

University of Nevada, Reno

**The Role of Symbiotic Nitrogen Fixation in Nitrogen Availability, Competition
and Plant Invasion into the Sagebrush Steppe**

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in
Ecology, Evolution, and Conservation Biology

by

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May, 2009



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prepared under our supervision by

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entitled

**The Role Of Symbiotic Nitrogen Fixation On Nitrogen Availability, Competition
And Plant Invasion Into The Sagebrush Steppe**

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ABSTRACT

In the semi-arid sagebrush steppe of the Northeastern Sierra Nevada, resources are both spatially and temporally variable, arguably making resource availability a primary factor determining invasion success. N fixing plant species, primarily native legumes, are often relatively abundant in sagebrush steppe and can contribute to ecosystem nitrogen budgets. *Lupinus argenteus* (Pursh), a native legume abundant in high elevation areas of western North America, is one of the most common native legumes in sagebrush steppe. *L. argenteus* responds positively to disturbance and prior studies indicate that it can increase the availability of soil nitrogen. Thus contribution of nitrogen by *L. argenteus* can potentially have a large effect on maintaining native species diversity and productivity of sagebrush ecosystems. However, if a non-native seed source is present, increased nitrogen associated with *L. argenteus* can create conditions favorable for invasion by non-native species. This study examined the role of *L. argenteus* on resource availability in the sagebrush steppe and the implications for invasion with four interrelated studies. Results indicate that *L. argenteus* can modify available soil N and increase productivity in sagebrush ecosystems both through rhizodeposition and litter decomposition. Further, modification of the local resource pool by *L. argenteus* can alter competitive outcomes among native and non-native species and can increase plant establishment and growth of both native and non-native species. However, higher establishment and growth rates give the non-native a greater advantage. The ability of *L. argenteus* to increase N availability can serve to promote resilience of native ecosystems, but also may create an avenue for invasion.

ACKNOWLEDGEMENTS

I would like to thank my advisor Jeanne Chambers for her guidance, support, and patience. I would also like to thank Dale Johnson, Jerry Qualls, Peter Weisberg, and Paul Verberg for providing feedback on my dissertation project and manuscripts and Bob Blank for the countless hours of help in his lab and his open door for all of my questions.

Most importantly, I need to thank my family for their unending support, love, and motivation that helped me make it through.

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INTRODUCTION

A fundamental goal of plant ecology is to understand processes that control plant species composition and organization within a community. Plant invasions can confound understanding of basic principles through the disruption of natural biogeochemical cycling and disturbance regimes. In addition, introduction of exotic species can alter the environmental filters that influence community composition and organization. These alterations can shift the trajectory of community development and lead to alternate ecosystem states. Aside from the basic scientific value of understanding how communities organize, this information can be applied to both restore and preserve native communities. Therefore, it is important to understand factors that promote the incorporation of exotic species into the native community matrix.

In the semi-arid sagebrush steppe of the Northeastern Sierra Nevada, resources are both spatially and temporally variable, arguably making resource availability a primary factor determining invasion success (Davis et al. 2000). Although moisture is often a key factor limiting plant growth in semi-arid systems, availability of nitrogen also strongly influences plant abundance and distribution (James and Jurinak 1978). Further, the strength of interactions among species varies depending on availability of resources. Spatially and temporally heterogeneous resources can therefore influence community composition and invasion through differential seedling establishment and subsequent resource competition. Whether interactions among seedlings and the existing vegetation are positive or negative will greatly influence seedling survival (Harper 1977). If competition for the limited resources between seedlings and the resident community is

high, establishment will depend on the seedlings ability to compete for those resources. In contrast, if members of the established community are able to modify the resource environment, seedling establishment may be facilitated. Although plant competition theory suggests that competition among species is not an important factor in harsh, low nutrient environments, research suggests that competition for limited resources in semi-arid environments is a dominant factor shaping community composition and influencing invasion events (Fowler 1986).

A number of biotic and abiotic factors can influence resource availability and species interactions within the sagebrush steppe. In semi-arid systems, fire can result in a pulse of available nitrogen that remains elevated for more than three years (Rau et al. 2007). In addition, nitrogen-fixing species are common in cold deserts, yet little is known about their role in this system. Legumes such as lupines can make up a large component of sagebrush systems, especially after fires. Although populations of lupines decrease in infrequently burned areas, they are still a dominant species on the landscape. Prior work suggests that amounts of nitrogen fixed by lupines can be substantial (Johnson and Rumbaugh 1981, Kenny and Cuany 1990, Rumbaugh and Johnson 1991), but the role of lupines and their ability to modify resource conditions has received little attention. The combination of a positive response to fire and the ability to persist in developed communities suggest that lupines may be a critical component affecting nutrient availability, and therefore community composition and invasion, in both disturbed and undisturbed areas.

One invasive species that has the potential to dramatically change these systems is the annual grass *Bromus tectorum* (cheatgrass), which can increase the frequency of fire

and increase in abundance after fire. Field studies examining the response of *B. tectorum* to resource availability suggest that optimal growth is limited by both water and nitrogen availability (Link et al. 1995). *B. tectorum* also benefits from N addition and can be an effective competitor for limited nutrients (Melgoza et al. 1990, Monaco et al. 2003, Lowe et al. 2002). The early germination and highly competitive nature of *B. tectorum* for limited water and nitrogen contributes to its formation of dominant stands, preventing re-establishment by native vegetation (Booth et al. 2003). Yet communities with well-established perennial grasses are more able to resist invasion by *B. tectorum* (Booth et al. 2003), suggesting that the presence of similar functional groups increases competition for these limiting resources. However, if resource availability is increased via disturbance or contribution from nitrogen fixing species, the balance of competition may shift from native to non-native.

Understanding conditions that modify resource availability in nutrient limited systems has important implications for both invasion by non-native species and for improving our ability to manage these sites to maintain or restore native diversity. These studies examine the role of lupines on resource availability in the sagebrush system and the implications for invasion with four interrelated studies (Figure 1). The underlying goal of this study is to gain a better understanding of the functional role of lupines, how their functional role is influenced by the resource environment, how they may modify species interactions, and how they influence seedling establishment, community composition and invasion within the sagebrush steppe.

In order to gain a better perspective of the functional role of the native legume, *Lupinus argenteus*, an observational field study was conducted. I examined the effects of

prescribed fire in the central Great Basin, Nevada, USA on density, biomass and nutrient content of a native legume, *L. argenteus*, and the effects of *L. argenteus* presence and prescribed fire on soil inorganic nitrogen and on neighboring plant functional groups. This was examined in three treatments - one year post-burn, three years post-burn and unburned control in three replicate blocks.

In addition to influencing the growth and distribution of *L. argenteus*, resource conditions may also influence nodule formation and nitrogen fixation. Therefore, the objective of the second study was to identify the response of *L. argenteus* to differing water and nutrient conditions in order to hypothesize how their role may change in the field under different environmental conditions. I conducted a greenhouse experiment to examine the separate and interacting effects of water and nitrogen availability on biomass production, tissue nitrogen concentration, nodulation, nodule activity, and rhizodeposition of *L. argenteus*. Plants were grown in a replicated, randomized block design with three levels of water and four levels of nitrogen.

Differential response of *L. argenteus* to the resource environment will influence how *L. argenteus* modifies competitive interactions among species. A second study investigated the effect of *L. argenteus* on competitive interactions among two functionally similar grasses. The objective of this study was to investigate the potential for seedlings of *L. argenteus* to facilitate invasion and expansion of *B. tectorum* by altering competitive interactions between seedlings of *E. multisetus* and *B. tectorum* over a gradient of N availability. In a greenhouse experiment, I used a randomized, factorial design with three levels of nitrogen availability, two target species (*B. tectorum* and *E. multisetus*), and three competitors (*B. tectorum*, *E. multisetus* and *L. argenteus*) grown in

two blocks. Six different species combinations were used to investigate both inter and intraspecific competition with and without *L. argenteus* under different nitrogen availability.

In addition to altering competitive interactions, *L. argenteus* may alter community composition by facilitating or inhibiting the emergence, growth, and survival of individual species. The objective of the last study was to determine the potential of *L. argenteus* to facilitate seedling establishment of *B. tectorum* versus native perennial herbaceous species in sagebrush steppe. A manipulative field experiment was set up as a completely randomized replicated block design with 5 replicate plots for each of six treatments in three blocks in both unburned (year 1) and burned (year 2) sagebrush steppe. Treatments were chosen to identify mechanisms by which *L. argenteus* may influence seedling establishment and community composition and included: 1) live lupine (LL) to examine modification of nutrient and physical environment of the whole, live plant; 2) dead lupine with litter in place (DL) to examine modification of nutrient environment by decomposing plant; 3) no lupine (NL) had no environmental modification; 4) no lupine with lupine litter (LLT) to examine modification of the soil surface and nutrient environment by decomposition of leaf tissue; 5) no lupine with inert litter (FLT) to examine modification of the soil surface; and 6) mock lupine (ML) to examine modification of the physical environment.

Overall, this research will increase our understanding of the role of native legumes on the nitrogen budget of sagebrush systems. It also will clarify the role that native legumes play in structuring plant communities and invasion within this system.

Further, knowledge gained from these experiments will assist in management of sagebrush ecosystems after disturbance.

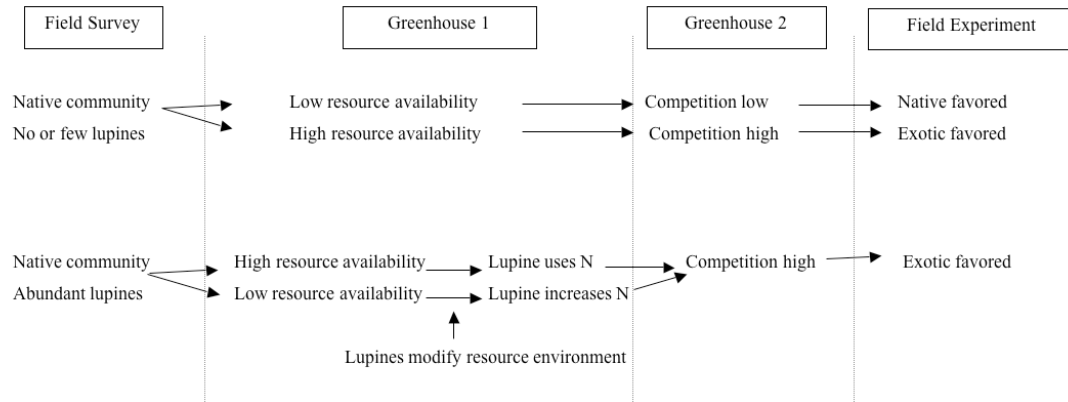


Figure 1. Diagram of the four different experiments and how they are related. The field study identifies patterns in the sagebrush steppe with and without lupines. The first greenhouse study indicates how lupines respond to high and low resource availability. The second greenhouse study takes information gained from the first study to look at the competitive interactions among native and exotic grasses with and without lupines and under different resource conditions. The field experiment builds on all of the prior projects and examines how lupines modify the resource environment to influence seedling establishment and community composition.

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Influence of a native legume on soil N and plant response following prescribed fire in sagebrush steppe

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Running head: Influence of legumes following prescribed fire

Additional key words: community recovery, disturbance, *Lupinus argenteus*, nitrogen

Abstract

Woodland expansion affects grasslands and shrublands on a global scale. Prescribed fire is a potential restoration tool, but recovery depends on nutrient availability and species responses after burning. Fire often leads to long-term losses in total nitrogen, but presence of native legumes can influence recovery through addition of fixed nitrogen. We examined the effects of prescribed fire in the central Great Basin, Nevada, USA on density, biomass and nutrient content of a native legume, *Lupinus argenteus* (Pursh), and the effects of *Lupinus* presence and prescribed fire on soil inorganic nitrogen and on neighboring plant functional groups. We examined three treatments - one year post-burn, three years post-burn and unburned control in three replicate blocks. Extractable soil inorganic nitrogen was variable, and despite a tendency towards increased inorganic nitrogen one year post-burn, differences among treatments were not significant. Extractable soil inorganic nitrogen was higher in *Lupinus* presence regardless of time since fire. *Lupinus* density increased after fire mainly due to increased seedling numbers three years post-burn. Fire did not affect *Lupinus* tissue N and P concentrations, but cover of perennial grasses and forbs was higher in *Lupinus* presence. The invasive annual grass, *Bromus tectorum*, had low abundance and was unaffected by treatments. Results indicate that *Lupinus* has the potential to influence succession through modification of the post-fire environment.

50 word summary

Woodland expansion affects grasslands and shrublands and prescribed fire is a potential restoration tool. Native legumes can influence recovery through addition of fixed nitrogen. We found higher extractable inorganic nitrogen and cover of perennial grasses and forbs in legume presence indicating legumes can influence post-fire succession through environment modification.

Introduction

Woodland expansion is affecting grasslands and shrublands on a global scale (Wessman et al. 2007). As woodlands expand into grasslands and shrublands, they increase shading and reduce resource availability (Breshears et al. 1997; Leffler and Caldwell 2005), leading to elimination of understory shrubs and herbaceous vegetation (Tausch and Tueller 1990; Miller et al. 2000) and depletion of the native understory species seedbank (Koniak and Everett 1982, Allen and Nowak 2008). Over time tree dominance results in higher fuel loads and can increase the risk of severe wildfires that leave areas susceptible to invasion by exotic species (Koniak 1985; Chambers et al. 2007). Higher elevation sagebrush steppe communities throughout the intermountain western US are affected by expansion of pinyon and juniper woodlands. Although fire is a natural, reoccurring disturbance in shrublands that prevents tree dominance, depletion of fine fuels due to overgrazing by livestock coupled with fire suppression has led to increased tree cover and woody fuel loads (Tausch et al. 1981). Prescribed fire has been proposed as a management tool for decreasing pinyon and juniper dominance and restoring sagebrush steppe communities (Miller et al. 2000). Fire return intervals in sagebrush steppe differ depending on community type, but range from about 60 to 110 years (Whisenant 1990). Recovery within sagebrush ecosystems is influenced by the post-fire resource environment and the responses of residual shrubs and herbaceous species.

Nutrients within woodlands are spatially and temporally variable (Chambers 2001). Fire significantly affects the availability of nutrients, especially nitrogen (N) (Blank et al. 2007; Rau et al. 2007). Over the long-term, fire can lead to losses in total N

through volatilization and through transfer of organic N from labile to recalcitrant pools (Gonzalez-Perez et al. 2004; Castro et al. 2006). Also, fire can negatively impact microbial biomass and activity that, in turn, can slow the cycling of plant available N (Choromansk and DeLuca 2002; Certini 2005; Guerrero et al. 2005). However, in the short-term fire often results in a pulse of available nutrients (Neary et al. 1999; Wan et al. 2001). Following low severity fires, increases in available nutrients occur due to deposition of ash onto the soil surface, release of ortho-P and NH_4^+ from organic matter, decomposition of below-ground biomass, and further oxidation of NH_4^+ to NO_3^- (Raison 1979; Hobbs and Schimel 1984; Covington et al. 1991; Blank and Zamudio 1998). For example, in the sagebrush steppe of the central Great Basin, Nevada, USA, inorganic N levels increased after a spring prescribed fire, and remained higher than a paired unburned control for three years (Rau et al. 2007). Fire also can influence N availability through its effect on N mineralization (Prieto-Fernandez et al. 1993; Guerrero et al. 2005). High fire temperatures often reduce N mineralization whereas low intensity fires can stimulate mineralization of N (Serrasolsas and Khanna 1995; Guerrero et al. 2005). For example, N mineralization increased one year after prescribed fire in both shrub and grassland communities in Colorado (Hobbs and Schimel 1984).

Reduction of competition and temporary elevation of available soil nutrients associated with prescribed fires may help promote increased growth of native understory vegetation (Moore et al. 1982; Rau et al. 2008). Biomass of perennial herbaceous sagebrush species was twice as high in burned plots as unburned controls in both Nevada (Rau et al. 2008) and Wyoming (Cook et al. 1994). In addition to increasing biomass production, post-fire conditions often increase N concentration of plant tissue (Grogan et

al. 2000; Metzger et al. 2006; Rau et al. 2008), plant reproductive potential (Wroblewski and Kauffman 2003), seed viability (Dyer 2002), and seed germination (Bradstick and Auld 1995; Romme et al. 1995; Williams et al. 2004). In combination, these factors influence post-fire succession and nutrient retention within sagebrush ecosystems.

Succession after fire can be strongly influenced by the identity of early seral plant species, especially if they are able to modify soil properties (Blank et al. 1994). One plant functional group with the ability to modify soil nutrient availability is legumes. Legumes can alter successional trajectories (Ritchie and Tilman 1995) by modifying the resource environment after disturbance (Morris and Wood 1989) or via facilitation and inhibition (Maron and Connors 1996). Legumes have the potential to affect community N budgets (Spehn et al. 2002) by increasing available soil N via higher N mineralization and nitrification (Vitousek and Walker 1989; Maron and Jefferies 1999; Myrold and Huss-Danell 2003; Johnson et al. 2004). If establishment and growth of native N₂-fixing species is promoted by fire, they may be able to replace N lost due to fire and facilitate community succession and stability over time. Also, input of organic N from legumes can stimulate recovery of microbial activity and N cycling.

However, disturbance and increased nutrient availability after fire combined with additional N input associated with legumes can provide conditions favorable for invasion by exotic species. N rich patches created by *L. arboreus* in California led to conversion of native shrubland to exotic annual grassland (Maron and Connors 1996). Similarly, facilitation by an exotic legume is believed to play a role in invasion by the exotic grass *Pennisetum cetaceum* in Hawaii (Carino and Daehler 2002). Although legumes are common in sagebrush steppe communities, little is known about their effects on N

availability or how their functional role changes following disturbance. Identifying the effect of legumes on N availability and retention in the post-fire environment can clarify the long-term effects of prescribed fire and the implications for maintenance and renewal of sagebrush systems.

Within the sagebrush steppe, one of the most common native legumes is *Lupinus argenteus* (Pursh), silver lupine (hereafter *Lupinus*). This species exhibits increases in cover and productivity in response to fire (Rau et al. 2008), and the amounts of N fixed can be substantial (Kenny and Cuany 1990; Rumbaugh and Johnson 1991) even in disturbed sites (Johnson and Rumbaugh 1986). This indicates that *Lupinus* may be important in influencing N availability, species interactions, and community recovery post-disturbance. The objectives of this study were to examine the interacting effects of the normal increase in mineral N after fire and *Lupinus* presence on N availability and community recovery in a sagebrush steppe ecosystem exhibiting pinyon-juniper expansion. Prescribed burns conducted in 2002 and 2004 at multiple locations along an elevation gradient within a Joint Fire Sciences Program demonstration watershed in the central Great Basin, Nevada, USA allowed us to develop a fully replicated study. We used a completely randomized design to examine replicate sites one year (2004) and three years (2002) post-burn and to compare them with unburned controls. We addressed three specific questions. (1) How does time since prescribed fire affect *Lupinus* density, biomass, and tissue nutrient concentration? (2) How is soil N affected by the separate and interacting effects of fire and *Lupinus* presence? (3) How is time since fire and *Lupinus* presence related to the response of other plant functional groups? We discuss the

implications of these effects for the recovery of sagebrush ecosystems after prescribed fire.

Methods

Study area

The study was part of a Joint Fire Sciences Program Demonstration Area established to examine the effects of prescribed fire on the soil and plant responses of sagebrush ecosystems exhibiting pinyon and juniper expansion (Chambers 2005; Rau et al. 2005, 2007, 2008). The study area is in Underdown Canyon (39°15'11" N 117°35'83"W) and is located in the Shoshone Mountain Range on the Humboldt-Toiyabe National Forest (Austin Ranger District) in Nye and Lander Counties, Nevada. Parent material at our sites in Underdown Canyon consist of welded and non-welded, Rhyolitic ash flow tuffs (Blank et al. 2007). The weathered parent rock itself is low in clay and therefore minimizes the potential for the host rock to contribute inorganic N contribution (Robert Blank, personal communication). Alluvial soils dominate the study sites and soils are classified as coarse loamy, mixed frigid, Typic Haploxerolls. Coarse mineral particles in the 0-15cm depth decrease from 73.7% to 42.8% and clay and sand particles generally increase with increasing elevation (Rau et al. 2005). Study sites were located on predominantly north-facing aspects and slopes ranged from 5 to 15%. Precipitation is mostly in the form of snow or spring rains, with mean annual amounts ranging from 230 mm at the bottom to 500 mm at the top of the drainage. Climate variables for the study period fell within the 33-year average (Table 1, Western Regional Climate Center 2007).

Dominant vegetation within the study area is mainly sagebrush (*Artemisia tridentata vasayana*) and single leaf pinyon (*Pinus monophylla*), but Utah juniper (*Juniperus osteosperma*) is also present. Based on vegetation structure and tree fire scars, it had been more than 100 years since a wildfire burned within the study area (Miller et al. 2008). Tree cover values ranged from 20 to 75% (Reiner 2004). Herbaceous species include perennial grasses (*Poa secunda*, *Elymus elymoides*, *Stipa commata*, *Festuca idahoensis*, and *Pseudoroegneria spicata*), perennial forbs (*Eriogonum species*, *Crepis acuminata*, *Phlox longifolia*, *Agoseris glauca*, and *Penstemon species*), and annual forbs (*Collinsia parviflora*, *Gayophytum ramosissimum* and *Phlox gracilis*). The invasive annual grass, *Bromus tectorum*, is present but its distribution is patchy. Cryptobiotic soil crusts are rare within this canyon. Legumes are present, with *Lupinus argenteus* being the most abundant species.

Experimental design

In 2002, three replicate burn sites (2103, 2225, and 2347 meters) and paired control sites (2073, 2195, and 2347 meters) were located along the elevation gradient within the canyon. In 2004, three additional burn sites were located adjacent to the previously established burn and control sites. All sites were located on north-facing alluvial fans with intermediate tree cover (30 to 50%). Prescribed burns were conducted in spring 2002 and 2004 and averaged 5-8 ha in size. The fires were cool burns that consumed trees, shrubs and herbaceous vegetation in a patchy nature. Surface fire temperatures averaged $206 \pm 24^{\circ}\text{C}$ in interspaces, $304 \pm 26^{\circ}\text{C}$ under trees and $369 \pm 33^{\circ}\text{C}$

under shrubs and decreased with soil depth to an average of 41°C at 5 cm for all microsites (Rau et al. 2005).

For this study, three replicate blocks were established in June 2005 along the elevation gradient within the watershed. Each block had similar vegetation and the same soil type, and contained one replicate of each of the three treatments 1) an unburned control site, 2) a site that was burned in 2002 (three years post-burn), and 3) a site that was burned in 2004 (one year post-burn). Blocks were separated by a minimum of 1 km. Within each treatment block, plots with and without *Lupinus* were surveyed, resulting in a completely randomized, split plot block design with subsampling.

Sampling methods and analysis

Variables related to *Lupinus* abundance, nutrient contribution, and community composition and biomass were sampled during peak growth using a restricted random sampling design. Two 50 m transects placed 25 m apart were established within each treatment-block combination in areas with similar vegetation, soils, and elevation. Location of the first quadrat for each transect was randomly assigned and additional quadrats were placed every two meters thereafter along the transect. Variables were surveyed by placing a 1 m² quadrat on the upslope side of the transect line in tree interspaces. The number of *Lupinus* plants within each 1 m² quadrat was recorded (n = 28-35 quadrats per treatment block depending on placement of first quadrat). Within a subsample of these quadrats (n = 10-25 per treatment block depending on the number of *Lupinus* present plots), the number of seedlings versus resprouting individuals was recorded. This sampling design captured the range of *Lupinus* density within blocks, but

variation in *Lupinus* density and distribution among blocks led to an unequal number of *Lupinus* present and absent plots.

To investigate community changes with time since fire, ocular estimates of *Lupinus* aerial cover and the cover of all other functional groups (annual grass and forb, perennial grass and forb, shrub) present within the 1 m² quadrat were made to the nearest percent. To minimize variation due to the individual observer, cover estimates were first calibrated using a test plot. Standing biomass of *Lupinus* and each functional group was harvested in a subsample of the quadrats (n = 6 per treatment block). Within three randomly selected quadrats along each transect, a 0.1 m² quadrat was nested within the 1 m² survey quadrat. Vegetation is fairly uniform across sites; therefore this size of quadrat provided a representative sample of herbaceous vegetation. For quadrats lacking *Lupinus*, the 0.1 m² quadrat was placed in the center of the plot to create a buffer area of at least 1 m² with no *Lupinus* plants. For quadrats containing *Lupinus*, the 0.1 m² quadrat was centered on a *Lupinus* plant to ensure that sampling was within the area affected by *Lupinus*. Within these quadrats, biomass was clipped to the ground, sorted by functional group, dried at 65°C for 48 h, and weighed.

Lupinus plants from each quadrat were composited, ground in a Wiley mill, and analyzed for N, carbon (C), and phosphorus (P) concentrations. Percentage of C and N were assessed by combustion of a 0.15 g subsample of ground tissue (LECO TruSpec CN analyzer, St. Joseph MI). Phosphorous concentrations (g g⁻¹) were obtained by ashing a 0.5 g subsample in a muffle furnace at 500°C for four hours. Ashed material was solublized with 20 mL of 0.1 N HCl and 0.5 mL nitric acid, diluted with de-ionized water in a 100 mL volumetric flask (Miller 1998), and analyzed colorimetrically (LACHAT

Instruments, Milwaukee WI). Plant N, C and P content were calculated by multiplying tissue concentration by plant weight. No individual forb species was present in enough sampling plots for an adequate comparison, therefore tissue of another common species, *Poa secunda* (hereafter *Poa*), also was ground and analyzed for total C and N to serve as a non-legume comparison.

To determine if *Lupinus* is altering extractable inorganic soil N, a soil sample was collected from the center of biomass quadrats ($n = 6$ per treatment block). All soil samples were located in tree/shrub interspaces, and included the 0 – 10 cm depth. Samples were homogenized, air dried, and sieved to remove particles >2 mm. Inorganic N (NH_4^+ -N + NO_3^- -N) levels were obtained by extraction with 2.0 M KCl and analyzed colorimetrically (Robertson et al. 1999, LACHAT Instruments, Milwaukee WI). Although measuring amounts of extractable inorganic N may suggest whether *Lupinus* presence and fire are influencing the amount of extractable inorganic soil N at this sampling period, it is only a static, one-time measure of N levels. Therefore, rates of potential net N mineralization were assessed with an aerobic lab incubation to examine the relative effect of *Lupinus* and fire on available soil N. A 10 g subsample of air dried, sieved soil was wet to 55% field capacity and incubated in the dark at 25°C for 30 days. Inorganic N (NH_4^+ -N + NO_3^- -N) levels after the 30-day incubation were obtained as above. Potential net N mineralization was calculated as final minus initial concentration of extractable inorganic N (NH_4^+ -N + NO_3^- -N). To determine total soil carbon and N, a 0.25 g subsample was ground and combusted (Sollins et al. 1999, LECO TruSpec CN analyzer, St. Joseph MI).

Data analysis

Differences in *Lupinus* density, cover, size, and tissue P concentration and content data were analyzed as a completely randomized block design with three treatments (control, 1 year post-burn and 3 years post-burn) and subsampling. A mixed effects model was used in which treatment was a fixed effect and block and treatment by block were random effects. Differences in tissue C and N concentration and content between *Lupinus* and *Poa* were analyzed with species as an additional fixed factor. To examine differences in soil ammonium, nitrate, potential net N mineralization, and total C and N, as well as differences in cover of functional groups, *Lupinus* presence/absence was included as a split plot within treatment and treated as a fixed factor. Due to differences in sampling, treatment effects on biomass were analyzed separately for *Lupinus* present and absent quadrats. In addition, relationships between *Lupinus* variables and soil variables also were examined using regression. All data were assessed and transformed as necessary to meet assumptions of normality and equality of variance. For results with significant effects, mean comparisons were assessed using Tukey adjusted least square means for multiple comparisons and considered significant at the 95% confidence level ($\alpha = 0.05$). All analyses were conducted using SASTM ver. 9.1.

Results

Soil nitrogen

We measured interspace environments, and although the one year old burn treatment tended to have higher concentrations of extractable soil NH_4^+ -N and NO_3^- -N, neither inorganic N or rates of potential net N mineralization differed among burn

treatments ($F_{2,4} = 0.36$, $p=0.716$, $F_{2,4} = 2.28$, $p=0.218$, and $F_{2,4} = 1.47$, $p=0.338$ for NH_4^+ -N, NO_3^- -N, and N mineralization, respectively). However, combining data across treatments indicated that higher levels of inorganic N occurred in quadrats that contained *Lupinus* than quadrats without *Lupinus* ($F_{1,33} = 4.13$, $p=0.05$ and $F_{1,33} = 9.32$, $p=0.005$ for NH_4^+ -N and NO_3^- -N, respectively). Although this trend is apparent in both control and burned treatments (Figure 1a), differences between *Lupinus* presence and absence within treatment were only significant in the unburned control plots ($p<0.05$). This trend also was present for rates of potential net N mineralization (Figure 1b), although differences were not significant ($F_{1,33} = 0.12$, $p = 0.739$). For both inorganic N amounts and N mineralization, differences for the three year post fire treatment should be treated with caution as the high abundance of *Lupinus* led to only one *Lupinus* absent quadrat being sampled in this treatment/block combination (Fig. 1a, b).

To determine what aspect of *Lupinus* presence may be influencing these results, the effects of *Lupinus* variables on soil variables were examined. Although *Lupinus* presence influenced the amount of extractable inorganic N, no measured *Lupinus* variable was significantly correlated with soil extractable N across all burn treatments. However, examining burn treatments individually indicated that *Lupinus* biomass was positively correlated with extractable NO_3^- -N in unburned treatments ($r^2=0.2015$, $p=0.06$). In contrast, *Lupinus* presence did not have a significant effect on N mineralization rates, but *Lupinus* biomass was positively correlated with N mineralization rates across both burned and unburned treatments ($r^2=0.2425$, $p=0.0106$).

Percent total N in the soils followed a similar pattern as extractable inorganic N, although there were no significant differences related to time since burn or *Lupinus*

presence (Table 2). Soil C:N ratios did not differ with time since fire or *Lupinus* presence and averaged 13 for all sites (Table 2).

Lupinus density, size distribution, cover, and standing biomass

Total *Lupinus* density was not affected by time since fire ($F_{2,4}=4.26$, $p=0.102$), although there was an increase in the proportion of seedlings to adults ($F_{2,4}=60.11$, $p=0.001$). Three years post fire, average *Lupinus* density was four times greater than in controls or the one year post-fire treatment, and approximately 40% of plots contained ten or more *Lupinus* plants per m^2 . The greatest density of seedlings occurred in the three year post-fire treatment (Fig. 2a).

Plants tended to increase in size with time since fire, but there was large variability in *Lupinus* standing biomass among treatments ($F_{2,4}=0.45$, $p=0.6663$) (Fig. 2b). One year post-fire, *Lupinus* biomass averaged greater than 50 g m^{-2} and made up 61% of herbaceous plant biomass. This amount was nearly twice that in unburned control treatments (25 g m^{-2} , 39%). Three years post-fire, *Lupinus* biomass averaged 150 g m^{-2} . All plant functional groups tended to have greater standing biomass production three years post-fire (see community productivity and composition section), but *Lupinus* still composed greater than 50% of the total herbaceous plant biomass.

Lupinus cover also tended to increase with prescribed fire but differences were not significant ($F_{2,4}=2.63$, $p=0.187$, Fig. 2c). One year after fire *Lupinus* cover averaged 5% per m^2 and made up 22.7% of total plant cover. This was almost twice that of unburned controls (3.5% cover per m^2 , 11.6% of total). By three years post-fire, mean *Lupinus* cover was more than double that in one year post-fire treatment. In these older burns

cover of all plant functional groups was greater, but *Lupinus* cover still made up roughly a quarter of the total plant cover (23.4%).

Tissue chemistry

Concentration of total C, N, and P in *Lupinus* tissue did not differ among treatments (Fig. 3a, b, c). *Lupinus* tissue from all sites had high concentrations of N leading to low C:N ratios (mean of 16) for all treatments (Fig. 4). The increase in standing biomass increased C, N and P content with time since fire although differences were not significant ($F_{2,4}=4.42$, $p<0.097$, $F_{2,4}=5.17$, $p<0.078$, and $F_{2,4}=6.52$, $p<0.055$ for C, N and P respectively) (Figure 3a, b, c). In contrast to *Lupinus*, *Poa* tissue N concentration followed the pattern for extractable soil N and was highest one year post-burn. Tissue N concentrations from *Poa* in the one year post-burn treatment contained almost twice as much N as those from the control and three year post-burn treatments (Fig. 3e).

The N concentrations of *Lupinus* were almost two times higher than those for *Poa* in all but the one year post-fire treatment. Due to higher plant biomass, *Lupinus* had 2-13 times higher N content than *Poa* for all treatments ($F_{1,23}=59.57$, $p<0.0001$). Greater standing biomass in *Lupinus* led to higher C content in *Lupinus* than *Poa* for all treatments ($F_{1,23}=31.61$, $p<0.0001$). *Lupinus* had significantly lower C:N ratios than *Poa* for all treatment combinations except the 2004 burn ($F_{2,23}=14.71$, $p<0.0001$, Fig. 4).

Community standing biomass and composition

Total herbaceous standing biomass tended to be greater with time since fire and in the presence of *Lupinus*. However, much of the observed increase in total biomass between the unburned control and the two burns was due to the increased growth of *Lupinus*. Community composition also was affected by prescribed fire (Fig. 5). Most functional groups tended to increase in cover with time since fire, but *Lupinus* became the dominant functional group post-fire. Among the other functional groups, the most notable change was the decrease in shrub cover following prescribed fire (14.5% to <1% one year post-fire, $F_{2,4}=6.36$ $p=0.057$). The cover of perennial grasses also tended to be influenced by time since fire ($F_{2,4}=6.23$, $p=0.059$). Cover of this functional group decreased by almost half in the year following fire (4.3% to 2.6%), but was more than triple that of the one year post-fire treatment by three years post-fire (2.6% to 8%, mean comparison, $p=0.069$). Cover of perennial grasses also tended to increase in *Lupinus* present plots ($F_{2,4}= 3.22$, $p=0.0739$), and differences were greatest three years post fire (Fig. 5). The cover of both annual and perennial forbs tended to increase with time since fire although differences were not significant. Cover of perennial forbs did increase in the presence of *Lupinus* ($F_{2,4}= 8.73$, $p=0.0034$) with differences being greatest one year after fire. The only annual grass in the study watershed was *Bromus tectorum*. The cover of this exotic, annual grass was highest on older burned sites, although the low frequency of occurrence led to no significant differences for treatment or *Lupinus* presence.

