ECOLOGICAL AND EVOLUTIONARY INSIGHTS INTO *ASCLEPIAS*: FROM SOIL SYMBIONTS TO DISTURBANCES

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ECOLOGICAL AND EVOLUTIONARY INSIGHTS INTO *ASCLEPIAS*: FROM SOIL SYMBIONTS TO DISTURBANCES

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Life is not meant to be done alone. Find those that will always be in corner.

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Abstract: Milkweeds (*Asclepias*) are a diverse genus of plants in the family Apocynaceae, with an estimated 130 species found in North America. Milkweed research has increased dramatically over the past two decades, due in part to its importance as the primary food source of monarch (*Danaus plexippus*) larvae. Reductions in milkweed availability in grasslands of central North America are contributing to *D. plexippus* population declines. While research assessing the biology of milkweeds has gained recent attention, the interactions of milkweeds, associated soil symbionts, and ecosystem disturbances on milkweed abundance remain unknown. I conducted two interrelated studies to assess the role of phylogenetic relatedness in plant-soil-microbial interactions of various milkweed species, and two additional studies assess the effects of disturbances on individual plants and on long-term abundances in tallgrass prairies. My data highlight the role of phylogenetic distance in influencing PSFs between sympatric congeners. Relative mycorrhizal responsiveness (rMR) of selected milkweeds varied greatly between species, ranging from 27 to 97%. While there was remarkable variation in the degree of responsiveness within the genus, twenty-four of thirty-five species produced significantly greater ($p \leq 0.05$) biomass following inoculation with arbuscular mycorrhizal (AM) fungi, compared to soil without AM fungi. Results also suggest that, due to longer shared evolutionary histories, it is likely that closely related species share a greater overlap of mutualist partners, compared to species with earlier divergence. My research also found productivity, plant defenses, and floral resources of tallgrass prairie forbs that are important for pollinating insects can be significantly reduced by drought, although responses to drought were species-specific. However, abundances of AM fungi associated with milkweeds were relatively unaffected by drought. My research shows long-term abundances of *Asclepias viridis*, the most abundant milkweed species in the region, are reduced by fire, grazing, and woody cover, while less common species were relatively unaffected. Generally, milkweeds are unaffected by drought at various temporal scales. Milkweeds are an important component of native plant communities, feeding not only monarch larvae, but also providing nectar for countless pollinator species. My research is a testament to the unseen interconnectedness within ecosystems, from soil symbionts to disturbances.

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CHAPTER I

INTERSPECIFIC VARIATION IN MILKWEED (*ASCLEPIAS* SPP.) RESPONSES TO ARBUSCULAR MYCORRHIZAL FUNGI: A PHYLOGENTIC APPROACH

ABSTRACT

Arbuscular mycorrhizal (AM) fungi are a common symbiont of most terrestrial vascular plant species and play a large role in influencing plant community structure and ecosystem processes. Plant responses to AM fungi can vary widely across functional groups with functionally similar species often displaying similar responses to AM symbioses. While plant functional group may explain much of the variation in mycorrhizal associations of plants within a community, variation in responses within genera has been observed and may be explained in part by phylogenetic relatedness. Milkweeds (*Asclepias*) are a diverse genus of plants in the milkweed and dogbane family (Apocynaceae), with an estimated 130 species found in North America. Milkweed research has increased dramatically over the past two decades, due in part to its importance as the primary food source of monarch larvae. However, little research exists examining the relationship between AM fungi and milkweeds. Understanding the responsiveness. or dependence, of milkweed species on this symbiosis may be vital to successful restoration efforts that include these species. Thirty-five species of milkweed from across the United States were selected to assess the relative mycorrhizal responsiveness (rMR). All seedlings were grown in steam-pasteurized soil collected from a native prairie site, and half of the plants were inoculated with 20 g of whole prairie soil with intact microbial communities. rMR of selected milkweeds varied greatly between species, ranging from 27 to 97%. While there was remarkable variation in the degree of responsiveness within the genus, twenty-four of thirty-five species produced significantly greater ($p \leq 0.05$) biomass following inoculation with whole prairie soil. However, AM fungal colonization of inoculated roots was not correlated with rMR. Our study presents the first phylogenetic analysis of *Asclepias* plastomes as a framework for the analysis of the evolutionary relationship between *Asclepias* and AM fungi. Our data indicate plant relatedness is an important determinant of responsiveness to AM fungi.

INTRODUCTION

Mycorrhizal fungi are common symbionts of vascular plant species, forming mutualisms with ~90% of earth's terrestrial flora (Wang and Qiu 2006; Brundrett and Tedersoo 2018). Arbuscular mycorrhizal (AM) fungi (Phylum Glomeromycota) are one of the most common mycorrhizas, associating with ~70% of terrestrial vascular plants (Brundrett and Tedersoo 2018), and play a large role in influencing plant community structure and function (Wilson and Hartnett 1998; Wilson et al. 2001). AM fungi have been shown to enhance nutrient uptake (Smith and Read 2008), increase productivity, improve water status of associated plants (Augé 2001) and play an important role in maintaining key ecosystem processes (Rillig 2004; Wilson et al. 2009). Although the relationship between plants and AM fungi is often described as mutualistic, where the plant receives key soil nutrients from the fungi in exchange for carbon, plant responses to AM fungi can also be neutral, or even negative (i.e. parasitism), depending on biotic and abiotic factors (Johnson et al. 1997; Wilson and Hartnett 1998). This relationship has been well-studied and established for many plant species and functional groups, however, there are many existing gaps in the literature, including information on plant taxa that are central to conservation concerns.

Plant responses to AM fungi can vary widely across species, although similarities have been reported among functional groups (e.g. C_4 and C_3 grasses; annual and perennial plants) (Wilson and Hartnett 1998; Hoeksema et al. 2010; Cortois et al. 2016), as functionally similar species often display similar responses to AM symbioses (Burns and Strauss 2011). While plant functional group may explain much of the variation in mycorrhizal associations of plants within a community, variation in responses within family or genera have been reported, and may be explained by phylogenetic relatedness (Burns and Strauss 2011; Reinhart et al. 2012; Lugo et al. 2014; Brundrett and Tedersoo 2018; Hoeksema et al. 2018; Koyama et al. 2019). For example, a phylogenetic analysis of one of the most extensive datasets on plant-AM fungal responses (Wilson and Hartnett 1998) revealed that grasses in the subfamily Pooideae do not typically benefit from AM fungi, while all other grass subfamilies examined did benefit from this relationship (Reinhart et al. 2012). However, these patterns are not universal, as there is also evidence that plant relatedness does not correlate with responses to AM fungi (Reinhart and Anacker 2014). Because of these inconsistencies, it should not be assumed that the responses of a few plant species to AM fungi represent the taxa as a whole.

Mycorrhizal responsiveness (MR) is a metric commonly used to describe the effect of mycorrhizal fungi on plant performance (Hetrick et al. 1996; Wilson and Hartnett 1998; Reinhart et al. 2012). The use of this metric offers a direct measurement of the influence of mycorrhizal fungi on a particular plant species, and this useful comparison is most often used to assess differences between or among species or genotypes (Johnson et al. 2010; Cobb et al. 2021). Despite being a useful metric for determining direct effects of AM fungi on host plants (Van Der Heijden 2003), this assay has been conducted on surprisingly few wild or non-agricultural species (Reinhart et al. 2012). Consequently, knowledge of the degree to which a particular species, or suite of species, rely on this ancient mutualism is often lacking, potentially hindering or delaying conservation efforts.

Milkweeds (*Asclepias*) are a diverse genus of plants in the milkweed and dogbane family (Apocynaceae), with 130 of the estimated 400 species found in North America (Fishbein et al. 2011). Milkweed research has increased dramatically over the past two decades, due in part to its importance as the primary food source of monarch (*Danaus plexippus* L.) larvae. However, relatively little research exists examining the relationship between AM fungi and milkweeds. For example, Wilson and Hartnett (1998) assessed the mycorrhizal responsiveness of a large number of grassland plants, including *Asclepias tuberosa* and *A. verticillata*, both of which displayed very high mycorrhizal responsiveness (> 90%), suggesting these particular species are highly reliant on this symbiosis for survival. However, negligible differences in above- or belowground biomass production were observed for several *Asclepias* species, when comparing mycorrhizal and corresponding non-mycorrhizal plants (Vanette and Rasmann 2012; Vanette et al. 2013). Due to the variation in life history, phenology, and site preference, it is plausible there is considerable variability in the response to AM fungi across the *Asclepias* genus.

Our current study assessed growth responses and intra-radical AM fungal root colonization of 35 *Asclepias* species with and without AM fungi. Due to the variation in growth form, habitat, and phenology, we hypothesized that biomass production in response to AM fungal inoculation would vary across *Asclepias* species, and responsiveness will likely differ due to phylogenetic relatedness. We also hypothesized, due to trade-offs between AM fungal demands (carbon) and plant resource requirements (nutrients), AM colonization levels will likely be phylogenetically constrained. To determine the relationship between *Asclepias* and AM fungal-related plant traits, we conducted a phylogenetic analysis of *Asclepias* as a framework to assess the evolutionary relationship between *Asclepias* and AM fungi.

MATERIALS AND METHODS

Experimental Design

Our complete experimental design was as follows: 35 species (Table 1.1) x 2 mycorrhizal treatments (mycorrhizal, non-mycorrhizal) x 8 replicates, resulting in 560 experimental units.

Seedling and Soil Preparation

Thirty-five milkweed species (Table 1.1) were selected to assess interspecific relative mycorrhizal responsiveness (rMR) and AM fungal colonization in a greenhouse experiment conducted at Oklahoma State University in Stillwater, Oklahoma, USA. Seeds of species from temperate regions were cold-moist stratified in vermiculite for 30 days, after which they were seeded into vermiculite. Seeds of desert and tropical/sub-tropical species were directly sown into vermiculite, with no pre-treatment. Seedlings were germinated in moist vermiculite to the two-leaf stage (~24 days) then transplanted into 0.6 L plastic pots (Deepots: Stuewe and Sons, Tangent, OR, USA) containing 500 g (dry wt) of steam-pasteurized soil. The soil was a native prairie soil (a Chase silty clay loam, fine montmorillonitic mesic Aquic Arguidoll), freshly collected from the Konza Prairie Biological Station, Manhattan, KS, transported to the greenhouse at Oklahoma State University, and hand-sorted to remove live roots and rhizomes. This soil had a pH of 6.8 and contained 5.2 mg/kg plant-available P (Mehlich), 240 mg/kg K, 15 mg/kg NO_3-N, 16 mg/kg NH_4-N, and 3.6% organic matter, as determined by the Soil, Water, and Forage Analytical Laboratory at Oklahoma State University. Half of the pots were inoculated with 20 grams of native whole soil containing local communities of AM fungi and other soil organisms, placed directly below the root system of each seedling at planting. The remaining half received 20 mL of non-mycorrhizal sievate. Non-mycorrhizal sievate was obtained by blending freshly-collected prairie soil with deionized water at a 1:1 ratio (by volume). Following blending, the slurry was then passed through a 38 μm sieve, trapping the relatively large AM fungal spores on the sieve, while allowing most other soil microbes to pass through (Johnson et al. 2010). The incorporation of non-mycorrhizal soil microbiota allows for a more accurate assessment of the effects of AM symbiosis.

Plant maintenance

Plants were maintained in an 18 – 22° C greenhouse for 24 weeks, at which time the plants began showing signs of senescence. Plants were watered daily and fertilized every other week with 35 mg N and 35 mg K, applied as Peter’s No-Phos Special Fertilizer solution (25:0:25, Robert B. Peter’s Co. Inc., Allentown, PA, USA). After 24 weeks, all individuals were destructively harvested, roots washed free of soil, and root and shoot biomass dried at 60° C for 48 hours and weighed. A subsection of roots was selected for assessment of intra-radical AM fungal root colonization.

Relative Mycorrhizal Responsiveness

Destructively harvested above- and belowground biomass was used to determine relative mycorrhizal responsiveness (rMR) of each species. Relative mycorrhizal responsiveness was calculated for each plant species as follows: percent mycorrhizal responsiveness = [(mean dry mass mycorrhizal plant – mean dry mass non-mycorrhizal plant)/mean dry mass mycorrhizal plant] x 100 (Wilson and Hartnett 1998).

AM Fungal Assessments

Three root subsamples from each individual plant were collected and stained with Trypan blue to determine percent mycorrhizal fungal root colonization (McGonigle et al. 1990). AM fungal root colonization was scored using the magnified gridline intersect method (McGonigle et al. 1990), using a digital microscope (Hirox KH 7700; 200x) to measure the percent of root colonized by AM fungal structures (hyphae + vesicles + arbuscules). For each root sample, three random sections of root length were scored separately, for a total of approximately 900 grids observed per sample (Moora et al. 2016). Reported colonization values of each sample were a mean of these three subsets.

Phylogenetic signal and Models of Evolution

Two of the most widely-used indices to test and measure phylogenetic signal are Blomberg's K (Blomberg et al. 2003) and Pagel's λ (Pagel 1999). To test whether significant interspecific variation in rMR and AM fungal colonization in *Asclepias* was influenced by phylogenetic relatedness, we calculated phylogenetic signal using Blomberg's K and Pagel's λ in the R 4.1.0 (R Core Team 2021) package phytools ver. 0.7-80 (Revell 2012) using the phylosig function. These are quantitatively different measures of similarities among species that are due to shared phylogenetic history. Blomberg's K measures phylogenetic signal through the ratio of the mean squared errors of observed and expected trait variance under Brownian motion evolution. Pagel's λ is a maximum likelihood estimator of the ratio of interspecific trait correlation to that expected under Brownian motion (Münkemüller et al. 2012; Kamilar and Cooper 2013). For both statistics, values of 0 indicate no phylogenetic signal in measured traits. Alternatively, a Pagel's λ value of 1 or Blomberg's K value of 1 indicate trait similarities as expected under neutral evolution (Brownian motion) along a diverging phylogeny. Because the two are distinct measures of phylogenetic signal, they do not always give similar values, and we evaluated both statistics (Münkemüller et al. 2012). We then fitted and compared three models of trait evolution along a diverging phylogeny using the fitContinuous function in the R package geiger ver. 2.0.7 (Pennell et al. 2014), including Brownian motion, early burst, and stable optimum modeled by an Ornstein-Uhlenbeck process (Butler and King 2004; Agrawal et al. 2009). To reconstruct ancestral states and visualize trait evolution along the phylogeny we used the contMap function in phytools. The underlying phylogeny was based on the plastid genome, using the data set of Fishbein et al. (2018). The data set was augmented with new plastid genome sequences from *Asclepias meadii, A. pumila, A. texana, and A. verticillata*, obtained by the same methods as Fishbein et al. (2018). The maximum likelihood (ML) phylogeny for the 116 taxa of *Asclepias* (including 112 previously published plastid genomes) was inferred using IQ-TREE ver. 2.1.2 (Nguyen et al. 2015), using ModelFinder (Kalyaanamoorthy et al. 2017) to estimate the optimal substitution model (TVM+F+R2). A time-calibrated phylogeny was obtained from the ML tree using penalized likelihood, implemented in treePL (Smith and O'Meara 2012). The phylogenetic sampling was matched to the mycorrhizal sampling by pruning unsampled taxa from the tree using the keep.tip function in the R package ape ver. 5.5 (Paradis & Schliep 2019).

Statistical Analyses

All biomass data were analyzed in R version 4.1.0 (R Core Team 2021). Prior to analysis, all data were tested for normality using the Shapiro-Wilk normality test. Statistical differences in biomass production between mycorrhizal and non-mycorrhizal conspecifics were determined using a one-way analysis of variance (ANOVA, $P \leq 0.05$) with mycorrhizal treatment as the independent variable, and a post-hoc Tukey's honest significant difference (HSD) test.

RESULTS

Based on rMR calculations, all 35 milkweed species can be designated as obligate mycotrophs (little growth in the absence of AM fungi) or facultative mycotrophs (able to grow in the absence of the symbiosis, although likely receive benefit) (Table 1.1). Of the 35 species tested, 23 species are considered obligate symbionts and 11 are facultative symbionts, as total plant biomass production of mycorrhizal plants did not significantly differ from corresponding non-mycorrhizal conspecific (Table 1.1). Roots of all plants grown in inoculated soil were colonized by AM fungi (Table 1.1), while roots of plants grown in soils inoculated with non-mycorrhizal sievate were not colonized at the conclusion of the experiment. Overall, rMR showed a weak negative relationship with AM fungal root colonization (Figure 1.1).

A strong phylogenetic signal was detected for interspecific rMR, as indicated by significant Blomberg's K and Pagel's λ values (Table 1.2). The greatest rMR values were consistently observed in the Incarnatae clade (Fishbein et al. 2011) (Figure 1.2), which consists of *A. nivea*, *A. curassavica*, *A. pumila*, *A. texana*, *A. subverticillata*, *A. verticillata*, *A. perennis*, *A. incarnata*, and *A. angustifolia*. With the exception of *A. subulata*, which belongs to the Sonoran Desert clade, all other species belong to the Temperate North American clade, many of which displayed moderate levels of rMR (Figure 1.2).

No phylogenetic signal was detected for interspecific AM fungal root colonization, (Figure 1.3). Further, the associated Blomberg's K value is larger than Pagel's λ and trending towards significance, suggesting a stronger, albeit non-significant, phylogenetic signal by this measure (Table 1.2).

