in 2019 as measured by final plot biomass, with invasive annuals impeding succession toward native perennial dominance (Fig. 2b). Focal annual biomass averaged three times greater in invasive plots relative to native and naturalized plots ($F_{3,96} = 29.9$, $P < 0.001$), with no difference between the latter treatments. In contrast, biomass of native perennials was depressed by > 60% in the invasive vs. native and naturalized treatments ($F_{3,96} = 5.3$, $P = 0.002$), again with no difference between the latter treatments. Hence, the invasive treatment remained dominated by annuals (invasive annuals had twice the biomass of perennials), while native and naturalized treatments had shifted strongly towards perennials (perennials had > 6 times the biomass of annuals). Focal annual biomass in controls was comparable to levels in native and naturalized treatments but lower than in invasive plots, while native perennial biomass in controls did not differ significantly from other treatments though trended towards the lower end of the continuum (Fig. 2b). As a result, composition in the control treatment was shifted only slightly towards perennials (perennials had 1.4 times the biomass of annuals), with prevalence of the latter limited primarily by poor recruitment relative to native and naturalized treatments in the prior year (Fig. 2a).

Treatment conditions and relationships with perennial recruitment

Both biotic and abiotic conditions differed among treatments, potentially influencing patterns of native perennial recruitment. Total focal annual cover accounted for the combined abundance of those annual species seeded into plots and those sown native and naturalized taxa colonizing from other plots for all but the invasive treatment, where colonization was negligible. Total focal annual cover was greatest in the invasive species treatment in two of three years despite the boost given to remaining treatments by colonizing taxa, and it was generally lowest in the control treatment due to the lack of initial seeding (Fig. 3a, Suppl. material 1: table S3). Total cover of focal annuals did not differ between native and naturalized treatments in any year.

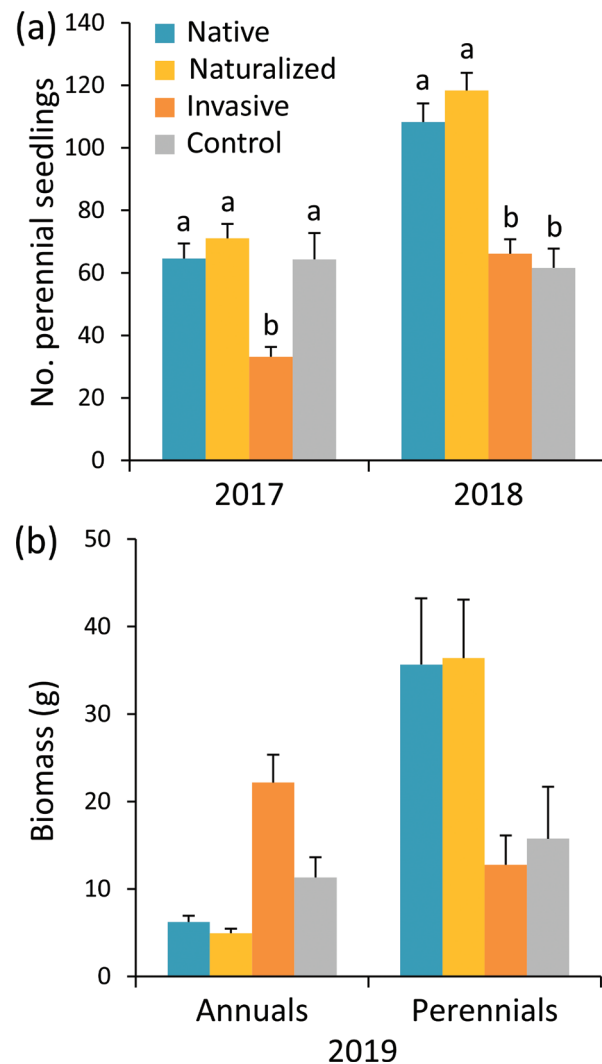


Figure 2. Recruitment of native perennials and final community composition by treatment. Native perennials were seeded into plots representing native, naturalized, or invasive annuals, and controls, and their mean abundance (± 1 SE) was measured by **a** seedling recruitment in 2017 and 2018, and **b** biomass relative to that of focal annuals at the end of the study in 2019. Sown native and naturalized annuals were allowed to colonize plots where they were not seeded and are included in focal annual biomass in all cases except the invasive treatment, where they were a minor component (Fig. 1). Native perennials were sown in fall 2016, counted and removed in spring 2017, and then sown again in 2017 and counted in 2018 to give two years of recruitment data. Perennial seedlings from 2018 were allowed to grow through the subsequent growing season to evaluate succession. Within-year patterns for each variable were evaluated with post-hoc comparisons when the treatment effect was significant ($P < 0.05$), and means that do not share letters are significantly different.

