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Using patchy plant invasions to understand how diffuse interactions modify facilitation and competition

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USING PATCHY PLANT INVASIONS TO UNDERSTAND HOW DIFFUSE

INTERACTIONS MODIFY FACILITATION AND COMPETITION

By

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ABSTRACT

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Using patchy plant invasions to understand how diffuse interactions modify facilitation and competition

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Indirect interactions among plants promote conditionality in competitive outcomes that affect plant community structure and function. I utilized spatially patchy distributions of two invasive exotic plants, *Centaurea stoebe* and *Bromus tectorum*, to explore conditionality in plant interactions and the implications of this conditionality for community invasibility. Additionally, I expanded this research to investigate how these two invaders interact with each other as they overrun native ecosystems. Throughout intermountain prairie of western Montana *Centaurea* was found at high abundances in open prairie, but was a relatively minor component of the plant community under isolated *Pinus ponderosa*. In contrast, *Bromus* was also common in open prairie, but it was most dominant under *Pinus* canopies.

I then experimentally investigated the complex dynamics potentially driving apparent biotic resistance by *Pinus* to one exotic species but facilitation of a second. I found that *Pinus* directly inhibited *Centaurea* growth through shade and litter effects and attenuated the competitive effects of *Centaurea*. While *Pinus* litter strongly suppressed *Centaurea* establishment, *Festuca* and *Bromus* where much less effected. The native plant community and *Bromus* were thereby indirectly facilitated. Additionally, the allelochemical (±)-catechin that is exuded by *Centaurea* roots was more phytotoxic to *Festuca* in open prairie than under *Pinus* canopies and in prairie soils than in conifer soils when tested in a greenhouse. Plant-soil feedbacks were important as well. When *Centaurea* was grown in full sunlight it "cultivated" the soil such that legacy effects inhibited recruitment of *Festuca* long after *Centaurea* had been removed, but these feedback effects did not occur when *Centaurea* cultivated soil in experimentally shaded plots. *Bromus* was directly facilitated by *Pinus* shade and soil but these effects were highly moderated by the native grass *Festuca idahoensis*. While many relatively straightforward pair-wise studies have shown direct facilitative effects of one species on another, these results demonstrate another form of biotic conditionality; strong facilitative effects manifest in pair-wise experiments can be eliminated or diminished by the presence of other competitors. In general, my results illustrate the importance of the competitive and facilitative interactions that occur among natives and exotics ultimately structuring plant communities on natural landscapes.

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PREFACE

In this dissertation I explore how the strongly contrasting biotic and abiotic conditions created by isolated *Pinus ponderosa* trees in intermountain grasslands affect the distributions and abundances of native and exotic plants and the interactions among them. The local abundance of two exotic plants, *Centaurea stoebe* and *Bromus tectorum* appear to be strongly affected by savanna pines, with *Centaurea* much less common under pines and *Bromus* much more common under pines. I used these spatial patterns to generate questions about the mechanisms that might determine local plant distributions and then tested those questions using experiments. This research has led to a keen interest in how plants respond to and manipulate their environment, as in Metlen *et al.* (2009), a review of plant behavioral plasticity and the role of plant secondary metabolites.

Direct effects of interactions between invaders and natives, such as biotic resistance to invasion (see Levine *et al.* 2004; Maron & Marler 2007) and competitive exclusion of natives (see Levine *et al.* 2003; Ortega & Pearson 2005) have been described at length in the literature. However, the role of indirect interactions among natives and invasive exotics has been less addressed (but see Parker & Muller 1982; Siemann & Rogers 2003; Weir *et al.* 2006; and a review by White *et al.* 2006). In Chapter 1, I investigate how *Pinus* alters direct and indirect interactions among the invasive exotic *Centaurea stoebe* and native grassland species. Many isolated *Pinus* trees embedded within grasslands in western Montana harbor relatively intact native communities within highly invaded grasslands. Through a series of field observations and manipulative experiments, I found that *Pinus* influenced soil and sunlight in such a way that competitive outcomes improved for native grasses. Plant-soil feedbacks of *Centaurea* inhibited native grass regeneration in open grassland conditions, but not in experimentally shaded plots. Finally, the phytotoxicity of (±)-catechin, an allelopathic chemical exuded by *Centaurea* roots was diminished in soil from under *Pinus* canopies. This detailed mechanistic examination of how a native tree shifts important interactions among natives and an exotic also provides a good demonstration of the importance of allelopathy and plant-soil feedbacks for the invasive process.

Facilitation can strongly promote exotic plant invasion (Maron & Connors 1996; Rice & Nagy 2000; Badano *et al.* 2007), but rarely are interactions among natives and invaders considered in the context of such facilitation. In Chapter 2, shade and fertile soil from under savanna pines facilitated the exotic annual grass *Bromus tectorum*. However, competition from a native grass substantially moderated these beneficial effects. Many relatively straightforward pair-wise studies have shown direct facilitative effects of one species on another (Callaway 2007). A smaller number have shown that by suppressing a competitor one species can indirectly facilitate another, subordinate species (Miller 1994, Levine 1999). My study is unique because strong facilitative effects were manifest in pair-wise experiments but they were eliminated or diminished by the presence of the native competitor, illustrating the importance of examining facilitation in a broader community context and the potential importance of intact native communities for resisting exotic plant invasion.

Competitive interactions among invaders have been minimally investigated (but see Piemeisel 1951; Rice & Nagy 2000; Belote & Weltzin 2006). In Chapter 3, I investigated how *Pinus* altered interactions between two strong invasive exotic plants, *Centaurea stoebe* and *Bromus tectorum*. I found that *Centaurea* dominated plant communities in open prairie but *Bromus* dominated under large isolated *Pinus* canopies, where *Centaurea* was much less abundant. Interestingly, *Bromus* abundance can increase dramatically after *Centaurea* removal in the prairie (Story *et al.* 2006; Ortega & Pearson, *in press*) suggesting that competition with *Centaurea* may be inhibiting greater *Bromus* invasion into prairie habitats. I found that *Pinus* indirectly facilitated *Bromus* in-part by allelopathically inhibiting *Centaurea* establishment. This is a unique contribution because while allelopathic effects of invaders have been well documented (e.g. "novel weapons" Callaway & Ridenour 2004), the reverse ecological interaction, allelopathic effects of natives on invaders, has been proposed (Verhoeven *et al.* 2009) but only rarely supported (Parker & Muller 1987; Weidenhamer & Romeo 2005). In addition to allelopathic effects on establishment, *Pinus* shifted competitive interactions between these two strong invaders to favor *Bromus*. While performance of both species was increased in fertile soil from under *Pinus* canopies, shade promoted *Bromus* growth and suppressed *Centaurea* growth. Additionally, *Pinus* litter eliminated the competitive effects of *Centaurea* on *Bromus*, but the competitive effects of *Bromus* were increased. In sum, I show that exotic invasions on natural landscapes are altered by interactions among invaders, as well as by the competitive and facilitative interactions that occur among natives and exotics.

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CHAPTER 3

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CHAPTER 1 - PONDEROSA PINE INDIRECTLY ALTERS COMPETITIVE AND ALLELOPATHIC INTERACTIONS AMONG NATIVES AND AN INVASIVE PLANT

Abstract

Invasive plants can have powerful effects on the communities they invade but these effects are invariably patchy at larger scales. *Centaurea stoebe* is an abundant and high-impact invader in intermountain grasslands of Montana, but in natural pine savannas we found that it is far less common under *Pinus ponderosa* canopies than in nearby open grassland.

Centaurea germination was reduced under *Pinus* and *Centaurea* recruitment was more inhibited by *Pinus* litter than was recruitment of the native grass *Festuca idahoensis*. In garden experiments, when *Centaurea* was grown in full sunlight it "cultivated" the soil such that legacy effects inhibited recruitment of *Festuca* long after *Centaurea* had been removed, but these feedback effects did not occur when *Centaurea* cultivated soil in experimentally shaded plots.

In reciprocal transplant experiments which bypassed the recruitment phase in the field we found that *Pinus* had no direct effects on *Centaurea* or the native grass *Pseudoroegneria spicata*, and that the strong competitive effects of *Centaurea* on *Pseudoroegneria* that occurred in open prairie disappeared under *Pinus* canopies. The allelochemical (±)-catechin was more phytotoxic in field experiments in open prairie than under canopies and similarly, the effect of catechin on *Festuca* was stronger in prairie soils than in conifer soils.

Our results show that *Pinus* enhances biotic resistance to *Centaurea* invasion directly through shade and litter, but also through attenuation of competitive effects of the invader through indirect mechanisms that are not easily predicted from the direct effects of *Pinus* on either the native or the invader. Along with generalized competitive effects, we explicitly show that shifting allelopathic effects and plant soil feedbacks are associated with the success of an invasive plant.

Keywords: biotic resistance, *Centaurea maculosa*, invasion, litter, plant community

"Clearly, there is no such thing as absolute competitive ability, nor any measure…that confers competitive ability under all conditions" Huston & Smith 1987

Introduction

Exotic invasive plant species can create unusually homogeneous and species-poor native communities and dramatically alter ecosystem processes (Vitousek *et al.* 1996*;* Liao *et al.* 2008). Many invasive species exhibit markedly strong competitive effects (Melgoza *et al.* 1990; D'Antonio & Mahall 1991; Ortega & Pearson 2005) and in some cases greater competitive effects in their invaded range than in their native range (Callaway & Aschehoug 2000; He *et al.* 2009; Thorpe *et al.* 2009). Exceptionally strong competitive ability has been discussed as a primary mechanism for invasive success and impact (reviewed by Levine *et al.* 2003), a perspective that is reinforced by the very high densities that invaders can reach in their non-native ranges. However, no species possess traits that confer competitive dominance under all conditions (Huston & Smith 1987) and the monospecific stands so emphasized in research on invasive plants are invariably less homogeneous and much patchier at larger scales (Kolb *et al.* 2002; Lortie & Cushman 2007; Melbourne *et al.* 2007). Spatial variability in the dominance of invaders may occur for many reasons, but patchiness associated with clear biotic or abiotic factors offers unique opportunities to explore the conditionality of competitive interactions in invasions (Kolb *et al.* 2002; Lortie & Cushman 2007).

Biotic resistance, based on mechanistic explanations for attenuated exotic invasion, was proposed by Elton (1958) to formalize the idea that some native organisms or systems possess biological traits that inhibit exotic invasion. Most studies of biotic resistance to exotic plant invasion have focused on native herbivores or predators rather than competition from other plants (e.g., Maron & Vilà 2001*;* Levine *et al.* 2004). However, biotic resistance can be an emergent property of plant community diversity *per se* (e.g. Levine *et al.* 2004*;* Maron & Marler 2007) suggesting that diversity increases whole-community utilization of, and competition for, resources.

Individual plant species can also have important effects on the invasibility of their communities; in fact, resistance to exotic invasion is often driven by the emergent traits of dominant species or species mixtures in communities (Zavaleta & Hulvey 2004*;* Emery & Gross 2006). This sort of biotic resistance to an invader could be driven by direct facilitation within the native community, direct inhibition of the invader, or by indirectly altering the way that the invader interacts with other native species. While biotic resistance is often studied in the context of direct effects on invader performance, indirect interactions (Levine 1976; Miller 1994; Callaway & Pennings 1998) are often underestimated even though they may be important aspects of understanding plant invasions (see Siemann & Rogers 2003; Weir *et al.* 2006; review by White *et al.* 2006).

Overstory trees exert significant direct and indirect competitive and facilitative influences on understory communities by altering above and belowground resource availability (Callaway *et al.* 1991; Barnes & Archer 1999), physical environment attributes (Callaway 2007), litter properties (Iason *et al.* 2005; Gundale *et al.* 2008) and nutrient cycling (Hibbard *et al.* 2001; Rich *et al.* 2003). Furthermore, invasive exotic species can be strongly inhibited (Von Holle *et al.* 2003; Chambers *et al.* 2007) or facilitated (Maron & Connors 1996; Holzapfel & Mahall 1999) by overstory canopies.

Centaurea stoebe L. ssp. *micranthos* (Gugler) Hayek (spotted knapweed; nee *C. maculosa* Lam.) can be a “strong invader” of native grassland communities, displacing native species and decreasing local biological diversity (Tyser & Key 1988; Ridenour & Callaway 2001; Ortega & Pearson 2005). Variation in *Centaurea* invasive success could be driven by changes in the many complementary mechanisms that have been shown to promote the competitive dominance of *Centaurea* including escape from specialist enemies (Story *et al.* 2000; but see Müller-Schärer & Schroeder 1993), escape from limiting soil biota (Callaway *et al.* 2004a), indirect competitive advantages from associations with arbuscular mycorrhizae (Marler *et al.* 1999; Carey *et al.* 2004; Callaway *et al.* 2004b), allelochemicals (Ridenour & Callaway 2001; He *et al.* 2009; Thorpe *et al.* 2009) and altering ecosystem processes (Thorpe *et al.* 2006; Liao *et al.* 2008).

Savannas co-dominated by *Pinus ponderosa* Dougl. ex Laws (ponderosa pine) are common in the northern Rocky Mountains and its canopies have striking effects on the abundance of some invasive herbaceous species, including *Centaurea*. Here we utilize patchiness in intermountain grasslands caused by *Pinus* trees to explore factors that 1) directly alter the performance of *Centaurea* and native species and 2) modify competitive interactions between *Centaurea* and native species. We also examine conditionality in plant-soil feedbacks and allelopathic interactions as mechanisms that modify interactions among natives and exotics.

Methods

Field patterns

Our research was conducted in intermountain grasslands of western Montana that were dominated by *Pseudoroegneria spicata* (Pursh) Á. Löve and *Festuca idahoensis* Elmer (Mueggler and Stewart 1980). The sites were predominantly grassland with widely spaced trees (>20 m apart) as a result of environmental conditions, not disturbance. The spatial relationship between *Pinus* and *Centaurea* abundance was assessed at eight sites, separated by a minimum of 1.2 km and a maximum of 80 km (see Appendix A). Five sites were heavily invaded by *Centaurea* ("invaded" sites), one of which had been harvested ≈30 years prior, allowing us to separate site effect from tree effect. Three of the sites had very little *Centaurea* invasion ("uninvaded" sites).

All sampling was conducted in the month of July, invaded sites in 2006, the invaded/harvested site in 2007 and the uninvaded sites in 2008. At each site 6-15 trees were selected as target trees, resulting in 36, 10 and 27 trees in the invaded, invaded/harvested and uninvaded sites, respectively. For each tree four transects were established, radiating from the bole in the four cardinal directions. Along these transects, vegetation was evaluated in seven 1-m^2 quadrats located in reference to the canopy edge (dripline): ¼ the distance from bole to dripline, ½ the distance from bole to dripline, five cm inside the dripline, then five cm, two m, four m and eight m from outside the dripline. When quadrats fell <2 m from the dripline of a non-target tree (>1.37 m tall) they were not measured, resulting in 1494 total quadrats. At the harvested site, "trees" were stumps remaining after harvest.

Cover of every species was estimated to the nearest percent in each quadrat with a lowest value of 0.1%. Duff depth and litter depth were measured in all quadrats. Aspect and slope were measured at each tree and then averaged for a site (Appendix A).

At the invaded sites, photosynthetically active radiation (PAR) and soil nutrient content were measured at all 27 trees. At each transect we measured PAR using a Li-Cor LI-250A© light meter and measurements were made ½ the distance from the tree bole to the dripline and in the open 8 m from the dripline. Light measurements were made 0.3 m above the ground and only when the sun was unobstructed by clouds on August 1, 2, 3 and 4 between 12:50 PM and 3:15 PM providing the greatest possible contrast in PAR between conifer and prairie habitats.

Nutrient availability was measured in the top 15 cm of mineral soil, sampled at one randomly chosen transect per tree at locations ½ the distance between the bole and the dripline (conifer habitat) and 8 m beyond the dripline (prairie habitat). Soil samples were placed in airtight plastic bags on ice and later analyzed for extractable NO_3^-, NH_4^+ and PO_4^{-3}. Samples were air dried at 30ºC for 48 hours and sieved through 2-mm mesh. From each sample, 25 g were placed in 200 ml French square bottles with 50 mL 2 molar KCl (for the NO_3^- and NH_4^+ extracts) or 50 mL 0.01 molar CaCl (for the PO_4^{-3} extract), then agitated for one hour. Samples were then filtered through Whatman no. 42 filter paper. All extracts were analyzed using a segmented flow analyzer (Auto Analyzer III, Bran Luebbe, Chicago, IL) using the Berthelot reaction for NH_4^+ analysis (Willis *et al.* 1993), the cadmium reduction method for NO_3^- analysis (Willis & Gentry 1987) and the molybdate method for the phosphate (Murphy & Riley 1962).

Abundance and relative cover of *Centaurea* and of all other species combined were analyzed using mixed model nested ANOVA with tree nested within site, and with tree and site classified as random variables. Invaded and uninvaded sites were analyzed separately, as was the harvested site. Initial tests were conducted with transect azimuth as a random variable. Azimuth was never significant, however, so we averaged all four transects per tree. Changes in absolute and relative cover in relation to pine trees were assessed with distance to tree bole as a fixed factor and an interaction term between distance to bole and site.

Differences in PAR, duff depth, litter depth, NH_4^+, NO_3^- and PO_4^{-3} were analyzed as above, but with the average of the measurements from outside the canopy pooled as prairie habitat and from under the canopy as conifer habitat. Habitat (conifer or prairie) was then treated as a fixed factor. In all instances, distributional assumptions of normality and homogeneity of variance were assessed and when necessary statistical tests were conducted with transformed data. Variance in relative cover could not be homogenized with transformations. However, nested ANOVA is robust to this assumption particularly with sample sizes greater than six (Underwood 1997) and so we reported these results. All statistical procedures were conducted in SPSS, 16.1.0 (SPSS, Chicago, Illinois, USA).

In order to gauge the severity of *Centaurea* invasion at our invaded sites and provide context with other studies of *Centaurea* impacts (e.g. Ortega & Pearson 2005), we calculated Pearson's correlation coefficients between *Centaurea* cover in invaded prairie plots and total plant cover, the Shannon-Weiner estimate of diversity and the cover of the two most abundant grasses, *Festuca* and *Pseudoroegneria*. We constrained the analysis of community measures to only those plots containing *Centaurea* and correlations with *Festuca* and *Pseudoroegneria* to only plots containing these species.

Effect of *Pinus ponderosa* on *Centaurea stoebe* germination

Centaurea stoebe germination rates were assessed by placing 36 experimental seed packets under and away from 18 isolated pines growing in grassland, nine at the Mount Jumbo site and nine at the Three-mile site (Appendix A) on 15 November 2006. Seed packets were 5 x 3 cm, contained 20 seeds each and were planted under the duff. Packets allowed the seeds to interact with their environment but allowed each seed to be accounted for. Seed packets in the field were collected on 8 May 2007. Germination rates were measured by counting seeds with radicles, then the viability of remaining seeds was tested by soaking for 48 hours at room temperature and assessing radicle emergence. Seeds that had still not germinated were soaked in 0.1% 2,3,5-Triphenyltertazaolium chloride for 24 hours and examined for CO_2 production (Cottrell 1947). Germination rates were analyzed using mixed model nested ANOVA with habitat as a fixed factor, replicate nested within site and with replicate and site as random variables.