Of the three investigated models of evolution, Brownian motion was the strongest model, accounting for approximately 62% of the variation in our rMR data (Table 1.3). Both the early burst model and Ornstein-Uhlenbeck process were comparable in respective ΔAIC scores (2.3 and 2.4, respectively) and relative model weights (19%), accounting for little of the explanation for interspecific variation. These results are consistent with rMR tracking the diversifying phylogeny at random, without evidence for strong selection. For AM fungal colonization, the Ornstein-Uhlenbeck process was the strongest model, accounting for nearly 88% of the variation in the model weights, relative to Brownian motion and early burst models (10% and 3%, respectively) (Table 1.3). These results suggest a strong constraint on interspecific AM fungal colonization of *Asclepias*, with an estimated optimum value of 0.36.

DISCUSSION

Host plant characteristics (i.e. morphology, phenology, taxonomic family) and soil characteristics (i.e. nutrients, texture) strongly influence plant-fungal responsiveness (Püschel et al. 2016; Hoeksema et al. 2018). Additionally, a growing body of literature suggests host plant phylogenetic relatedness is also a predictive metric of plant responsiveness to AM fungi (Burns and Strauss 2011; Reinhart et al. 2012; Lugo et al. 2014; Hoeksema et al. 2018). Plant species with shared evolutionary histories often exhibit phylogenetic signals, where related species tend to resemble one another to a greater degree than species randomly drawn from the same phylogenetic tree (Münkemüller et al. 2012). Phylogenetic relatedness may be especially important for interactions between plants and associated abiotic and biotic factors (Reinhart et al. 2012; Wooliver et al. 2016; Senior et al. 2018), however, while closely-related plant species often display similar responses to AM fungi (Wilson and Hartnett 1998; Hoeksema et al. 2010), empirical research examining suites of multiple congeners is surprisingly limited. This study, representing the most extensive analysis of *Asclepias*-AM fungal relationships, suggests *Asclepias* species generally benefit from symbiotic associations with AM fungi. Furthermore, benefit was associated with plant relatedness, as indicated by the strong phylogenetic signal for rMR (significant Blomberg's K and Pagel's λ values). These results suggest that mycorrhizal responsiveness of *Asclepias* species can be predicted by degree of relatedness under the Brownian motion model, and this pattern is significantly different than random. In our current study, 11 of 35 species were considered facultative mycotrophs, with no growth promotion following AM inoculation. Previous studies have also reported a lack of difference in biomass production between mycorrhizal and non-mycorrhizal *Asclepias* species (Vannette and Rasmann 2012; Vannette et al. 2013), including several species determined to be obligately mycotrophic in our study. However, Vannette and Rasmann (2012) and Vannette et al. (2013) were conducted using artificial potting media with an estimated 40 mg/kg soil plant-available P, whereas our current study was conducted using field-collected native prairie soils with 5.2 mg/kg plant-available P. Previous research indicates soil fertility modulates mycorrhizal benefits, and grassland AM mutualisms are likely most beneficial in P-limited systems (Johnson et al. 2010). A previous study examining rMR of warm-season grasses determined soil P-availability of 40 mg/kg resulted in the loss of all AM-derived benefits (Anderson et al. 1994), and Sylvia et al. (1993) observed that AM responses were most apparent in soils with less than 10 mg/kg.

Although mycotrophy of most vascular plant species fall along a continuum of facultative to obligate (Brundrett et al. 2002), specific assessments remain largely unexplored for many plant taxa, including those in ecosystems with prevalent AM fungal associations, such as grasslands. It is known that rMR varies substantially among higher level taxa and clades, e.g. between warm-season C_4 and cool-season C_3 grasses (Wilson and Hartnett 1998), suggesting that evolutionary history may strongly influence the degree of benefit from the mutualism. However, the lack of phylogenetic signal for AM fungal root colonization suggests phylogenetic relatedness itself is not a good predictor of AM fungal colonization. Rather, our results provide evidence that other processes, not phylogenetic relatedness, are likely driving levels of AM fungal colonization in *Asclepias* species.

Although AM fungal colonization serves as a good estimate of intra-radical AM fungal abundances, it is not always reflective of benefit received by the host plant (Hetrick et al. 1990; Wilson and Hartnett 1998; Gange and Ayres 1999). This may be due, at least in part, to abiotic characteristics in surrounding soils in which the plant is growing. Ganges and

Ayres (1999) proposed a conceptual model in which the relationship between AM fungal root colonization and plant benefit (i.e. biomass production) is curvilinear, with colonization and benefit increasing to a peak intercept, and benefits then decreasing as colonization levels surpass an optimum level. In our study, the evolutionary relationship between AM fungal root colonization and *Asclepias* was best explained by the stable optimum modeled by an Ornstein-Uhlenbeck process, suggesting an optimum range of AM fungal colonization levels exists for *Asclepias* from which species do not readily depart.

In addition to trade-offs between colonization levels and plant benefits, different AM fungal taxa have been shown to possess different functional traits (Chagnon et al. 2013; Powell and Rillig 2018) and may be an important consideration when assessing rMR. For example, some AM taxa are more effective at mining and transferring soil nutrients, while others may be more specialized at pathogen defense (Jin et al. 2017). Therefore, if AM fungal taxa with effective foraging capabilities are not present, as may occur in studies with single-species AM fungal inoculation, reduced plant productivity would not be unexpected (Köhl and van der Heijden 2016). It is well known most plant roots associate with a suite of AM fungal taxa (van der Heijden et al. 2015), and it is suggested that mutualist communities of closely-related plant species often overlap (Booth and Hoeksema 2010; Montesinos-Navarro et al. 2019). Therefore, it should be cautioned that variability in plant responses to AM symbiosis across studies may be an artifact of experimental design, as many studies include one or few AM taxa, often purchased from commercial sources (Vannette and Rasmann 2012; Vannette et al. 2013; Hart et al. 2018). In our study, the use of AM fungal inoculum from soils associated with native grassland plant communities allowed for the introduction of entire AM fungal communities, often consisting of at least twenty OTUs from multiple genera and families (Ji et al. 2013).

Although research surrounding the ecology and evolution of the genus *Asclepias* has increased dramatically over the past decade, the level of benefits conferred to the host plant by AM fungi remains unknown for many plant taxa, even those at the forefront of conservation issues. Filling these knowledge gaps has many practical applications, including, but not limited to, improved conservation and restoration of rare or endangered plant species. For example, inoculation with native AM fungi has been shown to improve productivity and survival of mid- and late-successional herbaceous plants (Koziol and Bever 2017; Koziol et al. 2018), many of which were determined to be highly responsive to AM fungi in earlier assays (Wilson and Hartnett 1998). Many the *Asclepias* spp. in our current study were highly-responsive to AM fungi, indicating use of these species in restorations of highly degraded soils may require inoculation with native fungal inoculum (Koziol et al. 2018). Alternatively, facultatively responsive *Asclepias* spp. may be useful in sites with a restoration goal of developing highly diverse communities of *Asclepias.* Understanding the dynamics of this relationship is critical, as *Asclepias* spp. provide food for larval monarch butterflies, as well as nectar for large suites of arthropods, including bees and wasps, which have also experienced recent precipitous declines (Bartomeus et al. 2013; Thomson 2016; Jacobson et al. 2018). Our results provide extensive insight into the interspecific diversity of this ancient relationship within *Asclepias*, providing crucial information that can help conservationists, restoration practitioners, and evolutionary ecologists.

TABLES

Table 1.1. Relative mycorrhizal responsiveness (rMR) and arbuscular mycorrhizal root colonization (RC) of selected *Asclepias* species.

Species	rMR[a]	AM fungal root colonization (%)
Obligately mycorrhizal		
A. texana	96.75**	29.84
A. perennis	95.74***	46.56
A. nivea	95.31***	24.61
A. speciosa	92.71***	38.12
A. subverticillata	89.86**	34.74
A. pumila	86.97**	14.62
A. verticillata	86.39***	42.40
A. angustifolia	86.00***	22.14
A. variegata	84.42***	26.10
A. linaria	83.68*	38.31
A. exaltata	83.66***	47.64
A. subulata	83.38***	22.15
A. oenotheroides	80.04**	31.59
A. humistrata	79.92***	28.66
A. tuberosa	78.82*	30.89
A. involucrata	76.29***	31.14
A. latifolia	75.23***	37.71
A. ovalifolia	73.25***	31.94
A. eriocarpa	70.61*	23.09
A. incarnata	69.87**	24.30
A. arenaria	68.63***	34.54
A. fascicularis	62.53*	27.16
A. stenophylla	52.52*	44.77
A. viridis	44.98*	40.19
Facultatively mycorrhizal		
A. syriaca	81.25	35.32
A. curassavica	79.38	20.50
A. purpurascens	67.19	39.39
A. sullivantii	61.29	52.19
A. engelmanniana	60.75	30.29
A. asperula	55.70	26.97
A. hirtella	53.62	38.25
A. meadii	43.89	22.25
A. cordifolia	43.69	27.39
A. viridiflora	36.53	41.46
A. vestita	27.04	33.27

[a] rMR = relative mycorrhizal responsiveness (%) = [(mean dry mass mycorrhizal plant – mean dry mass non-mycorrhizal plant)/mean dry mass mycorrhizal plant] x 100 (modified from Wilson and Hartnett 1998). Mean dry mass of inoculated plant significantly different from non-inoculated control as determined by one-way ANOVA (*** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$).

Table 1.2. Two measurements of phylogenetic signal (Blomberg's K and Pagel's λ) related to relative mycorrhizal responsiveness (rMR) and arbuscular mycorrhizal (AM) fungal root colonization in *Asclepias*. Bold *P* values indicate significant phylogenetic signals.

Trait	Blomberg's K	*P*	Pagel's λ	*P*
rMR	0.814	**0.009**	1.032	**0.003**
AM colonization	0.624	0.092	0.091	0.639

Table 1.3. Evolutionary model selection for *Asclepias* relative mycorrhizal responsiveness (rMR) and arbuscular mycorrhizal (AM) fungal root colonization.

Trait	AIC	ΔAIC	Weight
rMR			
Brownian motion	288.4	-	0.62
Ornstein-Uhlenbeck	290.7	2.3	0.19
Early burst	290.8	2.4	0.19
AM colonization			
Ornstein-Uhlenbeck	249.3	-	0.88
Brownian motion	253.7	4.4	0.10
Early burst	256.2	6.9	0.03

FIGURES

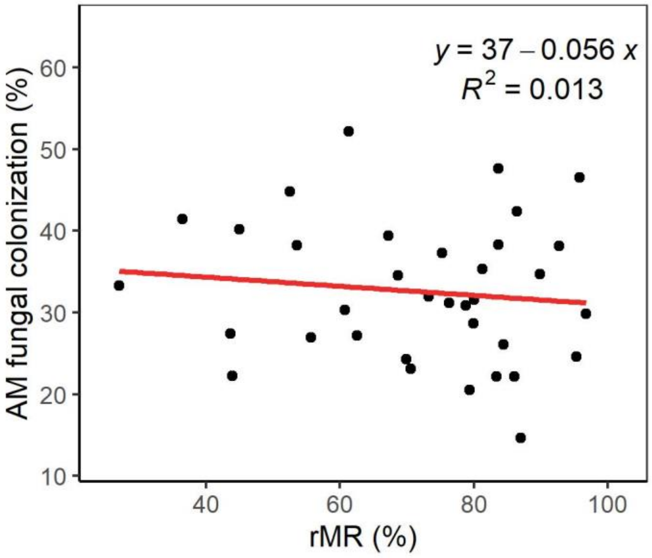

Figure 1.1. Linear regression showing the weakly negative relationship between relative mycorrhizal responsiveness (rMR) and arbuscular mycorrhizal (AM) fungal colonization for all sampled *Asclepias* species.

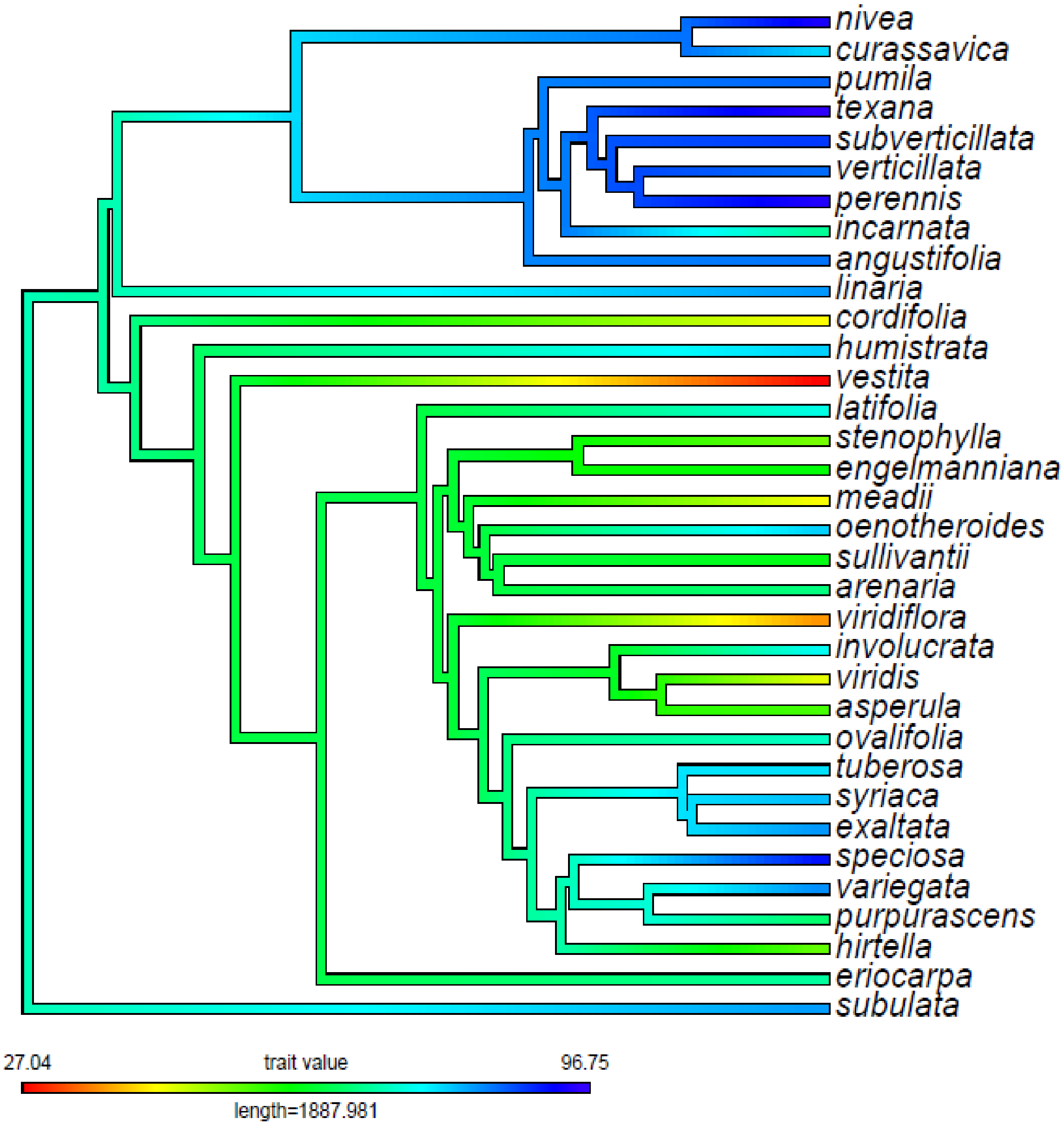

Figure 1.2. Maximum likelihood estimates of ancestral states of *Asclepias* relative mycorrhizal responsiveness (rMR) depicted on a chronogram obtained from penalized likelihood smoothing of substitution rates across the maximum likelihood phylogram in Fishbein et al. (2018). The scale bar indicates rMR values from low (hot colors) to high (cool colors).