Litter cover was limited to trace levels ($<1\%$) across treatments in 2016, the first year after annuals were seeded, but in subsequent years, treatments differed markedly (Fig. 3b, Suppl. material 1: table S3). In 2017 and 2018, litter cover was highest in the invasive annual treatment and lowest in controls, matching the overall pattern seen for total cover of focal annuals. Notably, litter biomass in invasive plots was five times that of other treatments by 2019 ($F_{3,96} = 79.1$, $P < 0.001$; Fig. 3c). This pattern was driven by greater litter depth in invasive plots, as litter cover differed only modestly among treatments by this point (Suppl. material 1: fig. S2).

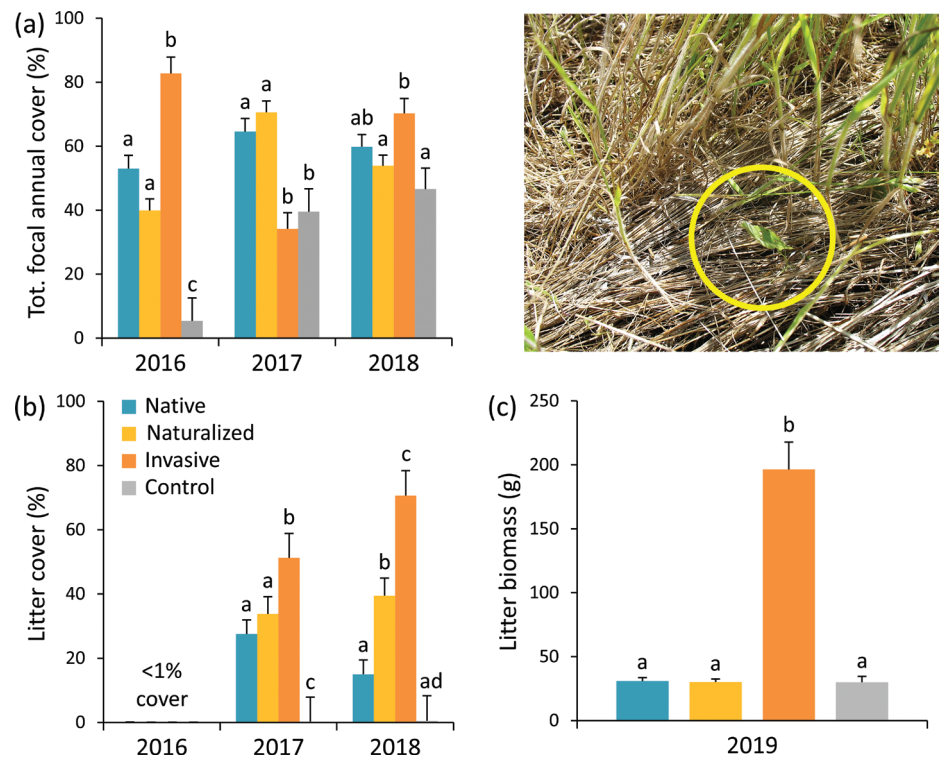


Figure 3. Total focal annual cover and litter in treatments representing native, naturalized, or invasive annuals, and controls. Given is mean (+ SE) **a** total cover of focal annual species, 2016–2018 **b** litter cover, 2016–2018 and **c** litter biomass at the end of the experiment in 2019. Sown native and naturalized annuals were allowed to colonize plots where they were not seeded and are included in total focal species cover in all cases except the invasive treatment, where they were a minor component (Fig. 1). Photo shows a perennial forb (*Balsamorhiza sagittata*) seedling (circled) emerging from the thick litter layer in an invasive plot. Within-year patterns for each variable were evaluated with post-hoc comparisons when the treatment effect was significant ($P < 0.05$), and means that do not share letters are significantly different.

Invasive annuals altered soil conditions, as measured in 2018. NH_4^+ concentration was > 50% higher on average in invasive annual plots relative to those established with native or naturalized annuals ($F_{3,94} = 6.4$, $P < 0.001$), and NO_3^- concentration was on average > 75% higher ($F_{3,93} = 7.8$, $P < 0.001$; Fig. 4a, b). For both nitrogen measures, control treatments generally fell at intermediate levels, though did not differ significantly from other treatments. Soil moisture was depressed in the invasive treatment, with a decline of 9% relative to naturalized plots and 16% relative to native and control plots ($F_{3,96} = 13.0$, $P < 0.001$; Fig. 4c). Comparison of additional soil properties showed minimal differences among treatments (Suppl. material 1: table S4).

