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Invasive warm-season grasses reduce mycorrhizal root colonization and biomass production of native prairie grasses

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Abstract Soil organisms play important roles in regulating ecosystem-level processes and the association of arbuscular mycorrhizal (AM) fungi with a plant species can be a central force shaping plant species' ecology. Understanding how mycorrhizal associations are affected by plant invasions may be a critical aspect of the conservation and restoration of native ecosystems. We examined the competitive ability of old world bluestem, a non-native grass (Caucasian bluestem [*Bothriochloa bladhii*]), and the influence of *B. bladhii* competition on AM root colonization of native warm-season prairie grasses (*Andropogon gerardii* or *Schizachyrium scoparium*), using a substitutive design greenhouse competition experiment. Competition by the non-native resulted in significantly reduced biomass production and AM colonization of the native grasses. To assess plant–soil feedbacks of *B. bladhii* and *Bothriochloa ischaemum*, we conducted a second greenhouse study which examined soil alterations indirectly by assessing biomass production and AM colonization of native warm-season grasses planted into soil collected beneath *Bothriochloa* spp. This study was conducted using soil from four replicate prairie sites throughout Kansas and Oklahoma, USA. Our results indicate that a major mechanism in plant growth suppression following invasion by *Bothriochloa* spp. is the alteration in soil microbial communities. Plant growth was tightly correlated with AM root colonization demonstrating that mycorrhizae play an important role in the invasion of these systems by *Bothriochloa* spp. and indicating that the restoration of native AM fungal communities may be a fundamental consideration for the successful establishment of native grasses into invaded sites.

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Introduction

Biological invasions by non-native plants incur tremendous economic and environmental impacts worldwide (Vitousek et al. 1997; Pimentel et al. 2000). Most previous studies describing invasibility by non-native species focus on aboveground features, with little attention given to the belowground environment, although soil organisms play important roles in regulating ecosystem-level processes (Levine et al. 2004). The association of arbuscular mycorrhizal (AM) fungi with a plant species can be the central force shaping the species' ecology (Herre et al. 1999), and introduced plant species have the potential to alter the density and/or composition of the mycorrhizal fungal community. These plant–fungal associations have been reported to both constrain (Vogelsang et al. 2004; Vogelsang and Bever 2009) and facilitate (Shah et al. 2009) the ability of a non-native species to successfully invade, and have been shown to influence the trajectory of the invasion process (Vogelsang and Bever 2009). Therefore, understanding how these associations influence plant invasions may be a critical aspect of the ecology and management of invasive plant species and the conservation and restoration of native ecosystems (Pringle et al. 2009).

The majority of invasions by non-native plants involve species that are functionally distinct from the dominant

native species (e.g., Vitousek and Walker 1989; Orr et al. 2005; Batten et al. 2006), and this can include their response to AM associations (Pringle et al. 2009; Seifert et al. 2009). Plants vary widely in their association with AM fungi, and Pringle et al. (2009) provide strong evidence that many invasive plants are facultatively mycorrhizal, forming associations with AM fungi or not, depending on the environment. This alternative dependency may be a successful strategy of some invasive plant species. In contrast, the growth and fitness of other invasive plant species are highly dependent on AM associations, and studies indicate there can be a promotion in plant invasibility through their association with native AM fungi (Shah et al. 2008, 2009).

Grasslands dominated by warm-season grasses have been shown to be highly resistant to invasion and establishment by non-native cool-season grasses and forb species (Smith and Knapp 1999; Fargione et al. 2003), functional groups that have been reported as facultatively mycotrophic (Wilson and Hartnett 1998). Therefore, the greatest threat to these grasslands may come from highly mycotrophic, non-native grasses belonging to the same ecosystem-level functional group as the dominant native species. Old world bluestems (*Bothriochloa bladhii* Retz. [Caucasian bluestem] and *Bothriochloa ischaemum* [yellow bluestem]) are a group of warm-season perennial grasses of Eurasian origin that are a great threat to native prairies of the southern and central Great Plains, as they are functionally similar to the dominant native warm-season grass species. In a study assessing AM colonization rates of tallgrass prairie species, warm-season prairie grasses were reported to be obligately mycorrhizal; the non-native nor the native seedlings were able to complete their life cycles in the absence of the AM symbiont when planted into low nutrient native prairie soil (Wilson and Hartnett 1998). In fact, *B. bladhii* AM root colonization rates were substantially higher than any of the 16 species of native warm-season grasses that this invasive frequently displaces.

Old world bluestem species have been seeded extensively in private land throughout the Great Plains through the USDA-NRCS Conservation Reserve Program. In addition, state Departments of Transportation have seeded *Bothriochloa* species along roadsides for soil erosion control. These species are known to occur in Texas, Oklahoma, Kansas, Missouri, Colorado, New Mexico, Ohio, Louisiana, and Florida (USDA; NRCS 2004). They are now common in roadsides and pastures, and are invading into adjacent native prairies (Harmoney et al. 2004). Previous studies have described a wide variety of invasive characteristics allowing *Bothriochloa* spp. (hereafter *Bothriochloa*) to out-compete dominant, native warm-season grasses (Harmoney and Hickman 2004; Reed et al. 2005; Schmidt et al. 2008). These grasses have multiple modes of reproduction, high fecundity, and the ability to make rapid adjustments to varying levels of nitrogen and water (Reed et al. 2005; Schmidt et al. 2008). To examine the competitive ability of two *Bothriochloa* species, *B. bladhii and B. ischaemum*, and assess the influence of these non-native species competition on AM symbiosis of native grasses, we conducted a study using a substitutive design greenhouse competition experiment. In this study (hereafter "competition study"), native grasses (big bluestem [*Andropogon gerardii*] or little bluestem [*Schizachyrium scoparium*]) were grown in monoculture or in combination with *B. bladhii* (Caucasian bluestem) in low nutrient native prairie soil. At harvest, we examined biomass production and percent AM root colonization of both native and exotic species. We hypothesized that *B. bladhii* would be highly colonized by AM fungi, whether grown in monoculture or with native grass seedlings, and would inhibit growth, reproduction, and AM colonization of the native grasses.

