

DID THE SELECTIVE BREEDING OF A NON-
NATIVE GRASS PROMOTE INVASIVENESS?

By

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NATIVE GRASS PROMOTE INVASIVENESS?

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Abstract: Concerns with invasive species have included exotic forage grasses that have undergone extensive breeding through development programs for greater success of establishment. These grasses have been widely distributed for soil erosion control and livestock forage production throughout the United States and many of these species have become invasive in native habitats. To determine whether plant breeding procedures increased the invasiveness of these exotic grasses relative to their native counterparts, we assessed the competitiveness of an old world bluestem (*Bothriochloa ischaemum*; OWB) a known invader of the Great Plains in the United States, relative to its "wildtype" from the Czech Republic, and two native tallgrass prairie grasses (big bluestem [*Andropogon gerardii*] and little bluestem [*Schizachyrium scoparium*]). To assess inter-and intraspecific competition a substitutive design greenhouse competition experiment was conducted in Stillwater, Oklahoma with partners from the Czech University of Life Sciences. Growth of the invasive *B. ischaemum* was enhanced when grown with the native *S. scoparium*. However, the Czech Republic wildtype did not exhibit increased production when grown in competition with natives compared to conspecifics, suggesting the extensive breeding of *B. ischaemum* may have enhanced its competitive abilities. To further assess plant-soil feedbacks we conducted a second greenhouse study which examined soil biotic communities using soil inoculums associated with either wildtype or invasive *B. ischaemum* cultivars. Growth and reproduction of native grass species, the wildtype, and invasive *B. ischaemum* were assessed 16 weeks following inoculation with soil microbial communities. Phospholipid and neutral lipid fatty acids were quantified at the end of the study to determine microbial biomass associated with each soil inoculum and species or cultivar combination. Total biomass production and abundance of AMF was enhanced when the wildtype was grown with inoculum associated with the invasive cultivar compared to wildtype inoculum, indicating the wildtype favors soil biotic communities associated with the invasive cultivar. When assessing responses of native grasses, *A. gerardii* illustrated greater production and abundance of AMF compared to *S. scoparium* suggesting seeding to *A. gerardii* may lead to greater success in grassland restorations following eradication of *B. ischaemum*, compared to restorations seeded to *S. scoparium*.

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CHAPTER I

DID THE SELECTIVE BREEDING OF A NON-NATIVE GRASS PROMOTE INVASIVENESS?

INTRODUCTION

Concerns over exotic plant invasions stem from their ability to become more abundant and dominant in their introduced ranges, out-competing the native plant communities present. Interestingly, these dominance patterns are often not exhibited by the invader in its native range (Callaway et al. 2012). This general observation has long interested ecologists (Darwin 1872; Elton 1958) and many different explanations have been proposed to better understand invasive species success (Mack et al. 2000; Hierro et al. 2005). For instance, successful invasions have been associated with the invader's capacity to competitively suppress native species in the introduced range (Ni et al. 2010). An invader can gain a superior competitive advantage over native species indirectly through the release of biotic constraints such as natural enemies, consistent with the enemy release hypothesis (Keane & Crawley 2002), or directly through the possession of particular traits that can lead to the ability to produce greater biomass, compared to resident native species (Callaway & Ridenour 2004; Maron & Marler 2008). To

understand the mechanisms for successful invasions, it is important to understand if invaders demonstrate different competitive abilities in their native home ranges as compared to their new invaded range. Most studies on invasive species have been conducted in their introduced ranges, focusing on native species responses within invaded and non-invaded sites (Leger 2008; Rojas-Sandoval et al. 2012; Gooden et al. 2014; Lee et al. 2012; Hill & Fischer 2014). Although important for understanding mechanisms of invasiveness, there is little information comparing relative dominance of invasive grasses in their native ranges compared to introduced ranges, and how the ability to dominate in a given area affects plant communities in both biogeographic areas (Callaway et al. 2012). Comparisons such as these, are essential for furthering our understanding of invasions as they provide additional insight as to how invasive species behave differently in their native and introduced regions (Callaway et al. 2012). However, competitive abilities of invasive species in both native and introduced areas, as well as impacts on native species in the introduced region has recently been reported (He et al. 2009; Ni et al. 2010; Callaway et al. 2012; 2011b). For instance, Russian knapweed, *Acroptilon repens* (L.), demonstrated a stronger competitive ability over neighboring plant species native to North America than native plant species from its home range in Uzbekistan (Ni et al. 2010). Garlic mustard, *Alliaria petiolata*, an aggressive invader in North American forest understories exhibited a stronger inhibitory effect on mycorrhizal mutualists in its invaded range in North American soils than in its native European soils (Callaway et al. 2008).

To help explain the reoccurring pattern of invasive species in their introduced ranges having apparent increased competitive abilities compared to their native ranges,

the "evolution of increased competitive ability" hypothesis was developed (Blossey & Nötzold 1995). This hypothesis revolves around existing data that indicate invasive species may undergo evolutionary changes in their new regions producing populations with greater biomass production and enhanced competitive abilities (Blossey & Nötzold 1995; Siemann & Rogers 2001; Zou et al. 2008), resulting in a stronger effect compared to congeners in their native range. There have been several studies providing evidence that invasive genotypes exhibited faster growth and greater fitness than their native counterparts (Jakobs et al. 2004; Kumschick et al. 2013; Lavergne & Molofsky 2007; Leiblein-Wild et al. 2014; Ross & Auge 2008). An increase in competitive ability, due to evolutionary or genotypic changes could explain the success of many invasive species. Here we compare the competitive ability of an invasive C₄ grass to its native counterpart from the Czech Republic to determine whether the invasive cultivar and its native counterpart demonstrate similar invasive characteristics.

Old World bluestems (OWB) (*Bothriochloa bladhii* Retz [Caucasian bluestem] and *Bothriochloa ischaemum* L. [yellow bluestem]) are a group of non-native, perennial, warm-season grasses that have been identified as a substantial threat to the stability of our native grasslands in the southern and central Great Plains (Wilson et al. 2012). The earliest records of OWB introduction in the United States was in 1917 (Celarier & Harlan 1995). Throughout the 19th century OWB became extensively planted for soil conservation and forage production purposes (Harmony et al. 2007). Additionally, OWB were heavily researched and many different cultivars were developed specifically suited for successful establishment in the southern Great Plains region (Dahl et al. 1988). Over the last 60 years 750 different OWB cultivars have been introduced into our native

grasslands (Berg et al. 1993). Old World bluestems are now common in roadsides and pastures, and are steadily invading native grasslands in the states of Alabama, Arkansas, California, Colorado, Florida, Georgia, Hawaii, Kansas, Louisiana, Mississippi, Missouri, Nebraska, New Mexico, New York, Ohio, Oklahoma, South Carolina, Tennessee, and Texas (USDA; NRCS 2014). Native grasslands, present in the Great Plains, are primarily dominated by warm-season grasses and the presence of an invader belonging to the same ecosystem-level functional group therefore, such as OWB, may pose the greatest threat to these native grasslands (Wilson et al. 2012). Compared with native prairie ecosystems, OWB dominated grasslands have been documented to contain lower bird species richness, abundance, and arthropod availability (Hickman et al. 2006); small mammal species richness and diversity (Sammon & Wilkins 2005); and native plant species diversity (Gabbard & Fowler 2007).

Recently, concerns about invasive species have expanded to include exotic species that were bred and released for agricultural purposes, such as OWB. Research indicates that the success of an invasive species and consequent loss of native species, cannot be explained by a single trait exhibited by the invader but rather as a combination of different factors (Weiher 2007). Previous studies on assessing OWB populations in invaded ranges have identified a multitude of characteristics enabling these species to be competitively superior to dominant native grasses of the Great Plains (Harmony and Hickman 2004; Reed et al. 2005; Schmidt et al. 2008). These invasive characteristics include: smaller seed size than natives (Coyne & Bradford 1985a), higher fecundity rates (Wilson et al. 2012), readily adaptable to varying levels of water and nitrogen deficiencies (Coyne and Bradford 1985b; Szente et al. 1996; Reed et al. 2005), maturing

earlier in the season compared to native warm-season grasses (Harmony & Hickman 2004), and multiple modes of reproduction (Schmidt & Hickman 2006).

Differences in aboveground factors between invasive and native species may not be the only factor in successful invasibility (Callaway et al. 2012). Another important factor to consider is the establishment of plant-soil feedbacks between invasive plants and native soil communities (Bever et al. 1997; Bever 2003). There is strong evidence suggesting that OWB, in addition to being competitively superior to native grasses in aboveground characteristics, alters soil biotic characteristics of native grassland ecosystems (Wilson et al. 2012). Biomass production of dominant Great Plains native grass species, (*Andropogon gerardii* and *Schizachyrium scoparium*) was consistently reduced when planted into soil collected from the rooting zone of OWB compared with native prairie soil. In addition, when living soil inoculum of native prairie was added to steam-pasteurized soil from invaded OWB sites, biomass production of the native grasses was significantly improved (Wilson et al. 2012).

The establishment of mutualisms between an invader and native soil organisms in the introduced range can play a critical role in facilitating successful invasions (Andonian & Hierro 2011; Callaway et al. 2011a; Cui & He 2009; Sun & He 2010), and in some cases can enhance the competitive ability of the invader (Aschehoug et al. 2012; Marler et al. 1999; Reinhart & Callaway 2004). Arbuscular mycorrhizal fungal associations, for example, can play a large role in a plant's ability to function (Herre et al. 1999). Introduced exotic plant species have the potential to alter these important plant-fungal communities (Vogelsang et al. 2004) and it has been acknowledged that AMF associations can influence the invasion process (Vogelsang & Bever 2009). Warm-season

native tallgrass prairie species, as well as OWB, are highly dependent upon these mycorrhizal interactions (Wilson & Hartnett 1998) and dominance in grasslands by warm-season grasses has been linked to their reliance upon their plant-fungal relationships (Wilson et al. 2012). The success of OWB, as an invader, could also be explained by the alteration of native mycorrhizal interactions since the absence or reduction in native AMF could potentially change the competitive outcome of grassland species (Hetrick et al. 1990; Wilson & Hartnett 1997; Hartnett & Wilson 1999; Hartnett & Wilson 2002). These alterations, or losses of symbiotic fungi may indirectly promote success of the invader.