Discussion

The presence of *Lupinus* had a greater effect on extractable inorganic and mineralizable soil N than time since fire at our site. Most studies investigating the effect of fire on soil N find a pulse in inorganic N after fire (Certini 2005), and an increase in available N was observed in a different study within the same watershed (Rau et al. 2007). Although our soils exhibited this characteristic trend of increased inorganic N post fire, only the effect of *Lupinus* presence was significant. The lack of a significant fire effect was likely due to the fact that we examined interspace soils which typically exhibit smaller differences in post-fire extractable inorganic N than under shrub and under tree microsites (Chambers et al. 2007; Rau et al. 2007). Numerous other studies, in a variety of systems, have found that the presence of N₂-fixing species results in elevated soil inorganic N (Vitousek and Walker 1989; Maron and Jefferies 1999; Johnson et al. 2004). However, few studies have examined the combined effect of fire on soil N and the presence/absence of N₂-fixing species. Our study showed that the presence of *Lupinus* increased inorganic soil N and N mineralization regardless of time since fire. This suggests that the combined effects of fire and legumes on soil N are greater than the effects of either fire or *Lupinus* alone. Thus, comparisons of soil N in relation to fire need to consider the presence of N₂-fixing species like *Lupinus*.

Fire did not directly influence the amount of extractable inorganic N in the soil, but it did have an indirect influence via its effect on *Lupinus*. Density of *Lupinus* tended to increase following fire and was greatest three years post-fire. In other ecosystems, including the tallgrass prairie and pine forests of the southeastern US, legumes also respond positively to fire (Towne and Knapp 1996; Hendricks and Boring 1999; Newland

and DeLuca 2000). In our study, recently burned sites consisted mostly of resprouting individuals. Germination of many legumes is stimulated by the heat and chemical cues associated with fire (Martin et al. 1975; Bradstock and Auld 1995; Hendricks and Boring 1999; Williams et al. 2004), but burning can result in seed mortality and harsher conditions for seedling establishment (Chambers and Linnerooth 2001 Williams et al. 2003). An increase in seed production the year following fire and favorable conditions for establishment likely resulted in high numbers of seedlings by the third year after fire. Prescribed fire in the Great Basin increased reproductive output of five out of nine species (Wroblewski and Kauffman 2003). Similarly, higher plant density, seed production, and reduced seed predation in recently burned areas increased rates of seedling establishment of *Liatris scariosa* in northeastern grasslands (Vickery 2003).

The increase of *Lupinus* post-fire may be in response to changes in availability of limiting resources other than N such as light, water, or P (Vitousek and Field 1999; Casals et al. 2005). In a companion study conducted at our field sites, Rau et al. (2007) found that P increased in burned plots within two years after the prescribed burn. Legumes are often limited by P (Vitousek and Field 1999), and increased availability of P by three years post-burn may have contributed to the increased density, biomass and cover of *Lupinus*. In contrast, increased soil N often reduces the competitive advantage to legumes and therefore their abundance (Lauenroth and Dodd 1979; Zahran 1999). In our study we observed slightly lower N mineralization one year after fire which may have benefited *Lupinus*. Also, N₂ fixation is not always negatively affected by increases in soil N following fire (Hiers et al. 2003). Casals et al. (2005) found that both seedling and resprouting legume species derived 52%-99% of their N from fixation after fire, despite

increased amounts of mineral N in burned plots. Similarly, N₂ fixation rates of *Macrozamia riedlei* in south-western Australia were greatest the year following fire and gradually decreased with time since fire (Grove et al. 1980). Although we did not directly measure N₂ fixation in this study, the high concentrations of N in *Lupinus* tissue suggest that fixation was occurring.

The ability of legume species to fix N₂ in the presence of elevated soil inorganic N may allow them to maintain high levels of tissue N concentration in the face of fluctuating resource availability (Marschner 1986). Although *Lupinus* biomass increased in burned treatments, tissue N concentrations did not decrease. It is possible that *Lupinus* tissue N is mostly derived from fixed N, making the amount of available soil N less influential than for other species. The three year post-fire treatment had the greatest amount of biomass production for *Lupinus* (and most other functional groups), but also the lowest amounts of available soil N. Although numerous studies have shown that tissue nutrient concentrations increase after fire (Anderson and Menges 1997; Bennett et al. 2002; Rau et al. 2008), studies examining response of legumes often find results similar to ours. Legume tissue N concentration was not affected by fire in pine forests, although other species did show an increase in tissue N concentrations (Lajeunesse et al. 2006; Metzger et al. 2006). In this study, only *Poa* tissue concentrations followed a pattern that resembled that of available soil N, again suggesting that *Lupinus* is supplementing its N requirement through fixation of atmospheric N.

Total standing biomass and plant cover tended to increase with time since fire, especially where *Lupinus* was present. Higher overall resource availability in the post-fire environment typically results in increased biomass and cover of most functional

groups. In a companion study, Rau et al. (2008) found that biomass of five out of six native species increased within two years after prescribed fire. Similarly, Hobbs and Schimel (1984) reported an increase in aboveground biomass within two years after fire in shrub and grassland ecosystems of Colorado, USA. In our study, much of the increase in standing biomass and cover after the fire could be attributed to *Lupinus* itself. In a high elevation ecosystem, biomass in patches of the N₂-fixing species *Trifolium dasyphyllum* was two times greater than in surrounding patches due to greater biomass of *Trifolium* rather than differences in the biomass of other plants (Thomas and Bowman 1998).

We found that the presence of *Lupinus* was associated with higher cover of certain functional groups. This association could be due to similar preferences for growing conditions; however, it also could indicate that *Lupinus* is facilitating the growth of neighboring plants in sagebrush ecosystems. Legumes often act as facilitators in harsh environments (Morris and Wood 1989; Pugnaire et al. 1996; Gosling 2005). In our system, *Lupinus* may facilitate other functional groups by modifying various aspects of the resource environment depending on time since fire. On both unburned and older burned sites with relatively low levels of N, *Lupinus* may be decreasing competition for soil N if they are fixing their own N. Decreased competition could result from reduced N uptake by *Lupinus* or from release of N due to decomposition of N-rich litter (Kenny and Cuany 1990; Hendricks and Boring 1992; Maron and Connors 1996), which may be increased by fire. Further, inputs of organic N by *Lupinus* via rhizodeposition (Goergen et al. unpublished data) and turnover of belowground tissue, especially nodules, can promote recovery of microbial biomass and activity, which is strongly related to soil

nutrient availability (Coleman et al. 2004; Booth et al. 2005). On recently burned sites with relatively high levels of extractable inorganic N, rapidly resprouting *Lupinus* also may modify microenvironmental conditions (light and temperature), facilitating seedling establishment and plant growth.

Results of this study indicate that *Lupinus* has the potential to both modify available soil N and increase productivity in sagebrush ecosystems like elsewhere (Vitousek and Walker 1989; Jacot et al. 2000; Spehn et al. 2002). The apparent independence of *Lupinus* tissue N concentrations from soil N concentrations suggests that the functional role of this species may be especially important in low nutrient systems. The ability of *Lupinus* to increase N availability can serve to promote resilience of native ecosystems, but also may create an avenue for invasion. Although cheatgrass was not yet a dominant component at our sites, differential resource use, faster growth rate, superior competitive ability and greater seed production of this aggressive annual grass under increased N availability compared to native species can influence recruitment into these systems (Melgoza et al. 1990, Lowe et al. 2002, Monaco et al. 2003). Although the increased N associated with *Lupinus* has the potential to shift community composition from native to alien dominance, ultimately, the effect of *Lupinus* presence will depend on the relative degree of competition and the composition of native perennial herbaceous species following fire.

Lupinus appears to affect compositional change through rapid establishment in open microsites following fire and facilitation of particular plant functional groups. Perennial grass and forb cover exhibited the greatest response to the presence of *Lupinus*. Recent studies suggest that relatively high cover of perennial herbaceous plant species

can increase the resilience of sagebrush ecosystems following fire, and increase resistance to invasion by exotics such as cheatgrass (Booth et al. 2003, Chambers et al. 2007). Further experimentation in this area is needed to gain a better understanding of the effects of *Lupinus* facilitation on community composition and invasion events in sagebrush systems.

Acknowledgements

We thank D. Board, E. Hoskins, K. Vicencio, and T. Morgan for valuable assistance in the field and lab, D. Board and D. Turner for statistical guidance, and D. Johnson, R. Qualls, P. Weisberg, P. Verburg, and anonymous reviewers for valuable comments that greatly improved this manuscript. Financial support was provided by the USDA Forest Service, Rocky Mountain Research station and from a Center for Invasive Plant Management seed money grant to J.C. Chambers and E.M. Goergen. Treatments and logistic support were provided by Humboldt-Toiyabe National Forest.

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Table 1. Climate data for the study period from nearby Reese River, O'Toole, Nevada (Station ID 266746). Values are annual mean $\pm$ standard deviation for 33-yr average.

33 - Year	Precipitation (mm)	Max Temp °C	Min Temp °C
	Oct-Jun	Apr-Jun	Apr-Jun
Average	145 $\pm$ 14	19.0 $\pm$ 2.6	-0.8 $\pm$ 1.3
2002	168	20.1	-1.1
2003	97	18.7	-1.3
2004	184	19.9	-1.2
2005	160	17.8	-1.2

Table 2. Total C, N and C:N ratio for control, 1 year post-fire, and 3 years post-fire soils in the presence and absence of *Lupinus*. Values are mean $\pm$ SE.

	Total C (%)	Total N (%)	C:N
Control			
+ <i>Lupinus</i>	3.78 $\pm$ 0.05	0.27 $\pm$ 0.01	13.9 $\pm$ 0.4
- <i>Lupinus</i>	2.31 $\pm$ 0.37	0.18 $\pm$ 0.04	13.2 $\pm$ 1.1
1 Year Post-fire			
+ <i>Lupinus</i>	4.97 $\pm$ 1.11	0.40 $\pm$ 0.10	12.8 $\pm$ 0.2
- <i>Lupinus</i>	3.44 $\pm$ 1.73	0.27 $\pm$ 0.14	13.4 $\pm$ 0.7
3 Years Post-fire			
+ <i>Lupinus</i>	3.62 $\pm$ 0.58	0.28 $\pm$ 0.05	13.2 $\pm$ 0.3
- <i>Lupinus</i>	5.07 $\pm$ 0.0	0.35 $\pm$ 0.0	14.7 $\pm$ 0.0

Figure Legends

Figure 1. Extractable soil NH_4^+ -N and NO_3^- -N (a) and potential net N mineralization (b) with time since fire in the absence and presence of *Lupinus*. The abundance of *Lupinus* at the three-year post-fire sites led to only one *Lupinus* absent quadrat being sampled. n = 6 plots per burn treatment block. Values are means $\pm$ SE; asterisk indicates significant differences in inorganic N between *Lupinus* present and absent plots at $p < 0.05$.

Figure 2. *Lupinus* response to time since fire as measured by (a) density of adult and * seedlings (m^{-2} ; n = 18 per treatment), (b) biomass production (n = 18 per treatment), and cover (n = 25-38 per treatment). Values are means $\pm$ SE; asterisk indicates significant differences in seedling density among burn treatments at $p < 0.05$.

Figure 3. Tissue concentrations and content with time since fire, including *Lupinus* (a) carbon, (b) nitrogen, (c) phosphorous, and *Poa* (d) carbon and (e) nitrogen. Due to small amounts of tissue, phosphorus analysis was only conducted on *Lupinus*. Phosphorus concentration is in different units due to analysis method. Values are mean $\pm$ SE. n = 9 per burn treatment.

Figure 4. C:N ratio of both *Lupinus* and *Poa*. Values are mean $\pm$ SE. n = 9 per burn treatment; asterisks indicate significant differences between species within burn treatment at $p < 0.001$.

Figure 5. Functional group response to time since fire in the absence and presence of *Lupinus* for the unburned control, 1 year post-fire and 3 years post-fire treatments. Values are mean cover $\pm$ SE. n = 28-35 plots per treatment per block; asterisk indicates significant differences between *Lupinus* absence/presence within burn treatment at $p < 0.05$.

Figure 1.

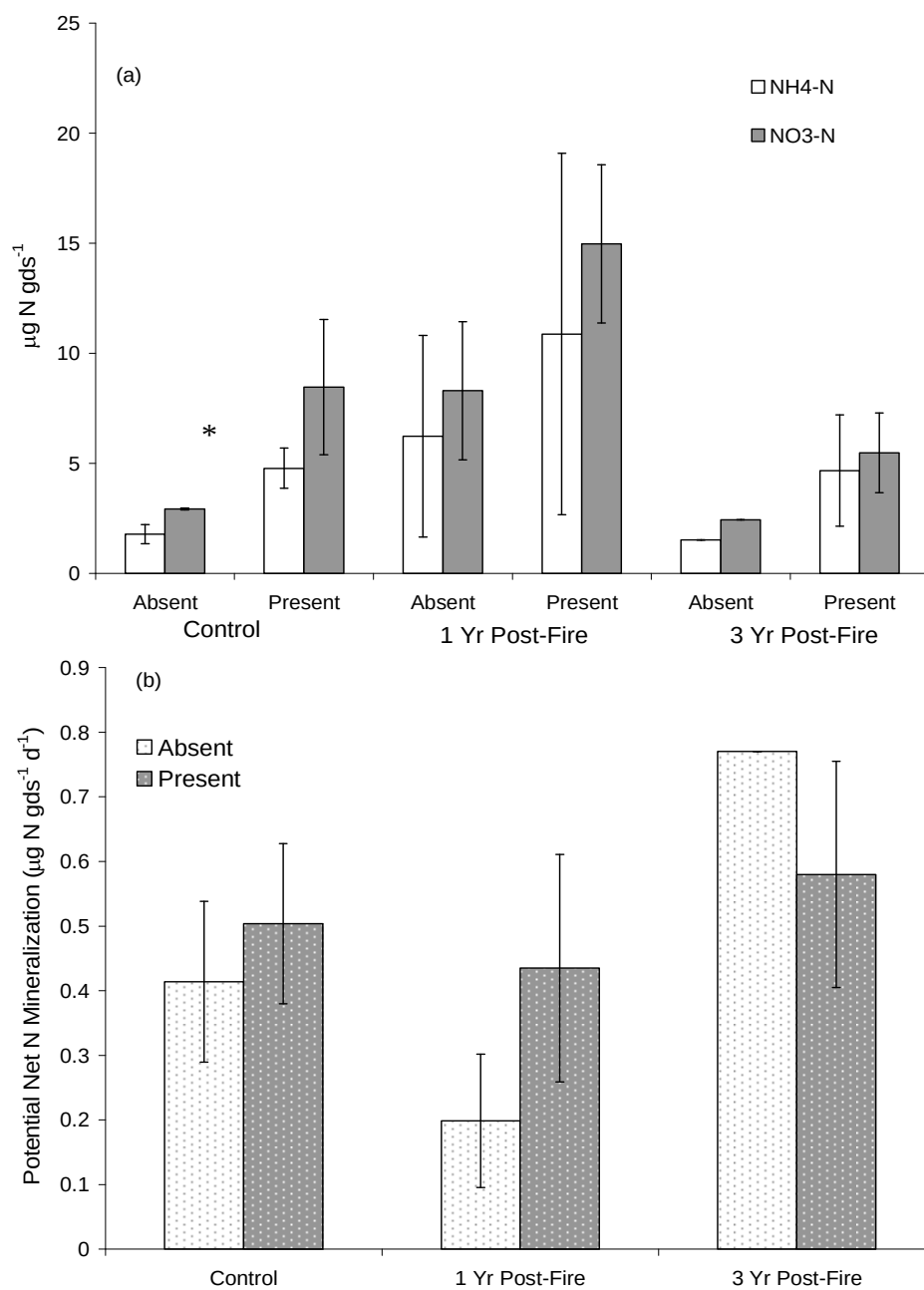


Figure 2.

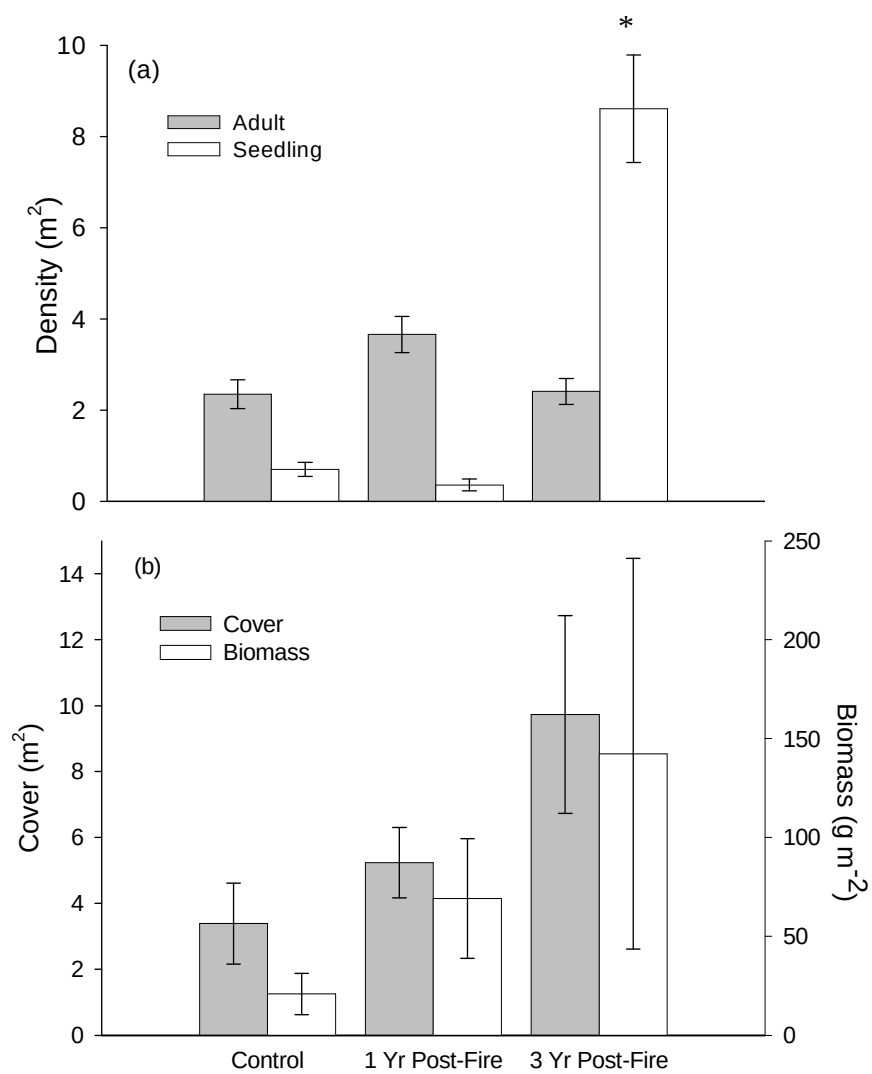


Figure 3.

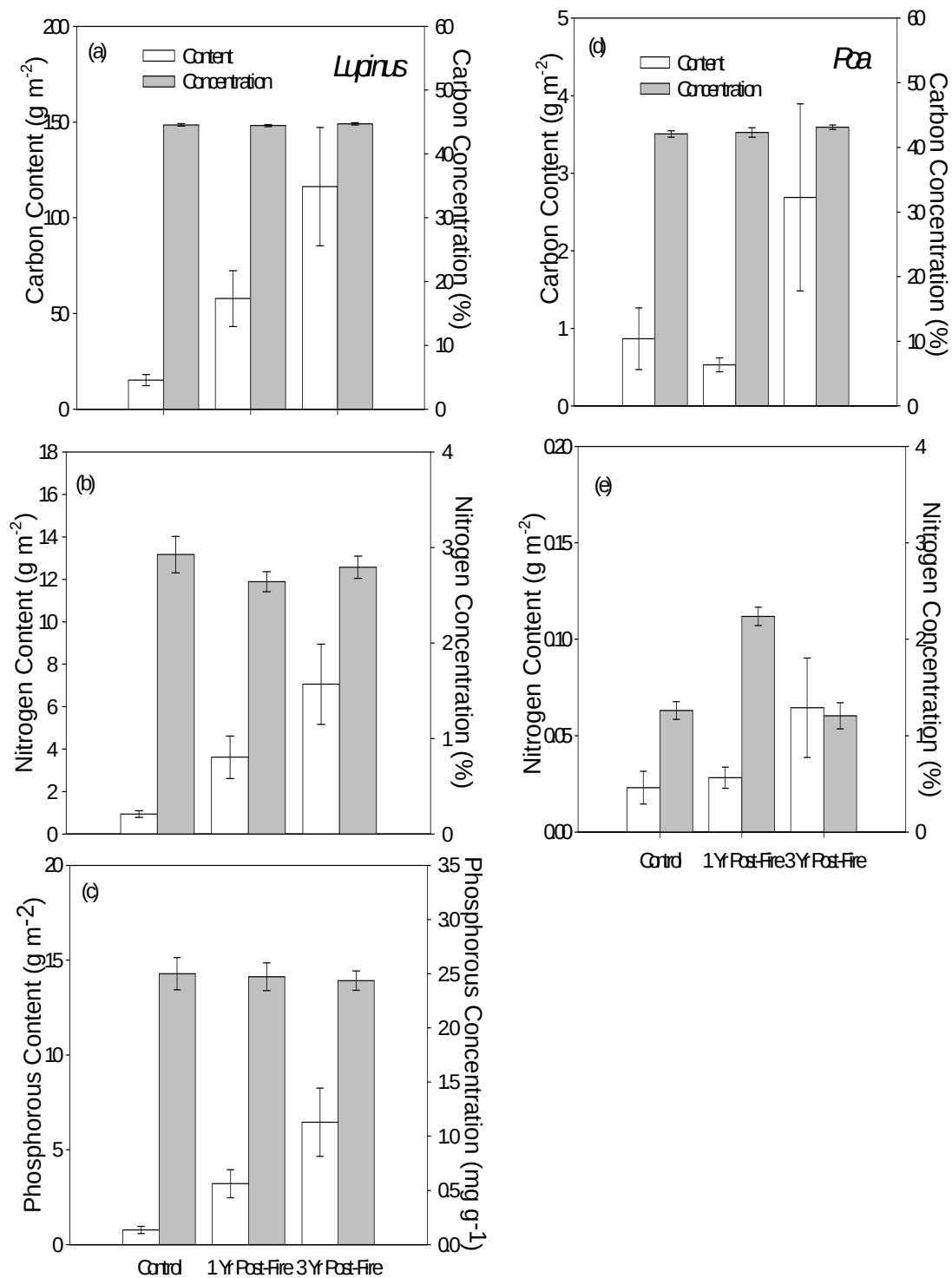


Figure 4.

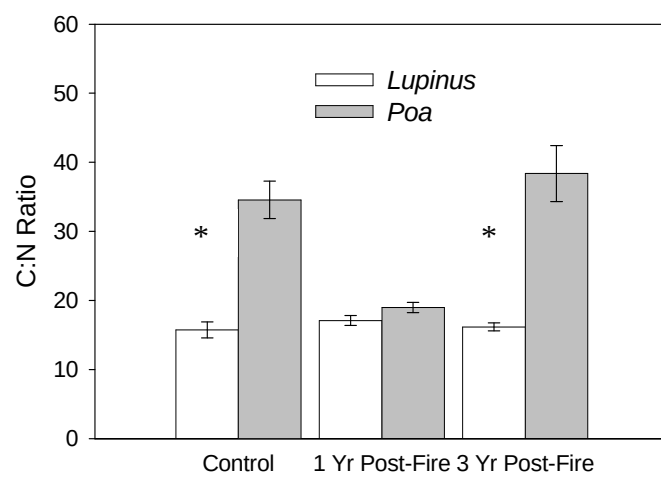
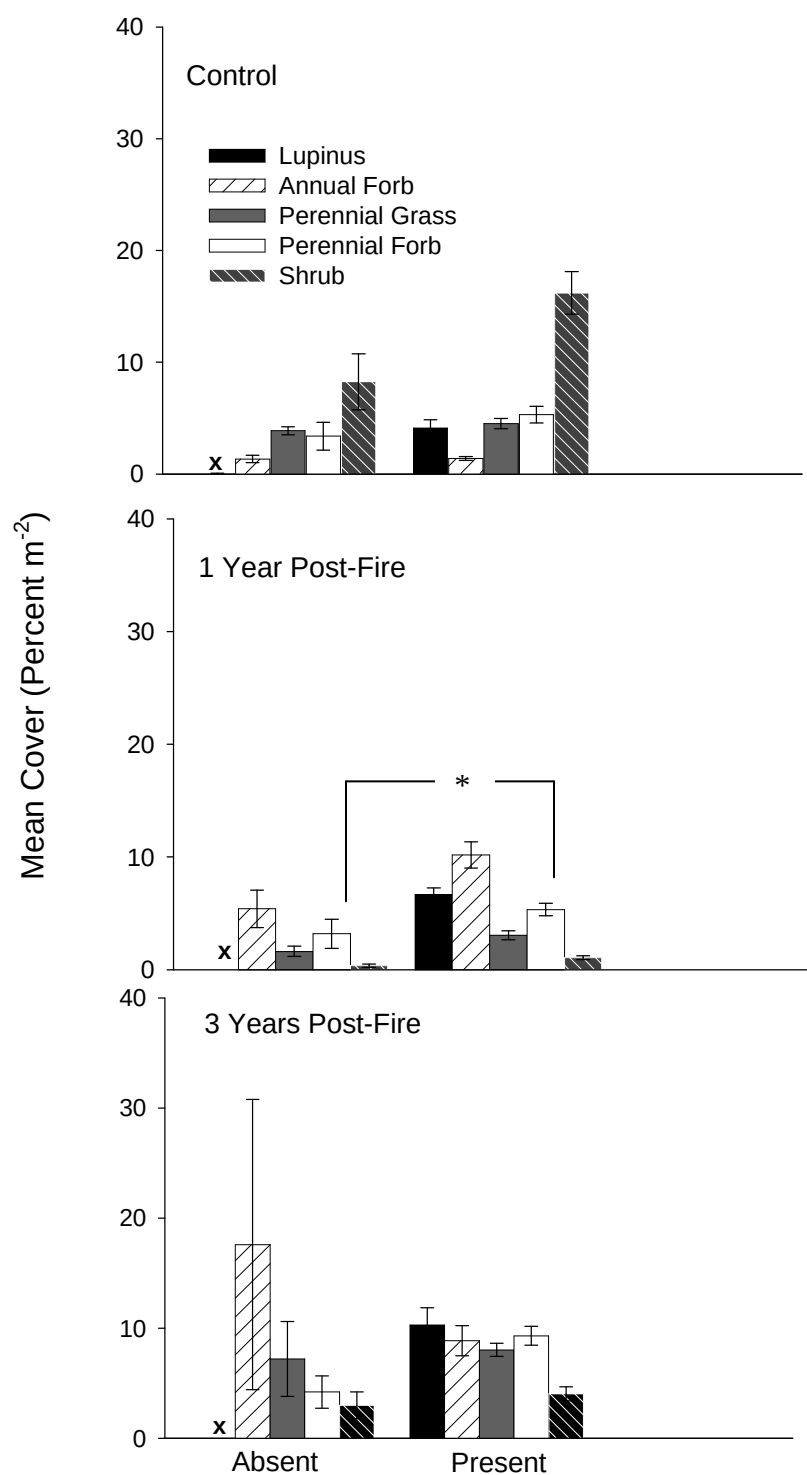


Figure 5.



Effects of Water and Nitrogen Availability on Nitrogen Contribution by the Legume,
Lupinus argenteus Pursh.

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Abstract

Nitrogen-fixing species contribute to ecosystem nitrogen budgets, but background resource levels influence nodulation, fixation, and plant growth. We conducted a greenhouse experiment to examine the separate and interacting effects of water and N availability on biomass production, tissue N concentration, nodulation, nodule activity, and rhizodeposition of *Lupinus argenteus* (Pursh), a legume native to sagebrush steppe. Plants were grown in a replicated, randomized block design with three levels of water and four levels of N. Additional water and N increased biomass except at the highest N level. All plants formed nodules regardless of treatment, but plants grown without N had the largest, most active nodules. Organic N was deposited into the rhizosphere of all plants, regardless of treatment, indicating that *Lupinus* can influence N availability while actively growing, even under water stress. High tissue N concentrations and low C:N ratios indicate that *Lupinus* also can provide substantial amounts of N through litter decomposition. The ability of *Lupinus* to affect N availability and cycling indicates that it has the potential to significantly influence community composition within the sagebrush steppe.

Key words: rhizodeposition, silver lupine, sagebrush steppe, symbiotic nitrogen fixation

Introduction

Nitrogen (N) often is a primary limiting nutrient in arid and semi-arid ecosystems. Total soil N ranges from 50 to 500 g m⁻², but much of this is present in forms unavailable to plants (West and Klemmedson 1978; Zak et al. 1994). Thus, processes influencing N availability in these ecosystems play an important role in determining plant productivity (Suding et al. 2005), species composition (Huenneke et al. 1990), and successional patterns (Paschke et al. 2000; Vinton and Burke 1995). Atmospheric deposition is an important source of N in some arid ecosystems (Fenn et al. 2003, Vourlitis et al. 2007). In portions of the California chaparral, atmospheric N deposition is sufficiently high that it influences species composition and plant invasions (Cione et al. 2002). In other arid ecosystems, N fixation is a main contributor of N. Fixation by legumes is known to be important for the N budget of the Sonoran Desert (Rundel et al. 1982; Shearer et al. 1983), the shrublands of Australia (Unkovich et al. 2000) and northern Mexico (Herrera-Arreola et al. 2007), and the Mediterranean region of Europe (Arianoutsou and Thanos 1996).

In sagebrush steppe ecosystems of the Intermountain West, N fixation by cryptobiotic crusts is often considered the main source of N (Belnap 2002a; Evans and Ehleringer 1993; Housman et al. 2006; Rychert et al. 1978). However, the presence of crusts is highly related to soil characteristics and ecological condition (Evans and Belnap 1999; Evans and Johansen 1999). In many areas overgrazing by livestock and other human disturbances have severely limited crust abundance (Belnap 2002b; Housman et al. 2006). In contrast, N fixing plant species, primarily native legumes, are often relatively abundant in sagebrush steppe (Crews 1999; Goergen and Chambers, *in press*;

Johnson and Rumbaugh 1986). Many of these species increase following disturbances such as overgrazing by livestock (Ralphs 2002) and fire (Goergen and Chambers, *in press*; Tracy and McNaughton 1997). Yet, despite the importance of N inputs from legumes in arid regions throughout the world, relatively little is known about N inputs by native legumes within sagebrush ecosystems.

Lupinus argenteus (Pursh) is a native legume abundant in high elevation areas of western North America, and is one of the most common native legumes in sagebrush steppe. *Lupinus argenteus* density can range from 3 to 10 plants m⁻² depending on time since disturbance (Goergen and Chambers, *in press*). Prior work on high elevation sites indicates that it can fix substantial amounts of N (Rumbaugh and Johnson 1991) even in disturbed sites (Johnson and Rumbaugh 1986), and that it can increase extractable inorganic soil N (Kenny and Cuany 1990). This indicates that *L. argenteus* has the potential to influence N availability in sagebrush ecosystems.

N fixation by legume species and, consequently, the potential contribution of fixed N, is influenced by resource availability. In arid and semi-arid ecosystems, soil water availability is generally the primary determinant of plant growth (Comstock and Ehleringer 1992; Loik et al. 2004) and can affect nodulation and symbiotic N fixation in legumes (Engin and Sprent 1973; Zahran 1999). For example, drought reduced nodule activity and nodule weight of *Glycine max* in a controlled greenhouse experiment (Streeter 2003). Likewise, water stress reduced N fixation by native legumes in burned pine forests of the southeastern US (Hendricks and Boring 1999). Plant growth also is greatly influenced by mineral N, and levels of available N affect initiation of root nodules in legume species as well as nodule activity (Arnone III et al. 1994; Chu et al. 2004). In

general, as N levels increase, nodule weight, density, and fixation rates decrease and dependence on mineral N increases (Zahran 1999). Nodule activity in *Glycine max* was reduced up to 73% under elevated (6.4 mM) nitrate (NO_3^-) (Streeter 1985). Similarly, high concentrations of NO_3^- (10 mM) decreased nodule mass, number, and nitrogenase activity in *Phaseolus vulgaris* (Leidi and Rodriguez-Navarro 2000). These studies indicate that both water and N influence N contribution by legumes, but these resources are often co-limiting. Thus, the interaction of these resources may have additional consequences for legume productivity and fixation.

Soil beneath canopies of N fixers often contains greater amounts of available inorganic N than soil from areas lacking N fixers (Kenny and Cuany 1990; Maron and Jefferies 1999; Myrold and Huss-Danell 2003; Thomas and Bowman 1998). However, the mechanisms by which legumes increase available soil N are still poorly understood. One potential mechanism is that N is added through decomposition of N-rich litter (Schlesinger 1991). Another potential mechanism through which N can be added is via rhizodeposition of amino acids (Cheng et al. 2003; Jones et al. 2004; Rovira 1969). For example, when grown under N free conditions, the leguminous tree *Robinia pseudoacacia* exuded 1-2% of fixed N as dissolved organic N (DON) from roots (Usselman et al. 1999). Many crop legumes have been found to exude even higher amounts of N. For example, rhizodeposition of N by unfertilized *Trifolium* amounted to 640-710 kg N ha⁻¹ depending on the species (Hogh-Jensen and Schjoerring 2001). However, information on N rhizodeposition by native herbaceous legumes is lacking.

Contribution of N by native legumes, either through rhizodeposition or decomposition, can potentially have a large effect on maintaining native species diversity

and productivity of sagebrush ecosystems. However, if an exotic seed source is present, N-rich patches created by native legumes can create conditions favorable for invasion by exotic species. Understanding how *L. argenteus* responds to varying water and N availability can provide insights into its influence on species interactions and community functioning under different environmental conditions and following disturbances such as fire or increased N deposition. Therefore, we examined the separate and interacting effects of water and N availability on N contribution by the native sagebrush legume *L. argenteus*. We addressed three questions. 1) How does *L. argenteus* biomass and tissue N change with resource availability? 2) How does nodulation and nodule activity change with resource availability? 3) Does *L. argenteus* exude detectable amounts of organic N and if so, how does exudation change with resource availability?

Materials and methods

Experimental Design

We conducted a greenhouse experiment to examine the effects of water and N on *L. argenteus* growth, tissue concentration, nodulation, nodule activity and root exudation. A factorial experiment with three levels of water and four levels of N was used. The study was implemented as a complete randomized block design with 12 replications of each treatment combination, although mortality over the course of the experiment resulted in lower sample sizes for some treatments (n = 6-12).

In spring 2006 *Lupinus argenteus* (hereafter *Lupinus*) seeds were obtained from a local Sierran source (Comstock Seeds, Gardnerville, NV). Seeds were scarified with sandpaper to promote germination. Scarified seeds were then coated with a commercial

inoculum (Nitragin, Lupine inoculum H) and allowed to dry before planting. Seeds were planted in 0.1 m diameter by 0.35 m tall PVC tubes (3 seeds/tube) containing washed sand (#16 mesh size). The bottom of each PVC tube was covered with mesh and pots were placed on an elevated platform to allow free drainage. Pots were watered with deionized (DI) water until the seedlings had emerged and had their first true leaf. Seedlings were then thinned to one plant per pot. Plants were grown in the University of Nevada – Reno greenhouses under natural light with a climate controlled maximum daily temperature of 27 °C and minimum nightly temperature of 7 °C.

