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RESEARCH ARTICLE

The Influence of Beneficial Fungi on Plant-Enemy Interactions and Plant Community Structure

Mycorrhizal-herbivore interactions and the competitive release of subdominant tallgrass prairie species

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Abstract

1. Plant-microbial-herbivore interactions play a crucial role in the structuring and maintenance of plant communities and biodiversity, yet these relationships are complex. In grassland ecosystems, herbivores have the potential to greatly influence the survival, growth and reproduction of plants. However, few studies examine interactions of above- and below-ground grazing and arbuscular mycorrhizal (AM) mycorrhizal symbiosis on plant community structure.
2. We established experimental mesocosms containing an assemblage of eight tallgrass prairie grass and forb species in native prairie soil, maintained under mycorrhizal and nonmycorrhizal conditions, with and without native herbivorous soil nematodes, and with and without grasshopper herbivory. Using factorial analysis of variance and principal component analysis, we examined: (a) the independent and interacting effects of above- and below-ground herbivores on AM symbiosis in tallgrass prairie mesocosms, (b) independent and interacting effects of above- and below-ground herbivores and mycorrhizal fungi on plant community structure and (c) potential influences of mycorrhizal responsiveness of host plants on herbivory tolerance and concomitant shifts in plant community composition.
3. Treatment effects were characterized by interactions between AM fungi and both above-ground and below-ground herbivores, while herbivore effects were additive. The dominance of mycorrhizal-dependent C_4 grasses in the presence of AM symbiosis was increased ($p < 0.0001$) by grasshopper herbivory but reduced ($p < 0.0001$) by nematode herbivory. Cool-season C_3 grasses exhibited a competitive release in the absence of AM symbiosis but this effect was largely reversed in the presence of grasshopper herbivory. Forbs showed species-specific responses to both AM fungal inoculation and the addition of herbivores. Biomass of the grazing-avoidant, facultatively mycotrophic forb *Brickellia eupatorioides* increased ($p < 0.0001$) in the absence of AM symbiosis and with grasshopper herbivory, while AM-related increases in the above-ground biomass of mycorrhizal-dependent forbs *Rudbeckia hirta* and *Salvia azurea* were eradicated ($p < 0.0001$) by grasshopper herbivory. In contrast, nematode herbivory enhanced ($p = 0.001$) the contribution of *Salvia azurea* to total biomass.

4. *Synthesis*. Our research indicates that arbuscular mycorrhizal symbiosis is the key driver of dominance of C_4 grasses in the tallgrass prairie, with foliar and root herbivory being two mechanisms for maintenance of plant diversity.

KEYWORDS
antagonism, arbuscular mycorrhizal (AM) fungi, herbivory, mutualism, mycorrhizal responsiveness, plant-herbivore interactions, plant-soil interactions

1 | INTRODUCTION

Plant–microbial interactions play a crucial role in the structuring and maintenance of plant communities and biodiversity in most terrestrial ecosystems, including grasslands (Bever et al., 2015; Hartnett & Wilson, 1999; Mangan et al., 2010). Arbuscular mycorrhizal (AM) fungi (Phylum Glomeromycota) are obligate root endosymbionts that are known to associate with >70% of all plant families (Brundrett & Tedersoo, 2018). In this relationship, host plants provide carbon to AM fungal symbionts in exchange for the mobilization and transfer of key nutrients, namely phosphorus. In addition to enhanced plant nutrient acquisition, AM fungi also play an important role in plant defence against pathogens (Newsham et al., 1995; Veresoglou & Rillig, 2012) and improvement of plant water status (Augé, 2001). Furthermore, AM fungi have been shown to play critical roles in the maintenance of several ecosystem services and functions, such as soil carbon storage and soil stability (van Der Heijden et al., 2015; Wilson et al., 2009). While the importance of the relationship between plants and AM fungi is well-studied, concomitant plant and AM fungal responses to multiple co-occurring interspecific interactions, such as herbivory, are less understood (Van der Putten et al., 2001).

In ecosystems across the globe, herbivores have the potential to greatly influence the survival, growth and reproduction of plants (Gehring & Whitham, 2002; Huntly, 1991; Limb et al., 2018). In response to above-ground herbivory, plants often increase carbon allocation to roots (Dyer et al., 1991; Holland et al., 1996), though this gradually shifts towards above-ground allocation over time or during periods of sustained defoliation (Bardgett et al., 1998). As AM fungi are wholly dependent on plant hosts for carbon, alterations to plant carbon budgets may impact the plant-AM fungal symbiosis. For instance, the carbon-limitation hypothesis (Gehring & Whitham, 1994) suggests that removal of photosynthetic plant material through herbivory would result in reduced carbon allocation to AM fungi, thereby depleting AM fungal carbon sources. However, previous research has shown AM fungi to have a wide range of responses to herbivory, indicating that AM fungal abundances can increase (Gehring & Whitham, 1994), decrease (Barber et al., 2012; Barto & Rillig, 2010; Gehring & Whitham, 1991, 2002) or exhibit no change (van der Heyde et al., 2017) in response to herbivory. Because of the variability and inevitable context-dependency of these complex biotic interactions (Alzarhani et al., 2019; Faghihinia et al., 2020; van der Heyde et al., 2019), further research is needed. The vast majority of existing research investigating the effects of herbivory on the plant-AM fungal symbiosis has focused almost exclusively on above-ground herbivory (Dyer et al., 1991; Gehring & Bennett, 2009; Holland et al., 1996). Consequently, many knowledge gaps remain considering the potential effects of below-ground herbivory on this intricate relationship, along with the subsequent responses of the local plant community.

Similar to above-ground defoliation, root herbivory by soil invertebrates can elicit a variety of responses by both plants and symbiotic AM fungi (Frew, 2022; Hol & Cook, 2005; Ingham & Detling, 1990). Soil nematodes are widespread, ubiquitous, and abundant constituents of the soil food web. Many soil-dwelling nematodes are herbivorous or fungivorous, feeding on fine plant roots and AM fungal hyphae, which, in turn affects plant (De Deyn et al., 2003) and soil microbial communities (Poll et al., 2007). Furthermore, soil nematode presence and activity in the rhizosphere has been linked to increased soil carbon and nitrogen turnover (Ferris, 2010; Standing et al., 2006), along with increased nutrient mineralization rates, and thereby enhancing AM fungal growth (Bakhtiar et al., 2001); however, other studies have found nematode herbivory of plant roots to be deleterious to AM fungal colonization (Hamel et al., 2008; Todd et al., 2001). Inconsistencies in the literature regarding plant and AM fungal responses to above- and below-ground herbivory are likely driven by several mechanisms, including plant mycorrhizal status.