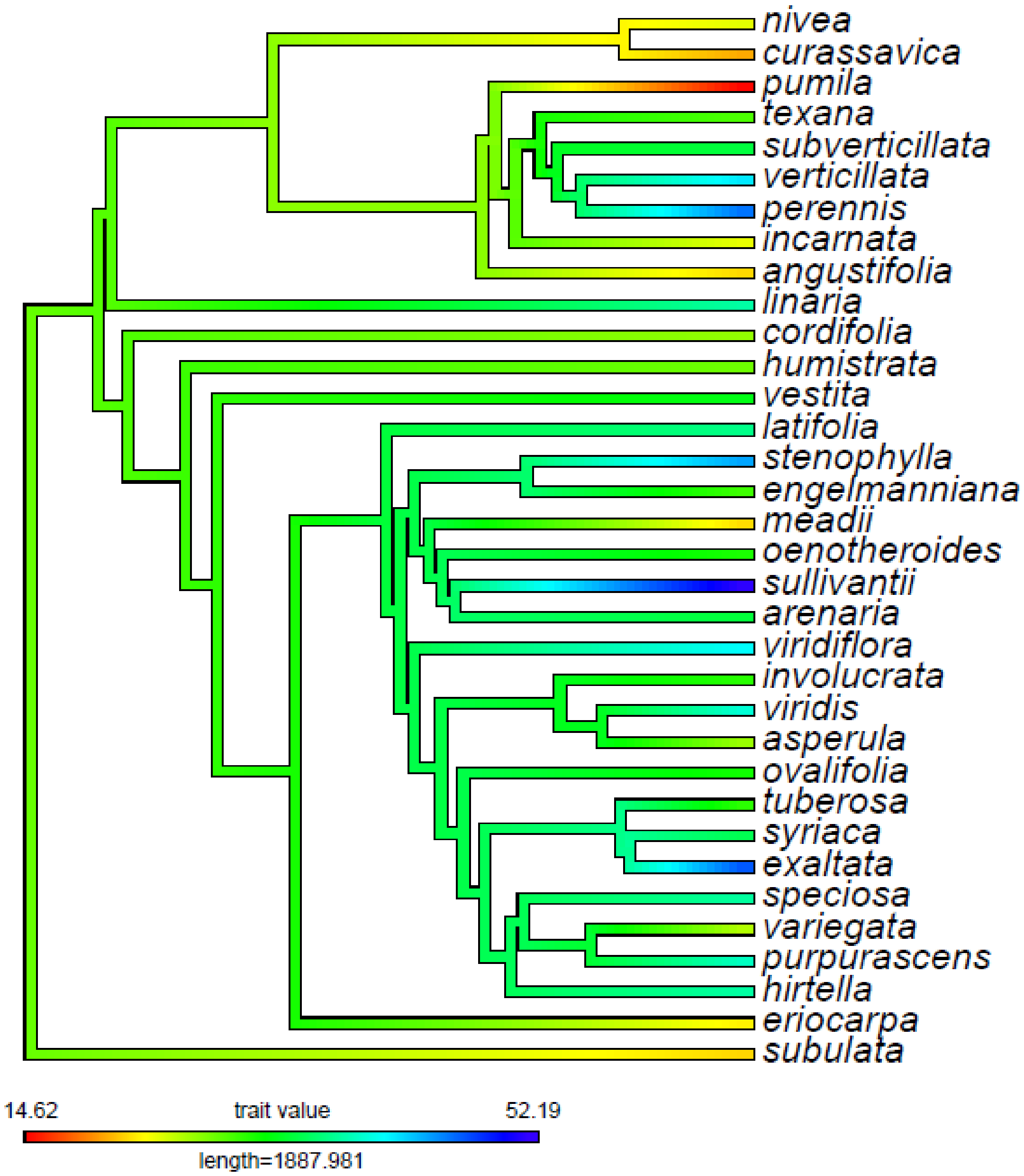

Figure 1.3. Maximum likelihood estimates of ancestral states of *Asclepias* intraradical arbuscular mycorrhizal fungal root colonization (%) depicted on a chronogram obtained from penalized likelihood smoothing of substitution rates across the maximum likelihood phylogram in Fishbein et al. (2018). The scale bar indicates colonization values from low (hot colors) to high (cool colors).

CHAPTER II

ROLE OF PHYLOGENETIC RELATEDNESS IN PLANT-SOIL FEEDBACK DYNAMICS OF SYMPATRIC *ASCLEPIAS* SPECIES

ABSTRACT

Plants affect associated biotic and abiotic edaphic factors, with reciprocal feedbacks from soil characteristics affecting plants. These two-way interactions between plants and soils are collectively known as plant-soil feedbacks (PSFs). The direction and magnitude of PSFs are influenced by several factors, including soil biota, above- and belowground herbivory, and the abiotic environment. The role of phylogenetic relatedness and evolutionary histories have recently emerged as a potential driver of PSFs, although the strength and direction of feedbacks among sympatric congeners is not well understood. We examined plant-soil feedback responses of *Asclepias syriaca*, a common clonal milkweed species, with several sympatric congeners across a gradient of increasing phylogenetic distances (*A. tuberosa*, *A. viridis*, *A. sullivantii*, and *A. verticillata*, respectively). *Asclepias syriaca* produced less biomass in soils conditioned by the most phylogenetically distant congener (*A. verticillata*), relative to conspecific-

conditioned soils. Similarly, arbuscular mycorrhizal (AM) fungal colonization of *A. syriaca* roots was reduced when grown in soils conditioned by *A. verticillata*, compared to colonization in plants grown in soil conditioned by any of the other three *Asclepias* species, indicating mycorrhizal associations are likely a mechanism of observed PSFs. Although these results suggest some overlap in mutualist communities between closely-related species, overall AM fungal-focused PSFs were significantly positive between con- and heterospecific *Asclepias* species, indicating *Asclepias* species condition host-specific AM fungal communities. Due to longer shared evolutionary histories, it is likely that closely-related species shared a greater overlap of mutualist partners, compared to species that diverged earlier in history. Overall, these results highlight the role of phylogenetic distance in influencing PSFs between sympatric congeners.

INTRODUCTION

Interactions between plants and associated soils play an important role in shaping and maintaining plant communities (Mangan et al. 2010b; Bever et al. 2015). Plant roots actively exude phytohormones, sugars, and other compounds, which directly and indirectly influence the biotic and abiotic properties of rhizosphere soil. The effects of plants on local soil properties, together with subsequent reciprocal interactions, are collectively known as plant-soil feedbacks (PSFs) (Kulmatiski et al. 2008; Bever et al. 2015). Plant-soil feedbacks span a continuum from negative to positive, with the direction and magnitude of feedbacks determined by the presence or absence, as well as abundance, of specific soil biota (Crawford et al. 2019). Negative PSFs occur when plant growth is reduced in soil previously occupied by plants of the same species (conspecific-conditioned), compared to growth in soil previously occupied by a different species (heterospecific-conditioned). Alternatively, positive PSFs occur when plants produce greater biomass in soil conditioned by conspecifics, relative to heterospecific-conditioned soil. In plant communities, negative PSFs encourage heterogeneity and co-existence between heterospecifics; thereby promoting greater plant diversity, whereas positive PSFs promote monotypic stands, reducing plant diversity (Bever et al. 1997; Mack et al. 2019). The direction and magnitude of PSFs depend on numerous biotic and abiotic edaphic factors, including but not limited to presence and abundance of mutualists, pathogens, herbivores, plant-available nutrients, and alterations in the environment (Duell et al. 2019; Crawford and Hawkes 2020). In native systems, PSFs are typically negative between co-evolved, phylogenetically unrelated species, resulting in greater overall plant community diversity (Meiners et al. 2017; Crawford et al. 2019). Although native plant communities typically consist of large suites of taxonomically unrelated species, many ecosystems support multiple sympatric congeners, and the role of PSFs among co-occurring congeners is poorly understood (Wilschut et al. 2019).

Closely-related species are more likely to share common traits or characteristics due to a longer shared evolutionary history, relative to species that diverged earlier in history (Pagel 1999). The tendency of closely-related species to resemble one another more closely than species drawn at random from the phylogeny is known as phylogenetic signal (Blomberg et al. 2003). Over the last decade, a growing body of research has begun exploring the role of phylogenetic relatedness in driving PSF direction and magnitude (Anacker et al. 2014). Previous studies investigating the role of phylogenic relatedness in PSF dynamics have reported increasing phylogenetic distance may lead to stronger negative PSF, relative to pairs of more recently diverged species (Tedersoo et al. 2013; Kempel et al. 2018; Stein and Mangan 2020; Wandrag et al. 2020), while other studies found PSFs could not be readily explained by phylogenetic distance (Mehrabi and Tuck 2014; Wilschut et al. 2019). However, as PSF dynamics are typically linked to the predominant forces driving feedbacks, differences in PSF dynamics are common. If a feedback is largely driven by the accumulation of host-specific pathogens or herbivores, phylogenetically distant species will likely exhibit a negative PSF due to the lack of shared antagonists (Gilbert and Webb 2007; Parker et al. 2015; Collins et al. 2020). Alternatively, PSFs may be driven by the presence or absence of mutualists. For example, plants that do not share mutualistic cohorts will likely exhibit positive PSFs if each plant species benefits equally from the host-specific mutualists (Reinhart et al. 2012; Hoeksema et al. 2018). In this scenario, if phylogenetically distant species are less likely to share mutualists due to earlier divergences, relative to more closely-related species, then PSFs will likely become more positive as phylogenetic distance increases (Crawford et al. 2019).

Milkweeds (*Asclepias* L., Apocynaceae) are a widely- recognized and diverse group of plants, with 130 of the estimated 400 species found in North America (Fishbein et al. 2011). Milkweed research has increased dramatically over the past two decades, due in part to the importance of milkweed as the primary food source of monarch (*Danaus plexippus* L.) larvae. Recently, Snyder and Harmon-Threatt (2019) assessed PSFs between two milkweed species (*A. syriaca* and *A. sullivantii*) in response to reduced soil moisture, but few studies to date have assessed the complex relationships between milkweeds and soil symbionts, particularly arbuscular mycorrhizal (AM) fungi, that likely contribute to PSFs. In grasslands of central North America, there are several *Asclepias* species that commonly grow within relatively close proximity to one another, including *Asclepias syriaca* L. (common milkweed), *A. tuberosa* L. (butterfly milkweed), *A. viridis* Walter (green antelopehorn), *A. sullivantii* Engelm. ex A. Gray (prairie milkweed), *A. verticillata* L. (whorled milkweed), as well as several others. However, the direction and magnitude of potential feedbacks occurring between sympatric *Asclepias* species is not known and understanding these relationships can provide insight into interspecific organization on landscapes where congeners co-occur, and how these dynamics may be mediated through PSFs.

To assess PSF strength and direction between sympatric *Asclepias* congeners, we conducted a greenhouse study examining *A. syriaca* (focal species) and four sympatric congeners across a gradient of increasing phylogenetic distances [*A. tuberosa*, *A. viridis*, *A. sullivantii*, and *A. verticillata*, respectively (collectively, peripheral species)]. *Asclepias syriaca* was selected as our focal species due to its importance as a monarch host plant throughout much of central and eastern North America. The influence of *A. syriaca* on sympatric congeners, as well as reciprocal effects, was measured through biomass production, root:shoot ratios, and AM fungal root colonization. Due to previous research suggesting native plant species often perform poorly in soil conditioned by conspecifics, we hypothesize that *A. syriaca* and each peripheral milkweed species will produce greater biomass and have greater AM fungal root colonization in soil conditioned by heterospecifics. Furthermore, we hypothesize that this difference will be amplified as phylogenetic distance of the peripheral species increases. While native plants often promote negative PSFs, previous research suggests more positive PSFs may occur between closely-related species (Liu et al. 2012). Therefore, we expect positive PSFs between *A. syriaca* and more closely-related species, such as *A. tuberosa* and *A. viridis*, with positive PSFs becoming weaker as phylogenetic distance increases, as with *A. sullivantii* and *A. verticillata*. Current literature suggests the role of phylogenetic relatedness in predicting PSF outcomes to be highly variable, and some of these inconsistencies are likely due to context-dependency and species or genus-specificity. Examination of PSFs of closely-related species is largely non-existent, including species at the center of current conservation concerns, such as *Asclepias* spp. Our research has implications for both conservation and evolutionary biologists/ecologists, as assessments of the influence of plant relatedness on PSF dynamics can provide key insights into species arrangement and organization on the landscape, as well as the degree to which evolutionary history dictates plant-soil-microbial interactions.

MATERIALS AND METHODS

Soil Conditioning Phase

We tested PSF dynamics of a focal plant species (*Asclepias syriaca*) and four sympatric congeners spanning across a phylogenetic gradient. *Asclepias syriaca* is a long-lived perennial, clonal milkweed species native across much of eastern and central North America. We selected four peripheral species, from decreasing to increasing phylogenetic distance from *A. syriaca*: *A. tuberosa* (non-clonal), *A. viridis* (non-clonal), *A. sullivantii* (clonal), and *A. verticillata* (clonal).

Seeds were purchased from Prairie Moon Nursery (Winona, MN, USA) and germinated in moist vermiculite to the two-leaf stage of development (~24 days). For the conditioning phase, soils were conditioned, or trained, by growing individual *Asclepias* seedlings in 650 mL dee-pots (Stuewe & Sons, Inc., Tangent, OR, USA) containing 600 g (dry wt) of native prairie soil. Soil was collected from the Konza Prairie Biological Station, Manhattan, KS, USA, and passed through a 10 mm sieve to remove rocks and coarse plant material. All five *Asclepias* species commonly occur on Konza Prairie Biological Station. For the conditioning phase of this study, seedlings of each species were transplanted at the onset of two sets of true leaves, with a single seedling per pot (Figure 1). Eighty replicate pots were established for soil conditioning by the focal species, *A. syriaca*, and twenty replicates were established for each of the four peripheral species. This ensured enough inoculum would be available for each pairwise feedback. Therefore, the soil conditioning phase consisted of 160 pots [(4 species x 20 replicates) + (1 species x 80 replicates)]. Plants were grown and maintained for 18 weeks, at which time the majority of individuals were beginning to senesce, and soil was collected for use as inoculum in the plant-soil feedback phase.

Plant-Soil Feedback (PSF) Phase

The experimental design of the PSF phase follows the approach described by Bever (1994). Seed treatment and soil collection followed the procedures described in the conditioning phase. In the PSF phase, soil was steam-pasteurized at 80°C for 2 h and allowed to cool for 72 h to eliminate biotic communities but retain soil abiotic traits (Wilson and Hartnett 1998, Johnson et al. 2010). Each pot was filled with 600 g of soil consisting of the following “layers”: 400 g of steam-pasteurized soil, followed by 100 g of soil inoculum (developed in soil conditioning phase), followed by 100 g of steam-pasteurized soil to protect from cross-contamination during the growing period (Duell et al. 2019). A single *A. syriaca* seedling was planted into individual pots with the *A. syriaca* (conspecific) inoculum or with the inoculum from a heterospecific peripheral species (Figure 2.1). A single seedling of each of the four peripheral species was planted into individual pots containing inoculum soil conditioned by *A. syriaca* or into pots with conspecific-conditioned soil, with a single seedling in each pot (Figure 2.1). Therefore, the PSF phase was established using a partial factorial design: *A. syriaca* x 5 inocula (*A. syriaca* conditioned soil or soil conditioned by each peripheral species) x 10 replicates + each peripheral species (*A. tuberosa*, *A. viridis*, *A. sullivantii*, *A. verticillata*) x 2 inocula (*A. syriaca* conditioned soil or soil conditioned by conspecific) x 10 replicates, for a total of 130 pots (Figure 2.1).

After 20 weeks, prior to shoot senescence, plants were harvested, root and shoot biomass was separated and roots were washed free of soil. Biomass was dried at 60°C for 48 h and weighed. Three root subsamples from each individual plant were collected and stained with trypan blue to determine percent mycorrhizal root colonization. AM fungal root colonization was scored by using the magnified gridline intersect method (McGonigle et al. 1990), using a digital microscope (Hirox KH 7700) at 200x magnification to measure the percentage of root colonized by AM fungal structures (hyphae + vesicles + arbuscules). For each root sample, three random sections of root length were scored separately, with a total of approximately 900 grids observed per sample. The reported colonization value of each sample was a mean of the three subsets.

Statistical Analyses

Feedbacks were calculated for total biomass, root:shoot ratio, and intra-radical AM fungal colonization. Interaction coefficients were calculated to quantify PSF dynamics between *A. syriaca* and peripheral congeners grown with inoculum trained by either conspecific or heterospecific plants: $I_s = \mathrm{G}(A)_\alpha - \mathrm{G}(A)_\beta - \mathrm{G}(B)_\alpha + \mathrm{G}(B)_\beta$, where I_s is the feedback interaction coefficient, $\mathrm{G}(A)_\alpha$ is growth of plant species *A* inoculated with conspecific soil, $\mathrm{G}(A)_\beta$ is growth of plant species *A* inoculated with heterospecific soil, $\mathrm{G}(B)_\alpha$ is growth of plant species *B* inoculated with heterospecific soil and, $G(B)_\beta$ is growth of plant species *B* inoculated with conspecific soil (Bever et al. 1997). When I_s values are positive ($I_s > 0$), a net positive feedback on plant growth is generated by the soil community, and coexistence between plant species is not likely. Conversely, when I_s values are negative ($I_s < 0$), a net negative feedback on plant growth is generated by the soil community, and coexistence between plant species is likely (Bever 2003). Interaction coefficient values were calculated for each combination of *A. syriaca* and each of the four peripheral species.

Using PROC-GLM in SAS, we constructed a general linear model using log-transformed (for normalization of data) biomass data, and logit-transformed root:shoot ratio and AM root colonization data as the dependent variables. Conditioning species (phase 1) identity and experimental species (phase 2) identity were used as factors with interactions for each focal-peripheral species pairing. Pairwise feedback was tested within the conditioning and experimental plant species interaction, where conditioning species represents the plant species used in the conditioning phase of the experiment and experimental species refers to the plant being assessed in the second experiment (Bever

1994). These analyses were conducted using the PROC GLM procedure in (SAS Institute, Cary, NC, U.S.A.), version 9.4 of the SAS System for Windows.

Prior to analysis, all data were tested for normality and homogeneity of variances using Shapiro-Wilk and Levene's tests, respectively. To test for differences in biomass production in response to soil conditioning, a one-way analysis of variance (ANOVA) was conducted, with soil inoculum as the main effect. Tukey's honest significant difference (HSD) test was conducted post-hoc to determine differences among conditioned soils, with significance assessed at $p = 0.05$. Similar analyses were conducted for root:shoot ratios and percent AM fungal root colonization. Due to lack of statistical differences and no clearly observed patterns, root:shoot ratios are not presented. All analyses were performed in R version 4.0.2 (R Core Team 2020).

RESULTS

Results

Asclepias syriaca produced significantly less biomass when grown in soils conditioned by *A. verticillata*, compared to conspecific-trained soils, or soils conditioned by *A. tuberosa*, *A. viridis*, or *A. sullivantii* (Figure 2.2). *Asclepias verticillata* produced significantly less biomass in conspecific-trained soil, relative to soil trained by *A. syriaca*, while growth associated with any other inoculum sources was not significantly different (Figure 2.3). Interaction coefficients revealed no significant feedbacks between *A. syriaca* and peripheral species in relation to biomass production (Figure 2.4).