Plants were randomly assigned to one of 12 water and N combinations. Plants received the assigned nutrient solution during each regular watering session. All nutrient solutions were based on a modified ¼ strength Hoagland's nutrient solution with only N varying. Concentration of N used was based on the range of N (as NH_4NO_3) found in soil extracts under field conditions: 0 N, 5 mM N (end of season conditions), 20 mM N (early season conditions), and 100 mM N (extreme post-fire conditions). To prevent accumulation of nutrients in the dried sand, pots were flushed with 1 L of DI at each scheduled watering and allowed to freely drain prior to receiving 200 ml of fertilizer. The water holding capacity of the sand filled pots was approximately 400 ml, and preliminary tests indicated that flushing pots with this amount was more than sufficient to remove any residual nutrient solution. This method allowed plants to be exposed to a more consistent nutrient environment throughout the experiment.

For water response, preliminary trials were conducted to determine the volume of water needed to bring pots to field capacity and at what frequency of watering plants exhibited different degrees of water stress. Pots were watered to field capacity (400 ml)

and allowed to freely drain. The number of days required for plant wilt to occur was recorded and served as the watering interval for the low water treatment. Water stress was verified by comparing stomatal conductance using a LI-6400 (LI-COR Lincoln, NE) between droughted and well watered plants. Based on these results, plants assigned the high water treatment were flushed and received the 200ml nutrient solution twice a week, plants under the moderate water treatment once a week, and plants in the low water treatment once every two weeks. PVC tubes without any seedlings were also set up and watered with DI water ($n = 6$) to determine any background N. Plants were grown under their respective treatments for three months (March –May 2006) and were randomized every two weeks to reduce edge and neighbor effects. To account for potential size bias at the conclusion of the experiment, height and number of leaves of all seedlings was recorded at initiation of the experiment.

Root Exudation

Before harvesting, all plants were flushed with 2 L of DI water and allowed to drain. Plants were then watered with the 0 N nutrient solution and incubated for 24 hours to examine root exudation. The incubation period was chosen to avoid any possible effect of diurnal fluctuations in root exudation. At the conclusion of incubation, all pots were flushed 3 times with 400 ml DI. The volume of water that drained after each flush was recorded and a 50 ml subsample was collected and immediately frozen for later analysis. The three subsamples for each pot were composited and divided into 2 samples: one for analysis of inorganic N and one for total N. Samples were centrifuged for 2 minutes at 5000 RPM before analysis (Sorvall RC 5C) to allow any particulates to settle.

Amounts of inorganic N ($\text{NH}_4^+ + \text{NO}_3^-$) were quantified using a LACHAT QuikChem® Flow Injection Analysis System (Milwaukee WI). Dissolved organic nitrogen (DON) was determined by subtracting total inorganic N from total N obtained via persulfate digestion (Sollins et al. 1999). In addition, for plants receiving the 0 N treatment for the duration of the experiment, amounts of organic N present in the rhizosphere was compared to N fixed to determine percentage of fixed N being exuded.

Plant harvesting and tissue analysis

At the conclusion of the experiment, above and belowground (roots + nodules) plant tissue was harvested, dried at 65 °C for 48 hr, and weighed to determine biomass. Above and belowground tissue were milled separately (UDY Corp, Fort Collins, CO) and analyzed for total N and carbon (C) concentrations using a LECO TruSpec CN analyzer (St. Joseph MI). Carbon and N concentrations were multiplied with plant biomass to determine C and N content. A small portion of total plant N originates from seed, thus the average amount of N present in a subsample *Lupinus* seeds was determined.

A subsample of aboveground plant tissue from each treatment also was analyzed for natural abundance ^{15}N and ^{13}C (UC Davis Stable Isotope Facility). The $\delta^{15}\text{N}$ signature reflects the N acquired over the life of the plant, and is the sum of N in the seed and N obtained from the soil plus any atmospheric N acquired through fixation. Therefore, in addition to analyzing leaf tissue from each treatment for natural abundance ^{15}N , a subsample of *Lupinus* seeds were analyzed for ^{15}N to determine the baseline signature ($\delta^{15}\text{N}_{\text{seed}} = -0.92 \text{ ‰}$). In order to identify uptake of fertilizer-derived N in the

5mM, 20mM and 100mM N treatments, the signature of the NH_4NO_3 fertilizer was also determined ($\delta^{15}\text{N}_{\text{fertilizer}} = -0.65\text{‰}$).

Tissue ^{13}C was used to verify plant stress according to water treatment, and also as an estimation of water use efficiency (WUE) under the different treatments (Ehleringer and Osmond 1983). Leaf $\delta^{13}\text{C}$ signature revealed that plants under low water experienced greater water stress than moderate or high water treatments, indicating that our treatment had the desired effect. Further, across all water treatments, plants increased WUE (leaf $\delta^{13}\text{C}$ became more positive) as N increased (data not shown), indicating that *Lupinus* responds to water and N as would be expected for a plants from a cold desert environment (Toft et al. 1988).

Nodulation and Acetylene Reduction Activity

At harvest, the number of nodules was recorded for each plant, and a subsample of nodules was weighed to determine mean specific nodule weight in each treatment. In addition, on a subsample of plants from each treatment, the acetylene reduction technique was used to determine treatment effect on acetylene reduction activity (ARA) as a measure of nodule activity. Although this method does not provide a direct measure of N_2 fixation or nitrogenase activity, it is a valuable tool to assess differences in fixation potential among treatments (Vessey 1994). Further, whereas natural abundance ^{15}N analysis provides a measure of fixation over the life of the plant, ARA provides a snapshot of nodule activity over a given period of time. A segment of root with intact nodules was cut and placed in a 40-cc vial filled with a 10 % acetylene atmosphere. Airtight vials were incubated for 1h in ambient temperatures and shielded from light.

Vials containing nodulated root segments without acetylene and vials with only acetylene also were included to examine any exogenous production of ethylene from root nodules and any contamination of ethylene in the acetylene. After the incubation period, a subsample of gas was removed with a syringe and placed in an evacuated container. 250 μ l gas samples were analyzed for ethylene content using a gas chromatograph (Shimadzu GC Mini Z) equipped with a hydrogen-flame ionization detector to determine the amount of ethylene evolved from nodulated root segments. Ultra high purity nitrogen (Sierra Airgas) was used as the carrier gas and was passed through an 8' column packed with 50-80 mesh Porapak "T". Column and injector temperatures were held at 80 and 100 °C respectively. Dry weight of nodules assayed was recorded, as well as number of nodules present to provide an estimate of acetylene reduced per nodule weight.

Data Analysis

The data were analyzed as a completely randomized block design with three levels of water and four levels of N treatment. Differences in lupine biomass, tissue N concentration and content, leaf $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, number and weight of nodules, nodule fixation, and root exudation were compared among treatments using a two-way ANOVA with water and N treatments as fixed effects and block as a random effect. For the 0 N treatment, percentage of plant fixed N exuded was compared among different water treatments using a one-way ANOVA. All data were transformed as necessary to meet assumptions of normality and equality of variance. For results with significant effects, mean comparisons were Tukey adjusted for multiple comparisons and considered

significant at the 95 % confidence level. All statistical analyses were conducted using a mixed model in SAS™ ver. 9.1 (SAS Institute 2004).

Results

Biomass production

At the initiation of the experiment, there was no difference in plant size (mean height of 2.7 cm and 1 leaf). However, at the conclusion of the experiment, there were significant differences in above and belowground biomass among water treatments (Table 1). Biomass was greater with increasing water for all but the 0 N treatment. Above and belowground biomass were both affected by N treatments, with biomass being highest in 5 and 20 mM N treatments (Fig. 1, Table 1). A water by N interaction indicated that increases in water level had positive effects on total biomass for all but the 0 N treatments (Fig. 1). *Lupinus* root:shoot ratio (R:S) decreased across all treatments as both water and N increased, with plants in the 100 mM N treatment having the lowest R:S (Table 2).

Tissue concentration

Aboveground plant tissue N concentrations were affected by both water and N treatment (Fig. 2a, Table 3). N concentration of aboveground tissue in 5 or 20 mM N treatments was significantly reduced under low water conditions, resulting in a water by N interaction. Root N concentrations increased with increasing N fertilization but were not affected by water treatments (Fig. 2a). Total plant C:N ratio followed the same pattern as tissue N concentration (Table 2). Total plant N content followed plant biomass

responses and was affected by water and N for all but the 0 N treatment, leading to a significant water by N interaction (Fig. 2b, Table 3). Seeds of *Lupinus* are relatively large and N rich, with an average N content of 0.118 mg N per seed, amounting to 0.04 – 0.9 % of total plant N content depending on treatment.

The $\delta^{15}\text{N}$ signature of *Lupinus* leaf tissue varied by both water and N treatment (Table 3). Although the fertilizer was not labeled, there was sufficient differentiation among treatments to assess relative reliance on fixation versus fertilization. Plants in the 0 N treatment had a lower (more negative) signature than *Lupinus* seeds, whereas plants in the 100 mM N treatment had a higher (more positive) signature than the N fertilizer (Fig. 3). Differential uptake of NH_4 vs. NO_3 by *Lupinus* and fractionation of N upon uptake likely contribute to the more positive signature in the plant tissue than the fertilizer. This difference indicates that plants in the 0 N treatment were relying on fixation, while plants in the 100 mM N treatment were relying more on fertilizer. Plants in the 5 and 20 mM N treatments had intermediate $\delta^{15}\text{N}$ values, indicating that the N source was a combination of both fixation and fertilizer N. Across N levels, plants in the high water treatment tended to have the least negative $\delta^{15}\text{N}$ values and plants in the low water treatment had the most negative $\delta^{15}\text{N}$ values, although differences between low and high water were only significant in the 20 mM N treatment (Fig. 3).

Nodulation and nodule activity

All plants were nodulated, with number of nodules being affected by both water and N treatment (Fig. 4a, Table 4). Plants grown under 0 or 5 mM N had the highest number of nodules and plants under 100 mM N the least. Additional water did not

increase nodule abundance at the lowest and highest N level, leading to a water by N interaction. Although increases in N from 0 to 5 mM increased nodule number, specific nodule weight significantly decreased with increasing N and was not affected by water availability (Fig. 4b, Table 4). Specific nodule activity as measured by ARA was affected by N but not by water treatment (Fig. 4c, Table 4). Plants in the 5 mM N treatment had the lowest ARA, suggesting that nodules were not very active. In contrast, plants grown in the absence of N produced nodules that were two times heavier than nodules in any other treatment and tended to be the most active.

Rhizodeposition

Substantial amounts of organic N, measured as DON, were deposited into the rhizosphere of all pots (Fig. 5). High variability within treatments resulted in a lack of significance for either the water or N treatment ($P > 0.1$), although plants in the 100 mM N treatment tended to have higher amounts of organic N in the rhizosphere.

Rhizodeposition by plants receiving 0 N was examined in relation to nitrogen fixation as assessed by ARA. To convert ARA to estimates of N fixed, we assumed a constant fixation rate over 24 h (Tricnick et al. 1976) and an ethylene to N_2 conversion factor of 4 (Hardy et al. 1973). Thus, percentage of N exuded was calculated as root exudation divided by the sum of N fixed over 24 h. Plants in low water exuded 58 % of fixed N, high water plants 26 %, and moderately watered plant 5 %. However, we acknowledge the limitations of using ARA to estimate fixation rates as conversion factors vary greatly with species and condition (Vessey 1994), thus values of fixation using ARA are at best a rough estimate that is likely on the low end, resulting in calculation of higher

percentages of fixed N being exuded. Another approach is to use tissue N content (minus N contribution from seed), which represents fixation over the life of the plant and therefore provides an upper limit of N fixed. Comparing root exudation rates using tissue N content rather than estimates of fixation based on ARA indicates that lower amounts of fixed N are exuded with plants grown in low water exuding 14 %, followed by high water plants (9 %) and moderately watered plants (3 %). Actual percentages of fixed N being deposited into the rhizosphere likely fall between the values obtained from these two different approaches.

Discussion

N fixing species can contribute to the N budgets of ecosystems, but this contribution varies with N and water availability. We examined the entire range of growth responses to N for this species over a range of water availability. *Lupinus* grew best under intermediate N and high water conditions, and exhibited reduced growth at the highest level of N (100 mM) indicating nitrogen toxicity (Goyal and Huffaker 1982). Additional water increased biomass at all N levels with the exception of the 0 N treatment indicating that under extremely N poor conditions, N is more limiting than water. In a shortgrass steppe field study, additional water led to an increase in legume density and biomass, and addition of both water and N (50 kg N ha⁻¹) resulted in a small, short-term increase in legume density and biomass, but addition of N alone had no effect (Lauenroth and Dodd 1979). Lack of similar responses from this field study to our greenhouse experiment are likely attributable to the effect of other limiting resources, the presence or absence of competition or differential responses of adults versus seedlings to

water and N availability. Regardless, both studies illustrate the importance of looking at the individual and synergistic effects of N and water on biomass production in legumes.

Lupinus exhibited luxury consumption of N, with tissue concentrations ranging from 2 to 8 % N. Field collections of *Lupinus* from NE Sierra Nevada, the central Great Basin (Goergen and Chambers submitted), and Rocky Mountains (Metzger et al. 2006) have average tissue N concentrations of 3 %. Resource availability, especially of N, is extremely variable in sagebrush ecosystems, and the ability to store N in excess of what is required for growth may be a factor that allows this species to persist at relatively high densities in intact, low nutrient systems (Chapin et al. 1986). Luxury consumption also may allow plants to sustain themselves during periods when nutrient resources are low either because of increased competition or reduced uptake caused by water limitation.

High tissue N concentrations combined with low C:N ratios indicate that upon senescence at the end of the growing season, *Lupinus*, like other N fixing species, can contribute substantial amounts of N through litter decomposition. Decomposition of *L. arboreus* tissue increased both soil available ammonium (NH_4^+) (~3 times) and NO_3^- (~5 times) relative to soil with no lupines (Maron and Connors 1996). Similarly, litter of the N fixing tree *Morella* (*Myrica*) *faya* decomposed faster and began releasing N sooner than did the native non-fixing tree *Meterosideros polymorpha* (Vitousek and Walker 1989). Results from our study on biomass and N content suggest that contribution of N to the soil by *Lupinus* tissue decomposition will likely be greatest under intermediate resource availability because plants grown at intermediate resource levels produced the most biomass. Aboveground N content of plants in our greenhouse experiment ranged from 0.04 g N/plant to 0.08 g N per plant. In lodgepole pine forests of Wyoming,

Lupinus contributes an average of 0.04 g N per plant (Metzger et al. 2006), and a study in the central Great Basin found an average of 0.18 g N per plant (Goergen and Chambers submitted).

Nodulation of *Lupinus* plants was reduced but not eliminated under elevated N, irrespective of water availability. Although many studies suggest that increased mineral N reduces nodule size and density, the response of legumes to N level is species specific and depends upon the particular Rhizobium-legume symbiosis (Harper and Gibson 1983; Manhart and Wong 1980), with some species showing stimulated fixation at moderate levels of N (Peoples et al. 1995; Bado et al. 2006). Nodules of *Lupinus angustifolius* infected with Rhizobium strain 127E15 were unaffected by addition of 15 mM NO_3^- , whereas all other combinations of Rhizobium-*Vigna unguiculata* used in the same study averaged a 30 % reduction in nodule weight (Manhart and Wong 1980). The effect of N on nodulation and nodule activity also depends on the timing of N addition. Consistently high levels of N can prevent root hair infection (Eaglesham 1989), whereas exposure to elevated N after nodule initiation has the greatest impact on nodule weight and activity (Arreseigor et al. 1997; Voisin et al. 2002). Since our plants did not receive any fertilization until after emergence, it is likely that all seedlings were initially nodulated and the N treatment affected the nodules that were already present and influenced the degree (density and size) of subsequent nodulation.

The acetylene reduction activity of *Lupinus* nodules also showed a greater effect of N on nodule activity than water. In the field, acetylene reduction by *Lupinus* increased with soil moisture and elevation (Rumbaugh and Johnson 1991). Although fixation tended to decrease with increasing N availability, nodule activity was present under

elevated levels of N. A similar result was seen with the *Lupinus* leaf $\delta^{15}\text{N}$ data - as N addition increased, plants shifted from relying on only N fixation to a combination of N fixation and fertilizer. In contrast to ARA, however, this method suggests that plants in the 100 mM N were relying almost entirely on fertilizer N. Regardless, the $\delta^{15}\text{N}$ value for the low water plants in the 20 mM N treatment indicates that fixation did occur under high N availability.

Our results suggest that when N is elevated following fire or other disturbances, *Lupinus* would likely rely mostly on inorganic N to increase growth, reproduction, and tissue concentrations while still maintaining active nodules. Other studies also have found that N_2 fixation is not always completely suppressed by increases in soil N associated with disturbance (Hiers et al. 2003). Casals et al. (2005) found that both seedling and resprouting legume species derived 52 - 99 % of their N from fixation after fire, despite increased amounts of mineral N in burned plots. Similarly, N_2 fixation rates of *Macrozamia riedlei* in southwestern Australia were greatest the year following fire and gradually decreased with time since fire (Grove et al. 1980). In these situations, reduced competition for other limiting resources (light, phosphorus, water) also may contribute to increases in fixation. *Lupinus* is a perennial species, and some work suggests that nodules also may be perennial (Johnson and Rumbaugh 1981). The ability to retain active nodules during conditions of high N availability would allow this species to return to fixing atmospheric N once N levels decrease or competition for N increases.

Substantial amounts of organic N were released from *Lupinus* roots to the soil, regardless of background resource levels. Another recent study also found that DON exudation by the legume *Trifolium repens* was unaffected by N fertilization even though

levels of N fixation decreased (Paynel et al. 2008). The presence of organic N within the rhizosphere could have multiple sources: 1) exudation of fixed N, 2) exudation of recycled N originating from inorganic fertilizer, or 3) turnover of roots and nodules. Plants were only grown for 3 months making a large contribution due to root turnover unlikely. Further, regular flushing of the soils probably removed potential contribution of DON from dead roots over the course of the experiment. The trend of increasing exudation with increasing N fertilizer suggests that exudation likely shifted from fixed N to recycled N as fertilization increased. Further, low rates of ARA and high $\delta^{15}\text{N}$ values for the higher N treatments suggest that plants were mainly relying on mineral N and the majority of exuded N was recycled inorganic fertilizer N. However, because the fertilizer was not labeled, it is only possible to distinguish the amount of fixed N being exuded in the 0 N treatment. For those plants, estimates range from 3 to 58 % of fixed N exuded depending on water treatment and method used to calculate amount of N fixed. Values of fixed N exuded based on N content (3-14%) fall within the range observed for other herbaceous crop legumes. For example, 13 % of *Vicia faba* and 16 % of *Lupinus albus* total plant N was added to the soil via rhizodeposition (Mayer et al. 2003).

Rhizodeposition is mainly regulated by passive diffusion, with exudation following a concentration gradient from root to soil (Jones et al. 2004). Although it may appear wasteful for *Lupinus* to be exuding costly N, it may not be an entirely passive process. For example, growth by legumes is often limited by phosphorus (Vitousek and Field 1999). To overcome this limitation, research suggests that many legumes exude N-rich enzymes such as phosphatase to increase availability of this limiting nutrient (Wang et al. 2007). For example, when grown in phosphorous limiting conditions, *Lupinus* spp.

increased acid phosphatase secretion 20 times compared to plants without phosphorus limitation (Bais et al. 2004). In addition, research has shown that root exudates can play an active role in plant-plant and plant-microbe communication (Bais et al. 2004).

Our results suggest that availability of water did not influence rhizodeposition, indicating that exudation may occur throughout the growing season under varying water availability and may potentially provide a novel source of N when this nutrient is most limiting to plant growth. Although some studies suggest that rhizosphere microbes have a greater ability to take up root exudates than other plant roots (Owen and Jones 2001), microbial turnover of exudates in soil is very fast (Jones et al. 2004), resulting in the quick conversion of this N source to plant-available mineral N. This would suggest that the high levels of N exudation observed in this species may contribute to plant-available N in these soils. Tissue decomposition also can contribute substantial amounts of N, but this input is temporally variable. DON input from litter is likely greatest in fall after plant senescence and when rain and snow allow for decomposition. Rhizodeposition, on the other hand, could potentially contribute plant-available N throughout the growing season.

Rhizodeposition has received little attention in desert ecosystems as a source of N input to total ecosystem N budgets. Taking the average exudation rate of 5.5 mg N d^{-1} and assuming an average of 3 plants m^{-2} (Goergen and Chambers, *in press*) and a constant exudation over a growing season of 3 months, our results indicate that *Lupinus* could contribute upwards of $15 \text{ kg of N ha}^{-1} \text{ yr}^{-1}$ via organic N rhizodeposition to sagebrush steppe under low N conditions. The contribution of N via decomposition of N rich tissue is an additional source which, based on results from this study and assuming a density of 3 plants m^{-2} , can range between 0.375 and $9 \text{ kg of N ha}^{-1} \text{ yr}^{-1}$ depending on the

resource environment. However, actual contributions of N by *Lupinus* in the field will depend not only on plant responses to the environment, but also on plant density, distribution, and phenology, which can vary greatly over the landscape. Even acknowledging that these values may be on the high end, in comparison to other sources of input such as atmospheric N deposition ($<2 \text{ kg of N ha}^{-1} \text{ yr}^{-1}$, CASTNET 2004-2006) or fixation by microbiotic crusts ($1\text{-}13 \text{ kg of N ha}^{-1} \text{ yr}^{-1}$, Belnap 2002a; Housman et al. 2006), these results indicate that *Lupinus* can contribute greatly to the N budget of the sagebrush steppe through rhizodeposition during the growing season and decomposition of N rich tissue after senescence.

Aside from the impact on N cycling and budgets in this system, the results of our study have implications for community composition and invasion potential. The sagebrush ecosystem is threatened by invasion of numerous exotic species, including both annual grasses and perennial forbs. Differential resource use, growth rates, and competitive abilities between native and exotic species influence recruitment into these systems and may shift community composition from native to alien dominance. Modification of the local resource pool by *Lupinus* combined with an exotic seed source may provide an avenue for expansion of highly competitive exotic species by modifying interactions among native and exotic species or by differentially influencing species establishment. Increases in N availability led to invasion by nonnative species and resulted in altered disturbance regimes and changes in successional trajectories in Mojave Desert (Brooks 2003), chaparral (Cione et al. 2002) and sagebrush ecosystems (Chambers et al. 2007). N rich patches created by *L. arboreus* in California led to a conversion of native shrubland to an exotic annual grassland (Maron and Connors 1996). Similarly,

facilitation by an exotic legume is believed to play a role in invasion by *Pennisetum* *cetaceum* in Hawaii (Carino and Daehler 2002). With exotic species introductions on the rise, it is important to understand factors that may promote invasion by exotic species. Although local N enrichment due to *Lupinus* has the potential to change species composition and incorporate exotic species into the native community matrix, ultimately the effect of *Lupinus* presence will depend on the composition of other native perennial herbaceous species and the relative degree of competition.

Acknowledgements

We thank K. Vicencio, F. Berthiaume, and T. Morgan for valuable assistance in the greenhouse and lab, C. Fritsen and J. Memmott for assistance with ARA analysis, and D. Johnson, J. Qualls, P. Weisberg, P. Verburg, S. Uselman and E. Allen for valuable comments on earlier drafts of this manuscript. Financial support was provided by the USDA Forest Service, Rocky Mountain Research Station.

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Table 1. ANOVA results for water and N treatment on total, above, and belowground biomass.

Source	Total			Above			Below		
	df	F	P	df	F	P	df	F	P
Water (W)	2,116	43.14	<0.0001	2,120	108.82	<0.0001	2,116	16.41	<0.0001
Nitrogen (N)	3,116	105.4	<0.0001	3,120	116.17	<0.0001	3,116	89.84	<0.0001
W*N	6,116	5.62	<0.0001	6,120	7.55	<0.0001	6,116	3.85	0.0015

Table 2. R:S and whole plant C:N values for all treatments. Different letters within R:S or C:N indicate significant differences across N and water treatments ($p < 0.05$).

	<u>R:S</u>			<u>C:N</u>		
	Low H ₂ O	Medium H ₂ O	High H ₂ O	Low H ₂ O	Medium H ₂ O	High H ₂ O
0N	3.81±0.49 ^a	2.21±0.33 ^{abc}	2.12±0.41 ^{bcd}	24.2±1.25 ^a	22.9±0.79 ^a	22.5±1.30 ^a
5mM N	3.32±0.34 ^a	2.41±0.29 ^{abc}	1.64±0.21 ^{cde}	18.2±0.41 ^b	17.5±0.43 ^{bc}	15.3±0.57 ^c
20mM N	3.13±0.40 ^{ab}	1.67±0.21 ^{cde}	1.15±0.11 ^{def}	10.1±0.28 ^d	9.7±0.24 ^d	10.2±0.28 ^d
100mM N	1.44±0.15 ^{cdef}	1.03±0.18 ^{ef}	0.74±0.09 ^f	5.7±0.15 ^e	5.7±0.08 ^e	5.6±0.28 ^e

Table 3. ANOVA results for water and N treatment on tissue N concentration, content, and leaf $\delta^{15}\text{N}$

	Source	Above			Below		
		df	F	P	df	F	P
N Concentration	Water (W)	2,114	25.88	<0.0001	2,115	2.09	0.1282
	Nitrogen (N)	3,114	602.05	<0.0001	3,115	166.19	<0.0001
	W*N	6,114	6.59	<0.0001	6,115	0.98	0.4427
N Content		df	F	P	df	F	P
	Water (W)	2,116	185.85	<0.0001	2,114	30.81	<0.0001
	Nitrogen (N)	3,116	202.95	<0.0001	3,114	173.33	<0.0001
	W*N	6,116	10.19	<0.0001	6,114	4.53	0.0004
Isotope $\delta^{15}\text{N}$		df	F	P			
	Water (W)	2, 24	11.21	0.0004			
	Nitrogen (N)	3, 24	35.71	<0.0001			
	W*N	6, 24	0.9126	0.4922			

Table 4. ANOVA results for water and N treatment on specific nodule density, weight, and ARA.

Source	Nodule Density			Nodule Wt			ARA		
	df	F	P	df	F	P	df	F	P
Water (W)	2,115	10.14	<0.0001	2,24	0.14	0.8709	2,28	1.2	0.3157
Nitrogen (N)	3,114	71.94	<0.0001	3,24	26.69	<0.0001	3,28	8.98	0.0003
W*N	6,114	5.87	<0.0001	6,24	1.95	0.1133	6,28	0.88	0.5256

Figure Legends

Figure 1. Above and belowground biomass of *Lupinus* plants in the different water and N treatments. Values are means $\pm$ SE. For 0N, n = 11 (L) or 12 (M and H); for 5mM N, n = 12; for 20mM N, n = 11 (L) or 12 (M and H); for 100mM N, n = 11 (L), 9 (M), or 6 (H). Different letters for above or belowground biomass indicate significant differences among N and water treatments ($p < 0.05$).

Figure 2. Above and belowground tissue N concentration and content for *Lupinus* plants in the different water-N treatments. Values are means $\pm$ SE. For 0N, n = 11 (L) or 12 (M and H); for 5mM N, n = 12; for 20mM N, n = 11 (L) or 12 (M and H); for 100mM N, n = 11 (L), 9 (M), or 6 (H). Different letters within above or belowground concentration and content indicate significant differences among N and water treatments ($p < 0.05$).

Figure 3. The $\delta^{15}\text{N}$ signature of *Lupinus* leaf tissue in the different water-N treatments. The solid line represents $\delta^{15}\text{N}$ of *Lupinus* seeds, and the dotted line represents $\delta^{15}\text{N}$ of the NH_4NO_3 fertilizer. Values below the seed line indicate reliance on fixed N and values above the fertilizer line indicate reliance on mineral N. N = 3 per treatment combination, and values are means $\pm$ SE. Different letters indicate significant differences among N and water treatments ($p < 0.05$).

Figure 4. Number of nodules per plant, specific weight per nodule, and specific activity per nodule weight per hour as measured by ARA for *Lupinus* in the different water-N treatments. Values are means $\pm$ SE. For nodule density, 0N, n = 11 (L) or 12 (M and H); for 5mM N, n = 12; for 20mM N, n = 11 (L) or 12 (M and H); for 100mM N, n = 11 (L), 9 (M), or 6 (H); for nodule weight and ARA, 0N, n = 3 (L) or 4 (M and H); for 5mM N, n = 4; for 20mM N, n = 3 (L) or 4 (M and H); for 100mM N, n = 3 (L) or 2 (M and H). Different letters indicate significant differences among N treatment (weight and ARA) or among N and water treatments (density) ($p < 0.05$).

Figure 5. Organic N exuded into the rhizosphere of *Lupinus* plants grown in the different water-N treatments. For 0N, n = 11 (L) or 12 (M and H); for 5mM N, n = 12; for 20mM N, n = 11 (L) or 12 (M and H); for 100mM N, n = 11 (L), 9 (M), or 6 (H). Values are means $\pm$ SE.

Figure 1.

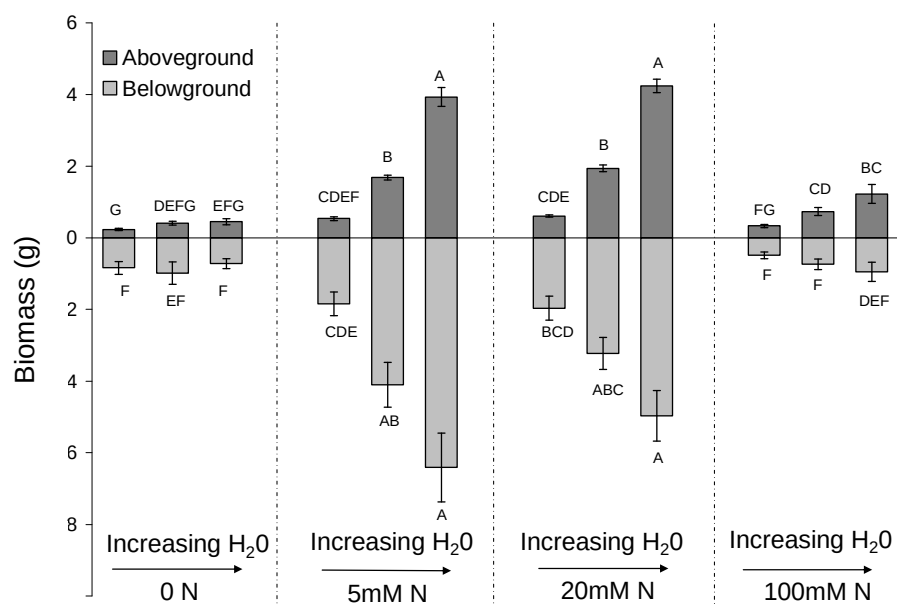


Figure 2.

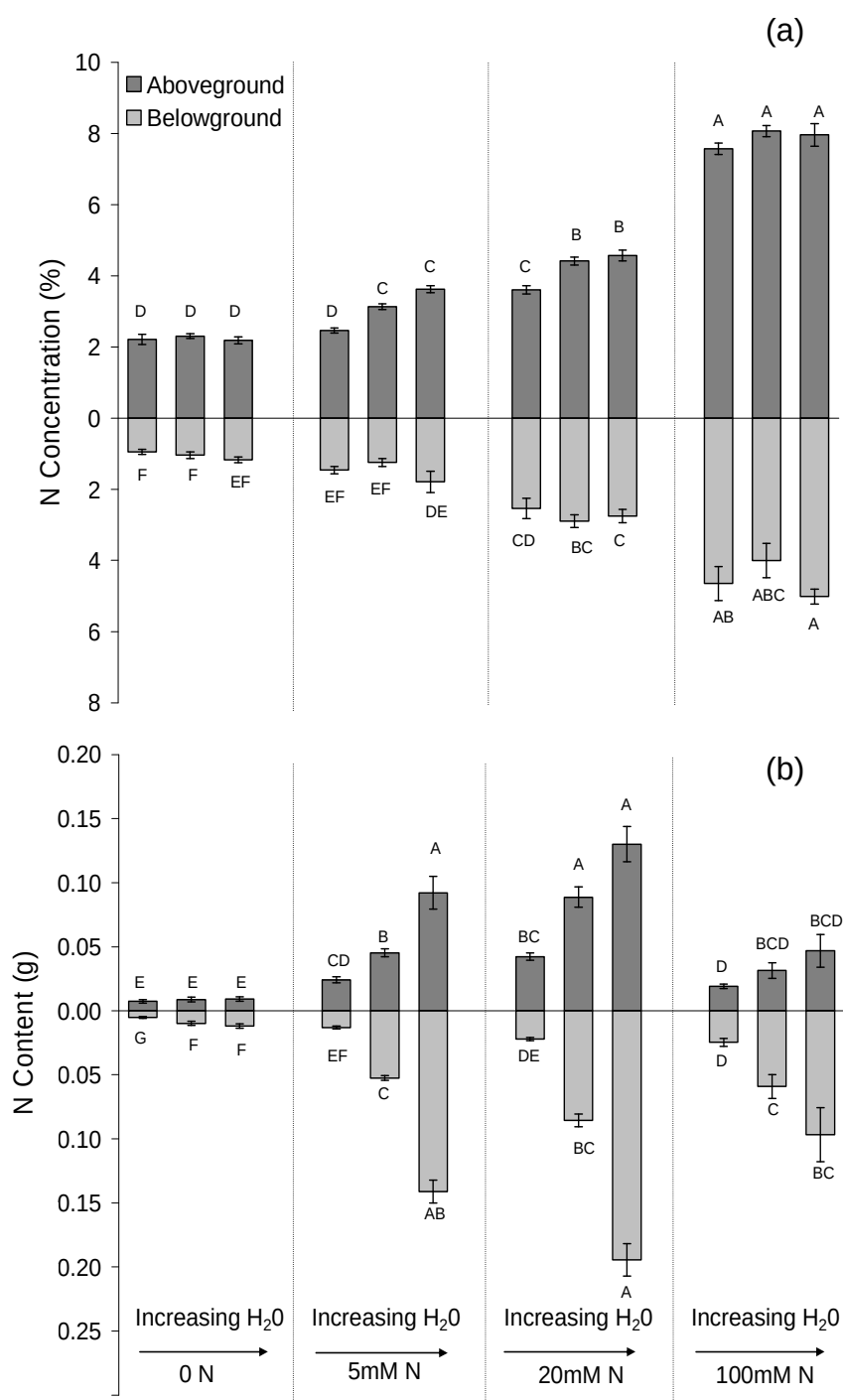


Figure 3.