Although the independent and interactive effects of herbivory and AM fungi on associated host plants have been examined extensively (Bennett et al., 2006), comparatively few studies have assessed interactions between concomitant above- and below-ground herbivory and their effects on plant-AM fungal relationships (Abdala-Roberts et al., 2019). These interactions are widespread and likely have different effects on plant communities than either herbivores or AM fungi acting alone. Within diverse plant communities, several functional groups often coexist, each of which may respond differently to herbivores (Deraison et al., 2015; Ibanez et al., 2013) or AM fungi (Hoeksema et al., 2018; Wilson & Hartnett, 1998). Because invertebrate herbivores (Kempel et al., 2015; Stein et al., 2010), as well as AM fungi, can drive and maintain plant community diversity and structure (Hartnett & Wilson, 1999; Lin et al., 2015), determining how these biotic interactions operate in conjunction with one another is critical to a more comprehensive understanding of plant community ecology (Bennett et al., 2006; Biere & Bennett, 2013).

In this study, we examined the effects of above- and below-ground grazing coupled with AM mycorrhizal symbiosis, and the potential interactions on experimental tallgrass prairie plant communities. An assemblage of eight tallgrass prairie grass and forb species was established in experimental mesocosms containing native prairie soil. Mesocosms were maintained under mycorrhizal and non-mycorrhizal conditions, with and without native soil nematodes, and with and without above-ground herbivory to elucidate the interactions and effects of these key species interactions. Our specific objectives were to (a) examine the independent and interacting effects of above- and below-ground herbivores on AM symbiosis in tallgrass prairie mesocosms; (b) determine how these key components of the soil community (mycorrhizal fungi and nematodes) influence plant community structure in the tallgrass prairie and; (c) assess the potential influence of mycorrhizal responsiveness of host plants on herbivory tolerance. We hypothesized that, due to reduction of above-ground biomass, and thus lower photosynthetic capacity, plants subjected to foliar herbivory would display lower intra-radical AM fungal colonization. Similarly, we hypothesized plants from mesocosms containing root-feeding nematodes would have lower AM fungal colonization, due to root herbivory, as well as the likelihood of direct hyphal consumption. Additionally, due to differing levels of AM fungal responsiveness and herbivore preference across plant species in our mesocosms, we hypothesized species-specific shifts in productivity depending on the presence or absence of AM fungi and herbivores.

2 | MATERIALS AND METHODS

Experimental tallgrass prairie mesocosms were established in plastic containers (40 cm length × 52 cm width × 32 cm depth) to address the interactions among mycorrhizal symbiosis, below-ground herbivory (nematodes), above-ground herbivory and their shared host plants. Above-ground herbivory was imposed with native invertebrate herbivory (grasshoppers; *Melanoplus bivittatus*). Experimental mesocosms were established in the greenhouse following the methods of Wilson and Hartnett (1997).

2.1 | Soil preparation

Native tallgrass prairie soil was collected from Konza Prairie Biological Station (KPBS), near Manhattan, KS. Soil was steam-pasteurized at 80°C for 2 h and allowed to cool for 72 h, at which time 64 replicate mesocosms were filled with 50 kg of soil. Sixteen replicate soil samples were randomly collected from the bulk soil prior to steam-pasteurization, and two samples were collected from each microcosm (16 total samples) following pasteurization, prior to seedling establishment. Soil samples were analysed for chemical composition by the Kansas State University Soil Testing Laboratory (Manhattan, KS). No measurable change in soil chemistry was observed due to pasteurization ($p > 0.05$). Soil had a pH of 7.1 and contained 8.0 μg/g plant-available P (Bray test *I*), 230 mg/kg K, 18 mg/kg NO_3^--N, 16 mg/kg NH_4^+-N, 0.8 mg/kg DTPA-extractable Zn, 22 mg/kg extractable Fe and 2.9% organic matter. All mesocosms were amended with non-sterile soil sievate (the suspension that passed through a 38 μm sieve) following the procedures of Koide and Li (1989). With this method, the vast majority of relatively large AM fungal spores are trapped on the sieve, while smaller soil microorganisms are allowed to pass through and can then be re-introduced into the steam-pasteurized soil; however, it is worth noting that some endosymbiotic fungal taxa may have small spores (<30 μm) that could through a sieve of this size (Aguilar-Trigueros et al., 2023). The microbial community of steam-pasteurized soil amended with sievate generally differs from that of whole prairie soil because the microbial community cannot be accurately reconstructed in steam-pasteurized soil. It is assumed, however, that microbes added to steam-pasteurized soil will reproduce and will equilibrate during the study (Koide & Li, 1989).

2.2 | AM fungal inoculum preparation

Mycorrhizal treatments were established in half of the mesocosms by incorporating a community of AM fungal spores isolated from KPBS tallgrass prairie soil. Spores were isolated from 1600 g soil by wet-sieving, decanting and centrifugation in a 20:40:60% sucrose density gradient (Daniels & Skipper, 1982). Based on taxonomic criteria of Schenck and Perez (1990), spores of 15 species were identified. In terms of spore densities, *Entrophospora etunicata* (syn. *Claroideoglomus etunicatum*), *Rhizophagus aggregatus* (formerly *Glomus aggregatum*), *Septoglomus constrictum* (formerly *Glomus constrictum*), *Acaulospora longula* and an unidentified *Glomus* sp. were the dominant species. Mycorrhizal spores were suspended into 16 L distilled water and 500 mL of inoculum were thoroughly mixed into top 25 kg of soil of each microcosm. This allowed for mycorrhizal spore densities of the upper half of each microcosm to approximate that of field densities.

2.3 | Nematode inoculum preparation

Half of the mesocosms were inoculated with nematode communities extracted from prairie soil freshly collected from KPBS using a modified Christie–Perry technique (Christie & Perry, 1951). Nematodes were added as 250 mL aliquots of a uniform suspension to the upper 25 kg of soil in each inoculated mesocosm at a rate equal to approximately 20% of natural field densities in tallgrass prairie (Seastedt et al., 1987; Todd, 1996; Todd et al., 1992). Herbivorous taxa represented 52% of the nematode inoculum, with the total number of herbivores added to each mesocosm averaging 22.5×10^3. *Helicotylenchus*, *Tylenchorhynchus* and *Gracilacus* spp. represented 52%, 20% and 13%, respectively, of herbivorous taxa. The remaining herbivores consisted of *Xiphinema*, *Pratylenchus* and *Mesocriconema* spp. Another 15%–20% of the inoculum consisted of fungivorous taxa in the families Tylenchidae (primarily *Filenchus*) and Aphelenchidae (*Aphelenchus*). The remaining taxa were bacterial-feeding (e.g. Cephalobidae, 11%) or omnivorous (e.g. Dorylaimida, 22%). A list of nematode taxa present at the collection site can be found in Ransom et al. (1998).