Whether the native conspecific of OWB from its home range demonstrates similar characteristics as the US invasive OWB present in the Great Plains has not been assessed. The purpose of our study was to compare the Great Plains invasive OWB species, *B. ischaemum*, to its native counterparts in the Czech Republic to determine if both varieties exhibit similar invasive characteristics. Given the detrimental effects invasive species can have on native biodiversity, and ecosystem structure and function, it is important to identify factors contributing to invasiveness. This study seeks to determine if the extensive and selective breeding of the *B. ischaemum* cultivar enhanced its competitive ability contributing to its success as an invader. Our project was conducted with partners from the Academy of Sciences of the Czech Republic. Two separate greenhouse studies were conducted in Stillwater, Oklahoma to assess the following objectives:

Our first greenhouse study, hereby referred to as "competition study", assessed the inter- and intraspecific competitive ability of an invasive *B. ischaemum* cultivar with a corresponding native wildtype from the Czech Republic, as well as with two

functionally similar dominant native tallgrass prairie grasses (big bluestem [*Andropogon gerardii* Vitman] and little bluestem [*Schizachyrium scoparium* Michx.]) present in the Great Plains. Our overall objective in this study was to evaluate the competitive ability of the invasive *B. ischaemum* to its wildtype from the Czech Republic relative to these two native warm-season grasses. Based upon previous studies, indicating *B. ischaemum*'s superior competitive ability over dominant native grasses (Schmidt et al. 2008; Wilson et al. 2012), we hypothesized that the invasive *B. ischaemum* cultivar from the Great Plains would be competitively superior to its wildtype from the Czech Republic due to breeder-selected characteristics. We further hypothesized that if both *B. ischaemum* varieties do share similar characteristics, the Czech Republic wildtype of *B. ischaemum* will also be competitively superior to the native grass species present in the Great Plains.

Previous studies have indicated that relations between resident native soil microorganisms and plant species can be influenced by invasive plant species where the success of the invader is promoted through establishment of plant-soil feedback mechanisms (Ehrenfeld 2004; Weidenhamer and Calloway 2010). To explore this as a potential mechanism behind the success of *B. ischaemum* as a superior competitor, the purpose of our second greenhouse study (hereby referred to "home vs. away") was to evaluate soil biotic communities using different soil inocula. Our objective was to assess soil microbial communities of wildtype *B. ischaemum* and invasive *B. ischaemum* cultivar following 16 weeks of growth in native prairie soil amended with 'home' vs 'away' whole soil inocula. We assessed the growth and reproduction of the two native US grass species from the Great Plains, the wildtype *B. ischaemum*, and invasive *B. ischaemum* cultivar, used in the competition study, following inoculation with soil

microbial communities associated with each grass species. Based on previous studies indicating *B. ischaemum's* ability to suppress growth of native plant species in the invaded range (Wilson et al. 2012), we hypothesized that when planted into soil with microbial communities associated with the wildtype variety from the Czech Republic, native seedling growth and reproduction would be reduced. We further hypothesized that the wildtype and the invasive *B. ischaemum* will also both exhibit a greater percent root colonization and abundance of AMF when paired with native grass species compared to conspecifics.

METHODS

Competition Study

A greenhouse study was conducted to assess the inter- and intraspecific competitive ability of the wildtype *B. ischaemum* and invasive *B. ischaemum* cultivar and two native Great Plains grasses (*A. gerardii* and *S. scoparium*). Seeds of the two native grasses and the invasive *B. ischaemum* were obtained from commercial sources from the Johnston Seed Company in Enid, Oklahoma. Commercial seeds of invasive *B. ischaemum* were used since we were examining if competitive abilities of *B. ischaemum* cultivars has been enhanced through plant breeding. The wildtype variety was hand-collected from three separate native grassland sites located in the Czech Republic (Drahobuz, Vedlice, and Prokopske Udoli) where *B. ischaemum* is a common sub-dominant grass native to these grassland ecosystems.

Seeds of *A. gerardii*, *S. scoparium*, invasive *B. ischaemum*, and the wildtype *B. ischaemum* were germinated in vermiculite. Fourteen days past emergence, when the

seedlings were in the second-leaf stage, they were transplanted into 4 L pots (21.5 cm diameter x 21.5 cm depth) containing 5.25kg (dry weight) of native prairie soil. Native prairie soil was collected from a site near the headquarters of the Konza Prairie Biological Station, Manhattan, Kansas that was dominated by native warm-season grasses such as, *A. gerardii*, *Sorghastrum nutans*, and *S. scoparium*. Before soil was placed into pots it was sieved through a 2-mm sieve to remove large plant roots, rhizomes, and small rocks. A subsample of native prairie soil, before experimental set up, was collected for chemical analyses to assess initial soil nutrient availability.

A substitutive design (Harper 1977) was used to assess the effects of intra- and interspecific competition on the growth of the two native species, the wildtype *B. ischaemum*, and invasive *B. ischaemum*. All possible pairwise combinations represent our treatments. For a control and to examine intraspecific competition, six seedlings of *A. gerardii*, *S. scoparium*, wildtype *B. ischaemum*, or invasive *B. ischaemum* were grown in monoculture and planted evenly spaced into each pot. To evaluate interspecific competition all pair-wise combinations were used. Each pot was planted with a pair of three seedlings of either *A. gerardii*, *S. scoparium*, wildtype *B. ischaemum*, or invasive *B. ischaemum* for a total of six seedlings per pot. In total, to assess both intra- and interspecific competition, ten different plant combinations (representing treatments) were used and replicated six times for a total of sixty pots. The pots were arranged in a randomized block design, watered daily, and kept in a greenhouse maintained at 20-25°C.

After fourteen weeks of growth, aboveground (vegetative and reproductive components) and belowground biomass were harvested. In each pot, plants were separated by species, the aboveground biomass was clipped at the soil surface and roots

washed free of soil. Plants, separated by above-and belowground biomass, were then dried for three days at 60°C and dry weights were measured to the nearest milligram. The competitive ability of both native species and both the wildtype *B. ischaemum* and invasive *B. ischaemum* cultivar was assessed by determining relative the total dry weight (shoot, root, and reproductive) of each of the two native grasses, the wildtype *B. ischaemum*, and the invasive *B. ischaemum* cultivar. Soil nutrients were assessed after experimental takedown. Soil tests were conducted by the Oklahoma State University Soil/Water/Forage Analytical Laboratory. Plant-available P (using a Mehlich 3 extraction), extractable NO₃-N, and pH were measured (Table 1). To determine percent AMF root colonization a sample of dried roots were stained with trypan blue. Arbuscular mycorrhizal fungi colonization in roots of the two native grasses, wildtype *B. ischaemum*, and invasive *B. ischaemum* cultivar, grown in monoculture and competition, was scored using the magnified gridline intersect method (McGonigle et al. 1990). A digital microscope (Hirox KH 7700) was used to measure the percentage of root length colonized by a combination of hyphae, vesicles, and arbuscules.

Home vs. Away Study

Our home vs. away greenhouse study assessed soil communities, and possible legacy effects, indirectly by quantifying growth and establishment of two native warm-season grasses, the invasive *B. ischaemum* cultivar, and the wildtype *B. ischaemum* following inoculation with whole soil microbial communities associated with either the two native grasses, or the wildtype and invasive *B. ischaemum*. A subsample of soil was collected before (Table 1) experimental setup and following takedown using marker

phospholipids and neutral lipids to quantify microbial biomass and relative abundance selected microbial functional groups: gram positive and negative bacteria, arbuscular mycorrhizal fungi, and saprophytic fungi. As a control, plant biomass production and soil communities of the two native grass species, the wildtype *B. ischaemum*, and invasive *B. ischaemum* were assessed following inoculation with their own native soil communities.

Consistent with the competition study, seeds used for this study were the same two native grasses, wildtype *B. ischaemum*, and invasive *B. ischaemum* from the same sources as described above (competition study). Soil was collected from native prairie at Konza Prairie Biological Station. In this study, soil was steam-pasteurized for 4 hours at 80°C to remove soil microorganisms, allowed to cool, and equilibrate for 14 days. Seeds were germinated in vermiculite. Fourteen days after emergence, at second leaf stage, seedlings were transplanted into plastic pots (6cm diameter x 25 cm deep) containing 600g (dry weight) of steam-pasteurized native prairie soil with one seedling per pot. To evaluate potential plant-soil feedbacks 5 different soil inoculums containing whole soil microorganisms associated with the two native grasses, wildtype *B. ischaemum*, and invasive *B. ischaemum* cultivar were added directly below seedlings roots during transplantation.

Inocula consisted of fresh whole soil associated with native warm-season grasses (collected from KPBS), invasive *B. ischaemum* (collected from Stillwater Research Range), and the wildtype *B. ischaemum* (collected from the Czech Republic). Soil associated with the invasive *B. ischaemum* was collected at three different sites in the Stillwater Research Range and homogenized into one treatment. Soil from the Czech Republic was also collected at three different sites (Drahobuz, Vedlice, and Prokopske

Udoli) but represented three different treatments of native Czech soil microorganisms. During transplantation of seedlings 20g (dry weight) of one of the 5 different soil inoculums (representing different treatments) were added directly below seedling roots. Thus the two native grasses, wildtype *B. ischaemum*, and invasive *B. ischaemum* were each grown separately in soil containing whole microorganisms associated with either native grasses, wildtype *B. ischaemum*, or invasive *B. ischaemum*. Each treatment was replicated 6 times giving a total of 120 pots. The pots were arranged in a randomized complete block design, watered daily, and kept in a greenhouse maintained at 20-25°C.

After fourteen weeks, aboveground (including reproductive components) and belowground biomass were harvested. Aboveground biomass was clipped at the soil surface and roots separated by species and washed free of soil. Plants, separated by above and belowground biomass, were then dried for 3 days at 60°C and both total biomass dry weights were measured to the nearest milligram. Soil nutrients were assessed before experimental setup on soil inocula used. Percent AMF root colonization was also determined. Methods used to quantify soil nutrients and percent AMF root colonization are consistent with the above competition study.