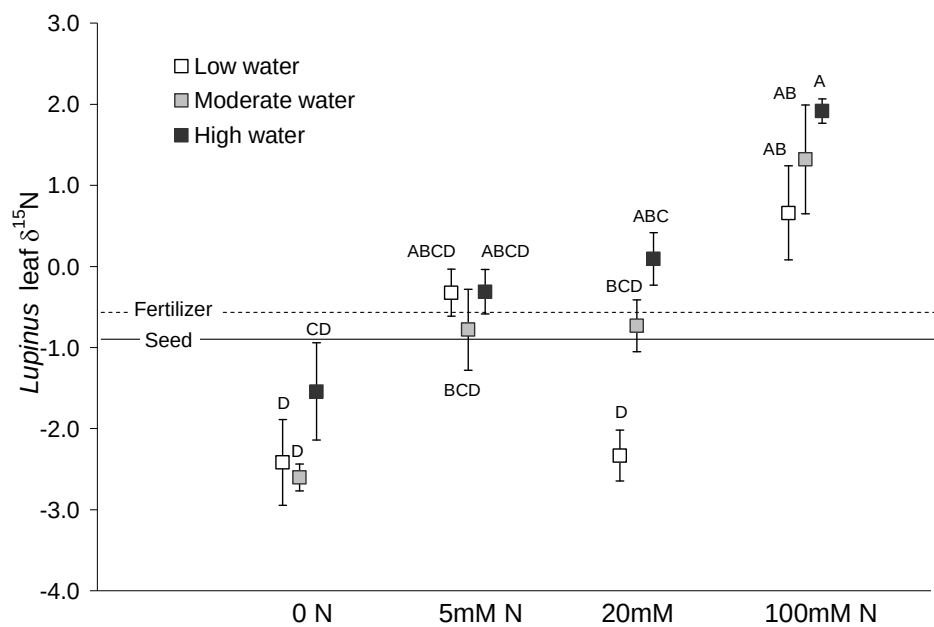


Figure 4.

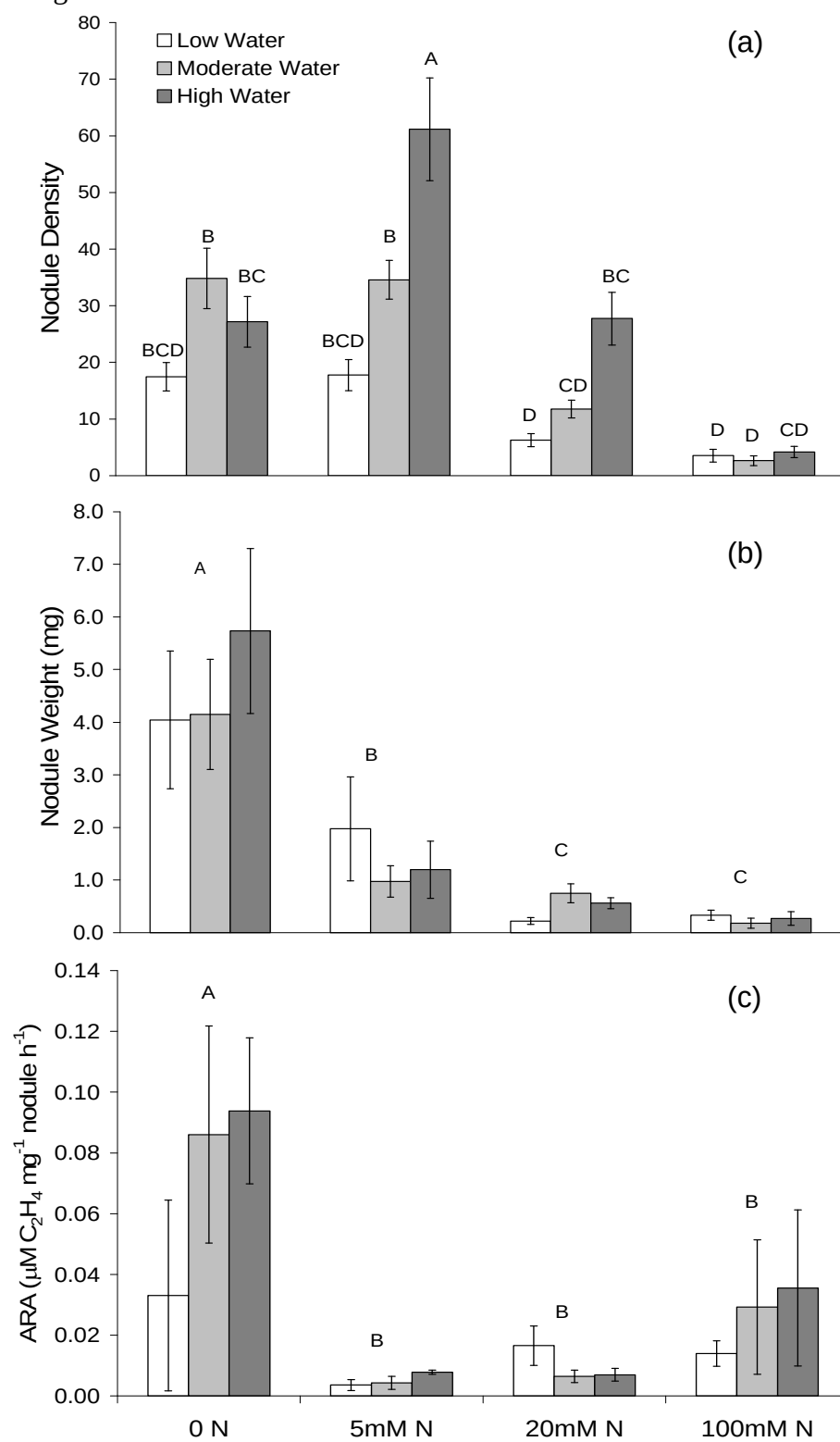
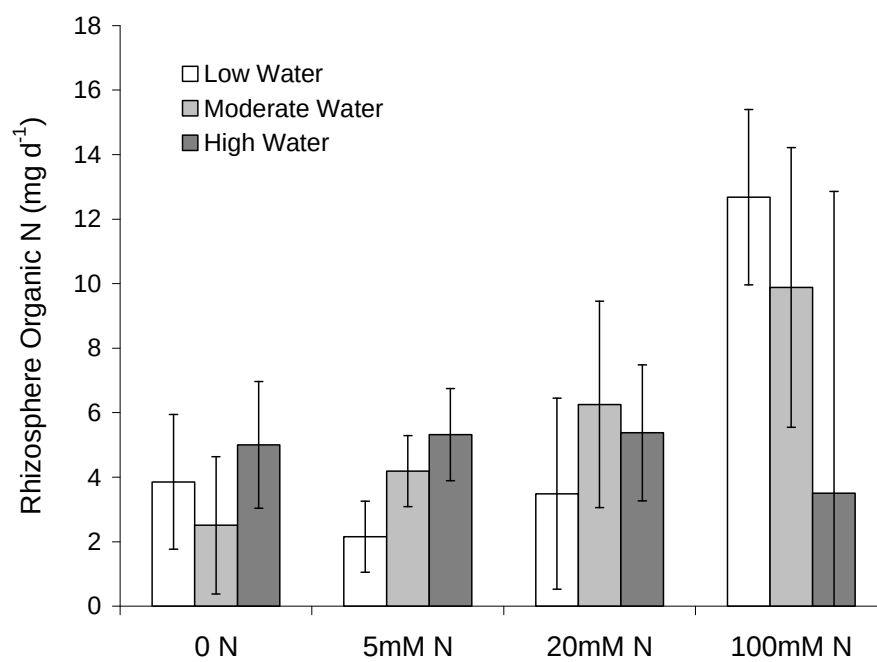


Figure 5.



Nitrogen Level and Legume Presence Affect Competitive Interactions between a Native
and Invasive Grass

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Abstract

Plant invasions are a global issue, but the exact mechanisms of invasion remain unclear. Increased resource availability often promotes expansion of invasive species by changing competitive interactions. N₂-fixing species are often abundant in N poor systems, and have the capacity to alter resource availability, particularly N, and, thus, to indirectly influence competitive interactions. Like many arid areas dominated by perennial grasses, the sagebrush steppe of the western U.S. is threatened by invasion of non-native species, especially annual grasses. N₂-fixing legumes are common, and are often used in restoration, but have the potential to facilitate invasion and expansion of invasive annual grasses. We conducted a greenhouse experiment to investigate effects of the native N₂-fixing legume, *Lupinus argenteus*, on competitive interactions between seedlings of the non-native annual grass, *Bromus tectorum*, and a native perennial grass, *Elymus multisetus*, over a gradient of N availability. The native and non-native species responded nearly identically to increasing N when grown in monoculture. *B. tectorum* biomass was more affected by intraspecific competition whereas *E. multisetus* was more affected by interspecific competition from *B. tectorum*. When grown in competition *B. tectorum* always outperformed *E. multisetus*, regardless of N level, although the degree of *E. multisetus* suppression by *B. tectorum* was least in the absence of additional N. Presence of *L. argenteus* increased biomass and tissue N content in both grasses, indicating that this native legume has the potential to facilitate both species. However, *B. tectorum* exhibited a greater positive response to the presence of *Lupinus*, which intensified competition between *E. multisetus* and *B. tectorum*. Thus, presence of *L. argenteus* was facilitative for *B. tectorum* but indirectly inhibitory for *E. multisetus*. Our results indicate

that modification of the local resource pool by *L. argenteus* can alter competitive outcomes among these competing native and non-native species and provide an avenue for expansion by *B. tectorum*.

Key words:

facilitation, invasion, nitrogen availability, *Bromus tectorum*, *Elymus multisetus*, *Lupinus argenteus*, sagebrush steppe

Introduction

Although plant invasions have been occurring for centuries (see Darwin 1859), globalization has increased the number and rate of plant introductions (Lodge 1993, Williamson 1996, Ludsin and Wolfe 2001). Disturbed ecosystems are particularly vulnerable to invasion, but the exact mechanisms are often complex. Increased resource availability and creation of physical space often promote invasion and expansion of invasive species following disturbance (Hobbs and Huenneke 1992, Mack et al. 2000, Daehler 2003). Interactions among recruiting species influence both successional trajectories and invasion potential.

In many N poor systems, leguminous species are early colonizers after disturbance (Tracy and McNaughton 1997, Ralphs 2002, Goergen and Chambers, *in press*). Their presence increases soil N availability (Morris and Wood 1980, Vitousek and Walker 1989, Johnson et al. 2004, Yelenik et al. 2004, Goergen and Chambers, *in press*) and can result in higher seedling growth and survival (Gosling 2005). In addition, legumes can facilitate seedling establishment by modifying microsite conditions via moisture, light, and temperature regulation (Pugnaire and Haas 1996, Pugnaire and Luque 2001). Although both native and non-native species can benefit from amelioration of harsh conditions by legumes, differential resource use and competitive abilities between seedlings determine which species receives the greatest benefit.

N₂-fixing species can have both positive and negative effects on neighboring species (Morris and Wood 1980, Vitousek and Walker 1989, Carino and Daehler 2001). The degree of facilitation by N₂-fixing species depends upon the legume's capacity to fix atmospheric N₂ and the ability of associated non-fixing species to gain an advantage from

the increased N (Temperton et al. 2007). Further, the facilitative effect of legumes can change depending on the resource environment (Armas and Pugnaire 2005, Brooker et al. 2008). The ability of N₂-fixing species to fix atmospheric N₂ is linked closely to mineral N in soil (Zahran 1999). Under low N conditions, fixation is typically high, potentially increasing resource availability (either through N addition or reduced N uptake) for non N-fixing species. As N availability increases, fixation of atmospheric N₂ is less efficient, and legumes typically rely more on soil N, potentially shifting their role from facilitative to competitive. Thus, understanding potential effects of legumes on plant competition and species invasions requires examining competitive interactions for varying N availability.

Like many arid areas of the western US, the sagebrush steppe is threatened by invasion of non-native annual grasses. Both field and greenhouse experiments indicate that elevated resources, especially N, accentuate differences between native and non-native grasses and are particularly important in shifting the balance of competition in favor of non-native annual grasses (Huenneke et al. 1990, Levine et al. 2003, Brooks 2003, Young and Mangold 2008, James 2008 a,b). Thus, increased resource availability after disturbance combined with N input from legumes can create conditions favorable for invasion and expansion of non-native species by shifting the balance of plant-plant interactions (Maron and Connors 1996, Carino and Daehler 2000). Although the effects of actively growing legumes on resource availability and productivity in cultivated pasture systems has been extensively studied (Trannin et al 2000, Høgh-Jensen and Schjoerring 2000, Paynel et al. 2001), the effects of legumes on uncultivated species in natural systems have received little attention.

In sagebrush steppe, invasion by the non-native annual grass, *Bromus tectorum* L. (cheatgrass) is leading to altered fire regimes and changes in community composition and ecosystem functioning (Mack 1981, D'Antonio and Vitousek 1992, Knapp 1996, Evans et al. 2001). *B. tectorum* benefits from additional N and is an effective competitor against native species for available nutrients (Melgoza et al. 1990, Lowe et al. 2002, Monaco et al. 2003, Beckstead and Auspurguer 2004), especially at the seedling stage (Humphrey and Schupp 2004).

Higher growth rates and earlier spring growth by *B. tectorum* compared to native perennial grass seedlings can result in greater biomass accumulation and pre-emption of resources (Knapp 1996, James 2008 a,b). Established perennial species that are morphologically and phenologically similar to *B. tectorum*, like *Elymus multisetus*, a short-lived perennial grass, compete for similar resources (Booth et al. 2003) and can limit *B. tectorum* establishment and reproduction (Stevens 1997, Humphrey and Schupp 2004). However, modification of the local resource pool by disturbance combined with N input by native legumes may alter interactions among seedlings of *B. tectorum* and native perennial grasses leading to invasion and dominance by *B. tectorum*.

Native legumes such as *Lupinus argenteus* can make up a large component of sagebrush ecosystems, especially after disturbances such as fire. Compared to unburned treatments, cover of this species nearly tripled by three years after fire and composed greater than 50% of the total herbaceous plant biomass in sagebrush steppe of the central Great Basin (Goergen and Chambers, *in press*). Further, in both burned and unburned sagebrush steppe, inorganic N availability doubled in *L. argenteus* presence (Goergen and Chambers, *in press*). Thus, this species has great potential to influence post-fire

establishment both directly and indirectly through its effect on system productivity and nitrogen availability. However, the ability of *L. argenteus* or other native legumes to modify plant-plant interactions and facilitate invasion into this system via resource modification has not been examined.

In this study we investigated the potential for seedlings of the native N₂-fixing legume, *L. argenteus*, to facilitate invasion and expansion of *B. tectorum* by altering competitive interactions between seedlings of *E. multisetus* and *B. tectorum* over a gradient of N availability. Our approach was to conduct a greenhouse experiment to evaluate the direct effect of increasing N, the indirect effect of *L. argenteus* on N availability, and the interacting effects of *L. argenteus* presence and N addition on *B. tectorum* and *E. multisetus* performance. We made three predictions. (1) Both species would exhibit a positive growth response to increasing N availability when grown individually, but the response would be greater for the nonnative species. (2) Due to its competitive nature, *B. tectorum* would produce more biomass than the native *E. multisetus* when the two grasses are grown together, and this response would be accentuated as fertilization increased. (3) The presence of *L. argenteus* seedlings would favor growth of the non-native species over the native. However, as resources increased, effects of *L. argenteus* would shift from facilitative to competitive for both the native and non-native species.

Materials and methods

Experimental design

In a greenhouse experiment, we used a replicated, randomized factorial design with three levels of N availability, two target species (*B. tectorum* and *E. multisetus*), and three competitors (*B. tectorum*, *E. multisetus* and *L. argenteus*) grown in two blocks. Six different species combinations were used to investigate both inter and intraspecific competition with and without *L. argenteus* under different N availability: 1) *E. multisetus* monoculture (E); 2) *B. tectorum* monoculture (B); 3) *L. argenteus* monoculture (L); 4) *E. multisetus* + *B. tectorum* (EB) 5) *E. multisetus* + *L. argenteus* (EL) 6) *B. tectorum* + *L. argenteus* (BL) 7) *E. multisetus* + *L. argenteus* + *B. tectorum* (ELB).

L. argenteus, *E. multisetus*, and *B. tectorum* seeds (hereafter *Lupinus*, *Elymus*, and *Bromus*) were obtained from a local Great Basin source (Comstock Seeds, Carson City, NV for *Lupinus* and *Elymus*, field collection for *Bromus*). *Lupinus* seeds were scarified with sandpaper to promote germination. Scarified seeds were coated with a commercial inoculum (Lupine inoculum 'H', Nitrogin) and allowed to dry before planting. Preliminary studies indicate that this inoculum promotes active nodules in this species. In February 2007 seeds of each species were planted in 1L pots containing a 1:2:1 mixture of sand:peat:perlite. *Lupinus* seeds were sown a week before the grass species to account for slower germination. The potting medium was chosen to provide a low nutrient, well draining soil. Plants were grown in the University of Nevada – Reno greenhouses under natural light with a climate controlled maximum daily temperature of 27 °C and minimum nightly temperature of 7 °C. Water was maintained at a level optimal for plant growth throughout the experiment. After seedling emergence, pots were thinned to four seedlings per pot in monocultures, two seedlings per species for two species treatments,

and one seedling per species for three species treatments, giving a density of 4 seedlings per pot for all but the ELB treatment.

N treatments were initiated after thinning. Three N levels were used based on the range of extractable soil inorganic N (as NH_4NO_3) found under field conditions in sagebrush steppe (West and Skujins 1978): 0 N, 5mM N (similar to growing season average), and 20mM N (similar to post-fire conditions). To prevent confounding effects from limitation by other nutrients, all N solutions were based on a modified $\frac{1}{4}$ strength Hoagland's nutrient solution with only the level of N varying. N treatments were randomly assigned and pots were separated into two blocks within the greenhouse (n=7 per treatment per block). Pots received their respective N solution once a week in aqueous form (100ml). Pots without seedlings also were placed within each block (n=3 per block) and watered with the 0N nutrient solution to serve as a background control for soil N in the absence of plant uptake (blank).

Plant combinations were grown for 3 months under their respective treatments and randomized on the bench every two weeks to prevent neighbor or edge bias. To account for any size bias, height and number of tillers for all seedlings were recorded at the initiation of the experiment (Gibson et al. 1999). At the conclusion of the experiment, plant height and number of tillers were recorded, and all plants were harvested by species to determine aboveground biomass. It was not possible to separate roots by species with certainty, especially for the grasses, so belowground results are only presented for monocultures. At harvest, *Lupinus* roots were examined for the presence of active nodules and a subsample of nodules from each treatment was weighed as an estimate of treatment effect on potential contribution of fixed N.

All plant tissue was dried at 65°C for 48 hr and weighed to determine biomass. Aboveground tissue was ground in a Wiley mill and analyzed for percent total N and C concentrations using a LECO TruSpec CN analyzer (St. Joseph MI). Plant N and C content were calculated by multiplying tissue concentration by plant weight.

To assess treatment effects on extractable N, soils from each pot were homogenized, and a 10 g subsample was extracted with 2.0M KCl. Concentrations of NH_4^+ and NO_3^- then were determined colorimetrically (Robertson et al. 1999, LACHAT Instruments, Milwaukee WI).

Data Analysis

Differences in tiller production, aboveground biomass, and tissue C and N were analyzed as a randomized factorial design with three levels of N treatment, two levels of target species (*Bromus* or *Elymus*) and three levels of competitor (*Lupinus*, *Bromus*, or *Elymus*). N treatment, competitor, and target plant were treated as fixed effects and block was treated as a random effect. Due to the potential for non-independence of growth among seedlings sharing the same pot, half of the pots in the EB and EBL treatments were randomly chosen for analysis of *Elymus*, and the remaining pots were used for the analysis of *Bromus*. The three species treatment pots were analyzed separately due to differences in total pot density, and target species and N were treated as fixed effects. Soil N was analyzed with species combination and N treatment as fixed factors. All data were assessed and transformed as necessary to meet assumptions of normality and equality of variance. For results with significant effects, mean comparisons were assessed using Tukey adjusted least square means for multiple comparisons and considered

significant at the 95% confidence level. All analyses were conducted using SAS™ ver. 9.1.

Results

Soil N

Total extractable inorganic N ($\text{NH}_4^+ - \text{N} + \text{NO}_3^- - \text{N}$) in soil was influenced by both N level ($F_{2, 249} = 2149.2$, $p < 0.0001$) and species ($F_{6, 249} = 38.2$, $p < 0.0001$; Fig 1).

Inorganic N increased with each increase in N fertilization for pots that contained *Elymus* and *Lupinus*. In contrast, extractable inorganic N in pots containing *Bromus* only increased under the highest N (20mM) treatment. This difference among species combinations response to N led to a significant species by N interaction ($F_{12, 249} = 10.49$, $p < 0.0001$). *Lupinus* monoculture pots had the highest amount of total inorganic N for each N treatment level. However, relative to the grass monocultures, *Lupinus* presence did not result in increased extractable soil N when grown with either *Bromus* or *Elymus* for any level of N.

Extractable inorganic N in the 0 and 5 mM N treatments was lower than in the 0 N blank indicating that even when fertilizing with 5 mM N, plants were N limited and depleted soil N (Fig 1). The only exception was *Lupinus* grown in the 5 mM N treatment. In contrast, soils in the 20 mM N treatment had significantly more extractable N than blanks indicating that this level of fertilization surpassed plant uptake. There also was a difference in extractable soil N between pots containing target species. For all but the 0 N treatment there was less total N in pots containing *Bromus*, indicating greater uptake than by *Elymus* and *Lupinus*. In the 5 and 20 mM N treatments, there was roughly twice as

much extractable inorganic N in *Elymus* than *Bromus* pots and about three to fifteen times more in *Lupinus* monoculture than *Bromus* pots. Also, the pattern of N uptake differed among species. Percent of extractable N as NH_4^+ - N was greater in pots containing *Bromus* than *Elymus* or *Lupinus* ($p < 0.05$) in the 5 and 20mM N treatments.

Treatment effects on *Lupinus*

Lupinus was on average 4-6 cm shorter than the grasses at the start of the experiment despite earlier planting. *Lupinus* plants grew best in monoculture but were significantly reduced in size when grown with either target grass species ($p < 0.05$), averaging only half the height of either grass species by the conclusion of the experiment. *Lupinus* biomass increased with additional N; however, compared to the target species, *Lupinus* produced less biomass and weighed on average 3 to 8 times less than *Elymus* and 4 to 44 times less than *Bromus* depending on N treatment and species combination. *Lupinus* biomass was most reduced when grown with *Bromus* under intermediate N and least when grown with *Elymus* under the highest N level (Table 1). Although *Lupinus* biomass was reduced when grown in interspecific competition, tissue N concentrations remained high (Table 1) and were nearly twice that of either target species in the absence of additional N. Under intermediate and high N addition, tissue N concentrations in *Lupinus* were similar to both target species. All *Lupinus* were nodulated, but weight of nodules decreased with increasing fertilization ($F_{2,45} = 173.56$, $p < 0.0001$). Nodules tended to be heaviest in *Lupinus* monocultures and lightest when grown with *Bromus* but differences were not significant (Table 1).

Treatment effects on target species

Elymus was initially taller ($F_{1,184} = 506.67$, $p < 0.0001$; mean of 7.6 cm) than *Bromus* (mean of 5.6 cm), but this difference disappeared within a week. There was no difference in number of tillers (mean of 1 tiller) between the two grass species at initiation of the experiment.

At harvest height and number of tillers for *Elymus* and *Bromus* differed by both N treatment and competitor (Table 2). For both species, addition of N increased both height and tillering. When grown in monoculture or in the presence of *Lupinus*, *Elymus* was consistently taller than *Bromus*, even after accounting for the initial difference in height. In contrast, when grown together in the absence of *Lupinus*, *Elymus* height was reduced and there was no difference between target species. *Bromus* produced more tillers than *Elymus* regardless of treatment, although differences were most pronounced when the target species were grown in competition (Table 2).

Aboveground biomass of both *Elymus* and *Bromus* was increased by N addition (Table 3A, Fig 2). However, biomass differences between the target species depended on the interaction of competitor and N treatment. Aboveground biomass did not differ between *Elymus* and *Bromus* when grown in monoculture. Increasing from 0 to 5 mM N almost tripled the biomass of both species but a further increase to 20 mM N did not result in higher biomass. In contrast, belowground biomass of grass monocultures decreased with N addition. *Bromus* monocultures had twice the belowground biomass of *Elymus* monocultures in all N treatments and, consequently, *Bromus* had larger R:S ratios in all N treatments (Table 2).

Relative to monocultures, mean aboveground biomass of both target species increased when grown with *Lupinus* (BL and EL) regardless of N treatment (Table 3A, Fig 2). The biomass increase was similar for the two species at 0 and 5 mM N, but at 20 mM N *Bromus* produced more biomass than *Elymus*.

When grown in competition, *Bromus* produced more biomass than *Elymus* regardless of N or *Lupinus* presence (EB Table 3A and ELB Table 3B; Fig 2), although *Elymus* appeared least affected by *Bromus* in the absence of additional N (Fig 3). On average, biomass of *Elymus* plants decreased by 50% of monoculture values, whereas *Bromus* biomass increased by 50% of monoculture values when grown together without *Lupinus* (Fig 3). Both *Bromus* and *Elymus* produced more biomass when grown with *Lupinus*, but the biomass response of *Bromus* was 3 to 5 times greater than that of *Elymus*.

Tissue N increased with N addition for both *Elymus* and *Bromus*. Differences between target species varied by competitor and N treatment (Table 3 C, D) but not the presence of *Lupinus* (Fig 4). *Bromus* tissue N concentration was unaffected by competitor. In contrast, *Elymus* tissue N concentration decreased relative to monoculture when grown with *Bromus* in all but the 0 N treatment (Fig 4). When grown in monoculture (E and B) and with *Lupinus* (EL and BL) *Elymus* had slightly higher tissue N concentrations than *Bromus* at 0 and 5 mM N but these differences were not significant (Fig 4). However, at 20 mM N *Bromus* had significantly greater tissue N concentrations than *Elymus*. When grown in competition without (EB) or with *Lupinus* (ELB), *Bromus* had higher tissue N than *Elymus* except for the 0 N treatment in the absence of *Lupinus* (Fig 4).

No significant differences in N content occurred between target species when grown in monoculture because of similar biomass (B and E, Table 3E, Fig 4). In contrast, increased biomass in the presence of *Lupinus*, regardless of N treatment, resulted in higher N content for both target species than in monocultures, but differences between species only occurred at 20mM N (BL and EL, Table 3E, F). When grown in competition *Bromus* had greater N content than *Elymus* for all treatments regardless of N or *Lupinus* treatment (Fig 4).

In both species C:N ratios decreased as N addition increased and was affected by competitor (Table 2). When grown in monoculture or with *Lupinus*, C:N ratios were similar for the target species except in the 20mM N treatment where *Bromus* had a significantly lower C:N ratio. In contrast, when grown in competition, the C:N ratio was lower for *Bromus* in all treatments with the exception of EB without N added (Table 2).

Discussion

Lupinus effect on N availability

In our greenhouse experiment, we examined the potential for *Lupinus* seedlings to facilitate invasion by altering the competitive outcome between seedlings of a native and non-native grass. Plant recruitment, establishment, and invasion potential in low resource environments can be strongly influenced by elevation of a primary limiting resource (Davis et al. 2000), and in our study N was a key limiting factor under all but the highest level of N. Therefore, the importance of *Lupinus* as a facilitator rests mainly on the assumption that its presence increases plant available N, and that this increase translates to a benefit for co-occurring species. We found that total extractable inorganic N was

greatest in monocultures of *Lupinus* seedlings, with most of this N present as NO₃. This is consistent with other studies of legumes where the main effect is an increase in plant available NO₃ (Herridge et al. 1995, Chu et al. 2004). We did not observe an increase in extractable soil inorganic N when the target seedlings were grown with *Lupinus* seedlings; however, this is likely a result of greater N uptake by the target species rather than a lack of *Lupinus* effect on soil N. Both *Elymus* and *Bromus* seedlings were larger in *Lupinus* presence indicating that they were benefiting from either the conservative use of N by *Lupinus* seedlings (due to low competitive ability or N sparing) or through addition of rhizosphere N. Given that N limited biomass of both grass species under 0 or 5mM N in our experiment, any additional N availability due to *Lupinus* presence has the potential to influence plant-plant interactions in these treatments through a facilitative fertilization effect.

Effect of fertilizer N on target species

Contrary to our first prediction, the native and non-native species responded nearly identically to increasing N when grown in monoculture. There was no difference in biomass between species as both grasses increased aboveground biomass with addition of 5mM N but exhibited no further increase with additional N. As nutrients increase, competition frequently shifts from predominantly belowground to aboveground (Wilson and Tilman 1991), and allocation to roots tends to decrease (Lamb et al. 2007). In our experiment, both species had reduced R:S ratios with N addition, although *Bromus* consistently had a lower R:S ratio. Additionally, *Bromus* and *Elymus* exhibited equal increases in tissue N concentration with increasing N addition. Other studies also have

found that monocultures of native and non-native species growing under increasing N availability show no difference in biomass production, N concentration, or R:S ratio (Lowe et al. 2002). However, the response of species in monocultures does not always provide a clear picture of how species will respond when grown in competition.

Bromus biomass was affected more by intraspecific competition whereas *Elymus* was affected more by interspecific competition from *Bromus*. Consistent with our second prediction, *Bromus* always outperformed *Elymus* when grown in competition regardless of N level. The amount of *Elymus* suppression by *Bromus* depended upon N treatment and was least in the absence of additional N. In a similar study, competitive pressure by *Bromus* on *Bouteloua gracilis* was reduced under low N availability (Lowe et al. 2003). As in our study, low N did not give the native species an advantage, but it did reduce competition, resulting in the smallest decrease in *Elymus* relative to monocultures. As nitrogen availability increased, the higher growth rate and resource capture of *Bromus* allowed increased competitive suppression of *Elymus* as has been shown for other natives (Monaco et al. 2003, Vasquez et al. 2008). Higher tiller production, greater root length and earlier branching of adventitious roots by *Bromus* results in both pre-emption of space and increased resource capture (Melgoza and Nowak 1990, Aguirre and Johnson 1991, Knapp 1996, Monaco et al. 2003).

Effect of *Lupinus* seedlings on target species

The presence of *Lupinus* seedlings increased biomass of both grass species at each N level, indicating that *Lupinus* presence was providing a facilitative effect. Increased biomass of non-legumes in the presence of legumes has been found in a variety of

systems (Thomas and Bowman 1998, Sphen et al. 2002, Warembourg et al. 2004). In our study, the presence of *Lupinus* seedlings also resulted in greater N content for both target species. Although *Lupinus* seedlings did not directly influence tissue N concentrations in either target species, percent tissue N remained constant for both grasses as biomass increased indicating that in N limiting treatments (0 and 5 mM N) there was greater availability of N in *Lupinus* presence. Increased N availability in legume presence could be from rhizodeposition of organic N (Rovira 1969), turnover of N-rich root and nodule tissue (Wardle and Greenfield 1991), or N sparing of soil N due to acquiring of N from fixation (Trannin et al. 2000). In our short greenhouse study it is unlikely that there was a large contribution from root exudation or root turnover. However, over time as *Lupinus* increases in size, contribution of fixed N via exudation or tissue decomposition would be expected to increase.

There was an obvious competitive hierarchy among seedlings with *Bromus* being most competitive and *Lupinus* least competitive. Other studies investigating interactions among grass and legume seedlings also found poor performance of legumes due to the competitive nature of grasses, especially during initial growth stages (Trannin et al. 2000, Warembourg et al. 2004, Nguluve et al. 2004). Yet despite the fact that *Lupinus* plants were roughly half the size of target grasses, tissue N concentrations were equal to or greater than that found in the grasses, especially in the absence of additional N. This indicates that *Lupinus* was obtaining at least a portion of its N from fixation, and reducing demand on the soil N pool to the benefit of both grass species. At the highest N level where N was not limiting, the presence of *Lupinus* seedlings was still facilitative, but likely via a different mechanism. The slower growth rate of this seedling compared to

the grasses resulted in reduced competitive pressure for space and or light in this treatment.

Although both grasses had a positive response to *Lupinus* presence, consistent with our third prediction *Bromus* exhibited a greater response, especially when grown in competition with *Elymus*. Compared to *Elymus*, *Bromus* had greater N uptake and tended to dominate the soil N uptake pattern. The greater root production and belowground competitive ability of *Bromus* likely gave it an advantage in acquiring additional N associated with *Lupinus*. Further, the dominant form of N utilized differed between species. *Bromus* pots contained more N as NH_4 compared to *Elymus* suggesting that *Bromus* preferentially takes up more N as NO_3 . The presence of more NO_3 in *Lupinus* pots may be another reason why *Bromus* received a greater benefit than *Elymus* when growing with *Lupinus*. Monaco et al. (2003) also found that the response of *Bromus* as well as another non-native annual grass, *Taeniatherum caput-medusae*, was greater to NO_3 than NH_4 .

The stress-gradient hypothesis predicts that facilitation is important under conditions of high environmental stress and decreases as stress is reduced (Brooker et al. 2008). Thus, if only N is limiting, the presence of *Lupinus* should shift from being facilitative to competitive as N addition increases. Contrary to our prediction, the presence of *Lupinus* remained positive over the entire gradient of N addition. Further, the magnitude of the facilitative effect did not change with increasing N for either grass species. Differences in the growth rate, rooting pattern, and morphology between the grass seedlings and *Lupinus* seedlings likely contributed to the facilitative effect by reducing the competitive pressure by *Lupinus* for other limiting resources. Additionally,

at the low and intermediate levels of N, the effects of *Lupinus* and N appeared to be additive for both *Bromus* and *Elymus* monocultures and mixtures. In contrast, at the highest level of N, the effect of N and *Lupinus* were only additive for *Bromus* when in competition with *Elymus*. These results suggest that after disturbances such as fire that increase N availability, the presence of *Lupinus* seedlings may create conditions that accentuate the competitive ability of *Bromus* over native perennial grass seedlings.

The balance of plant-plant interactions often depends on the life stage examined (Callaway and Walker 1997), and it is possible that in our experiment the use of seedlings negated the effect of increasing N on the outcome. If *Lupinus* had established before introducing the target grasses it may have been more competitive for soil N, resulting in a negative effect of *Lupinus* presence as N availability increased. Further, other competition studies between *Elymus* and *Bromus* found that different outcomes were obtained when competition was between resprouting *Elymus* and *Bromus* seedlings (Booth et al. 2003, Humphrey and Schupp 2004).

Conclusions

In our greenhouse study, the presence of *Lupinus* seedlings increased biomass and tissue N content in both grass species, indicating that the presence of this native legume has the potential to facilitate establishment of both grass species. However, *Bromus* exhibited a greater positive response to the presence of *Lupinus*, which intensified competition between *Elymus* and *Bromus* seedlings. Ultimately, the presence of *Lupinus* was facilitative for *Bromus* but indirectly inhibitory for *Elymus* in the presence of *Bromus*.

This study cannot isolate the exact mechanism by which *Lupinus* is acting as a facilitator; however, it is likely that its low demand on soil mineral N (N sparing) provided increased availability for the grass species. In the field, facilitation may occur via multiple direct and indirect mechanisms such as improving harsh environmental conditions, modifying the substrate, reducing competition, fostering beneficial soil microorganisms, or providing protection from herbivores (Callaway 1995). Although our greenhouse experiment is not able to mimic all possible effects that occur in the field, it does provide insight into the possible facilitative role of *Lupinus* through its effect on soil N availability. Modification of the local resource pool by *Lupinus* seedlings combined with a non-native seed source may provide conditions that favor highly competitive non-native species by modifying interspecific interactions. Our results indicate that the effects of elevated N and *Lupinus* are additive for *Bromus*, further promoting the growth of this aggressive invader. In the absence of disturbance, the presence of N fixing species also may provide localized patches of elevated N that promotes *Bromus* over native species. With non-native species introductions on the rise, it is important to understand factors that may promote invasion and incorporation of non-native species into the native community matrix.