To consider how these changes in biotic and abiotic conditions might influence patterns of native perennial recruitment, we modeled the relationship between seedling counts from 2018 and measured covariates (Suppl. material 1: table S5). For treatments established with non-invasive annuals (native or naturalized taxa) or eventually colonized by these species (controls), recruitment varied positively with litter cover in the multivariate model that included the full set of covariates ($P = 0.025$; Fig. 5a). In contrast, for the invasive annual treatment, where litter abundance was relatively high, recruitment correlated negatively with litter biomass ($P < 0.001$; Fig. 5b), while also correlating negatively with both focal annual cover

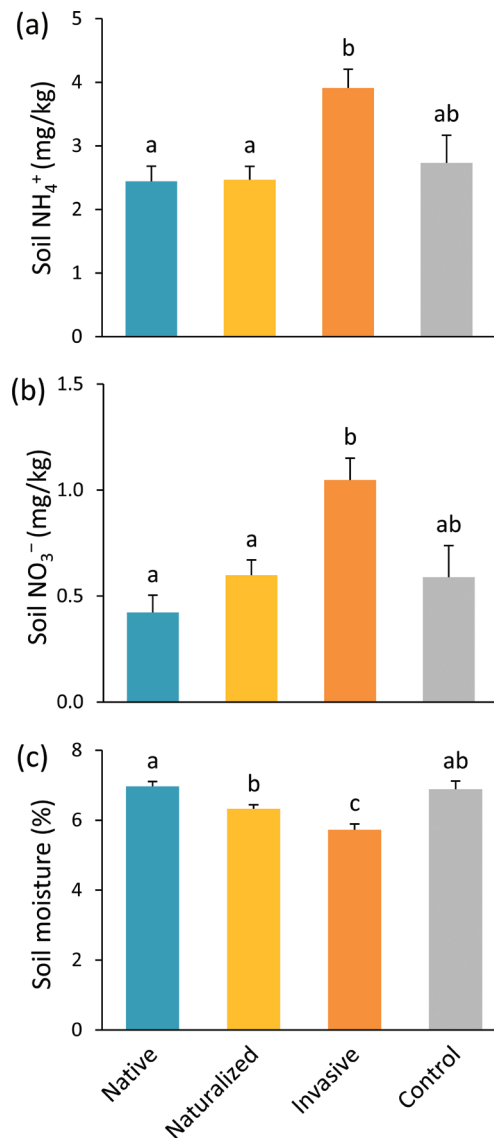


Figure 4. Soil conditions in treatments representing native, naturalized, or invasive annuals, and controls. Given is mean (+ SE) **a** available ammonium (NH_4^+) content **b** available nitrate (NO_3^-) content, and **c** soil moisture (volumetric water content); as measured in 2018. For each variable, means that do not share letters are significantly different, as evaluated with post-hoc comparisons when the treatment effect was significant ($P < 0.05$).

($P = 0.026$) and NH_4^+ ($P < 0.001$) vs. positively with NO_3^- ($P = 0.019$). When we individually removed covariates from the multivariate model for non-invasive/control plots, only removal of litter cover reduced model quality ($\Delta\text{AICc} = 2.6$). For invasive plots, removal of litter biomass and NH_4^+ from the multivariate model reduced model quality ($\Delta\text{AICc} = 14.0$ and 18.7 , respectively), whereas removal of NO_3^- had relatively little effect ($\Delta\text{AICc} = 1.2$). In remaining cases, covariate removal improved model quality ($\Delta\text{AICc} = -0.2$ to -6.7), i.e., these covariates were not important in explaining variation in recruitment.