2.4 | Plant preparation

Seeds of C_4 grasses (*Andropogon gerardii* Vitman and *Sorghastrum nutans* L.) and C_3 grasses [*Elymus canadensis* L. and *Pascopyrum smithii* (Rydb.) Love] were provided by the Natural Resources Conservation Service Plant Materials Center, Manhattan, KS. Seeds of forb species [*Rudbeckia hirta* (L.) Br. Ex Aiton, *Brickellia eupatorioides* (L.) Shinners, *Lespedeza capitata* Michx., and *Salvia azurea* Michx. Ex Lam.] were supplied by CRM Ecosystems, Prairie Ridge Nursery, Mt. Horeb, WI. These species were selected for this study because they are common, co-occurring species of the tallgrass prairie (Towne, 2002). Additionally, both obligately and facultatively mycorrhizal dependent species (Table 1) were selected (Wilson & Hartnett, 1998). Mycorrhizal responsiveness was quantified using the following formula: [(mean dry mass mycorrhizal plant − mean dry mass non-mycorrhizal plant)/(mean dry mass mycorrhizal plant)] × 100 (Wilson & Hartnett, 1998).

Seeds were germinated in vermiculite in a 22°C greenhouse. Three weeks after grass seedling emergence and 8 weeks after forb emergence seedlings were transplanted randomly into the mesocosms at approximate natural relative abundances and field densities (Table 1) (Towne, 2002). At the time of seedling transplant, rhizosphere zones of the transplanted seedlings were inoculated with AM fungi and nematodes described above.

2.5 | Above-ground herbivory

The effects of above-ground invertebrate herbivory were assessed using grasshoppers. Grasshoppers (Acrididae) are abundant on tallgrass prairie and, among invertebrate herbivores, have the largest potential impacts on plants and on ecosystem dynamics (Scott et al., 1979). Grasshoppers were collected from KPBS by sweep-netting in the field and sorted when they were at an intermediate instar stage (late June). The two-striped grasshopper (*Melanoplus bivittatus* Say) was selected due to its abundance at KPBS, its widespread range across the Great Plains, and its non-specific feeding preferences (Capinera & Sechrist, 1982; Jonas & Joern, 2008). Screen enclosures were constructed over each microcosm 6 weeks after seedling transplant. Eight weeks after transplant, six adult grasshoppers were placed into each of the screened mesocosms designated as an herbivory treatment. This rate simulated the typical peak KPBS field density for this species (Evans, 1988). Non-grazed control mesocosms were placed within similar enclosures containing no grasshoppers. Grasshoppers were allowed to feed for 24 days, at which time grasshoppers were removed from all mesocosms. Grasshopper survival was assessed daily, and dead grasshoppers were removed and replaced with freshly collected live individuals. Grasshopper death was rare and was not correlated with mycorrhizal or nematode treatments. At the time of grasshopper removal from the mesocosms, herbivory levels were estimated for each plant, using a scale of 0–4 based on proportion of leaves damaged and approximate percent leaf area removed [where 0 = no damage; 1 = <25% leaf area removed; 2 = >25% but <75% leaf area removed; 3 = >75% but less than 90% leaf area removed; 4 = >90% leaf area removed (Table S1)].

2.6 | Experimental design and maintenance

Mesocosms were arranged in a complete block design with eight replications for each treatment, where the greenhouse was maintained at 18–22°C. This temperature was selected because it allows growth of both C_4 and C_3 grasses (Wilson & Hartnett, 1997, 1998). Plants were watered as needed and fertilized every 14 days with 35 μg/g (dry weight of soil) N and 35 μg/g K by adding 1000 mL of a Peter's No-Phos Special fertilizer solution (25:0:25, N-P-K; Robert B. Peter's Co., Allentown, PA). No-phos fertilizer (i.e. containing 0 ppm phosphorus) is preferred in studies assessing plant-AM fungal

TABLE 1 Species used in mesocosm experiment, including taxonomic family, life history and mycorrhizal responsiveness (adapted from Wilson & Hartnett, 1998).

Species	Common name	Family	Fnct. group[a]	MR[b]	Density[c]
Andropogon gerardii	Big bluestem	Poaceae	C_4 grass	99.1	20
Sorghastrum nutans	Indiangrass	Poaceae	C_4 grass	99.5	20
Elymus canadensis	Canada wild-rye	Poaceae	C_3 grass	5.3	8
Pascopyrum smithii	Western wheatgrass	Poaceae	C_3 grass	11.9	8
Brickellia eupatorioides	False boneset	Asteraceae	Forb	21.7	6
Rudbeckia hirta	Black-eyed Susan	Asteraceae	Forb	97.8	6
Salvia azurea	Azure blue sage	Lamiaceae	Forb	87.8	6
Lespedeza capitata	Roundhead bushclover	Fabaceae	Legume	98.0	6

[a] Functional group (note: all forbs are perennial forbs).

[b] MR = Mycorrhizal responsiveness (%) = [(mean dry mass mycorrhizal plant − mean dry mass non-mycorrhizal plant)/mean dry mass mycorrhizal plant] × 100 (modified from Wilson & Hartnett, 1998) (from Wilson & Hartnett, 1998).

[c] Number of seedlings per mesocosm, based on average field densities from Konza Prairie Biological Station (from Towne, 2002).

relationships, as addition of inorganic phosphorus has been shown to decouple the plant-AM fungal symbiosis. This study was conducted in a full-factorial manner, with the complete experimental design as follows: 2 mycorrhizal treatments × 2 nematode treatments × 2 grasshopper treatments × 8 replicates, for a total of 64 mesocosms.

After 18 weeks (allowing 7 weeks of plant re-growth following defoliation) shoots were harvested, separated by species, and oven dried for 72 h at 80°C and dry masses of vegetative and reproductive components (i.e. "above-ground biomass") were determined for each plant species. At 18 weeks, the above-ground shoots of the majority of the plant species were senescent, although most grasses and all forbs failed to flower and set seed. Numbers of surviving plants of each species were recorded at harvest, with few plant seedlings experiencing death prior harvest. To determine mycorrhizal colonization, four soil cores (2.5 cm diameter × 14 cm deep) were removed from each of the mesocosms and roots were extracted from the soil. Roots from each of the four soil cores were washed free of soil, and stained with Trypan blue to determine percent AM fungal root colonization (McGonigle et al., 1990). Root colonization by AM fungi was scored by using the magnified gridline intersect method (McGonigle et al., 1990), using a compound microscope (200–400×) to measure the percentage of root colonized by total (hyphae + vesicles + arbuscules) AM fungi. Four additional soil cores were collected from each microcosm to assess the final nematode populations. Nematodes were extracted from 100 cm^3 subsamples using a modified Christie–Perry technique (Christie & Perry, 1951). The modification consisted of replacing Baermann funnels with 4-inch diameter pots. Nematodes were identified to genus based on morphological characters observable at 100× magnification.