Acknowledgements

We thank E. Hoskins, L. Davis, F. Berthiaume, T. Morgan, and B. Blank for valuable assistance in the greenhouse and lab and D. Johnson, J. Qualls, P. Weisberg, P. Verburg, E. Leger, and E. Espeland for valuable comments on earlier drafts of this manuscript. Financial support was provided by the USDA Forest Service, Rocky Mountain Research Station. The experiments comply with the current laws of the United States where they were performed.

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Table 1. Treatment effect on height, biomass, mean nodule weight, and tissue N concentrations of *Lupinus* plants in monoculture, *Elymus* + *Lupinus* (EL), *Bromus* + *Lupinus* (BL), and *Elymus* + *Bromus* + *Lupinus* (ELB) treatments and under the 3 different N levels.

	Height (cm)	Biomass (g)	Nodule Wt (mg)	% Tissue N
Monoculture				
0 N	16.48 ± 1.44	0.47 ± 0.06	4.58 ± 0.71	1.91 ± 0.09
5mM N	21.20 ± 0.93	0.93 ± 0.04	0.60 ± 0.14	3.01 ± 0.10
20mM N	23.45 ± 1.61	1.10 ± 0.05	0.24 ± 0.01	4.26 ± 0.15
EL				
0 N	12.23 ± 0.34	0.16 ± 0.01	2.50 ± 0.54	1.76 ± 0.11
5mM N	14.62 ± 0.72	0.37 ± 0.05	0.85 ± 0.13	2.37 ± 0.09
20mM N	13.85 ± 0.94	0.66 ± 0.08	0.25 ± 0.03	4.72 ± 0.28
BL				
0 N	11.46 ± 0.47	0.12 ± 0.02	2.57 ± 0.68	1.87 ± 0.20
5mM N	8.23 ± 1.08	0.05 ± 0.01	0.34 ± 0.08	1.85 ± 0.00
20mM N	9.19 ± 0.96	0.11 ± 0.01	0.24 ± 0.04	3.79 ± 0.15
ELB				
0 N	11.71 ± 0.30	0.13 ± 0.02	3.18 ± 0.57	1.71 ± 0.02
5mM N	8.79 ± 1.33	0.12 ± 0.01	0.57 ± 0.08	1.84 ± 0.05
20mM N	10.07 ± 1.08	0.14 ± 0.03	0.21 ± 0.03	4.37 ± 0.72

Table 2. Mean height (1), number of tillers (2), C:N ratio (3), and R:S ratio (4) for *Elymus* (E) and *Bromus* (B) plants at harvest under the different N and competitor treatments. Single target species columns refer to monoculture and target species mix refers to *Elymus* and *Bromus* grown in competition under the different N treatments and in the presence of *Lupinus* (+L). Values are mean $\pm$ SE. Different letters indicate significant differences across N, target species, and competitor ($P < 0.05$). Shaded cells represent the three species mixtures. Data for these pots were analyzed separately due to differences in total pot density.

	Single Target Species		Target Species Mix	
	(E)	(B)	(E)	(B)
(1) Height (cm)				
0 N	21.38 $\pm$ 0.93 ^{efg}	13.91 $\pm$ 0.68 ⁱ	17.43 $\pm$ 1.07 ^{ghi}	14.07 $\pm$ 0.47 ^{hi}
5 N	29.30 $\pm$ 0.88 ^{bcd}	18.80 $\pm$ 0.79 ^{gh}	23.21 $\pm$ 0.76 ^{efg}	19.36 $\pm$ 0.71 ^{fghi}
20 N	33.05 $\pm$ 1.03 ^b	22.16 $\pm$ 0.50 ^{efg}	26.93 $\pm$ 2.41 ^{cde}	21.93 $\pm$ 1.74 ^{efg}
0 N + L	25.96 $\pm$ 0.41 ^{de}	15.00 $\pm$ 1.17 ^{hi}	23.57 $\pm$ 2.76 ^{ABC}	16.43 $\pm$ 2.21 ^C
5 N + L	32.00 $\pm$ 1.74 ^{bc}	19.58 $\pm$ 0.57 ^{fgh}	25.86 $\pm$ 0.55 ^{AB}	20.29 $\pm$ 0.84 ^{BC}
20 N + L	39.81 $\pm$ 0.81 ^a	24.12 $\pm$ 0.73 ^{ef}	29.86 $\pm$ 1.96 ^A	23.86 $\pm$ 1.43 ^{ABC}
(2) Tiller Number				
0 N	5.41 $\pm$ 0.40 ^{hi}	8.88 $\pm$ 0.41 ^{gh}	3.57 $\pm$ 0.66 ⁱ	9.50 $\pm$ 0.73 ^{fgh}
5 N	11.48 $\pm$ 0.61 ^{efg}	16.45 $\pm$ 0.79 ^{cd}	5.50 $\pm$ 0.53 ^{hi}	22.93 $\pm$ 0.81 ^b
20 N	11.23 $\pm$ 0.63 ^{efg}	16.54 $\pm$ 0.49 ^{cd}	5.86 $\pm$ 0.91 ^{hi}	20.71 $\pm$ 2.43 ^{bc}
0 N + L	6.96 $\pm$ 0.41 ^{hi}	12.85 $\pm$ 0.82 ^{defg}	5.29 $\pm$ 0.87 ^D	19.57 $\pm$ 2.89 ^B
5 N + L	15.15 $\pm$ 0.79 ^{de}	28.15 $\pm$ 1.89 ^a	9.00 $\pm$ 1.02 ^{CD}	41.14 $\pm$ 7.47 ^A
20 N + L	14.81 $\pm$ 0.81 ^{def}	23.88 $\pm$ 1.32 ^{ab}	11.00 $\pm$ 1.53 ^{BC}	43.86 $\pm$ 5.23 ^A
(3) C:N ratio				
0 N	34.96 $\pm$ 0.99 ^{ab}	37.15 $\pm$ 0.96 ^{ab}	40.92 $\pm$ 0.82 ^a	36.39 $\pm$ 1.59 ^{ab}
5 N	18.89 $\pm$ 0.46 ^d	20.08 $\pm$ 0.42 ^d	25.49 $\pm$ 0.21 ^c	19.24 $\pm$ 0.45 ^d
20 N	12.56 $\pm$ 0.20 ^e	9.27 $\pm$ 0.23 ^f	13.64 $\pm$ 0.30 ^e	8.43 $\pm$ 0.14 ^f
0 N + L	32.85 $\pm$ 1.08 ^b	37.81 $\pm$ 1.18 ^{ab}	42.32 $\pm$ 1.77 ^A	33.71 $\pm$ 1.01 ^B
5 N + L	18.63 $\pm$ 0.43 ^d	19.77 $\pm$ 0.57 ^d	24.85 $\pm$ 1.40 ^C	17.72 $\pm$ 0.55 ^D
20 N + L	12.59 $\pm$ 0.28 ^e	9.95 $\pm$ 0.92 ^f	13.63 $\pm$ 0.71 ^E	8.58 $\pm$ 0.28 ^F
(4) R:S ratio				
0 N	61.83 $\pm$ 6.67 ^b	106.69 $\pm$ 8.70 ^a		
5 N	19.05 $\pm$ 1.24 ^d	39.63 $\pm$ 3.53 ^c		
20 N	10.39 $\pm$ 0.72 ^e	22.94 $\pm$ 1.59 ^d		

Table 3. ANOVA results for aboveground biomass (A and B), tissue N concentration (C and D), and N content (E and F). Fixed effects were N level (0, 5, 20 mM N), target species (*Elymus* or *Bromus*) and competitor (*Elymus*, *Bromus*, or *Lupinus*). Block was treated as a random effect. Shaded cells represent the three species mixtures (ELB). Data for these pots were analyzed separately due to differences in total pot density with only N and target species as fixed effects.

Response Variable	df	F	P
(A) Biomass			
Nitrogen (N)	2,185	523.33	<.0001
Target (T)	1,185	229.95	<.0001
Competitor (C)	2,185	318.60	<.0001
N x T	2,185	7.43	0.0008
N x C	4,185	13.28	<.0001
T x C	2,185	33.99	<.0001
N x T x C	4,185	5.76	0.0002
(B) Biomass (ELB)			
Nitrogen (N)	2,35	40.94	<.0001
Target (T)	1,35	227.14	<.0001
N x T	2,25	5.89	0.0081
(C) N Concentration			
Nitrogen (N)	2,184	2013.58	<.0001
Target (T)	1,184	24.79	<.0001
Competitor (C)	2,184	16.02	<.0001
N x T	2,184	48.47	<.0001
N x C	4,184	3.01	0.0194
T x C	2,184	12.94	<.0001
N x T x C	4,184	1.94	0.1055
(D) N Concentration (ELB)			
Nitrogen (N)	2,27	647.45	<.0001
Target (T)	1,27	86.27	<.0001
N x T	2,27	5.54	0.010
(E) N Content			
Nitrogen (N)	2,184	1817.82	<.0001
Target (T)	1,184	265.37	<.0001
Competitor (C)	2,184	305.10	<.0001
N x T	2,184	21.57	<.0001
N x C	4,184	4.52	0.0017
T x C	2,184	63.31	<.0001
N x T x C	4,184	4.82	0.001
(F) N Content (ELB)			
Nitrogen (N)	2,27	116.18	<.0001
Target (T)	1,27	230.26	<.0001
N x T	2,27	5.36	0.011

Figure Legends

Figure 1. Total extractable inorganic soil nitrogen ($\text{NH}_4\text{-N} + \text{NO}_3\text{-N}$) for each species combination under 0, 5, or 20 mM N addition. Horizontal line (level of blank) indicates amount of extractable N in soil with no fertilizer added (0N) and in the absence of plants. For species combinations, B= *Bromus*, E = *Elymus*, and L = *Lupinus*, BL = *Bromus* + *Lupinus*, EL = *Elymus* + *Lupinus*, EB= *Elymus* + *Bromus*, ELB= *Elymus* + *Lupinus* + *Bromus*. N = 14. Values are mean $\pm$ SE for total inorganic nitrogen ($\text{NH}_4\text{-N} + \text{NO}_3\text{-N}$). Different letters indicate significant differences across all species combinations and nitrogen treatments ($P < 0.05$).

Figure 2. Mean aboveground biomass for *Bromus* and *Elymus* plants in monoculture, two species mixtures, and 3 species mixture under 0, 5, or 20 mM N. Due to differences in pot density, data in panel (a) and (b) were analyzed separately. Different letters in panel (a) indicate significant differences at $P < 0.05$ between target species and across all competitor and N treatments. Different letters in panel (b) indicate significant differences at $P < 0.05$ between target species and across N treatment. Values are mean $\pm$ SE. N = 7 (EB or ELB) or 14 (BB, EE, EL, BL).

Figure 3. Mean percent increase or decrease in aboveground biomass per plant over monoculture values for *Bromus* and *Elymus* when grown with *Lupinus*, in the two target species mixture, or in mixture plus *Lupinus* under 0, 5, or 20 mM N. Values are mean $\pm$ SE. N = 7 (EB or ELB) or 14 (BB, EE, EL, BL).

Figure 4. Mean N concentration and content of *Bromus* and *Elymus* plants in monoculture, two species mixtures, and 3 species mixture under 0, 5, or 20 mM N. Due to differences in pot density, data in panel (a) and (b) were analyzed separately for both N concentration and content. Different letters in panel (a) indicate significant differences at $p < 0.05$ across target species, competitor, and N treatment. Different letters in panel (b) indicate significant differences at $p < 0.05$ across target species and N treatment. Values are mean $\pm$ SE. N = 7 (EB or ELB) or 14 (BB, EE, EL, BL).

Figure 1.

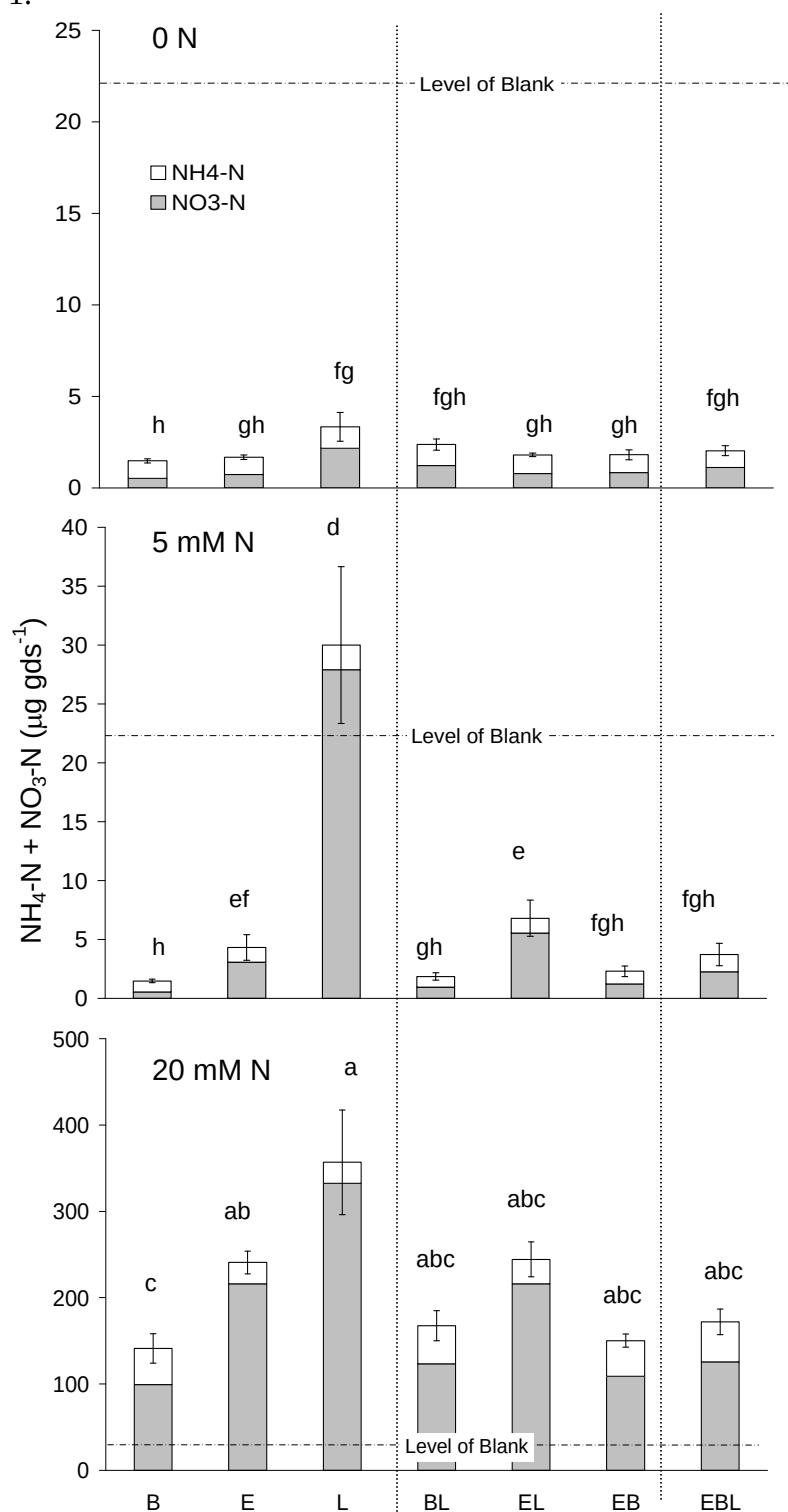


Figure 2.

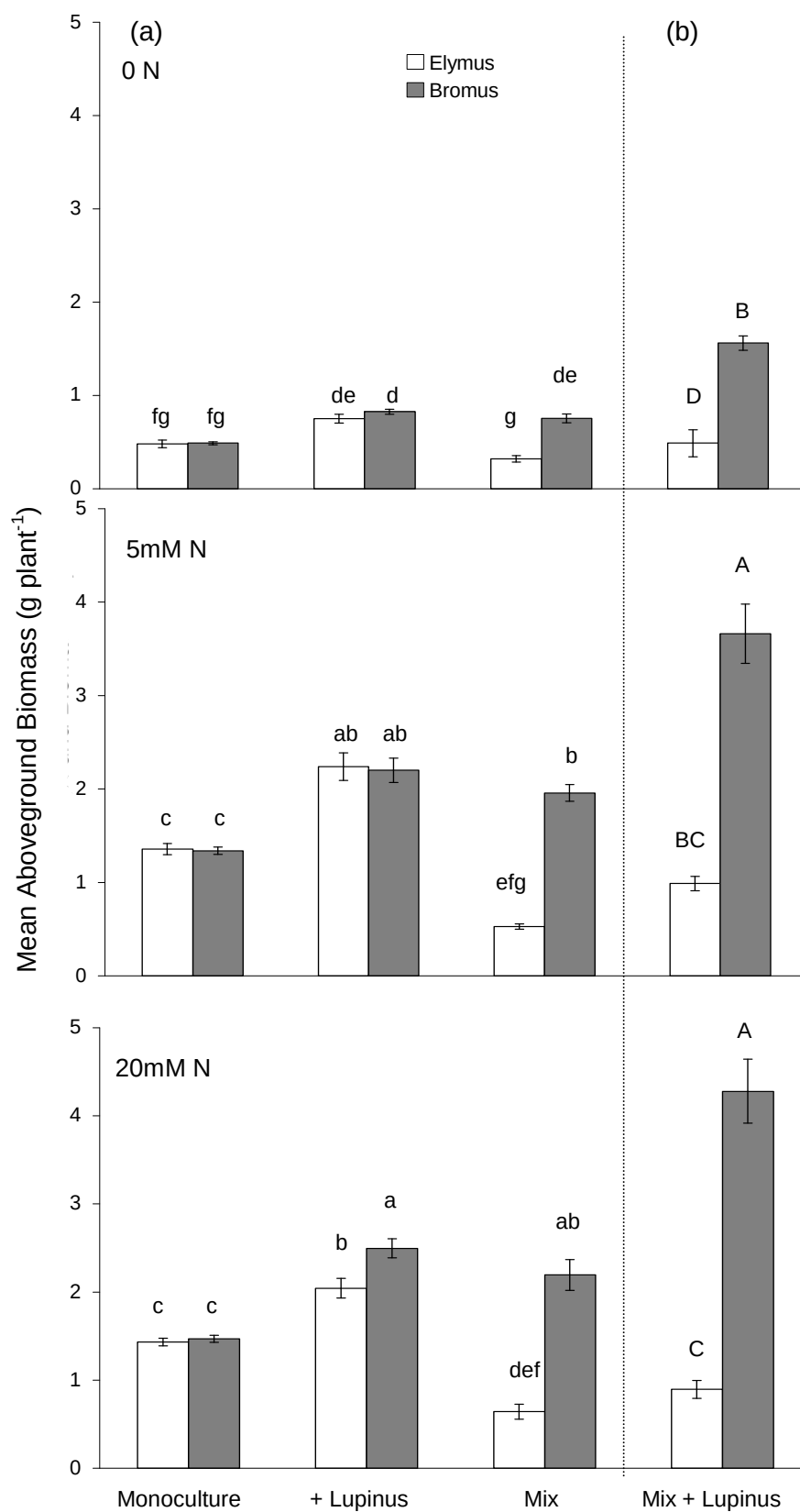


Figure 3.

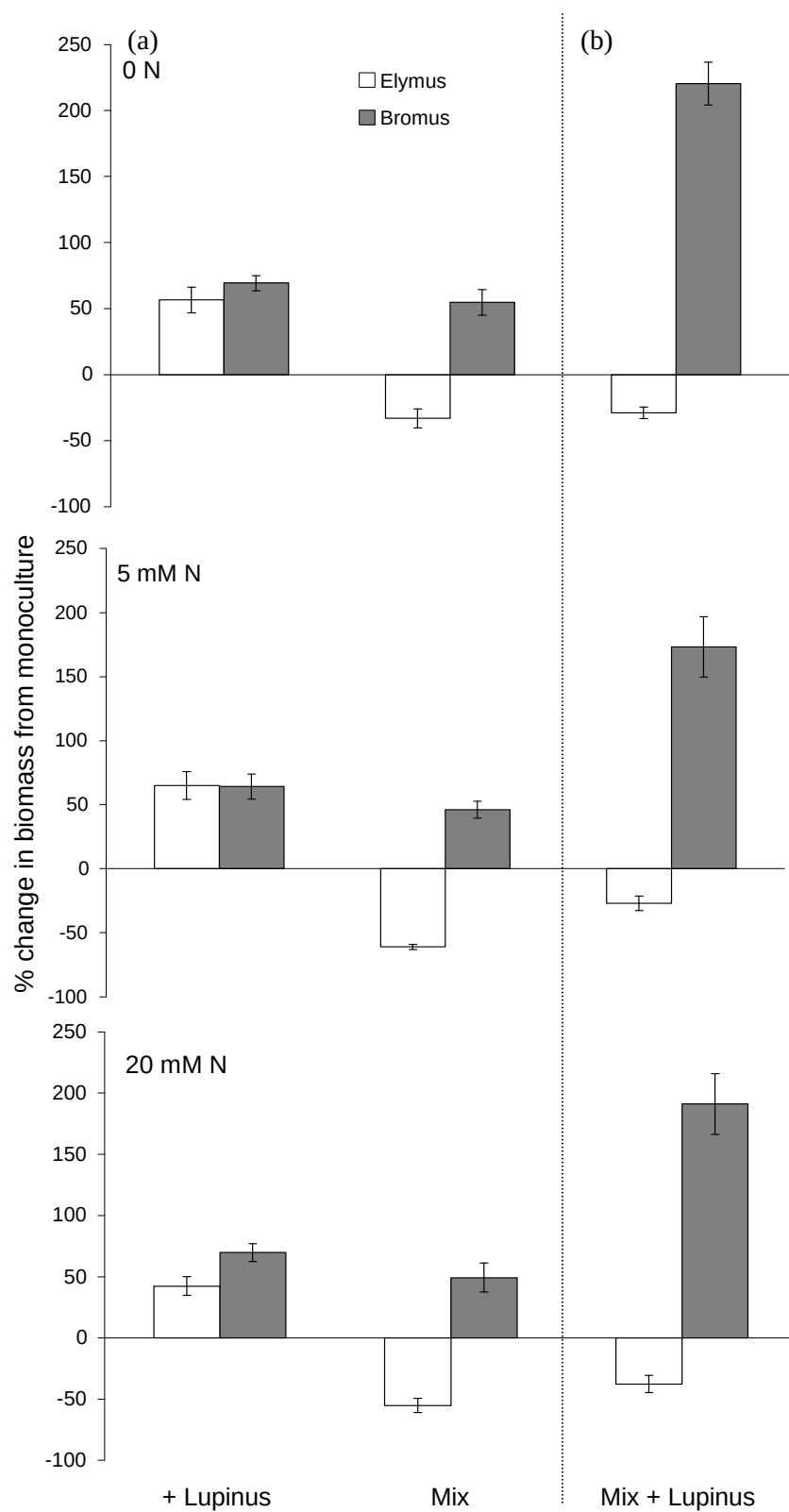
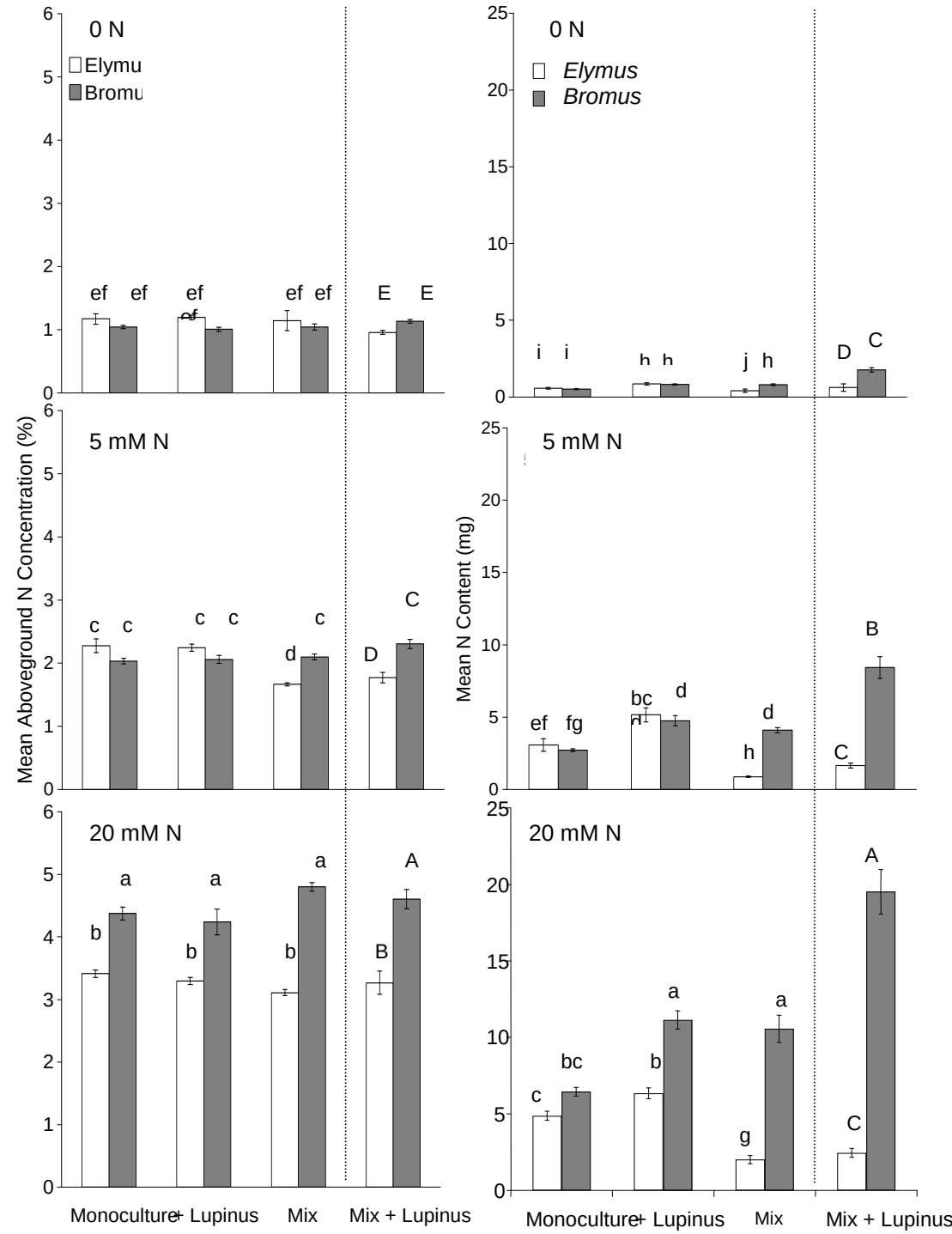


Figure 4.



Role of a Native Legume in Facilitating Native vs. Invasive Species in Sagebrush Steppe
Before and After Fire

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Summary

Interactions among species can have multiple consequences for communities, especially when non-native invasive species are involved. We examined facilitation of seedling establishment of native vs. invasive species by a native legume, *Lupinus argenteus*, in unburned and burned sagebrush steppe. We chose six treatments to identify specific mechanisms by which *L. argenteus* potentially influences establishment and community composition: 1) live lupine; 2) dead lupine; 3) no lupine; 4) no lupine with lupine litter; 5) no lupine with inert litter; and 6) mock lupine. We examined burn and treatment effects on environmental variables (soil nutrient availability, soil moisture, soil temperature, and light), overall community composition, and seedling establishment of the native perennial grass *Elymus multisetus*, native perennial forb, *Eriogonum umbellatum*, and non-native invasive annual grass, *Bromus tectorum*. In both unburned and burned communities, temperature fluctuations and light levels were highest in no lupine plots and least in mock lupine and live lupine plots. Soil N was highest in the burned community, but decreased over the growing season and was highest in lupine litter plots at the start of the growing season in both communities. In unburned communities, emergence of seeded species was unaffected by treatment. Increased N availability from lupine litter increased *B. tectorum* survival, biomass and seed number. Survival of native species was unaffected by lupine treatment, but height of *E. multisetus* was higher in both lupine litter and fake lupine treatments. In burned communities litter treatments reduced emergence of *E. umbellatum* and increased emergence of *E. multisetus*, but treatment still had no effect on emergence of *B. tectorum*. Similarly, reduced light and temperature fluctuation in litter treatments increased *E. multisetus*

survival and height. Plants of all species were larger in burned communities, but higher N levels likely masked treatment differences. *B. tectorum* had higher emergence and survival than either native species regardless of treatment or community type.

Modification of the resource environment by native legumes can increase plant establishment and growth of both native and non-native species, but higher establishment and growth rates give the non-native, *B. tectorum*, a greater advantage. Fire can mask the effects of N augmentation by legumes and modification of the physical environment can become relatively more important than N addition.

Key words: *Bromus tectorum*, *Elymus multisetus*, *Eriogonum umbellatum*, invasion, sagebrush steppe, seedling establishment

Introduction

Organization within plant communities is a function of the combined positive (i.e. facilitation) and negative (i.e. competition) interactions among species (Lortie et al. 2004, Brooker et al. 2008). The type of interaction that dominates is particularly important for seedling establishment, which is limited not only by availability of propagules, but also by availability of safe sites and resources (i.e. light, water, nutrients) (Harper 1977). In arid and semi-arid ecosystems, resources are spatially and temporally heterogeneous (Jackson and Caldwell 1993, Schlesinger and Pilmanis 1998) and can lead to differential recruitment over time. Interactions among native and non-native seedlings also can lead to compositional shifts in vegetation (Tilman 1997, Mack et al. 2000), but in these harsh, low nutrient environments, seedling establishment of both native (Callaway 1995, Pugnaire and Haas 1996, Moro et al. 1997) and non-native species (Lenz and Facelli 2003, Cavieres et al. 2005) can be facilitated by existing vegetation. Facilitation may be direct by improving harsh environmental conditions, modifying the substrate, or elevating resource availability, or indirect via reduced competition, introduction of beneficial soil microorganisms, or protection from herbivores (Callaway 1995). The mechanism of facilitation and degree of benefit received by the facilitated plant depends upon species life histories and ecophysiological characteristics (Brooker et al. 2008), and may differ significantly for native versus invasive species despite similarities among species.

Numerous examples of facilitation among native species exist for natural communities in arid and semi-arid ecosystems (Valiente-Banuet and Ezcurra 1991, Chambers 2001, Flores and Jurado 2003, Pugnaire et al. 2004, Maestre and Cortina 2004, Tirado and Pugnaire 2005). The majority of these examples of facilitation indicate that

trees and shrubs are most often the benefactors, but other perennial vegetation also can facilitate seedling establishment by ameliorating harsh environmental conditions (Fowler 1988, Franco and Nobel 1988, Greenlee and Callaway 1996). Herbaceous, perennial nitrogen-fixing legumes are often abundant in arid and semi-arid ecosystems and can play an important role in altering resource conditions (e.g. Maron and Connors 1996, Wood and del Moral 1987, Morris and Wood 1989). Legumes can facilitate seedling establishment by modifying microsite conditions (via moisture, light, and temperature regulation) or fostering different microbial communities; however, most studies suggest that facilitation is due to elevation of available soil N (Maron and Connors 1996, Maron and Jefferies 1999, Carino and Daehler 2002). The mechanisms for this increase are still largely unknown and could occur through a number of pathways. For example, N can be added through decomposition of nutrient rich tissue following death of the plant (Wardle and Greenfield 1991, Maron and Connors 1996), “leaking” of fixed N from live plant roots (Usleman et al. 1999, Spehn et al. 2002, Goergen et al.), or more rapid cycling and release of N (Vitousek and Walker 1989).

The balance between facilitation and competition can change whenever resource conditions are modified such as occurs over gradients of elevation (Callaway 1995, Choler et al. 2001), moisture (Maestre and Cortina 2004) or productivity (Goldberg et al. 1999). Fire is a dominant disturbance in arid and semi-arid systems that can drastically alter environmental conditions and influence both facilitative and competitive interactions. After fire available light and soil moisture increase due to reduced plant presence, and soil temperatures increases as a result of blackened soil surfaces (Keeley and Fotheringham 2000, Chambers and Linnerooth 2001, Paula and Pausas 2008). Fire

also results in short term increases in nutrient availability, especially N (Neary et al. 1999; Wan et al. 2001, Blank et al. 2007, Rau et al. 2007). Although the post-fire condition may favor seedling establishment of certain species, it also can result in harsher conditions that reduce seedling establishment of other species (Chambers and Linnerooth 2001, Williams et al. 2003). Species that establish soon after fire can modify the environment to make it more hospitable. Germination of many legumes is stimulated by the heat and chemical cues associated with fire (Martin et al. 1975; Bradstock and Auld 1995; Hendricks and Boring 1999; Williams et al. 2004), and abundance of legumes often increases after fire (Goergen and Chambers *in press*; Tracy and McNaughton 1997). In the post-fire environment, the functional role of legumes may differ from that of the pre-burned environment due to differences in resources limiting to establishment (Goergen and Chambers *in press*). Although it is widely recognized that the prevalence of facilitation versus inhibition often depends upon environmental conditions (Pugnaire and Luque 2001, Brooker et al. 2008), few studies have investigated the effects of fire on interactions among benefactors and seedlings or the mechanisms involved.

In sagebrush steppe the native perennial nitrogen-fixing legume *Lupinus argenteus* can make up a large component of the vegetation, especially after fire (Goergen and Chambers, *in press*). High tissue N concentrations and low C:N ratios indicate that *L. argenteus* can provide substantial amounts of N through rapid litter decomposition (Goergen et al. in prep, Metzger et al. 2006). Exudation of organic N into the rhizosphere by *L. argenteus* also indicates that this species can influence N availability while actively growing (Goergen et al. in prep). Prior work indicates that available soil N is elevated beneath *L. argenteus* (Kenny and Cuany 1990) even in

disturbed environments (Johnson and Rumbaugh 1986, Goergen and Chambers, *in press*). *L. argenteus* also has the potential to alter resource availability through modification of the microclimate beneath its canopy. Although legumes are known to facilitate establishment and invasion of seedlings in other systems (Morris and Wood 1989, Maron and Connors 1996), facilitation by legumes has not been examined in sagebrush steppe. The ability to persist in developed communities and positively respond to fire, combined with the capacity to modify the resource environment, indicate that *L. argenteus* may exert a substantial influence over plant recruitment in both burned and unburned sagebrush steppe.

L. argenteus can have both direct and indirect effects on the resource environment, and these effects likely differ in unburned and burned ecosystems. The ability of *L. argenteus* to modify resource availability and microsite conditions may promote recruitment of native species, but also may create an avenue for invasion. Modification of resource availability, especially N, is particularly important for promoting establishment and spread of non-native annual grasses (Huenneke et al. 1990, Levine et al. 2003, Brooks 2003, Young and Mangold 2008, James 2008 a, b). Sagebrush ecosystems are threatened by invasion of numerous non-native species, especially the annual grass *Bromus tectorum*. Early spring growth of *B. tectorum* combined with high growth rates can result in pre-emption of resources (James 2008 a, b, Knapp 1996). Rapid accumulation of biomass by *B. tectorum* can increase fine fuels and result in more frequent and larger fires (D'Antonio and Vitousek 1992, Knapp 1996). *B. tectorum* benefits from additional N (Monaco et al. 2003, Lowe et al. 2002), and increased nutrient availability after fire combined with additional N from legumes may increase both

establishment and population growth of *B. tectorum*. Perennial herbaceous plant species within the native community can increase the resilience of sagebrush ecosystems following fire, and increase resistance to invasion by *B. tectorum* (Booth et al. 2003, Chambers et al. 2007). Maintaining native diversity and minimizing impacts of *B. tectorum* invasion thus requires an understanding of how the presence of *L. argenteus* influences recruitment for native and non-native species in both unburned and burned communities.