To depict the linkage between native perennial recruitment and litter abundance across all treatments, we combined litter cover and litter biomass measures into a principal component and treated this as covariate in a model that also included a quadratic term to account for the observed shift in pattern at low vs high litter abundance. This exercise showed a significant unimodal relationship wherein perennial

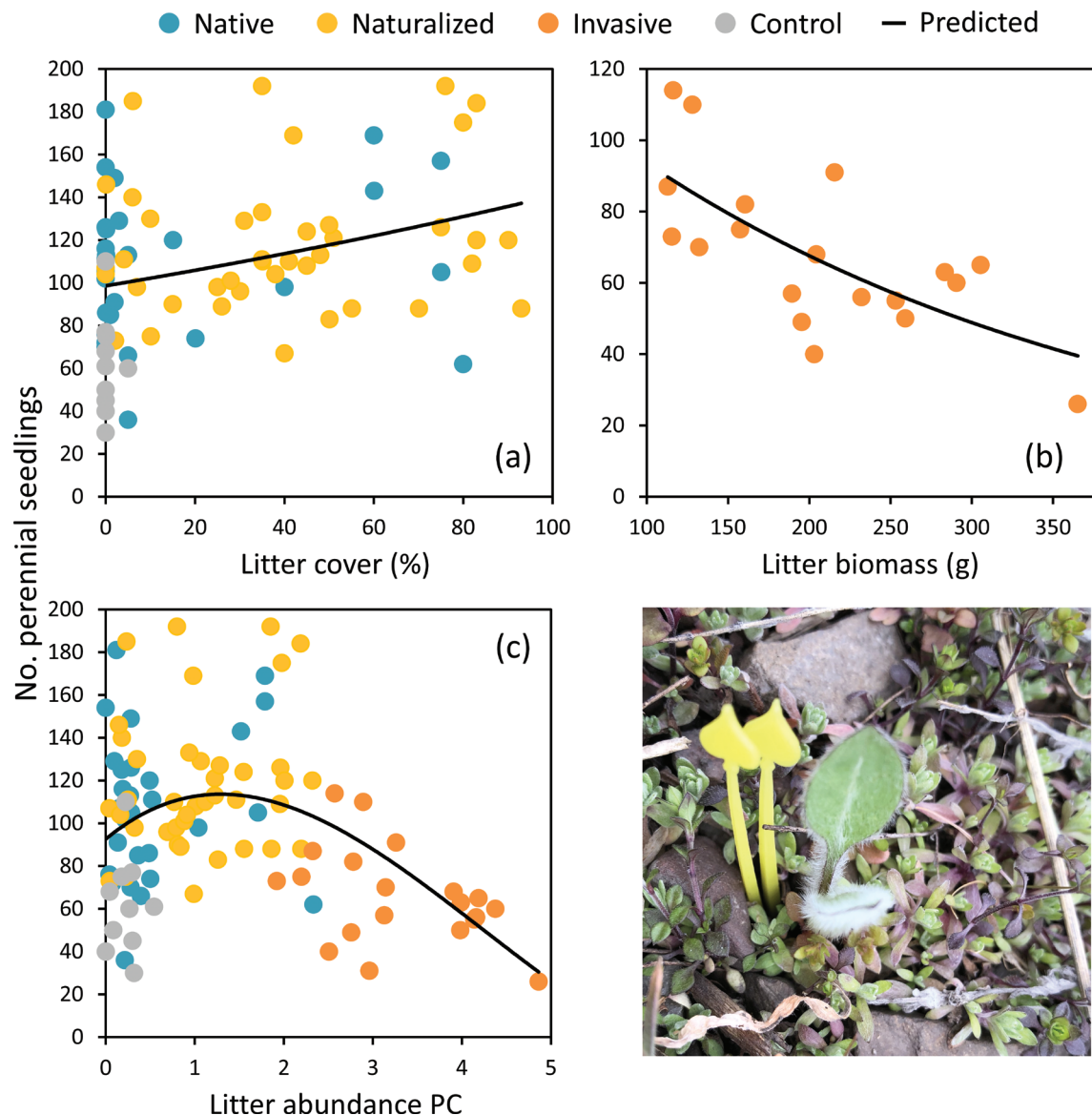


Figure 5. Relationships between recruitment of perennial seedlings in 2018 and litter abundance. Litter abundance was measured by **a** litter cover for treatments representing native or naturalized annuals and controls, **b** litter biomass for the invasive annual treatment, and **c** a principal component combining both litter metrics to visualize the unimodal relationship across all treatments (this relationship was also significant when we ran a parallel model using 2017 data: $F_{1,97} = 17.5$, $P < 0.001$). Photo shows a perennial forb (*Balsamorhiza sagittata*) seedling emerging amidst sparse litter in a naturalized plot. Note that recruitment was modeled with a negative binomial distribution in all cases and associated predicted relationships have been back-transformed to the original scale.

seedling recruitment increased at lower litter abundances represented by non-invasive annual and control treatments but decreased at higher litter abundances represented by the invasive annual treatment ($F_{1,97} = 21.4$, $P < 0.001$; Fig. 5c).