Given the high dependency of *Bothriochloa* on AM symbiotic associations, an important mechanism for these species' successful invasibility may be self-facilitation through modification of the mycorrhizal associations, leading to positive plant–soil feedbacks (Bever et al. 1997; Bever 2002, 2003). Feedback between soil organisms and plant growth has been shown to be an important force in structuring plant communities (Bever 2003; Reynolds et al. 2003). Positive feedback occurs when the soil organisms which proliferate in a plant's rhizosphere improve the growth of that particular plant, and negative feedback happens when the soil organisms reduce the growth of the plant in which they proliferate. To examine potential plant–soil feedbacks following *Bothriochloa* invasion, we conducted a second greenhouse study (hereafter "feedback study"). Feedbacks were evaluated by assessing biomass production of native grasses planted into *Bothriochloa* soil collected from *Bothriochloa* invaded sites. We hypothesized plant–soil feedbacks associated with these invasive grasses function as an alteration in biotic communities, including AM fungi, and native seedling growth would be inhibited when planted into soils collected from soils beneath *Bothriochloa*. We further hypothesized that if the feedback functions as an alteration in biotic communities, plant growth suppression would be alleviated if the soil was sterilized and re-inoculated with freshly collected soil from beneath native grasses.

Materials and methods

Competition study

A greenhouse study was conducted to assess inter- and intraspecific competitive ability of native grasses and *B.*

bladhii (Caucasian bluestem) and to quantify arbuscular mycorrhizal root colonization of the invasive and native grasses. Seeds of non-native *B. bladhii* and two native tallgrass prairie grasses (big bluestem [*A. gerardii*], and little bluestem [*S. scoparium*]) were germinated in vermiculite. Seeds of the native grasses were obtained from the USDA Natural Resources Conservation Service Plant Materials Center, Manhattan, Kansas. The seeds of *B. bladhii* were hand-collected from Konza Prairie Biological Station (KPBS), Manhattan, Kansas. Fourteen days (second-leaf stage) after emergence seedlings were transplanted into 4 L pots (21.5 cm diameter×21.5 cm depth) containing 5.25 kg (dry weight) of native prairie soil. Native prairie soil was collected from a site near the headquarters area of KPBS that was dominated by native warm-season grasses (*A. gerardii*, *Sorghastrum nutans*, and *S. scoparium*), sieved through a 2-mm sieve to remove large plant roots, rhizomes, and stones, and transported to greenhouses at Oklahoma State University. The soil contained 12.0 mg kg^{-1} plant-available P (Mehlich test 3), 14.4 mg kg^{-1} NH_4, and 9.2 mg kg^{-1} NO_3. Soil samples were analyzed by the Oklahoma State University Soil, Water and Forage Analytical Laboratory, Stillwater, OK.

To determine the effects of intraspecific and interspecific competition on the growth of these three grass species (*A. gerardii*, *S. scoparium*, and *B. bladhii*), a substitutive design (Harper 1977) was utilized. This study consisted of an intraspecific control with eight seedlings (*A. gerardii*, *S. scoparium*, or *B. bladhii*) planted evenly spaced into each pot. To examine the interspecific competition between native grasses and the invasive, four native seedlings were paired with four *B. bladhii* seedlings. Each consisted of six replicate pots.

Pots were arranged in a randomized complete block design in a greenhouse maintained at 20–25°C, watered daily, and fertilized every 3 weeks with 0.59 g Peter's No-Phos Special Fertilizer solution (25–0–25) in 100 ml water to give 35 mg N g^{-1} soil. After 14 weeks, shoots were harvested and vegetative and reproductive components were determined. Reproductive structures included the stalk supporting the seed head, as well as the inflorescence itself. Roots were separated by species and washed free of soil. Plants were dried in a 60°C oven for 3 days, and root, shoot, and reproductive dry weights were measured to the nearest milligram. Subsamples of dried roots were stained in trypan blue using the method of Koske and Gemma (1989) and scored for AM root colonization using the magnified gridline intersect method developed by McGonigle et al. (1990). This method uses a compound microscope (200–400×) to quantify cortical root length colonized by intraradical hyphae, vesicles, arbuscules, and coil structures.

Feedback study

Our feedback study examined soil alterations indirectly by assessing growth and establishment of native warm-season grass species planted into soil collected from beneath plants of monoculture stands of two species of non-native grasses (*B. bladhii* or *B. ischaemum*), or from interstitial areas between *B. bladhii*. As a control, plant biomass production was assessed in plants grown in soils collected beneath native warm-season grasses in adjacent, non-invaded areas at each site.

Soil collection We collected soil from four prairie sites; two sites in Kansas—(1) Fort Hays State University Albertson Pasture, Hays, KS (FHSU) and (2) Konza Prairie Biological Station, Manhattan, KS (KPBS); and two in Oklahoma—(3) Marvin Klemme Range Research Area, Bessie, OK (MKRR) and (4) Stillwater Research Range, Stillwater, OK (SRR; Table 1). All of these sites are frequently burned, native warm-season prairies that have experienced invasion by either one or both of the *Bothriochloa* species over the previous 10–20 years. However, *Bothriochloa* species varied between the four prairie locations due to invasion history (Table 1).

Invasive *Bothriochloa* create easily identifiable patches where they invade and establish so that areas dominated by these species are well delineated from adjacent non-invaded native prairie. Native soils were collected from these adjacent sites dominated by warm-season native prairie grasses (*A. gerardii*, *S. scoparium*, and *S. nutans*). At KPBS and SRR, areas dominated by *B. bladhii* had large spaces of bare soil between individual bunches of the non-native grass (as described by Reed et al. 2005). Therefore, in these two sites, we established plots beneath bunches and between bunches (interstitial) of *B. bladhii*. At FHSU, *B. bladhii* grows predominantly as a rhizomatous grass and does not have the caespitose morphology observed at KPBS and SRR; therefore, no interstitial sites were available for soil collection at FHSU. *B. ischaemum* grows as a rhizomatous grass and does not form interstitial bare areas. At each site, a 12×12-m plot was established in the invaded areas (*B. ischaemum* and/or *B. bladhii*; Table 1) and adjacent native prairie. A 10-m transect was established diagonally through the center of each 12×12 m plot and at each 1 m interval the closest *Bothriochloa* or native plant was marked, for a total of ten selected plants per plot. Soil was collected from the top 15 cm beneath each of these ten plants and a 50-g subsample of soil from each plant was placed into a plastic bag for soil chemical analyses (soil pH, inorganic NH_4^+-N, NO_3-N, and H_2PO_4; Table 2); the remaining soil from each transect was homogenized. For the interstitial plots, at each 1 m interval the closest bare soil area was designated and the soil was collected to a

Table 1 Soil was collected from beneath native and non-native plants at each prairie site

Plant species varied between prairie sites due to presence of the invasive *Bothriochloa bladhii* or *Bothriochloa ischaemum*

Site	Soil collection site			
	Native species	*B. bladhii*	Soil between *B. bladhii*	*B. ischaemum*
FHSU, KS	X	X		X
KPBS, KS	X	X	X	
MKRR, OK	X			X
SRR, OK	X	X	X	X

depth of 15 cm, for a total of ten interstitial areas per plot. Soil was transported to a laboratory at Oklahoma State University for processing and experimental set-up. One half of the soil from each target site was steam pasteurized for 2 h at 80°C and allowed to cool and equilibrate for 14 days. All soil analyses were conducted by the Oklahoma State University Soil, Water and Forage Analytical Laboratory, as previously described.