The objective of this study was to determine the potential of a native legume, *L. argenteus*, to facilitate seedling establishment of *B. tectorum* versus native perennial herbaceous species in sagebrush steppe. A wildfire burned through the study area during the second year of the study, providing a unique opportunity to examine direct and indirect mechanisms of facilitation in unburned and burned sagebrush steppe. We evaluated whether facilitative mechanisms differed for *B. tectorum* versus native species with varying life forms (grass or forb) in both the unburned and burned communities. We addressed four questions: (1) How does *L. argenteus* modify the resource environment, and for N, what is the mechanism responsible for modification? (2) How does resource modification by *L. argenteus* influence community composition and diversity? (3) Does resource modification by *L. argenteus* facilitate seedling establishment? (4) Does facilitation by *L. argenteus* differ for native species versus non-native invasive species?

Methods

STUDY AREA

The study area is 25 km NW of Reno, Nevada, USA (39°40'N, 120°03'W, elevation ~1615 m) at the east face of the Sierra Nevada foothills (slope 2-3%). Study sites were located in 3 small watersheds on predominantly north-facing aspects. Soils are classified as well draining, very stony, sandy loam Xerollic Durargids of the Trosi series (Sketchley 1975). Mean temperatures range from -6°C in January to 31°C in July. Mean annual precipitation is 316 mm and arrives mostly in the form of snow or spring rains. Precipitation at the study area was below average for both years examined (Table 1, Western Regional Climate Center 2007). Ownership is divided among the BLM, US Forest Service, and California Department of Fish and Game. The area is grazed during summer, but our study sites were not grazed for one or more years prior to our study.

Vegetation is typical sagebrush steppe dominated by *Artemisia tridentata wyomingensis*. Dominant herbaceous species include the perennial grasses *Poa secunda* and *Elymus multisetus*, and the perennial forbs *Wyethia mollis*, *Balsamorhiza sagitata*, *Crepis acuminata*, and *Phlox speciosa*. The invasive, annual grass *B. tectorum*, is present but it is not a dominant component on study sites. *Lupinus argenteus* is the most abundant legume although other species, including *Astragalus* and *Trifolium* occur in the study area.

EXPERIMENTAL DESIGN

A manipulative field experiment was set up as a completely randomized replicated block design with 5 replicate plots for each of six treatments in three blocks in

both unburned (year 1) and burned (year 2). Treatments were chosen to identify mechanisms by which *L. argenteus* may influence seedling establishment and community composition and included: 1) live lupine (LL) to examine modification of nutrient and physical environment of the whole, live plant; 2) dead lupine with litter in place (DL) to examine modification of nutrient environment by decomposing plant; 3) no lupine (NL) had no environmental modification; 4) no lupine with lupine litter (LLT) to examine modification of the soil surface and nutrient environment by decomposition of leaf tissue; 5) no lupine with inert litter (FLT) to examine modification of the soil surface; and 6) mock lupine (ML) to examine modification of the physical environment (Table 2). Blocks were established in three small watersheds on sites with similar slope, elevation, aspect, soils, vegetation, and fire history. Two types of plots were established within each block: seeding plots to examine seedling establishment and community plots to examine community level effects. Each block contained 5 replicates of each treatment for community plots (n=30) and seeding plots for 2 years (n=30 per year) for a total of 90 plots per site.

All plots were located in spring 2006 and treatments were randomly assigned. Treatments were initiated in late summer 2006 for community and year 1 seeding plots, and in summer 2007 for year 2 seeding plots. In DL plots, all *L. argenteus* plants in plots and within 0.5 m of plots were treated with Round-up™ herbicide during June. *L. argenteus* litter and inert litter (fabric of similar color and reflective properties as *L. argenteus* tissue cut into 1' strips) were added to LLT and FLT plots in October to simulate natural litter fall and kept in place using 1" vinyl coated poultry netting and staples. The amount of tissue added was determined by obtaining the average dry mass of

L. argenteus plants contained within 10 randomly located 1m² quadrats. ML structures (artificial plants of similar size and shape staked in place with rebar) were placed in plots in the following spring to correspond with the growth of *L. argenteus* in the field.

Lupinus argenteus were absent from NL plots, and LL plots had an average *L. argenteus* cover of 25%.

A wildfire burned through the study area in July 2007, and all three replicate blocks were burned. The fire moved rapidly through the area consuming most standing vegetation. The fire made it impossible to collect a second year of data for the unburned condition, but allowed us to examine the same question, at the same location, under different ecological conditions - unburned (year 1) and burned (year 2). The dead lupine (DL) treatments had been initiated for the second growing season, but none of the other treatments had been implemented and the plots had not been seeded. Thus, the pre-selected plots were treated and seeded in fall of year 2 to examine the effect of lupine presence on seedling establishment and community composition following wildfire. Some community plots (ML, FLT and LLT) were not used in year 2 because of potential effects of melted plastic from treatments on soil properties and plant growth.

RESOURCE ENVIRONMENT

Treatment modification of the physical environment was assessed by measuring soil moisture, temperature, and light. For each of these variables, samples were collected from directly beneath the treatment structure. For example, soil or light interception was collected beneath a live *L. argenteus* plant, a ML structure, beneath litter, or adjacent to a dead *L. argenteus* plant to ensure a representative measure of the treatment effect.

Gravimetric soil water content was measured monthly over the growing season (30 March, 30 April, 24 May, and 11 June 2007 and 25 March, 22 April, 20 May, 17 June, and 19 August 2008). Soil samples were collected from 3 replicate plots per treatment per site ($n = 9$ per treatment) from the 0-10cm depth. Soil water content was determined based on wet and oven dried (100°C) weight. In addition, temperature buttons (1 button, Maxim, Sunnyvale CA) were buried at 5 cm within community plots (mid March 2007) or seeding plots (mid March 2008), and temperature was recorded every 2-4 hours over the growing season ($n=15$ per treatment in year 1 and $n=9$ in year 2). The percentage of photosynthetically active radiation (PAR) reaching the soil surface was recorded in both years during peak plant growth (mid-June) using a LiCor 250 (LICOR, Lincoln NE) within a subsample of each treatment ($n=9$ per treatment).

In order to assess the effect of *L. argenteus* treatments on soil nutrient availability, soil extractable inorganic N status was examined in each treatment (30 March and 11 June 2007 and 25 March, 17 June, and 19 August 2008). Additionally, soils were characterized for potential net N mineralization, and total carbon (C) and N at the end of the 2007 and 2008 field seasons. For all soil nutrient analyses, 3 soil samples were taken per treatment per site from the 0-10cm depth. Samples were air dried and sieved to remove particles >2 mm. Inorganic N ($\text{NH}_4^+ + \text{NO}_3^-$) levels were obtained by extraction with 2.0 M KCl and analyzed colorimetrically (Robertson et al. 1999, LACHAT Instruments, Milwaukee WI). Rates of potential net N mineralization were assessed with an aerobic lab incubation. A 10 g subsample of air dried, sieved soil was wet to 55% field capacity and incubated in the dark at 25°C for 30 days. Inorganic N ($\text{NH}_4^+ - \text{N} + \text{NO}_3^- - \text{N}$) levels after the 30-day incubation were obtained as above. Potential net N mineralization

was calculated as final minus initial concentration of extractable inorganic N ($\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N}$). To determine total soil C and N, a 0.25 g subsample was ground and combusted (Sollins et al. 1999, LECO TruSpec CN analyzer, St. Joseph MI).

Treatment and species effects on soil nutrients over the growing season (26 March to 15 June, 2007 and 25 March to 18 June, 2008) also were assessed using ion exchange resin stakes (PRSTM Probes, Western Ag Innovations). Results from the probes represent the cumulative availability of nutrients over the period of analysis and more closely reflect plant available nutrients than do extraction with KCl as adsorption of ions onto the probes depend upon field moisture and temperature. One pair of PRSTM probes was placed vertically in the soil (0-12 cm depth) within 3 replicates of each treatment per species per site. After removal, PRSTM probes were washed with deionized water, placed in plastic bags and sent for analysis to Western Ag Innovations (Saskatchewan, Canada). Macronutrients were determined colorimetrically (Hangs et al. 2004). Nutrient availability is reported as amount of nutrient adsorbed per amount of adsorbing surface area (i.e. μg nutrient per 10 cm^2) during the time period probes were in the soil (90 and 86 days).

There is little information on rates and timing of decomposition within cold deserts; however this is an integral component to understanding how *L. argenteus* contributes N to the environment. Aboveground tissue (leaves plus stems) of *L. argenteus* and *Crepis acuminata* (a non-leguminous comparison species) were collected in June 2006 from near each field site and 10 g placed in mesh litterbags. 40 litter bags per species were placed in interspace microsites along a transect in the field near each of the 3 sites in October 2006. Interspace microsites were used in order to determine rates of

decomposition and N release similar to that experienced by the seedlings. 5 litter bags of each species per site were collected in November 2006, February, and May 2007. Any remaining litterbags that survived the fire were collected in early August 2007. All samples were weighed to determine percent mass remaining. Percent carbon and nitrogen of ground tissue was assessed by combustion of a 0.15g subsample (LECO TruSpec CN analyzer, St. Joseph MI). Tissue decomposition is influenced by environmental conditions, thus in order to examine the contribution of N via decomposition of *L. argenteus* litter in a burned environment, an additional 24 litterbags of *L. argenteus* were placed near each site in October 2007. Due to the unexpected nature of the fire, tissue of a non-legume was unavailable for comparison. 6 litterbags were collected in March, April, May, and June 2008 and analyzed as above.

COMMUNITY RESPONSE

L. argenteus can influence seedling establishment indirectly via effects on existing vegetation. Community plots (1 m²) were used to examine treatment effects on plant composition. Ocular estimates of aerial cover for all species present were made to the nearest percent. To minimize variation due to the individual observer, observations were conducted by the same individual at each census. In addition, density of each species and functional diversity within each plot was assessed. Variables were quantified before treatment application in mid June 2006 and again after year 1 (mid June 2007) and year 2 (mid June 2008). In year 2, only LL, DL, and NL plots were re-surveyed due to loss of ML, LLT, and FLT plots to fire.

Lupinus argenteus also may indirectly affect plant composition, and thus seedling

establishment, by fostering different microbial communities. Therefore, soil samples were collected in mid June 2007 and 2008 during peak plant production and a community level substrate utilization approach was used to examine differences in microbial communities relating to treatment (Ecoplates, Biolog, Inc.). The plates contain 3 replicates of 31 different carbon sources that can provide information on the composition of the microbial community. Differences in number of positive responses and in level of reaction to the different carbon sources indicates differences among treatments (Garland and Mills 1991, Garland 1996a,b). Three replicate soil samples per treatment were collected at each site (0-10cm depth, n=9 per treatment). In 2007 soil was collected from the community plots; however, due to loss of some community plots to fire, soil was collected adjacent to seeding plots in 2008. For LL, ML, DL, and NL treatments, soil samples were taken opposite from seed grids. For LLT and FLT treatments, extra plots were established for soil collection and were placed adjacent to seed grids. Soil was kept at 4° C for approximately 1 week until analysis. 5g of soil was blended in 50 ml of 0.85% saline, shaken for an hour, and allowed to settle. 100 µl of solution was pipetted to each well and plates were incubated in the dark at 25°C for 5 days. Plates were then scored for a positive response and each positive well was scored on a scale from 1 to ten to assess the relative level of response to each carbon source within treatments. Although this method does not provide quantitative data about microbial communities, it does provide a qualitative measure of differences among treatments.

SEEDLING ESTABLISHMENT

Native species with different life form and life history characteristics, a perennial grass and forb, and the invasive annual grass, *B. tectorum*, were chosen to evaluate treatment effects on seedling establishment. *Elymus multisetus*, a short-lived perennial grass, competes for resources similar to those used by *B. tectorum* (Booth et al. 2003) and can limit *B. tectorum* establishment and reproduction (Stevens 1997, Humphrey and Schupp 2004). *Eriogonum umbellatum* is a perennial forb with an intermediate life span. Seeds of *E. umbellatum*, *E. multisetus*, and *B. tectorum* (hereafter *Eriogonum*, *Elymus*, and *Bromus*) were obtained from a local Great Basin source (Comstock Seeds, Carson City, NV for *Eriogonum* and *Elymus*, field collection for *Bromus*). A standard tetrazolium viability test (Moore 1972) indicated that seeds of *Bromus* were 93% and 99% viable in 2006 and 2007, respectively whereas seeds of *Elymus* were 81% and 82% and *Eriogonum* seeds were 80% and 79% viable in 2006 and 2007, respectively. Seeding was conducted in September 2006 and 2007 to ensure that the seeds received wet-cold stratification over winter. Two seeding grids for each of the 3 species (n=6) were located within localized areas that contained the necessary treatment conditions (i.e. beneath a *L. argenteus*, in an interspace, etc.) for all grids. Each grid was seeded with 25 filled seeds of the appropriate species at a 4 cm spacing. Treatments were applied over each seeded grid.

Seedling emergence and survival were assessed monthly over the growing season (30 March, 30 April, 29 May, and 13 June 2007 and 25 March, 22 April, 20 May, and 17 June, and 19 August 2008). Individual seedlings were marked with toothpicks upon emergence and recorded as alive or dead at each census. Emergence was calculated as

cumulative number of seedlings observed in each growing season. Percent survival was evaluated as number of seedlings alive at the end of each growing season divided by the number that emerged. Seedlings of *Bromus* were harvested at maturity (mid-June), dried at 60° C for 48hr, sorted by vegetative versus reproductive tissue, and weighed. Biomass was ground and analyzed for percent total carbon and nitrogen (LECO TruSpec CN analyzer, St. Joseph MI). Biomass and tissue concentration data are presented as mean per plant based on final grid density. Plant N and C content were calculated by multiplying tissue concentration by plant weight. Unlike *Bromus*, the perennial species tend to remain green longer into the summer. Thus, biomass of perennial species was not collected, but height of *Elymus* and number of leaves of *Eriogonum* were recorded as an estimate of biomass in mid June 2007 and 2008 for comparison with *Bromus*. At the seedling stage, *Elymus* height is strongly correlated to biomass ($r^2=0.7$, Goergen unpublished data). In 2008 a final survey was conducted in August to assess perennial seedling survival. Due to the wildfire in July of 2007, we were unable to conduct an additional survey in that year.

DATA ANALYSIS

The study was analyzed as a completely randomized, replicated block design with six treatments. Due to differences in baseline condition among years (burned versus unburned) measured variables for each year were analyzed separately. Analyses of variance (ANOVA) were performed using SAS PROC MIXED procedures (SAS Institute 2002). All data were assessed to verify model assumptions of normality and equality of variance. For results with significant effects, mean comparisons were assessed using

Tukey adjusted least square means for multiple comparisons and considered significant at the 95% confidence level.

For resource environment data (soil moisture, extractable inorganic N, and temperature) we tested for the effect of sampling date (time) and treatment with repeated measures ANOVA. Treatment was treated as a fixed effect, block and treatment by block as random effects, and time as the repeated measure. For PAR we tested for an effect of treatment. For nutrient probe data, species was added as a fixed effect. Seedling emergence and survival were assessed with treatment and species as fixed factors and block and treatment by block as random factors. Due to differences in measurement of size among seeded species, each species was analyzed separately. Treatment effect on plant and microbial community composition was evaluated with Multi-response Permutation Procedure (MRPP) using PCord. MRPP is a non-parametric procedure for testing for differences among groups (McCune and Grace 2002). Distance was calculated using Euclidean distance and results are presented as an effect size (chance-corrected within-group agreement, A) and p-value. A p-value of 0.05 or less indicates a treatment effect as similarities among communities are greater than would be expected by chance.

Results

RESOURCE ENVIRONMENT

In both unburned (2007) and burned (2008) sagebrush steppe, soil moisture at 0-10 cm was influenced by pattern of precipitation and decreased over the growing season ($F_{3,143} = 770.86$, $p < 0.0001$ and $F_{4,192} = 173.75$, $p < 0.0001$ for unburned and burned respectively, Fig.1). In unburned sagebrush steppe, soil moisture rapidly decreased from

late March (19.79%) to late April (1.37%) when values were about 14 times lower. Soil moisture decreased again in late May (0.8%) but then increased following precipitation in mid June (3.5%). Differences in soil moisture were unaffected by treatment for all sampling dates. In burned sagebrush steppe, soil moisture also was highest in late March (11.8%) and decreased in late April (5.1%) but was similar in late May, mid June and mid August (1.1%). Difference among treatments only occurred in March resulting in a treatment by sampling date interaction ($F_{20,192} = 1.6$, $p = 0.056$) when LL plots had higher soil moisture than any other treatment ($p < 0.05$, Fig. 1).

Temperature and light reaching the soil surface were affected by treatment in both unburned and burned sagebrush steppe. In unburned communities, the maximum soil temperature increased over the growing season ($F_{4,284} = 1912.73$, $p < 0.0001$) and differed among treatments ($F_{5,74} = 4.75$, $p = 0.0008$). For all but the first month sampled (March), NL plots were warmest and LL plots were coolest (treatment x time; $F_{20,284} = 4.53$, $p < 0.0001$, Fig. 2). Minimum soil temperatures also increased over the growing season ($F_{4,284} = 1928.02$, $p < 0.0001$) but were unaffected by treatment (Fig. 2). As the season progressed, the difference between minimum and maximum temperatures increased for all treatments ($F_{4,284} = 4424.04$, $p < 0.0001$), but differences in temperature fluctuation among treatments varied by sampling month (treatment x time; $F_{20,284} = 3.28$, $p < 0.0001$). In general, NL plots experienced the greatest temperature fluctuation and LL plots the least (Fig. 2). Light level also differed among treatments in unburned steppe ($F_{5,9,6} = 205.12$, $p < 0.0001$), with NL plots having twice as much light reaching the soil surface as DL plots, and on average almost 4 times more than remaining treatments. The least amount of light occurred in ML plots (Table 3).

In burned steppe, both maximum and minimum temperatures also increased over the growing season ($F_{5,200} = 1108.87$, $p < 0.0001$ and $F_{5,200} = 3621.78$, $p < 0.0001$ for maximum and minimum temperature, respectively), with only maximum temperatures being influenced by treatment ($F_{5,38} = 5.76$, $p = 0.0005$). At the beginning of the growing season there were no differences in temperature among treatments. From April through August, ML plots had lower maximum temperatures than the other treatments (treatment x time; $F_{25,200} = 3.38$, $p < 0.0001$, Fig. 2). Differences in temperature fluctuations follow the same pattern as maximum temperatures, with ML plots having the least fluctuation except in March (treatment x time; $F_{25,200} = 3.2$, $p < 0.0001$). Treatment affected PAR interception ($F_{5,9.14} = 22.45$, $p < 0.0001$), and reflected differences in temperature fluctuations among treatments in burned steppe. NL and DL plots had the highest amount of light reaching the soil surface, whereas ML and LL had the least ($p < 0.05$, Table 3).

Total KCl extractable soil inorganic nitrogen in unburned sagebrush steppe decreased over the growing season ($F_{1,82} = 171.42$, $p < 0.0001$) and was influenced by treatment ($F_{5,82} = 3.01$, $p = 0.015$), although differences only occurred at the beginning of the growing season (Fig 3a). LLT plots had the greatest amount of total inorganic N ($\text{NH}_4 + \text{NO}_3$) while ML plots had the least (Fig 3a). NO_3 was the dominant form of N present, although the proportion of N as NH_4 increased in June likely in response to precipitation. There was no difference in treatment for potential net N mineralization, but the pattern resembled that of extractable N (Table 4). Similarly, there was no effect of treatment on soil C:N ratio (Table 4). Results from resin exchange probes, which are an estimate of plant available N over the duration of the growing season, indicate that neither treatment nor species affected amount of total N, P or K. However, similar to KCl

extractable soil N, LLT plots tended to have more total inorganic N. LL plots tended to have more P and DL plots more K (Fig. 4). Observed differences in soil nitrogen in unburned sagebrush steppe are likely due to tissue chemistry of *Lupinus*. In litter bags, *Lupinus* tissue had greater N concentration and lower C:N ratio, but the non-legume *Crepis* initially decomposed faster. However, after 8 months both species had lost approximately half of their tissue mass (data not shown). Over time, the C:N ratio of *Crepis* decreased (from 32 to 23) whereas the C:N ratio of *Lupinus* stayed at a constant, lower level (19) for the duration of the experiment.

In burned sagebrush steppe, total KCl extractable soil inorganic N differed among sampling times ($F_{2,96}=111.6$, $p<0.0001$, Fig 3b). Values were greatest in late March and then decreased by mid June before increasing again in mid August ($p < 0.05$). Treatment only affected amount of total inorganic nitrogen in late March (treatment x time; $F_{10,96}=1.98$ $p=0.044$) when NL and ML had the least N. NH_4 was the dominant form of N at the start of the growing season, but over time more N was present as NO_3 (Fig 3b). Potential net N mineralization and C:N of burned soil were unaffected by treatment (Table 4). Resin exchange probes indicated that neither treatment nor species affected cumulative amount of total N or P over the growing season. However, treatment did influence amount of K ($F_{5,47}=2.79$, $p=0.032$), with LLT plots having higher amounts than LL, ML, NL, and FLT plots (Fig. 4).

Decomposition of *Lupinus* tissue was slow in burned sagebrush steppe. After approximately 8 months there was only an average of 30% mass lost even though the C:N ratio of *Lupinus* tissue remained relatively constant at 15 over the growing season.

COMMUNITY RESPONSE

Before treatment initiation, *Lupinus* was the most dominant plant in the LL and DL plots (mean 22%). In 2007 and 2008, *Lupinus* remained the dominant species within in LL plots (mean 22% and 28% for unburned and burned, respectively) but was reduced to less than 1% in DL plots in both years. Thus, due to inherent differences in plots with and without lupines, percent cover of *Lupinus* was removed before analysis to identify differences attributable to species other than *Lupinus*. Before treatment initiation in 2006, plant cover was most similar between plots containing *Lupinus* (LL and DL) or among plots without *Lupinus* (ML, NL, FLT, LLT, Table 5, $A=0.058$, $p=0.0003$). However, by summer 2007 community composition was more alike within ML and LL plots than in any of the other treatments ($A=0.152$, $p<0.0001$, Table 5). In both years, the dominant plant in each plot was *Poa secunda*, but the next dominant plant varied by treatment (Table 6). *Elymus mutisetus* was second in dominance in ML, NL, and FLT plots while the introduced grass *Poa bulbosa* was dominant in LLT plots. The shrub *Artemisia tridentata* was dominant in DL plots and *Balsamorhiza sagittata* in LL plots. Mean cover of *Bromus* was greater in 2007 than in 2006 but did not differ among treatments.

Plant cover in burned sagebrush steppe was not influenced by treatment ($A=0.0178$, $p=0.071$, Table 5), although plant composition in DL and LL plots tended to be more similar than plant composition within NL plots. In the post-fire environment, *Poa secunda* remained the most dominant plant. *Elymus mutisetus* was second in dominance in NL plots, but the perennial *Balsamorhiza sagittata* was second in DL and LL plots (Table 6).

In both unburned and burned steppe, microbial communities, as assessed by carbon source utilization, were similar among treatments ($A=0.007$, $p=0.232$ and $A=0.0186$, $p=0.088$ for unburned and burned steppe, respectively). All treatments had a positive response for the majority of the different carbon substrates. In unburned steppe, treatments containing *Lupinus* tissue (LL, DL, and LLT treatments) had a positive response for all 31 different carbon sources, ML plots responded to 30 and FLT and NL plots responded to 29 of the carbon sources. Similarly, in burned steppe, LL and DL plots had a positive response for all 31 different carbon sources, ML, NL, and LLT plots responded to 30 and FLT plots responded to 29 of the carbon sources. In both unburned and burned sagebrush steppe, carbon sources that had the greatest response were the same among all treatments and included D-Mannitol, L-Asparagine, and D-Xylose (Table 7).

SEEDED SPECIES RESPONSE

In unburned sagebrush steppe, all species had greater than 80% emergence by the April sampling (Fig. 5), but emergence was not affected by treatment. However, there was a difference among the seeded species ($F_{2, 168}=160.42$, $p<0.0001$). Non-native *Bromus* consistently had higher emergence than either native species. Between native perennial species, emergence was greater in the grass, *Elymus*, than in the forb, *Eriogonum* (Fig. 5).

There also was a difference among the seeded species in survival ($F_{2, 168}=151.14$, $p<0.0001$, Fig. 6). *Bromus* had roughly twice the number of plants surviving until June compared to either native species (*Bromus* 83%, *Elymus* 50%, and *Eriogonum* 45%). Plant survival did not differ among native species. Survival of *Bromus* and *Eriogonum*

was dependent upon number of seedlings that emerged ($r^2 = 84.2\%$, $p < 0.001$ and $r^2 = 71\%$, $p < 0.001$ for *Bromus* and *Eriogonum*, respectively). However, emergence only partially explained survival in *Elymus* ($r^2 = 52\%$, $p < 0.001$). Survival also was affected by treatments ($F_{5, 83} = 2.4$, $p = 0.044$). The highest survival for *Bromus* was in LLT plots, while *Elymus* had higher survival in FLT, LL, and LLT plots (Fig. 7). In contrast, *Eriogonum* tended to have the highest survival in DL plots (Fig. 7).

Treatment also influenced height, number of leaves, or leaf biomass and seed number of seeded species within unburned steppe ($F_{5, 75} = 2.4$, $p = 0.048$, $F_{5, 82} = 10.1$, $p < 0.0001$, $F_{5, 80} = 3.11$, $p = 0.013$, $F_{5, 80} = 2.60$, $p = 0.032$ for *Eriogonum*, *Elymus*, *Bromus* leaves and *Bromus* seeds, respectively; Fig. 7). The native forb, *Eriogonum*, produced significantly more leaves in the ML treatment, while *Elymus* was tallest in the LLT and FLT treatments. Similar to *Elymus*, weight of *Bromus* plants and number of seeds were higher in the LLT plots than in the NL or ML plots (Fig. 8). The N concentration of *Bromus* tissue (seeds plus biomass) averaged $0.81 \pm 0.01\%$, but was not affected by treatment. However, treatment affected N content ($F_{5, 75.3} = 3.13$, $p = 0.013$) due to greater tissue and seed production in the LLT treatment (data not shown).

In burned sagebrush steppe, all species again had greater than 80% emergence by the April sampling (Fig. 5) and emergence differed among the seeded species ($F_{2, 166} = 116.33$, $p < 0.0001$). *Bromus* had higher emergence than either of the native species in all but DL and LL plots. Emergence was similar between the native species, but was higher in *Elymus* than *Eriogonum* in FLT and LLT treatments ($p < 0.05$). The differential effect of treatment on seedling emergence among species created a species by treatment interaction ($F_{10, 166} = 10.35$, $p < 0.0001$). Total emergence in both *Bromus* and *Elymus*

tended to be greater in LLT plots but differences were not significant. In contrast, emergence of *Eriogonum* was reduced in LLT and FLT plots (Fig 5).

Survival differed among seeded species in burned steppe ($F_{2, 166}=149.84$, $p<0.0001$, Fig. 6). At the June harvest, *Bromus* had more plants surviving than either of the native species (*Bromus* 86%, *Elymus* 66%, and *Eriogonum* 42%; Fig. 7), but this was highly dependent upon the number of seedlings that emerged ($r^2 = 92.4\%$, $p<0.001$). Although treatment did not increase survival for any species, they responded differently to treatments leading to a species by treatment effect ($F_{10, 166}=4.0$, $p<0.0001$, Fig 9). Although *Bromus* had consistently greater survival than *Eriogonum*, survival did not differ between the two grasses in FLT, LL, LLT, and ML plots (Fig 7). By August, survival of both *Elymus* and *Eriogonum* decreased by half (*Elymus* 34%, and *Eriogonum* 23%) and the number of seedlings that emerged only partially explained survival at the end of the growing season ($r^2 = 38\%$, $p<0.001$ $r^2 = 46\%$, $p<0.001$ for *Elymus* and *Eriogonum*, respectively).

For all seeded species, measures of plant growth were generally higher in the burned community. Higher variability occurred in the post burn environment, where the only effect of treatment on native plant growth was an increase in the height of *Elymus* seedlings ($F_{5, 81}=7.94$, $p<0.0001$, Fig. 7). *Elymus* was tallest in the FLT treatment, and although *Eriogonum* plants in the DL, ML, and NL plots tended to have more leaves differences were not significant. Biomass of *Bromus* did not differ among treatments but the number of seeds produced per plant was affected by treatment ($F_{5, 81}=2.73$, $p=0.025$ *Bromus* seeds), with plants in the LLT plots producing more seeds than in the LL plots (Fig. 7). The N concentration of *Bromus* seeds and leaf tissue averaged $0.66 \pm 0.02\%$ and

1.68± 0.02% respectively, but was unaffected by treatment. However, treatment affected N content ($F_{5, 76.3}=2.36$, $p=0.048$ and ($F_{5, 76.3}=2.77$, $p=0.024$) due to differences in tissue and seed production between LLT and LL treatments (data not shown).

Discussion

RESOURCE MODIFICATION BY *LUPINUS*

Lupinus modified several aspects of the resource environment in both unburned and burned communities, but these effects likely were influenced by growing season conditions (Table 8). Below average precipitation resulted in rapid depletion of soil water over the growing season in 2007 and 2008, potentially decreasing the ability to detect differences among treatments in both unburned and burned communities. However, in burned communities soil moisture was greatest in LL plots early in the season. Although facilitation in semi-arid environments frequently involves modification of soil water availability (Zhao et al. 2007, Holzapfel et al. 2006), decreases in solar radiation and temperature also can facilitate seedling establishment by lowering water stress due to high temperatures or low available soil water (Callaway 1995, Ludwig et al. 2001, Hastwell and Facelli 2003). We found that relative to NL plots, all other treatments decreased amount of PAR reaching the soil surface. Differences in soil temperature also reflected treatment effects on interception of solar radiation on unburned sites. NL plots had both the greatest maximum temperature and largest temperature fluctuations. In contrast, on burned sites, there was less correlation between amount of PAR reaching the soil surface and soil temperatures, likely due to increased soil heating from blackened

soil. In both unburned and burned sagebrush steppe, plots with shade creating structures (LL or ML) remained cooler and had lower temperature fluctuations.

In addition to influencing microenvironmental factors, *Lupinus* also modified nutrient availability in unburned steppe. Addition of *Lupinus* litter (LLT) increased amount of KCl extractable inorganic N in the soil, indicating that the main mechanism responsible for the legume associated N increase in the unburned sagebrush ecosystems (e.g., Goergen and Chambers *in press*) is decomposition of nitrogen rich tissue.

Decomposition via photodegradation and subsequent release of N can be rapid in arid and semi-arid environments (Facelli and Pickett 1991, Koukoura et al. 2003, Austin and Vivanco 2006). High tissue N concentrations and low C:N ratios, as compared to the non-legume, *Crepis acuminata*, indicate that *Lupinus* can contribute substantial amounts of N through litter decomposition as observed for other N-fixing species (Vitousek and Walker 1989). In a similar study, soil under dead *L. arboreus* had higher available ammonium (NH_4^+) (~3x) and NO_3^- (~5x) relative to soil with no lupines (Maron and Connors 1989). This suggests that our DL plots also should have high amounts of available nitrogen due to decomposition of dead tissues. Total KCl extractable N was higher early in the growing season for LLT plots, but we did not find more soil N in our DL than NL plots. *Lupinus* in the DL plots were killed the summer prior to sampling, and it is possible that any pulse in soil N due to belowground tissue decomposition may have been shorter-lived than anticipated.

Inorganic N as measured by KCl extraction was higher for burned steppe than unburned steppe for both the March and June sampling dates. Fire increases available nitrogen due to deposition of ash onto the soil surface, release of NH_4^+ from organic

matter, decomposition of below-ground biomass, and further oxidation of NH_4^+ to NO_3^- (Raison 1979; Hobbs and Schimel 1984; Covington et al. 1991). We found that treatment also influenced N availability early in the growing season with more KCl extractable inorganic N in LL, DL, and litter treatments than in ML or NL plots. In addition to N inputs from tissue turnover, *Lupinus* exudes substantial amounts of organic nitrogen into the rhizosphere, even under water stress (Goergen et al. in prep), and could contribute to higher amounts of N in the LL treatment. These results suggest that in the post-fire environment, N contribution from *Lupinus* is via both tissue decomposition and rhizodeposition, although decomposition may still be the more dominant mechanism. Late in the growing season FLT plots tended to have the highest KCl extractable inorganic N. At our sites, wind is an important factor influencing redistribution of nutrients post-fire. Eroded topsoil tended to accumulate in the FLT plots, likely resulting in concentration of N-rich soil in this treatment.

COMMUNITY COMPOSITION

Diversity within the microbial community is known to influence aboveground plant diversity (van der Heijden et al. 1998, 2008). In our study, the belowground community composition, as assessed by carbon source utilization, did not vary among treatments, although all plots that contained *Lupinus* tissue had microbial communities that utilized all of the carbon sources, possibly indicating greater diversity. Our results suggest that presence of specific plants also may influence microbial communities. Removal of specific functional groups in old-field plant communities affected various trophic levels of soil foodwebs – microbial diversity increased or decreased depending on

the functional group removed (Wardle et al. 1999). Further, plots containing *Lupinus* had higher level of reaction to most substrates suggesting greater population sizes. Higher microbial diversity and larger population sizes of microbes can lead to faster nutrient cycling and greater plant available nutrients.

Modification of the resource environment by *Lupinus* also affected aboveground aspects of the existing community. Even before initiating treatments, plots containing *Lupinus* (LL and DL) were more similar in species cover to one another than to plots that lacked *Lupinus*, which is likely due to similar abundances of the most dominant species, *Poa secunda*. However, within 1 year after starting treatments, similarities among plots changed. The closer resemblance of LL plots to that of ML suggests that effects of the physical environment were influential in shaping cover of species. However, after fire, plots that contained *Lupinus* (LL and DL) were once again more similar to one another than to plots that never contained *Lupinus*. The fluctuating resource hypothesis suggests that changes in invasion success are due to changes in the competitive intensity of the recipient vegetation (Davis and Pelsor 2001). The ability of *Lupinus* to influence composition of the existing vegetation suggests that *Lupinus* may indirectly influence plant establishment and invasion potential at our sites within the sagebrush steppe.