Roots were washed free of soil, oven dried for 72 h at 80°C and dry mass determined. Total biomass of the mesocosm was quantified by taking the sum of all above-ground structures (i.e. vegetative and reproductive) and below-ground biomass. At the conclusion of the study, individual roots of mesocosm plants had become intertwined, making separation of below-ground biomass by species impossible. Therefore, below-ground biomass and AM fungal root colonization were assessed and are presented at the community-level.

2.7 | Statistical analyses

Prior to analysis, all data were tested for normality and homogeneity of variances using Shapiro–Wilk and Levene's tests, respectively. To assess the effects of AM fungi, below-ground herbivory (nematodes), and above-ground herbivory (grasshoppers) on biomass production of individual species, as well as entire plant communities, generalized linear models (GLM) were employed. Due to the non-normal, right-skewed nature of our data, biomass production of the whole plant community, as well as individual species, were assessed using GLM with gamma error distributions (log link). Because above- and below-ground biomass was likely to be differentially affected by their respective herbivores, biomass was separated into above- and below-ground and analysed separately. Models for biomass response variables, including both whole plant community and single species, included AM fungi, below-ground herbivory (nematodes) and above-ground herbivory (grasshoppers) as fixed effects, as well as all possible interactions. Due to differences in life history, growth form, functional group, and mycorrhizal responsiveness, identical models were constructed to elucidate species-specific effects AM fungi, nematodes, and grasshoppers, as well as all possible interactions. For mesocosms inoculated with AM fungi, the effects of nematode and grasshopper herbivory on AM fungal colonization was assessed using GLM with Gaussian error distributions due to the normal data distribution. In our AM fungal colonization model, we included above- and below-ground herbivory as fixed effects, as well as interactions between the two. Among-group comparisons were made using pairwise linear contrasts with Tukey adjustments ($p \leq 0.05$) using the package emmeans (Lenth et al., 2019). All other analysis were performed using base R version 4.1.0 (R Core Team, 2021).

3 | RESULTS

3.1 | Total mesocosm productivity

A strong significant three-way interaction between AM fungi, grasshoppers and nematodes was detected for total biomass production of mesocosms (Table 2; Figure 1). Generally, greater total biomass was produced when mesocosms were inoculated with AM fungi, but the magnitude of AM fungal effects diminished with the inclusion of grasshopper herbivory (Figure 1). Also, nematode root herbivory generally resulted in greater mesocosm productivity, but this effect was most apparent in the absence of simultaneous above-ground herbivory by grasshoppers (Figure 1). When only considering above-ground biomass, a significant two-way interaction between AM fungi and grasshoppers was detected (Table 2; Figure 2a). Specifically, above-ground biomass was greatest when mesocosms were inoculated with AM fungi and in the absence of grasshopper herbivory, although the magnitude of the effect of grasshoppers was reduced dramatically in the absence of AM fungi (Figure 2a). Interestingly, a strong significant two-way interaction was detected between grasshopper and nematode herbivory when considering mesocosm below-ground biomass, when AM fungi are not considered in the interaction (Table 2; Figure 2b). Specifically, the presence of grasshopper herbivory resulted in reduced below-ground productivity, and the inclusion of nematode herbivory further exacerbated these effects, with the lowest below-ground biomass being found in mesocosms containing both grasshoppers and nematodes (Figure 2b).

3.2 | C_4 grass productivity

A significant three-way interaction between AM fungi, nematode herbivory and grasshopper herbivory was detected for *Andropogon gerardii* above-ground biomass production [$F = 5.74$, $df = 1$, $p = 0.02$

TABLE 2 ANOVA table displaying the effects of arbuscular mycorrhizal (AM) fungi, below-ground herbivores (nematodes), and above-ground herbivores (grasshoppers) on total (vegetative + reproductive + below-ground), above- (vegetative + reproductive), and below-ground biomass production of experimental tallgrass prairie mesocosms.

Source	*F* statistic	df	*p*-Value
Total biomass			
AM fungi (AMF)	96.79	1	<0.0001***
Nematode	37.54	1	<0.0001***
Grasshopper	253.68	1	<0.0001***
AMF × Nematode	12.98	1	0.0006**
AMF × Grasshopper	7.01	1	0.01*
Nematode × Grasshopper	0.55	1	0.45
AMF × Nematode × Grasshopper	11.43	1	0.001**
Above-ground biomass			
AM fungi (AMF)	240.86	1	<0.0001***
Nematode	3.91	1	0.05*
Grasshopper	102.86	1	<0.0001***
AMF × Nematode	0.11	1	0.74
AMF × Grasshopper	6.83	1	0.01*
Nematode × Grasshopper	0.64	1	0.42
AMF × Nematode × Grasshopper	2.46	1	0.12
Below-ground biomass			
AM fungi (AMF)	7.18	1	0.009**
Nematode	167.09	1	<0.0001***
Grasshopper	226.23	1	<0.0001***
AMF × Nematode	0.36	1	0.54
AMF × Grasshopper	2.60	1	0.11
Nematode × Grasshopper	41.67	1	<0.0001***
AMF × Nematode × Grasshopper	0.86	1	0.35
AM fungal colonization			
Nematode	5.56	1	0.025*
Grasshopper	45.31	1	<0.0001***
Nematode × Grasshopper	0.031	1	0.86

Note: Also shown are the effects of below- and above-ground herbivores on colonization of prairie plant roots by arbuscular mycorrhizal (AM) fungi. Asterisks indicate significant effects at $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$.

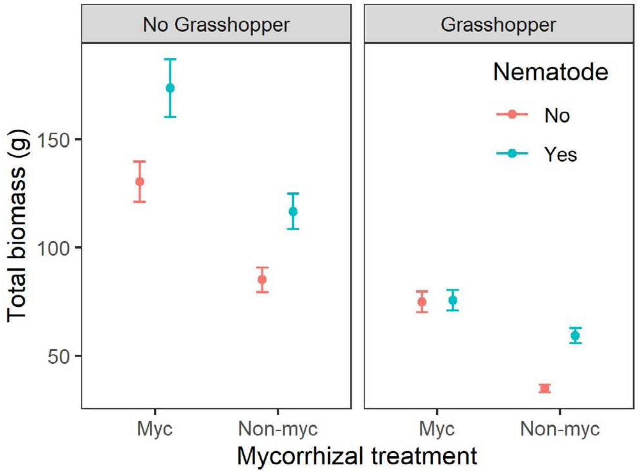

FIGURE 1 The effects of above-ground herbivory (left panel = no herbivory; right panel = herbivory), below-ground herbivory (red = no herbivory; blue = herbivory), and arbuscular mycorrhizal fungi on total biomass production of experimental tallgrass prairie communities within mesocosms.

(Figure S1)]. Specifically, greater productivity was exhibited by plants in mesocosms inoculated with AM fungi, relative to non-inoculated mesocosms; however, plants growing in mesocosms inoculated with AM fungi also exhibited a far greater degree of above-ground herbivory by grasshoppers, as opposed to plants grown in non-inoculated mesocosms (Figure S1). Finally, *A. gerardii* plants produced greater biomass in AM fungal-inoculated mesocosms in the presence of nematode root herbivory, compared to mesocosms absent of nematodes (Figure S1).