Selected phospholipid fatty acid (PLFA) and neutral lipid fatty acid (NLFA ω) biomarkers were used to determine abundances of gram positive and negative bacteria, AMF, saprophytic fungi, and total microbial biomass. Soil samples containing 5g were taken from whole soil inoculum before experimental set up (Table 1) to determine relative abundance of selected functional groups and total microbial biomass. Soil was collected after takedown from each experimental pot to assess soil microbial communities. PLFA/NLFA's were extracted from the soil using a modification of the

Bligh and Dyer (1959) extraction (White & Ringelberg 1998). Total lipid extracts were separated into PLFA's and NLFA's using silicic acid chromatography; the fatty acids cleaved from the glycerol backbone using KOH saponification; and the harvested fatty acids methylated to form fatty acid methyl esters (FAME) (White & Ringelberg 1998; Allison & Miller 2005). The FAME's were then analyzed by gas chromatography and mass selection detection using a GCMS unit Agilent MS 5975C/GC 7890A. Biomarkers used to select for the functional group of gram positive bacteria included i-15:0, a-15:0, i-17:0, and i-16:0. For gram negative bacteria biomarkers used were 16:1 ω 7, cy19:0, and 2-OH 16:0. For the functional group of AMF biomarkers selected for consisted of 16:1 ω 5c, 20:1 ω 9, and 22:1 ω 13. Biomarkers selected for the functional group of saprophytic fungi included 18:2 ω 9,12 and 18:1 ω 9c.

Statistical Analysis

Data from both greenhouse studies were analyzed using the program SigmaPlot 12.5 (SigmaStat). Total dry weight biomass per plant, including reproductive, above-and belowground components, from each greenhouse study was analyzed using a two-way Analysis of Variance (ANOVA) along with the Hom-Sidak test for all pairwise multiple comparisons. For the competition study, responses assessed consisted of grass species (*A. gerardii*, *S. scoparium*, wildtype *B. ischaemum*, and invasive *B. ischaemum*), treatment (grass species combinations), and the interactions among species x treatment with responses being total plant biomass (avg. g per individual plant). The linear model was,

$$Y_{ijk} = \mu + SPP_i + TRT_j + (SxT)_{ij} + \epsilon_{ijk} \quad [1]$$

where Y was plant biomass, μ was the overall mean, SPP was an overall effect for species i , TRT was an overall effect of the alternate j -th species pairing, SxT was the specific species pairing effects including conspecific pairings, and ϵ was residual error for replication k . Biomass means and standard errors for species in conspecific or interspecific pairings were computed from raw data, which was appropriate for this balanced complete block design. Significance of pairwise comparisons were assessed through the Hom-Sidak tests. In the competition study because interactions involving plant species reproductive biomass x treatment were not significant, the data were reanalyzed as a one-way analysis of variance to compare reproductive biomass in general.

For the home vs. away study, treatments included grass species (*A. gerardii*, *S. scoparium*, wildtype *B. ischaemum*, and invasive *B. ischaemum*), soil inoculum treatment, and the interactions between species x soil inoculum was analyzed as a two-way ANOVA. Separate response variables included total plant biomass, and percent AMF colonization in roots. The linear model was,

$$Y_{ijk} = \mu + \text{SPP}_i + \text{SOIL}_j + (\text{SPPxSOIL})_{ij} + \epsilon_{ijk} \quad [1]$$

where Y was the response, μ was the overall mean, SPP was an overall effect for species i , SOIL was an overall effect of the j -th soil inoculum, SPPxSOIL was the species by soil inoculum interaction effect, and ϵ was residual error for replication k . Biomass and AMF means and standard errors were computed from raw data for each species and inoculum pairing. Significance of pairwise comparisons were assessed through the Hom-Sidak tests. For the competition study however, because interactions involving plant species AMF root colonization percent x treatment were not significant, the data were reanalyzed as a one-way ANOVA to compare average species AMF root colonization overall.

For soil nutrients a one-way ANOVA was used for both greenhouse studies to examine any differences in soil characteristics among treatments. Mean soil characteristic values are presented for each soil type (Table 1). For the home vs. away study when analyzing the PLFA/NLFA data, a two way ANOVA was also utilized along with the Hom-Sidak test for all pairwise multiple comparisons. PLFA data were analyzed separately from the NLFA data. Factors in the analysis were grass species, soil treatment, and the interaction among species x soil treatment with the data consisting of either gram positive bacteria, gram negative bacteria, AMF, and saprophytic fungi. For the NLFA data only AMF and saprophytic fungi were included in the analysis. Because interactions involving the 3 Czech soil sources were not significant, data for these soils were pooled together representing one treatment and data were reanalyzed using a two-way ANOVA.

RESULTS

Competition Study

In this study, we assessed the inter- and intraspecific competitive ability of two native grasses (*A. gerardii* and *S. scoparium*) with two *B. ischaemum* varieties; the wildtype from the Czech Republic and the invasive cultivar present in the Great Plains of the US. The invasive cultivar *B. ischaemum* exhibited a significant increase in total biomass production when grown with the native *S. scoparium*, relative to *A. gerardii*, or when grown with conspecifics (Figure 1). Additionally, the invasive *B. ischaemum* did not exhibit an increase in total biomass produced when paired with its Czech wildtype

(Figure 1). The wildtype *B. ischaemum* when paired with native grasses exhibited no increase in total biomass production compared to when grown with conspecifics (Figure 2). Each plant species produced reproductive structures in all treatments. However, among the two native grasses, wildtype *B. ischaemum*, and invasive *B. ischaemum* the invasive *B. ischaemum* cultivar had greater reproductive biomass overall compared to the wildtype *B. ischaemum* and native grasses (Figure 3).

Percent AMF root colonization among the two *B. ischaemum* varieties were not significantly different regardless of whether they were grown in competition with conspecifics or the native grasses of *S. scoparium* and *A. gerardii* (24.56 ± 2.05). Additionally, there were no statistical differences in percent AMF root colonization between the two native grasses when grown with either the wildtype or invasive cultivar of *B. ischaemum*. Across treatments percent AMF root colonization was on average $26.38\% \pm 1.36$ in the invasive *B. ischaemum* cultivar, $22.75\% \pm 3.95$ in the wildtype *B. ischaemum*, $36.56\% \pm 1.61$ in *S. scoparium*, and $42.06\% \pm 4.27$ in *A. gerardii*. However, when assessing the average percent root colonization of each species overall, regardless of treatment, the native *A. gerardii* had significantly higher colonization compared to the wildtype and invasive *B. ischaemum* (Figure 4). Native *S. scoparium*, regardless of treatment interaction, had significantly greater root colonization compared to the wildtype *B. ischaemum* cultivar (Figure 4).

Home Vs. Away

In this study we evaluated responses of the native, or wildtype, *B. ischaemum* from the Czech Republic and the invasive cultivar of *B. ischaemum*, developed in the

United States, to different soil microbial communities. We assessed the growth and reproduction of native US grass species from the Great Plains and both the *B. ischaemum* varieties following inoculation with soil microbial communities associated with two native species (*A. gerardii* and *S. scoparium*), the invasive *B. ischaemum*, and the wildtype *B. ischaemum*. When the wildtype *B. ischaemum* was inoculated with soil inoculum associated with the invasive cultivar of *B. ischaemum*, it produced significantly more total biomass as compared to when it was grown in soil inoculated with native prairie (Figure 5). However, when the wildtype *B. ischaemum* was grown in soil associated with soil inoculum consisting of native soil communities from the Czech Republic, total biomass production was not significantly different compared to when the wildtype *B. ischaemum* was grown in soil associated with native US species and the invasive *B. ischaemum* (Figure 5). In contrast, the invasive cultivar of *B. ischaemum* exhibited no significant differences in total biomass production regardless of what soil microbial community with which it was inoculated (Figure 6). When comparing the responses of the native grasses, to different soil microbial communities, *S. scoparium* did not have significant differences in total biomass regardless of what soil microbial communities with which it was inoculated (Figure 7). However, *A. gerardii* exhibited an increase in overall total biomass when grown in soil inoculum associated with both the wildtype and invasive *B. ischaemum* as compared to when it was grown with microbes associated with its own native prairie (Figure 8).

Additionally, we wanted to evaluate percent AMF root colonization to determine any responses of microbial communities. There were no significant differences in the percentage of AMF root colonization among the different grass species used and the soil

inoculum treatments. Across treatments the average percentage of AMF root colonization was $29.67\% \pm 3.36$ in the invasive *B. ischaemum* cultivar, $35.32\% \pm 2.91$ in the wildtype *B. ischaemum*, $29.72\% \pm 2.89$ in *S. scoparium*, and $32.08\% \pm 3.14$ in *A. gerardii*.

Further analyses of soil microbial communities included PLFA/NLFA extractions to quantify relative abundances of gram negative and positive bacteria, AMF, and saprophytic fungi across treatments. For PLFAs there were no statistical differences in relative abundance of microbial communities assessed throughout the different treatments (Table 2). For NLFAs the two main microbial communities quantified were abundances of AMF and saprophytic fungi. Throughout the different treatments, within each grass species, there were no statistical differences in the abundance of saprophytic fungi. However, when assessing the abundance of AMF the Czech wildtype *B. ischaemum* exhibited a significantly higher abundance of AMF when planted into soil associated with the invasive *B. ischaemum* cultivar as compared to when it was grown in soil inoculated with native prairie or wildtype *B. ischaemum* (Figure 9). In contrast, the invasive *B. ischaemum* cultivar experienced no significant differences in abundance of AMF regardless of soil inoculum (Figure 10). When examining the responses of the two native grasses *S. scoparium* exhibited a significant increase in abundance of AMF when it was grown in soil associated with the wildtype *B. ischaemum* relative to when it was grown in soil associated with its own native prairie or the invasive *B. ischaemum* (Figure 11). In comparison *A. gerardii* experienced no differences in abundance of AMF regardless of soil inoculum (Figure 12).

DISCUSSION

Competition Study

A trademark of successful invaders is their ability to establish high population numbers through out-competing resident native communities in their invaded ranges. However this pattern of competitive superiority, exhibited by the invader over native species in the invaded range, may not be displayed by the invaders in their native home range (Ni et al. 2010). We hypothesized the *B. ischaemum* invader present in the Great Plains would exhibit enhanced competitive abilities when compared with the wildtype *B. ischaemum* from its home range in the Czech Republic. Further, we hypothesized enhanced competitive abilities were a reflection of selective and extensive breeding of the invasive *B. ischaemum* cultivar.