Effect of *Pinus ponderosa* on establishment

Field litter experiment

We tested the effects of prairie and conifer habitat, *Pinus* litter and competitive effects of intact plant communities on *Centaurea* establishment in a fully factorial field experiment at the Cyr Ridgeline site. We used 10 trees as replicates, with 4 randomly placed 1 m^2 plots under each tree and another 4 in open prairie surrounding each tree. Litter and duff were removed from all 80 plots, but one half of the plots in each habitat and each neighbor removal treatment were subsequently covered with 7 cm of *Pinus* litter and duff. Neighbors were removed with Roundup© (50 mL Roundup/1000 mL water) applied on 26 September 2008. Entire 1 m^2 plots received these combinations of treatments, but to avoid edge effects only a central 0.25 m^2 subplot was used for seeding and sampling. Each subplot was sowed with 500 *Centaurea* seeds on 3 October 2008. The numbers and biomass of new *Centaurea* seedlings were assessed on 17 October 2009. These data were analyzed using a saturated mixed model ANOVA with habitat, litter and neighbors as fixed factors and replicate as a random factor.

Greenhouse litter experiment

The effect of *Pinus* litter on the establishment of *Festuca* and *Centaurea* was examined in more detail in the greenhouse. All greenhouse experiments were conducted at the University of Montana (Missoula, Montana, USA) Diettert greenhouse (lat. 46.842°, long. -114.093°, 990 m elevation). Greenhouse temperatures during experiments ranged from 15 to 30ºC, similar to early summer temperatures outside. Natural light in the greenhouses was supplemented by metal halide bulbs, and total photosynthetically active radiation (PAR) during the day remained above 1200 $\mu mol/m^2/s$ with a day length of 13 hours.

Field soil from under conifers and from open prairie was placed into 2.4 L pots; 18 cm diameter, 22 cm deep (n=10). Treatments included prairie soil with no litter, conifer soil with no litter, 20 g of pine needles (7 cm deep) scattered on the surface of conifer soil, or 20 g pine litter chopped finely and mixed into conifer soil (litter effects in prairie soil were not investigated). The chopped litter treatment was designed to exaggerate chemical litter effects while minimizing the physical effects of litter. Each pot was planted with ten seeds of either *Festuca* or *Centaurea* on 25 January 2008. We counted the number of plants that established in each pot on 29 May 2008. We tested for differences in establishment rates among species and treatments with a general linear model (GLM) with pairwise tests for differences between *Centaurea* and *Festuca* within treatments.

Plant-soil feedbacks in shade and sun

Centaurea has been shown to affect native species through its effects on soils (Olson & Wallander 2002; Callaway *et al.* 2004; Thorpe *et al.* 2006). We tested the potential for shade to influence the "soil legacy" effect of *Centaurea* on the establishment of *Festuca*, in a garden at The University of Montana's Fort Missoula (latitude 46.842º, longitude -113.993º, 962 m elevation). Twenty 5 x 2 m replicates were established, 10 of which were randomly selected for a shade treatment, created with a single shadecloth extending 0.5 m in each direction from all pots. Shade cloths were 4.35 m x 1.0 m and 0.5 m high and reduced PAR by 48%, 862.8±10.1 $\mu mol/m^2/s$, less than the maximum effects of *Pinus* canopies (Appendix B) but an estimate of the effects of canopies over the course of a day. To prevent soil treatments from mixing with field soil we buried 9 L (15 x 15 x 40 cm) black plastic pots with the bottoms removed to allow water to travel through the soil, filled them with field soil and planted *Centaurea* and *Festuca* (n=10). After 44 weeks of growth, all pots were sprayed with Roundup© (18% glyphosate) mixed at the rate of 50 mL Roundup/1000 mL water on 15 October 2007. On 26 March 2008 we planted 50 *Festuca* seeds in all 40 pots. We scored pots as having established *Festuca* or not on 17 November 2008. The effect of prior cultivation of soil by either *Centaurea* or *Festuca* was tested using a χ^2 statistic in the crosstabs function of SPSS with *Festuca* presence as the rows, soil cultivation as columns and shade as a layer. We report Fischer's Exact test statistic for two tails, a robust test to low observed counts in some cells. Because the size of the plant training the soil could affect outcomes, we tested for differences in biomass of the plant that had trained the soil between open and shaded treatments with shade as a fixed factor and with biomass natural log-transformed to homogenize variance.

Effects of *Pinus ponderosa* on growth

Reciprocal transplants in the field

The direct and indirect effects of conifer canopies and prairie and conifer soil on the growth of individual *Centaurea, Festuca* and *Pseudoroegneria* and interactions among the invader and the natives were tested in the field with a reciprocal transplant experiment at the Cyr Ridgeline site (see Appendix A). Four different treatments were applied along a random azimuth from each of ten trees: 1) prairie soil moved under a conifer, 2) prairie soil removed but replaced in the prairie, 3) conifer soil moved to the prairie and 4) conifer soil removed but replaced under a conifer. Prairie plots were located 20 m from the nearest tree. Treatments were initiated and two-month-old seedlings of all three species were planted alone and each native grass was planted in pairwise competition with *Centaurea* (5 cm apart) on 24 April 2008. The experiment was periodically monitored for herbivory and mortality throughout the year and plants were harvested at maximum annual growth, 15 months after planting on 15 July 2009. Above ground biomass was harvested, dried in an oven at 60ºC for 36 hours and weighed (as for all other harvests reported below).

Herbivory eliminated *Festuca* from this experiment. Soil effects on the growth of *Pseudoroegneria* and *Centaurea* and their competitive interactions were analyzed separately by habitat using a GLM with soil source and competition as fixed factors. Data for *Pseudoroegneria* in the prairie habitat were natural log-transformed prior to analysis.

Garden experiment

We isolated the effects of shade and conifer/prairie soil on growth and competition between *Centaurea* and *Festuca* in a split-plot garden experiment. The setup was as for the plant-soil feedback experiment (see above) but with each shaded or unshaded replicate containing *Centaurea and Festuca* alone and in interspecific competition in both conifer and prairie field soil (n=10). Plants were started from seed in 125 mL rocket pots in the greenhouse, in the same soil type they would experience in the experiment and then transplanted into the experiment as 3-month old seedlings on 17 August 2006. Aboveground biomass was harvested on 10 July 2007. The data for each species were analyzed separately using a fully saturated GLM with soil origin, shade and competition as fixed factors. The effect of replicate nested within shade (split-plot design) was not significant, so the data were analyzed as if this was a factorial experiment. *Festuca* biomass was square root-transformed prior to analysis.

Greenhouse litter experiment

We tested the effects of intact pine litter on *Festuca* and *Centaurea* growth and competitive interactions in a greenhouse experiment. Treatments were as for the greenhouse litter establishment experiment described above, but without the prairie soil treatment. We seeded on

25 January 2008 as described above, but also with pots seeded with 10 seeds of each species. Aboveground biomass was harvested 29 May 2008. Direct and indirect effects of intact litter on *Centaurea* and *Festuca* growth and competitive dynamics were tested using separate GLM's for each species with litter and competition as fixed effects. The biomass of *Festuca* was squared to homogenize variance. When significant interactions were identified in the global model (Appendix H), we further explored relationships between variables using pairwise tests. Differences in competitive effect could be driven exclusively by direct effects on the size of the competitors, so we also evaluated biomass of the competitor separately by species with treatment as a fixed factor for all pots containing competitive pairings. Heteroscedasticity of *Centaurea* competitor biomass was eliminated by squaring.

<u>Catechin</u>

Roots of *C. stoebe* exude the polyphenol catechin (Blair *et al.* 2005; Pollock *et al.* 2009; Tharayil & Triebwasser, *in press*), but early reports of exudation have not been reproducible under conditions similar to the original experiment (see Bais *et al.* 2003 vs. Stermitz *et al.* 2009). Catechin has been reported at very low concentrations in soil in the rhizospheres of *C. stoebe* (Blair *et al.* 2006) but high concentrations may occur periodically (Perry *et al.* 2007; Schultz 2008). The originally reported "(±)-catechin" form has been identified in the rhizospheres of *C. stoebe* (Perry *et al.* 2007) but whether or not this entantiomeric form is exuded from the roots of *C. stoebe* remains to be resolved (Stermitz *et al.* 2009). However, the phytotoxic effects of both (±)-catechin and the (+) form have been repeatedly demonstrated in vitro, in sand culture, in controlled experiments with field soils and in the field (He *et al.* 2009; Pollock *et al.* 2009; Thorpe et el. 2009 and citations within, but see Schultze 2008; Duke 2009).

We applied 2 ml of aqueous solution containing (±)-catechin (Shivambu International, Himachel Pradesh, India) at a concentration of 100 µg/ml H_2O in field and greenhouse experiments. The 2 ml solution wetted ≈4 g of the soil in these habitats resulting in an estimated initial bulk concentration in soils of ≈25 µg g^2, much lower than the pulse reported by Perry *et al.* (2007). Bulk soil concentrations such as these are suggested target concentrations for soil experiments but represent an "averaging" of the measured concentration of the chemical in bulk soil. Such measures substantially underestimate realistic concentrations of solutions at the surfaces of interacting roots (Inderjit *et al.* 2008). In other experiments, this concentration in the bulk soil likely decreased by an order of magnitude within 24 hours (Pollock *et al.* 2009).

In the field, catechin was applied to 15 individuals of *Festuca* and 15 individuals of *Pseudoroegneria* under *Pinus* canopies and in the open prairie around each of six different trees on 9 May 2008 at the Cyr Ridgeline site. The solution was applied using a pipette directly to the rhizosphere of target plants. Control plants received an equivalent volume (2 mL) of milleque water. Leaf number was assessed at the time of treatment and again on 6 June 2008. Data were analyzed separately by species, using mixed model GLM's with habitat, catechin and catechin x habitat as fixed factors, replicate as a random factor and pretreatment leaf number as a covariate to adjust for pretreatment variability in plant size.

The effects of catechin were also compared in conifer and prairie soils in the greenhouse. Field soils were sieved (<1 inch) and placed in 250 ml "rocket pots". On 3 April 2008 we seeded *Achillea millefolium* L., *Festuca idahoensis*, *Pseudoroegneria*, *Geum triflorum* Pursh and *Bromus tectorum* in pots, with 10 pots of each species being treated with catechin and 10 used as controls. Three ml of catechin solution (100 µg catechin/ml water) was applied using a pipette directly to the rhizosphere of the target plants on 8 May 2008 and the plants were measured the next day for leaf number and height. These values were used as a covariate to adjust for pretreatment plant size. The plants were harvested on 27 May 2008. Catechin effects were analyzed for each species separately using GLM's with soil source and catechin application as fixed factors as well as a soil x catechin effect. Measurements for *Geum* and *Pseudoroegneria* were natural log-transformed and *B. tectorum* was square root-transformed to homogenize variances.

Results

Field patterns

Centaurea was far more abundant in open prairie than under *Pinus* at highly invaded sites, increasing from a relative cover of 2.3±1.3% under trees to 43±1.4% at 12 m from trees (Fig. 1; Appendix C; $F_{6, 18}$=24.8; P<0.001). Concomitantly, the relative cover of native species as a group declined as the cover of *Centaurea* increased (Fig. 1). This pattern was also significant for non-relativized values (Appendix C). The interaction between site and distance to tree was significant in uninvaded and highly invaded sites, indicating that the abundance of *Centaurea* in the open prairie, relative to its abundance under trees, increased with invasion intensity.

At the highly invaded site where trees had been removed ≈30 years prior to sampling there was no effect of distance from tree (stump) on the relative abundance of *Centaurea* (Appendix C; $F_{6, 257}$=1.3; P=0.280). However, the absolute cover of *Centaurea* was 16% *higher* where canopies had been previously than in prairie (Appendix C; $F_{9, 257}$=12.1; P<0.0001). Thus the low abundance of *Centaurea* under *Pinus* was caused by trees, rather than by special microsites occupied by trees that are less suitable for *Centaurea*.

At invaded sites, *Centaurea* cover was negatively correlated with the cover (r= -0.29; P<0.001) and diversity (r= -0.29; P<0.001) of all native species combined and the cover of *Pseudoroegneria* (r= -0.30; P<0.001) and *Festuca* (r= -0.18; P=0.008) individually. Trees reduced PAR by 84%, increased duff and litter depth by 66 and 57% (respectively) and increased

PO_4^{-3} in soil by 97% relative to open prairie (Appendix B). Nitrate and ammonium concentrations tended to be higher under *Pinus*, but concentrations were highly variable and not significantly different under canopies versus in prairie.

Effect of *Pinus ponderosa* on germination

In the field, conifer canopies reduced the germination of *Centaurea* seeds in packets by 23% relative to open prairie (Appendix D; $F_{1, 1}$=17720.6; P=0.005). The viability of ungerminated seeds was 84% in both the subcanopy and open habitats.

Effect of *Pinus ponderosa* on establishment

Field litter experiment

Pine litter reduced the density of *Centaurea* seedlings by 94% (Appendix E; $F_{1, 63}$=15.3; P<0.0001) but there was no effect of neighbor removal or habitat. Despite the slow growth of the year-old seedlings, without litter *Centaurea* biomass was 1.04±0.20 g/m^2, 84% greater in plots without litter than in plots with litter ($F_{1, 63}$=9.4; P=0.003). In prairie plots, the biomass of year-old seedlings was 1.00±0.20 g/m^2 compared to 0.20±0.20 g/m^2 under *Pinus* (Appendix E; $F_{1, 63}$=7.3; P=0.009). In bare plots, neighbor removal increased biomass 81% (litter x neighbor $F_{1, 63}$=9.2; P=0.004). Both litter and neighbor removal were more effective in prairie than under *Pinus* (habitat x litter x neighbors $F_{1, 63}$=6.3; P=0.015), with the greatest biomass of year-old *Centaurea* in the open, prairie habitats without neighbors and without litter (2.92±0.44 g/m^2).

Greenhouse litter experiment

Centaurea established at 1.9 and 1.3 times higher densities than *Festuca* in conifer and prairie soil without litter (Table 1). Intact pine litter reduced seedling establishment of both species to similarly low levels around 10%. Eliminating the physical effect of the litter (as inferred by adding chopped litter to the soil) eliminated the inhibitory effect on *Festuca*, but chopped litter still reduced *Centaurea* establishment by 74% (Table 1).

Plant-soil feedbacks

Soil from the field in which *Centaurea* had been grown for 11 months, and then removed, had strong inhibitory effects on the establishment of *Festuca* (Fisher's Exact Test; N=40; p=0.008), but only when *Centaurea* had been grown in full sunlight (Fig. 2). When soil was cultivated by *Centaurea* in the shade the effect decreased to that of the training by *Festuca* itself. The biomass of the plants that had trained the soil did not significantly vary between shaded and open treatments for either *Centaurea* ($F_{1, 36}$=1.5; P=0.234) or *Festuca* ($F_{1, 37}$=1.6; P=0.211).

Post-establishment effects of *Pinus ponderosa*

Reciprocal transplants in the field

Grown alone, the biomass of *Centaurea* and *Pseudoroegneria* did not differ under *Pinus* or in the open prairie, and did not differ by soil type (Fig. 3; Appendix F). However, in the open prairie *Pseudoroegneria* produced 66% less aboveground biomass when grown in competition with *Centaurea* than when grown alone irrespective of soil origin ($F_{1, 21}$=5.2; P=0.033). This strong competitive effect disappeared under canopies where *Centaurea* had no effect on *Pseudoroegneria* (Fig. 3a). The biomass of *Centaurea* was not affected by competition with *Pseudoroegneria* under any conditions (Fig. 3b).

Garden experiment

We isolated the effects of shade and soil in a garden and found that *Centaurea* reduced *Festuca* biomass by 45% ($F_{1,76}$=22.6; P<0.0001) irrespective of treatment. *Festuca* biomass was not affected by shade or soil origin when grown alone (Fig. 4a; Appendix G). *Centaurea* did not respond to *Festuca* competition in any treatment (Fig. 4b; Appendix G), but *Centaurea* was 36% larger in conifer soil ($F_{1,77}$=5.0; P=0.029) and shade diminished *Centaurea* biomass by 31% ($F_{1,77}$=7.7; P=0.007).

Greenhouse litter experiment

For both *Festuca* and *Centaurea* intact litter eliminated the competitive effect of the interspecific neighbor (Appendix H; litter x competition; $F_{1,30}$=11.7; P=0.002 and $F_{1,30}$=5.6; P=0.021 respectively). Without litter, *Centaurea* reduced *Festuca* biomass by 46% from 1.3±0.1 g to 0.7±0.1 g per pot (pairwise test; $F_{1,18}$=54.5; P<0.001). Similarly, *Festuca* reduced *Centaurea* biomass by 45% in unammended conifer soil; 1.1±0.1 g to 0.6±0.1 g per pot (pairwise test; $F_{1,18}$=19.7; P<0.001). When grown alone, the intact-litter treatment directly suppressed the growth of *Festuca* to 0.6±0.2 g and *Centaurea* to 0.4±0.1 g per pot (Pairwise tests; $F_{1,14}$=28.4; P<0.001; $F_{1,14}$=51.7; P<0.001), but when grown together in the litter treatment neither species had a competitive effect on the other. This was not because the plants were too small to interact; total species biomass in competition was the same with or without litter.

Catechin

In the field, naturally established *Festuca* and *Pseudoroegneria* seedlings were inhibited by catechin in both habitats (Fig. 5; Appendix I; $F_{1,43}$=26.6; P<0.0001; $F_{1,43}$=27.6; P<0.0001). For *Festuca* the effect of catechin was diminished under *Pinus*, reduced from a 56% inhibition in prairie to 31% in conifer habitats (catechin x habitat $F_{1, 43}$=5.0; P=0.030). *Pseudoroegneria spicata* leaf number was reduced by 44% with catechin application but this effect did not differ between understory and prairie habitats.

Corresponding with the field results, in the greenhouse catechin effects varied by species and, for *Festuca,* by soil origin. Catechin reduced total biomass of *Festuca* by 30% in prairie soil, but not in conifer soil (catechin x soil $F_{1, 36}$=6.5; P=0.015). *Geum triflorum* biomass was reduced by catechin in both soil types, and *B. tectorum* and *Pseudoroegneria* were unaffected by catechin application. Catechin increased the size of *A. millefolium* (Appendix J for ANOVA tables).

Discussion

The abundance of *Centaurea* and its competitive effects on native species were greater in open prairie than under isolated *Pinus* due to increased biotic resistance, caused in part by the indirect effect of the pine on interactions between the invader and natives (Fig. 6). In addition to modification of *Centaurea's* competitive effect, *Pinus* shade and litter directly inhibited *Centaurea*, indirectly facilitating natives. Diminished *Centaurea* competitive effects were most strikingly demonstrated in the field experiment where we found no direct effects of tree canopies or soils on the growth of either target species, but a much stronger competitive effect of *Centaurea* on the native in the open grassland than under *Pinus*. Thus, the most remarkable effect of *Pinus* was to indirectly diminish the intensity of competitive effects of *Centaurea* on native grasses in ways that were not easily predicted from the direct effects of *Pinus* on the invader or on native species (Fig. 6). There appeared to be multiple potential causes of this shift: pine litter strongly reduced competitive intensity between *Centaurea* and natives, *Centaurea* inhibition of *Festuca* establishment was weaker in shade and the phytotoxic effect of catechin was reduced under *Pinus* canopies in the field and in conifer soil. Because invasion is far more intense in the open prairie than under *Pinus* canopies, and competitive effects also are far stronger in the open prairie; it seems that strong competitive effects may be a fundamental process by which *Centaurea* successfully invades intermountain prairie.