Pathogen spillover experiment

For the dominant native perennial grass, *P. spicata*, emergence of marked seeds planted into field plots in fall 2018 was reduced by > 25% in invasive annual vs other treatments ($F_{3,61} = 10.0$, $P < 0.001$), with no differences among the latter (Suppl. material 1: fig. S3). Similarly, survival of these emerging seedlings from fall 2018 to spring 2019 was depressed by > 55% in invasive relative to remaining

treatments ($F_{3,61} = 8.2$, $P < 0.001$), again with no differences among the latter (Suppl. material 1: fig. S3). Examination of unemerged *P. spicata* seeds ($n = 289$) in the lab revealed that significantly more plots had evidence of attack by the pathogen *Pyrenophora* in the invasive annual treatment (20% of $n = 20$ invasive plots) relative to remaining treatments (0% of $n = 40$ plots; Fisher's exact test: $P = 0.001$, $n = 60$), but this pathogen accounted for the fate of only 1% of seeds examined overall. We found no evidence of attack by *Fusarium* pathogens.

PSF experiments

In the greenhouse experiment designed to evaluate PSFs of individual focal annuals, growth across all species in round 2 was 33% greater than in round 1 ($F_{1,68} = 94.1$, $P < 0.001$) while growth in inert soil did not differ between rounds overall ($F_{1,16} = 0.7$, $P = 0.41$), indicating that plants tended to generate soil conditions that favored subsequent growth. However, this effect on focal annual growth did not differ among native, naturalized, and invasive annuals (round $\times$ annual type: $F_{2,68} = 0.7$, $P = 0.41$; Suppl. material 1: fig. S4a). Similarly, growth of six native perennial species did not differ in soil trained with the invasive annual, *B. tectorum*, vs soil trained with native or naturalized annuals in our second greenhouse experiment (soil type: $F_{1,71} = 2.4$, $P = 0.13$; soil type $\times$ species: $F_{5,71} = 0.2$, $P = 0.94$; Suppl. material 1: fig. S4b).

Discussion

Explaining how some introduced plants become problematic pests is a central question in invasion ecology. Here, we explored this question by contrasting the roles that native, naturalized, and invasive annuals play in the context of local succession rules in perennial grasslands. We found that invasive annual bromes, including both cheatgrass and Japanese brome behaved quite differently from native and naturalized annuals. Native and naturalized annuals both developed ephemeral populations and facilitated establishment of the climax perennial natives, transitioning the community from early seral annuals to perennial dominance, consistent with succession theory. In contrast, the invasive bromes established robust populations that strongly inhibited recruitment of native perennials, thereby impeding succession. In essence, the invasive annuals acted as both early seral and climax species, thereby breaking local assembly “rules” for succession. As we discuss below, framing our experiment in the context of local successional processes allowed us to identify traits like litter accumulation that may facilitate invasiveness but are not readily apparent using traditional trait comparisons.

In establishing the baseline for our system, we found that native annuals exhibited two functional behaviors that transitioned the community toward climax. First, they failed to maintain community dominance where sown, readily ceding space to other species (Fig. 1). Second, they facilitated establishment of perennial seedlings relative to unseeded control plots (Fig. 2). In sum, early seral natives actively transitioned the system toward climax in a Clementsian fashion (Clements 1916). Yet, naturalized annuals behaved in the very same manner, implying that the mechanisms underlying these patterns are generic in our system, more consistent with a Gleasonian perspective (Gleason 1926). This behavior of ceding space to other species is consistent with the idea that early seral species may experience stronger negative PSFs

(Kardol et al. 2006), which could provide a generic mechanism for this pattern, but we found no evidence for such PSFs in greenhouse experiments (see below). In sharp contrast, our invasive annual bromes maintained dominant populations at high biomass and suppressed establishment of native perennial seedlings, thereby impeding succession toward the native perennial state. This divergent behavior between invasive and naturalized annuals prevailed despite interannual variation in cover of sown annuals (Fig. 1) and recruitment of sown perennial seedlings (Fig. 2a) attributable to variation in weather conditions. While our experiment was relatively short, there was sufficient time for succession to progress to perennial dominance in the native and naturalized plots. Importantly, these findings are consistent with widespread accounts of annual bromes overtaking and dominating perennial grassland communities over vast expanses of the Intermountain West and Great Plains (Daubenmire 1942; Humphrey 1945; Mack 1981; Knapp 1996; Ogle et al. 2003; Germino et al. 2016; Pearson et al. 2016). Our results beg the question, how do these two groups of superficially similar introduced plants (all fast-growing, high-fecundity, annuals) generate such divergent outcomes in the recipient community?