Invasions by *B. ischaemum* have been shown to substantially reduce production of dominant native grass species (*S. scoparium* and *A. gerardii*) and exhibit enhanced biomass when grown in competition with natives (Schmidt et al. 2008; Wilson et al. 2012). In our study, consistent with previous research, when the invasive *B. ischaemum* cultivar was grown in competition with the native *S. scoparium* growth of the invader was substantially increased compared to conspecifics, most likely due to reduced intraspecific competition (Schmidt et al. 2008). When grown with *A. gerardii* however, total biomass production of the invasive *B. ischaemum* was not enhanced suggesting that *B. ischaemum* is a superior competitor when grown in close proximity to the native grass species *S. scoparium* but not *A. gerardii*. When assessing the competitive abilities of the wildtype *B. ischaemum* with native grass species, contrary to our hypothesis, there was no increase in biomass produced by the wildtype, and native grass species production was

not reduced. Additionally, when comparing the two *B. ischaemum* varieties together, although the invasive *B. ischaemum* cultivar did not experience an increase in above-or belowground biomass when paired with its wildtype, it did produce significantly more reproductive biomass than its wildtype.

The ability to produce more seeds is a characteristic attributed to invasive species success, as competitive abilities of the invader can be enhanced promoting displacement of native communities (Blossey & Notzold 1995). However, this trait may not be exhibited by the invaders in their native environment due to certain constraints which may not be present in the invaders introduced region (Elton 1958; Keane & Crawley 2002). In our study, the wildtype *B. ischaemum* not only produced significantly less reproductive biomass than the invasive cultivar but also produced significantly less reproductive structures than the two native grasses. Compared to its wildtype, the invasive *B. ischaemum* produced significantly more reproductive biomass than the wildtype and the two native grasses suggesting this is a trait developed through selective breeding of the invasive *B. ischaemum*.

In accordance with the "evolution of increased competitive ability" hypothesis evolutionary changes in invasive species have been attributed to the success of several exotic plant invasions (Blossey & Notzold 1995; Siemann & Rogers 2001; Ridenour et al. 2008; Zou et al. 2008), however the degree to which evolutionary changes drive invasion success is unclear (Garcia et al. 2013). We sought to determine if the extensive and selective breeding of the invasive *B. ischaemum* cultivar inadvertently produced evolutionary changes enhancing its competitive ability and contributing to its success as an invader in the southern and central Great Plains region. Successful invasion of

Bothriochloa spp. in general, has been attributed to its rapid growth and ability to reach sexual maturity before native grass species (Harmony & Hickman 2004; Wilson et al. 2012) which was not observed in the wildtype *B. ischaemum* in our study. Our results suggest that the superior competitiveness of the invasive *B. ischaemum* cultivar over the native *S. scoparium* is a trait that is not present in its wildtype suggesting that the selective and extensive breeding of *B. ischaemum* may have inadvertently developed certain characteristics (e.g. increased growth and reproductive capabilities) enhancing its competitive abilities and leading to its success as an invader.

In addition to invaders exhibiting strong competitive abilities, there is increasing evidence suggesting that alterations in soil microbial communities help facilitate successful invasions of exotic plants (Callaway et al. 2008; Sanon et al. 2009; Andonian et al. 2011b). Garlic mustard, *Alliaria petiolata*, an aggressive invader of North American forest understories for example, exhibited strong suppression of native mycorrhizas in invaded North American soils inhibiting growth of native North American plant species and enhancing its own growth. However, presence of *A. petiolata* in its native soil from Europe produced no inhibitory effects on fungal mutualists and European native plants were unaffected (Callaway et al. 2008). There is strong evidence suggesting that *Bothriochloa* spp. alters soil biotic characteristics, specifically fungal mutualists, in native grassland ecosystems (Wilson et al. 2012) and there is the question of whether this characteristic would also be exhibited in the wildtype. When evaluating the percent of AMF root colonization among the wildtype *B. ischaemum* there were no significant differences regardless of whether the wildtype *B. ischaemum* was grown in competition with conspecifics or native grasses. Additionally, percent AMF root colonization of

native grasses was not reduced when grown in close proximity to either *B. ischaemum* variety. In a previous study percent AMF root colonization of native grasses was significantly reduced when grown with the congener of *B. bladhii*. (Wilson et al. 2012). This pattern was not observed in our study although, the use of *B. ischaemum* rather than *B. bladhii* in our study could account for the differences in percent AMF root colonization in the *Bothriochloa* spp. compared to native prairie species.

Since *B. bladhii* and *B. ischaemum* are both invasive in the United States and only *B. ischaemum* was used in our study, future studies should encompass additional populations of *Bothriochloa* spp. from their native environments to further our understanding of characteristics enabling their superior competitiveness and potential to alter soil biotic communities. Additionally, impacts of the invasive cultivar and the wildtype *B. ischaemum* on native plant species in the Czech Republic were not assessed. Further comparisons of the invasive *Bothriochloa* spp. competitive impacts on native species in its native range would provide additional valuable information on behaviors exhibited by the invader in its invaded and native region.

Home vs. Away

Since success of multiple invasive plant species has been attributed to their ability to influence plant-soil feedback relationships enhancing their own successful establishment over native plant species in their invaded region (Ehrenfeld 2004; Weidenhamer & Calloway 2010), an important question to consider is whether these invasive plant species encounter new soil microbial communities in their new environments compared to their native home ranges (Rout & Callaway 2012). Assessing

responses of soil biotic communities in the native and non-native ranges of invasive plants can give us a better understanding of how soil communities in relation to invasive plants can influence invasion success (Reinhart & Callaway 2004).

In this study our objective was to determine potential responses of the two *B. ischaemum* varieties and two native grass species to different soil microbial communities as previous research indicates growth of dominant native grass species is consistently reduced when grown in soil associated with *Bothriochloa* spp. (Wilson et al. 2012). Overall, the invasive *B. ischaemum* cultivar and wildtype *B. ischaemum* exhibited no significant differences from one another in total biomass production or percent AMF root colonization, when grown in soil associated with either *B. ischaemum* variety. However, the wildtype *B. ischaemum* experienced a significant increase in total biomass production when it was inoculated with soil associated with the invasive *B. ischaemum* cultivar as compared to when it was grown in soil associated with native prairie from the Great Plains. Additionally, data from the NFLA fraction indicated a significantly higher abundance of AMF when the wildtype *B. ischaemum* was grown in soil associated with the invasive *B. ischaemum* cultivar compared to when it was inoculated with soil associated with native prairie. Neutral lipid fatty acids associated with AMF are primarily found in storage structures such as spores (Cooper & Lösel 1978) and there is a direct correlation between the abundance of neutral lipids and spore formation by mycelium (Olsson et al. 1997). The high abundance of AMF exhibited by the wildtype *B. ischaemum* when grown in soil associated with the invasive cultivar indicates more energy was allocated towards spore formation by AMF compared to when the wildtype

was grown in native prairie soil suggesting the wildtype favored mycorrhizal communities associated with the invasive *B. ischaemum* cultivar.

Invasive plants in their newly invaded environments may not only lack their original soil mutualists but also may become exposed to new novel soil organisms with which they are not adapted (Klironomos 2002; Callaway et al. 2004; Pringle et al. 2009). As a result, invasive plant species in their new environments could establish interactions with novel mutualists that are more beneficial than mutualists in their native home range (Reinhart & Callaway 2006). For instance, enhancement of invasive plant success through presence of native soil biota in the invaded range has been observed in facilitating invasion of Asian knotweeds (*Fallopia japonica*, *F. sachalinensis*, and *Fallopia x bohemica*) in North America and Europe. When compared to resident native plant species in the invaded range native soil biota was found to be more beneficial to Asian knotweeds than the resident native plant species, thereby shifting the competitive balance in favor of the invader (Parepa et al. 2013). In our study total biomass production and abundance of AMF were significantly enhanced when the wildtype *B. ischaemum* was grown in soil associated with the invasive cultivar, compared to native prairie soil from the Great Plains, indicating that the wildtype favored soil biotic communities associated with the invasive *B. ischaemum* and not the US native prairie, suggesting that the invasive cultivar may have altered soil biotic communities promoting growth of conspecifics.

Contrary to our hypothesis, native seedling growth and reproductive effort were not significantly reduced when planted into soil inoculated with microbial communities associated with the wildtype variety. Interestingly, the responses of the two native grasses

to soil microbial communities associated with the wildtype were vastly different from one another. When compared to growth in native prairie soil the native *S. scoparium* exhibited no differences in total biomass produced when inoculated with soil communities associated with the wildtype, while *A. gerardii* experienced a significant increase in total biomass suggesting *A. gerardii* favors soil associated with the wildtype. When examining the abundance of AMF in the NLFA fraction, *S. scoparium* had substantially higher abundance of AMF when grown in soil associated with the wildtype compared to its own native prairie soil or the invasive *B. ischaemum* cultivar, while *A. gerardii* experienced no differences in abundance of AMF. Our results suggest that the *S. scoparium* also favors soil associated with the wildtype, as the high abundance of AMF indicates more energy is available to be stored in the NLFA fraction primarily used for spore development (Olsson et al. 1997).

Growth of dominant native grass species has been documented to consistently be reduced when grown in soil associated with the invasive *Bothriochloa* spp. (Wilson et al. 2012). In our study this pattern was not observed with the wildtype *B. ischaemum*. In contrast *A. gerardii* experienced an increase in total biomass production. Although, the wildtype did not reduce growth of the two native grasses, the response of increased growth by *A. gerardii* and abundance of AMF in *S. scoparium* indicates that the wildtype *B. ischaemum* had an influence on soil microbial communities. Our study indicates that both *B. ischaemum* varieties alter soil biota characteristics differently as the wildtype *B. ischaemum* exhibited enhanced growth in soil associated with the invasive *B. ischaemum* and the two native grasses favored soil associated with the wildtype. The different responses between the *B. ischaemum* varieties indicate that the invasive *B. ischaemum*

cultivar may have undergone evolutionary changes through selective breeding from its wildtype from the Czech Republic.