Mycorrhizal root colonization of *A. syriaca* following growth in *A. verticillata*-conditioned soils was significantly less relative to soils conditioned by *A. sullivantii*

(Figure 2.5). AM fungal root colonization of *A. sullivantii* and *A. verticillata* (the two most phylogenetically distant peripheral species) was significantly less following growth in *A. syriaca*-conditioned soil, relative to conspecific-conditioned soil (Figure 2.6). Additionally, a strong positive feedback was observed between *A. syriaca* and the distantly related *A. verticillata* (Figure 2.7), indicating each of these species promotes greater AM fungal root colonization following growth in soil conditioned by conspecifics, compared to growth in heterospecific-conditioned soil. A weakly negative PSF occurred for overall interaction coefficients related to *Asclepias* biomass production, indicating that *Asclepias* species generally produce greater biomass following growth in soils conditioned by heterospecifics, compared to growth in soil conditioned by the conspecific (Figure 2.8). Alternatively, overall PSF dynamics related to AM fungal root colonization indicate a strong positive interaction coefficient, suggesting *Asclepias* species generally had greater AM fungal colonization in soils previously occupied by the conspecific (Figure 2.8).

DISCUSSION

We investigated the biomass- and AM fungal-focused PSF dynamics between *A. syriaca* and several sympatric congeners spanning a phylogenetic gradient (*A. tuberosa*, *A. viridis*, *A. sullivantii*, and *A. verticillata*, respectively). Overall, three clear patterns emerged. First, biomass-focused PSFs between *A. syriaca* and each of the four peripheral species were largely neutral, suggesting a high likelihood of coexistence; second, AM fungal-focused interaction coefficients suggest positive PSFs between *A. syriaca* and the two more phylogenetically distant peripheral species (*A. sullivantii* and *A. verticillata*);

and third, overall PSF between con- and heterospecific *Asclepias* species revealed weakly negative biomass PSF and strong positive AM fungal root colonization PSF.

Phylogenetically-driven PSFs are often linked to the presence, abundance, and degree of overlap of host-specific pathogens (Gilbert and Webb 2007; Parker et al. 2015; Collins et al. 2020) or mutualists (Mangan et al. 2010a; Bever et al. 2015; Wang et al. 2017). In our study, biomass production of the focal species, *A. syriaca*, was less when grown in *A. verticillata*-conditioned soils, compared to growth in soil conditioned by the other species. This growth reduction is likely driven by a lack of shared mutualists, such as AM fungi, due to the strong positive PSF observed for AM fungal root colonization. Alternatively, *A. verticillata* performed much better in soils previously occupied by *A. syriaca*, relative to conspecific-trained soils, suggesting a build-up of host-specific pathogens. These results are similar to those of Mangan et al. (2010a), where seedlings of tropical tree species performed better in soils and AM fungal communities conditioned by conspecifics, compared to those of heterospecific trees.

While PSF studies generally assess the influence of plants, soils, and reciprocal interactions on biomass production, the flexibility of the interaction coefficient calculation allows for testing of any quantified traits. In our study, a positive AM fungal PSF was observed; AM fungal colonization of *A. sullivantii* and *A. verticillata* was greater in conspecific-trained soils, compared to *A. syriaca*-conditioned soils. This is likely due to the absence of mutualists that easily reciprocate with *A. sullivantii* or *A. verticillata* in soils conditioned by *A. syriaca*, which is likely due to the phylogenetic distances of these species. These results align with previous studies that have found greater mutualist abundances in soils previously occupied by conspecifics, relative to heterospecifics (Haskins and Gehring 2005; Kulmatiski et al. 2017; Wang et al. 2019). Our results also suggest these patterns likely exist across a gradient with increasing phylogenetic distances, as species with longer shared evolutionary histories likely exhibit greater overlap of mutualists, relative to those that diverged earlier in time. The positive AM fungal PSFs observed in our study may be due, at least in part, to the clonal nature of *A. syriaca*, *A. sullivantii*, and *A. verticillata*, as weaker positive PSFs were observed for non-clonal *A. tuberosa* and *A. viridis*. The positive PSFs created by these clonal species may facilitate outward spread of new ramets and associated AM fungal propagules into the surrounding plant community.

It is likely that AM fungal host-specificity exists within closely-related plant species, and previous research has shown that as plant community succession advances temporally, species within those communities often display greater host-specificity of AM fungal symbionts (Koziol and Bever 2015, 2016). Although successional stages of *Asclepias* are often species-specific and context-dependent (Middleton and Bever 2012; Bauer et al. 2018), the majority are mid-successional. Therefore, *Asclepias* spp. likely develop close associations with selected AM fungal partners, developing positive PSFs. Further research is needed to determine the identity of associated AM taxa. However, rapid advancement in molecular tools and techniques provides identification of AM fungal taxa in soils and host roots, accelerating an interest in host-symbiont specificity within plant communities (Sepp et al. 2019; Wang et al. 2019), and determination of microbial community similarities between related species is now possible.

Interactions between plants and their associated soils are recognized as key drivers of plant community diversity and organization (Mangan et al. 2010b; Bever et al. 2015) and may be especially important for sympatric species (Mangan et al. 2010a). Although previous studies suggest closely-related species exhibit positive PSFs due to shared pathogens, our results suggest PSF dynamics between *Asclepias* species are driven by associated mutualist communities. Our results also suggest this relationship may weaken with increased phylogenetic distance, as was observed with *A. syriaca* and *A. verticillata*, as earlier diverging species with less shared evolutionary histories are less likely to select for similar symbiotic communities, compared to more recently diverged plant species.

FIGURES

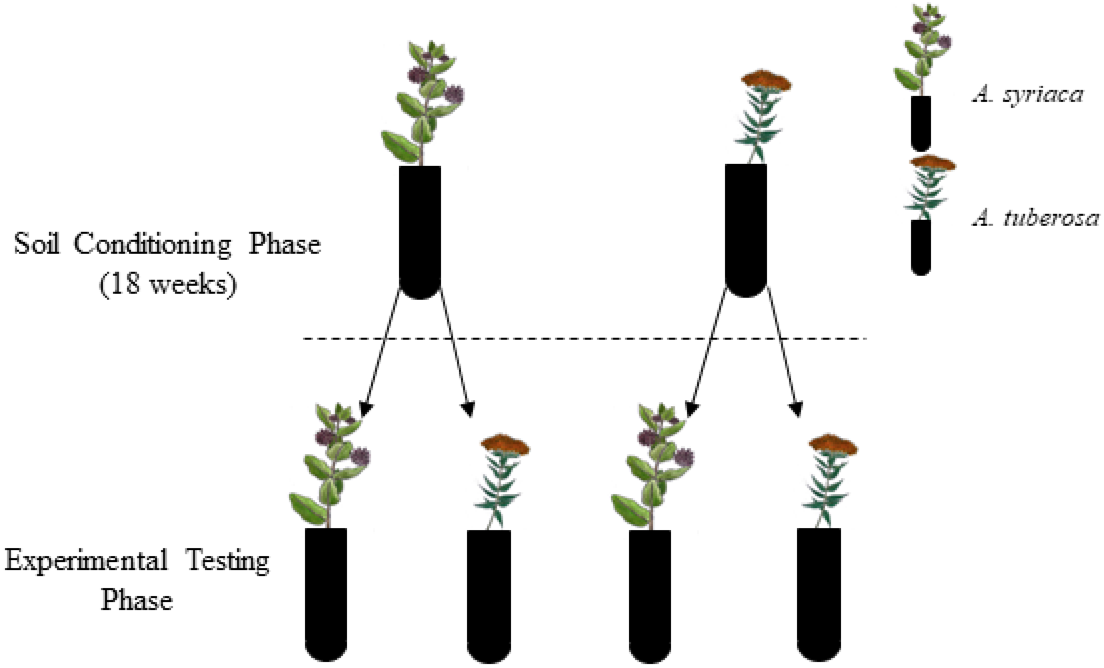

Figure 2.1. Schematic diagram for the experimental design of the conditioning and experimental testing phases of this research. In the conditioning phase, one focal (*Asclepias syriaca*) or one of the four peripheral species spanning a gradient of phylogenetic distances (*A. tuberosa*, *A. viridis*, *A. sullivantii*, and *A. verticillata*) were grown individually in separate pots. Two different soil communities conditioned in the conditioning phase were used as inocula in the testing of PSF between each pairing of *A. syriaca* and the four congeners. The schematic shows only one iteration of paired focal (*A. syriaca*) and peripheral species (*A. tuberosa*). As shown by our schematic, each soil treatment from the conditioning phase was divided twice times and used as inoculum for conspecific or heterospecific seedlings. This experimental design was replicated using each of the three remaining peripheral species (*A. viridis*, *A. sullivantii*, and *A. verticillata*).

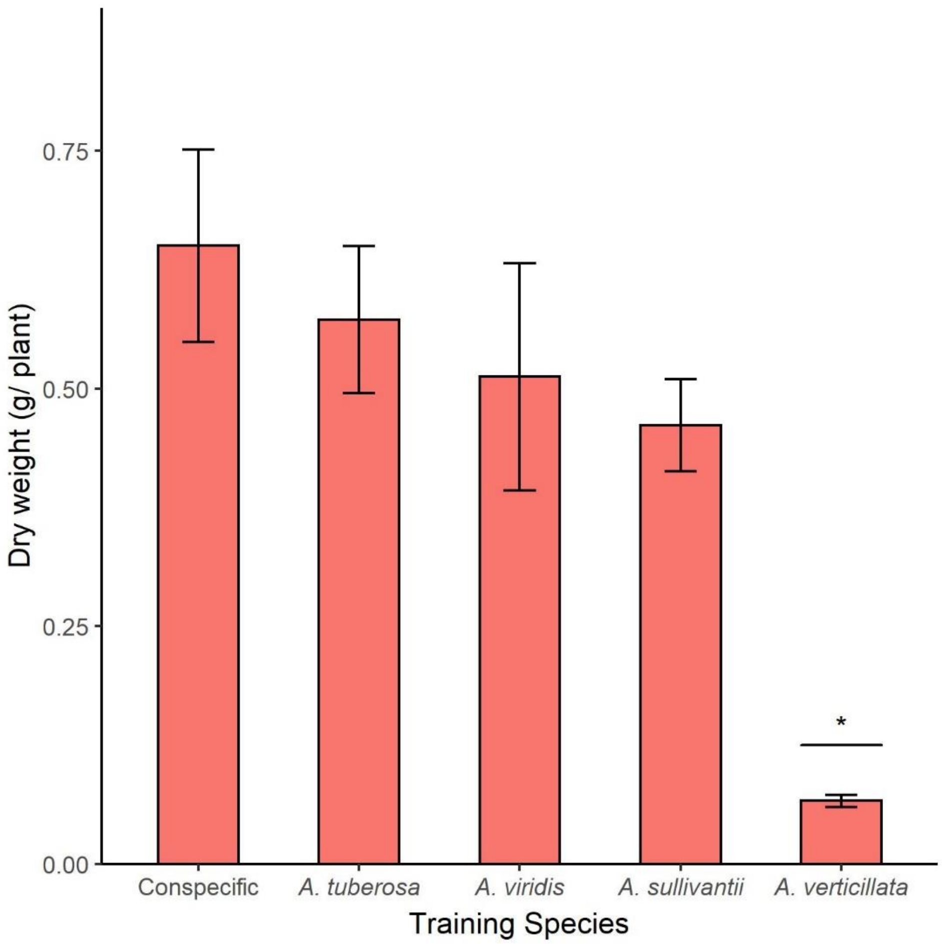

Figure 2.2. Biomass production (mean ± SE) of *Asclepias syriaca* in response to soil conditioning by sympatric congeners spanning a phylogenetic gradient. Phylogenetic distance from *A. syriaca* increases from left to right. Asterisk denotes significant difference between *A. verticillata*-conditioned soils and all other soils, with significance assessed at $p \leq 0.05$.

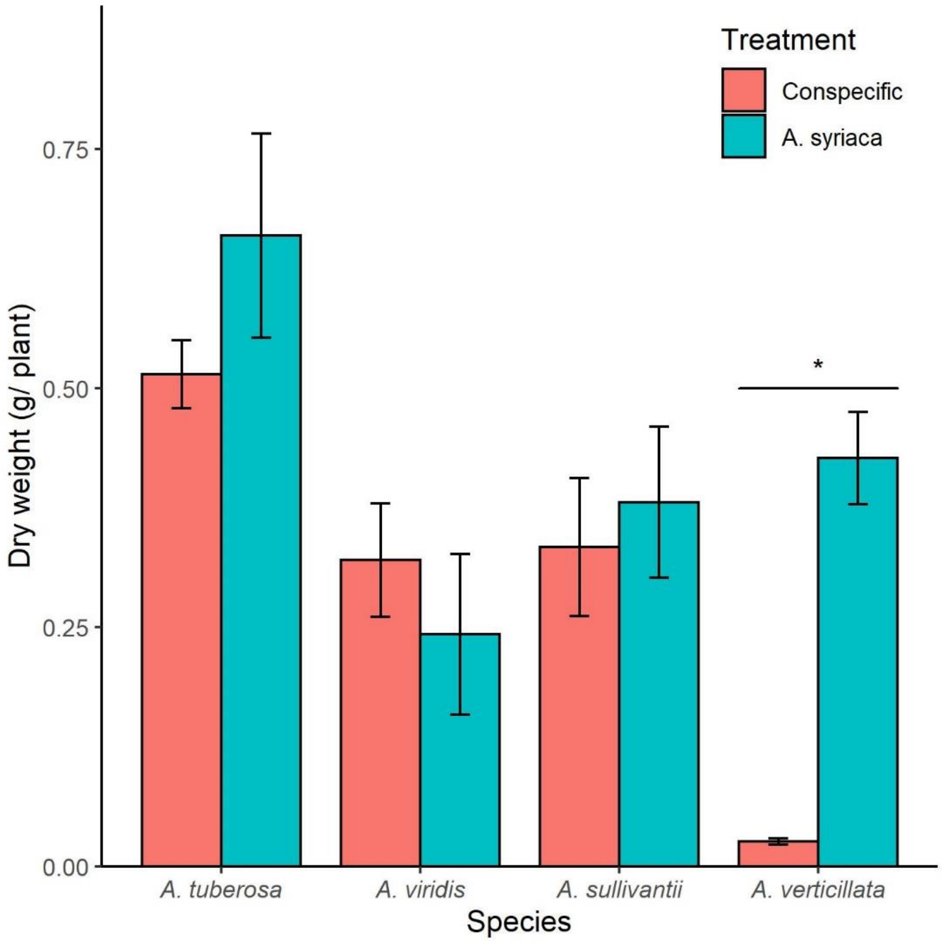

Figure 2.3. Biomass production (mean ± SE) of peripheral species in conspecific- (red bar) and *Asclepias syriaca*-trained (blue bar) soils. Asterisks denote significant differences within a species, with significance assessed at $p \leq 0.05$.

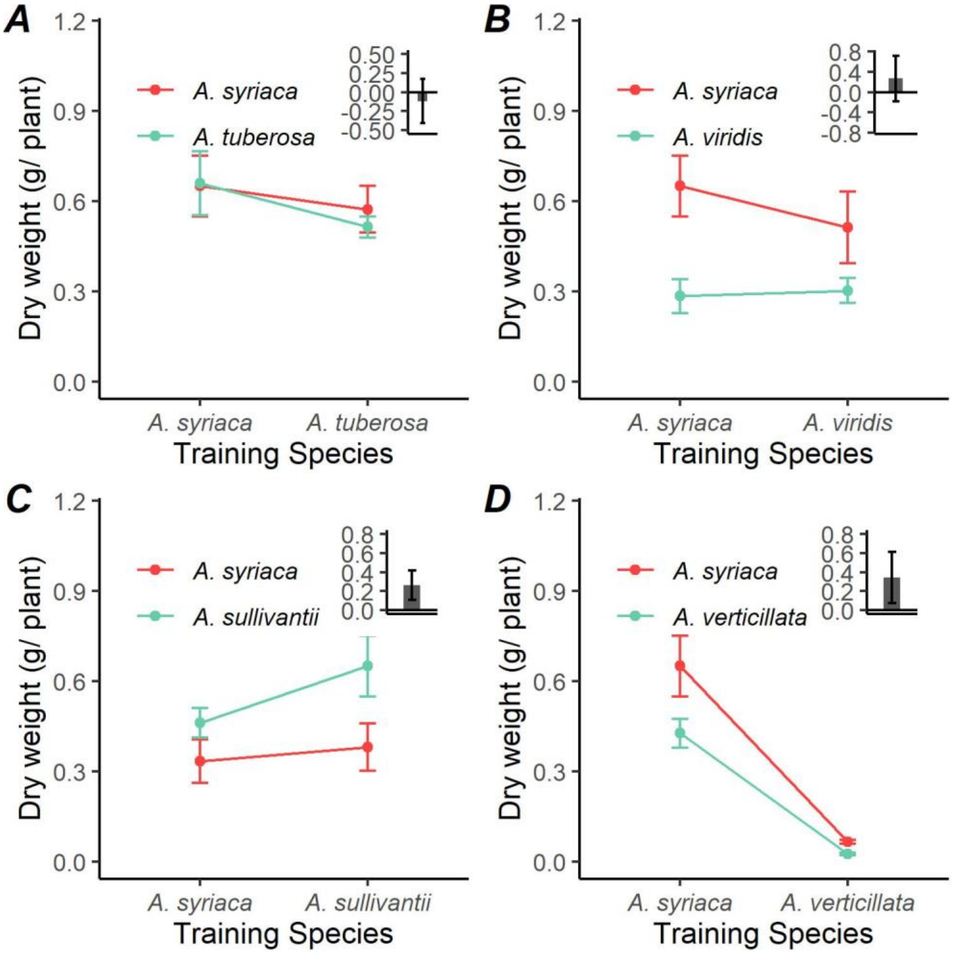

Figure 2.4. Interaction coefficient for PSF related to biomass production (mean ± SE) of *Asclepias syriaca* and four peripheral species across a phylogenetic gradient. Phylogenetic distance from *A. syriaca* increases from A–D.