In evaluating plausible explanations for these patterns, we examined covariates in the succession experiment and conducted additional experiments testing for pathogen spillover and PSFs (Fig. 6). Within our succession experiment, the invasive bromes generated higher live plant cover/biomass, litter cover/biomass, and available N (NH_4^+ and NO_3^-) and modestly reduced soil moisture relative to other treatments. A multivariate model including these covariates indicated that native perennial recruitment had strong negative relationships with litter abundance and NH_4^+ in brome plots, with minimal evidence for live cover/biomass or soil moisture effects when accounting for these other factors. Interestingly, litter exhibited positive correlations with seedling recruitment at the low abundances generated by the native and naturalized annuals and negative correlations at the high abundances generated by the invasive annuals. The result was a unimodal relationship between litter abundance and native perennial seedling recruitment across treatments (Fig. 5). This pattern could be driven by physical properties of litter affecting

Invasive annuals inhibit grassland succession

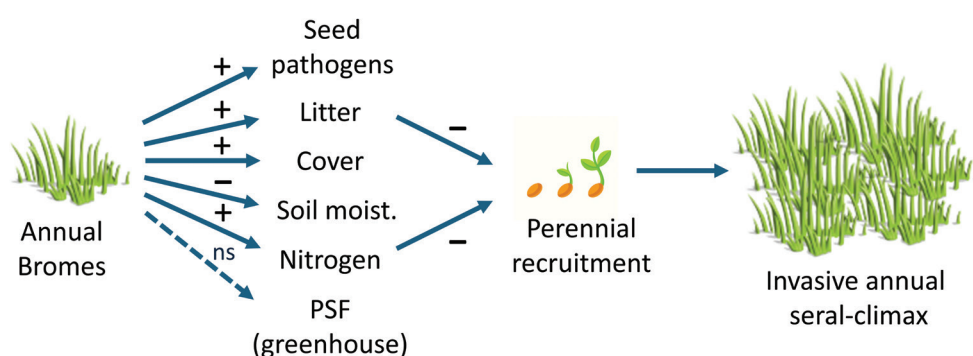


Figure 6. Potential mechanisms allowing invasive annual bromes to inhibit grassland succession, as explored in our study. Summarized are 1) significant effects of invasive bromes (*Bromus japonicus* and *B. tectorum*) on biotic and abiotic factors, measured relative to treatments established with native and naturalized annuals, and 2) primary linkages between studied factors and recruitment of sown native perennials revealed through multivariable modeling and secondary experiments testing for pathogen spillover and PSFs (greenhouse only). Signs indicate direction of effects. Note that pathways are not necessarily independent. Collective effects of invasive annual bromes, including excessive litter buildup, prevented the transition to native climax perennials and maintained communities in a novel seral-climax state (see Discussion for details).

seedling germination and/or survival in ways that could be beneficial at low litter levels, explaining observed facilitative effects of native and naturalized annuals, but detrimental at higher levels (Carson and Peterson 1990; Meyer et al. 2014; see reviews in Facelli and Pickett 1991; Loydi et al. 2013). While litter abundance could influence soil moisture in ways that affect seedlings, our analysis of recruitment patterns indicated that litter abundance was important even when soil moisture was controlled for, with the latter explaining no significant variation in multivariate models (Suppl. material 1: table S5). Hence, it was unclear from the factors that we measured by what mechanism litter might influence native perennial seedlings. Studies documenting negative effects of *Bromus* litter on native recruitment in other systems have implicated light limitation (Meyer et al. 2014; Molinari and D'Antonio 2020), but thick litter may also form a mechanical barrier to seedling establishment (Facelli and Pickett 1991; Jessen et al. 2023). In contrast, litter manipulation studies show that establishment and performance of annual bromes are facilitated by litter buildup (Evans and Young 1970; Meyer et al. 2014; Molinari and D'Antonio 2020). Whatever the specific mechanism, high litter abundance appeared to favor brome dominance by inhibiting native perennial establishment while allowing bromes to recruit sufficiently to maintain robust populations.