Seedling preparation and experimental set-up Seeds of *A. gerardii* and *S. scoparium* were germinated in vermiculite and transplanted 14 days (second-leaf stage) after emergence. Seeds of the native grasses were obtained from the USDA Natural Resources Conservation Service Plant Materials Center, Manhattan, Kansas. Twelve plastic pots (6 cm diameter×25 cm deep) were individually filled with 600 g (dry weight) of steam-pasteurized soil collected from each soil collection site, and 12 pots were filled with 600 g (dry weight) of non-sterile soil from the respective site. The pots containing steamed soil were inoculated with 15 g of fresh (living) soil collected beneath native grasses (dominated by *A. gerardii* and *S. scoparium*) in non-invaded prairie from each respective site. The living soil inoculum was added directly below the seedling roots during transplantation. Six seedlings of each species were individually transplanted into pots containing one of two soil treatments, non-sterile or amended soil from each prairie site. Thus, this study consisted of 12 soil collection sites (see Table 1)×2 soil treatments×2 native host plant species×6 replicate pots per treatment for a total of 288 pots.

Experimental design and maintenance Pots were arranged in a randomized complete block design in a greenhouse maintained at 20–25°C. Plants were harvested after 14 weeks. Roots were washed free of soil and shoot, and root biomass was oven-dried for 72 h at 60°C. Shoot, root, and total dry weights were determined. Roots were subsampled, stained with trypan blue, and scored for intraradical AM colonization using the magnified gridline intersect method (McGonigle et al. 1990).

Statistical analysis Data from both greenhouse studies were analyzed using ANOVA with a complete block design (SAS Institute Inc., Cary, NC). Variances were determined to be homogeneous according to Levene's test for homogeneity of variance prior to analysis. No clear trends were observed following assessment of individual types of AM fungal structures (hyphae, vesicles, arbuscules, and coil structures); therefore total AM colonization is presented. Shoot and root dry weights were each highly correlated with total dry weight for each study (competition and feedback). Thus, for simplification of data presentation, only total dry weights are presented. Root/shoot ratios were assessed, but no clear trends were observed and these data are not shown.

For the feedback study, soil characteristics of the potted soil included pH, inorganic NH_4^+-N, NO_3-N, and H_2PO_4. These characteristics were analyzed using analysis of variance for each site (FHSU, KPBS, MKRR, SRR) × soil source (plant species that soil was collected beneath). Because interactions involving soil source were not significant, the data were reanalyzed as a one-way analysis of variance to compare sites. Mean soil characteristic values are presented for each site. Prior to transplantation of seedlings into greenhouse pots, soil chemical analyses (pH, inorganic NH_4^+-N, NO_3-N, and H_2PO_4) were conducted before and after steam pasteurization. Soil chemical

Table 2 Soil N and P availability and pH at the four prairie sites

Within site, soil characteristics of native, non-native, and interstitial sites were not significantly different ($P>0.05$), and means (±SE) are averaged within site

Site	Soil nutrient characteristics			
	pH	N-NO_3	N-NH_4	P-PO_4
FHSU, KS ($n=18$)	7.3 (±0.51)	3.89 (±0.50)	11.64 (±1.55)	21.2 (±1.81)
KPBS, KS ($n=18$)	6.2 (±0.23)	1.57 (±0.29)	5.82 (±0.73)	8.6 (±2.93)
MKRR, OK ($n=12$)	6.7 (±0.51)	1.80 (±0.28)	8.62 (±1.71)	13.9 (±2.60)
SRR, OK ($n=24$)	6.1 (±0.31)	1.47 (±0.32)	4.43 (±0.58)	16.9 (±3.11)

analyses did not differ significantly between non-sterile soil and soil that was steam pasteurized and allowed to rest and equilibrate for 14 days.

Analysis of plant biomass production was conducted using a four-way analysis of variance for species (*A. gerardii*, *S. scoparium*) × soil source (plant species that soil was collected beneath) × soil treatment × site. Because the interactions involving species were not significant, the data were reanalyzed as three-way analyses of variance: soil source × soil treatment × site. Correlation and stepwise regression analyses were used to examine the relationships between mycorrhizal root colonization and total plant biomass production. Root colonization was highly correlated with total plant biomass for each of the two native species for each of the soil collection sites (soil collected beneath native plant species, non-native plant species, or interstitial areas). Slopes for the two native species at each of the collection sites were compared to potentially combine sites for additional correlation and regression analysis (SAS Institute Inc., Cary, NC).

Results

Competition study In this study, we assessed inter- and intraspecific competitive ability of native grasses (*A. gerardii* and *S. scoparium*) with a non-native grass (*B. bladhii*). Both native grasses failed to grow beyond germination when planted with the non-native grass (Fig. 1 a, b). Biomass production of individual *B. bladhii* plants was greater when grown with either of the native grasses, as compared to growth with conspecifics (Fig. 1 a, b). Non-native plants produced reproductive structures in all treatments, while the native plants did not sexually reproduce in any treatment (Fig. 1 a, b). Percent AM root colonization of the native grasses was not significantly different from *B. bladhii* when the species were grown with their own conspecifics (Fig. 1 c, d). However, colonization of both native species was significantly reduced when these grasses were grown with *B. bladhii*.