Tree canopies can facilitate understory species by creating cooler and more mesic environments (Callaway 2007), or inhibit understory species by reducing light and through root competition (Callaway *et al.* 1991; Barnes & Archer 1999). Siemann and Rogers (2003) found that shade from native shrubs indirectly facilitated *Sapium sebiferum* invasion by diminishing the competitive effects of herbaceous native species, thereby reducing biotic resistance. Conversely, we found shade from *Pinus* canopies to be an important component of resistance to invasion, but the mechanism may be complex. In the garden, *Centaurea* growth was reduced by 33% in shade, but the competitive effects of these smaller *Centaurea* on established neighbors were not reduced. In the field, we found no direct effects of conifer canopies on either *Centaurea* or *Pseudoroegneria*, but competitive effects were eliminated in the conifer habitat, suggesting indirect interactions may be more important than direct effects. Shade also altered the effects of *Centaurea* on the establishment of *Festuca* by eliminating the long-term effects of the invader on soil. Olson and Wallander (2002) also found that soil collected from *Centaurea* infested prairie inhibited the germination of *Pseudoroegneria* by 11%. We found that soil training by *Centaurea* reduced *Festuca* establishment by 75% when *Centaurea* was grown in the open, but that this effect was eliminated in the shade. Feedbacks between *Centaurea* and the soil may involve altered soil biota (Callaway *et al.* 2004a; Thorpe *et al.* 2006) or diminished phytotoxic effects of allelopathic chemicals (Pollock *et al.* 2009; Thorpe *et al.* 2009).

The production and function of secondary metabolites varies for many reasons (see reviews by Karban & Myers 1989; Karban 2008; Metlen *et al.* 2009). Tannins and phenolics (such as catechin) are produced at higher rates with greater light intensity in some plant species

(Hofland-Zijlstra & Berendse 2009a). Tharayil and Triebwasser (*in press*) found that high light intensity led to pulses of catechin release from the roots of *C. stoebe* seedlings which did not occur under low light conditions. Soil characteristics are also known to alter catechin production. For example, production of catechin in the roots of *Zea mays* can be triggered by adding aluminum or silicon to soils (Kidd *et al.* 2001) with aluminum-resistant varieties responding more strongly to aluminum additions.

In addition to variable metabolite production, catechin is capable of rapid oxidation and/or sorption (Tharayil *et al.* 2008) and as a result phytotoxic effects are dependent on soil chemistry (Tharayil *et al.* 2008; Kaku & Nakagawa 2009; Pollock *et al.* 2009). We found catechin to be phytotoxic to the two most abundant native grasses, *Pseudoroegneria* and *Festuca* in the field. However, under pine trees, we found the negative effects of catechin on *Festuca* were ameliorated by 40%. Furthermore, catechin had phytotoxic effects on *Festuca* in prairie soils but not in conifer soils. At alkaline pH, such as local prairie soils, catechin can form catechenic acid derivatives and quinones (Jensen *et al.* 1983) while in more acidic pH (as in conifer soils) catechin rapidly forms dimmers (Chen *et al.* 2006), creating the potential for different phytotoxic effects. Our work provides ecological context for these studies that hint at important interactions between secondary metabolites and field conditions but rarely are tested in the field (but see Weir *et al.* 2006).

Many other studies show that plant litter can modify soil chemistry in ways that affect plant growth and competitive interactions. Decomposing litter can enrich soil nutrients (Callaway 2007) and increased nutrients can benefit species that are good competitors under high resource conditions (Rice & Nagy 2000), including some exotic plant invaders (Davis *et al.* 2000; Siemann & Rogers 2007). We found that *Centaurea* growth increased in nutrient rich conifer soil in the garden, but this effect was not observed in the field experiment, and more importantly greater growth did not translate into increased competitive effects on natives. However, years after trees had been harvested; *Centaurea* cover was higher near the stumps. Without shade and after the decomposition of *Pinus* litter, *Centaurea* may benefit from soil nutrient enrichment by the trees.

Litter has been shown to alter competitive outcomes by reducing competitor densities and delaying emergence, and by altering the timing and intensity of competitive interactions (Bosy & Reader 2005; Ladd & Facelli 2008). Intraspecific variation in the chemical signature of leaves of *Pinus sylvestris* correlates with the composition of understory plant communities (Iason *et al.* 2005) suggesting that the chemical effects of litter can be quite species specific (Barritt & Facelli 2001; Hofland-Zijlstra & Berendse 2009b). In the field, the germination and establishment of *Centaurea* was strongly inhibited by *Pinus* and most strikingly by litter regardless of habitat. Additionally, we found that intact native communities inhibited the recruitment of *Centaurea* seedlings, but only in the absence of litter. In the greenhouse, the presence of intact litter had disproportionally strong effects on *Centaurea* relative to *Festuca* resulting in no difference in establishment between the native and invader. However, when litter was chopped and mixed into the soil, minimizing physical litter effects while promoting leachates, *Centaurea* was far more suppressed than *Festuca*.

Litter can indirectly alter competitive outcomes in ways that are not predictable from direct effects on plants grown alone. For example, when grown alone, *Calluna vulgaris* performs best with litter. Despite this, when grown in competition with *Deschampsia flexuosa, C. vulgaris* is more competitive without litter (Hofland-Zijlstra & Berendse (2009b). In our greenhouse experiment competitive effects and responses were eliminated for both *Centaurea* and *Festuca* by intact pine litter. Given the strong competitive effects exhibited by *Centaurea* in the field, greenhouse, and garden, the opportunity for native species to grow with *Centaurea* in an environment where plant-plant interactions are attenuated may shift the balance of interactions to allow coexistence with an otherwise strong invader. By examining how species interact, rather than only their performance, along gradients of resources or abiotic conditions we can better understand conditionality in the net effect of species on each other (Callaway *et al.* 1991) and better evaluate the general importance of competition for community organization and invasion.

Biotic resistance to exotic plant invasion was driven by the direct effects of *Pinus ponderosa* on *Centaurea stoebe*, but also by indirect amelioration of the strong competitive effects of the invader on native species (Fig. 6). Our results suggest three interrelated mechanisms by which *Pinus* litter chemistry, shade, and soil effects reduce invasion by *Centaurea*: 1) direct inhibition of *Centaurea* establishment and growth, 2) reduced competitive effects of *Centaurea* on established natives, and 3) reduced toxicity of *Centaurea* root exudates on native plants.

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Table 1: The proportion of *Festuca* and *Centaurea* seeds that established in a greenhouse experiment. The treatments were unammended soil from prairie and conifer habitats as well as conifer soil with intact litter on top of it (intact litter) or an equivalent volume of litter chopped up and mixed into the soil (chopped litter).

Treatment	Species	Establishment (%)*		df	F	*P*†
Prairie soil	*Festuca idahoensis*	36	(4)	1,18	31.2	<0.001
	Centaurea stoebe	71	(4)			
Conifer soil	*Festuca idahoensis*	47	(4)	1,18	6.5	0.020
	Centaurea stoebe	61	(4)			
Intact litter	*Festuca idahoensis*	11	(4)	1,18	0.4	0.538
	Centaurea stoebe	8	(4)			
Chopped litter	*Festuca idahoensis*	50	(4)	1,18	25.7	<0.001
	Centaurea stoebe	16	(4)			

Notes: *Adjusted marginal means (SE), †pairwise tests between species within treatments.

Global model: Species ($F_{1, 72}$=1.1, *P*=0.308), Treatment ($F_{3, 72}$=51.9, *P*<0.001), Treatment x Species ($F_{3, 72}$=24.9, *P*<0.001)

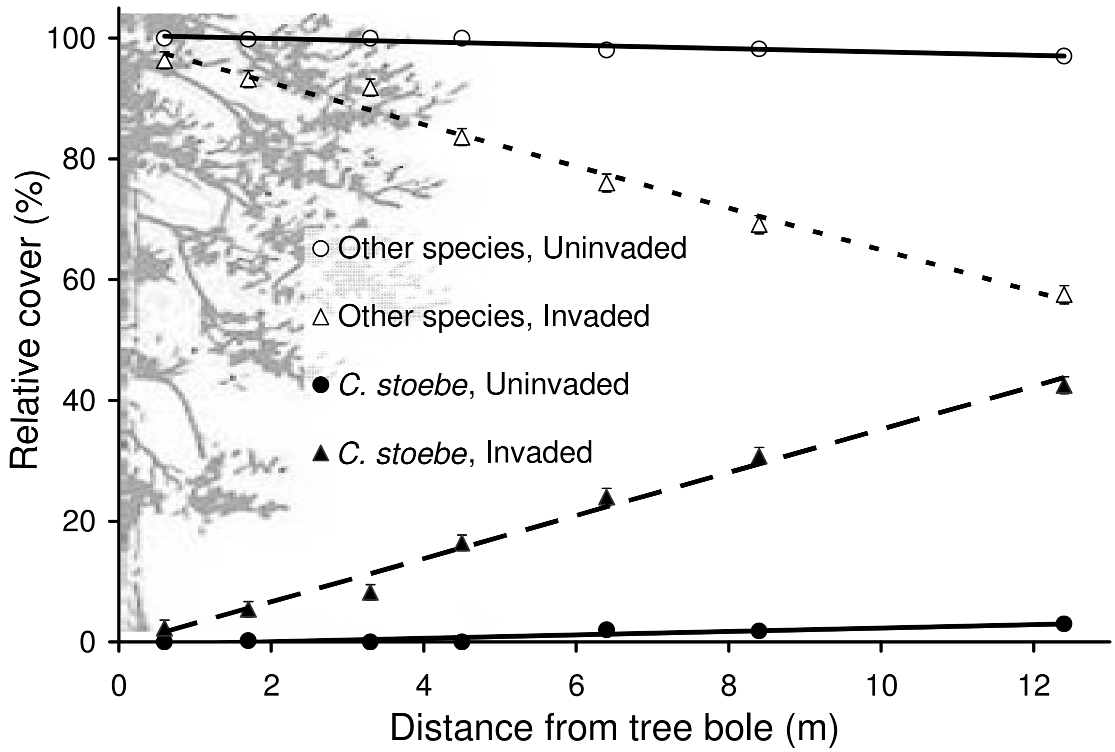

Figure 1: Relative cover of *Centaurea stoebe* (solid symbols) and other species (open symbols) with increasing distance from savanna trees in uninvaded (solid lines) and invaded (dashed lines) sites. The canopy edge averaged 4.4 m. Estimated marginal means and standard error from ANOVA presented in Appendix C.

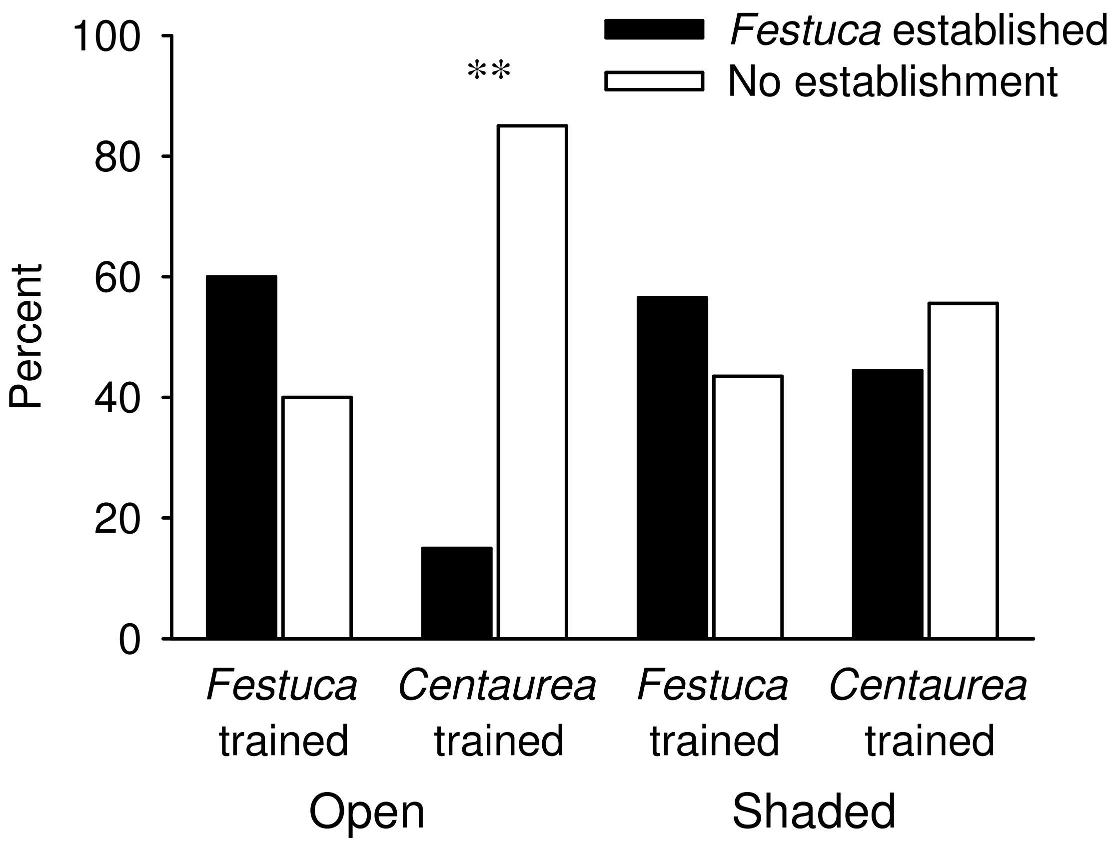

Figure 2: Percent of pots seeded with *Festuca idahoensis* that contained established seedlings (occupied) or for which no seeds germinated (empty). Pots contained either *Centaurea stoebe* or *Festuca idahoensis* for 11 months prior to harvest and subsequent reseeding. ** Fisher's exact χ^2 test, N=40, p=0.008

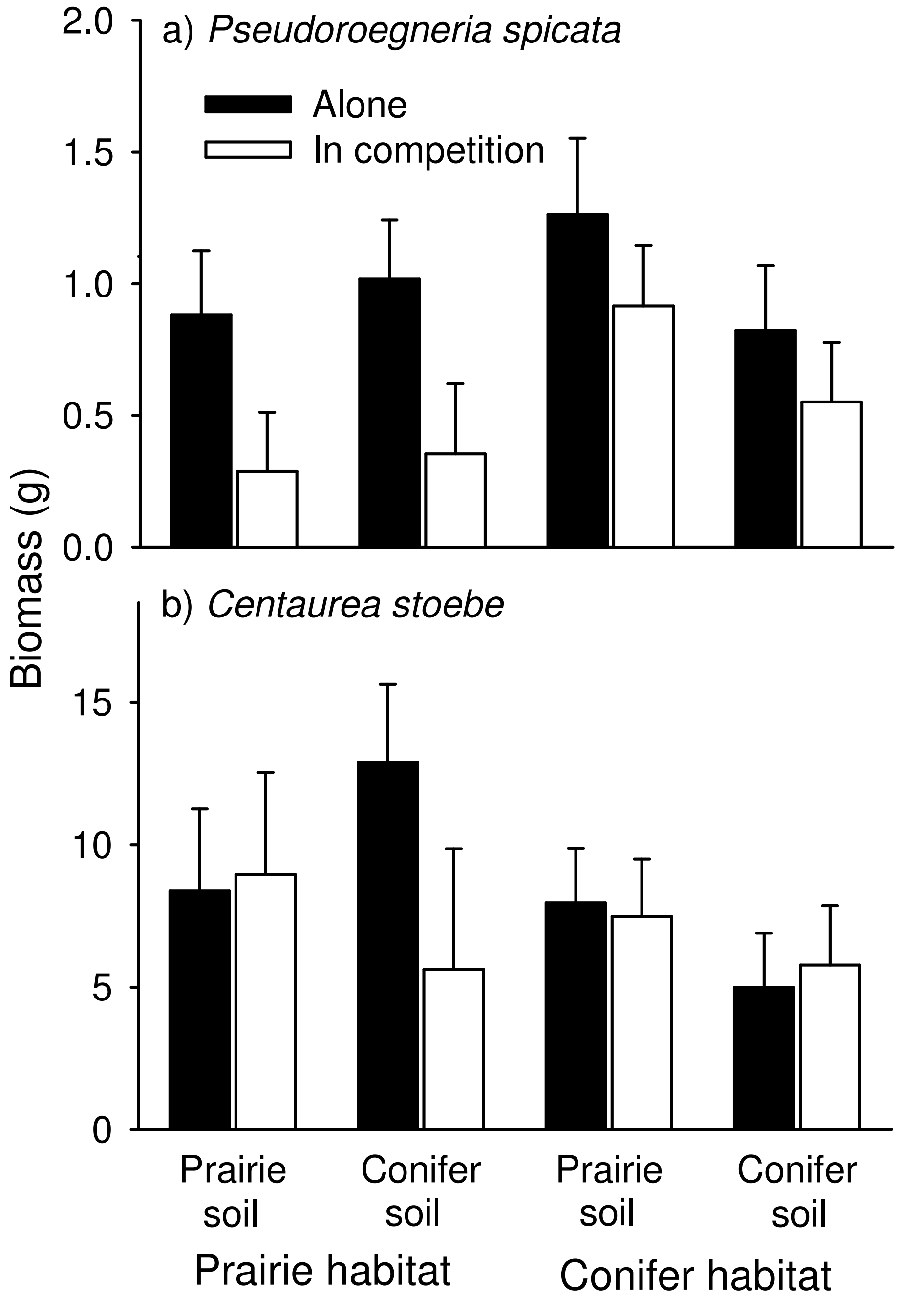

Figure 3: Biomass (g) of a) *Pseudoroegneria spicata* and b) *Centaurea stoebe* transplanted into conifer and prairie soil in prairie and conifer habitats both alone and in competition (mean ± SE). See Appendix F for ANOVA results.