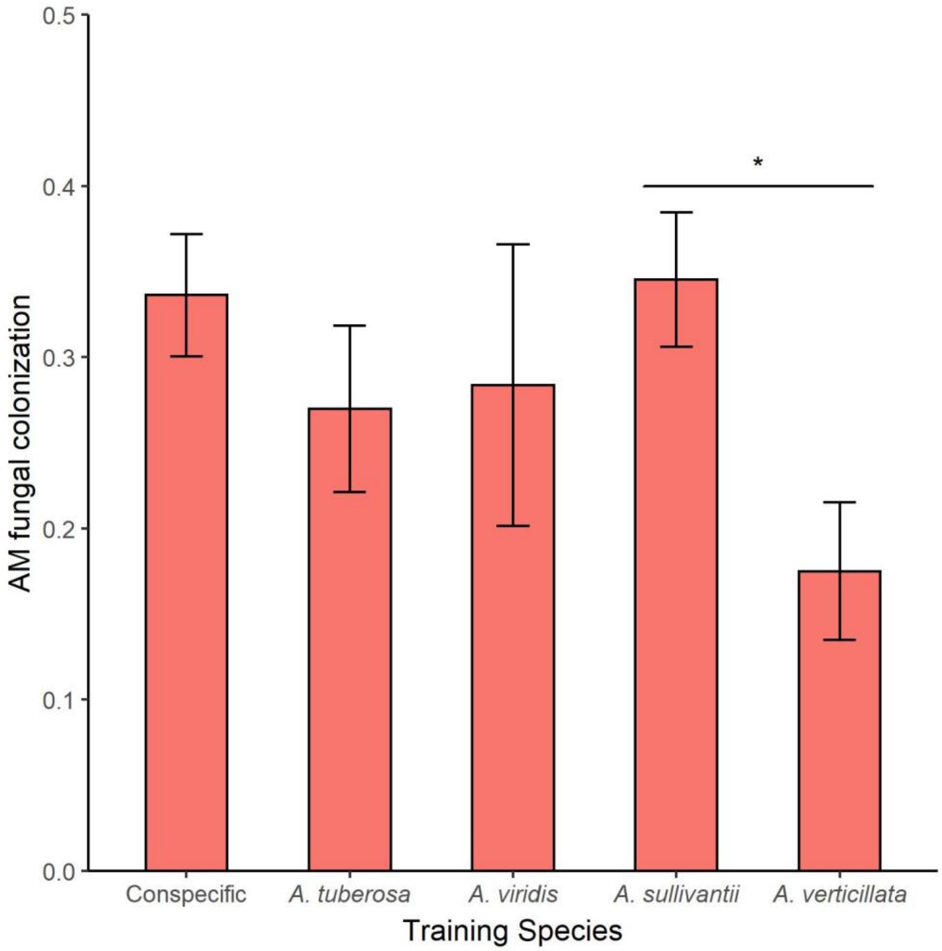

Figure 2.5. Arbuscular mycorrhizal (AM) fungal colonization (mean ± SE) of *Asclepias syriaca* in response to soil conditioning by sympatric congeners spanning a phylogenetic gradient. Phylogenetic distance from *A. syriaca* increases from left to right. Asterisks denote significant differences between conditioning species treatments, with significance assessed at $p \leq 0.05$.

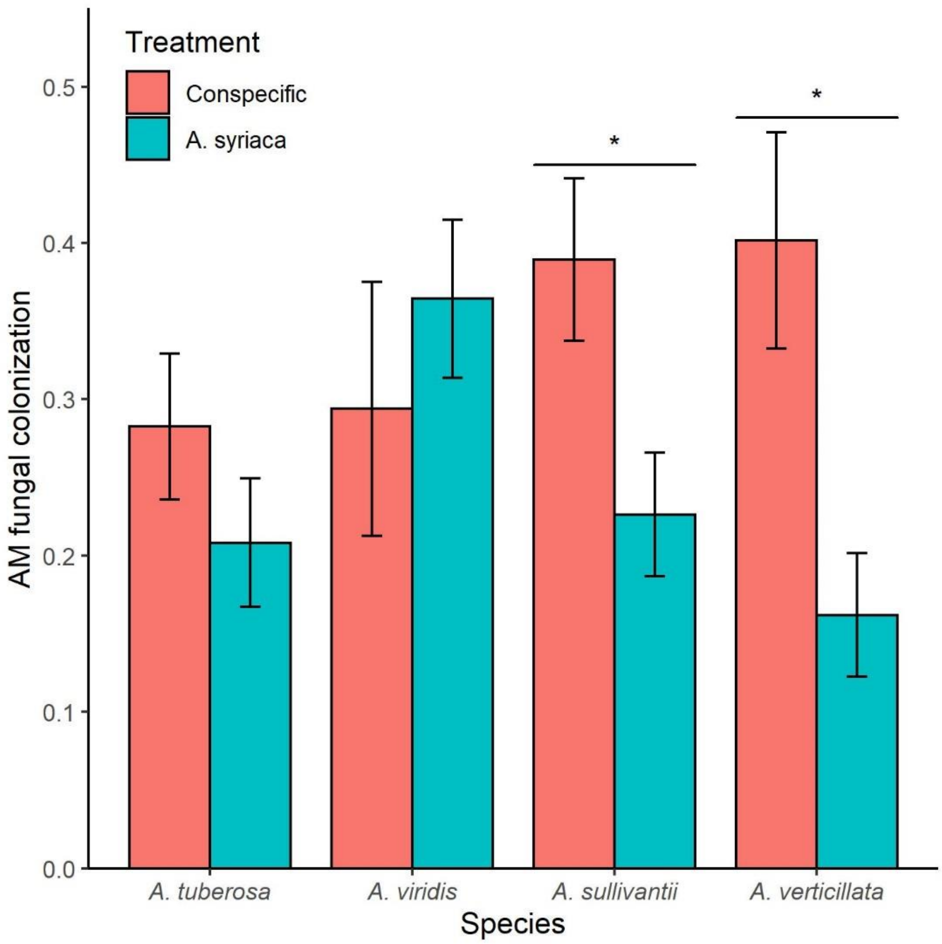

Figure 2.6. Arbuscular mycorrhizal (AM) fungal colonization (mean ± SE) of peripheral species in conspecific- (red bar) and *Asclepias syriaca*-trained (blue bar) soils. Asterisks denote significant differences within a species, with significance assessed at $p \leq 0.05$.

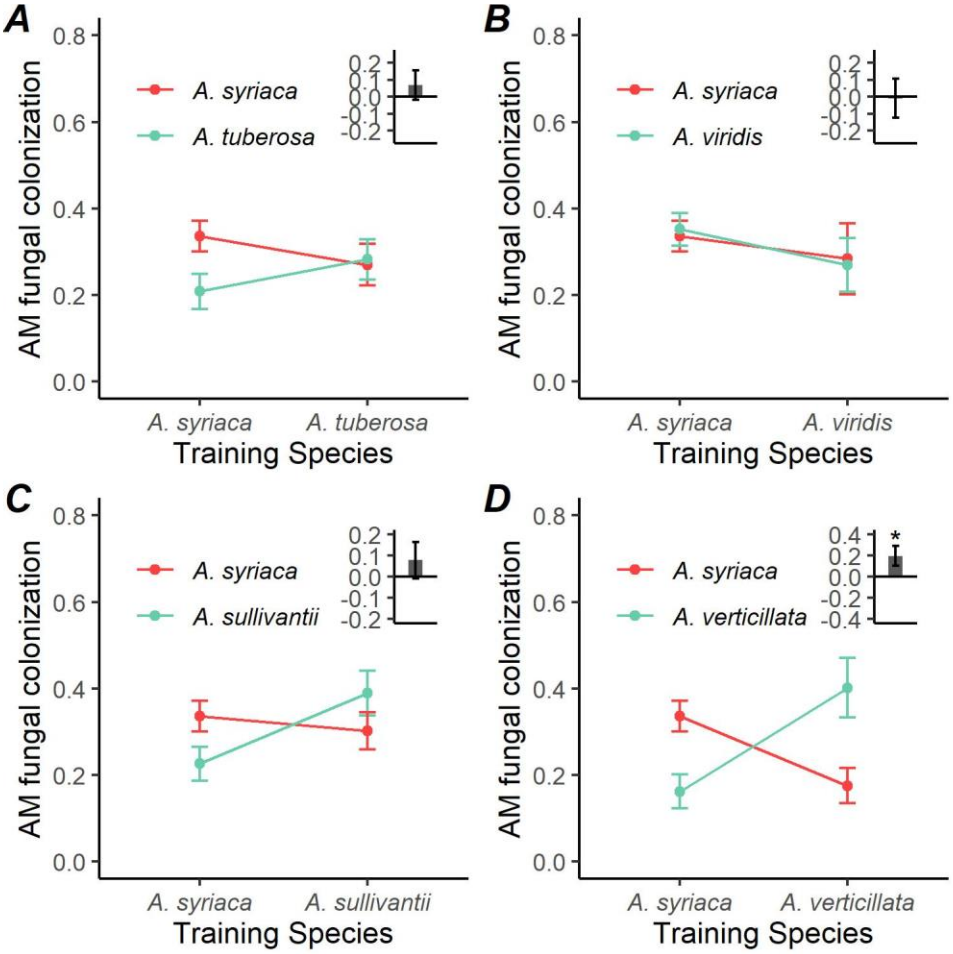

Figure 2.7. Interaction coefficient for PSF related to AM fungal colonization (mean ± SE) of *Asclepias syriaca* and four peripheral species across a phylogenetic gradient. Phylogenetic distance from *A. syriaca* increases from A–D. Asterisks in figure inset denotes feedback coefficient that is statistically different from zero, with significance assessed at $p \leq 0.05$.

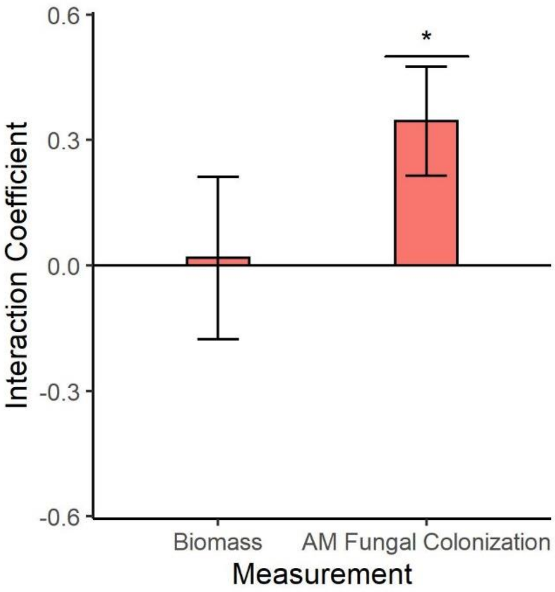

Figure 2.8. Overall interaction coefficients (I_S) (mean ± SE) related to each of total biomass production and AM fungal colonization between con- and heterospecific *Asclepias* species. Asterisks denote a feedback coefficient is statistically different from zero, with significance assessed at $p \leq 0.05$.

CHAPTER III

DROUGHT REDUCES BIOMASS PRODUCTION AND HERBIVORE DEFENSES OF SELECTED TALLGRASS PRAIRIE FORBS: IMPLICATIONS FOR BIOTIC INTERACTIONS

ABSTRACT

Drought frequency, severity, and duration is projected to increase globally, likely incurring negative impacts on ecosystem structure and functioning. For example, severe drought often reduces plant productivity, potentially influencing plant secondary metabolites, such as floral resources and herbivore defenses. These metabolic alterations are context-dependent, and can increase, be reduced, or remain unaffected, depending on plant species and drought severity. Drought may also decrease important symbiotic functions between plants and soil microbes such as arbuscular mycorrhizal (AM) fungi, potentially reducing nutrient acquisition of important entomophilous plants, with possible concomitant losses of key biotic interactions. We examined effects of drought on biomass production, physical defense properties, nectar production, and associated AM fungal abundance of five common tall- and mixed-grass prairie forb species (*Asclepias syriaca*, *A. viridis*, *A. tuberosa*, *Ruellia humilis,* and *Salvia azurea*) in a greenhouse study. In three species, reduced soil moisture decreased vegetative biomass production, while in two species, vegetative biomass did not differ between well-watered and droughted conditions. *Ruellia humilis* produced reproductive biomass under drought, however, flowers produced significantly less nectar, relative to well-watered conditions. Generally, production of physical defenses decreased under drought, relative to well-watered conditions. Intra-radical AM fungal abundances were not significantly affected by drought, although extra-radical AM fungal biomass decreased in *S. azurea*. Overall, grassland forb species were negatively impacted by moderate drought, with resultant negative implications for biotic interactions. For example, reduced flowering biomass and nectar production would likely result in fewer pollinator visitors, with resultant seed limitation for many forb species. Reduced physical defenses increases the likelihood of herbivory, further decreasing the ability to store energy for essential functions, such as reproduction. Additionally, drought has the potential for decoupling associated mutualisms, with a concomitant loss in nutrient acquisition and biomass production, thereby increasing susceptibility to additional environmental and biotic stressors.

INTRODUCTION

Regional drought is likely to continue, as alterations in precipitation regimes are expected to increase in frequency and intensity, especially in arid and semi-arid regions of the world (Bradford et al. 2020; Luo et al. 2020). While native plant species and communities in arid and semi-arid regions are well-adapted to periodic drought, more frequent and intense events will likely have direct impacts on primary productivity, individual plant performance, and ecosystem services (Knapp et al. 2015; Duell et al. 2016). Additionally, drought may indirectly affect plant performance due to alterations in belowground microbial communities (Kaisermann et al. 2017; Duell et al. 2019). The majority of previous drought studies focus on dominant plant species of a specific ecosystem (Nippert et al. 2009; Connor and Hawkes 2018), with substantially less research examining effects of drought on sub-dominant plant species. It is critical to understand the impacts of drought on the overall plant community, as less common or rare species are often vital contributors to community-level diversity, and provide important floral resources to pollinators and other nectar-feeding species.

During drought events, many perennial plant species allocate resources to aboveground biomass production and belowground carbohydrate reserves, often at the expense of production of secondary metabolites, such as floral resources and defense compounds, as well as physical defense traits. However, while drought stress often results in alterations to secondary compounds and herbivore defenses, the direction and magnitude of these changes is likely dependent on the plant species and associated compounds or defense structures. In previous studies, nectar quantity was generally found to increase with water-availability (Wyatt et al. 1992; Carroll et al. 2001; Halpern et al. 2010), however, the impacts on defense traits and other secondary metabolites appear to be highly variable (Halpern et al. 2010; Gutbrodt et al. 2011; Metz et al. 2014; Kleine and Müller 2014; Pineda et al. 2016). In the event of extreme drought, alterations in allocation of resources to secondary metabolites are not only important for individual plants, but also the large suite of herbivores, pollinators, and belowground symbionts that depend on them.

Almost without exception, perennial plant species in grassland ecosystems form at least some degree of association with arbuscular mycorrhizal (AM) fungi, with the majority highly dependent on the symbioses (Wilson and Hartnett 1998). In this typically mutualistic relationship, AM fungi penetrate the root cortex and extend hyphal structures into the surrounding soil, increasing plant root surface area and enhancing soil nutrient acquisition. In exchange, the plant provides photosynthetic carbon to the fungi. In addition to enhancing nutrient uptake and acquisition by the plant, AM fungi are known to benefit host plants in many other ways, including increased pathogen resistance and mitigating effects of drought stress through greater water-use efficiency and photosynthetic efficiency (Ruiz-Sánchez et al. 2010; Boyer et al. 2015; Bowles et al. 2018; Symanczik et al. 2018). However, following periods of extreme drought, AM fungal abundance in rhizosphere soil can decrease, likely due to hyphal desiccation (Duell et al. 2016).

Our current study assessed effects of drought on several North American grassland forb species. Specifically, we examined the above- and belowground biomass production following periods of drought. As greater root: shoot ratios are indicative of greater drought tolerance (Schenk and Jackson 2002; Wang et al. 2018), this parameter was assessed, as well. Soil microbial communities were assessed, with a focus on AM fungi, as many grassland species are dependent on the symbiosis for resource, and possibly water, acquisition. We examined production of physical defenses (trichomes or latex production) when applicable. Two species (*Asclepias syriaca* and *A. tuberosa*) produce a large number of foliar trichomes which function as the first line of defense against potential herbivores. Two species (*A. syriaca* and *A. viridis*) typically produce large amounts of latex in defense against potential herbivores. We hypothesized reduced soil moisture would: 1) decrease biomass production for all five grassland forb species, 2) reduce extra- and intra-radical AM fungal abundances, 3) reduce latex production in *A. syriaca* and *A. viridis*, as water is the primary constituent of plant latex, 4) reduce trichome production, as physical defenses are often resource-limited, and 5) reduce nectar production, as water is the primary constituent of nectar.