Feedback study We examined (1) the potential for native seedlings to establish in soil collected beneath *B. ischaemum* or *B. bladhii* and (2) the influence of native microbial communities added to sterilized soil collected beneath non-native grasses. Seedlings were also planted into native prairie soil as a control. Native soil was collected from beneath native warm-season grasses directly adjacent to the invaded areas. Because the presence of the non-native grasses varied among our four sites, treatments were not consistent across all sites (Table 1) making comparisons among sites difficult. Therefore, plant growth (biomass production) and AM root colonization from each of our four sites are presented separately (Fig. 2 a–d). Soil nutrient data indicated all four sites contained relatively low plant-available N and P and are typical of tallgrass prairie soils within the Great Plains (Johnson et al. 2010; Wilson et al. 2009; Hartnett and Wilson 1999). Soil pH for all sites was in the neutral range (6.1–7.3), also typical of soils from prairie sites (Johnson et al. 2010; Wilson et al. 2009). Soil pH, inorganic NH_4^+-N, NO_3-N, and H_2PO_4 were not significantly different between invaded and native areas at

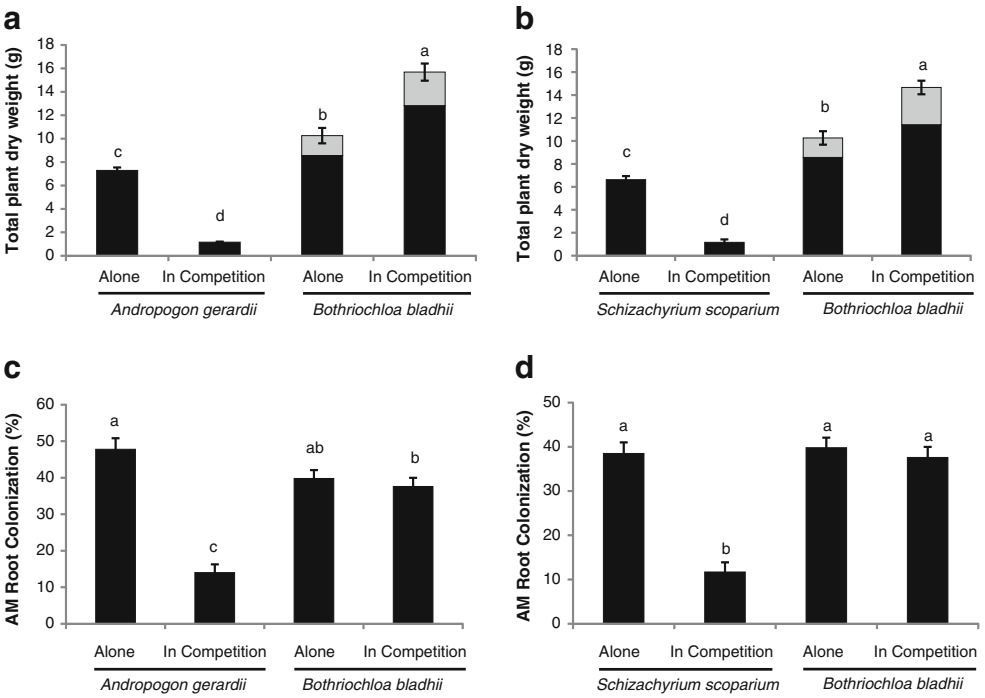

Fig. 1 Total vegetative plant dry weight (shoot plus root; *solid bars*) and reproductive dry weight (*shaded bars*) of **a** *Andropogon gerardii* and **b** *Schizachyrium scoparium* grown in monoculture and in combination with *Bothriochloa bladhii*; and percent arbuscular root colonization of **c** *A. gerardii* and **d** *S. scoparium* grown in monoculture and in combination with *B. bladhii*. *Error bars* show ±1 standard error. *Bars with the same letter* are not significantly different ($P \leq 0.05$)

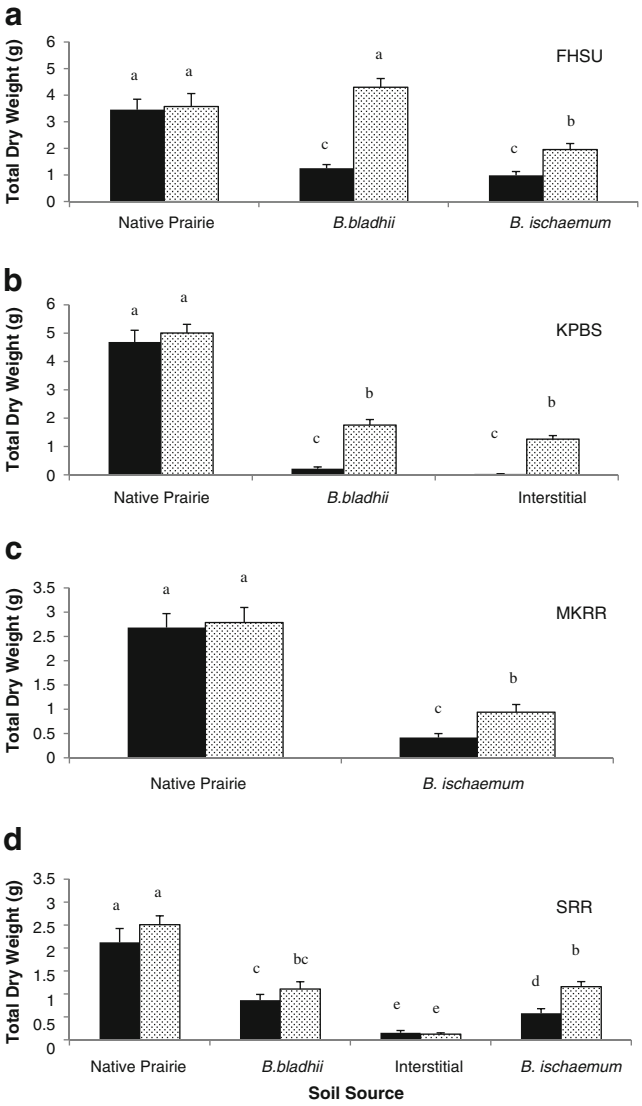

Fig. 2 Total plant dry weight (shoot plus root) of native warm-season grasses (combined mean of *Andropogon gerardii* and *Schizachyrium scoparium* biomass production) grown in soil collected from native, non-native, or interstitial areas at **a** Fort Hays State University Albertson Pasture, Hays, KS (FHSU); **b** Konza Prairie Biological Station, Manhattan, KS (KPBS); **c** Marvin Klemme Range Research Area, Bessie, OK (MKRR); and **d** Stillwater Research Range, Stillwater, OK (SRR). Prior to planting, soil was left non-sterile and non-inoculated (*solid bars*) or steam pasteurized and inoculated with15 g native prairie soil (*shaded bars*). *Error bars* show ±1 standard error

any site, and soil characteristics were not significantly different among the four prairie sites (Table 2).