A strong significant effect of AM fungi was detected for *Sorghastrum nutans*, with greater productivity exhibited by plants grown in mesocosms inoculated with AM fungi [$F = 363.90$, df = 1, $p < 0.0001$ (Figure S1)]. Furthermore, a marginal effect of nematodes was found, with greater productivity generally associated with plants grown in mesocosms containing nematodes, compared to mesocosms absent of nematodes (Figure S1).

3.3 | C_3 grass productivity

For both *Elymus canadensis* and *Pascopyrum smithii*, significant independent effects of AM fungi and grasshoppers were found. Both *E. canadensis* and *P. smithii* displayed greater above-ground productivity in the absence of AM fungi, compared to mesocosms inoculated with AM fungi [$F = 4.25$, df = 1, $p = 0.04$ and $F = 4.33$, df = 1, $p = 0.04$, respectively (Figure S1)]. Similarly, *E. canadensis* and *P. smithii* each produced greater above-ground biomass in the absence of grasshopper herbivory, compared to plants grown in mesocosms without grasshoppers [$F = 48.28$, df = 1, $p < 0.0001$ and $F = 39.50$, df = 1, $p < 0.0001$, respectively (Figure S1)]. Additionally, a marginal two-way interaction between AM fungi and nematodes was observed for *P. smithii* productivity ($F = 2.89$, df = 1, $p = 0.09$), with nematode herbivory relating to increased productivity in mesocosms inoculated with AM fungi, compared to mesocosms absent of nematodes (Figure S1). Alternatively, productivity of *P. smithii* was greater in non-mycorrhizal mesocosms without nematodes, compared to non-mycorrhizal mesocosms with nematodes (Figure S1).

3.4 | Forb productivity

Above-ground productivity of *Brickellia eupatorioides* was significantly affected by a three-way interaction between AM fungi, nematode herbivory and grasshopper herbivory [$F = 4.47$, df = 1, $p = 0.04$ (Figure S2)]. Overall, *B. eupatorioides* produced greater biomass in

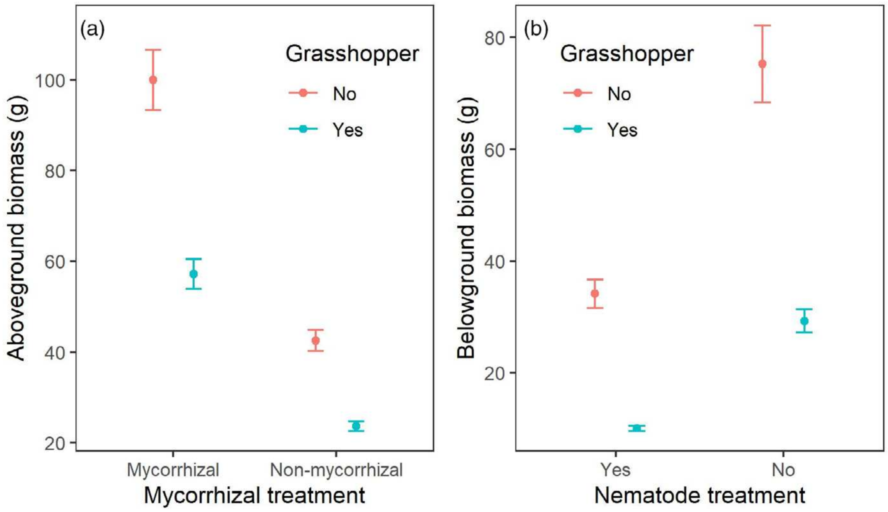

FIGURE 2 The (a) interactive effects of above-ground herbivory and arbuscular mycorrhizal fungi on above-ground biomass production within mesocosms and (b) the interactive effects of above- and below-ground herbivory on below-ground biomass production in mesocosms.

non-AM fungal mesocosms, compared to mesocosms inoculated with AM fungi (Figure 4). Furthermore, *B. eupatorioides* biomass generally decreased in the absence of concurrent nematode and grasshopper herbivory, compared to conspecific productivity in mesocosms containing both nematodes and grasshoppers (Figure S2).

Rudbeckia hirta productivity was significantly affected by independent main effects AM fungi and grasshopper herbivory ($F=64.80$, df$=1$, $p<0.0001$ and $F=79.18$, df$=1$, $p<0.0001$, respectively), but not the interaction between the two (Table 2; Figure S2). Specifically, *R. hirta* biomass was greater in mesocosms absent of AM fungi, compared to mesocosms which had been inoculated with AM fungi (Figure S2). Additionally, *R. hirta* biomass was greater in the absence of grasshopper herbivory (Figure S2).

Above-ground productivity of *Salvia azurea* was significantly affected by a three-way interaction between AM fungi, nematode herbivory, and grasshopper herbivory [$F=4.85$, df$=1$, $p=0.03$ (Figure S2)]. Biomass of *S. azurea* was greatest in mesocosms inoculated with AM fungi and nematodes but absent of grasshopper herbivory (Figure 4). Interestingly, in the absence of AM fungi, nematode herbivory decreased *S. azurea* biomass, though this pattern was reversed in mesocosms inoculated with AM fungi (Figure S2).

A significant two-way interaction between AM fungi and grasshopper herbivory was detected for above-ground biomass of *Lespedeza capitata* [$F=11.20$, df$=1$, $p=0.001$ (Table 1; Figure S2)]. Specifically, productivity of *L. capitata* was greater in mesocosms inoculated with AM fungi, compared to non-inoculated mesocosms (Figure 4). However, grasshopper herbivory resulted in decreased productivity in the absence of AM fungi, with a far less pronounced effect when AM fungi were present (Figure S2).