MATERIALS AND METHODS

This greenhouse experiment was conducted at Oklahoma State University in Stillwater, Oklahoma, USA. Five common grassland forb species were selected to determine the effect of drought on plant productivity, secondary metabolite production, and associated soil microbial communities. These species were selected as each has been identified as high importance to butterflies and other pollinators. In addition, three of the selected species belong to the genus *Asclepias*, many of which have gained much recent attention due at least in part to their importance as monarch (*Danaus plexippus* L.) larval host plants. Selected forb species included: *A. syriaca* L., *A. tuberosa* L., *A. viridis* Walter, *Ruellia humilis* Nutt., and *Salvia azurea* Michx. ex Lam. Soil was collected from Konza Prairie Biological Station, Manhattan, Kansas, from areas dominated by perennial warm-season grasses, including *Andropogon gerardii* Vitman (big bluestem), *Schizachyrium scoparium* (Michx.) Nash (little bluestem), and *Sorghastrum nutans* (L.) Nash (Indiangrass), with intermixed sub-dominant forbs and cool-season grasses. All forb species used in this experiment can be found in relatively high numbers on the surrounding landscape. Soil was transported to Oklahoma State University greenhouses and sieved through a 10 mm sieve to remove rocks and coarse plant material. Four-liter pots were filled with 9 kg of homogenized soil.

Seeds of all species were obtained from Prairie Moon Nursery, Winona, Minnesota, USA. In the spring of 2017, seeds were cold-moist stratified in vermiculite for 30 days, after which the vermiculite was transferred to seedling trays in greenhouses to facilitate germination. After approximately 3 weeks, at the two-leaf stage, seedlings were transplanted, with a single seedling in each pot. Plants were maintained at well-watered conditions March – October, 2017 to allow for successful establishment to maturity before implementing drought treatments. November 2017, aboveground biomass was removed, pots were placed outdoors, and covered with USDA-certified weed-free hay to provide insulation and allow plants to over-winter. March 2018, plants were returned to the greenhouse and allowed to grow for 3 weeks, after which drought treatments were implemented.

Following initiation of drought treatments, soil moisture of well-watered control plants was maintained between 85 and 90% field capacity (FC), and soil of droughted plants was maintained between 70 and 75% FC (i.e. near permanent wilt point, moderate drought). Permanent wilt point for these soils was determined by the pressure-plate apparatus method, in which 1.5 MPa of pressure was exerted onto soil samples until equilibrium was reached (following methods by Richards and Weaver 1943; Duell et al. 2016, 2019). Soil moisture was monitored throughout the study, with water added as needed based on gravimetric water content, to maintain appropriate field capacities. The complete experimental design consisted of 10 treatment combinations: 5 plant species x 2 water-availability treatments, arranged in a complete block design with eight replicates for a total of 80 pots.

After drought treatments were initiated, plants were maintained for 20 weeks, at which time plants were harvested, and roots washed free of soil. Root, shoot, and reproductive (inflorescence) biomass (if available) were separated, dried for 48 h at 60°C, and weighed. Subsamples of roots were collected for assessment of intra-radical AM fungal root colonization, and rhizosphere soil was collected for analysis of extra-radical AM fungal biomass.

Plant Defense Traits

Prior to harvest, production of anti-herbivore defense traits was measured in *A. syriaca*, *A. tuberosa*, and *A. viridis*. Leaf trichome densities of *A. syriaca* and *A. tuberosa* were estimated by counting the number of trichomes on both sides of a leaf disc (28 mm^2), divided by the area of the disc (Agrawal et al. 2008). Trichomes were not assessed on *A. viridis* as the leaf surfaces are glabrous. Following methods of Agrawal (2005), latex production in *A. syriaca* and *A. viridis* was quantified by cutting the tip of the youngest fully-expanded leaf, and collecting the exuded latex on a pre-weighed 1 cm filter paper disc. Filter paper discs were then dried at 60°C for 24 h, and weighed to the nearest microgram. Latex production was not quantified in *A. tuberosa*, as this species does not produce the white, sticky latex characteristic of many asclepiads.

Nectar Sampling

After flowers had fully-opened, nectar was sampled between the hours of 7:00 and 8:00 am to reduce the effects of temperature on nectar production. Nectar production was quantified using 5-µL glass microcapillary tubes (Gardener and Gillman 2001;

Power et al. 2018). Nectar is easily drawn into tubes through capillary action when placed at or near the flower nectary. Nectar volume can then be quantified by measuring the length of capillary tube filled by withdrawn nectar, divided by the total length of the capillary tube.

AM Fungal Biomass

Intra-radical AM fungal colonization was assessed microscopically using a modified gridline intersect method (McGonigle et al. 1990). Small subsamples (≤0.05 g) of fresh roots from each individual plant were collected, oven-dried at 60°C for 48 h, stained with trypan blue, and scored for colonization. Phospholipid fatty acid (PLFA) and neutral lipid fatty acid (NLFA) biomarkers 16:1ω5c, 20:1ω9, and 22:1ω13 were used to determine relative abundances of extra-radical AM fungal biomass (Olsson 1999). As major components of biological membranes, PLFAs can be used to estimate fungal biomass due to the strong correlation between biovolume and cell surface area (Tunlid and White 1992). Alternatively, NLFAs serve as the primary energy reserve and basic storage product of fungi, allowing for estimates of spore biomass for AM fungi (Larsen and Bødker 2001).

Using a modification of the Bligh and Dyer (1959) extraction method (Allison and Miller 2005), PLFA and NLFA were extracted from the soil. Utilizing silicic acid chromatography, PLFAs and NLFAs were separated, after which fatty acids were cleaved from the glycerol backbone using KOH saponification. The harvested fatty acids were methylated to form fatty acid methyl esters (FAMEs), which were then analyzed using a gas chromatography-mass spectrometry (GCMS) unit (Agilent MS 5975C/GC 7890A).

Statistical Analyses

Prior to analyses, all data were checked for normality and homogeneity of variances using Shapiro-Wilk and Levene's tests, respectively. To assess the effects of soil moisture on biomass production, root: shoot ratios, and intra- and extra-radical fungal abundances for each species, one-way analysis of variance (ANOVA) tests were employed with soil moisture as the sole factor in the analysis, with a Tukey post-hoc test and significance assessed at $p \leq 0.05$. One-way ANOVAs were also used to assess latex exudation and trichome densities of milkweeds under well-watered and drought conditions, and similar analyses were performed for nectar production of our three species which flowered. All analyses were performed using R-software version 3.6.1 (R Core Team 2019).

RESULTS

Reduced soil moisture generally resulted in decreased biomass production, relative to well-watered conditions, but the magnitude of this decrease was species-specific. For example, biomass reduction was significant for *A. viridis*, *R. humilis*, and *S. azurea*, but was not significant for *A. syriaca* ($F_{1,13} = 0.247$, $p = 0.63$) or *A. tuberosa* ($F_{1,10} = 0.322$, $p = 0.59$) (Figure 3.1). Regardless of species, no differences in root: shoot ratios were observed following exposure to drought (data not presented). Following two years of growth, *A. syriaca* and *A. viridis* failed to produce reproductive biomass (Figure 3.2). This is not unexpected, as long-lived perennial *Asclepias* species may require multiple years to produce inflorescences under field conditions (Wilbur et al. 1976). Of the three species that produced reproductive biomass, both *R. humilis* and *S. azurea* displayed greater inflorescence production under well-watered conditions, compared to plants exposed to drought. *Asclepias tuberosa* produced few inflorescences, and neither *A. tuberosa* nor *S. azurea* produced reproductive biomass under drought conditions (Figure 3.2). Only *R. humilis* produced reproductive biomass under both well-watered and drought conditions. However, when subjected to drought, *R. humilis* produced significantly less nectar (0.8 ± 0.41 μg nectar per flower), compared to well-watered conditions (2.9 ± 0.13 μg nectar per flower) ($F_{1,12} = 16.28$, $p \leq 0.002$; data not shown).

Drought differentially affected the expression of anti-herbivore defense traits in each of the three milkweed species. Individual *A. syriaca* and *A. viridis* plants produced significantly less latex when subjected to drought conditions, compared to individuals grown under well-watered conditions (Table 3.1). When subjected to drought conditions, *A. syriaca* trichome densities were significantly lower, compared to well-watered plants, while trichome densities of *A. tuberosa* were unaffected (Table 3.1).

Drought did not significantly affect intra-radical AM fungal abundance, regardless of host species (Table 3.2). However, while inter-radical AM fungal biomass was generally reduced following drought, effects were dependent on host plant species (Table 3.2). For example, inter-radical fungal abundance was significantly and substantially reduced in droughted *S. azurea*, yet marginally increased in soils associated with *A. tuberosa* (Table 3.2).

DISCUSSION

There is increasing evidence that rapidly changing precipitation regimes will have tremendous impacts on plant productivity (Brookshire and Weaver 2015), anti-herbivore defenses (Pineda et al. 2016), and floral resources (Waser and Price 2016; Phillips et al. 2018), though relatively few studies have assessed responses to drought in co-occurring forb species. In addition, a growing body of evidence suggests that plant-associated soil microbial communities, such as AM fungi, will experience substantial losses in abundance following severe drought (Duell et al. 2016; Ochoa-Hueso et al. 2018), potentially decoupling these mutualistic relationships and further affecting plant performance. In our study, we found decreases in plant biomass across all species, with significant decreases for *A. viridis, R. humilis,* and *S. azurea*. Reductions in biomass production often influence the ability to capture and store energy for important functions, such as reproduction and defense. We found dramatic reductions or complete lack of reproductive output in response to drought conditions, with negative implications for nectarivorous biota, including pollinators. Of the five prairie forb species in our study, only *R. humilis* produced inflorescences under drought conditions.

Anti-herbivore defenses of milkweeds generally decreased in response to drought treatments. Latex production was consistently significantly reduced, regardless of species, and trichome densities were reduced in *A. syriaca*, although not *A. tuberosa*. Reductions in defense mechanisms often result in greater plant palatability, thus increasing herbivore pressure (Gutbrodt et al. 2011), with implications for plant resource allocation (Cole et al. 2021).

Finally, while intra-radical AM fungal hyphae did not differ in roots subjected to drought treatments, compared to well-watered, relative extra-radical fungal abundances were reduced in *A. viridis* and *S. azurea*. Extra-radical hyphae serve a critical role in nutrient and water acquisition, therefore, loss of hyphae may result in indirect reductions in biomass production, as well as plant defenses (Jung et al. 2012). Reductions in extra-radical hyphal biomass likely extend to ecosystem-level services, as AM hyphae have been shown to improve soil aggregate stability and soil carbon storage (Wilson et al. 2009).

In our current study, *A. viridis*, *R. humilis*, and *S. azurea* exhibited a significant decrease in biomass production when subjected to drought conditions, relative to well-watered conspecifics. It is possible that the simulated drought imposed was not severe enough to elicit a response in either *A. syriaca* or *A. tuberosa*, as the soil moisture we selected was well above permanent wilt point determined for plants growing in similar soils (Duell et al. 2021). As a result, the responses observed in our study may underestimate the effects of more prolonged or intense drought on grassland plants. However, grasslands have been shown to be resilient and relatively unaffected by moderate drought. For example, rainfall exclusion experiments at Konza Prairie Biological Station suggest ANPP often did not differ between droughted rainout shelters and control plots, depending on the timing of the imposed drought (Denton et al. 2017). While biomass is frequently reduced immediately following drought, this decrease can be short-term, with biomass production returning to levels similar to well-watered controls shortly after re-wetting (Mackie et al. 2019). However, even if biomass is not significantly reduced, drought is likely to inhibit reproduction. In our current study, three forb species produced flowers under well-watered conditions, yet only one species (*R. humilis*) produced flowers when subjected to drought. The five forb species selected for our experiment exhibit a perennial life cycle and may reduce inflorescence numbers or persist in a vegetative state under frequent water-limited conditions (Karban and Pezzola

2017; Barnett et al. 2018), as reproduction in flowering plants is extremely energy-demanding. These alterations have negative implications for pollinating and nectarivorous fauna, with potential for further negative feedbacks in the context of plant reproduction. For example, reduced reproductive biomass (i.e., flowers) and nectar production will likely result in fewer pollinator visitors, with resultant exacerbation of pollen and reproduction limitation exhibited by grassland forb species (Bennett et al. 2020; Richardson et al. 2021).

The effects of drought on anti-herbivore defenses has been well-studied over the past two decades, as many plant species rely on anti-herbivore defenses. These studies indicate responses to drought are species-specific and context-dependent, as drought has been shown to reduce (Gutbrodt et al. 2011; Metz et al. 2014), increase (Kleine and Müller 2014; Pineda et al. 2016), or have no effect on concentrations of defense compounds in plants (Halpern et al. 2010). For example, following drought, growth and latex production were decreased in *A. syriaca* in our study and Couture et al. (2015), while Hahn and Maron (2018) reported no change in *A. syriaca* latex production following drought. In our study, *A. syriaca* also decreased foliar trichome densities following persistent one-year drought. Plants with reduced defenses become considerably more palatable to herbivores (Arany et al. 2008; Gutbrodt et al. 2011), leading to increased herbivore pressure. Potential cascading affects could further impact resource trade-offs between growth and defenses (Cole et al. 2021), promoting negative feedbacks as plants shift resource allocation to growth and maintenance, rather than secondary metabolites that serve as herbivore defenses or pollinator attractants.

Above- and belowground communities are inextricably linked, and it is well documented that soil organisms play important roles in regulating plant growth and herbivory resistance in native systems (Wilson and Hartnett 1998; Jung et al. 2012; Tao et al. 2016). Additionally, plants can alter soil characteristics in ways that feed back to affect the performance of that species or other plant species (Bever et al. 1997). In the event of extreme drought, negative consequences to individual plants and plant communities will likely impact mutualistic soil microbes, such as AM fungi (Duell et al. 2016; de Nijs et al. 2019). AM fungi, well-known for their role in nutrient uptake and pathogen defense (Sikes et al. 2009; Wehner et al. 2010), can also play an important role in the production and regulation of plant anti-herbivore defenses (Rasmann et al. 2017; Malik et al. 2018). Specifically, milkweeds in the genus *Asclepias* have been shown to increase production of latex, cardenolides, and volatile organic compounds in response to inoculation with AM fungi (Tao et al. 2016). Following moderate or extreme drought, reductions in AM fungal abundance could result in indirect reductions in biomass, floral resources, and anti-herbivore defenses of host plants. In our study, intra-radical AM fungal colonization was not affected in any forb species. A previous drought study on native prairie grasses (Duell et al. 2016) also found few effects on AM colonization, suggesting colonization of plant roots may be relatively unaffected by reductions in soil moisture. However, extra-radical AM fungal abundances associated with *A. viridis* and *S. azurea* were significantly decreased when subjected to drought conditions. That *S. azurea* experienced a significant decrease in extra-radical AM fungi, as well as biomass production under drought conditions, provides evidence that drought may decouple plant-AM fungal relationships, as *S. azurea* is highly dependent on the AM fungal mutualism for growth in native soils (Wilson and Hartnett 1998). However, relative abundances of extra-radical AM fungi associated with *A. tuberosa* exhibited a slight increase in response to drought conditions, further suggesting plant-fungal responses to drought to be complex and species-specific.

Although vegetative biomass production is not reduced in all grassland species subjected to short-term drought events, our results suggest that reproductive effort can be reduced independently of biomass production. In addition to reductions or loss of flower production, floral resources may be severely impacted; these losses can negatively impact local arthropod communities. Furthermore, loss of extra-radical AM fungal hyphae associated with highly-responsive plant species indicates possible drought-induced de-coupling of these important mutualistic associations, with cascading effects through losses in plant productivity and anti-herbivore defenses. Our study examined five sub-dominant grassland forb species that provide important floral resources to pollinators and other nectar-feeding species, and across these species, we observed a clear indication that drought effects are species- and context-dependent. Because pollinators and other arthropods dependent on floral resources rely on a large suite of forbs, further examination, especially for host-specific species of conservation concern, is clearly needed. Furthermore, while current drought models suggest persistent and increasingly severe drought across numerous ecosystems, our results suggest that even short-term drought can negatively affect such traits as biomass production, reproduction, floral resources, and plant-fungal symbioses in long-lived perennial species. Our study provides additional empirical support for the growing interest in understanding how environmental changes, such as drought, can mediate interactions between plants and herbivores, with important implications for conservation efforts to improve native biodiversity and ecosystem services.

TABLES

Table 3.1. Defense traits [latex production and trichome density (mean ± SE)] of *Asclepias syriaca*, *A. viridis*, and *A. tuberosa* grown under well-watered (85-90% field capacity [FC]) or droughted (70-75% FC) conditions. Across each row, means that do not share a letter are significantly different ($p \leq 0.05$).

	Latex (µg)		Trichomes/cm^{2}	
	Well-watered	Drought	Well-watered	Drought
A. syriaca	3.32 ± .18^{a}	1.78 ± .13^{b}	244.45 ± 27^{a}	170.14 ± 16^{b}
A. viridis	2.29 ± .16^{a}	1.22 ± .15^{b}	-	-
A. tuberosa	-	-	365.5 ± 21	390.33 ± 15

Table 3.2. Extra- and intra-radical arbuscular mycorrhizal (AM) fungal abundances (mean ± SE) associated with grassland forb species (*Asclepias syriaca*, *A. viridis*, *A. tuberosa*, *Ruellia humilis*, and *Salvia azurea*) grown under well-watered or droughted conditions. Asterisks denote significant difference within species between soil moistures ($p \leq 0.05$).