Biomass production and AM root colonization of the native species were consistently reduced when seedlings were planted into soil collected from beneath or between the non-native grasses, as compared to biomass and root colonization of plants grown in soils from adjacent non-invaded prairie. At each site, re-inoculating sterilized native soils with native inoculum did not affect biomass production or AM colonization of either native species, indicating the process of sterilization (steam pasteurization) did not induce adverse chemical or physical effects. However, additions of native soil to sterilized soil collected beneath *Bothriochloa* consistently increased biomass production and AM root colonization, compared to growth in non-sterile soil collected beneath *Bothriochloa*. Total plant dry weight of each native grass was strongly correlated with percent AM root colonization (Fig. 3a, b) across collection sites, soil sources, and soil treatment.

At two of our sites, KPBS and SRR, areas dominated by *B. bladhii* were characterized by interstitial areas between *B. bladhii* grass bunches, with large spaces of bare soil between individual bunches of the non-native grass. Seedlings were unable to grow and did not survive beyond the seedling stage when planted into soil collected from interstitial areas of either KPBS or SRR site (Fig. 2 b, d), and roots of seedlings grown in the interstitial soil were not colonized by AM fungi (colonization was closely correlated with biomass and these results are shown in Fig. 3 a, b). Inoculation with living native soil inoculum resulted in a significant increase in biomass production (Fig. 2 b) and an increase in AM root colonization in the KPBS soil, as

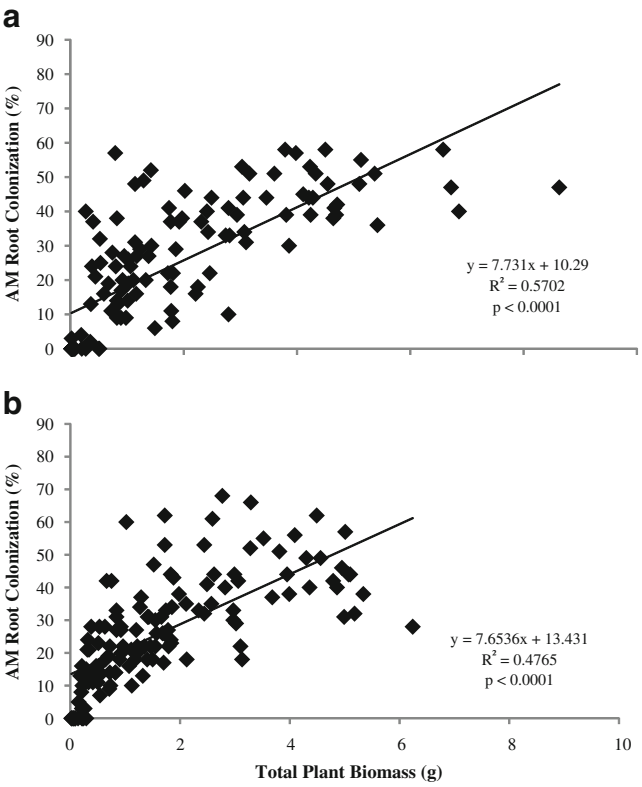

Fig. 3 Relationship between arbuscular mycorrhizal root colonization and total dry weight (shoot plus root) of **a** *Andropogon gerardii* and **b** *Schizachyrium scoparium* across all treatments (n=144: soil collected beneath native and non-native warm-season grasses; non-sterile and sterilized/inoculated soils)

compared to growth in non-sterile interstitial soil. However, seedlings planted into interstitial soil collected from SRR were unable to grow with or without the addition of native inoculum (Fig. 2 d) and none of the roots from this treatment were colonized with AM fungi.

Discussion

The presence of *B. bladhii* resulted in reduced above- and belowground growth of both native grasses. In fact, the native species (*A. gerardii* or *S. scoparium*) were unable to grow beyond the seedling stage when paired with *B. bladhii* and did not competitively inhibit growth of the invasive (*B. bladhii*). Instead, the invasive plants produced significantly greater above- and belowground biomass when grown in combination with the native species, presumably due to reduced intraspecific competition. Examining dry weight on an individual plant basis, growth of the invasive was significantly greater when paired with the native species, as compared to growth when planted in monoculture. Because the native species were unable to grow in the presence of the invasive grass, there were essentially fewer *B. bladhii* plants per pot (4) competing for resources when planted with native seedlings than when planted with conspecifics (8). In a target-neighbor greenhouse study conducted in high nutrient soil, similar inhibition of *S. scoparium* growth was observed when *B. ischaemum* was included as a neighbor (Schmidt et al. 2008). In that study, above- and belowground biomass production of *S. scoparium* grown in competition with *B. ischaemum* were significantly reduced by 18% and 22%, respectively, and the authors concluded that this invasive grass was competitively superior, resulting in the competitive exclusion of native species and potentially a successful invader of *S. scoparium* dominated grasslands.

In our greenhouse study, plants were grown for 14 weeks, by which time all of the *B. bladhii* plants had produced inflorescences. However, the native species (*A. gerardii* or *S. scoparium*) exhibited no evidence of inflorescence initiation at the time of harvest. One characteristic previously attributed to successful invasibility of *B. bladhii* is rapid growth and the ability to reach sexual maturity before native grass species (Harmoney and Hickman 2004). Our study clearly indicates that when planted into native prairie soils with native seedlings, *B. bladhii* successfully inhibited growth of the native species with rapid growth beyond the seedling stage, and reached sexual maturity earlier than the native grass species. Therefore, our study concurs with Harmoney and Hickman (2004) that rapid growth and early flowering may be mechanisms for the superior competitive advantage of *Bothriochloa*.

Percent mycorrhizal root colonization of the native grasses (both *A. gerardii* and *S. scoparium*) was significantly higher than the colonization of *B. bladhii* roots, when the plants were grown in pots with conspecifics. However, colonization of the native grasses was substantially reduced when planted with the non-native grass. *B. bladhii* is highly dependent on AM symbiosis (Wilson and Hartnett 1998) and, therefore, may be pre-adapted to successfully establish with native AM fungal communities. Schmidt et al. (2008) and our current study observed rapid growth and earlier flowering of the invasive grasses, compared with the natives. This rapid growth might allow for the non-native to preempt AM fungal communities more favorable to its own growth. Additionally, establishment of a direct transfer of resources or fixed carbon between plant species via AMF connections has been reported (Grime et al. 1987; Watkins et al. 1996), and nutrient transfer via AM mycelium has been shown to be unequal between neighboring species (Wilson et al. 2006). Therefore, rapid growth of the non-native might also allow for the establishment of a species-specific network between the exotic grasses at the expense of the native seedlings.