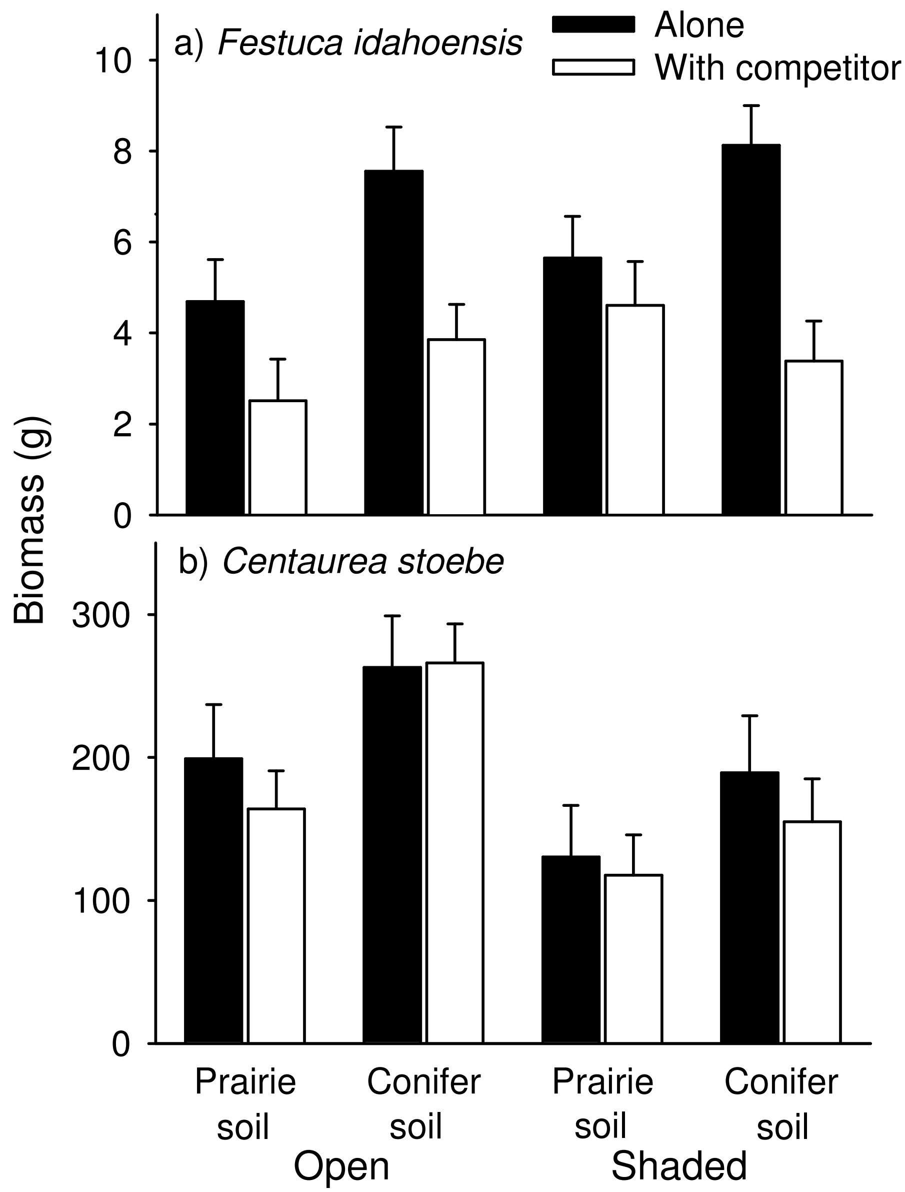

Figure 4: Biomass (g) of a) *Festuca idahoensis* and b) *Centaurea stoebe* grown alone and in competition in prairie and conifer soils with and without shade (mean ±SE). See Appendix G for ANOVA results.

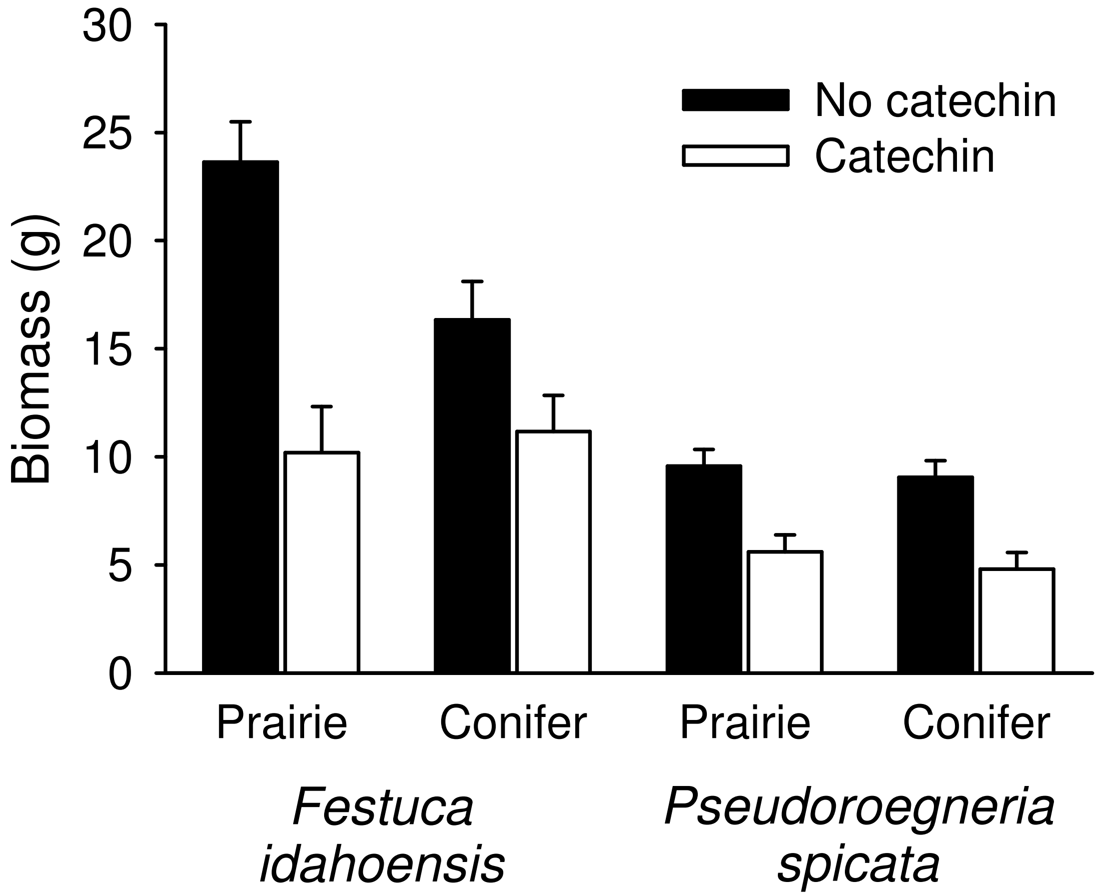

Figure 5: Catechin inhibited growth of both *Festuca idahoensis* and *Pseudoroegneria spicata* when administered in the field in both open prairie and conifer habitats (mean +/- SE). Values adjusted to a covariate pretreatment leaf number of 11.2 for *Festuca* and 6.2 leaves for *Pseudoroegneria*. See Appendix I for full ANOVA.

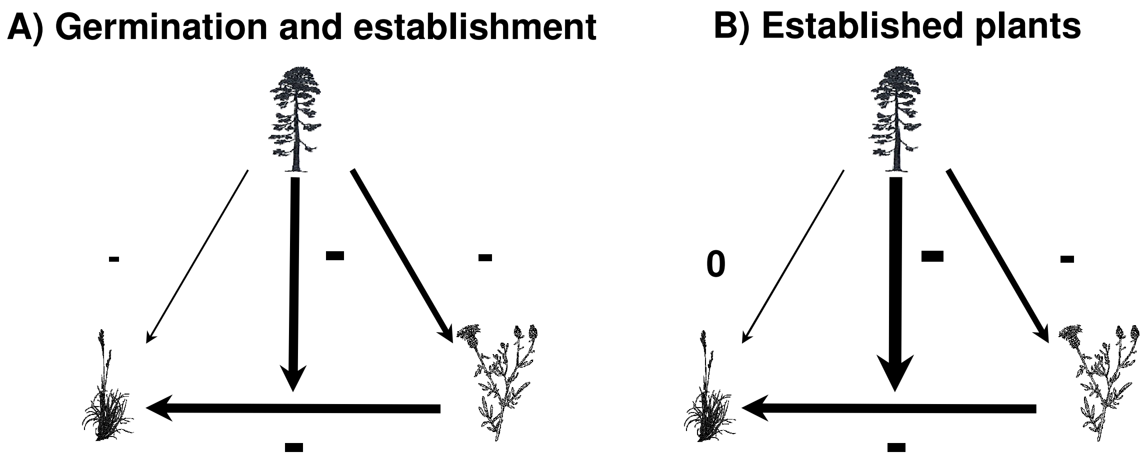

Figure 6: Schematic representation of the direct and indirect interactions among *Pinus ponderosa, Centaurea stoebe* and native plants that modify competitive interactions between native plants and *Centaurea*. Biotic resistance to plant invasion occurs when A) *Pinus* reduces germination and establishment of *Centaurea* more than native species or reduces the effects of the invader on native establishment, and B) *Pinus* directly inhibits the growth and competitive effects of *Centaurea* but not of natives.

Appendix A: List of sites with mean site characteristics.

Site	Aspect	Slope (degrees)	Soil type*	Latitude†	Longitude‡	Elevation (m)	Purpose	*Centaurea stoebe* cover (%)‡
Albert Creek	195	24	Repp very gravelly loam	46.977	-174.267	1173	Uninvaded	0
Beavertail	175	25	Whitecow gravelly loam	46.740	-113.565	1382	Invaded/ harvested	17
Blue Mountain	189	25	Winkler very gravelly sandy loam	46.809	-114.110	1197	Invaded	5
Calf Creek	272	13	Sawicki-Whitlash, stony complex	46.269	-113.986	1482	Invaded	17
Cyr Creek	226	39	Repp very gravelly loam	46.943	-114.223	1135	Uninvaded	0
Cyr Ridgeline	220	30	Repp very gravelly loam	46.947	-114.227	1200	Field experiments	NA
Jumbo	206	19	Bigarm gravelly loam	46.902	-113.942	1320	Invaded	9
Rock Creek	180	35	Repp very gravelly loam	46.965	-114.265	1130	Uninvaded	0
Three Mile	181	32	Holter-Repp-Sharrott families complex	46.620	-113.894	1376	Invaded	9

*USDA, NRCS, Web Soil Survey, http://websoilsurvey.nrcs.usda.gov/, †Decimal degrees, WGS84 datum, ‡Mean for prairie plots only

Appendix B: Environmental characteristics associated with conifer and open prairie habitats. All models include habitat (conifer or prairie), site, replicate nested with site and habitat x site interactions.

	Habitat	Mean	(SE)*	ANOVA	df	F	*P*
PAR (μmol/m^2/s)	Prairie	1879.7	(21.7)	Habitat	1, 3	639.8	*<0.001*
	Conifer	291.6	(21.7)	Site	3, 3	1.2	0.431
				Replicate(Site)	32, 32	1.0	0.501
				Habitat x Site	3, 32	4.2	*0.013*
Duff and litter (mm)	Prairie	26.5	(1.6)	Habitat	1, 6	46.2	*<0.001*
	Conifer	68.6	(1.6)	Site	6, 13	0.8	0.598
				Replicate(Site)	56, 56	2.4	*0.001*
				Habitat x Site	6, 56	7.3	*<0.001*
NH_4^+ (μg/g)	Prairie	1.7	(0.3)	Habitat	1, 3	8.1	0.065
	Conifer	2.2	(0.3)	Site	3, 1	122.3	0.971
				Replicate(Site)	32, 32	0.8	0.695
				Habitat x Site	3, 32	0.2	0.906
NO_3^- (μg/g)	Prairie	0.7	(0.5)	Habitat	1, 3	2.5	0.209
	Conifer	1.9	(0.5)	Site	3, 2	2.1	0.326
				Replicate(Site)	32, 32	0.9	0.636
				Habitat x Site	3, 32	1.2	0.317
PO_4^{-3} (μg/g)	Prairie	0.1	(0.5)	Habitat	1, 3	5.2	0.108
	Conifer	3.4	(0.5)	Site	3, 3	1.0	0.500
				Replicate(Site)	32, 32	1.0	0.496
				Habitat x Site	3, 32	5.0	*0.006*

Notes: *Adjusted marginal means

Appendix C: Mixed model nested ANOVA for *Centaurea stoebe* absolute cover and relative cover at uninvaded and invaded sites. Replicate and site are random variables while distance to tree is a fixed variable.

	Absolute cover (%)			Relative cover (%)		
	df	F	*P*	df	F	*P*
Uninvaded						
Distance to tree	6, 12.9	2.0	0.149	6, 12.9	2.1	0.117
Site	2, 14.9	0.7	0.497	2, 18.9	0.8	0.458
Replicate(Site)	24, 235	1.2	0.204	24, 235	1.7	*0.020*
Distance x Site	12, 235	1.9	*0.039*	12, 235	1.9	*0.031*
Invaded						
Distance to tree	6, 18.0	14.9	*<0.001*	6, 18	24.8	*<0.001*
Site	3, 40.8	4.8	*0.006*	3, 41.5	4.0	*0.014*
Replicate(Site)	32, 881	8.1	*<0.001*	32, 881	6.9	*<0.001*
Distance x Site	18, 881	7.9	*<0.001*	18, 881	4.9	*<0.001*
Invaded/Harvested						
Distance to tree	6, 257	3.4	*0.003*	6, 257	1.3	0.280
Replicate	9,257	12.1	*<0.001*	9, 257	7.3	*<0.001*

Appendix D: Nested GLM for the percentage of *Centaurea stoebe* seeds that germinated in seed packets placed in conifer and prairie habitats. Habitat is fixed while replicate and site are random variables.

	df	F	*P*
Habitat	1, 1	17720.6	*0.005*
Site	1, 0.3	6.3	0.504
Replicate(Site)	15, 14	1.2	0.389
Habitat x Site	1, 14	0.1	0.977

Appendix E: Analysis of variance for the effect of habitat, pine litter and neighbors on *Centaurea stoebe* establishment and subsequent biomass in the field. Habitat (prairie or conifer), litter (presence or absence) and neighbors (present or not) are fixed factors and replicate is random.

	Establishment			Biomass		
	df	F	*P*	df	F	*P*
Habitat	1, 63	0.1	0.947	1, 63	7.4	*0.009*
Litter	1, 63	15.3	*<0.001*	1, 63	9.4	*0.003*
Neighbors	1, 63	0.2	0.668	1, 63	3.5	0.064
Replicate	9, 63	0.8	0.614	9, 63	1.2	0.291
Habitat x Litter	1, 63	0.1	0.868	1, 63	2.4	0.123
Habitat x Neighbors	1, 63	0.3	0.586	1, 63	1.8	0.180
Litter x Neighbors	1, 63	0.4	0.509	1, 63	9.2	*0.004*
Habitat x Litter x Neighbors	1, 63	1.0	0.315	1, 63	6.3	*0.015*

Appendix F: Analysis of variance for biomass (g) of *Pseudoroegneria spicata* and *Centaurea stoebe* transplanted into conifer and prairie soil in prairie and conifer habitats both alone and in competition.

	Pseudoroegneria spicata			*Centaurea stoebe*		
	df	F	*P*	df	F	*P*
Prairie habitat						
Soil	1, 21	1.0	0.332	1, 31	0.1	0.865
Competition	1, 21	5.2	*0.033*	1, 31	1.0	0.332
Soil x Competition	1, 21	0.1	0.940	1, 31	1.3	0.260
Conifer habitat						
Soil	1, 24	2.7	0.114	1, 30	1.3	0.260
Competition	1, 24	1.6	0.220	1, 30	0.1	0.942
Soil x Competition	1, 24	0.1	0.881	1, 30	0.1	0.756

Notes: Data for *Pseudoroegneria* in the prairie habitat were natural log-transformed prior to analysis.

Appendix G: Analysis of variance for *Festuca idahoensis* and *Centaurea stoebe* grown alone and in competition in prairie and conifer soils with and without shade.

	Festuca idahoensis			*Centaurea stoebe*		
	df	F	*P*	df	F	*P*
Soil	1, 76	2.5	0.117	1, 77	5.0	*0.029*
Shade	1, 76	1.9	0.166	1, 77	7.7	0.*007*
Competition	1, 76	22.6	<*0.001*	1, 77	0.2	0.632
Soil x Competition	1, 76	3.4	0.069	1, 77	0.1	0.922
Shade x Competition	1, 76	0.1	0.730	1, 77	0.1	0.771
Soil x Shade	1, 76	2.2	0.143	1, 77	0.2	0.619
Soil x Shade x Competition	1, 76	0.9	0.354	1, 77	0.2	0.680

Appendix H: Analysis of variance for the effect of intact litter on shoot biomass of *Festuca idahoensis* and *Centaurea stoebe* grow in conifer soil alone and in competition. Data for *Festuca* are squared to homogenize variance.

	Festuca idahoensis			*Centaurea stoebe*		
	df	F	*P*	df	F	*P*
Litter	1, 31	1.6	0.210	1, 34	27.3	*0.012*
Competition	1, 31	1.4	0.242	1, 34	7.1	*<0.001*
Litter x Competition	1, 31	11.7	*0.002*	1, 34	6.0	*0.021*

Appendix I: Mixed model ANCOVA for the effect of catechin application on *Festuca idahoensis* and *Pseudoroegneria spicata* when administered in the field in both open prairie and conifer habitats. Pretreatment leaf number is the covariate, catechin and habitat are fixed factors and replicate is random.

	Festuca idahoensis			*Pseudoroegneria spicata*		
	df	F	*P*	df	F	*P*
Pretreatment leaf number	1, 43	74.3	*<0.001*	1, 50	56.4	*<0.001*
Catechin	1, 43	26.6	*<0.001*	1, 50	27.6	*<0.001*
Habitat	1, 43	2.7	0.110	1, 50	0.7	0.400
Replicate	4, 43	4.5	*0.004*	4, 50	3.5	*0.014*
Catechin x Habitat	1, 43	5.0	*0.030*	1, 50	0.034	0.855

Appendix J: Biomass of five species with catechin addition in conifer and prairie soil (SE), with ANOVA conducted within each species.

Species	Soil	Amendment	Biomass (mg)*		ANOVA	F	df	*P*
Achillea millefolium	Prairie	None	90	(14)	Soil	15.2	1, 36	<0.001
		Catechin	117	(14)	Catechin	4.9	1, 36	0.034
	Conifer	None	255	(14)	Soil x catechin	0.1	1, 36	0.834
		Catechin	287	(14)				
Bromus tectorum	Prairie	None	315	(48)	Soil	111.2	1, 36	<0.001
		Catechin	310	(48)	Catechin	1.0	1, 36	0.324
	Conifer	None	848	(48)	Soil x catechin	0.4	1, 36	0.532
		Catechin	750	(48)				
Festuca idahoensis	Prairie	None	130	(21)	Soil	11.0	1, 36	0.002
		Catechin	91	(21)	Catechin	0.6	1, 36	0.435
	Conifer	None	146	(21)	Soil x catechin	6.5	1, 36	0.015
		Catechin	220	(21)				

Geum triflorum	Prairie	None	41	(11)	Soil	25.2	1, 34	<0.001
		Catechin	36	(10)	Catechin	5.5	1, 34	0.025
	Conifer	None	137	(12)	Soil x catechin	0.9	1, 34	0.340
		Catechin	82	(9)				
Pseudoroegneria spicata	Prairie	None	146	(32)	Soil	39.1	1, 35	<0.001
		Catechin	155	(32)	Catechin	0.4	1, 35	0.510
	Conifer	None	368	(32)	Soil x catechin	0.3	1, 35	0.600
		Catechin	381	(34)				

Notes: *Adjusted marginal mean (standard error)

CHAPTER 2 - FACILITATIVE EFFECTS OF *PINUS PONDEROSA* ON *BROMUS TECTORUM* ARE REDUCED BY A NATIVE COMPETITOR

Abstract

Native plants can facilitate exotic invaders, but how direct facilitative effects are indirectly modified by more complex interactions among invaders and native communities is less understood. We found that the annual grass *Bromus tectorum,* one of the most widespread invaders in North America, was 2.3 times more abundant under *Pinus ponderosa* canopies than in prairie, a pattern suggesting a net facilitative effect under natural conditions. When grown alone *Bromus* was facilitated by shade and by soil from under *Pinus*, and these two factors in combination promoted an even greater positive response from the invader. However, competition from the perennial native *Festuca idahoensis* eliminated the facilitative effects of *Pinus* soil on *Bromus*, and reduced the positive effects of shade. High levels of soil fertility, as found under *Pinus* canopies, commonly promote competitive dominance and invasion by *Bromus* and other exotic annual grasses. But while experimentally exploring this common process we found that nutrient-rich conifer soil and fertilized prairie soil promoted both the invasive and the native, and in both cases the magnitude of the facilitative effects of nutrient enrichment on *Bromus* was attenuated by competition with *Festuca*. Our results provide a unique perspective on facilitation. Many relatively straightforward pair-wise studies have shown direct facilitative effects of one species on another. A smaller number have shown that by suppressing a competitor one species can indirectly facilitate another, subordinate species. Our results demonstrate another form of biotic conditionality; strong

facilitative effects manifest in pair-wise experiments can be eliminated or diminished by the presence of other competitors.