Interactions between plants and soil microbial communities are often times species-specific and changes in soil biota can alter competitive interactions between plant species (Parepa et al. 2013). Our study suggests that native grass species respond differently to soil communities previously occupied by *B. ischaemum*. On average *A. gerardii* had greater total biomass production and abundance of AMF when grown in soil associated with both the *B. ischaemum* varieties compared to *S. scoparium* indicating that it may be a superior competitor over *S. scoparium* in *B. ischaemum* invaded landscapes. Successful establishment of native species is essential when restoring areas previously eradicated of invasive species such as *B. ischaemum*. However, native species may have difficulties establishing in areas previously dominated by *B. ischaemum* especially if *B. ischaemum* alters native soil microbial communities (Wilson et al. 2012). Other grassland restorations have been met with difficulties in establishing native plant species following the alteration of native resident soil characteristics by invasive plant species (Jordan et al. 2011). However, when assessing the responses of multiple invasive and native plant species to soil microbial communities associated with the invaders Jordan et al. (2011) found that some native species were unaffected by such soil legacies left behind by invaders. These native plants therefore, may provide valuable services, restoring soil conditions needed for successful establishment of additional desirable native species in targeted restoration areas (Jordan et al. 2011). From a restoration standpoint selection and replanting of native species, such as *A. gerardii*, that have a greater tolerance to

alterations in soil microbial communities may lead to more successful restorations in *B. ischaemum* invaded landscapes.

CONCLUSION

The invasive *B. ischaemum* cultivar and the wildtype of *B. ischaemum* from the Czech Republic did exhibit differences in competitive abilities when compared to native grass species from the Great Plains, where the invasive cultivar is present. Contrary to our hypothesis, there were no overall increases in biomass production of the wildtype, nor reduction of native grass species biomass when the wildtype and native grass species were grown in competition with each other. However the invasive *B. ischaemum* exhibited a competitive advantage over the native grass *S. scoparium*. Our results suggest that the superior competitiveness of the invasive *B. ischaemum* cultivar over native grass species is a trait that is not present in its wildtype indicating that the selective and extensive breeding of *B. ischaemum* may have inadvertently developed certain characteristics enhancing its competitive abilities and leading to its success as an invader.

Additionally our results indicated both *B. ischaemum* varieties exhibited differential responses to dissimilar soil microbial communities relative to native grass species. Interestingly the wildtype was found to favor soil biotic communities associated with the invasive *B. ischaemum*. Total biomass production and abundance of AMF was significantly enhanced when the wildtype *B. ischaemum* was grown in soil associated with the invasive cultivar compared to native prairie soil from the Great Plains,

supporting preexisting evidence that the invasive cultivar somehow alters soil biotic communities promoting growth of conspecifics.

Furthermore, contrary to our hypothesis, growth and reproductive effort of natives were not significantly reduced when planted into soil associated with the wildtype variety and exhibited vastly different responses. Most notable is when compared to growth in native prairie soil the native *S. scoparium* exhibited no differences in total biomass produced when inoculated with soil communities associated with the wildtype while *A. gerardii* experienced a significant increase in total biomass. Future restoration efforts following eradication of *B. ischaemum* therefore, may have greater success in native species establishment by selecting native species, such as *A. gerardii*, that have a greater tolerance to soil legacy effects of invasive grasses.

REFERENCES

- Allison V.J., and R.M. Miller. 2005. Soil grinding increases the relative abundance of eukaryotic phospholipid fatty acids. *Soil Science Society of America Journal*. 69:423-426.
- Andonian K., and Hierro J.L. 2011a. Species interactions contribute to the success of a global plant invader. *Biological Invasions*. 13:2957-2965.
- Andonian K., Hierro J.L., Khetsuriani L., Becerra P., Janoyan G., Villarreal D., Cavieres L., Fox L.R., and Callaway R.M. 2011b. Range-expanding populations of a globally introduced weed experience negative plant-soil feedbacks. *PLOS one*. 6:e20117.
- Aschehoug E.T., Metlen K.L., Callaway R.M., and Newcombe G. 2012. Fungal endophytes directly increase the competitive effects of an invasive forb. *Ecology*. 93:3-8.
- Berg W.A., C.L. Dewald, R.L. Gillen, and P.L. Sims. 1993. Old World bluestem selection, establishment, and management. U.S. Department of Agriculture, Agricultural Research Service, Southern Plains Range Restoration Station, Woodward, Oklahoma.
- Bever J.D. 2003. Soil community feedback and the coexistence of competitors: conceptual frameworks and empirical tests. *New Phytologist*. 157:465-473.
- Bever J.D., Westover K.M., and Antonovics J. 1997. Incorporating the soil community into plant population dynamics: the utility of the feedback approach. *Journal of Ecology*. 85:561-573.
- Blossey B., and Nötzold R. 1995. Evolution of increased competitive ability in invasive non-indigenous plants: a hypothesis. *J Ecol* 83:887-889.
- Callaway R.M., and W.M. Ridenour. 2004. Novel weapons: invasive success and the evolution of increased competitive ability. *Front Ecol Environ*. 2:436-443.
- Callaway R.M., Thelen G.C., Rodriguez A., and Holben W.E. 2004. Soil biota and exotic plant invasion. *Nature*. 427:731-733.
- Callaway R.M., D. Cipollini, K. Barto, G.C. Thelen, S.G. Hallett, D. Prati, K. Stinson, and J. Klironomos. 2008. Novel weapons: invasive plant suppresses fungal mutualists in America but not in its native Europe. *Ecology*. 89:1043-1055.
- Callaway R.M., Bedmar E.J., Reinhart K.O., Silvan C.G., and Klironomos J. 2011a. Effects of soil biota from different ranges on *Robinia* invasion: acquiring mutualists and escaping pathogens. *Ecology* 92:1027-1035.

- Callaway R.M., L.P. Waller, A. Diaconu, R. Pal, A.R. Collins, H. Mueller-Schaerer, and J.L. Maron. 2011b. Escape from competition: Neighbors reduce *Centaurea stoebe* performance at home but not away. *Ecology* 92:2208-2213.
- Callaway R.M., U. Schaffner, G.C. Thelen, A. Khamraev, T. Juginisov, and J. L. Maron. 2012. Impact of *Acroptilon repens* on co-occurring native plants is greater in the invaders's non-native range. *Biological Invasions*. 14:1143-1155.
- Celarié R.P. and J.R. Harlan. 1995. Studies on Old World bluestems. 31p. Oklahoma Agr. Exp. Sta. Tech. Bull. T-58.
- Cooper K., and Lösel D. 1978. Lipid physiology of VA mycorrhiza. I. Composition of lipids in roots of onion, clover and ryegrass infected with *Glomus mosseae*. *New Phytol.* 80:143-151.
- Coyne P., and J. Bradford. 1985a. Morphology and growth in seedlings of several C₄ perennial grasses. *J Range Manage* 38:504-512.
- Coyne P., and J. Bradford. 1985b. Some growth characteristics of four Old World bluestems. *Journal of Range Management*. 38:27-33.
- Cui Q-G, and He W-M. 2009. Soil biota, but not soil nutrients, facilitate the invasion of *Bidens pilosa* relative to a native species *Saussurea deltoidea*. *Weed Research*. 49:201-206.
- Dahl B.E., P.F. Cotter, D.B. Wester, and C.M. Britton. 1988. Range plant establishment in the southern plains region, p. 42-46. *In: J.E. Mitcheel (ed.) Impacts of the Conservation Reserve Program in the Great Plains, USDA For. Serv. Rocky Mtn. For. and Range Exp. Sta. Gen. Tech. Rep. RM-158.*
- Darwin C. 1872. *The origin of species by means of natural selection.* John Murray, UK.
- Ehrenfeld J.G. 2004. Implications of invasive species for belowground community and nutrient processes. *Weed Technology*. 18:1236-1239.
- Elton C.S. 1958. *The ecology of invasions.* Methuen, London.
- Gabbard B.L., and N.L. Fowler. 2007. Wide ecological amplitude of a diversity-reducing invasive grass. *Biological Invasions*. 9:149-160.
- García Y., Callaway R.M., Diaconu A., and Montesinos D. 2013. Invasive and non-invasive congeners show similar trait shifts between their same native and non-native ranges. *PLOS one*. 8:e82281.
- Gooden B., French K., and Robinson S.A. 2014. Alien grass disrupts reproduction and post-settlement recruitment of co-occurring native vegetation: a mechanism for diversity decline in invaded forest? *Plant Ecology* 215:567-580.

- Harmoney K.R., and K.R. Hickman. 2004. Comparative morphology of Caucasian old world bluestem and native grasses. *Agronomy Journal*. 96:1540-1544.
- Harmoney K.R., P.W. Stahlman, and K.R. Hickman. 2007. Suppression of Caucasian old world bluestem with split application of herbicides. *Weed Technology*. 21:573-577.
- Harper J.L. 1977. *Population biology of plants*. Academic Press, San Diego.
- Hartnett D.C., and G.W.T. Wilson. 1999. Mycorrhizal mediation of plant species composition and diversity in tallgrass prairie. *Ecology* 80:122-130.
- Hartnett D.C., and G.W.T. Wilson. 2002. The role of mycorrhizas in plant community structure and dynamics: lessons from the grasslands. *Plant Soil* 244:319-331.
- He W-M., Y. Feng, W.M. Ridenour, G.C. Thelen, J.L. Pollock, A.Diaconu, and R.M. Callaway. 2009. Novel weapons and invasion: biogeographic differences in the competitive effects of *Centaurea maculosa* and its root exudate (\pm)-catechin. *Oecologia* 159:803-815.
- Herre E.H., N. Knowlton, U.G. Mueller, and S.A. Rehner. 1999. The evolution of mutualisms: exploring the paths between conflict and cooperation. *Trends Ecol Evol*. 14:49-53.
- Hetrick B.A.D., and G.W.T. Wilson. 1990. Differential responses of C₃ and C₄ grasses to mycorrhizal symbiosis, P fertilization, and soil microorganisms. *Can J Bot* 68:461-467.
- Hickman K.R., G.H. Farley, R. Channell, and J.E. Steier. 2006. Effects of old world bluestem (*Bothriochloa ischaemum*) on food availability and avian community composition within the mixed-grass prairie. *The Southwestern Association of Naturalists*. 51:524-530.
- Hierro J.L., Maron J.L., and Callaway R.M. 2005. A biogeographic approach to plant invasions: the importance of studying exotics in their introduced and native range. *Journal of Ecology*. 93:5-15.
- Hill K.C., and Fischer D.G. 2014. Native-exotic species richness relationships across spatial scales in a prairie restoration matrix. *Restoration Ecology* 22:204-213.
- Jakobs G., Weber E., and Edwards P.J. 2004. Introduced plants of the invasive *Solidago gigantea* (Asteraceae) are larger and grow denser than conspecifics in the native range. *Diversity and Distributions*. 10:11-19.
- Jordan N.R., Larson D.L., and Huerd S.C. 2011. Evidence of qualitative differences between soil-occupancy effects of invasive vs. native grassland plant species. *Invasive Plant Science and Management*. 4:11-21