3.5 | Plant community structure

The first principal component (PCA) of above-ground biomass, with separation of mesocosm treatments on the first two principal

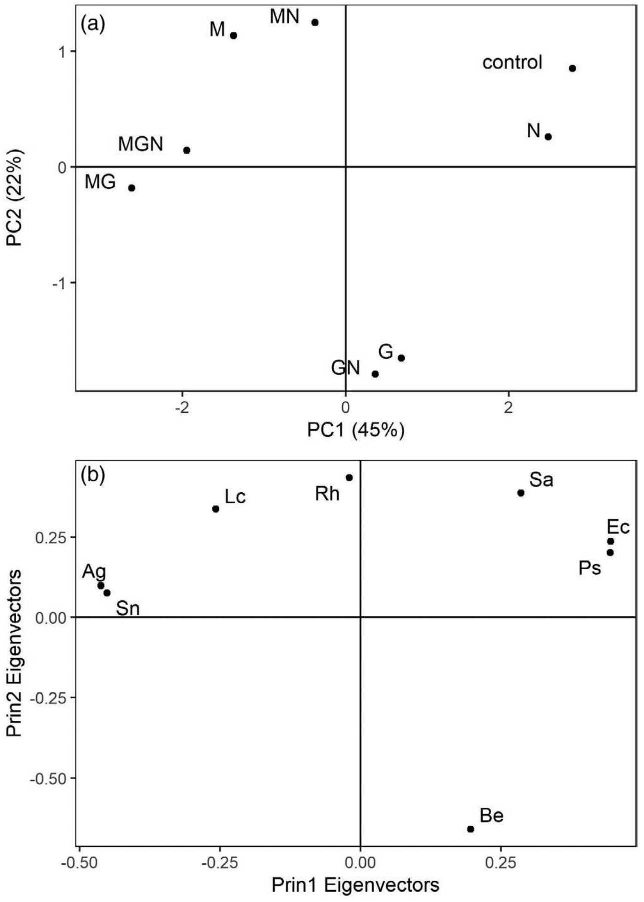

FIGURE 3 Principal component analysis of total above-ground mesocosm biomass of eight prairie species. (a) Separation of mesocosm treatments on the first two principal components: M = arbuscular mycorrhizal fungal-inoculated; G = grasshopper-presence; N = nematode-inoculated. (b) Eigenvector weightings of prairie species: Ag = *Andropogon gerardii*; Sn = *Sorghastrum nutans*; Ec = *Elymus canadensis*; Ps = *Pascopyrum smithii*; Rh = *Rudbeckia hirta*; Be = *Brickellia eupatorioides*; Lc = *Lespedeza capitata*; Sa = *Salvia azurea*. Error bars represent 95% confidence intervals.

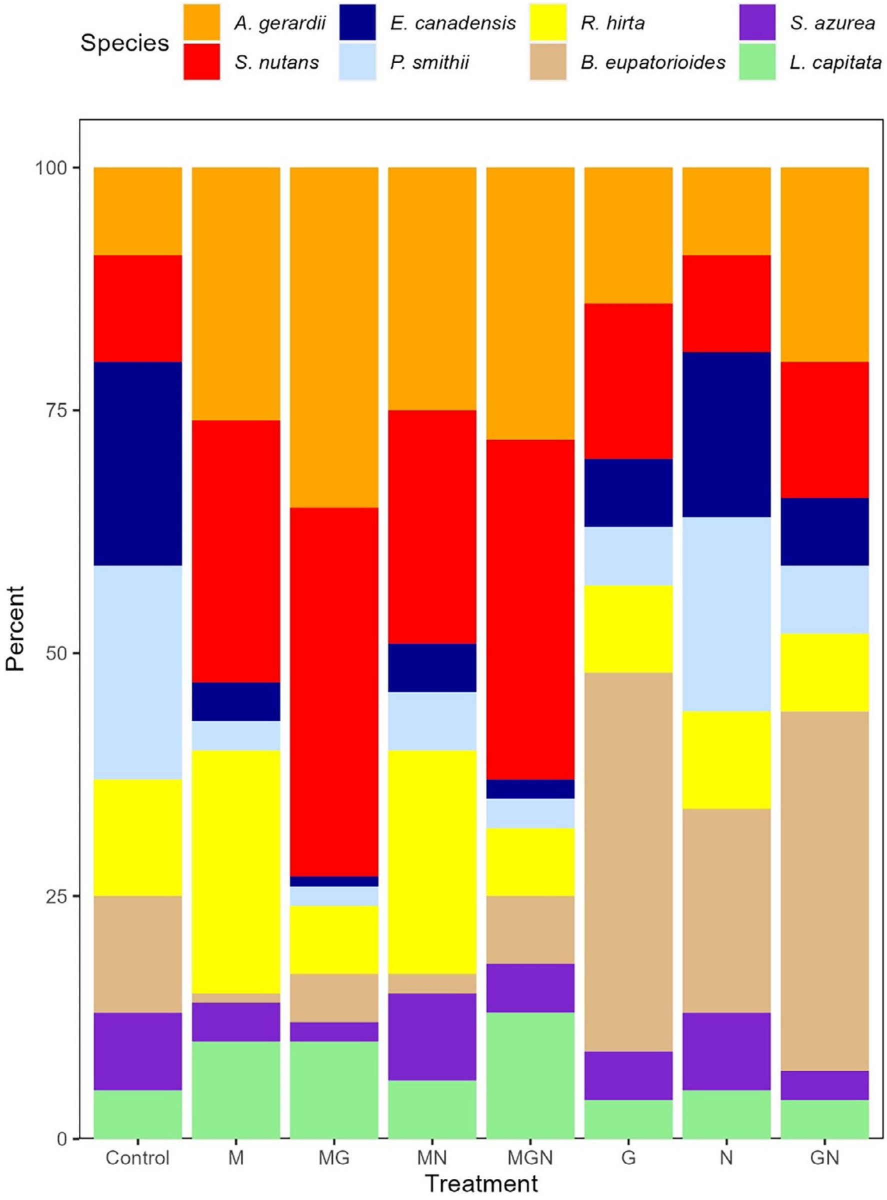

FIGURE 4 Proportional changes in above-ground biomass production of eight representative tallgrass prairie plant species, denoted by the colours within bars, in response to inoculation with arbuscular mycorrhizal fungi (M), grasshopper herbivory (G), and nematode herbivory (N). Combined letters on the *x*-axis represent treatment combinations.

components, represents a contrast of above-ground biomass production by obligately versus facultatively mycorrhizal dependent species (Figure 3a), explaining 45% of the total variation. The second principal component contrasts above-ground biomass production of facultatively mycorrhizal dependent *Brickellia eupatorioides* versus all other grass and forb species (Figure 3b), explaining 22% of the total variation. Mycorrhizal fungal × grasshopper treatment interactions ($p < 0.0001$) were observed for both principal components, with above-ground herbivory effects larger in the absence than in the presence of AM fungal symbiosis. An AM fungal × nematode treatment interaction ($p < 0.0001$) was also observed for the first principal component, with below-ground herbivory effects observed only in the presence of AM fungal symbiosis. The effects of above-ground and below-ground herbivory differed, however, not only in degree but also in the plant functional group affected. Above-ground grazing by grasshoppers reduced the proportional biomass of the C_3 grasses, resulting in increased C_4 grass biomass, while below-ground grazing by nematodes tended to reduce the dominance of the C_4 grasses in the presence of AM fungal symbiosis.