Keywords: biotic resistance, invasion, indirect interactions, plant community, soil fertility

Introduction

Plant community composition, diversity, and productivity are strongly influenced by the shifting balance of facilitation and competition within plant communities (Callaway et al. 1996, Holzapfel & Mahall 1999; Michalet et al. 2006). Indirect interactions among species can also cause shifts in the outcomes of interactions but these are often investigated in the context of a dominant species suppressing another dominant species, thereby promoting a less competitive species (Miller 1994, Levine 1999). But facilitation can also indirectly alter competitive outcomes among species by increasing the competitive ability of a previously subordinate species (Rice and Nagy 2000, Callaway 2007). Such indirect interactions have not been well studied, but may have important implications for communities. Furthermore, because of the unusually strong competitive abilities of some invasive species, indirect effects on competitive interactions with natives may yield important insight into invasions.

Interactions between native plants and invasive exotic species are typically investigated in the context of competitive exclusion of natives (e.g. Ortega and Pearson 2005) or biotic resistance by natives (Levine et al. 2004, Zavaleta and Hulvey 2004, Maron and Marler 2007). Some native species, however, directly facilitate exotic plants through increased nitrogen availability (Maron and Connors 1996, Rice and Nagy 2000) or amelioration of stressful conditions (Freeman and Emlen 1995, Von Holle 2005, Badano et al. 2007). But as with interactions among native species, changing environmental conditions can shift these interactions among natives and exotics along the continuum from facilitative to competitive (Bertness and Callaway 1994; Holzapfel and Mahall 1999, Von Holle 2005).

Savanna trees are classic examples of facilitators, often benefiting grassland species through increased soil nutrient availability and buffering harsh aspects of the physical environment (e.g. Parker and Muller 1982, Archer 1988, Callaway et al. 1991, Tewksbury and Lloyd 2001). Community invasibility often increases with availability of soil nutrients (Burke and Grime 1996, Davis et al. 2000) and with reduced environmental stress (Von Holle 2005, Chambers et al. 2007). Therefore as one might predict, overstory trees can facilitate exotic plant invasion (Von Holle 2005, Rice and Nagy 2000, Gundale et al. 2008).

Bromus tectorum L. (cheatgrass; hereafter *Bromus*) is an exotic annual grass that was introduced to western North America around 1890 and has subsequently transformed shrublands across the American West into annual grasslands, vastly altering disturbance regimes (Harris 1967, Mack 1981, D'Antonio and Vitousek 1992) and nutrient cycling (Evans et al. 2001, Sperry et al. 2006). *Bromus* invasion has been correlated with elevated levels of soil nitrogen and phosphorus (Bashkin et al. 2003, Gundale et al. 2008) and increased competitive effects of *Bromus* have been observed with added nitrogen (Lowe et al. 2003, Vasquez et al. 2008). Neighboring plants can facilitate *Bromus*, when grown without other potential competitors, through canopy effects (Freeman and Emlen

1995, Griffith 2010), increased soil fertility (Gundale et al. 2008), and litter effects (Adair et al. 2008), but how facilitation influences the competitive dynamic between *Bromus* and other members of the plant community has yet to be explored.

In intermountain grasslands, *Bromus* is a "strong" invader capable of invading plant communities and excluding natives (Ortega and Pearson 2005). Gundale et al. (2008) found that *Bromus* abundance is substantially higher under the canopies of isolated *Pinus ponderosa* Dougl. ex Laws (ponderosa pine; hereafter *Pinus*) where soil nitrogen and phosphorus concentrations are higher.

We have pursued a more mechanistic understanding of this system through a series of field observations and manipulative experiments. Specifically, we investigated the potential for soil and shade conditions found under *Pinus* canopies to 1) increase performance of *Bromus* and 2) for increased performance to alter the competitive effect of *Bromus* on the native perennial grass *Festuca idahoensis* Elmer (hereafter *Festuca*). Further, we explored how competition from *Festuca* modifies the facilitative effects of *Pinus* on *Bromus*.

Methods

Field patterns

Our field sites were intermountain grasslands in western Montana dominated by *Pseudoroegneria spicata* (Pursh) Á. Löve and *Festuca idahoensis* (Mueggler and Stewart 1980) with scattered *Pinus ponderosa* trees and heavily invaded by *Bromus*. Spatial patterns of exotic and native species were assessed at three savanna sites at a mean elevation of 1250 m and were located at lat. 46.809º, long. -114.110º; lat. 46.902 º, long. -

113.942º; and lat. 46.620º, long. -113.894. Site aspects were mostly south and west facing, ranging from 181-206º with slopes ranging from 19-32º. Soil types at the sites were Winkler very gravelly sandy loam, Bigarm gravelly loam, and the Holter-Repp-Sharrott families complex.

Pinus trees were widely spaced (>20 m apart) as a result of environmental conditions, not disturbance. At each site nine trees were selected as replicates, resulting in 27 sampled trees. At each tree four transects were established, radiating from the bole in the four cardinal directions and the abundance of all understory species was assessed in 1-m^2 quadrats located ¼ the distance from bole to dripline, ½ the distance from bole to dripline, five cm inside the dripline, then five cm, two m, four m, and eight m from outside the dripline. For each transect quadrats were averaged to provide representative "conifer" and "prairie" quadrats (located under or outside of *Pinus* canopies respectively). For more detailed methods and sites, conifer, and prairie habitat characteristics see Metlen and Callaway (*in review*).

Bromus abundance in relation to pines was analyzed using mixed model nested ANOVA with habitat (conifer or prairie) and azimuth as fixed effects, and the random variables, site, and replicate nested within site. Variance in relative cover could not be homogenized with transformations. However, nested ANOVA is robust to this assumption particularly with sample sizes greater than six (Underwood 1997) and so we reported these results. The relationship between *Bromus* and native species was assessed as in Ortega and Pearson (2005) by adding *Bromus* cover as a covariate to a GLM for native cover with site and replicate nested within site as random variables, then reporting the slope and F-statistic as an estimate of the strength of the interaction. All statistics were conducted with SPSS, 16.1.0 (SPSS, Chicago, Illinois, USA). Assumptions of normality and homogeneity of variance were assessed for each ANOVA and when necessary statistical tests were conducted with transformed data.

Soil and shade effects

Tree canopies usually increase soil fertility and always provide shade (Callaway 2007). Therefore, in a garden at The University of Montana's Fort Missoula (latitude 46.842º, longitude -113.993º, 962 m elevation), we conducted a split-plot experiment to test how shade and soil collected from open prairie or from under *Pinus* canopies might influence *Bromus* and affect the competitive responses of the invader to *Festuca*. Twenty 5 x 2 m experimental replicates were established, 10 of which were randomly selected for a shade treatment, created with a single shadecloth extending 0.5 m in each direction from all pots. Shade cloths were 4.35 m x 1.0 m and 0.5 m high and reduced PAR by 48%, to 862.8±10.1 $\mu mol/m^2/s$. In this experiment, PAR was higher than that measured for the maximum effect of *Pinus* at the center of the canopies, midday in late summer in the field (291.6±21.7 $\mu mol/m^2/s$; Metlen and Callaway, *in review*). However, this measurement of shade substantially underestimates the light available under canopies over time. To minimize mixing of field-collected conifer and prairie soils with soil in the garden, we buried 9 L (15 x 15 x 40 cm) black plastic pots with the bottoms removed to allow drainage. We planted 10 *Bromus* seeds alone or with 50 *Festuca* seeds, with the *Festuca* seeds planted on 26 March 2008 and the *Bromus* seeds planted on 20 April 2008. The aboveground biomass of all plants was harvested on 17 November 2008. The effect of replicate nested within shade (split-plot design) was not significant, so the data were analyzed as if this was a factorial experiment. Two separate analyses were conducted utilizing univariate fixed factor general linear models (GLM). One GLM evaluated *Bromus* response to soil and shade when alone or when competing with *Festuca*, and the interactions between these factors, and the second evaluated *Festuca* response to soil and shade when competing with *Bromus.*

Increasing soil fertility commonly benefits annual exotic grasses, including *B. tectorum*, more than perennial natives (Huenneke et al. 1990, Kolb et al. 2002, Vasquez et al. 2008). We therefore compared competitive interactions between *Bromus* and *Festuca* in fertile soil from under conifer canopies and less fertile prairie soil in a greenhouse experiment. Greenhouse temperatures during experiments ranged from 15 to 30ºC, similar to early summer temperatures outside. Natural light in the greenhouse was supplemented by metal halide bulbs, and total photosynthetically active radiation (PAR) during the day remained above 1200 µmol/m^2/s with a day length of 13 hours.

Field soils were put into 2.4 L pots; 18 cm diameter, 22 cm deep (n=10 for all six treatment combinations). Ten seeds of *Bromus* and 10 seeds of *Festuca* were planted either in monoculture or in interspecific competition. *Festuca* seeds were planted in 24 November 2008 and *Bromus* seeds were planted on 12 December 2008. All plants were harvested on 19 February 2009, dried at 60ºC for three days, and then weighed. Data were square root-transformed and analyzed separately by species with univariate fixed factor GLM's. The relative interaction index (RII, see Armas and Pugnaire 2004) ranges from competitive exclusion (-1) to complete facilitation (+1) and was used to illustrate competitive effects.

We further isolated the effects of elevated N and P and growth and competitive dynamics of *Bromus* and *Festuca* by fertilizing field-collected prairie soil with NO_3^- and PO_4^+ to mimic levels reported for soil under *Pinus* by Gundale et al. (2008) and Metlen and Callaway (*in review*). We found that in the prairie KCl extractible NO_3^- averaged ≈0.7 µg/g soil and PO_4^+ averaged ≈0.1 µg/g soil in a 15 cm deep soil sample. In contrast, in soil under *Pinus* KCl extractible NO_3^- averaged ≈1.9 µg NO_3^-/g soil and PO_4^+ averaged ≈3.4 µg PO_4^+/g soil (Metlen and Callaway, *in review*). While Gundale et al. (2008) used different techniques for quantifying nutrient availability, they found ~3x as much plant available N and P in conifer soil compared to prairie soil. We experimentally added N and P in two pulses which together totaled 0.004 g (39.6 µmol) KNO_3 and 0.10 g (574.1 µmol) K_2HPO_4 dissolved in 1 mL H_2O. Because we added nutrients from the surface (300 mg NO_3^-/m^2 and 6900 mg PO_4^+/m^2), establishing whole-pot concentrations at field levels would result in much higher concentrations in the upper several centimeters and so we chose to use conservative nutrient addition treatments. Rocket pots (500 mL) were filled with prairie soil (n=10 for all six treatment combinations). *Festuca* germinates and initially grows more slowly than *Bromus*, thus *Festuca* was seeded into the pots for *Festuca* grown alone and in interspecific competition with *Bromus* on 24 November 2008 and *Bromus* was seeded into pots for *Bromus* grown alone and in interspecific competition with *Festuca* on 20 December 2008. Nutrient solutions were added on 1, January 2009 and 22 January 2009. All plants were harvested on 24 February 2009, dried at 60ºC for three days, and then weighed. We analyzed the data with separate univariate fixed factor GLM's for each species, and with pairwise t-tests for the effect of competition within soil treatments if significant effects of fertilization were determined in the full model.

Results

Field patterns

Bromus tectorum was 2.3 times more abundant under pines than in the prairie (Table 1) comprising 21% of the plant community under conifers but only 9% of the plant community in open prairie (Fig. 1). While the strength of this pattern varied by site (Table 1), for each site *Bromus* relative cover was 45%, 61%, and 71% greater under pines than in the prairie. There was a negative relationship between *Bromus* cover and native cover (slope=-0.28; $F_{1, 184}$ =9.7; P=0.002) and this relationship was even stronger when conifer plots were excluded from the analysis (slope=-0.58; $F_{1, 76}$ =11.5; P=0.001).

Soil and shade effects

In the garden, both conifer soil and shade facilitated the growth of *Bromus* when the invader was grown alone, but the combination of these variables resulted in 5.5 times larger *Bromus* plants than any other treatment (Fig. 2). *Festuca* did not have significant overall competitive effects on *Bromus* in this experiment (Fig. 2), but the positive effect of conifer soil on *Bromus* was eliminated when *Bromus* was grown in competition with *Festuca* (pairwise test; $F_{1, 19}$=1.1, P=0.304). Shade still marginally facilitated *Bromus* (pairwise test; $F_{1, 19}$=4.0, P=0.061), but much less than when *Bromus* was grown alone. Shoot biomass of *Festuca* grown in competition with *Bromus* did not vary with soil or shade (Soil $F_{1, 29}$=1.5, *P*=0.227; Shade $F_{1, 29}$=0.3, *P=0*.586; Shade x Soil $F_{1, 29}$=0.1, *P*=0.834).

In greenhouse experiments, *Bromus* grew 170% larger in conifer than in prairie soil, but *Festuca* also grew 190% larger in conifer soil (Fig. 3). Neither species had a competitive effect on the other in prairie soil, but in conifer soil *Bromus* and *Festuca* were both 33% smaller when grown in interspecific competition. The RII for the effect of *Bromus* on *Festuca* changed from -0.11 in prairie soil to -0.22 in *Pinus* soil, and the effect of *Festuca* on *Bromus* changed from +0.13 to -0.23.

In the second greenhouse experiment (with smaller pots and with *Festuca* given a longer time to establish prior to competition) competition was more consistent and more intense. Adding NO_3^- and PO_4^+ to prairie soil increased the growth of both *Bromus* and *Festuca* by 74% (Fig. 4). RII for the effect of *Bromus* on *Festuca* in unfertilized prairie soils was -0.50 but decreased to -0.23 with fertilization, and the RII for the effect of *Festuca* on *Bromus* was -0.24 regardless of nutrient availability. But across all treatments *Bromus* was a better competitor than *Festuca*, reducing the latter's biomass by 55%; whereas *Festuca* reduced *Bromus* biomass by 39%.

Discussion

Bromus tectorum was more abundant under *Pinus ponderosa* canopies than in open prairie, and shade and higher nutrient availability under *Pinus* canopies facilitated *Bromus* growth. Throughout invaded savannas, *Bromus* cover was negatively correlated with the total cover of natives suggesting that competitive interactions may be an important component of *Bromus* invasion (see Harris 1967, Freeman and Emlen 1995).

In experiments, *Bromus* grown with *Festuca* was consistently the dominant species based on biomass. However, the presence of *Festuca*, whether the competitive effects of the native were significant or not, reduced the otherwise very strong facilitative effects of *Pinus* on *Bromus*. These results emphasize the importance of examining facilitation in a broader community context and the potential for complex interactions among natives to resist invasion even when invaders are facilitated. In other words, in the absence of competitive resistance from native grasses, the facilitative effects of *Pinus* might be expected to facilitate far greater abundances of *Bromus*.

Canopies often facilitate establishment and growth of plants by reducing photoinhibition, moderating temperatures and increasing moisture availability (Archer et al. 1988, Greenlee and Callaway 1996, Holzapfel and Mahall 1999, Tewksbury and Lloyd 2001, Callaway 2007), but competitive dynamics among native and exotic species in the understory community have rarely been considered. In a notable exception, Siemann and Rogers (2003) showed that the invasion of *Sapium sebiferum* was facilitated by the shade of native shrubs because shade enhanced the competitive effects of the invader on native tallgrass prairie species. Also, Parker and Muller (1982) found that *Quercus agrifolia* canopies directly facilitated the native forb *Pholistima auritum*, but *Pholistima* then suppressed exotic annual grasses through allelopathic effects. Interestingly, in the absence of *Pholistima* some of these annual grasses are facilitated by *Quercus* species (Callaway et al. 1991). Native canopies can facilitate *Bromus* (Freeman and Emlen 1995, Griffith 2010) and neighbor removal can have negative effects on *Bromus* despite concomitant increases in nutrient availability (Adair et al. 2008).

Bromus is an aggressive invader of open semi-arid grasslands under many conditions (Harris 1967, Mack 1981, D'Antonio and Vitousek 1992, Evans et al. 2001, Bashkin et al. 2003), and we do not interpret our results as indicating that *Bromus* is particularly shade tolerant. However, *Bromus* is plastic with respect to light availability. Pierson and Mack (1990) found that light interception by forest overstory did not limit *Bromus* invasion, even though forest canopies reduced PAR to 463 and 340 $\mu mol/m^2/s$, respectively. In the greenhouse, Pierson et al. (1990) found that *Bromus* plants grown at 128 $\mu mol/m^2/s$ were much smaller, but were still physiologically capable of responding to increased PAR as rapidly as plants grown in the open. *Bromus* may efficiently use temporally sporadic light, typical of subcanopies, while benefiting from protection from temperature extremes, desiccation, and intense sunlight.

Nutrient availability was the highest under savanna pines, corresponding with peak *Bromus* abundance and performance when grown alone, but perennial neighbors strongly diminished this facilitative effect. Other studies have clearly shown correlations between *Bromus* abundance and nutrient rich sites (Bashkin et al. 2003, Gundale et al. 2008), and increased nutrient availability generally favors exotic annuals over native perennials in competition (Huenneke et al. 1990, Claassen and Marler 1998, Kolb et al. 2002, Vasquez et al. 2008). For example, Huenneke et al. (1990) found that infertile serpentine soils were relatively uninvaded, but when they experimentally increased soil N to 20 $\mu g\ NO_3^-/g$ soil and soil P to 50 $\mu g\ PO_4^+/g$ soil, annual grass invasion was significant. Studies that show strong shifts to annual competitive dominance tend to involve much higher nutrient concentrations than those found in our system; by way of comparison, we found that levels of NO_3^- and plant available PO_4^+ are 1.9 and 3.4$\mu g/g$

soil respectively in soil from under *Pinus* (Metlen and Callaway, *in review*). As for Lowe et al. (2003) and Claassen and Marler (1998), we found that perennials and annuals alike benefited when <14 g µN/g soil were added. In our system, the natural concentrations of soil N and P may be lower than those which strongly shift competitive advantages to annual grasses.