Of course, the link between litter and perennial recruitment is potentially confounded with litter chemistry since the species producing the litter differed among treatments. In terms of litter quality, many introduced plants have higher leaf N (low C:N ratio) than co-occurring natives, which has been linked to increased litter decomposition rates and elevated soil N (Liao et al. 2008). However, *B. tectorum* litter has relatively low N (high C:N ratio; McLeod et al. 2021), a trait likely common to annual brome grasses and linked to slower decomposition, greater litter accumulation, and reduced soil N (Evans et al. 2001; Bansal et al. 2014). Although such a reduction in N inputs from litter could cause reduced seedling performance, we found that soil N was actually elevated in brome plots (see below), and effects of litter abundance on perennial recruitment were independent of soil N (Suppl. material 1: table S5). Alternatively, litter could suppress seedlings by leaching phytotoxins (Facelli and Pickett 1991), but field and greenhouse experiments evaluating the chemical effects of introduced annual *Bromus* litter on grassland species emergence have shown negligible effects (Amatangelo et al. 2008; Chen et al. 2018; Molinari and D'Antonio 2020). Collectively, these studies in combination with our results suggest that chemical properties of *Bromus* litter were unlikely to play a direct role in suppressing native plant recruitment, although they likely influenced litter buildup.

Numerous studies have linked annual bromes to elevated soil N (Paschke et al. 2000; Stark and Norton 2015; McLeod et al. 2016), but how they elevate N has been unclear. Recent work in our system indicates that *B. tectorum* is associated with a greater abundance of ammonia-oxidizing bacteria, which convert NH_4^+ to NO_3^- (McLeod et al. 2016). If annual bromes elevate N over long time periods, this can generate soil legacies that inhibit succession and impede restoration because elevated N favors annuals over perennials (see Paschke et al. 2000; Mazzola et al. 2011). However, the N levels we measured in brome plots were comparable to those observed in native grasslands in our system (McLeod et al. 2016), and hence would not be predicted to reduce seedling establishment. Yet NH_4^+ correlated negatively with native perennial recruitment in brome plots (evident even when we controlled for other measured factors). We found no plausible explanation for this pattern (NH_4^+ was not correlated with measured variables other than NO_3^-), but

we note that elevated N likely promoted *Bromus* populations (e.g., Paschke et al. 2000; Mazzola et al. 2011; Piper et al. 2015; Stark and Norton 2015) in a positive feedback process that fed the production of large quantities of litter (biomass of *Bromus* species and litter were positively correlated: $r = 0.63$, $P = 0.003$), which in turn suppressed perennial seedling recruitment.

Despite the potential for PSFs to strongly influence both succession and invasion processes, we found no evidence in our greenhouse experiments that PSFs differentially affected growth of *Bromus* relative to other annuals or that growth of native perennials was suppressed in soil trained by cheatgrass vs. other annuals. Whereas we might expect all the annuals to have negative feedbacks as predicted in the context of succession (Kardol et al. 2006; Kulmatiski et al. 2008) or the invasives alone to have strong positive feedbacks due to reduced enemies and/or more beneficial associations with mutualists (Klironomos 2002; Callaway et al. 2004; Levine et al. 2006), if anything, we found evidence of positive feedbacks that were comparable among native, naturalized, and invasive annuals (Suppl. material 1: fig. S4a). Likewise, while the highly invasive bromes could inhibit succession by reducing the abundance of arbuscular mycorrhizal fungi (Lekberg et al. 2013) that benefit late successional perennial species more than ruderal annuals (Wilson and Hartnett 1998), we did not find evidence for feedbacks on native perennials (Suppl. material 1: fig. S4b). Although our greenhouse results suggest that PSFs did not explain patterns seen in our field experiment, such lack of concordance could be attributed to complexities linked to PSF experiments (Brinkman et al. 2010; Forero et al. 2019).

Pathogen spillover from annual bromes by black fingers of death (*Pyrenophora semeniperda*) may periodically suppress native grass establishment in invaded stands (Beckstead et al. 2010, 2016; Meyer et al. 2014; but see Mordecai 2013). Although examination of marked seeds of *P. spicata*, the dominant native perennial grass in our system, revealed that attack by *Pyrenophora* was more likely in *Bromus* plots, providing evidence of pathogen spillover, this pathogen accounted for failed emergence of very few recovered seeds. Similarly, we did not find evidence of attack by other pathogens like *Fusarium* spp. Yet, the carefully monitored *P. spicata* seeds revealed that both seedling emergence and survival were strongly suppressed in brome plots. Moreover, most seeds that failed to emerge also showed no evidence of germination (93% of $n = 289$) upon inspection in the lab. These results suggest that factors such as excessive litter levels, which can influence both germination cues and seedling survival, were more important than seed pathogen spillover in explaining *Bromus* impacts on perennial recruitment in our experiment.