One mechanism for the loss of the ability for the native seedlings to be colonized by AM fungi could be the result of a shift in AM fungal species due to fungal selection by the invasive. Mycorrhizal fungi can contribute to competitive exclusion and decrease native plant fitness if AM fungi favor a superior, non-native competitor (Allen and Allen 1990). Non-native plant species have the potential to alter the density and/or composition of the mycorrhizal fungal community, which may lead to positive feedback and subsequent spread of the introduced plant species (Bever et al. 1997, 2002; Bever 2003). Competition occurs among AM fungal species (Hepper et al. 1988; Cano and Bago 2005) and competition between two or more fungi can result in the exclusion of an AM fungal species from host roots and a resultant alteration in AM fungal species community (Bennett and Bever 2009). The recent discovery that plants preferentially allocate carbon to individual AM fungi that benefit them the most (Bever et al. 2009) suggests that host plants are capable of exerting sanctions on fungal strains that are not useful trading partners (Kiers and Denison 2008). Changes in the composition of the AM fungal community have been observed for several introduced plant species in Canada (Klironomos 2002) and have been implicated as an important factor in the success of Asian knapweed (*Centaurea maculosa*) when invading native prairies in North America (Marler et al. 1999). Hawkes et al. (2006) and Mummey and Rillig (2006) provide further evidence as the composition of AM fungi in roots of native plants is different than that of *Centaurea*, and when grown together, AM fungal community composition was altered in roots of native plants neighboring *Centaurea* (Mummey et al. 2005).

Previous research on plant invasions make it clear that there is no single mechanism to explain invasibility, and invasions by non-natives are likely to be dependent on a multitude of factors such as reproductive potential, competitive ability, allelopathic chemical production, and soil or other habitat characteristics (Leger and Rice 2003; Blair and Wolfe 2004; Mitchell et al. 2006; Inderjit and van der Putten 2010). The subsequent study was initiated to further examine potential plant–soil feedbacks driven through modifications of the soil microbial community following *Bothriochloa* invasions.

Data from the feedback study indicate that native species biomass production was consistently reduced when planted into soil collected from beneath or between the non-native warm-season grasses, as compared with native prairie soil collected from adjacent areas. This is true regardless of the species of *Bothriochloa* (*B. bladhii* or *B. ischaemum*) and regardless of the prairie collection site (FHSU, KPBS, MKRR, SRR). We also observed a strong positive relationship between AM colonization and plant biomass production, which was consistent for both native species (*A. gerardii* and *S. scoparium*) and across all four prairie sites. These native seedlings grew very poorly, or were unable to grow, in soil collected from beneath *Bothriochloa*, and roots were poorly colonized by AM fungi. There is strong evidence from these data that the non-native grasses altered soil biotic characteristics. When additions of live (non-sterile) soil collected from adjacent native prairie sites were added to sterilized soil from the invaded sites, increased biomass production of native grasses was consistently observed. Across all sites, AM root colonization of the native grasses was strongly correlated with biomass production, indicating that alterations in AM fungal communities may be contributing to the lack of growth in *Bothriochloa* soil. However, due to the many soil microorganisms that can affect plant dynamics (Bever 2003), we cannot definitively attribute our increase in plant biomass production to any one group of organisms. Indeed, exotic plant species have been reported to alter both the composition and functional properties of soil biota in their rhizosphere, and changes in microbial communities have been observed that are directly ascribed to differences in exotic plant root systems, compared with native plants (Ehrenfeld 2004; Weidenhamer and Calloway 2010). However, the close positive relationship between biomass production and AM root colonization is consistent with our hypothesis that mycorrhizae play an important role in the re-establishment of native prairie grasses, indicating the native AM fungal community may be a fundamental consideration to the successful establishment of native grasses into invaded sites.

The important role that AM symbiosis has on the survival and competition of native prairie grasses has been well documented; and the absence of the mycorrhizal symbiosis can alter the competitive outcome of these dominant native grassland species (Hetrick et al. 1990; Wilson and Hartnett 1997; Hartnett and Wilson 1999; Hartnett and Wilson 2002). Therefore, the dominance that the native warm-season grasses hold in these grassland ecosystems is highly dependent on their mycorrhizal status. These functionally similar non-natives appear to use similar, but more effective, methods as those used by the native grasses to achieve dominance in these ecosystems. This results in the transformation of fundamental ecosystem traits, or changing the competitive dynamics of the native community, rather than large ecosystem-level changes. This may be an exceptionally insidious invasion technique, with these invasive grasses disrupting the evolutionary stable relationships native grasses rely on for dominance and survival, thereby "beating them at their own game." Because rangelands dominated by warm-season grasses have been shown to be highly resistant to invasion and establishment of non-native cool-season grasses and invasive forb species (Smith and Knapp 1999; Fargione et al. 2003), the greatest threat may come from non-native species belonging to the same ecosystem-level functional group as the dominant native species.

While plant biomass production was significantly improved following re-inoculation with native soil, as compared with the soil collected directly beneath or between *Bothriochloa* plants, adverse effects of the *Bothriochloa* were not completely ameliorated in this 14-week study. In all sites, dry weight of plants grown in re-inoculated soil was significantly less than plant growth in soils collected from the native prairie. One explanation might be that re-inoculation of the native soil microbial communities was accomplished by the addition of 15 g of native soil into 600 g sterilized field soil. It is likely the soil microbial population densities of the re-inoculated treatment did not reach that of the native, non-sterilized soil treatment in our 14-week study.