Established native perennial species are capable of strong competitive effects on annuals (Seabloom et al. 2003, Corbin and D'Antonio 2004), and priority effects may help to explain the competitive performance of the native perennial *Festuca* in our fertilization experiments, as well as in nature. *Bromus tectorum* is a winter annual that benefits from a life history strategy that allows several months of growth before native species germinate, so while priority effects benefit established native species, when seedlings compete *Bromus* often has the priority advantage (Harris 1967, Freeman and Emlen 1995). We gave the native perennial a 3-4 week head start in all experiments. Abraham et al. (2009) found that a priority effect of only 14 days was sufficient to dramatically increase perennial competitive performance when grown with the annual grass *Bromus diandrus* at both high and low nitrogen availability. Claassen and Marler (1998) found that with a growth advantage of 50 days the competitive effect of a perennial grass on an annual grass was increased by as much as 55%, but observed only weak competitive effects of the perennial when both species were seeded simultaneously. Freeman and Emlen (1995) found that established perennials were weakly affected by competition with *Bromus* but competition between seedlings was often intense. Indeed, the exceptionally strong competitive ability of *Bromus* led them to state; "perhaps the most disturbing result of our study is that this introduced annual seems to be oblivious to the presence or absence of other species." Thus, under natural conditions competition among seedlings may be more intense than what we measured, and the longer term effects of high nutrient supply under pines could promote *Bromus* invasion more than our short-term experiments suggest.

Facilitation can strongly promote exotic plant invasion, but rarely are interactions among natives and invaders considered in the context of such facilitation. We show that *Pinus ponderosa* soil and shade facilitates the exotic annual grass *Bromus tectorum* and that *Pinus* soil facilitates the native perennial grass *Festuca idahoensis*. While competitive outcomes between the native and the exotic grasses were unchanged by conditions found under *Pinus* canopies, *Festuca* mitigated otherwise strong facilitation of *Pinus* on *Bromus*. Even within intact native communities, *Bromus* has successfully invaded intermountain savannas, but our results demonstrate the importance of biotic resistance to invasion, even when highly competitive exotic annuals are being facilitated.

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Table 1: The absolute cover of *Bromus tectorum* by habitat and azimuth from tree bole with associated mixed model ANOVA.

Habitat	Azimuth	Percent (SE)†		ANOVA‡	F	df	*P*
Absolute cover							
Prairie	All azimuths	2.9	(0.7)	Habitat	6.9	1, 2	0.119
	North	1.2	(1.5)	Azimuth	0.2	3, 6	0.870
	East	2.9	(1.4)	Site	0.3	2, 16	0.723
	South	5.5	(1.4)	Replicate(Site)	5.0	24, 164	*<0.0001*
	West	2.1	(1.5)	Habitat x Azimuth	4.3	3, 6	0.060
Conifer	All azimuths	6.6	(0.7)	Habitat x Site	4.1	2, 6	0.075
	North	6.1	(1.4)	Azimuth x Site	6.5	6, 6	*0.019*
	East	6.2	(1.4)	Habitat x Azimuth x Site	0.5	6, 164	0.839
	South	5.4	(1.4)				
	West	8.6	(1.4)				

Notes: †Adjusted marginal means.

‡ Site and replicate are random variables, replicate is nested within site.

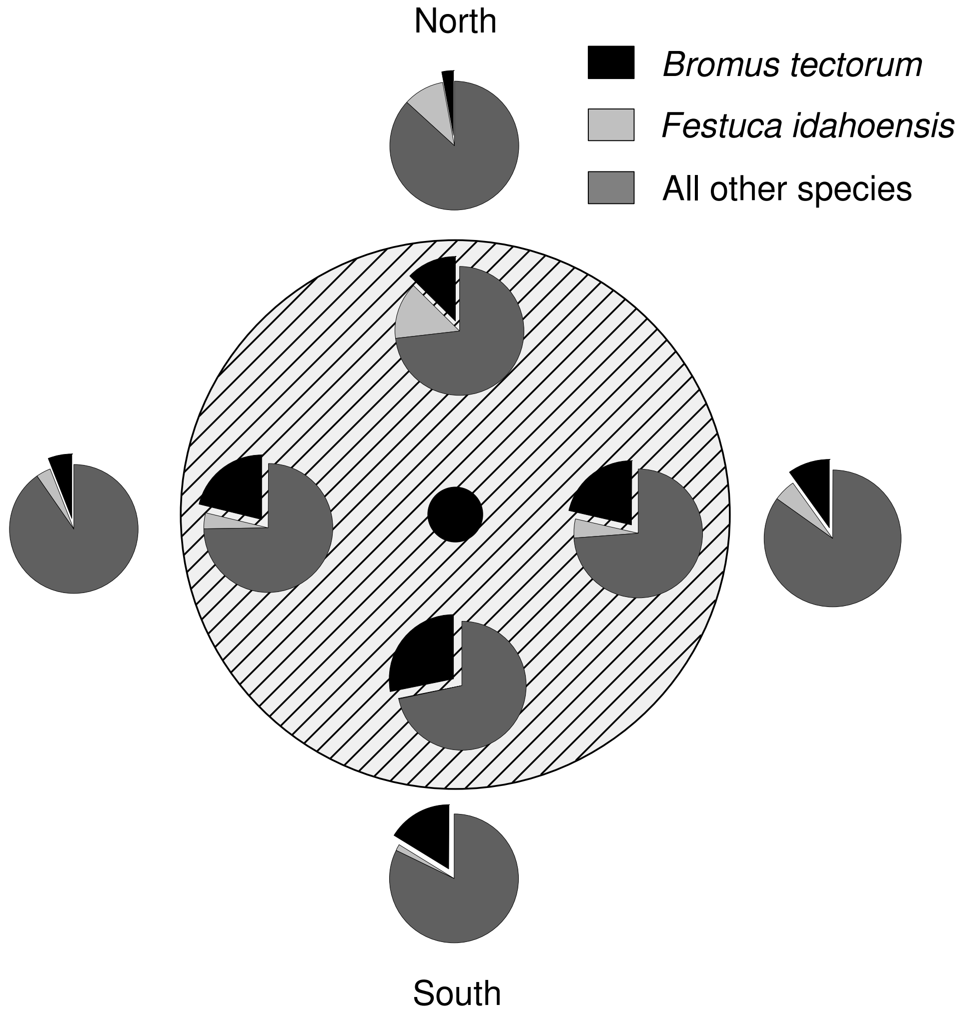

Figure 1: Proportion of the plant community composed of *Bromus tectorum* and *Festuca idahoensis* under *Pinus ponderosa* and in open prairie. Mixed model ANOVA for *Bromus* relative cover: Habitat $F_{1, 2}$=39.8, *P=0.024*; Azimuth $F_{3, 6}$=1.7, *P*=0.259; Site $F_{2,13}$=1.0, *P*=0.405; Replicate(Site) $F_{24, 164}$=4.7, *P<0.0001;* Habitat x Site $F_{2, 6}$=0.7, *P*=0.526; Habitat x Azimuth $F_{3, 6}$=0.3, *P*=0.823; Site x Azimuth $F_{6, 6}$=3.9, *P*=0.061; Habitat x Azimuth x Site $F_{6, 164}$=1.1, *P*=0.375.

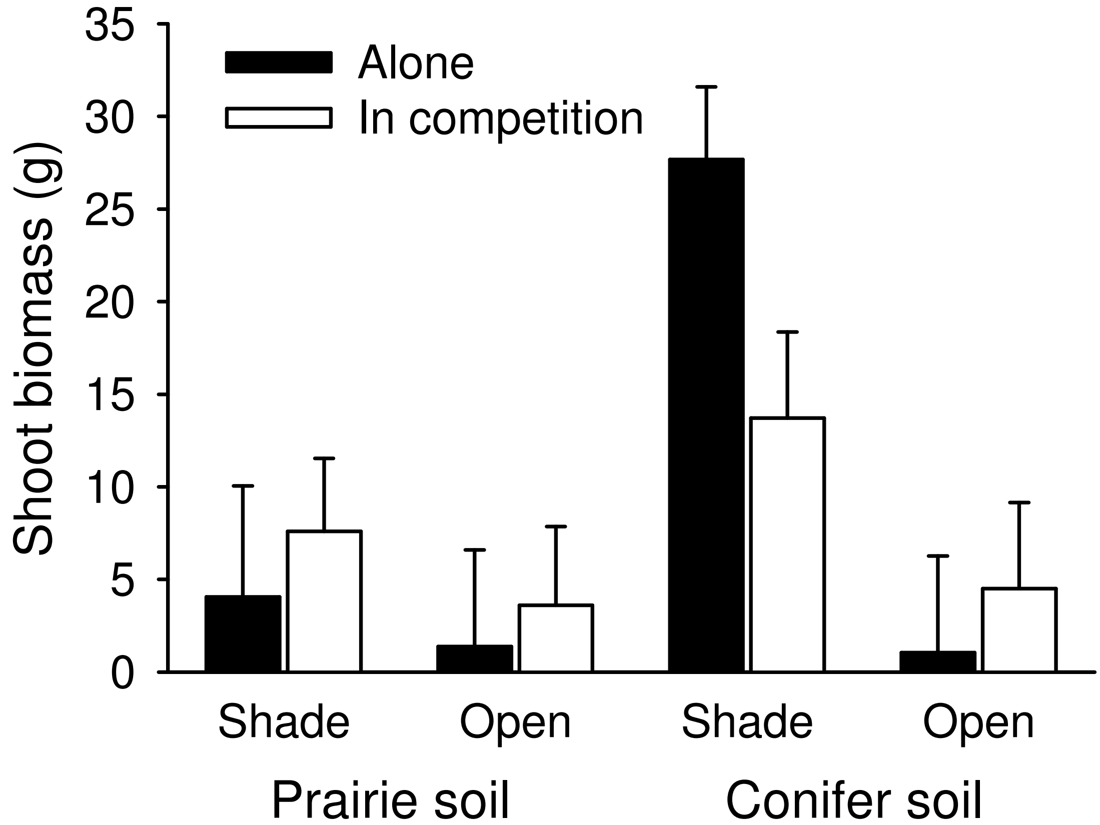

Figure 2: *Bromus tectorum* grown alone or in competition with *Festuca idahoensis* in shaded or open plots, and in prairie or conifer soil. Adjusted marginal means ±SE. ANOVA: Competition $F_{1, 33}$=0.1, *P*=0.729; Soil $F_{1, 33}$=5.0, *P=0.032*; Shade $F_{1, 33}$=9.9, *P=0.004*; Competition x Soil $F_{1, 33}$=1.5, *P*=0.242; Competition x Shade $F_{1, 33}$=1.4, *P*=0.242; Shade x Soil $F_{1, 33}$=4.7, *P=0.038*; Competition x Shade x Soil $F_{1, 33}$=1.9, *P*=0.175.

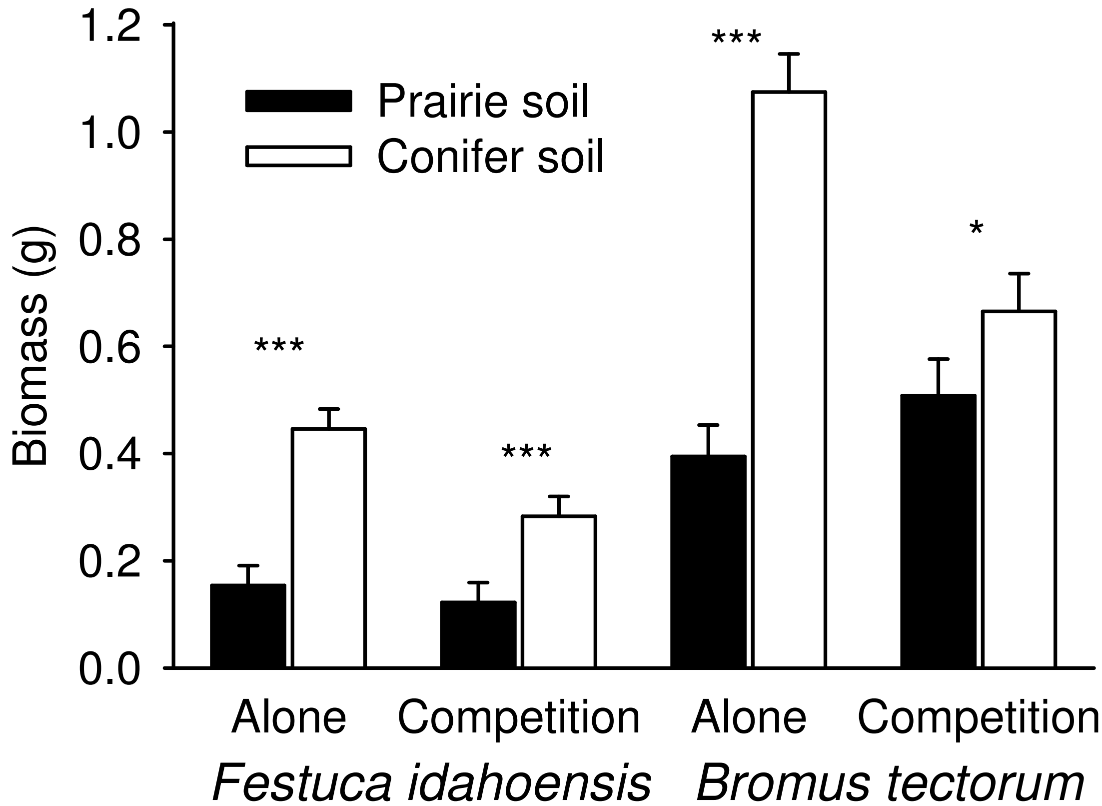

Figure 3: *Festuca idahoensis* and *Bromus tectorum* grown alone and in competition in field collected conifer and prairie soil. Adjusted marginal means ±SE. *Festuca* ANOVA: Soil $F_{1, 36}$=48.1, *P<0.0001*; Competitor $F_{1, 36}$=6.0, *P=0.019*; Soil x Competitor $F_{1, 36}$=1.7, *P*=0.199. *Bromus* ANOVA: Soil $F_{1, 42}$=40.6, *P<0.0001*; Competitor $F_{1, 42}$=2.3, *P*=0.135; Soil x Competitor $F_{1, 42}$=15.3, *P<0.0001*. For pairwise tests, * 0.05>*P*>0.01 and ****P*<0.001.

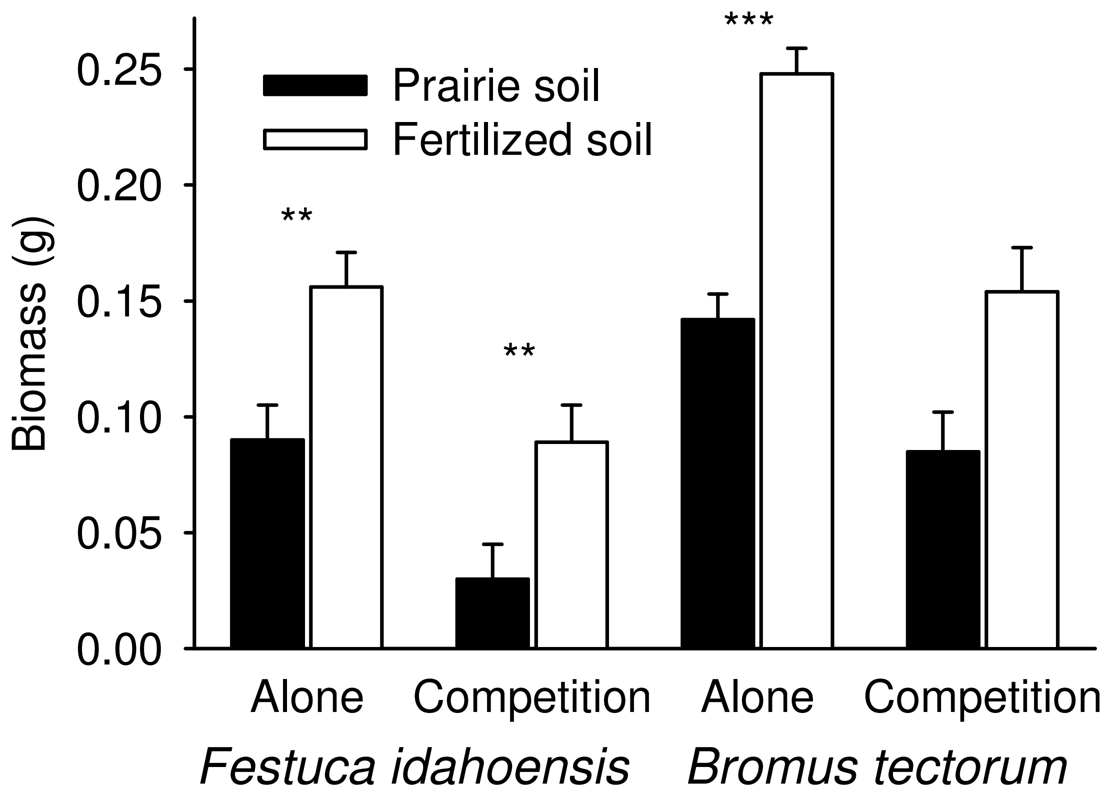

Figure 4: *Festuca idahoensis* and *Bromus tectorum* grown alone and in competition in prairie soil with no fertilizer, or with supplemental NO_3^- and PO_4^+ to mimic nutrient conditions found in conifer soil. Adjusted marginal mean ±SE. *Festuca* ANOVA: Nutrients $F_{1,33}$=17.0, *P<0.0001*; Competitor $F_{1,33}$=17.5, *P<0.0001*; Nutrients x Competitor $F_{1,33}$=0.1, *P*=0.821. *Bromus* ANOVA: Nutrients $F_{1,67}$=34.4, *P<0.0001*; Competitor $F_{1,67}$=25.1, *P<0.0001*; Nutrients x Competitor $F_{1,67}$=1.4, *P*=0.233. For pairwise tests, **0.01>*P*>0.001 and ****P*<0.001.

CHAPTER 3 - *PINUS PONDEROSA* INDIRECTLY FACILITATES *BROMUS TECTORUM* BY SUPPRESSING *CENTAUREA STOEBE*

Abstract

Native communities are commonly invaded by more than one exotic species, yet we know little about how invaders interact with each other as they overrun native ecosystems. *Centaurea stoebe* and *Bromus tectorum* are strong invaders in North America, and in prairie of the Northern Rockies they appear to displace each other under some conditions. We experimentally explored the mechanisms behind a striking spatial pattern in which *Centaurea* dominates plant communities in open prairie but *Bromus* dominates under large isolated *Pinus ponderosa* canopies where *Centaurea* is much less abundant. These spatial patterns correspond with increased soil nutrients, shade, and *Pinus* litter under *Pinus* canopies. Nutrient-rich soil from under *Pinus* and experimentally fertilized prairie soil improved the growth of both species similarly, and did not give the annual *Bromus* a competitive advantage over the perennial *Centaurea*. Intact *Pinus* litter reduced *Bromus* biomass by 35%, but reduced *Centaurea* biomass by 60% and strongly shifted competitive interactions in favor of *Bromus*. We also chopped litter and mixed it into the soil to minimize physical effects, and in this experiment there were no inhibitory effects on *Bromus* but *Centaurea* establishment was reduced by 76%. Experimental shade promoted *Bromus* growth, but decreased *Centaurea* growth. Thus, we found strong indirect facilitative effects of *Pinus* on *Bromus* via the suppression of *Centaurea,* as well as direct facilitative effects of *Pinus* on *Bromus*. Our results illustrate the importance of studying interactions among invaders, as well as the competitive and

facilitative interactions that occur among natives and exotics, to better understand patterns of exotic invasion on natural landscapes.