Cheatgrass is the most notorious invasive plant in the western United States (Mack 1981; Knapp 1996; Bradley et al. 2018). Hence, understanding how this species and other annual bromes achieve community dominance is critical to mitigating their impacts. Cheatgrass has been linked to “grass-fire” cycles in the western United States wherein it generates abundant fine fuels that increase fire frequency, thereby favoring cheatgrass over natives, which are poorly adapted to frequent disturbance (D’Antonio and Vitousek 1992; Balch et al. 2013; Bradley et al. 2018). Such fire feedbacks could explain cheatgrass dominance if fire return intervals increased sufficiently to lock the system into an early seral state, such as the 3–5 year fire return intervals described for 9 sites in Idaho by Whisenant (1990). However, modeling approaches applied over vast regions of the Intermountain West have reported fire return intervals of 50–78 years in cheatgrass-invaded sites (Balch et al. 2013). Although these intervals translate to a marked increase in fire frequencies,

the period between fires should be more than sufficient for intermountain grasslands to reach climax. In our plots, perennial dominance over native and naturalized annuals was achieved in a few growing seasons. While cheatgrass's influence over fire regimes undoubtedly favors its expansion over large landscapes, this process is insufficient to explain how this annual achieves dominance over perennial grasslands. We hypothesize that cheatgrass overtakes native communities by way of a “ratcheting” effect wherein it invades recent disturbances and then dominates newly claimed ground by inhibiting succession. One study sowing cheatgrass into experimentally disturbed vs. control plots demonstrates this species' initial reliance on disturbance, showing that while cheatgrass thrives in disturbed plots, it performs very poorly in undisturbed plots (Pearson et al. 2023). Our hypothesis is consistent with historical accounts describing the progression of cheatgrass invasion as a ratcheting effect wherein disturbances like grazing and human activities facilitate initial invasions that then shift systems from perennial to annual dominance (note that early accounts do not invoke fire, see Mack 1981 and citations therein). We propose that cheatgrass overtakes perennial intermountain grasslands, shifting them to a novel alternative equilibrium state (*sensu* Hobbs et al. 2006) that we refer to as a seral-climax invasive community, by impeding succession following disturbance via processes linked to litter buildup.

Traditional approaches to understanding how some introduced species become problematic pests have compared traits among invasive, naturalized, and native species without reference to the local assembly rules that define recipient communities. Such studies have broadly linked invasive species to traits like high fecundity and rapid growth (Pysek and Richardson 2007; Van Kleunen et al. 2010; Jelbert et al. 2015; Moracová et al. 2015), but they do not explain why many introduced plants bearing similar traits are benign. While the introduced plants in our study exhibit superficially similar traits in that they are all ruderal annuals with rapid growth and high fecundity, the invasive and naturalized taxa demonstrated highly divergent behaviors when evaluated in the context of local assembly rules. The naturalized annuals closely followed local assembly rules to behave like the natives, explaining why these species do not overtake recipient communities. In contrast, the invasive annuals broke local assembly rules by impeding succession to act as both early seral and climax, or “seral-climax,” species. The invasive bromes appeared to inhibit succession by producing large quantities of litter that suppressed native perennial seedling establishment. While we were unable to identify the specific mechanisms underlying these inhibitory effects, they likely involve some aspect of ecosystem engineering. Notably, many of the worst invaders (including *B. tectorum*) have been linked to ecosystem engineering effects wherein the invader alters availability of resources like nutrients, moisture, light, etc. for other species (Crooks 2002). While obviously important, engineering effects may not always be amenable to *ex situ* comparisons typically applied to classic traits like seed mass, plant height, specific leaf area, etc. because the relevance and strength of engineering effects are explicitly measured at the local community scale. More generally, litter production may be considered an extended phenotype (*sensu* Dawkins 2016) much like spider webs and other engineering traits that are not conducive to traditional *ex situ* trait comparisons because they manifest as a function of environmental context. Contrasting native, naturalized, and invasive plants in the context of local assembly processes (*sensu* Pearson et al. 2018a) provides a powerful means for elucidating invader advantages, including mechanisms not readily amenable to traditional *ex situ* trait comparisons.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

DEP and YKO initiated the project and developed and implemented the field experiment. YL led in developing the plant-soil-feedback experiments, soil nutrient sampling, and evaluation of seed pathogens. YKO analyzed the data. All authors contributed to the writing.

Data availability

Data is available in Dryad: <https://doi.org/10.5061/dryad.9ghx3fft6>.

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Supplementary material 1

Additional results and study design information for field and greenhouse experiments

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