Alternatively, the suppression of native plant growth may include other mechanisms. An alternative or additive mechanism could be the production of allelopathic compounds by the non-native grass. Some exotic plant species have been shown to produce novel chemicals that can be phytotoxic to plants that are native to the invaded site and these compounds can influence microbial communities and their functioning (Inderjit and van der Putten 2010). Production of allelopathic compounds by non-natives has been reported to directly inhibit the ability of AM fungi to colonize these native grasses, or indirectly reduce AMF colonization by suppressing the growth of the native grasses, thereby reducing carbon allocations to the symbiont (Calloway and Ridenour 2004; Abhilasha et al. 2008; Inderjit et al. 2008). Inderjit and van der Putten (2010) theorize that native plant species associate with soil

microbes that have evolved with native plant-produced compounds, resulting in the selection of microbial species capable of degrading these compounds. However, if native soil microbes are exposed to novel chemicals they have not evolved with, such as those produced by non-native plant species, they might be unable to degrade these chemicals, which could then accumulate to toxic levels. In our current study, soil sterilization and re-inoculation with native soil microbial communities resulted in an increase in plant biomass production. If the allelopathic chemicals produced by the non-native are not able to be degraded by native microbes, sterilizing and re-inoculating with native microbial communities would not be expected to result in a positive growth response by our native grasses. However, the inability to achieve equivalent biomass production between sterilized/re-inoculated soil and native soil may indicate multiple mechanisms are contributing to native plant growth suppression following invasion by these non-natives. For example, the production of allelopathic compounds could directly or indirectly be responsible for alterations in soil microbial communities (Inderjit and van der Putten 2010). Further studies, especially those involving field studies, will be essential for providing information concerning successful restoration of invaded sites following inoculation with native field soils.

Successful establishment of native species can be an integral component to the overall success of areas replanted following eradication of monoculture stands of exotic grasses, such as *Bothriochloa*. The establishment of native species has been shown to decrease establishment of exotic plant species in prairie restorations (Middleton et al. 2009). In a comparison of post-agricultural fields and adjacent unplowed prairie, Bever et al. (2003) reported a shift in AM fungal species composition that favored weedy fungal species in the post-agricultural sites and hypothesized that this shift in fungal species may impede restoration and establishment of native grasses of the post-agricultural lands. The applications of native prairie soil have been shown to increase the establishment success of native plant species, at least in part, due to restoration of the native AM fungal communities (Smith et al. 1998; White et al. 2008; Bever et al. 2009). If *Bothriochloa* alters the AM fungal communities, providing a competitive advantage and inhibiting the obligate symbiosis of the native grasses, restoring AM fungal communities through additions of native prairie inoculum that promote the success of native species may be an important management consideration in areas where *Bothriochloa* is eradicated and the restoration of native species is in progress. Our current greenhouse studies indicate that the additions of native prairie inoculum may be a useful management tool for successful establishment of native warm-season grass species in areas previously invaded by *Bothriochloa* species.

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References

Abhilasha D, Quintana N, Vivanco J, Joshi J (2008) Do alleopathic compounds in invasive *Solidago canadensis* s.l. restrain the native European flora? J Ecol 96:993–1001

Allen MF, Allen EB (1990) Carbon source of VA mycorrhizal fungi associated with chenopodiaceae from a semiarid shrub-steppe. Ecology 71:2019–2021

Batten KM, Scow KM, Davies KF, Harrison SP (2006) Two invasive plants alter soil microbial community composition in serpentine grasslands. Biol Invasions 8:217–230

Bennett AE, Bever JD (2009) Trade-offs between arbuscular mycorrhizal fungal competitive ability and host growth promotion in *Plantago lanceolata*. Oecologia 160:807–816

Bever JD (2002) Host-specificity of AM fungal population growth rates can generate feedback on plant growth. Plant Soil 244:281–290

Bever JD (2003) Soil community feedback and the coexistence of competitors: conceptual frameworks and empirical tests. New Phytol 157:465–473

Bever JD, Westover KM, Antonovics J (1997) Incorporating the soil community into plant population dynamics: the utility of the feedback approach. J Ecol 85:561–573

Bever JD, Schultz PA, Miller RM, Gades L, Jastrow JD (2003) Prairie mycorrhizal fungi inoculant may increase native plant diversity on restored sites. Ecol Restoration 21:311–312

Bever JD, Richardson SC, Lawrence BM, Holmes J, Watson M (2009) Preferential allocation to beneficial symbiont with spatial structure maintains mycorrhizal mutualism. Ecol Let 12:13–21

Blair AC, Wolfe LM (2004) The evolution of an invasive plant: an experimental study with *Silene latifolia*. Ecology 85:3035–3042

Calloway RM, Ridenour WM (2004) Novel weapons: invasive success and the evolution of increased competitive ability. Front Ecol Environ 2:436–443

Cano C, Bago A (2005) Competition and substrate colonization strategies of three polyxenically grown arbuscular mycorrhizal fungi. Mycologia 97:1201–1214

Ehrenfeld JG (2004) Implications of invasive species for belowground community and nutrient process. Weed Technol 18:1232–1235

Fargione J, Brown CS, Tilman D (2003) Community assembly and invasion: an experimental test of neutral versus niche processes. PNAS 100:8916–8920

Grime JP, Macky JM, Hiller SH, Read DJ (1987) Mechanisms of floristic diversity: evidence from microcosm. Nature 328:420–422

Harmoney KR, Hickman KR (2004) Comparative morphology of Caucasian old world bluestem and native grasses. Agron J 96:1540–1544

Harmoney KR, Stahlman PW, Hickman KR (2004) Herbicide effects on established yellow old world bluestem (*Bothriochloa ischaemum*). Weed Technol 18:545–550

Harper JL (1977) Population biology of plants. Academic Press, San Diego

Hartnett DC, Wilson GWT (1999) Mycorrhizal mediation of plant species composition and diversity in tallgrass prairie. Ecology 80:122–130

Hartnett DC, Wilson GWT (2002) The role of mycorrhizas in plant community structure and dynamics: lessons from the grasslands. Plant Soil 244:319–331

Hawkes CV, Belnap J, D'Antonio C, Firestone MK (2006) Arbuscular mycorrhizal assemblages in native plant roots change in the presence of invasive exotic grasses. Plant Soil 281:369–380

Hepper CM, Sen R, Azconaguilar C, Grace C (1988) Variation in certain isozymes amongst different geographical isolates of the vesicular arbuscular mycorrhizal fungi *Glomus clarum*, *Glomus monosporum* and *Glomus mosseae*. Soil Biol Biochem 20:51–59

Herre EA, Knowlton N, Mueller UG, Rehner SA (1999) The evolution of mutualisms: exploring the paths between conflict and cooperation. Trends Ecol Evol 14:49–53

Hetrick BAD, Wilson GWT, Todd TC (1990) Differential responses of C_3 and C_4 grasses to mycorrhizal symbiosis, P fertilization, and soil microorganisms. Can J Bot 68:461–467

Inderjit, van der Putten WH (2010) Impacts of soil communities on exotic plant invasions. Trends Ecol Evol 25:512–519