Keywords: (<13 words) allelopathy, biotic resistance, invasion, litter, plant community, soil nutrients, *Centaurea maculosa*

Introduction

Competitive and facilitative interactions are important processes in native plant communities (Callaway et al. 1996; Holzapfel & Mahall 1999; Callaway 2007). As exotic plant invasions rapidly transform and reorganize native communities, it becomes crucial to better understand the importance of competition and facilitation among native and exotic species (e.g. Freeman & Emlen 1995; Holzapfel & Mahall 1999) and among the invaders themselves. The competitive exclusion of native species by encroaching exotics may be the most conspicuous interaction in invasions (e.g. Levine et al. 2003; Ortega & Pearson 2005; Maron & Marler 2008), but native species can facilitate exotic invasion (Maron & Connors 1996; Siemann & Rogers 2003; Badano et al. 2007) or strongly resist invasion through competition (Elton 1958; Levine et al. 2003; Maron & Marler 2007).

In other cases exotic species promote other exotics, ecological interactions dubbed "invasional meltdown" (Simberloff & Von Holle 1999; O'Dowd et al. 2003; Grosholz 2005). Meltdown may occur when exotic plant species alter disturbance regimes or nutrient cycling (Vitousek et al. 1987; Mack et al. 2001), eliminate natives that are good competitors against the new exotics (Alverez & Cushman 2002; Ortega &

Pearson 2005; Kulmatiski 2006), or alter soil biota such that exotics are favored over natives (Richardson et al. 2000; Jordan et al. 2008; Grman et al., *in press*). In contrast to meltdown, invasive species can also competitively exclude other invaders (Piemeisel 1951; Kolb et al. 2002; Belote & Weltzin 2006).

In intermountain grasslands of the Rocky Mountains, the annual grass *Bromus tectorum* L. (cheatgrass; hereafter *Bromus*) and the perennial forb *Centaurea stoebe* L. ssp. *micranthos* (Gugler) Hayek (spotted knapweed; nee *C. maculosa* Lam.; hereafter *Centaurea*) are both "strong" invaders capable of invading plant communities and excluding natives (Piemeisel 1951; Harris 1967; Mack 1981; Ridenour & Callaway 2001; Ortega & Pearson 2005). The invasive success of *Bromus* has been correlated with relatively high nutrient availability, particularly phosphorus (Bashkin et al. 2003; Newingham & Belnap 2006; Gundale et al. 2008) and altered disturbance regimes (Harris 1967; Mack 1981). *Centaurea* is a well studied invader for whom success has been attributed to many complementary mechanisms, including escape from specialist enemies (Story *et al.* 2000; but see Müller-Schärer & Schroeder 1993), escape from limiting soil biota (Callaway *et al.* 2004b), indirect competitive advantages through arbuscular mycorrhizae (Marler *et al.* 1999; Carey *et al.* 2004; Callaway *et al.* 2004a), allelochemicals (Ridenour & Callaway 2001; He *et al.* 2009; Thorpe *et al.* 2009; Pollock et al. 2009), and altering ecosystem processes (Thorpe *et al.* 2006).

Centaurea and *Bromus* appear to compete strongly with each other as they invade. Declines in *Centaurea* abundance due to herbicide and biocontrol agents have led to dramatic increases in *Bromus* abundance (Story et al. 2006; Ortega & Pearson, *in press*). *Pinus ponderosa* Dougl. ex Laws (ponderosa pine; hereafter *Pinus*), a common savanna tree in intermountain grassland, may have strong effects on the competitive interactions between *Centaurea* and *Bromus*. Gundale et al. (2008) found that in intermountain prairie *Bromus* was much more abundant under the canopies of isolated *Pinus*. Plant-available nitrogen and phosphorus concentrations are higher under *Pinus* than in prairie soil, and the abundance of *Bromus* under *Pinus* was attributed to the facilitative effects of higher nutrient availability. Metlen & Callaway (*in review*) found that, in similar intermountain savannas, *Centaurea* was much less abundant under *Pinus* than in open grassland, clearly contrasting with the spatial pattern of *Bromus*.

We utilized these sharply contrasting spatial patterns of *Centaurea* and *Bromus* to investigate direct and indirect facilitative and competitive mechanisms at work in these invasions, with a focus on how competition between these two exotic species might determine their distributions. Specifically, we ask: 1) Does *Pinus* directly facilitate *Bromus*, thereby modifying competitive outcomes to favor *Bromus* over *Centaurea*? 2) Does *Pinus* indirectly facilitate *Bromus* by suppressing *Centaurea*?

Methods

Field observations

Our field sites were intermountain grasslands in western Montana dominated by *Pseudoroegneria spicata* (Pursh) Á. Löve and *Festuca idahoensis* Elmer (Mueggler & Stewart 1980). *Pinus* trees were widely spaced (>20 m apart) as a result of natural environmental conditions, not human disturbance. Spatial patterns of exotic and native species were assessed at three savanna sites at a mean elevation of 1250 m and were located at lat. 46.809º, long. -114.110º; lat. 46.902º, long. -113.942º; and lat. 46.620º, long. -113.894º. Site aspects were mostly south and west facing, ranging from 181-206º with slopes ranging from 19-32º. Soil types at the sites were Winkler very gravelly sandy loam, Bigarm gravelly loam, and the Holter-Repp-Sharrott families complex.

At each site nine trees were selected as replicates, resulting in 27 sampled trees. At each tree four transects were established, radiating from the bole in the four cardinal directions and the abundance of all understory species was assessed in 1-m^2 quadrats located ¼ the distance from bole to dripline, ½ the distance from bole to dripline, five cm inside the dripline, then five cm, two m, four m, and eight m from outside the dripline. On each transect "conifer" and "prairie" plots were represented statistically by an average of all quadrats from under or outside the canopy. For more detailed methods and sites, conifer, and prairie habitat characteristics see Metlen and Callaway (*in review*).

The proportion of the plant community consisting of *Bromus* or *Centaurea* was analyzed separately using mixed model nested ANOVA with habitat (conifer or prairie) as a fixed effect, and the random variables, site, and replicate nested within site. Variance in relative cover could not be homogenized with transformations. However, nested ANOVA is robust to this assumption particularly with sample sizes greater than six (Underwood 1997) and so we report these results. The relationship between *Bromus* and *Centaurea* was assessed in the plots that contained *Centaurea* and *Bromus* by constructing a general linear model (GLM) for each species cover with cover of the other species as a covariate, and site and tree nested within site as random variables (as in Ortega and Pearson 2005). We then report the slope and F-statistic as an estimate of the strength of the interaction. All statistics were conducted with SPSS, 16.1.0 (SPSS, Chicago, Illinois, USA). Assumptions of normality and homogeneity of variance were assessed for each ANOVA and when necessary statistical tests were conducted with transformed data.

Pinus and prairie soil

Increasing soil fertility often benefits annual life histories more than perennial life histories (Grime 1977; Huston & Smith 1987; Kolb et al. 2002). We therefore compared competitive interactions between *Bromus* and *Centaurea* in fertile soil from under *Pinus* canopies and less fertile prairie soil in a greenhouse experiment. Greenhouse temperatures during experiments ranged from 15 to 30ºC, similar to early summer temperatures outside. Natural light in the greenhouse was supplemented by metal halide bulbs, and total photosynthetically active radiation (PAR) during the day remained above 1200 µmol/m^2/s with a day length of 13 hours.

Field soils were put into 2.4 L pots; 18 cm diameter, 22 cm deep (n=10 for all six treatment combinations). Ten seeds of *Bromus* and 10 seeds of *Centaurea* were planted either in monoculture or in interspecific competition. *Centaurea* seeds were planted in 24 November 2008 and *Bromus* seeds were planted on 12 December 2008. All plants were harvested on 19 February 2009 and, as in all subsequent experiments, dried at 60ºC for three days, and then weighed. The data were analyzed separately by species with univariate fixed factor GLM's. Data for *Bromus* were square root-transformed to homogenize variance. The relative interaction index (RII, see Armas & Pugnaire 2004) ranges from competitive exclusion (-1) to complete facilitation (1) and was used to illustrate competitive effects. The results for the performance of *Bromus* grown alone were reported in Metlen & Callaway (*in prep)* to contrast with performance of *Festuca,* but are presented here to contrast with the growth of *Centaurea* and performance of *Bromus* when grown in interspecific competition with *Centaurea*.

We further isolated the effects of elevated nitrogen (N) and phosphorus (P) on the growth of *Bromus* and *Centaurea* and competition between the invaders by fertilizing field-collected prairie soil with NO_3^- and PO_4^+ to mimic levels reported for soil under *Pinus* by Gundale et al. (2008) and Metlen and Callaway (*in review*). We found that in the prairie KCl extractible NO_3^- averaged ≈0.7 µg/g soil and PO_4^+ averaged ≈0.1 µg/g soil in a 15 cm deep soil sample. In contrast, in soil under *Pinus* KCl extractible NO_3^- averaged ≈1.9 µg NO_3^-/g soil and PO_4^+ averaged ≈3.4 µg PO_4^+/g soil (Metlen and Callaway, *in review*). Gundale et al. (2008) used different techniques for quantifying nutrient availability, and they found ≈3x as much plant available N and P in *Pinus* soil compared to prairie soil. We experimentally added N and P in two pulses which together totaled 0.004 g (39.6 µmol) KNO_3 and 0.10 g (574.1 µmol) K_2HPO_4 dissolved in 1 mL H_2O. Because we added nutrients from the surface (300 mg NO_3^-/m^2 and 6900 mg PO_4^+/m^2), establishing whole-pot concentrations at field levels would result in much higher concentrations in the upper several centimeters and so we chose to use conservative nutrient addition treatments calculated to increase soil NO_3^- and PO_4^+ to in 30 µg/g soil and 600 µg/g soil respectively, in the upper 2.5 cm of the pots. Rocket pots (500 mL) were filled with prairie soil (n=10 for all six treatment combinations). *Centaurea* was seeded into the pots for *Centaurea* grown alone and pots for interspecific competition with *Bromus* on 24 November 2008, and *Bromus* was seeded into pots for *Bromus* grown alone and pots for interspecific competition with *Centaurea* on 20 December 2008. Nutrient solutions were added on 1 January 2009 and 22 January 2009 and the plants were harvested on 24 February 2009. We analyzed the data with separate univariate fixed factor GLM's for each species. The data for *Bromus* grown alone were reported in Metlen & Callaway (*in prep)* but are presented here to show the competitive effect of *Centaurea* and to contrast with *Centaurea* performance.

<u>Litter effects</u>

We tested the effects of intact pine litter on *Bromus* and *Centaurea* growth and competitive interactions in a greenhouse experiment. Field soil from under *Pinus* and from open prairie was placed into 2.4 L pots; 18 cm diameter, 22 cm deep and either left bare or 20 g of pine needles (7 cm deep) were added to the soil surface (n=10 for all treatments). Each pot was planted with ten seeds of each species alone or in interspecific competition on 25 January 2008. Aboveground biomass was harvested 29 May 2008. Direct and indirect effects of whole litter on *Bromus* and *Centaurea* were tested using separate GLM's for each species with litter and competition as fixed effects. Variance in *Centaurea* biomass could not be homogenized with transformations. When significant interactions were identified in the global model we further explored relationships between variables using pairwise tests. A square root transformation removed heteroscedasity for the pairwise tests with *Centaurea* biomass.

The effect of *Pinus* litter on the establishment of *Bromus* and *Centaurea* was further examined by chopping 20 g of pine needles into fine pieces and stirring them into the soil in order to minimize the physical effects of litter on germinating seedlings at the soil surface. This was done in the greenhouse in 2.4 L pots in field soil from under *Pinus* (n=10 for both treatments). Each pot was planted with ten seeds of either *Bromus* or

Centaurea on 25 January 2008. We counted the number of plants that established in each pot on 29 May 2008. The data were analyzed with a global GLM containing species, treatment, and the interaction term, and then pairwise tests were used to determine differences between treatments within species.

Shade

We conducted two separate experiments to test how shade, mimicking that under *Pinus* canopies, affects *Bromus* and *Centaurea.* In the garden at The University of Montana's Fort Missoula (latitude 46.842º, longitude -113.993º, 962 m elevation) twenty 5 x 2 m replicates were established, 10 of which were randomly selected for a shade treatment, created with a single shadecloth extending 0.5 m in each direction from all pots. Shade cloths were 4.35 m x 1.0 m and 0.5 m high and reduced PAR by 48%, to 862.8±10.1 $\mu mol/m^2/s$. In this experiment PAR was higher than that measured for the maximum effect of *Pinus* at the center of the canopies, midday in late summer in the field (291.6±21.7 $\mu mol/m^2/s$). However, this measurement of shade substantially underestimates the light available under canopies over time. To minimize mixing of field-collected soils with soil in the garden, we buried 9 L (15 x 15 x 40 cm) black plastic pots with the bottoms removed to allow drainage. To evaluate the effect of shade on *Bromus* we planted 10 *Bromus* seeds in each pot on 20 April 2008 (n=10) and harvested the aboveground biomass on 17 November 2008. *Centaurea* were started from seed in 125 mL rocket pots in the greenhouse, transplanted into the experiment as 3-month old seedlings on 17 August 2006 (n=20), and harvested on 10 July 2007. Separate pairwise GLM's of the effect of shade were conducted for both species. The effect of replicate nested within shade (split-plot design) was not significant, so the data were analyzed as if this was a factorial experiment. *Bromus* biomass was natural log-transformed to homogenize variance.

Results

Field observations

Bromus tectorum and *Centaurea stoebe* were both found throughout the intermountain grasslands we sampled. The relative cover of *Bromus* was 2.3 times greater under *Pinus* canopies than in open prairie, but the relative cover of *Centaurea* was 5 times greater in the open prairie than under *Pinus* canopies (Fig. 1). Where both *Centaurea* and *Bromus* were present, *Bromus* cover was highly negatively correlated with *Centaurea* cover (slope=-0.46; $F_{1, 125}$ =8.9; *P=0.003*) and *Centaurea* cover was negatively but more weakly correlated with *Bromus* cover (slope=-0.15; $F_{1, 125}$ =8.9; *P=0.003*).

Soil effects

When grown in field collected *Pinus* soil *Bromus* grew 2.7 times larger than when grown in prairie soil, whereas *Centaurea* grew 4.3 times larger in *Pinus* soil than in prairie soil (Fig. 2). *Centaurea* was seeded 18 days prior to seeding *Bromus*, but there were no competitive effects of either species in prairie soil in this experiment. In contrast we measured strong competitive effects in *Pinus* soil, and competition eliminated the facilitative effects of *Pinus* soil on both invasive species. In *Pinus* soil, RII for the competitive effect of *Bromus* on *Centaurea* (-0.30) was smaller than the effect of *Centaurea* on *Bromus* (-0.50).

In the second experiment (with smaller pots and with *Centaurea* given 26 days to establish prior to seeding *Bromus*), adding NO_3^- and PO_4^+ to prairie soil increased the growth of *Bromus* by 1.8 times, but increased *Centaurea* growth by 2.2 times (Fig. 3). Surprisingly, we found that fertilization did not change the competitive effects between *Bromus* and *Centaurea* in this experiment.

<u>Litter effects</u>

Intact *Pinus* litter inhibited *Bromus* growth by 34%, but reduced *Centaurea* growth by 60% (Fig. 4). In this experiment, both species were seeded simultaneously and in the absence of litter *Centaurea* competitive effects on *Bromus* were weak (RII -0.15) while competitive effects of *Bromus* on *Centaurea* were strong (RII -0.73). Litter eliminated the competitive effect of *Centaurea* on *Bromus*, but RII for the effect of *Bromus* on *Centaurea* increased to -0.87, resulting in very little *Centaurea* growth when in competition with *Bromus* in the treatment with *Pinus* litter. Chopped *Pinus* litter mixed into *Pinus* soil did not affect the establishment of *Bromus,* but reduced *Centaurea* establishment by 74% (Fig. 5).

Shade

Shade promoted growth of *Bromus* ($F_{1, 17}$=8.9, *P=0.008*) which grew to 21.8 ±4.5 g in shaded pots but only grew to 1.2 ±5.2 g in pots open to the sun. In a separate experiment which ran for twice as long but used the same shade structures, *Centaurea* was inhibited by shade ($F_{1, 39}$=103.4, *P=0.055*) and grew to 232.5 ±26.7 g in the open but only to 156.8±27.4 g in the shade.

Discussion

Our results suggest that performance of *Bromus tectorum,* one of the most successful invaders of North America, is facilitated by the soil and shade conditions found under *Pinus ponderosa.* In addition, *Pinus* litter provides strong biotic resistance to *Centaurea stoebe*, a second strong invader, thereby facilitating *Bromus* indirectly. Resistance to *Centaurea* appears to occur, at least in part, from the chemical effects of *Pinus* litter which is interesting because of the potential allelopathic effects of *Centaurea* itself (Ridenour & Callaway 2001; He *et al.* 2009; Thorpe *et al.* 2009; Pollock et al. 2009). Shade also inhibited *Centaurea*. In contrast to strong litter and shade effects, fertile soil under *Pinus* canopies facilitated both species and did not promote the annual exotic over the perennial exotic. We did not directly measure the competitive effects of *Centaurea* on *Bromus* in prairie but others have documented dramatic increases in *Bromus* after *Centaurea* removal by specialist biological control insects or herbicide (Story et al. 2006; Ortega & Pearson, *in press*), suggesting that *Centaurea* may competitively exclude *Bromus* in open prairie.

Other native species can inhibit highly competitive exotic species allelopathically (Parker & Muller 1979; Weidenhamer & Romeo 2005), but mechanisms driving these processes have not been thoroughly developed. A growing, well-supported hypothesis for successful exotic invasion posits that some invaders may possess allelopathic, anti-herbivore, or antimicrobial secondary metabolites that are novel in the invaded ranges of the species, resulting in stronger biological impacts in the non-native ranges (Rabotnov 1982; Callaway & Aschehoug 2000; Mallik & Pellissier 2000; Cappuccino & Arnason 2006). However, in a recent review, Verhoeven et al. (2009) pointed out exotic species have equal chances of encountering novel traits in their new ranges to which they themselves are naive. Perhaps chemical effects of *Pinus* litter on *Centaurea* provide evidence for the importance of this ecological interaction.

Centaurea may compete well with *Bromus* under some conditions, but *Bromus* is a highly invasive annual grass that can invade communities of other annual exotics and exclude them to form persistent monocultures (Piemeisel 1959) and that can have strong competitive effects on native perennials (Ortega & Pearson 2005), particularly at the seedling stage (Harris 1967; Freeman & Emlen 1995). In soils experimentally manipulated to contain N and P concentrations far greater than observed under *Pinus* canopies, *Bromus* can be a competitive dominant over *Centaurea* (L. Besaw, K.L. Metlen, R.M. Callaway, *unpublished data*). However, annual species are often replaced by species with perennial life strategies over the course of succession (Grime 1977; Huston & Smith 1987) in part because of “priority effects” that favor established perennials over annuals that must establish from seed (Seabloom et al. 2003; Corbin & D’Antonio 2004). Importantly, a priority effect of only 14 days can strongly shift the outcome of competition between exotic perennials and annual grasses in favor of the perennials (Abraham et al. 2009) and a growth advantage of 50 days increased the competitive effect of a native perennial grass on an annual grass by as much as 55% (Claassen and Marler 1998).