Treatment effects in terms of changes in proportional above-ground biomass of individual plant taxa indicate the biomass of C_4 and C_3 grasses generally increased and decreased, respectively, relative to control (non-mycorrhizal and non-grazed) mesocosms, with the largest effects observed for above-ground grazers in the presence of AM fungi (Figure 4). In contrast, mycorrhizal and grazing effects on forb biomass were more varied. The largest effects were observed for *Brickellia eupatorioides*, with both above- and below-ground herbivory resulting in increased ($p < 0.0001$) biomass in the absence of AM fungi, while biomass reductions in the presence of AM fungi similarly declined when subjected to grazing (Figure 4). However, grasshopper grazing reversed the AM fungal-associated growth response of obligately mycorrhizal dependent *Rudbeckia hirta*.

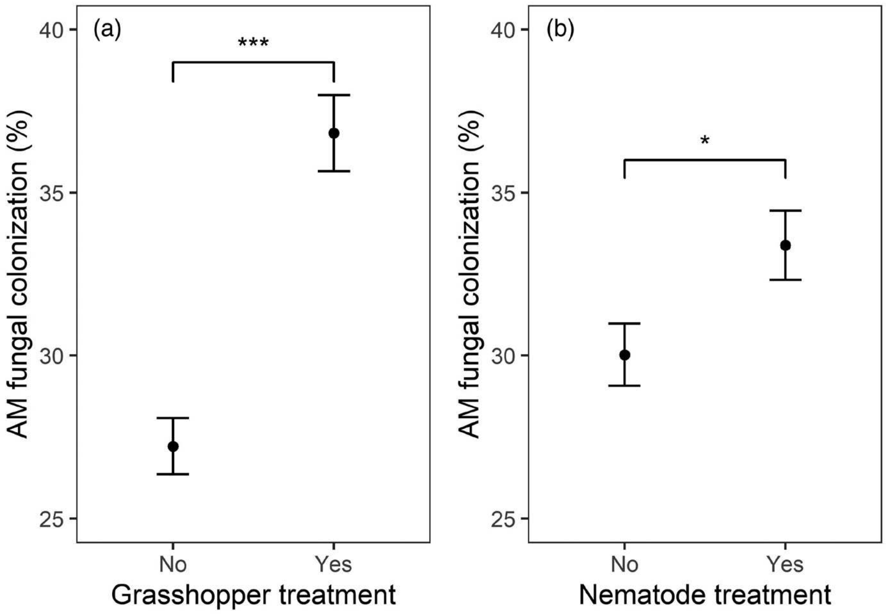

FIGURE 5 Independent effects of (a) above- and (b) below-ground herbivory on intra-radical arbuscular mycorrhizal (AM) fungal colonization of prairie mesocosms (***$p \le 0.0001$; *$p \le 0.05$).

3.6 | Arbuscular mycorrhizal fungal colonization

Significant independent effects of nematode ($F = 5.56$, $\text{df} = 1$, $p = 0.025$) and grasshopper ($F = 45.31$, $\text{df} = 1$, $p \le 0.0001$) herbivory, but not the interaction between the two, were detected for intra-radical AM fungal colonization of roots sampled from mesocosms (Table 2; Figure 5). When subjected to either grasshopper or nematode herbivory, AM fungal colonization was significantly greater when mesocosms were subjected to herbivory, compared to mesocosms in the absence of herbivory (Figure 5a,b).

3.7 | Nematode final population densities

Final herbivore population densities averaged 27.7×10^3 versus 5.4×10^3 in nematode-inoculated and noninoculated mesocosms, respectively ($p < 0.0001$). The genus *Tylenchorhynchus* comprised the majority of herbivorous nematodes (46%), followed by *Helicotylenchus* (28%) and *Mesocriconema* (26%). Fungivorous Tylenchidae (*Filenchus*) and Aphelenchidae (*Aphelenchus*) population densities averaged 11.1×10^3 versus 5.0×10^3 in nematode-inoculated and noninoculated mesocosms, respectively ($p = 0.002$), while bacterivorous Cephalobidae (primarily *Acrobeles*, *Acrobeloides* and *Eucephalobus*) and Rhabditidae (primarily *Mesorhabditis*) population densities averaged 63.3×10^3 versus 93.6×10^3 in nematode-inoculated and noninoculated mesocosms, respectively ($p = 0.15$). Evidence ($p < 0.05$) for AM fungal and grasshopper treatment effects and interactions was not observed.

4 | DISCUSSION

Our study demonstrates not only the intricate linkages between plants and AM fungi, but also the species-specific and context-dependent effects of above- and below-ground herbivory on this well-known relationship. Plants, as primary producers, also influence soil microbial community structure, as a substantial proportion of their fixed carbon is allocated to the rhizosphere. Primary consumers of the above-ground food web (i.e. foliar herbivores) may influence both the ecophysiology of individual plants and the overall structure of the plant community. These in turn influence the quality and quantity of resources that are returned to the soil, thereby influencing the below-ground food web and root-associated symbionts (Gange & Brown, 2002; Hamilton & Frank, 2001). Thus, as above- and below-ground herbivory is inextricably linked, likely interacting with other symbioses or interactions involving plant hosts. Overall, several clear patterns emerged from our research. First, total biomass production of tallgrass prairie mesocosms was strongly influenced by the presence of foliar herbivores, root herbivores and AM fungi. Second, closer investigation revealed shifts in species-specific biomass allocation, often depending on mycorrhizal status of the host plant, or feeding preferences of grasshoppers. Furthermore, differential effects were demonstrated for partitioned above- and below-ground productivity. Finally, intra-radical AM fungal colonization of host plant roots suggests plant-AM fungal symbioses are likely affected by both foliar and root herbivory, but not the interaction between the two. Complex, multi-trophic studies, such as presented here, are critical for gaining further insight into intricately linked ecological communities.

The herbivory intensities of this study reflect estimations of natural grazing levels by grasshopper and nematode densities at Konza Prairie Biological Station (Evans, 1988; Seastedt et al., 1987; Todd, 1996; Todd et al., 1992). Although *M. bivittatus* is considered a generalist herbivore (Jonas & Joern, 2008), feeding on a variety of grasses and forbs, clear patterns were observed within established mesocosms, suggesting preferential selection of certain plant species. For example, C_3 grasses *E. canadensis* and *P. smithii* produced significantly less biomass when subjected to foliar herbivory, compared to the non-grazed controls, regardless of AM fungal or nematode treatments. Grazing of the forbs *R. hirta* and *S. azurea* also resulted in reductions of their individual final above-ground biomass production, compared to non-grazed counterparts. However, the facultative mycotrophic forb *B. eupatorioides* responded favourably to above-ground herbivory in our experiment. This was attributed to proliferation of lateral shoots in response to defoliation (personal observation), low leaf damage by grasshopper herbivory, and the competitive release from the majority of plant species in this study that were unable to re-grow following intense defoliation. The presence of AM fungi provided grazing tolerance to highly mycotrophic C_4 grasses, likely facilitating re-growth following intense defoliation, whereas nonmycorrhizal counterparts were unable to recover from defoliation. However, the growth of these dominant grasses, and thus their community dominance, was decreased following defoliation, allowing competitive release and subsequent increase in community composition of the grazing tolerant, and likely unpalatable, forb *B. eupatorioides*.