SEEDLING EMERGENCE, BIOMASS AND SURVIVAL

In unburned sagebrush steppe, *Lupinus* has the potential to facilitate both native and non-native species, although the variable affected (emergence, survival, or biomass) and mechanism differed among species (Table 8). Seedling emergence and survival in semi-arid ecosystems are highly dependent on soil microenvironmental characteristics

(Chambers 2000, Linnerooth and Chambers 2001, Chambers et al. 2007). We found that in unburned steppe, treatment did not affect emergence of any seeded species, even though treatment did modify both microclimatic variables and the soil surface (litter plots). Other studies indicate that emergence of *Bromus* and other species may be increased by plant canopies or litter if they result in more favorable water relations (Suding and Goldberg 1999, Newingham et al. 2007). However, in our experiment, litter treatments (FLT, LLT) tended to have the least amount of soil water. In our study, emergence was moderate for all species, with *Bromus* having the highest emergence (55%) followed by *Elymus* (45%) and *Eriogonum* (26%). A companion study conducted over 2 years of higher precipitation (Mazzola 2008), also found that *Bromus* had higher emergence than *Elymus*, but that herbaceous interspaces had lower emergence than bare interspaces (28-65% for *Bromus* versus 6-38% for *Elymus* in herbaceous and bare interspaces, respectively).

Although treatment did not influence emergence in unburned steppe, certain treatments did facilitate plant growth and survival of the seeded species. In our study, the mechanism underlying the facilitative effect on survival and plant growth also varied by species. Survival, biomass, and reproductive effort of *Bromus* were greatest in the LLT plots, presumably due to greater N availability in this treatment. Survival and seedling height of *Elymus* also were high in LLT and LL plots, but were similar in FLT plots indicating that modification of the soil surface and microclimate also were important facilitative mechanisms. Unlike the two grass species, the highest survival for the native forb *Eriogonum* was unaffected by treatment. In contrast, leaf number was greatest in the ML plot, indicating that reduced PAR, and likely reduced water and temperature stress,

contributed to greater plant growth in this species.

In burned sagebrush steppe, *Lupinus* treatments did not facilitate emergence, leaf biomass or survival in *Bromus*, but did affect the number of seeds per plant. Even though *Lupinus* did increase N availability at the beginning of the growing season, seed production was lower in the LL plots. This result suggests that competition from established *Lupinus* plants outweighed the benefit of increased soil N for *Bromus* seed production. Treatment also influenced emergence, survival and plant growth of natives. Emergence and height of *Elymus* were increased by presence of litter, but the same treatment decreased emergence and survival of *Eriogonum*. Combined over treatments, *Bromus* had the highest survival (86%) followed by *Elymus* (66%) and *Eriogonum* (42%). Survival results from our study are higher than those from a similar study investigating establishment of *Bromus* in burned steppe (50% survival, Chambers et al. 2007). However, seeded species responded differently to treatments leading to conditions where survival was not different between *Bromus* and *Elymus*. In all but the DL and NL plots, survival did not differ between these two species.

CONCLUSIONS

Overall, this study indicates that in sagebrush steppe the native legume *L. argenteus* can facilitate plant establishment and growth of both the native and non-native species studied by modification of the resource environment. At our sites within the sagebrush steppe, *Lupinus* modified temperature, availability of nutrients, and light. However, the role of *Lupinus* as a facilitator and the mechanisms involved differed for unburned and burned communities. In unburned communities, both the native and non-

native species were facilitated, but non-native *Bromus* received a greater benefit. Increased N availability from decomposing *Lupinus* tissue resulted in higher survival, plant size and seed production for *Bromus*. In contrast, in the burned community fire masked the effect of N augmentation, and modification of the physical environment became more important. Emergence, height, and survival of *Elymus* and seed production in *Bromus* were enhanced in the litter treatments. Survival was similar between *Elymus* and *Bromus* in the post-fire environment, but *Bromus* still had higher densities in most treatments. In addition, in both unburned and burned communities above and belowground community composition was influenced by *Lupinus* presence, suggesting that *Lupinus* may indirectly influence plant establishment and invasion by affecting the existing community. Understanding conditions that modify resource availability in nutrient limited systems thus has important implications for both understanding invasion by *B. tectorum* and for improving our ability to manage these sites to maintain or restore native diversity.

Acknowledgements

We thank K. Vicencio, E. Hoskins, S. Li, and T. Morgan for valuable assistance in the greenhouse and lab, D. Board for statistical assistance, and D. Johnson, J. Qualls, P. Weisberg, and P. Verburg for valuable comments on earlier drafts of this manuscript. Financial support was provided by the USDA Forest Service, Rocky Mountain Research Station.

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Table 1. Average precipitation for the period of record (22 years) from Stead, Nevada (Station ID 267820, 1560m elevation), approximately 10 miles south of the field sites, and recorded precipitation for 2007 and 2008 from rain gauges at field sites.

	Avg Precip (mm)	2007 Precip (mm)	2008 Precip (mm)
Nov-Mar	200.91	100.33	161.33
April	15.24	10.67	0.00
May	15.24	23.67	2.00
June	14.22	7.00	13.00
Annual	245.62	141.67	176.33

Table 2.
Experimental treatments and mechanism being tested by each treatment.

<u>Treatment</u>	<u>Mechanism tested</u>
No lupine (NL)	Control – no modification of either nutrient or physical environment
Live lupine (LL)	Modification of nutrient environment by whole, live plant, modification of physical environment (light, temp, moisture)
Dead Lupine (DL)	Modification of nutrient environment by decomposition of whole plant
Mock lupine (ML)	Modification of physical environment only (light, temp, moisture)
Lupine litter (LLT)	Modification of nutrient environment by decomposition of aboveground tissue only, modification at soil surface (light, temp, moisture)
Inert litter (FLT)	Modification of physical environment at soil surface (light, temp, moisture)

Table 3. Light levels reaching the soil surface in each treatment in both unburned and burned sagebrush steppe. Measurements were taken in mid June 2007 and 2008 within 2 hours of solar noon. Values are means $\pm$ SE, n= 9 per treatment. Unlike letters indicate significant differences among treatments ($p < 0.05$).

YEAR 1 - Unburned Steppe		YEAR 2 - Burned Steppe	
Treatment	PAR ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	Treatment	PAR ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)
NL	1621.04 $\pm$ 66 a	NL	1612.19 $\pm$ 82 a
LL	490.15 $\pm$ 41 c	LL	858.65 $\pm$ 59 b
DL	812.35 $\pm$ 68 b	DL	1698.18 $\pm$ 56 a
ML	253.46 $\pm$ 57 d	ML	183.60 $\pm$ 35 c
LLT	525.33 $\pm$ 33 c	LLT	734.74 $\pm$ 43 b
FLT	468.04 $\pm$ 38 c	FLT	497.51 $\pm$ 67 bc

Table 4. Potential net N mineralization and C:N ratio for soil from each treatment at the end of year 1 and year 2. Values are mean $\pm$ SE, n = 9.

Year 1 – Unburned Steppe			Year 2 – Burned Steppe		
Treatment	Potential Net N Mineralization ($\mu\text{g N gds}^{-1} \text{ d}^{-1}$)	C:N Ratio	Treatment	Potential Net N Mineralization ($\mu\text{g N gds}^{-1} \text{ d}^{-1}$)	C:N Ratio
NL	8.18 $\pm$ 0.88	10.59 $\pm$ 0.53	NL	0.72 $\pm$ 0.14	13.60 $\pm$ 0.27
LL	6.88 $\pm$ 1.30	10.79 $\pm$ 0.47	LL	0.82 $\pm$ 0.04	14.69 $\pm$ 0.51
DL	7.71 $\pm$ 0.92	11.24 $\pm$ 0.49	DL	0.86 $\pm$ 0.16	13.98 $\pm$ 0.41
ML	7.24 $\pm$ 1.25	10.06 $\pm$ 0.59	ML	0.78 $\pm$ 0.07	13.98 $\pm$ 0.16
LLT	8.69 $\pm$ 0.81	10.35 $\pm$ 0.48	LLT	0.85 $\pm$ 0.05	14.01 $\pm$ 0.34
FLT	6.84 $\pm$ 1.15	12.69 $\pm$ 1.80	FLT	0.83 $\pm$ 0.09	14.31 $\pm$ 0.36

Table 5. MRPP results for treatment differences among species composition in 2006 before treatment, 2007 unburned, and 2008 burned sagebrush steppe.

MRPP results 2006		MRPP results 2007		MRPP results 2008	
	Distance		Distance		Distance
Lupine	27.82	DL	31.01	DL	25.07
No Lupine	31.75	ML	24.25	LL	23.42
		LL	23.64	NL	28.37
		LLT	29.27		
		NL	30.10		
		FLT	27.18		

Table 6. Species with greater than 1% cover in at least one treatment in community plots for unburned steppe (2006 and 2007) and burned steppe (2008). *Poa secunda* was the dominant species in all treatments, but the next most abundant species is in bold. In 2008 only DL, LL, and NL treatments were resurveyed due to loss of other treatments to fire. N = 15 per treatment.

Species	2006						2007						2008					
	DL	ML	LL	LLT	NL	FLT	DL	ML	LL	LLT	NL	FLT	DL	ML	LL	LLT	NL	FLT
<i>Artemisia tridentata</i>	4.9	1.7	1.7	2.3	1.0	1.4	8.0	1.4	3.3	1.4	2.7	2.3	0.0		0.0		0.0	
<i>Aster spp.</i>	0.0	1.7	0.3	1.4	0.0	0.3	0.0	2.0	0.7	1.3	0.0	1.2	0.0		0.0		0.0	
<i>Balsamorhiza sagittata</i>	5.7	6.4	4.7	3.4	2.7	5.7	6.7	3.0	5.4	3.7	4.1	7.3	9.2		8.3		7.3	
<i>Bromus tectorum</i>	0.1	0.1	0.1	0.0	0.1	0.1	0.9	0.3	0.1	0.8	0.2	1.1	0.7		0.3		0.3	
<i>Chrysothamnus nauseosus</i>	0.0	1.0	0.4	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0		0.0	
<i>Chrysothamnus viscidiflorus</i>	2.4	0.1	3.0	1.4	0.4	0.7	2.1	2.3	5.0	1.7	1.0	0.0	0.9		4.7		1.9	
<i>Cordylanthus ramosus</i>	0.2	1.0	0.1	0.5	0.4	0.7	0.8	0.2	0.4	0.4	0.2	0.4	0.5		0.1		0.0	
<i>Crepis acuminata</i>	0.2	0.8	1.7	0.5	2.1	1.0	1.5	1.1	1.7	2.2	2.7	0.7	3.8		3.2		2.8	
<i>Elymus multisetus</i>	6.7	4.1	2.7	4.0	5.4	7.7	7.7	5.7	4.4	4.0	9.4	8.4	7.7		5.5		7.9	
<i>Erigeron bloomeri</i>	0.0	0.0	0.0	0.0	1.0	0.3	0.0	0.0	0.0	0.0	1.3	0.0	0.0		0.0		1.9	
<i>Eriogonum elatum</i>	0.0	0.0	0.0	0.0	1.0	0.3	0.0	0.0	0.0	0.0	0.7	0.0	0.0		0.0		0.0	
<i>Festuca idahoensis</i>	0.7	0.3	0.3	0.0	0.0	1.3	0.3	0.3	0.3	0.0	0.0	1.3	0.0		0.0		0.0	
<i>Phlox speciosa</i>	0.8	1.6	1.4	1.8	0.7	0.9	1.8	2.5	1.8	2.9	1.1	2.7	3.5		4.8		1.4	
<i>Poa bulbosa</i>	0.0	2.0	1.0	2.0	2.4	0.7	0.0	1.0	1.0	9.0	1.4	1.7	0.5		0.8		3.9	
<i>Poa secunda</i>	10.7	18.0	13.0	21.0	19.7	15.3	15.0	19.0	19.0	17.3	22.0	20.7	20.0		14.8		25.4	
<i>Wyethia mollis</i>	2.0	0.3	0.0	0.7	0.7	1.6	2.0	0.3	0.0	0.3	1.0	1.0	3.8		0.0		0.4	

Table 7. Carbon substrates with a reaction level of 7 or higher in at least one treatment in unburned and burned sagebrush steppe as scored after 5 day incubation. Soil was collected in mid June 2007 and 2008. Values are on a scale of 0 (no reaction) to 10 (high). N = 9 per treatment.

SUBSTRATE	YEAR 1 - Intact Steppe						YEAR 2 - Burned Steppe					
	NL	DL	LL	ML	LLT	FLT	NL	DL	LL	ML	LLT	FLT
D-Cellobiose	5.54	5.67	6.22	6.58	7.11	6.11	4.73	5.11	5.92	5.42	5.92	3.93
D-Galacturonic	7.76	7.67	7.7	7.3	7.56	6.52	7.70	7.22	7.67	7.83	7.13	7.33
D-Mannitol	9.13	9.29	8.81	8.96	9.22	9.04	7.30	7.19	7.50	7.50	7.54	6.89
D-Xylose	7.92	9.13	8	8.37	8.41	7.3	7.27	6.81	7.50	7.46	7.42	6.52
L-Arginine	6.77	7.17	6.96	7.22	6.94	6.56	5.20	5.41	6.00	6.08	5.79	5.15
L-Asparagine	9.33	9.46	9	8.78	9.48	8.52	7.87	8.07	8.04	8.25	8.00	7.85
L-Serine	7.17	7.29	7.07	7.11	7.26	7.15	6.43	6.89	7.58	7.04	7.17	6.44
N-Acetyl-												
DGlucosamine	5.79	7.71	7.42	8.15	7.59	6.37	4.80	5.30	6.04	6.08	6.83	4.85
Pyruvic Acid	6	7.04	6	6.59	7.15	6.33	6.70	6.59	6.71	6.79	7.00	6.00

Table 8. Summary table of effect of *L. argenteus* treatment on resource environment and seedling establishment. + indicates an increase, - a decrease, and o no difference relative to NL plots that had no modification of the physical or nutrient environment.

	DL	LL	ML	LLT	FLT
Year 1 Unburned					
Soil Moisture	o	o	o	o	o
Soil Temperature	o	-	-	o	o
PAR	-	-	-	-	-
Inorganic N	o	o	-	+	o
Emergence					
<i>Bromus</i>	o	o	o	o	o
<i>Elymus</i>	o	o	o	o	o
<i>Eriogonum</i>	o	o	o	o	o
Survival					
<i>Bromus</i>	o	o	o	+	o
<i>Elymus</i>	o	+	o	+	+
<i>Eriogonum</i>	o	o	o	o	o
Growth					
<i>Bromus</i>	o	o	o	+	o
<i>Elymus</i>	+	+	o	+	+
<i>Eriogonum</i>	o	o	+	o	o
Year 2 Burned					
Soil Moisture	o	+	o	o	o
Soil Temperature	o	-	-	o	o
PAR	o	-	-	-	-
Inorganic N	+	+	o	+	+
Emergence					
<i>Bromus</i>	o	o	o	o	o
<i>Elymus</i>	o	o	o	o	o
<i>Eriogonum</i>	o	o	o	-	-
Survival					
<i>Bromus</i>	o	o	o	o	o
<i>Elymus</i>	o	o	o	o	o
<i>Eriogonum</i>	o	o	o	-	-
Growth					
<i>Bromus</i>	o	o	o	o	o
<i>Elymus</i>	o	o	o	o	+
<i>Eriogonum</i>	o	o	o	o	o

Figure 1. Soil moisture for each treatment over the growing season in unburned and burned sagebrush steppe. Values are mean $\pm$ SE, $n = 9$ per treatment per sampling time. Asterisk indicates significant difference among treatments within sampling time ($p < 0.05$). Dead lupine (DL), fake lupine litter (FLT), live lupine (LL), lupine litter (LLT), mock lupine (ML) and no lupine (NL).

Figure 2. Maximum (top lines) and minimum (bottom lines) temperature in unburned and burned sagebrush steppe. Values are mean $\pm$ SE, $n = 15$ per treatment for year 1 and 9 per treatment for year 2. Dead lupine (DL), fake lupine litter (FLT), live lupine (LL), lupine litter (LLT), mock lupine (ML) and no lupine (NL).

Figure 3. KCl extractable soil inorganic N ($\text{NO}_3 + \text{NH}_4$) for each treatment in unburned and burned sagebrush steppe. Values are mean $\pm$ SE of total N, $n = 15$ per treatment per sampling time for year 1 and 9 per treatment per sampling time for year 2. Unlike letters indicate significant differences among treatments ($p < 0.05$). Dead lupine (DL), fake lupine litter (FLT), live lupine (LL), lupine litter (LLT), mock lupine (ML) and no lupine (NL).

Figure 4. Amounts of total N, potassium (K), and phosphorus (P) from resin exchange probes in unburned and burned sagebrush steppe. Values are treatment mean $\pm$ SE, $n = 27$ per treatment. Dead lupine (DL), fake lupine litter (FLT), live lupine (LL), lupine litter (LLT), mock lupine (ML) and no lupine (NL).

Figure 5. Cumulative seedling emergence over time in unburned and burned sagebrush steppe for *Eriogonum umbellatum*, *Elymus multisetus*, and *Bromus tectorum*. Values are mean $\pm$ SE, $n = 15$ per species per treatment. Dead lupine (DL), fake lupine litter (FLT), live lupine (LL), lupine litter (LLT), mock lupine (ML) and no lupine (NL).

Figure 6. Proportional seedling survival for *Eriogonum umbellatum*, *Elymus multisetus*, and *Bromus tectorum* under each treatment in unburned and burned sagebrush steppe (a) Values are mean $\pm$ SE and survival curves (b) for each species. $n = 15$ per species per treatment. Dead lupine (DL), fake lupine litter (FLT), live lupine (LL), lupine litter (LLT), mock lupine (ML) and no lupine (NL).

Figure 7. Proportional seedling survival for *Eriogonum umbellatum*, *Elymus multisetus*, and *Bromus tectorum* at each sampling date under each treatment in unburned and burned sagebrush steppe (a) Values are mean $\pm$ SE and survival curves (b) for each species. $n = 15$ per species per treatment. Dead lupine (DL), fake lupine litter (FLT), live lupine (LL), lupine litter (LLT), mock lupine (ML) and no lupine (NL).

Figure 8. Mean height (cm) for *E. multisetus* plants and mean number of leaves for *E. umbellatum* plants for each treatment in unburned and burned sagebrush steppe. Values are mean $\pm$ SE, $n = 15$ per species per treatment. Unlike letters indicate significant differences among treatments ($p < 0.05$). Dead lupine (DL), fake lupine litter (FLT), live lupine (LL), lupine litter (LLT), mock lupine (ML) and no lupine (NL).

Figure 9. Mean weight (g) of *B. tectorum* plants for each treatment in burned and unburned sagebrush steppe. Values are mean $\pm$ SE, n = 15 per species per treatment. Unlike letters indicate significant differences among treatments ($p < 0.05$). Dead lupine (DL), fake lupine litter (FLT), live lupine (LL), lupine litter (LLT), mock lupine (ML) and no lupine (NL).

Figure 1.

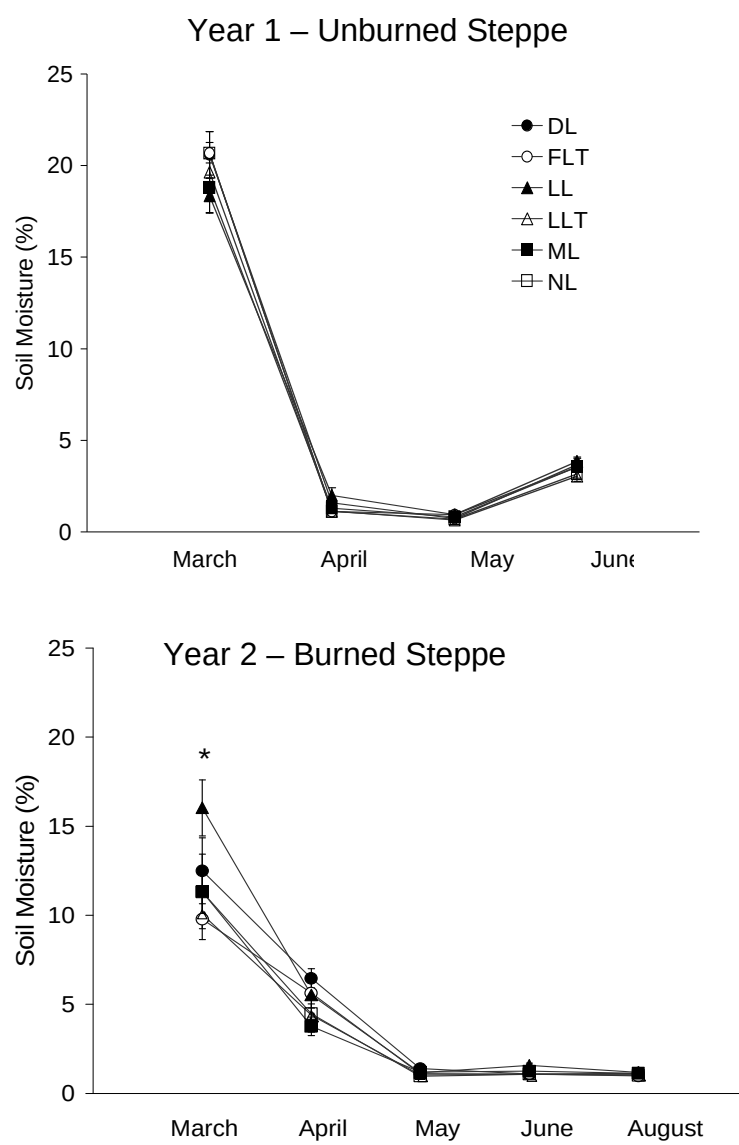


Figure 2.

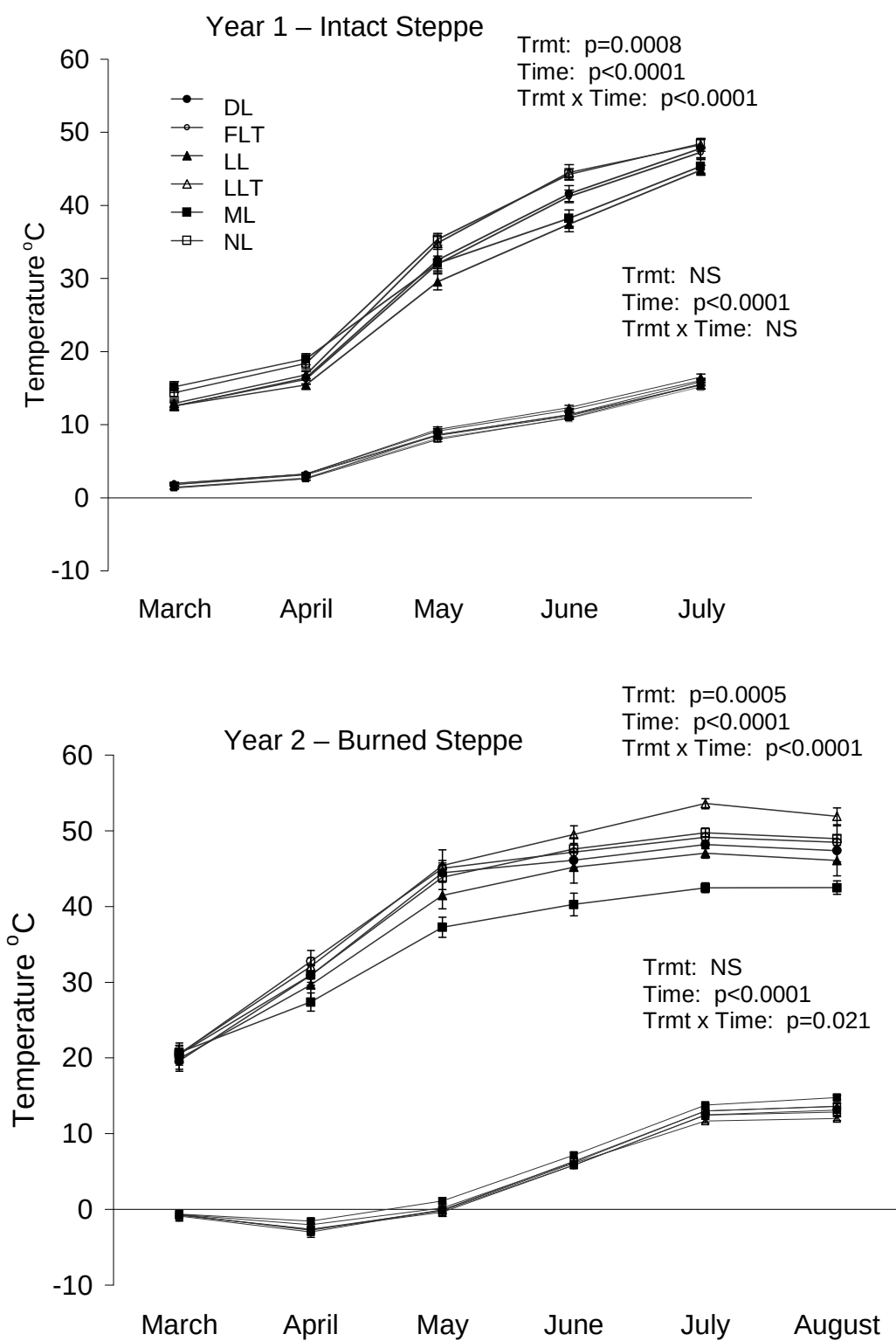


Figure 3.

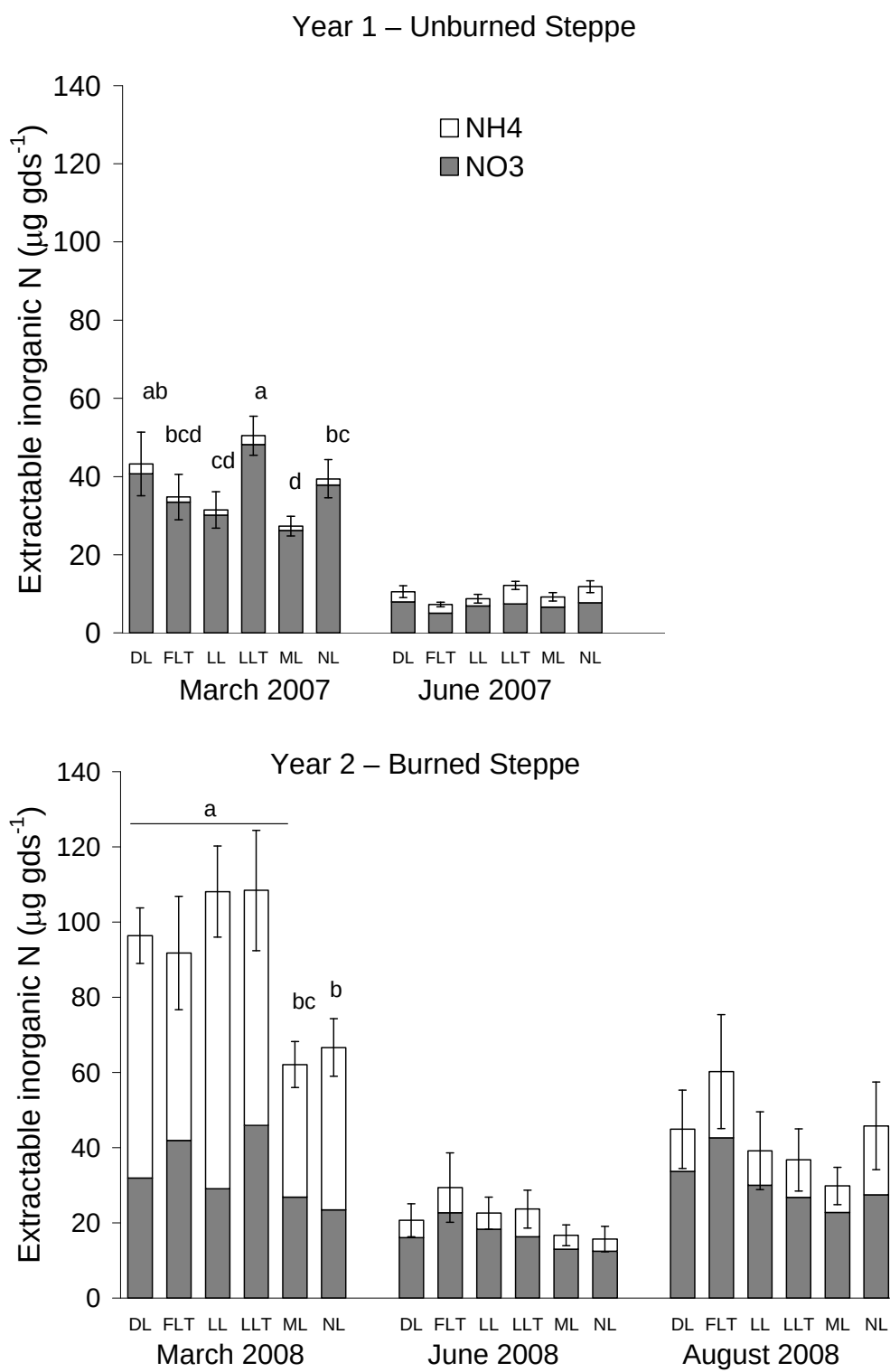


Figure 4.

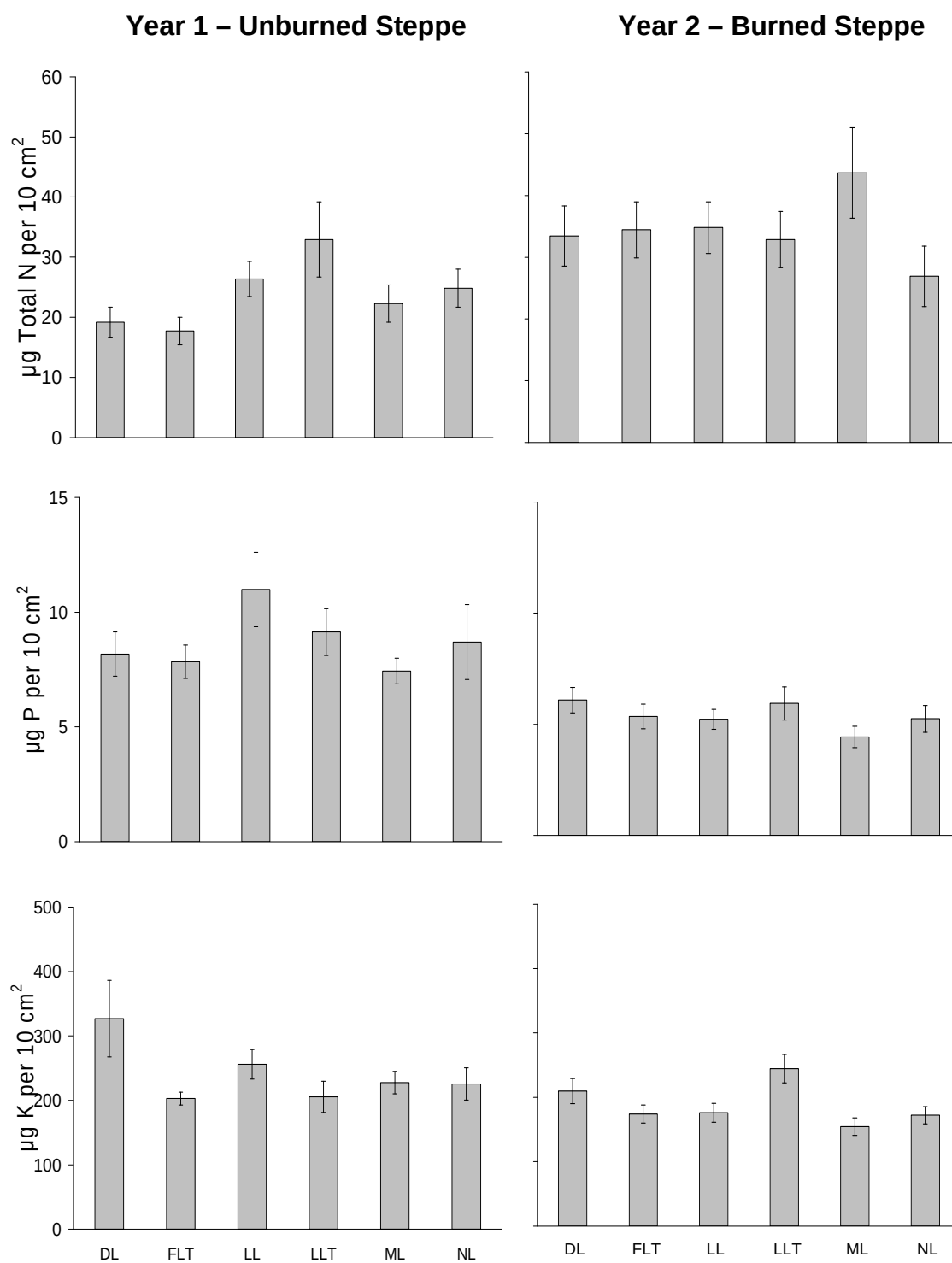


Figure 5.

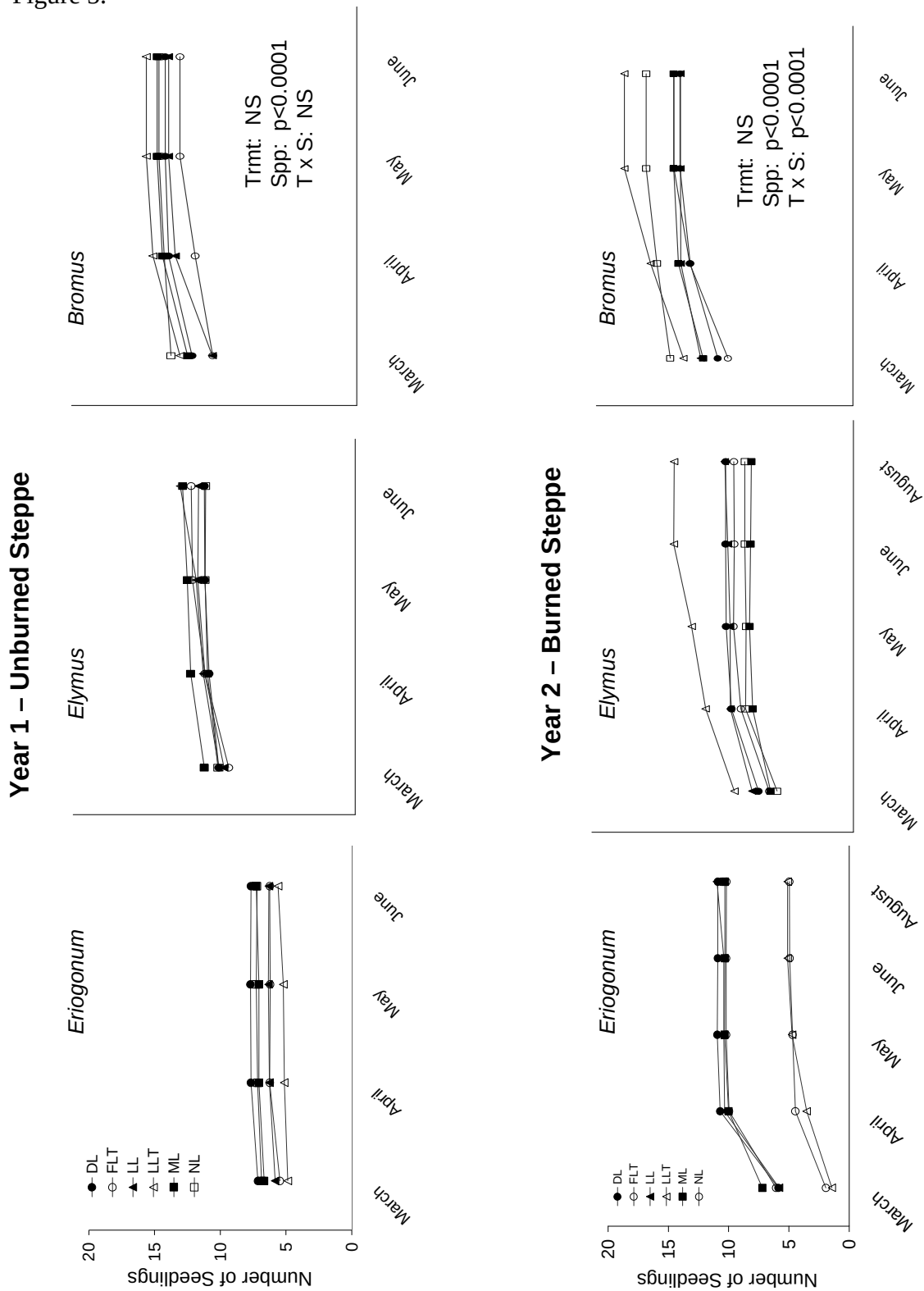


Figure 6.