Our results reflect the importance of priority effects, as determined by planting order, on competitive outcomes. In our experiments with *Pinus* and prairie soil, we gave *Centaurea* a head start of 18 days and in the fertilization experiment *Centaurea* was seeded 26 days before seeding *Bromus*. In these experiments, the effects of competition were modest. In the litter experiment there were no priority effects; we seeded both species together, leading to the strongest observed competitive effects of *Bromus* on *Centaurea* with an RII of -0.73 without litter and an RII of -0.87 with litter. Thus, competition among seedlings may favor the annual exotic while development of the exotic dominated plant community may favor the perennial exotic over time.

Soil and shade conditions found under *Pinus ponderosa* canopies facilitated *Bromus tectorum* performance. In addition, litter inhibited *Centaurea stoebe* establishment, possibly allelopathically, and intact *Pinus* litter altered the competitive ability of *Centaurea*, resulting in indirect facilitation of a second invader, *Bromus tectorum*. It is particularly notable that in this case a native species was shown to allelopathically inhibit a purportedly allelopathic exotic invader. Complex interactions among invasive exotic species are not frequently investigated but our results suggest that interactions among *Bromus* and *Centaurea* have important effects which can be modified by the native community; emphasizing the importance of studying invasions in the context of realistic communities comprised of natives and exotics.

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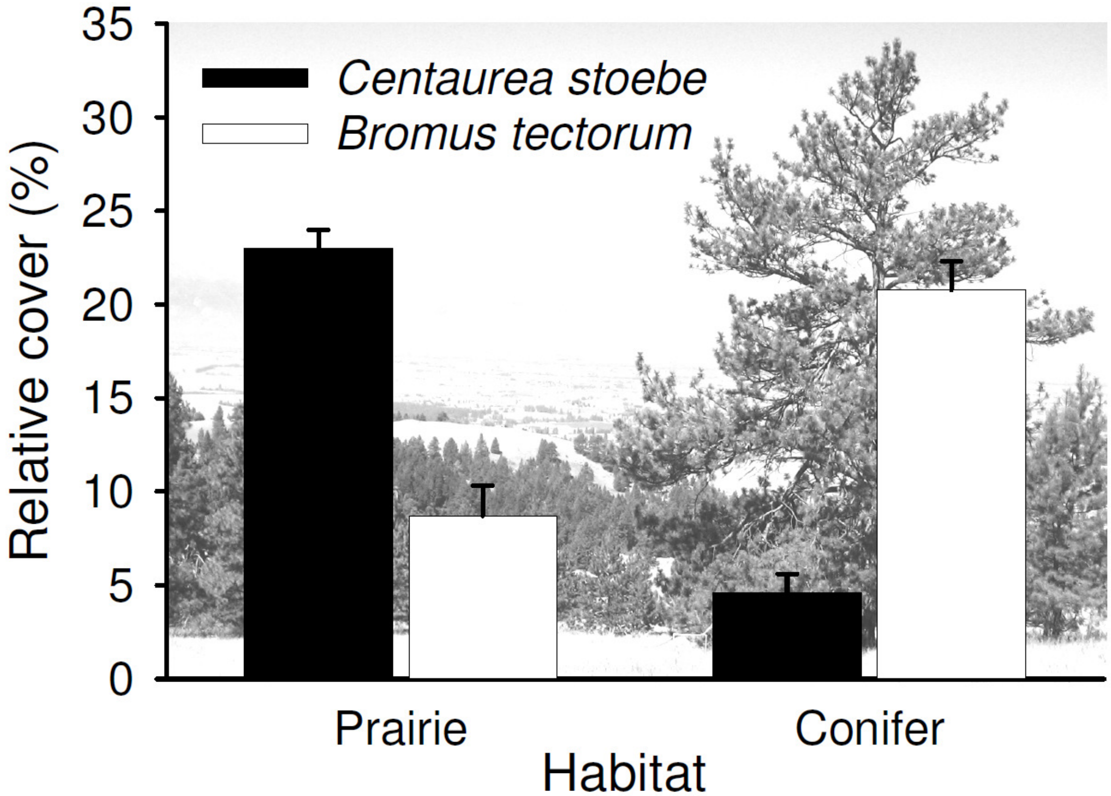

Figure 1: *Bromus tectorum* and *Centaurea stoebe* relative cover in open prairie or under canopies of *Pinus ponderosa*. Adjusted marginal means ± standard error. Mixed model ANOVA for *Bromus:* Habitat $F_{1, 2}$=39.1, *P=0.025*; Site $F_{2,15}$=1.9, *P*=0.180; Replicate(Site) $F_{24, 182}$=3.8, *P<0.0001;* Habitat x Site $F_{2, 182}$=0.6, *P*=0.538. Mixed model ANOVA for *Centaurea:* Habitat $F_{1, 2}$=133.3, *P=0.007*; Site $F_{2, 8}$=0.4, *P*=0.711; Replicate(Site) $F_{24, 182}$=2.7, *P<0.0001;* Habitat x Site $F_{2, 182}$=1.2, *P*=0.314.

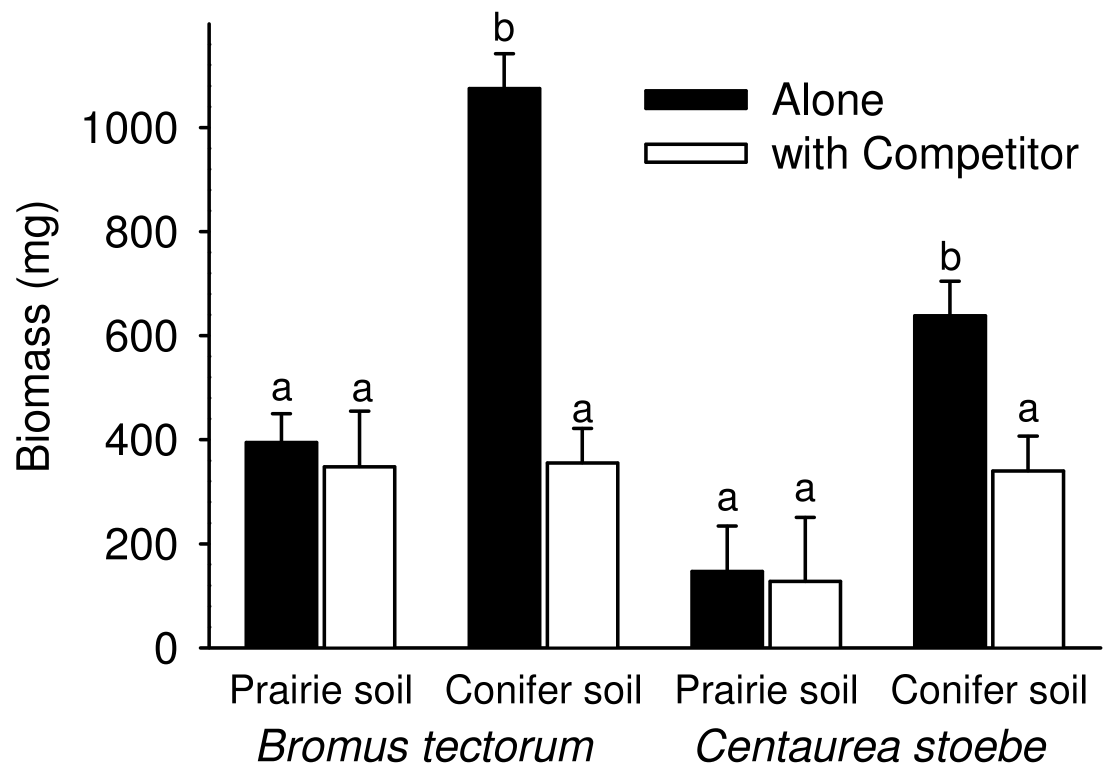

Figure 2: *Bromus tectorum* and *Centaurea stoebe* biomass when grown alone or in interspecific competition in soil collected in open prairie or from under *Pinus* canopies. Adjusted marginal means ± standard error. Letters indicate significant differences within a species (pairwise tests; $P<0.05$). ANOVA for *Bromus*: Soil $F_{1, 35}=15.9$, $P<0.0001$; Competition $F_{1, 35}=20.7$, $P<0.0001$; Soil x Competition $F_{1, 35}=16.9$, $P<0.0001$. ANOVA for *Centaurea*: Soil $F_{1, 25}=20.5$, $P<0.0001$; Competition $F_{1, 25}=4.2$, $P=0.051$; Soil x Competition $F_{1, 25}=3.2$, $P=0.085$.

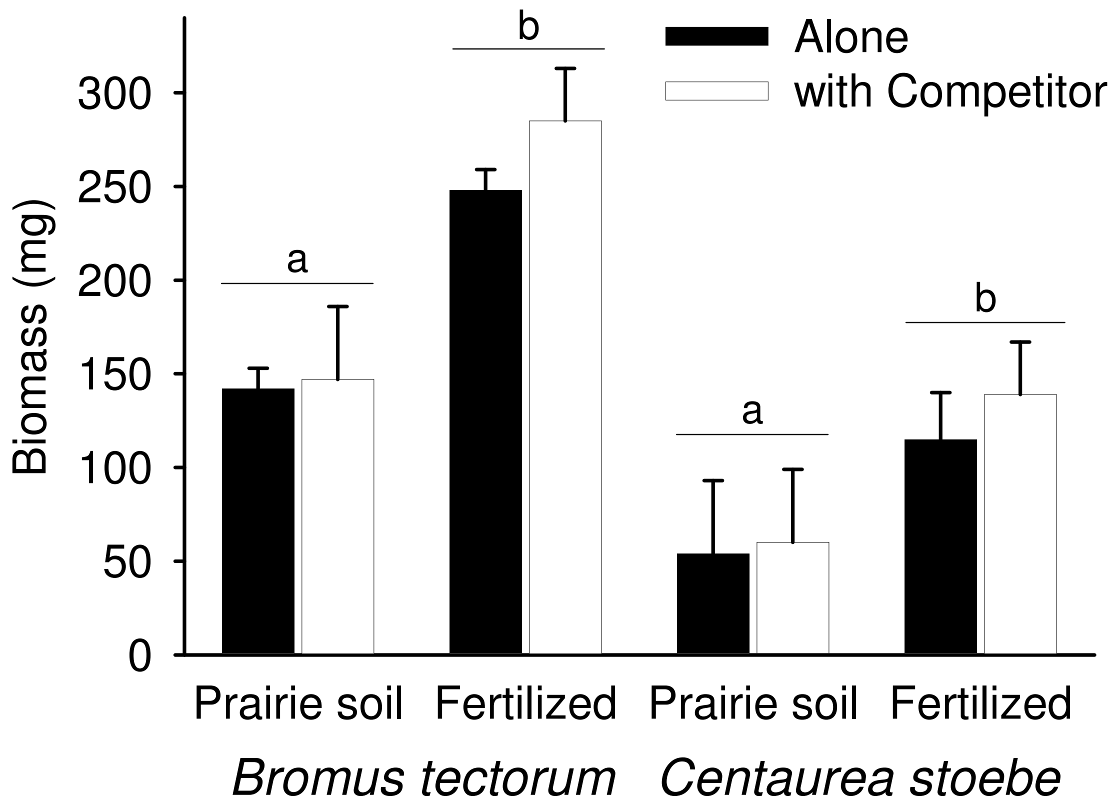

Figure 3: *Bromus tectorum* and *Centaurea stoebe* biomass when grown alone and in competition in prairie soil with no fertilizer, or with supplemental NO_3^- and PO_4^+ to mimic nutrient conditions found in *Pinus* soil. Adjusted marginal means ±SE. Different letters represent significant differences between fertilization treatments (pairwise tests; $P<0.05$). ANOVA for *Bromus*: Nutrients $F_{1, 55}=22.2$, $P<0.0001$; Competitor $F_{1, 55}=0.7$, $P=0.422$; Nutrients x Competitor $F_{1, 55}=0.4$, $P=0.534$. ANOVA for *Centaurea*: Nutrients $F_{1, 13}=5.9$, $P=0.037$; Competitor $F_{1, 13}=0.3$, $P=0.606$; Nutrients x Competitor $F_{1, 13}=0.1$, $P=0.765$.

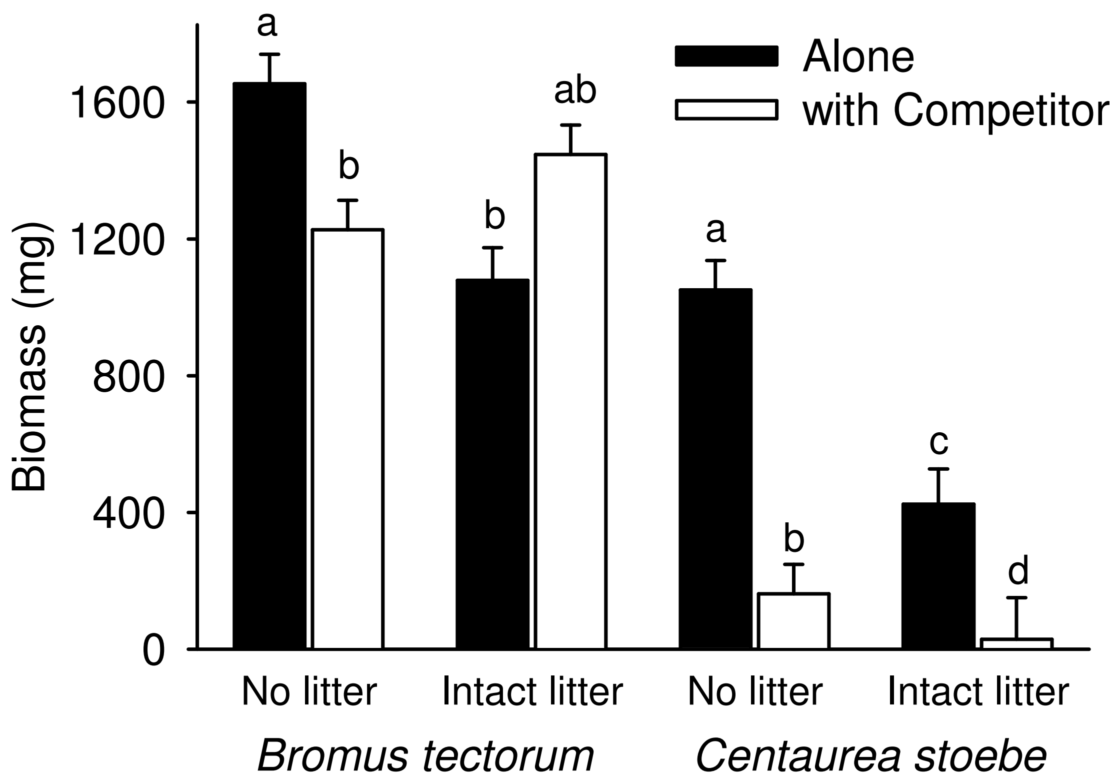

Figure 4: *Bromus tectorum* and *Centaurea stoebe* biomass when grown alone or in interspecific competition in soil from under *Pinus* canopies with or without *Pinus* litter on the soil surface. Adjusted marginal means ± standard error. Letters indicate significant differences within a species (pairwise tests; $P<0.05$). ANOVA for *Bromus*: Litter $F_{1, 34}=2.5$, $P=0.123$; Competition $F_{1, 34}=0.1$, $P=0.790$; Litter x Competition $F_{1, 34}=12.5$, $P=0.001$. ANOVA for *Centaurea*: Litter $F_{1, 28}=55.5$, $P<0.0001$; Competition $F_{1, 28}=158.6$, $P<0.0001$; Litter x Competition $F_{1, 28}=23.4$, $P<0.0001$.

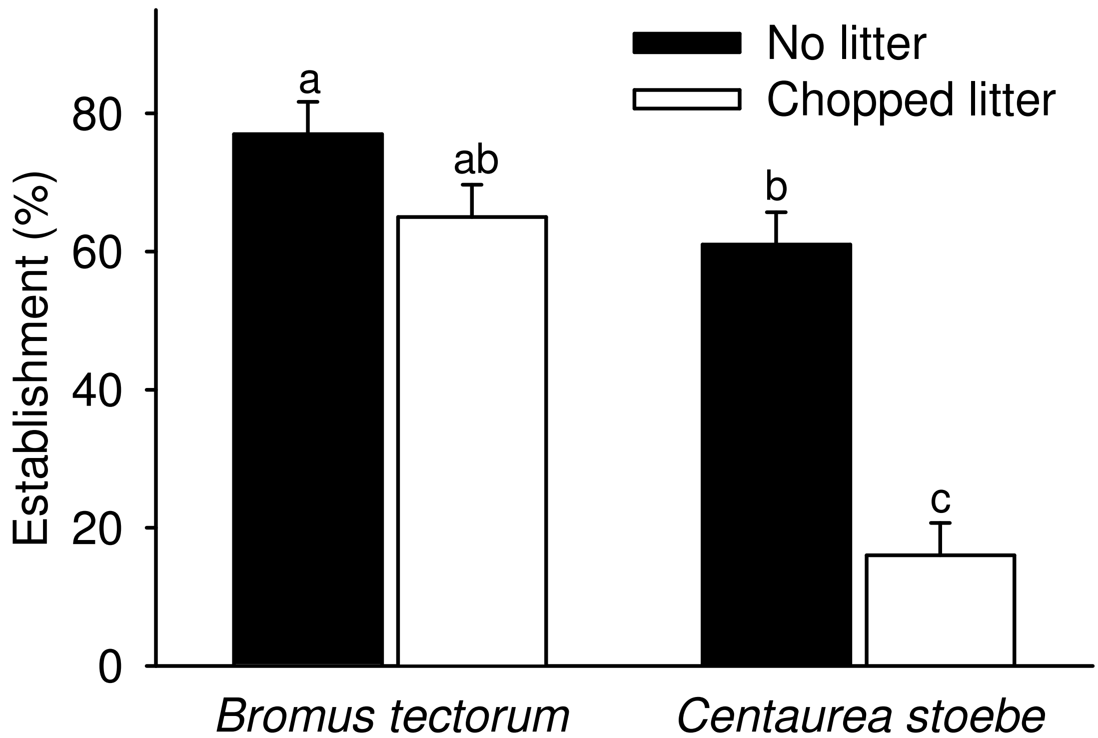

Figure 5: The proportion of *Bromus tectorum* and *Centaurea stoebe* seeds that established in soil from under *Pinus ponderosa* canopies or in *Pinus* soil with *Pinus* litter chopped up and mixed in to minimize physical effects (chopped litter). Adjusted marginal means ± standard error. Letters indicate significant differences (pairwise tests; $P<0.05$). Global ANOVA: Species ($F_{1, 36}=48.8$, $P<0.0001$), Litter ($F_{1, 36}=37.5$, $P<0.0001$), Litter x Species ($F_{1, 36}=12.6$, $P=0.001$).