- Keane R.M., and Crawley M.J. 2002. Exotic plant invasions and the enemy release hypothesis. *Trends Ecol Evol* 17:164-169.
- Klironomos J.N. 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature*. 417:67-70.
- Kumschick S., Hufbauer R.A., and Alba C., et al. 2013. Evolution of fast-growing and more resistant phenotypes in introduced common mullein (*Verbascum thapsus*). *Journal of Ecology*. 101:378-387.
- Lavergne S., and Molofsky J. 2007. Increased genetic variation and evolutionary potential drive the success of an invasive grass. *Proceedings of the National Academy of Sciences of the United States of America*. 104:3883-3888.
- Lee M.R., Flory S.L., and Phillips R.P. 2012. Positive feedbacks to growth of an invasive grass through alteration of nitrogen cycling. *Oecologia*. 170:457-465.
- Leiblein-Wild M.C., Kaviani R., and Tackenberg O. 2014. Germination and seedling frost tolerance differ between the native and invasive range in common ragweed. *Oecologia*. 174:739-750.
- Leger E.A. 2008. The adaptive value of remnant native plants in invaded communities: an example from the Great Basin. *Ecological Applications* 18:1226-1235.
- Mack R.N., Simberloff D, Lonsdale W.M., Evans H., Clout M., and Bazzaz F.A. 2000. Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological Applications*. 10:689-710.
- Marler M.J., Zabinski C.A., and Callaway R.M. 1999. Mycorrhizae indirectly enhance competitive effects of an invasive forb on a native bunchgrass. *Ecology* 80:1180-1186.
- Maron J.L., and M.J. Marler. 2008. Effects of native species diversity and resource additions on invader impact. *American Naturalist*. 172:S18-S33.
- McGonigle T.O., Miller M.H., Evans D.G., Fairchild G.L., and Swan J.A. 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytologist*. 115:495-501.
- Ni G-Y., U. Schaffner, S-L. Peng, and R.M. Callaway. 2010. *Acroptilon repens*, an Asian invader, has stronger competitive effects on species from America than species from its native range. *Biological Invasion*. 12:3653-3663.
- Olsson P.A., Baath E., and Jakobsen I. 1997. Phosphorus effects on mycelium and storage structures of an arbuscular mycorrhizal fungus as studied in the soil and roots by fatty acid signatures. *Appl. Environ. Microbiol.* 63:3531-3538.

- Pringle A., Bever J.D., Gardes M., Parrent J.L., Rillig M.C., and Klironomos J.N. 2009. Mycorrhizal symbioses and plant invasions. *Annual Review of Ecology, Evolution & Systematics*. 40:699-715.
- Reed H.E., T.R. Seastedt, and J.M. Blair. 2005. Ecological consequences of a C₄ grassland: a dilemma for management. *Ecological Applications*. 14:1560-1569.
- Reinhart K., and Callaway R.M. 2004. Soil biota facilitate exotic *Acer* invasions in Europe and North America. *Ecological Applications*. 14:1737-1745.
- Reinhart K.O., and Callaway R.M. 2006. Soil biota and invasive plants. *New Phytologist*. 170:445-457.
- Rojas-Sandoval J., Melendez-Ackerman E., and Fernandez D.S. 2012. Plant community dynamics of a tropical semi-arid system following experimental removals of an exotic grass. *Applied Vegetation Science* 15:513-524.
- Rout M.E., and Callaway R.M. 2012. Interactions between exotic invasive plants and soil microbes in the rhizosphere suggest that "everything is not everywhere". *Annals of Botany*. 110:213-222.
- Ross C.A., and Auge H. 2008. Invasive Mahonia plants outgrow their native relatives. *Plant Ecology*. 199:21-31.
- Sammon J.G. and K.T. Wilkins. 2005. Effects of an invasive grass (*Bothriochloa ischaemum*) on a grassland rodent community. *Texas Journal of Science*. 57:371-382.
- Sanon A., Beguiristain T., Cebon A., Berthelin J., Ndoye I., Leyval C., Sylla S., and Duponnois R. 2009. Changes in soil diversity and global activieites following invasions of the exotic invasive plant, *Amaranthus viridis* L. decrease the growth of native sahelian Acacia species. *FEMS Microbiology Ecology*. 70:118-131.
- Schmidt C.D., and K.R. Hickman. 2006. Stolon production by caucasian bluestem (*Bothriochloa bladhii*). *Trans Kansas Acad Sci*. 109:74-76.
- Schmidt C.D., K.R. Hickman, R. Channell, K. Harmony, W. Stark. 2008. Competitive abilities of native grasses and non-native (*Bothriochloa* spp.) grasses. *Plant Ecology*. 197:69-80.
- Siemann E., and Rogers W.E. 2001. Genetic differences in growth of an invasive tree species. *Ecol Lett* 4:514-518.
- Sun S-K. and He W-H. 2010. Evidence for enhanced mutualism hypothesis: *Solidago canadensis* plants from regular soils perform better. *PLOS One*. 5:e15418.

- Szente K, Z. Nagy, Z. Tuba et al. 1996. Photosynthesis of *Festuca rupicola* and *Bothriochloa ischaemum* under degradation and cutting pressure in a semiarid loess grassland. *Photosynthetica*. 32:399-407.
- Vogelsang K.M., J.D. Bever, M. Griswold, and P.A. Schultz. 2004. The use of mycorrhizal fungi in erosion control applications. Final report for Caltrans. California Department of Transportation Contract no. 65A0070. Sacramento, California.
- Vogelsang K.M., and J.D. Bever. 2009. Mycorrhizal densities decline in association with nonnative plants and contribute to plant invasion. *Ecology*. 90:399-407.
- Weidenhamer J.D, and Callaway R.M. 2010. Direct and indirect effects of invasive plants on soil chemistry and ecosystem function. *Journal of Chemical Ecology*. 36:59-69.
- Weihner E. 2007. On the status of restoration science: Obstacles and opportunities. *Restoration Ecology*. 15:340-343.
- White, D.C. and D.B. Ringelberg. 1998. Signature lipid biomarker analysis. *Techniques in Microbial Ecology*.
- Wilson G.W.T., and D.C. Hartnett. 1997. Effects of mycorrhizas on plant growth and dynamics in experimental tallgrass prairie microcosms. *Am J Bot* 84:478-482.
- Wilson G.W.T, and D.C. Hartnett. 1998. Interspecific variation in plant responses to mycorrhizal colonization in prairie grasses and forbs. *Am J Bot*. 85:1732-1738.
- Wilson G.W.T., K.R. Hickman, and M.M. Williamson. 2012. Invasive warm-season grasses reduce mycorrhizal root colonization and biomass production of native prairie grasses. *Mycorrhiza*. 22:327-336.
- Zou J.W., Rogers W.E., and Siemann E. 2008. Increased competitive ability and herbivory in the invasive plant *Sapindus sebiferum*. *Biol Invasion* 10:291-302.

TABLES

Table 1

Soil	Soil nutrient characteristics			PLFA abundance values (nmol/g)			
	pH	NO ₃ -N (lbs/A)	P (ppm)	AMF	Sap. fungi	Gram -	Gram +
Native Prairie	7.8	9.5	13.5	2.95	7.30	1.20	3.93
Steam Pasteurized Native Prairie	7.9	20.5	15	ND	ND	0.07	0.03
Invasive <i>B. ischaemum</i>	6.1	11	13.5	1.23	2.83	0.56	3.01
Vedlice Czech 1	7.9	2	5.83	1.24	3.60	2.06	10.82
Drahobuz Czech2	7.8	2	4	1.70	4.51	3.77	13.74
Prokopske Udoli Czech 3	7.8	4.67	14.17	0.87	3.97	4.95	16.54

Table 2

Species	Soil inoculum	PLFA abundance value (nmol/g)			
		AMF	Sap. Fungi	Gram -	Gram +
Invasive <i>B. ischaemum</i>	Invasive <i>B. ischaemum</i>	1.23	2.83	0.56	3.01
	Wildtype <i>B. ischaemum</i>	1.83	3.90	0.48	2.04
	Native Prairie	1.94	1.29	0.46	1.40
Wildtype <i>B. ischaemum</i>	Invasive <i>B. ischaemum</i>	0.92	3.17	0.65	4.07
	Wildtype <i>B. ischaemum</i>	1.90	2.05	0.39	2.03
	Native Prairie	3.19	1.67	0.70	2.34
<i>S. scoparium</i>	Invasive <i>B. ischaemum</i>	3.17	6.63	0.89	4.98
	Wildtype <i>B. ischaemum</i>	2.01	6.82	0.79	4.84
	Native Prairie	2.42	8.01	0.89	4.98
<i>A. gerardii</i>	Invasive <i>B. ischaemum</i>	2.44	2.42	0.45	1.99
	Wildtype <i>B. ischaemum</i>	1.29	3.00	0.55	3.13
	Native Prairie	3.01	1.51	0.54	2.24