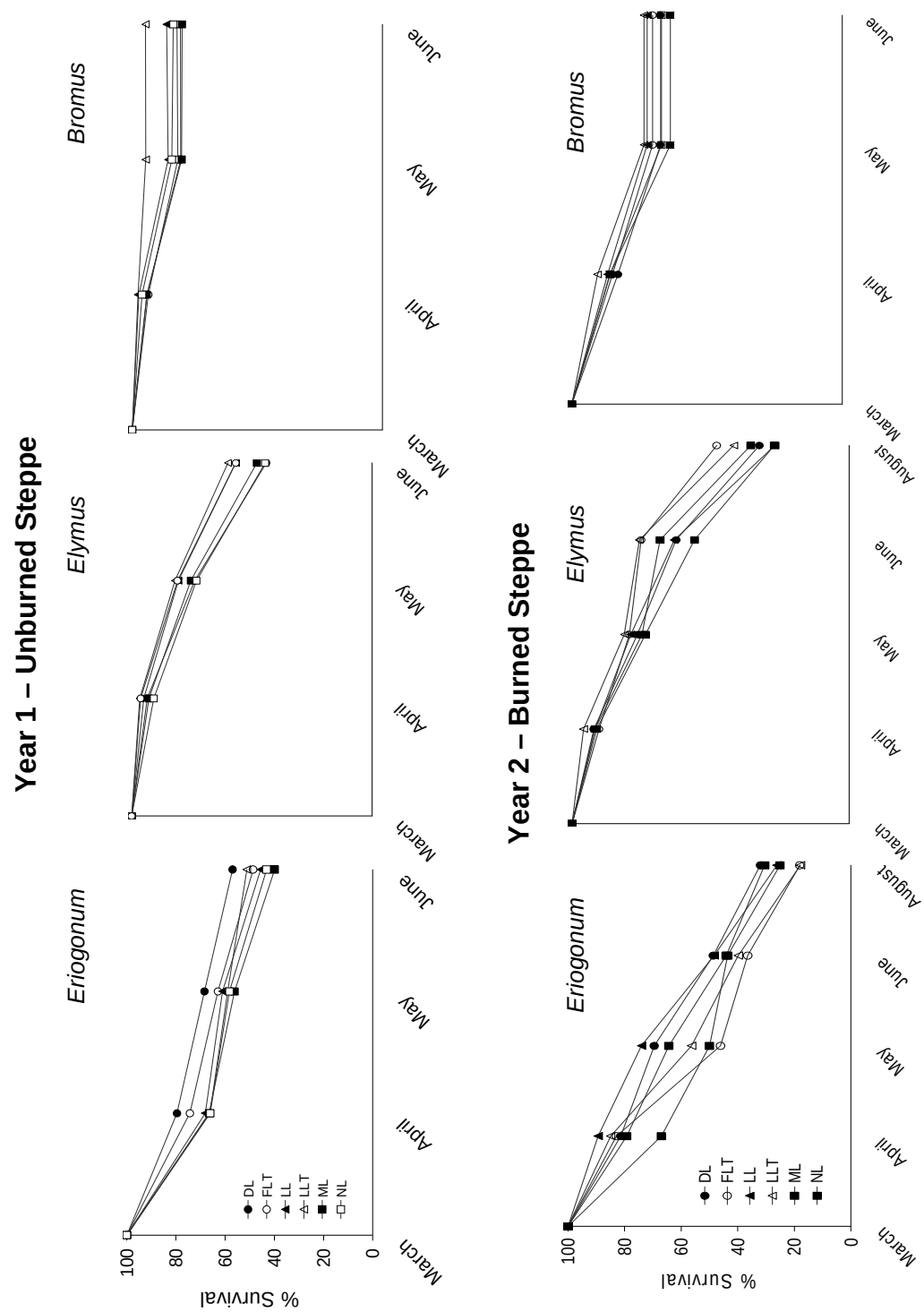


Figure 7.

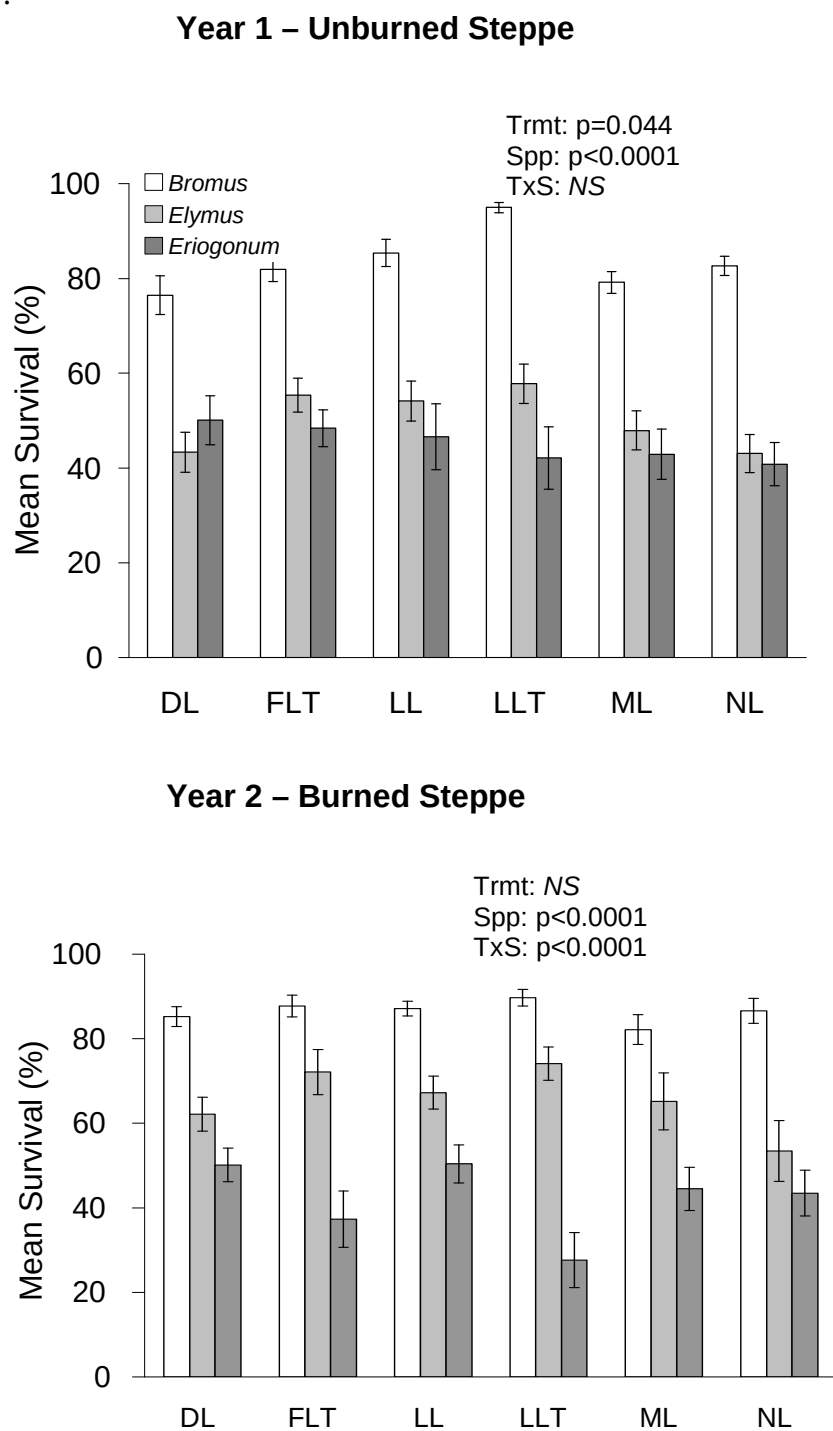


Figure 8.

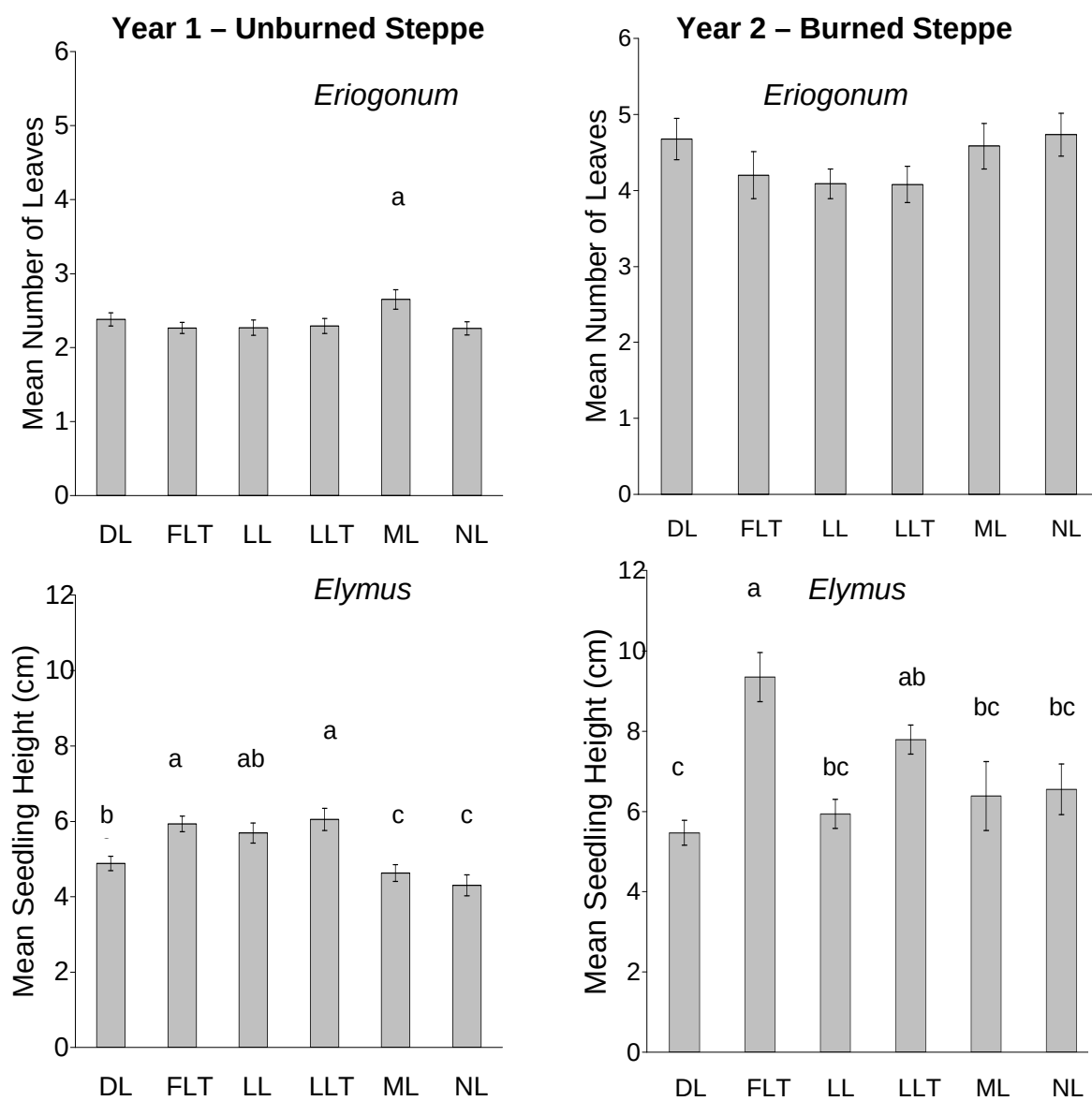
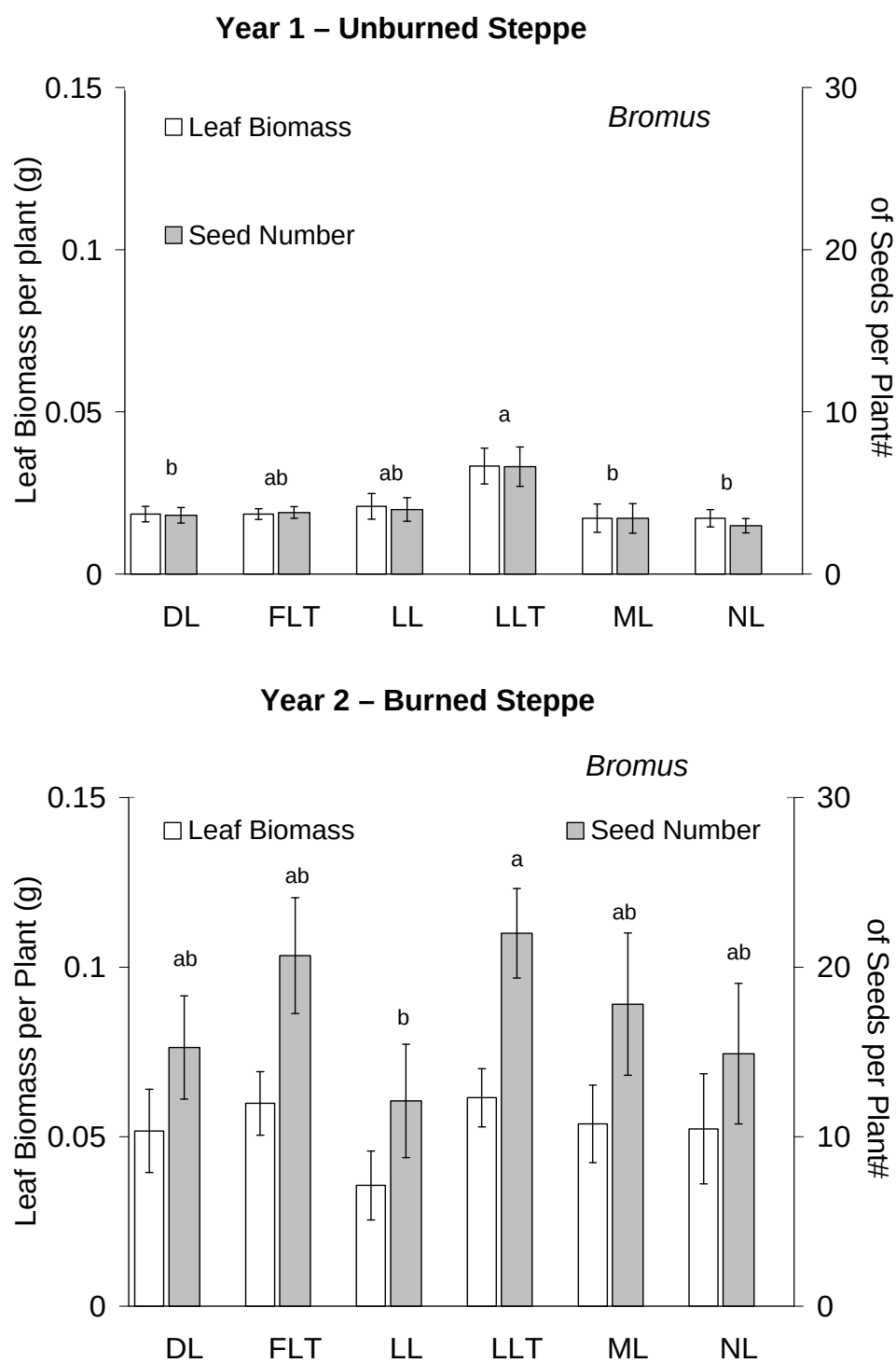


Figure 9.



APPENDIX

ANOVA tables for Role of a Native Legume in Facilitating Native vs. Invasive Species in Sagebrush Steppe Before and After Fire – Abiotic variables 2007.

2007				
Fixed Effect Tests for Soil Moisture				
Source	DF	<u>DFDen</u>	F Ratio	<u>Prob > F</u>
Treatment	5	46	0.7	0.628
Time	3	143	770.86	<0.0001
Treatment x Time	15	143	0.63	0.848
Fixed Effect Tests for Temperature Max				
Source	DF	<u>DFDen</u>	F Ratio	<u>Prob > F</u>
Treatment	5	74	4.75	0.0008
Time	4	284	1912.73	<0.0001
Treatment x Time	20	284	4.53	<0.0001
Effect Tests for Temperature Min				
Source	DF	<u>DFDen</u>	F Ratio	<u>Prob > F</u>
Treatment	5	74	1.88	0.108
Time	4	284	1928.02	<0.0001
Treatment x Time	20	284	0.7	0.829
Effect Tests for Temperature Difference				
Source	DF	<u>DFDen</u>	F Ratio	<u>Prob > F</u>
Treatment	5	74	4.59	0.0011
Time	4	284	424.04	<0.0001
Treatment x Time	20	284	3.28	<0.0001
Effect Tests for Temperature PAR				
Source	DF	<u>DFDen</u>	F Ratio	<u>Prob > F</u>
Treatment	5	79	109.6	<0.0001

ANOVA tables for Role of a Native Legume in Facilitating Native vs. Invasive Species in Sagebrush Steppe Before and After Fire – Abiotic variables 2007.

2007				
Fixed Effect Tests for Total Soil Extractable Nitrogen				
Source	DF	DFDen	F Ratio	Prob > F
Treatment	5	82	3.01	0.0152
Time	1	82	171.42	<0.0001
Treatment x Time	5	82	1.85	0.112
Fixed Effect Tests for N mineralization				
Source	DF	DFDen	F Ratio	Prob > F
Treatment	5	10	0.47	0.794
Effect Tests for Soil Total %N				
Source	DF	DFDen	F Ratio	Prob > F
Treatment	5	10	0.248	0.912
Effect Tests for Soil total %C				
Source	DF	DFDen	F Ratio	Prob > F
Treatment	5	10	0.213	0.949
Effect Tests for Soil C:N				
Source	DF	DFDen	F Ratio	Prob > F
Treatment	5	10	2.36	0.116
Effect Tests for Soil Probe (N)				
Source	DF	DFDen	F Ratio	Prob > F
Treatment	5	44	1.32	0.273
SPP	2	92	0.393	0.676
Treatment x Spp	10	92	0.517	0.874
Effect Tests for Soil Probe (P)				
Source	DF	DFDen	F Ratio	Prob > F
Treatment	5	44	2.33	0.058
SPP	2	92	0.799	0.453
Treatment x Spp	10	92	0.411	0.938
Effect Tests for Soil Probe (K)				
Source	DF	DFDen	F Ratio	Prob > F
Treatment	5	44	1.17	0.338
SPP	2	92	0.029	0.971
Treatment x Spp	10	92	1.23	0.283

ANOVA tables for Role of a Native Legume in Facilitating Native vs. Invasive Species in Sagebrush Steppe Before and After Fire – Abiotic variables 2008.

2008				
Fixed Effect Tests for Soil Moisture				
Source	DF	<u>DFDen</u>	F Ratio	<u>Prob > F</u>
Treatment	5	46	2	0.1
Time	4	192	173.75	<0.0001
Treatment x Time	20	192	1.6	0.056
Fixed Effect Tests for Temperature Max				
Source	DF	<u>DFDen</u>	F Ratio	<u>Prob > F</u>
Treatment	5	38	5.76	0.0005
Time	5	200	1108.87	<0.0001
Treatment x Time	25	200	3.38	<0.0001
Effect Tests for Temperature Min				
Source	DF	<u>DFDen</u>	F Ratio	<u>Prob > F</u>
Treatment	5	38	2.04	0.095
Time	5	200	3621.78	<0.0001
Treatment x Time	25	200	1.73	0.0208
Effect Tests for Temperature Difference				
Source	DF	<u>DFDen</u>	F Ratio	<u>Prob > F</u>
Treatment	5	38	5.22	0.001
Time	5	200	367.7	<0.0001
Treatment x Time	25	200	3.2	<0.0001
Effect Tests for Temperature PAR				
Source	DF	<u>DFDen</u>	F Ratio	<u>Prob > F</u>
Treatment	5	9.14	22.45	<0.0001

ANOVA tables for Role of a Native Legume in Facilitating Native vs. Invasive Species in Sagebrush Steppe Before and After Fire – Abiotic variables 2008.

2008				
Fixed Effect Tests for Total Soil Extractable Nitrogen				
Source	DF	<u>DFDen</u>	F Ratio	<u>Prob > F</u>
Treatment	5	46	3.06	0.018
Time	2	96	111.6	<0.0001
Treatment x Time	10	96	1.98	0.044
Fixed Effect Tests for N mineralization				
Source	DF	<u>DFDen</u>	F Ratio	<u>Prob > F</u>
Treatment	5	10	0.383	0.85
Effect Tests for Soil Total %N				
Source	DF	<u>DFDen</u>	F Ratio	<u>Prob > F</u>
Treatment	5	10	0.545	0.739
Effect Tests for Soil total %C				
Source	DF	<u>DFDen</u>	F Ratio	<u>Prob > F</u>
Treatment	5	10	0.796	0.576
Effect Tests for Soil C:N				
Source	DF	<u>DFDen</u>	F Ratio	<u>Prob > F</u>
Treatment	5	10	0.738	0.612
Effect Tests for Soil Probe (N)				
Source	DF	<u>DFDen</u>	F Ratio	<u>Prob > F</u>
Treatment	5	46	0.964	0.499
SPP	2	95	0.297	0.744
Treatment x Spp	10	95	1.92	0.0514
Effect Tests for Soil Probe (P)				
Source	DF	<u>DFDen</u>	F Ratio	<u>Prob > F</u>
Treatment	5	46	1.38	0.25
SPP	2	95	0.763	0.47
Treatment x Spp	10	95	0.476	0.9
Effect Tests for Soil Probe (K)				
Source	DF	<u>DFDen</u>	F Ratio	<u>Prob > F</u>
Treatment	5	46	2.7	0.032
SPP	2	95	0.528	0.592
Treatment x Spp	10	95	1.53	0.124

ANOVA tables for Role of a Native Legume in Facilitating Native vs. Invasive Species in Sagebrush Steppe Before and After Fire – Biotic variables 2007.

2007				
Fixed Effect Tests for Seedling Emergence				
Source	DF	<u>DEDen</u>	F Ratio	<u>Prob ></u> F
Treatment	5	84	0.32	0.902
Spp	2	168	160.43	<0.0001
Treatment x Spp	10	168	1.31	0.232
Fixed Effect Tests for Seedling Survival				
Source	DF	<u>DEDen</u>	F Ratio	<u>Prob ></u> F
Treatment	5	84	2.4	0.044
Spp	2	168	151.14	<0.0001
Treatment x Spp	10	168	1.34	0.214
Effect Tests for Elymus Ht				
Source	DF	<u>DEDen</u>	F Ratio	<u>Prob ></u> F
Treatment	5	82	10.1	<0.0001
Effect Tests for Eriogonum Leaves				
Source	DF	<u>DEDen</u>	F Ratio	<u>Prob ></u> F
Treatment	5	75.3	2.36	0.048
Effect Tests for Bromus Vegetative Biomass				
Source	DF	<u>DEDen</u>	F Ratio	<u>Prob ></u> F
Treatment	5	80	3.11	0.013
Effect Tests for Bromus Reproductive Biomass				
Source	DF	<u>DEDen</u>	F Ratio	<u>Prob ></u> F
Treatment	5	80	3.19	0.011
Effect Tests for Bromus N Concentration				
Source	DF	<u>DEDen</u>	F Ratio	<u>Prob ></u> F
Treatment	5	75.3	0.95	0.454
Effect Tests for Bromus N Content				
Source	DF	<u>DEDen</u>	F Ratio	<u>Prob ></u> F
Treatment	5	75.3	3.13	0.013
Effect Tests for Bromus C:N				
Source	DF	<u>DEDen</u>	F Ratio	<u>Prob ></u> F
Treatment	5	75.3	1.49	0.202

ANOVA tables for Role of a Native Legume in Facilitating Native vs. Invasive Species in Sagebrush Steppe Before and After Fire – Biotic variables 2008.

2008				
Fixed Effect Tests for Seedling Emergence				
Source	DF	<u>DEDen</u>	F Ratio	<u>Prob > F</u>
Treatment	5	81	2	0.087
Spp	2	166	116.33	<0.0001
Treatment x Spp	10	166	10.35	<0.0001
Fixed Effect Tests for Seedling Survival				
Source	DF	<u>DEDen</u>	F Ratio	<u>Prob > F</u>
Treatment	5	81	0.615	0.688
Spp	2	166	149.84	<0.0001
Treatment x Spp	10	166	4	<0.0001
Effect Tests for Elymus Ht				
Source	DF	<u>DEDen</u>	F Ratio	<u>Prob > F</u>
Treatment	5	81.4	7.94	<0.0001
Effect Tests for Eriogonum Leaves				
Source	DF	<u>DEDen</u>	F Ratio	<u>Prob > F</u>
Treatment	5	76.2	1.25	0.292
Effect Tests for Bromus Vegetative Biomass				
Source	DF	<u>DEDen</u>	F Ratio	<u>Prob > F</u>
Treatment	5	76.1	2.19	0.064
Effect Tests for Bromus Reproductive Biomass				
Source	DF	<u>DEDen</u>	F Ratio	<u>Prob > F</u>
Treatment	5	76.1	2.04	0.082
Effect Tests for Bromus N Concentration (seed)				
Source	DF	<u>DEDen</u>	F Ratio	<u>Prob > F</u>
Treatment	5	80	0.10	0.992
Effect Tests for Bromus N Concentration (leaf)				
Source	DF	<u>DEDen</u>	F Ratio	<u>Prob > F</u>
Treatment	5	77	1.31	0.267

MRPP results for Role of a Native Legume in Facilitating Native vs. Invasive Species in Sagebrush Steppe Before and After Fire – Microbial data.

Microbial MRPP results 2007

Test statistic: T = -0.66799037
 Observed delta = 11.667490
 Expected delta = 11.755463
 Variance of delta = 0.17344500E-01
 Skewness of delta = -0.64044862

A = 0.00748361

p = 0.23246069

Distance

DL	9.9443465
FLT	13.042881
LL	11.412634
LLT	11.050454
ML	11.631822
NL	12.859865

Microbial MRPP Results 2008

Test statistic: T = -1.4250530
 Observed delta = 11.586384
 Expected delta = 11.806331
 Variance of delta = 0.23821737E-01
 Skewness of delta = -0.73875058

A = 0.01862957

p = 0.08842738

Distance

LLT	11.193144
DL	11.860494
LL	9.7168749
ML	15.034415
NL	11.602864
FLT	10.030827

CONCLUSIONS

Understanding factors that modify resource availability in nutrient limited systems has important implications for community assembly and invasion by non-native species, and can improve our ability to manage these systems to maintain or restore native diversity. The overall goal of this study was to gain a better understanding of the functional role of the nitrogen fixing species *Lupinus argenteus*, a native perennial herb that is broadly distributed in sagebrush ecosystems. The specific goals were to determine how the functional role of this species is influenced by the resource environment, how it may modify species interactions, and how it influences seedling establishment, community composition and invasion within the sagebrush steppe. Results from these four interrelated studies help clarify the role of *L. argenteus* in both burned and unburned sagebrush ecosystems (Figure 1).

Woodland expansion affects grasslands and shrublands on a global scale. Prescribed fire is a potential restoration tool, but recovery depends on nutrient availability and species responses after burning. Fire often leads to long-term losses in total nitrogen, but presence of native legumes can influence recovery through addition of fixed nitrogen. In a field survey conducted in sagebrush steppe of central Nevada, extractable soil inorganic nitrogen was higher in *L. argenteus* presence regardless of time since fire. *Lupinus* density increased after fire mainly due to increased seedling numbers three years post-burn. Fire did not affect *L. argenteus* tissue N and P concentrations, but cover of perennial grasses and forbs was higher in *L. argenteus* presence. The invasive annual grass, *Bromus tectorum*, had low abundance and was unaffected by treatments. Results

indicate that *L. argenteus* has the potential to influence succession through modification of the post-fire environment.

The contribution of *L. argenteus* to the nitrogen budget of sagebrush ecosystems, and thus its potential impact on community composition depends upon the resource environment. *L. argenteus* grew largest in intermediate N and high water, indicating that plants growing in this condition likely would contribute the most N to the system via decomposition of N-rich tissue. Additionally, organic N was deposited into the rhizosphere of all plants, regardless of treatment, indicating that *L. argenteus* can influence N availability while actively growing, even under water stress. The ability of *L. argenteus* to affect N availability and cycling indicates that it has the potential to significantly influence community composition and plant-plant interactions within the sagebrush steppe.

Like many arid areas dominated by perennial grasses, the sagebrush steppe of the western U.S. is threatened by invasion of non-native species, especially the annual grass *B. tectorum*. Increased resource availability can promote expansion of *B. tectorum* by changing interactions among *B. tectorum* and native perennial grass seedlings. Results from my competition experiment indicated that native *Elymus multisetus* and *B. tectorum* responded nearly identically to increasing N when grown in monoculture. *B. tectorum* biomass was more affected by intraspecific competition whereas *E. multisetus* was more affected by interspecific competition from *B. tectorum*. When grown in competition *B. tectorum* always outperformed *E. multisetus*, regardless of N level, although the degree of *E. multisetus* suppression by *B. tectorum* was least in the absence of additional N. Presence of *L. argenteus* increased biomass and tissue N content in both grasses,

indicating that this native legume has the potential to facilitate both species. However, *B. tectorum* exhibited a greater positive response to the presence of *L. argenteus*, which intensified competition between *E. multisetus* and *B. tectorum*. Thus, presence of *L. argenteus* was facilitative for *B. tectorum* but indirectly inhibitory for *E. multisetus* when grown with *B. tectorum*. Thus, this study indicates that modification of the local resource pool by *L. argenteus* can alter competitive outcomes among these native and non-native seedlings and can promote dominance by non-native *B. tectorum*.

Facilitation by *L. argenteus* also can impact community composition through differentially influencing seedling establishment of *B. tectorum* and the natives *E. multisetus* and *Eriogonum umbellatum*. In the field I examined six treatments to identify specific mechanisms by which *L. argenteus* potentially influences establishment: 1) live lupine; 2) dead lupine; 3) no lupine; 4) no lupine with lupine litter; 5) no lupine with inert litter; and 6) mock lupine. In both unburned and burned communities, temperature fluctuations and light levels were highest in no lupine plots and least in mock lupine and live lupine plots. Soil N was highest in the burned community, but decreased over the growing season and was highest in lupine litter plots at the start of the growing season in both communities. In unburned communities, emergence of seeded species was unaffected by treatment. Increased N availability from lupine litter increased *B. tectorum* survival, biomass and seed number. Survival of native species was unaffected by lupine treatment, but height of *E. multisetus* was higher in both lupine litter and fake lupine treatments. In burned communities litter treatments reduced emergence of *E. umbellatum* and increased emergence of *E. multisetus*, but treatment still had no effect on emergence of *B. tectorum*. Similarly, reduced light and temperature fluctuation in litter treatments

facilitated *E. multisetus* survival and height. Plants of all species were larger in the burned communities, but higher N levels likely masked treatment differences. *B. tectorum* had higher emergence and survival than either native species regardless of treatment or community type. Modification of the resource environment by *L. argenteus* can increase plant establishment and growth of both the native and non-native species studied, but higher establishment and growth rates gave the non-native, *B. tectorum*, a greater advantage. Fire can mask the effects of N augmentation by legumes and modification of the physical environment can become relatively more important than N addition.

This research indicates that *L. argenteus* has the potential to modify sagebrush steppe ecosystems at multiple levels – nitrogen availability and cycling, species interactions, and recruitment of both native and non-native species. Thus this research also has implications for management of sagebrush ecosystems. The ability of *L. argenteus* to increase N availability can serve to promote resilience of native ecosystems, but also may create an avenue for invasion. In burned communities *L. argenteus* appears to affect compositional change through rapid establishment in open microsites following fire and facilitation of particular plant functional groups. *L. argenteus* can replace N that is lost to fire, and initially may promote regrowth and establishment of native perennial grasses and forbs that enhances ecosystem resilience. However, *L. argenteus* may create localized patches of increased N that favor establishment of *B. tectorum*. Communities with well established perennial grasses and forbs may not be as affected by increased N because of greater competitive ability of adult perennial plants. However, in the absence of established perennial herbaceous vegetation, such as might be found in heavily grazed

sagebrush steppe, increased N availability associated with *L. argenteus* may allow replacement of natives via enhanced competitive ability of *B. tectorum*.

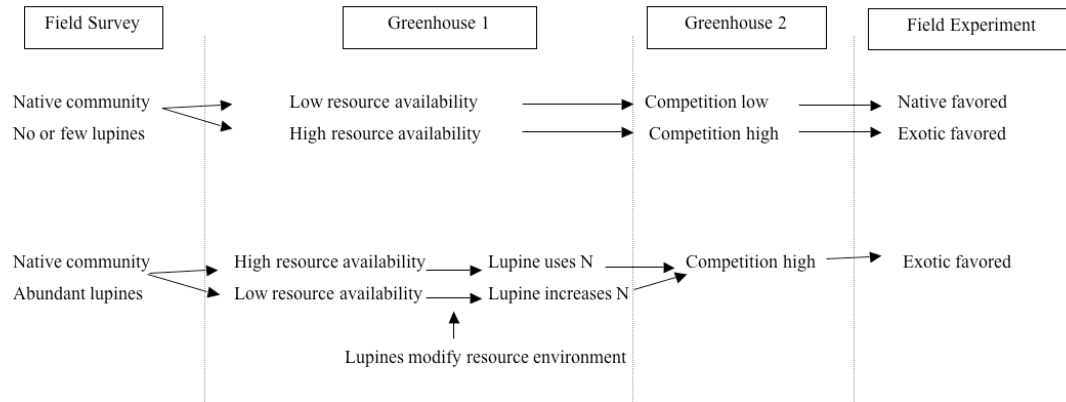


Figure 1. Diagram of the four different experiments and how they are related. The field study identifies patterns in the sagebrush steppe with and without lupines. The first greenhouse study indicates how lupines respond to high and low resource availability. The second greenhouse study takes information gained from the first study to look at the competitive interactions among native and exotic grasses with and without lupines and under different resource conditions. The field experiment builds on all of the prior projects and examines how lupines modify the resource environment to influence seedling establishment and community composition.