	Extra-radical				Intra-radical	
	PLFA		NLFA		Colonization (%)	
	Well-watered	Drought	Well-watered	Drought	Well-watered	Drought
A. syriaca	0.74 ± .08	0.64 ± .03	15.95 ± 2.9	10.22 ± 1.4	33.2 ± 2.1	34.8 ± 2.7
A. viridis	0.85 ± .24*	0.41 ± .01	5.82 ± 1.4	2.54 ± .24	22.1 ± 2.3	19.6 ± 2.8
A. tuberosa	0.62 ± .08	0.92 ± .19	5.56 ± 1.5	10.13 ± 2.4	20.2 ± 1.8	25.6 ± 1.7
R. humilis	0.45 ± .05	0.49 ± .06	8.47 ± 2.2	7.59 ± 3.7	17.9 ± 2.2	20.9 ± 2.1
S. azurea	0.52 ± .05*	0.31 ± .08	13.0 ± 2.6*	3.79 ± .90	18.2 ± 4.7	13.1 ± 2.0

FIGURES

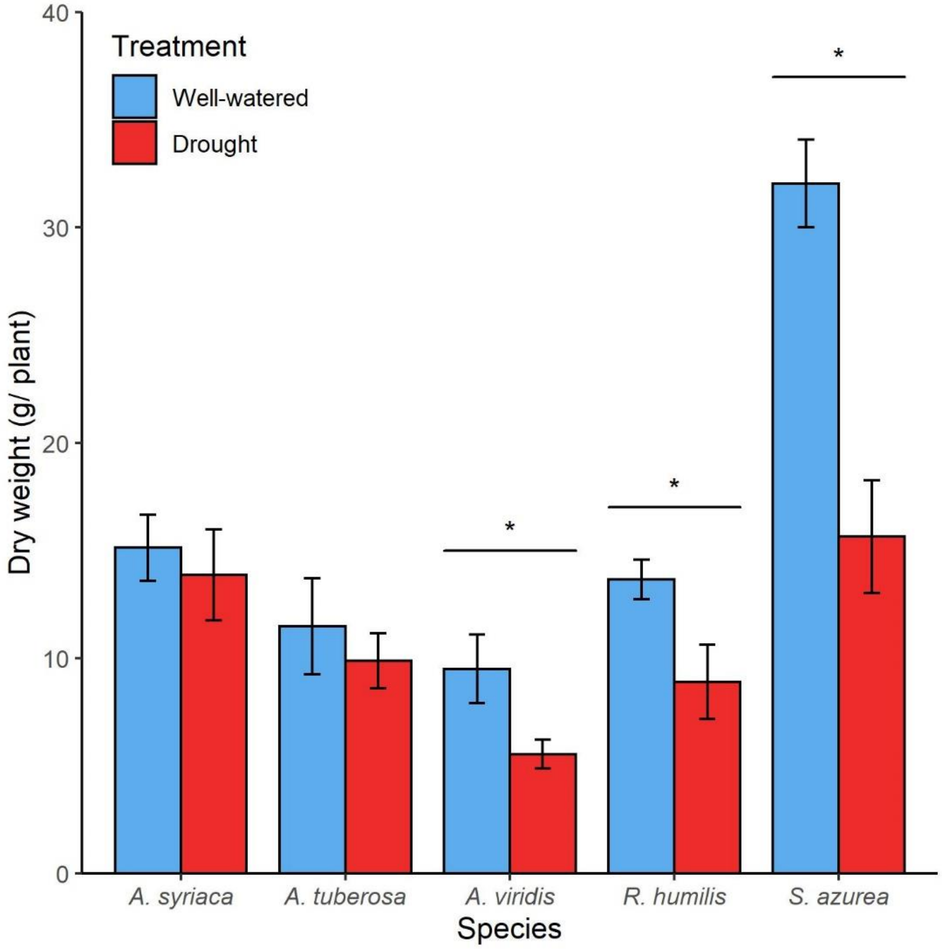

Figure 3.1. Total biomass production (mean ± SE) of five grassland forb species (*Asclepias syriaca*, *A. viridis*, *A. tuberosa*, *Ruellia humilis*, and *Salvia azurea*) grown under well-watered (light bars) or droughted conditions (dark bars). Asterisks above bars denote significant differences within species between the two soil moistures ($p \leq 0.05$).

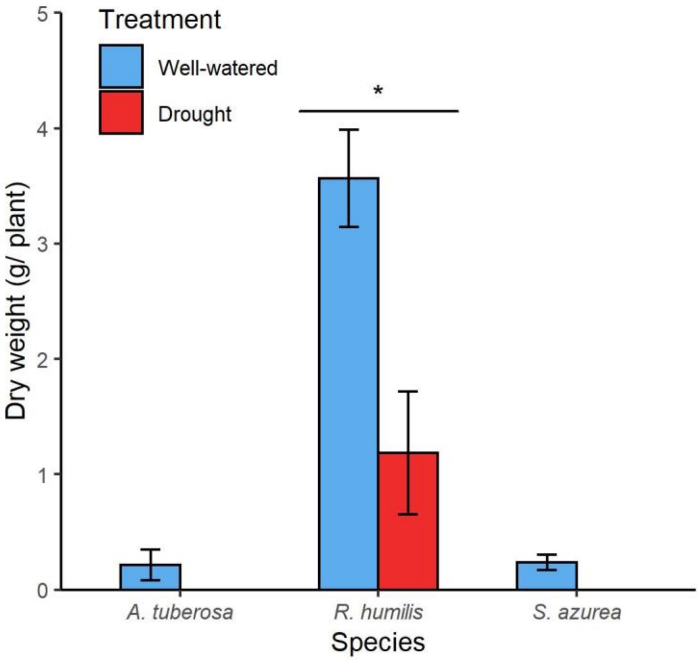

Figure 3.2. Reproductive biomass production (mean ± SE) of grassland forb species (*Asclepias tuberosa*, *Ruellia humilis*, and *Salvia azurea*) grown under well-watered (light bars) or droughted conditions (dark bars). Asterisks above bars denote significant differences within species between the two soil moistures ($p \leq 0.05$).

CHAPTER IV

INFLUENCES OF BIOTIC AND ABIOTIC DISTURBANCES ON LONG-TERM MILKWEED ABUNDANCES IN TALLGRASS PRAIRIE: IMPLICATIONS FOR MONARCH CONSERVATION

ABSTRACT

Grasslands in central North America evolved with three primary disturbances: fire, grazing, and periodic drought. Alterations to these key processes have resulted in subsequent loss of grasslands to woody encroachment, affecting not only the local plant communities, but also the organisms which depend on them. Monarch butterfly (*Danaus plexippus*) populations have decreased substantially over the past two decades. Reductions in host plant (*Asclepias* spp.) availability in grasslands of central North America are contributing to *D. plexippus* population declines, although few studies have assessed the effects of long-term disturbance regimes on *Asclepias* abundances. We used a 30-year plant community dataset to assess the effects of fire frequency (1-, 4-, and 20-year), bison (*Bison bison*) grazing (grazed, non-grazed), woody cover, and drought on the abundances of three *Asclepias* species (*A. viridis, A. syriaca,* and *A. tuberosa*). Asclepias viridis occurred in greater abundances in watersheds with 1-year fire frequencies, compared to 4- and 20-year frequencies, and decreased in relationship to increasing woody cover. Abundance was initially greater in non-grazed watersheds, but declined throughout the study, while increasing through time in grazed watersheds. Regardless of temporal grain or window, drought did not affect *A. viridis* abundance. At the beginning of the study, *A. syriaca* abundances were greatest in watersheds with 20-year fire frequencies, relative to 1- and 4-year frequencies, and exhibited a positive relationship with increasing woody cover. *Asclepias syriaca* abundances were generally greater in non-grazed watersheds but declined in abundance regardless of bison grazing. Drought had no effect on *A. syriaca* abundances across the 30-year sample period. Overall abundance of *A. tuberosa* was not affected by fire frequency, grazing, woody cover, or drought. Our results suggest that *A. syriaca* and *A. tuberosa* are relatively unaffected by historic disturbances that shape grasslands, or consequences of alterations to these disturbance regimes. However, *A. viridis*, which is found almost exclusively in open grasslands, is extremely sensitive to removal or alterations in these disturbances. Because *A. viridis* is the most common monarch host plant across much of the central and southern Great Plains, further alterations to historic disturbances such as fire and grazing could have severe indirect consequences for monarch butterflies.

INTRODUCTION

Monarch butterflies (*Danaus plexippus* L.) of the eastern North America population have experienced precipitous declines over the past two decades (Brower et al. 2011; Pleasants and Oberhauser 2013). These declines are largely due to degradation of over-wintering habitats of central Mexico, as well as reductions in host plant availability on breeding grounds in central North American grasslands (Pleasants and Oberhauser 2013). Monarch larvae feed exclusively on milkweeds (family Apocynaceae, subfamily Asclepiadoideae), primarily in the genus *Asclepias*. Approximately 130 of the estimated 400 members of the genus are found in North America (Fishbein et al. 2011). Although comprising a small component of the overall plant community (≤ 1% of cover), several *Asclepias* species are common to central North American grasslands and frequently grow in close proximity to one another. Common sympatric species in the region include *A. viridis* Walter (green antelopehorn), *Asclepias syriaca* L. (common milkweed), and *A. tuberosa* L. (butterfly milkweed). To date, little research exists assessing the effects of alterations in common disturbances, such as fire and grazing (but see Ricono et al. 2018) or influences of drought on long-term abundance of milkweeds in these grassland systems.

Disturbances play a critical role in maintaining grassland systems across the globe. Fire and grazing are principal disturbances in grasslands, and both are implicated as critical to the stability of these systems (Gibson 2009). These disturbance regimes have been anthropogenically altered, threatening grassland plant communities as well as organisms that depend on grassland plant species for food or shelter. Fire plays a key role in the maintenance of plant community structure and diversity in grassland ecosystems, as fire removes detritus, provides canopy openings, stimulates germination or spring re-growth of native forbs and grasses (Winter et al. 2015), and limits woody encroachment (Wilcox et al. 2018; Pierce et al. 2019). However, annual fire can lead to grass-dominated ecosystems (Collins and Calabrese 2012), as most dominant grasses are long-lived perennials adapted to frequent fire, often with resultant decreases in forb abundance and richness (Veen et al. 2008). Fire suppression or low fire frequency leads to increased woody cover, which has also been linked to reduced herbaceous species richness and diversity (Schlesinger et al. 1990; Knapp et al. 2008; Ratajczak et al. 2012; Alofs and Fowler 2013). Thus, intermediate fire frequencies are often critical for the maintenance of grassland forb communities.

Grazing by large ungulates also plays an important role in maintaining plant community structure and plant diversity in grassland ecosystems. Herbivores alter vegetation structure through consumption of biomass, trampling, and deposition of nutrients through urination and defecation (Gibson 2009). In addition, grazers and browsers, especially large ungulates, influence local colonization and extinction of some plant species through selective grazing (Johnson and Cushman 2006; Allred et al. 2012). For example, in mixed- and tallgrass prairies of central North America, grasses comprise the majority of *Bison bison* L. (bison) diets, with resultant increases in forb abundance and diversity (Collins and Calabrese 2012; Welti and Joern 2018) through competitive release, as reductions in graminoid biomass and cover increases resource availability to subdominant forbs and woody species (Elson and Hartnett 2017). Increases in grass biomass in non-grazed grasslands provide further evidence that competition with grasses may prevent the survival and establishment of forb species (Koerner and Collins 2013; Elson and Hartnett 2017). Because many insect species, including those of conservation concern such as monarchs, are dependent on floral resources provided by forbs, limiting woody encroachment and dominance of grasses by fire and/or grazing are therefore highly important to monarch conservation.

A third important disturbance for grassland community structure is drought. Regional drought has recently increased and is expected to continue to increase in frequency and intensity, especially in arid and semi-arid regions of the world (Bradford et al. 2020; Luo et al. 2020). While native plant species and communities in arid and semi-arid regions are well-adapted to periodic drought, more frequent and intense events will likely have direct impacts on primary productivity, individual species performance, and ecosystem services (Knapp et al. 2015; Duell et al. 2016). Previous research assessing the impacts of drought on grassland plant communities have focused on dominant plant species (Nippert et al. 2009; Connor and Hawkes 2018), while substantially less research has examined effects of drought on sub-dominant forb species. However, it is critical to understand the impacts of drought on the overall plant community, as less common or rare species are often vital contributors to community-level diversity, based on their role as host plants and providing important floral resources to pollinators and other nectar-feeding species.

Our current study assessed the long-term effects of large native ungulate grazers, fire frequency, and drought on the abundances of three common *Asclepias* species, all of which are used by monarch butterflies as larval host plants and adult nectar plants. Using long-term plant community data, we also assessed the relationship between woody cover and the three sympatric *Asclepias* species. Many grassland forb species are well-adapted to periodic fire, however, annual burning as a management practice may result in grass-dominated systems, with a reduction in forbs as well as woody species (Collins and Calabrese 2012). Thus, we hypothesize that *Asclepias* abundances would be greatest in watersheds with intermediate (4-year) fire frequencies, with the lowest abundances in watersheds with 20-year fire frequencies, as these areas are heavily encroached by native woody species. Bison are selective grazers, especially during the growing season, and thereby reduce graminoid cover. Due to expected decreased competition with warm-season grasses as a result of bison grazing, we hypothesized there would be greater abundances of all three *Asclepias* species in grazed watersheds. As many grassland plant species are relatively drought-tolerant, we hypothesized that drought would not have a significant effect on *Asclepias* abundances, although early growing season drought may result in a reduction in overall abundance. We also hypothesized abundances of all *Asclepias* species would decrease with increasing woody cover, largely due to interspecific competition. This extensive analysis of long-term abundance of *Asclepias* species in response to several biotic and abiotic factors provides critical information about the long-term plant abundances important for supporting conservation efforts for monarch butterflies.

MATERIALS AND METHODS

Site description

Konza Prairie Biological Station (KPBS) is a 3847 ha prairie preserve located in northeastern Kansas, USA, and is located in the Flint Hills ecoregion, which contains the largest remaining area of unplowed tallgrass prairie in North America. Average monthly temperatures range from a low of -1.6 °C in January to 26.1 °C in July, and mean annual precipitation is 835 mm (Collins et al. 2021). The topography of KPBS is characterized by rocky hillslopes separating shallow-soil uplands (Florence cherty silt loam) from deep-soil lowlands (Tully silt clay loam). KPBS is divided into watersheds based on topography, with each watershed being assigned varying fire (1, 2, 4, and 20-year fire frequencies) and grazing regimes [native (*B. bison*), domesticated (*Bos taurus* L.), and ungrazed], totaling 57 watersheds (average area: 0.55 ha).

The vegetation community of KPBS is characterized by a dominance of perennial C_4 grasses, including *Andropogon gerardii* Vitman (big bluestem), *Schizachyrium scoparium* Michx. (little bluestem), *Sorghastrum nutans* (L.) Nash (indiangrass), and *Panicum virgatum* L. (switchgrass). The remaining plant community consists of a diverse mixture of C_3 grasses, forbs, legumes, and native woody species (Freeman and Hulbert 1985).

Experimental design

We selected ten KPBS watersheds to investigate the effects of fire frequency, bison grazing, and woody cover on the abundance of three milkweed species (*A. syriaca*, *A. tuberosa*, and *A. viridis*). While other *Asclepias* species occur on the surrounding landscape, these three are the most abundant commonly used as larval host plants in the region, while also providing nectar for adult monarchs. Of the available treatments we selected all possible combinations of 1, 4, and 20-year fire frequencies and bison grazing (grazed, non-grazed) for this study (plant community data can be found in Hartnett & Collins 2019). Bison were introduced to Konza in 1987, with all current bison-grazed watersheds having bison present since 1991. Therefore, we focused on plant community data from 1991-2020. Because data from sloped areas is limited in this dataset, only upland and lowland vegetation data were used.

Vegetation sampling

Plant species composition was sampled in May and August from 1983-2020 to capture within season plant phenological dynamics. Bison were introduced to Konza in 1987, with all current bison-grazed watersheds having bison present since 1991. Therefore, we included plant community data from 1991-2020. Because data from sloped areas is limited in this dataset, only upland and lowland vegetation data were used. Plant communities were sampled in five 10-m^2 permanent circular plots evenly spaced along each of four 50-m transects in each soil type within each watershed, for a total of four transects and twenty plots per soil type within each watershed. Cover class to the species-level was estimated using the following modified Daubenmire scale: 1 = < 1%, 2 = 2-5%, 3 = 6-25%, 4 = 26-50%, 5 = 51-75%, 6 = 76-95%, 7 = 96-100%. We determined abundance by first converting the Daubenmire cover class of selected species in each plot to the associated midpoint of the cover range, and then averaging the values of the five plots along a transect. Because we were interested in abundance of particular species, any plot without a recorded Daubenmire value was assigned a zero, and the species was assumed absent. Using methods identical to those above, we also calculated the abundance of woody cover for each transect by averaging and summing the cover of the following woody species across transect plots: *Cornus drummondii* C.A. Mey. (roughleaf dogwood), *Gleditsia triacanthos* L. (honey locust), *Juniperus virginiana* L. (eastern redcedar), *Rhus copallinum* L. (winged sumac), *R. glabra* L. (smooth sumac), and *Symphoricarpos orbiculatus* Moench (coralberry or buckbrush). To reduce the effects of plant phenology (e.g., plants being absent in May but present in August), maximum canopy cover within each year was used for each species, which in some cases results in total cover exceeding 100% for woody cover in our analyses (Collins et al. 2021).

Drought quantification

Following methods by Cady et al. (2019), drought was quantified using standardized precipitation evapotranspiration index (SPEI). SPEI was selected because it allows temporal grain and window flexibility, while also incorporating evapotranspiration, which is especially important for quantifying drought at fine temporal resolutions (Vincente-Serrano et al. 2010). A total of four SPEI metrics were calculated for 1990-2020 at varying temporal grains and windows. The three temporal grains and windows were determined *a priori*, and SPEI was calculated for early growing season [April-June (3-month)], growing season [April-September (6-month)], and the previous year [January-December (12-month lag)].