FIGURES

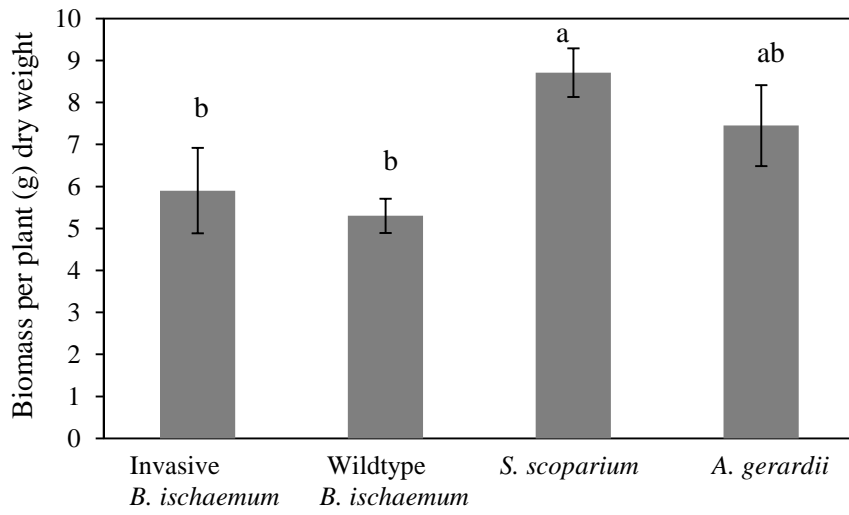


Figure 1

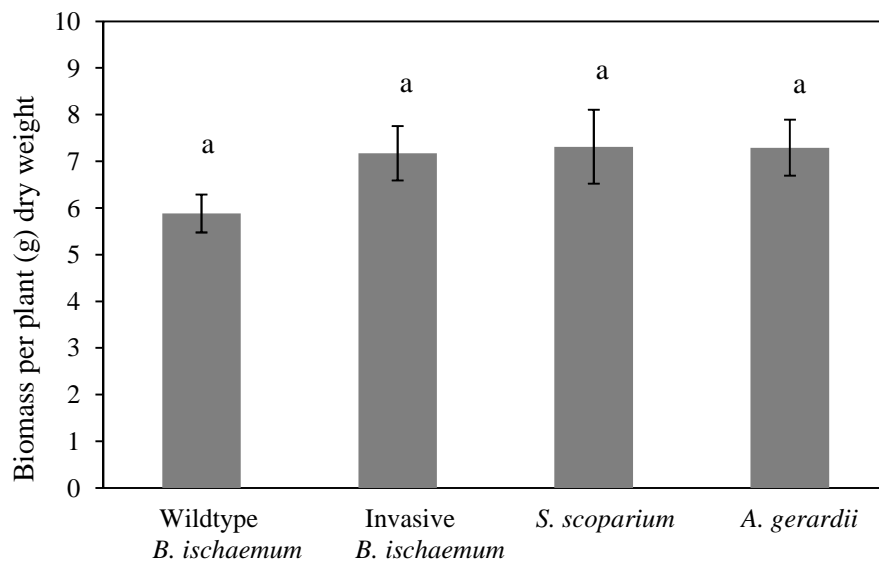


Figure 2

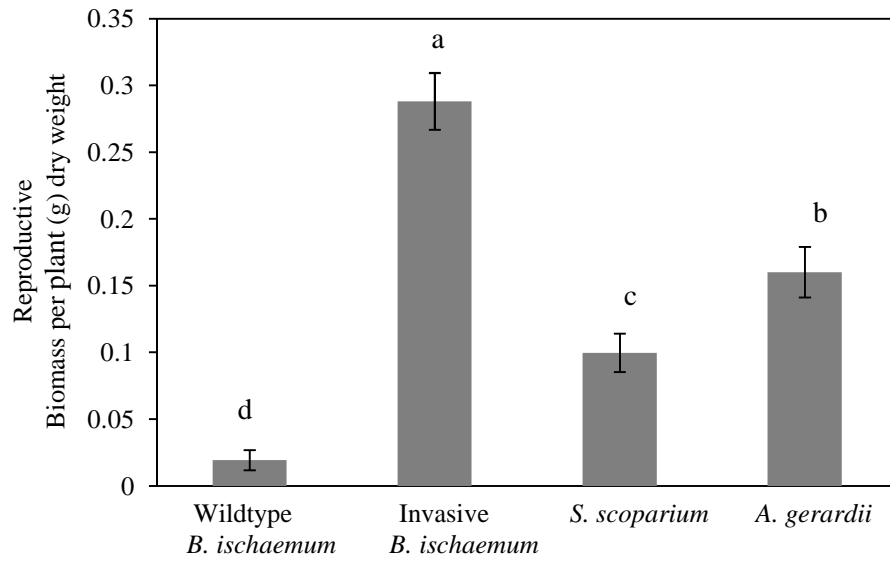


Figure 3

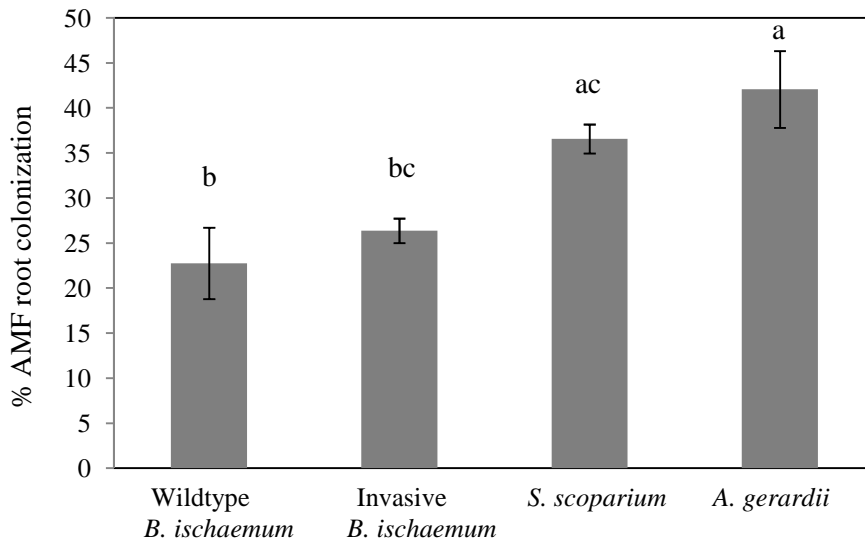


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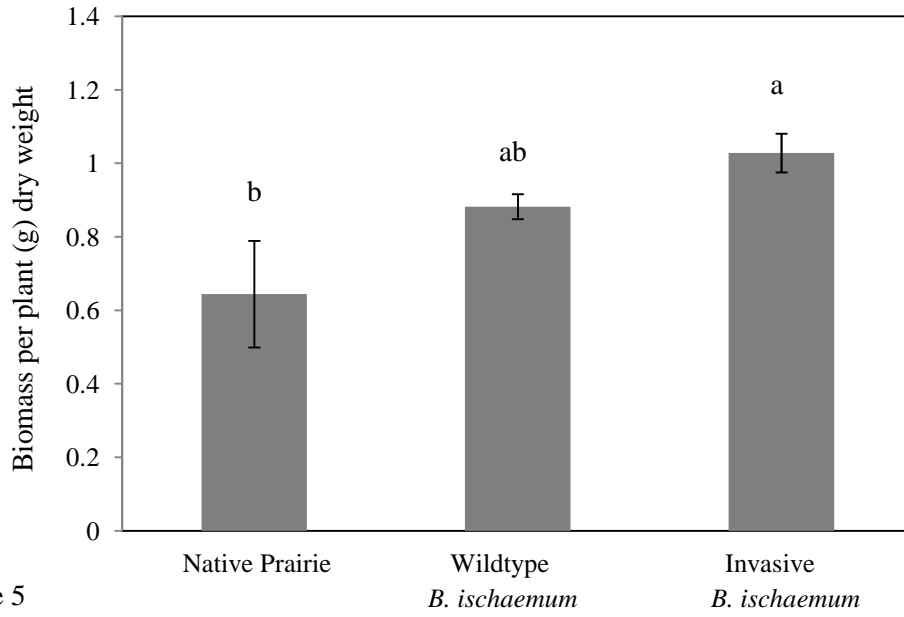


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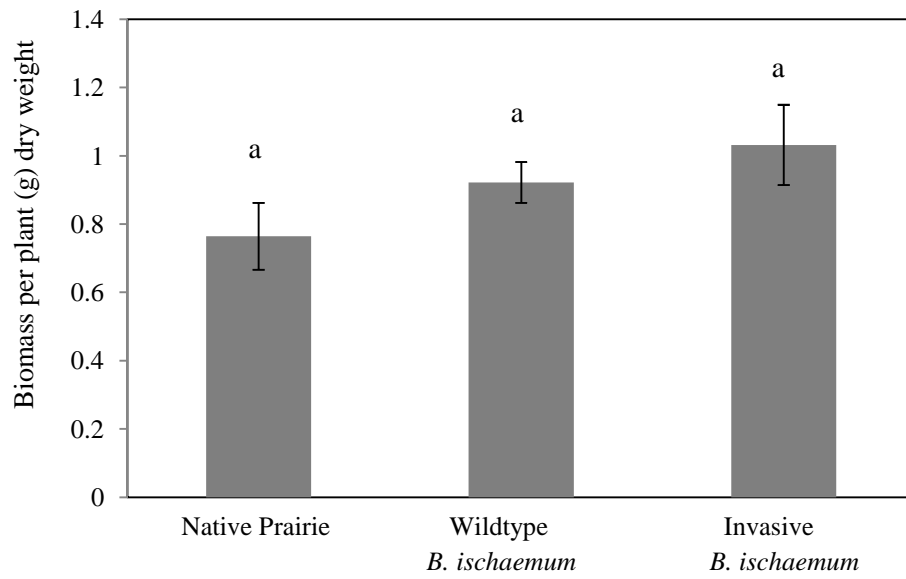


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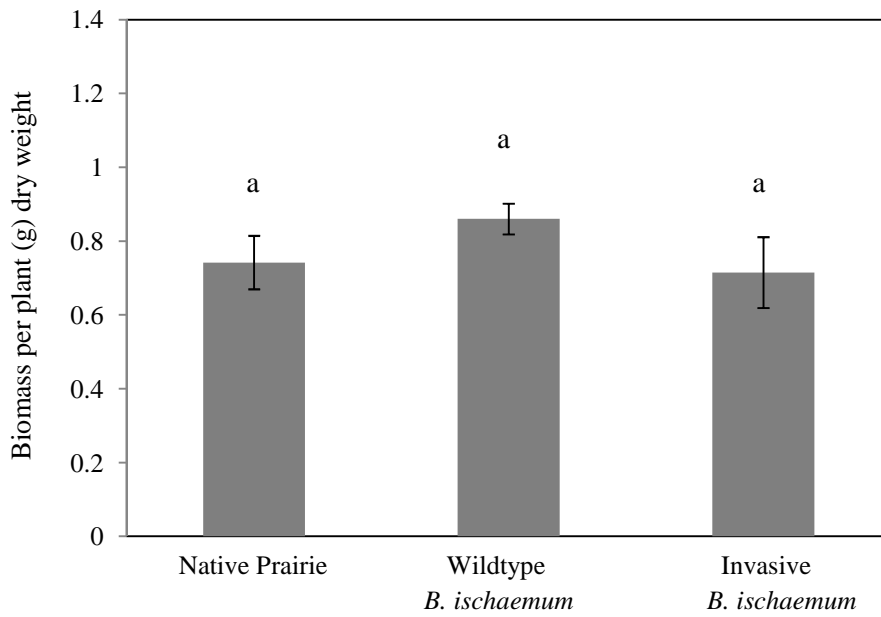