The nematode effects on plant biomass observed in this study were likely due, either directly or indirectly (e.g. through competitive release), to root herbivory. Fungivorous and microbivorous nematode densities were similar for all mesocosms (data not shown), suggesting that any effects due to nematode-microbial interactions were constant across treatments. The limited role of root herbivory in plant community dynamics has been suggested as a key component of plant succession through time (Bardgett & Wardle, 2003). In our present study, the evidence suggests a further role for root herbivory in reducing the competitive advantage of dominant plant species in a climax ecosystem, as demonstrated by the competitive release of the C_3 grasses and the forb *B. eupatorioides*. Although root herbivory is extremely difficult to study under field conditions due to a number of constraints (Körner et al., 2014), it has been suggested to complement the role of above-ground herbivores in the maintenance of species richness and diversity (Collins et al., 1998; Stein et al., 2010).

Bardgett and Wardle (2003) have suggested that the mechanisms responsible for herbivore effects on ecosystems (changes in resource quantity, quality, and functional composition) are broadly similar for foliar and root herbivory. Results from our study generally support this assertion based on short-term changes in above- and below-ground primary productivity of an experimental assemblage of tallgrass prairie plants. Foliar and root herbivory reduced the competitive dominance of the C_4 grasses in approximately additive fashion; plant species responding to competitive release, however, were determined by species-specific levels of grazing tolerance and mycorrhizal dependency. Both C_3 grasses and three of the four forb species, for example, were intolerant of foliar herbivory, rendering them unable to respond to competitive release. There were differential above- versus below-ground grazing responses for some plant species in this study, but these could be interpreted as differences in specialization among herbivores rather than differences in effects of foliar versus root herbivory. While strong feeding preferences were observed for grasshoppers, however, we were unable to demonstrate similar preferences among the dominant nematode herbivores in single-plant-species tests of host suitability, with the exception of *Tylenchorhynchus* sp., which exhibited greater population increase on the C_4 grasses compared to all other plant species (data not shown).

Along with antagonists, such as herbivores, symbionts have long been recognized for their importance in the context of plant community structure and function, as is the case with AM fungi (Hartnett & Wilson, 2002; Wilson & Hartnett, 1998). There is strong evidence that the effects of AM fungi on plant communities are driven by plant mycorrhizal dependence and position in the local dominance hierarchy (Hartnett & Wilson, 2002; Urcelay & Díaz, 2003; Van Der Heijden, 2002). Hartnett and Wilson (2002) hypothesized that given interspecific differences among plants in mycorrhizal effects on growth and competitive ability, any changes in mycorrhizal colonization or symbiotic function will alter plant species competitive effects and responses, resulting in shifts in species dominance, coexistence and diversity. The plant species of our present study can be easily divided into two groups based on their growth responses to mycorrhizal colonization in the absence of herbivory. The first group is composed of highly mycorrhizal-dependent species (*A. gerardii*, *S. nutans*, *L. capitata*, *S. azurea* and *R. hirta*). The second group contains the facultative mycotrophs, which showed negative or neutral growth responses in response to AM fungal colonization (*E. canadensis*, *P. smithii* and *B. eupatorioides*). In non-grazed mesocosms, highly mycotrophic plant species (former group) had significantly greater biomass in mesocosms inoculated with AM fungi, as compared to conspecifics grown in mesocosms absent of AM fungi. Conversely, plants from the latter group produced greater biomass in the absence of AM fungi. Earlier studies of multispecies tallgrass prairie mesocosms conducted under greenhouse conditions (Kula et al., 2005; Wilson & Hartnett, 1997), field studies of mycorrhizal-suppressed tallgrass prairie plots (Hartnett & Wilson, 1999; Smith et al., 1999; Wilson et al., 2001), and this study indicate that AM fungal symbioses greatly increase competitiveness of dominant, C_4 grasses. These studies also indicate that subordinate facultative mycotrophs competing with highly mycorrhizal-dependent species experience competitive release when AM fungi are suppressed or eliminated. In our study, roots were sampled at random, and likely consisted of roots of the dominant plant species in the respective mesocosm. Additionally, it is likely that below-ground herbivory by nematodes affected some plant species disproportionately. Thus, it is possible that colonization levels in certain plant species increased or decreased depending on neighbour responses, yet these dynamics could not be disentangled with our study design. Further research using mesh barriers are needed to further understand species-specific plant-herbivore-AM fungal dynamics within experimental communities.

Given a local species pool, species diversity of a given area depends on the existence of mechanisms that prevent a competitive dominance from excluding all other species (Reynolds et al., 2003). Mycorrhizal fungi are key players in the dominance of the highly mycotrophic C_4 grasses of the tallgrass prairie, and can be viewed as the ultimate positive feedback for these grasses. These feedbacks, operating alone, would be expected to lead to monocultures of C_4 grasses. In contrast, the process of negative impacts through herbivory (above- and below-ground) can generate a pattern of increased species diversity through competitive release of dominant species. Foliar herbivory has been shown to have significant effects on plant performance, plant community dynamics and ecosystem processes (Gehring & Whitham, 2002; Heinen et al., 2022) and increases in plant species diversity were reported in response to herbivory by large herbivores (Collins & Steinauer, 1998; Hartnett et al., 1996). We suggest that AM symbiosis is a key mechanism providing the positive feedbacks for dominance of the C_4 grasses in the tallgrass prairie, with foliar and root herbivory being two mechanisms for limiting dominance, thereby facilitating increased plant diversity. The concept of plant-symbiont-herbivore interactions as drivers of vegetation dynamics deserves more attention and has the potential to enrich our understanding of vegetation diversity and plant community structure on the landscape.

AUTHOR CONTRIBUTIONS

Gail W. T. Wilson and Timothy C. Todd conceived and designed the experiment. Gail W. T. Wilson maintained the experiment and was responsible for data curation. Eric B. Duell and Timothy C. Todd conducted data analyses. Eric B. Duell wrote the initial draft, and all authors contributed equally to the final manuscript draft.

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CONFLICT OF INTEREST STATEMENT

Gail W. T. Wilson is an Associate Editor for the *Journal of Ecology* but took no part in the peer review and decision-making processes for this paper.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/1365-2745.14351.

DATA AVAILABILITY STATEMENT

All data are available in the Dryad Digital Repository: https://doi.org/10.5061/dryad.5x69p8dck (Duell et al., 2024).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1: The effects of aboveground herbivory, belowground herbivory, and AM fungi on aboveground productivity of prairie grasses.

Figure S2: The effects of aboveground herbivory, belowground herbivory, and AM fungi on aboveground productivity of prairie forbs.

Table S1: Herbivory levels on mycorrhizal and non-mycorrhizal prairie grasses and prairie forbs 24 days after introduction of grasshoppers.

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