Inderjit STR, Callaway RM, Pollock JL, Jasleen K (2008) Allelopathy and plant invasions: traditional, congeneric, and bio-geographical approaches. Biol Invasions 10:875–890

Johnson NC, Wilson GWT, Bowker MA, Wilson JA, Miller RM (2010) Resource limitation as a driver of local adaptation in mycorrhizal symbioses. PNAS 107:2093–2098

Kiers ET, Denison RF (2008) Sanctions, cooperation, and the stability of plantrhizosphere mutualisms. Ann Rev Ecol Evol Syst 39:215–236

Klironomos JN (2002) Feedback with soil biota contributes to plant rarity and invasiveness in communities. Nature 417:67–70

Koske RE, Gemma JN (1989) A modified procedure for staining roots to detect VA-mycorrhizas. Mycol Res 92:486–505

Leger EA, Rice KJ (2003) Invasive California poppies (*Eschscholzia californica* Cham.) grow larger than native individuals under reduced competition. Ecol Lett 6:257–264

Levine JM, Adler PB, Yelenik SG (2004) A meta-analysis of biotic resistance to exotic plant invasions. Ecol Lett 7:975–989

Marler MJ, Zabinski CA, Callaway RM (1999) Mycorrhizae indirectly enhance competitive effects of an invasive forb on a native bunchgrass. Ecology 80:1180–1186

McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA (1990) A new method which gives an objective measure of colonization of roots by vesicular arbuscular mycorrhizal fungi. New Phyt 115:495–501

Middleton EL, Bever JD, Schultz PA (2009) The effect of restoration methods on the quality of the restoration and resistance to invasion by exotics. Restor Ecol 18:181–187

Mitchell CE, Agrawal AA, Bever JD, Gilbert GS, Hufbauer RA, Klironomos JN et al (2006) Biotic interactions and plant invasions. Ecol Lett 9:726–740

Mummey DL, Rillig MC (2006) The invasive plant species *Centaurea maculosa* alters arbuscular mycorrhizal fungal communities in the field. Plant Soil 288:81–90

Mummey DL, Rillig MC, Holben WE (2005) Neighboring plant influences on arbuscular mycorrhizal fungal community composition as assessed by T-RFLP analysis. Plant Soil 271:83–90

Orr SP, Rudgersm JA, Clay K (2005) Invasive plants can inhibit native tree seedlings: testing potential allelopathic mechanisms. Plant Ecol 181:153–165

Pimentel D, Lach L, Zuniga R, Morrison D (2000) Environmental and economic costs of nonindigenous species in the United States. Bioscience 50:53–65

Pringle A, Bever JD, Gardes M, Parrent JL, Rillig MC, Klironomos JN (2009) Mycorrhizal symbioses and plant invasions. Annu Rev Ecol Evol Syst 40:699–715

Reed HE, Seastedt TR, Blair JM (2005) Ecological consequences of C_4 grass invasion of a C_4 grassland: a dilemma for management. Ecol Appl 15:1560–1569

Reynolds HL, Packer A, Bever JD, Clay K (2003) Grassroots ecology: plant-microbesoil interactions as drivers of plant community structure and dynamics. Ecology 84:2281–2291

Schmidt CD, Hickman KR, Channell R, Harmoney K, Stark W (2008) Competitive abilities of native grasses and non-native (*Bothriochloa spp*.) grasses. Plant Ecol 197:69–80

Seifert EK, Bever JD, Maron JL (2009) Evidence for the evolution of reduced mycorrhizal dependence during plant invasion. Ecology 90:1055–1062

Shah MA, Reshi Z, Reshi I (2008) Mycorrhizosphere mediated mayweed chamomile invasion in the Kashmir Himalaya, India. Plant Soil 312:219–225

Shah MA, Reshi ZA, Khasa D (2009) Arbuscular mycorrhizal status of some Kashmir Himalayan alien invasive plants. Mycorrhiza 20:67–72

Smith MD, Knapp AK (1999) Exotic plant species in a C_4-dominated grassland: invasibility, disturbance, and community structure. Oecologia 120:605–612

Smith MR, Charvat I, Jacobson RL (1998) Arbuscular mycorrhizae promote establishment of prairie species in a tallgrass prairie restoration. Can J Bot 76:1947–1954

USDA NRCS (U.S. Department of Agriculture, Natural Resource Conservation Service) (2004) The PLANTS database: national plant data center, Baton Rouge, Louisiana, USA

Vitousek PM, D'Antonio CM, Loope LL, Rejmanek M, Westbrooks R (1997) Introduced species: a significant component of human-caused global change. New Zeal J Ecol 21:1–16

Vitousek PM, Walker LR (1989) Biological invasions by Myrica-faya in Hawaii-plant demography, nitrogen-fixation, ecosystem effects. Ecol Monogr 59:247–265

Vogelsang KM, Bever JD (2009) Mycorrhizal densities decline in association with nonnative plants and contribute to plant invasion. Ecology 90:399–407

Vogelsang KM, Bever JD, Griswold M, Schultz PA (2004) The use of mycorrhizal fungi in erosion control applications. Final report for Caltrans. California Department of Transportation Contract no. 65A0070. Sacramento, California

Watkins NK, Fitter AH, Graves JD, Robinson D (1996) Carbon transfer between C_3 and C_4 plants linked by a common mycorrhizal network, quantified using stable carbon isotopes. Soil Biol Biochem 28:471–477

Weidenhamer JD, Calloway RM (2010) Direct and indirect effects of invasive plants on soil chemistry and ecosystem function. J Chem Ecol 36:59–69

White JA, Tallaksen J, Charvat I (2008) The effects of arbuscular mycorrhizal fungal inoculation at a roadside prairie restoration site. Mycologia 100:6–11

Wilson GWT, Hartnett DC (1997) Effects of mycorrhizas on plant growth and dynamics in experimental tallgrass prairie microcosms. Am J Bot 84:478–482

Wilson GWT, Hartnett DC (1998) Interspecific variation in plant responses to mycorrhizal colonization in prairie grasses and forbs. Am J Bot 85:1732–1738

Wilson GWT, Hartnett DC, Rice CW (2006) Mycorrhizal-mediated phosphorus transfer between the tallgrass prairie plants *Sorghastrum nutans* and *Artemisia ludoviciana*. Funct Ecol 20:427–435

Wilson GWT, Rice CW, Rillig MC, Springer A, and Hartnett DC (2009) Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: results from long-term field experiments. Ecol Lett 12:452–461