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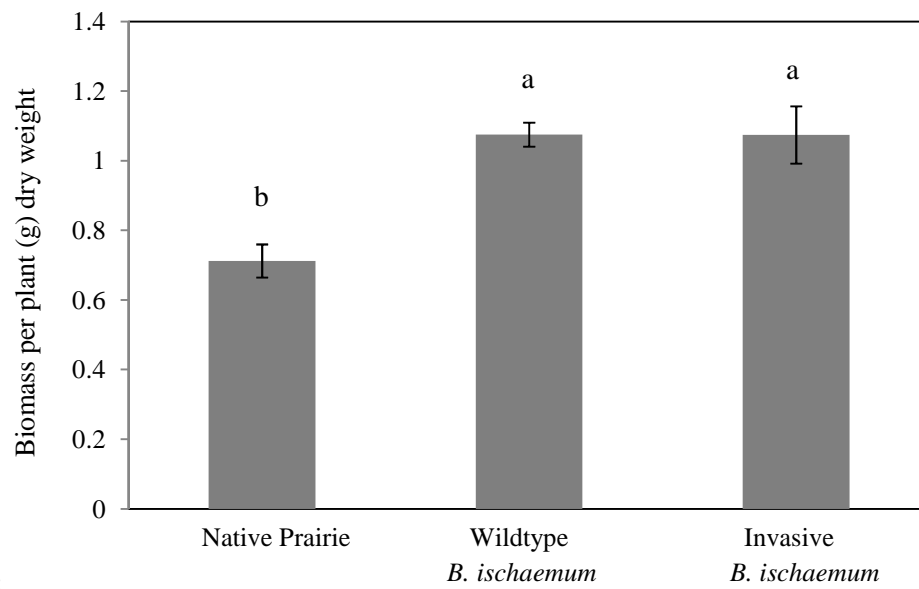


Figure 8

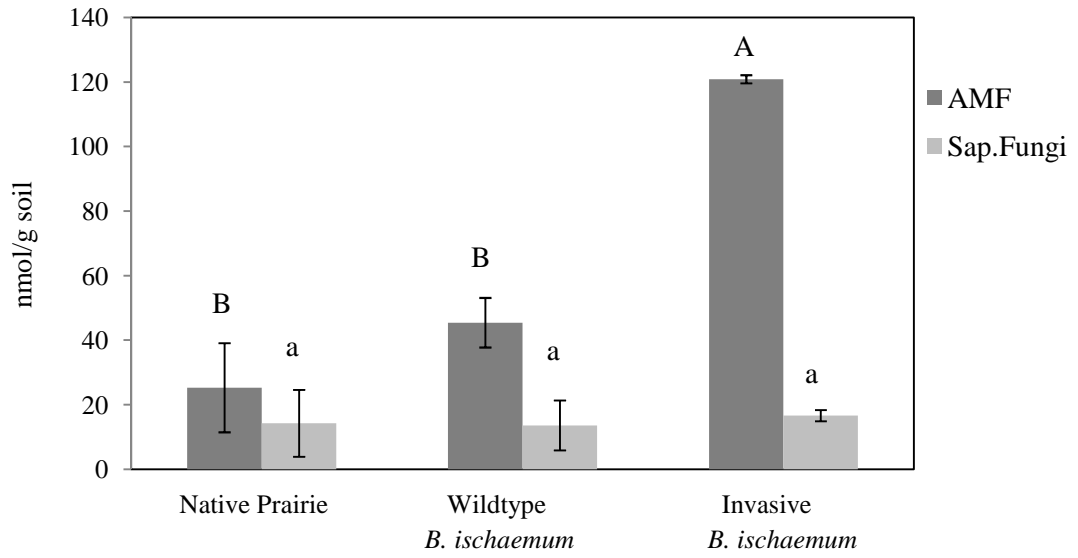


Figure 9

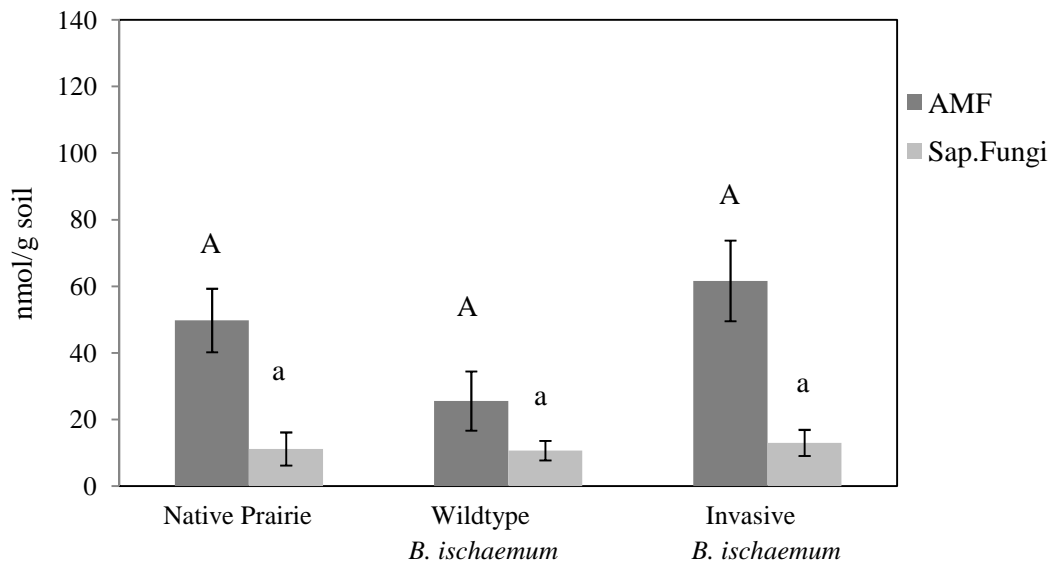


Figure 10

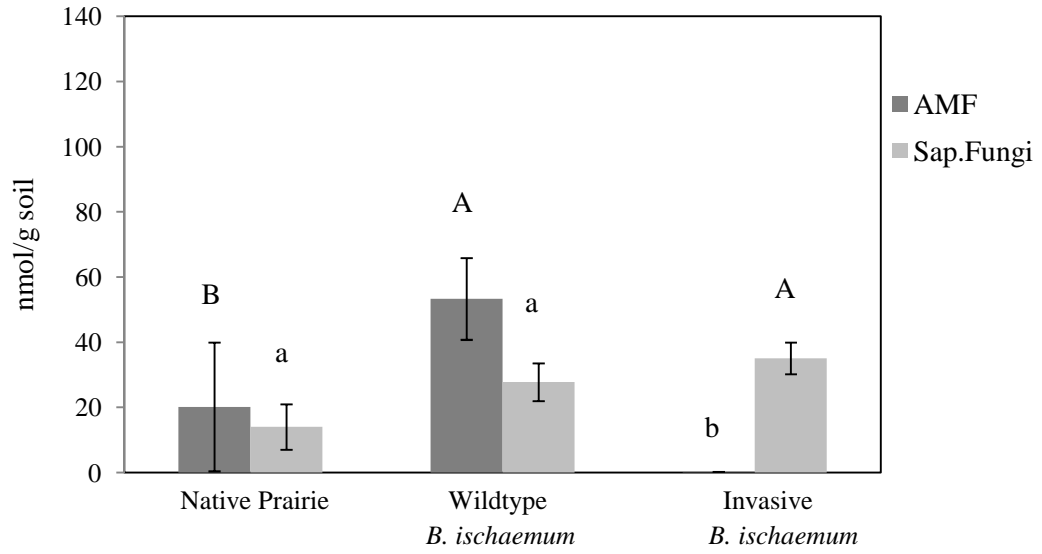


Figure 11

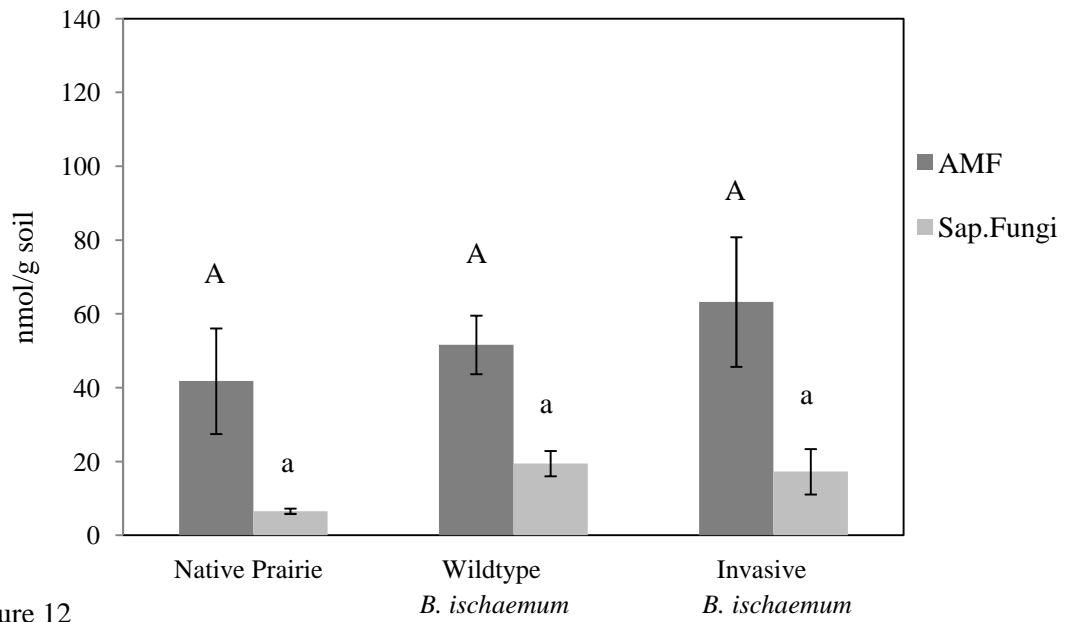


Figure 12

VITA

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