Statistical analyses

Following Collins et al. (2021) and Wilmer et al. (2020), we used generalized additive models (GAMs; Wood 2017) to assess changes in abundance of the selected *Asclepias* species through time in response to grazing, fire frequency, woody cover, and drought at four temporal grains and windows. GAMs allow for combining factors and smoothing functions, in our case time-variant estimators of error, which are useful when analyzing complex time-series data across multiple treatments and have been used to analyze similar data (Wilmer et al. 2020; Collins et al. 2021). For each species, a series of competing models were constructed including the respective explanatory variable as the singular main effect, as well as the interaction of the main effect and year as a smoothing function. This allowed us to capture the change of abundance through time in response to the main effect. Because we were interested in singular drivers of *Asclepias* abundances, interactions between main effects were not considered. Our link function for the response variable was log with a beta distribution due to proportional response variable values between 0 and 1. All models were implemented using the ‘mgcv’ package in R version 4.1.0 (R Core Team 2021). When significance was detected within a model for categorical variables, post-hoc comparisons across categorical levels were conducted using the ‘emmeans’ package in R (Lenth 2021).

RESULTS

Asclepias viridis

The greatest abundances of *A. viridis* throughout the 30-year sample period were associated with annual fire frequencies, with significantly lower abundances associated with 4- or 20-year fire frequencies ($p \leq 0.001$ and 0.001, respectively; Figure 4.1A). In watersheds managed under 20-year fire frequencies, abundances of *A. viridis* declined throughout the 30-year study, while remaining relatively constant across time when managed under 4-year fire frequencies (Figure 4.1A). Abundances of *A. viridis* were initially greater in watersheds not managed under grazing. However, abundance of *A. viridis* exhibited a sharp decline through time in non-grazed watersheds, while *A. viridis* abundance in grazed watersheds remained consistent across the 30-year sample period (Figure 4.1B). *Asclepias viridis* abundances were strongly negatively associated with increasing woody cover ($p = 0.005$; Figure 4.1C), as woody cover of native encroaching species increased under 20-year fire frequencies throughout the study (Figure 4.2). Regardless of temporal grain or window, *A. viridis* abundance was unaffected by drought (Figure 4.3).

Asclepias syriaca

At the beginning of the 30-year study, *A. syriaca* abundance was greatest in watersheds burned in 20-year fire frequencies (Figure 4.4A) yet the abundance of *A. syriaca* decreased over time, with no difference in abundance among fire frequency treatments by the end of the study (Figure 4.4A). Throughout most of the 30-year period, *A. syriaca* abundances were significantly greater in the absence of bison grazing, compared to grazed sites ($p \leq 0.001$) (Figure 4.4B). Abundance of *A. syriaca* was positively associated with increased woody cover (Figure 4.4C). Similar to *A. viridis*, *A. syriaca* was unaffected by drought, regardless of temporal grain or window (Figure 4.5).

Asclepias tuberosa

While abundance of *A. tuberosa* was generally greater on watersheds managed under annual fire frequencies, overall, there were no significant differences between the three fire frequencies (Figure 4.6A). Furthermore, abundance of *A. tuberosa* was not affected by the presence of bison grazing ($p = 0.175$) (Figure 4.6B) or following increases in woody cover (Figure 4.6C). Similar to *A. viridis* and *A. syriaca*, *A. tuberosa* was not affected by drought, regardless of temporal grain or window (Figure 4.7).

DISCUSSION

Disturbances have played a crucial role in the evolution and shaping of grassland ecosystems across the globe. However, both biotic and abiotic disturbance regimes of grasslands have been altered by anthropogenically-driven global changes, such as row-crop agriculture, urban expansion, and climate change. For example, urban expansion and alterations in grassland management have greatly reduced the frequency and prevalence of fire as a key ecosystem process, typically resulting in increased woody cover (Briggs et al. 2005; Ratajczak et al. 2011). Expansion of woody species has led to a decline in native herbaceous plant communities (Ratajczak et al. 2012; Alofs and Fowler 2013), as well as many grassland-dependent vertebrate and invertebrate species. Historically present throughout the Great Plains, bison now occupy only a few small, isolated pockets across the region. Although domestic cattle (*Bos taurus* L.) are present across much of the historic range of bison, many areas lack large ungulate grazers. Alterations to historic disturbance regimes represent some of the greatest threats to grassland biodiversity (Augustine et al. 2021).

Monarch butterflies have been at the center of insect conservation goals for nearly two decades, fueling a growing body of research focusing on drivers of their declines. In central North American breeding grounds, monarchs rely on several species of *Asclepias,* including *A. viridis, A. syriaca,* and *A. tuberosa,* as primary larval food sources (Pocius et al. 2017). However, current land-use changes and widespread use of herbicides along roadsides and in pasture management have further reduced abundances of these already relatively uncommon plant species (Pleasants and Oberhauser 2013; Zaya et al. 2017).

However, effects of alterations in disturbances that shape the ecosystem and plant communities in which *Asclepias* occurs has not been well investigated.

While we hypothesized intermediate fire frequencies (4-year) would result in the greatest abundance of each of the three *Asclepias* species, positive effects of frequent fire on *A. viridis* have been suggested (Baum and Sharber 2012). However, annual burning in these systems has been shown to increase dominance of warm-season perennial grasses, often to the detriment of less common forb species (Koerner and Collins 2013; Elson and Hartnett 2017). Although historic fire frequencies in the central Great Plains were estimated at 3-4 years (Knapp et al. 1998), some research suggests that 4-year fire frequencies and grazing combined are not able to limit encroachment by woody species (reviewed in Briggs et al. 2005; Ratajczak et al. 2014), which is likely reflected in the lack of overall abundance of *Asclepias viridis* under infrequent burning observed in our study. In fact, *A. viridis* showed a strong negative relationship with woody cover, exhibiting a steep decline in abundance as woody cover increased. *Asclepias viridis* abundances were initially greater in non-grazed watersheds, relative to grazed areas. However, throughout the study, *A. viridis* abundance declined sharply in watersheds absent of bison. In grasslands of central North America, bison diets consist primarily of graminoids (Coppedge et al. 1998; Collins and Calabrese 2012), with resultant increases in forb diversity and richness, likely translating to consistent *A. viridis* abundances. However, abundance of *A. viridis* remained relatively constant in bison-grazed watersheds across the entire 30-year period, and by the end of the study abundances were greater compared to watersheds without bison, possibly due to suppression of dominant warm-season grasses, and because bison activity increased with time following introduction at the initiation of this study.

Most of previous research examining the ecology of milkweeds is focused on *A. syriaca*. For example, *A. syriaca* has shown increased germinability when treated with smoke (Mozjes and Kalapos 2015), likely a mechanism for post-fire recruitment and colonization suggesting recruitment may be promoted under frequent burning regimes. Indeed, *A. syriaca* has been reported to decrease in abundance in the absence of fire (Ricono et al. 2018), further suggesting the importance of frequent fire for this species. However, season of burn may play an important role, as summer fires may decrease abundance (Towne and Kemp 2008). In our study, *A. syriaca* abundance was initially greatest in 20-year fire frequencies, but a sharp decline was evident across the 30-year study, with no differences among the three fire frequencies by the end of the study. The loss of *A. syriaca* abundance is not linked with increases in woody cover, as we found a positive relationship between *A. syriaca* abundance and woody cover, indicating it is likely that other exogenous factors, such as climate, interspecific competition, and recruitment constraints, are at play. Our data do not support our hypothesis that stated grazing would increase abundance of each of the *Asclepias* species, as abundances decreased over time in both the presence and absence of grazers. Furthermore, *A. syriaca* abundances were greater in non-grazed watersheds, indicating competitive release from warm-season grasses under grazing pressure does not play an important role in facilitating abundance of *A. syriaca.*

Effects of disturbances on *A. tuberosa* abundances have not been previously assessed; our study is the first to examine the long-term effects of fire frequencies or grazing on abundance of *A. tuberosa*. Contrary to our hypotheses, this species was unaffected by fire frequency or grazing, although throughout the study overall abundances were generally greater in watersheds subjected to bison grazing. Furthermore, there was no effect of woody cover on *A. tuberosa*. It is worth noting that *A. tuberosa* was the least abundant of our three species of interest, accounting for only 0.025-0.035% of canopy cover across watersheds used in this study. Thus, it is possible that the low abundances are at least partially responsible for the lack of observed differences, or that peak *A. tuberosa* phenology does not match the sampling windows.

There is increasing evidence that rapidly changing precipitation regimes will have tremendous impacts on plant productivity (Brookshire and Weaver 2015; Knapp et al. 2016; Felton et al. 2021; Leizeaga et al. 2021). However, no studies have paired drought data and long-term abundance data of *Asclepias* species. Throughout our 30-year study, there were no effects of drought on *Asclepias* abundances. However, this may not be unsurprising, as grassland plant species are typically drought resistant or resilient. For example, rainfall exclusion experiments at KPBS, suggest annual net primary productivity (ANPP) often does not differ between droughted and control (receive ambient rainfall) plots, depending on the timing of the imposed drought and overall annual rainfall (Denton et al. 2017). Biomass production of grassland plant communities in a multisite study was reduced immediately following drought, although productivity returned to levels similar to well-watered controls shortly after re-wetting (Mackie et al. 2019).

Our results highlight effects of common grassland disturbances on abundances of plant species at the center of conservation concern. The grassland forbs examined in our study provide important resources to monarch butterflies, but also a suite of specialist foliage-feeding and generalist nectarivorous insects. Overall, *A. syriaca* and *A. tuberosa* remained relatively unaffected by grazing, fire, and drought. However, our research indicates abundance of *A. viridis*, the most common *Asclepias* species in much of the central and southern Great Plains, may be severely limited by lack of grazing pressure, reduced fire frequencies, and increased woody cover. Our study provides additional empirical support for the growing interest in understanding how alterations to historic disturbance regimes can influence the abundances of certain plant species, with important implications for conservation efforts to improve native biodiversity and ecosystem services.

FIGURES

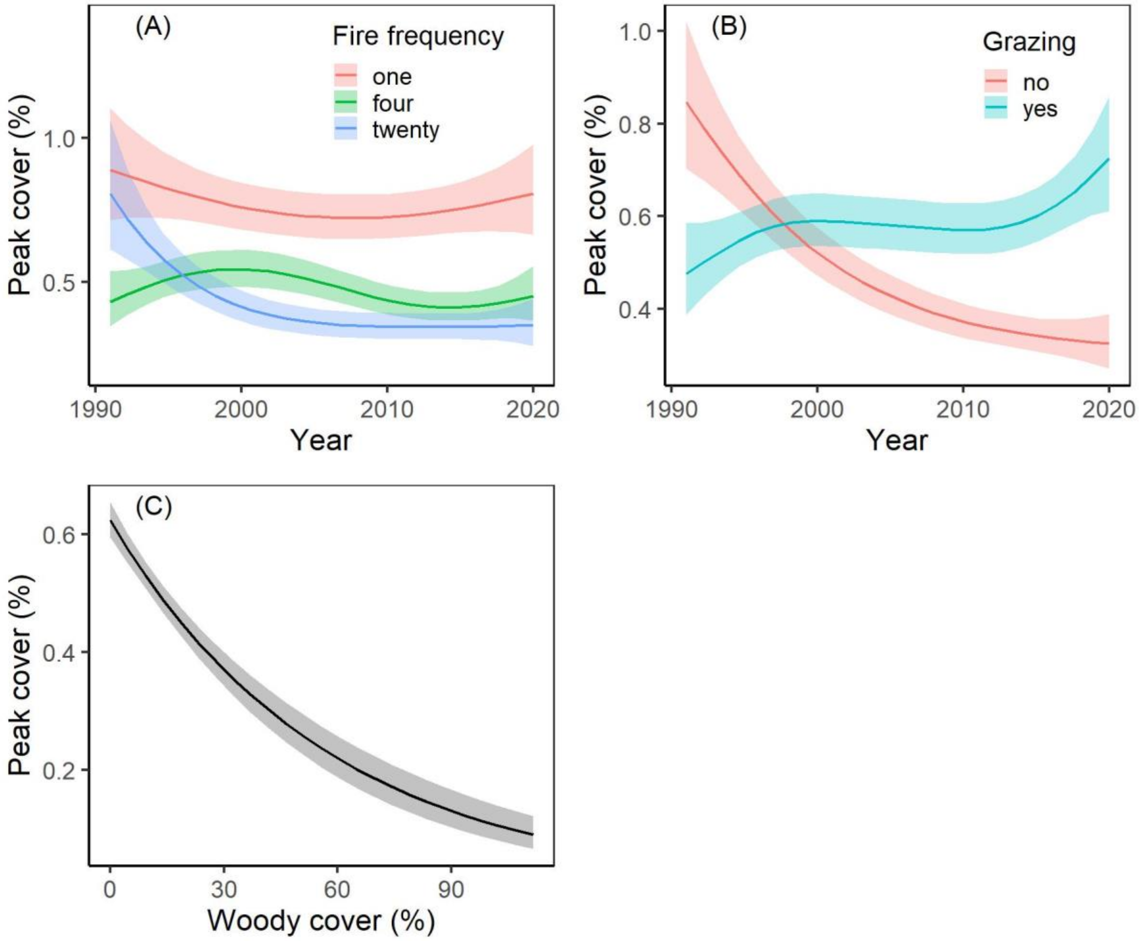

Figure 4.1. The long-term effects of (A) fire frequency, (B) bison grazing, and (C) woody cover on abundance of *Asclepias viridis*. For fire frequency (A), the red line and ribbon represents annual fire frequency, the green line and ribbon represent four-year (intermediate) fire frequency, and the blue line and ribbon represent 20-year fire frequency. For bison grazing (B), the red line and ribbon represent non-grazed watersheds, while the blue line represents grazed watersheds. For all lines in panels A-C, ribbons represent 95% confidence intervals.

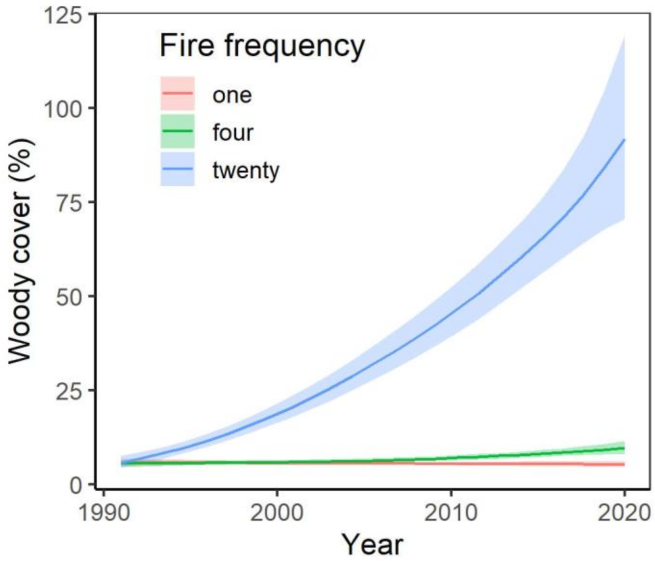

Figure 4.2. The effects of fire frequency on summed cover of six encroaching woody species [(*Cornus drummondii* (roughleaf dogwood), *Gleditsia triacanthos* (honey locust), *Juniperus virginiana* (eastern redcedar), *Rhus copallinum* (winged sumac), *R. glabra* (smooth sumac), and *Symphoricarpos orbiculatus* (coralberry)] across the 30-year study period (1991-2020). For fire frequency, the red line and ribbon represents annual fire frequency, the green line and ribbon represent four-year (intermediate) fire frequency, and the blue line and ribbon represent 20-year fire frequency. Ribbons represent 95% confidence intervals.

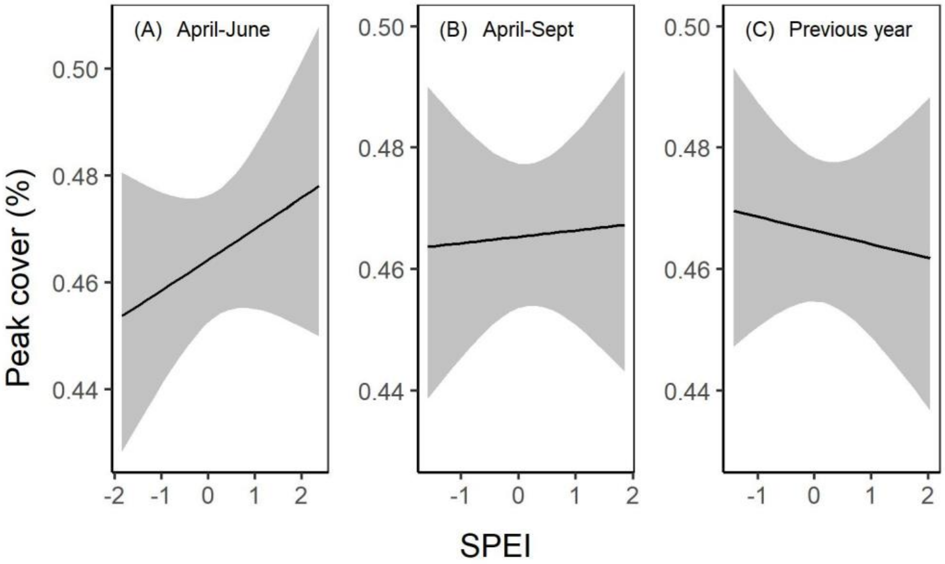

Figure 4.3. *Asclepias viridis* abundances in relation to drought across early growing season (A), growing season (B), and the previous year (C).

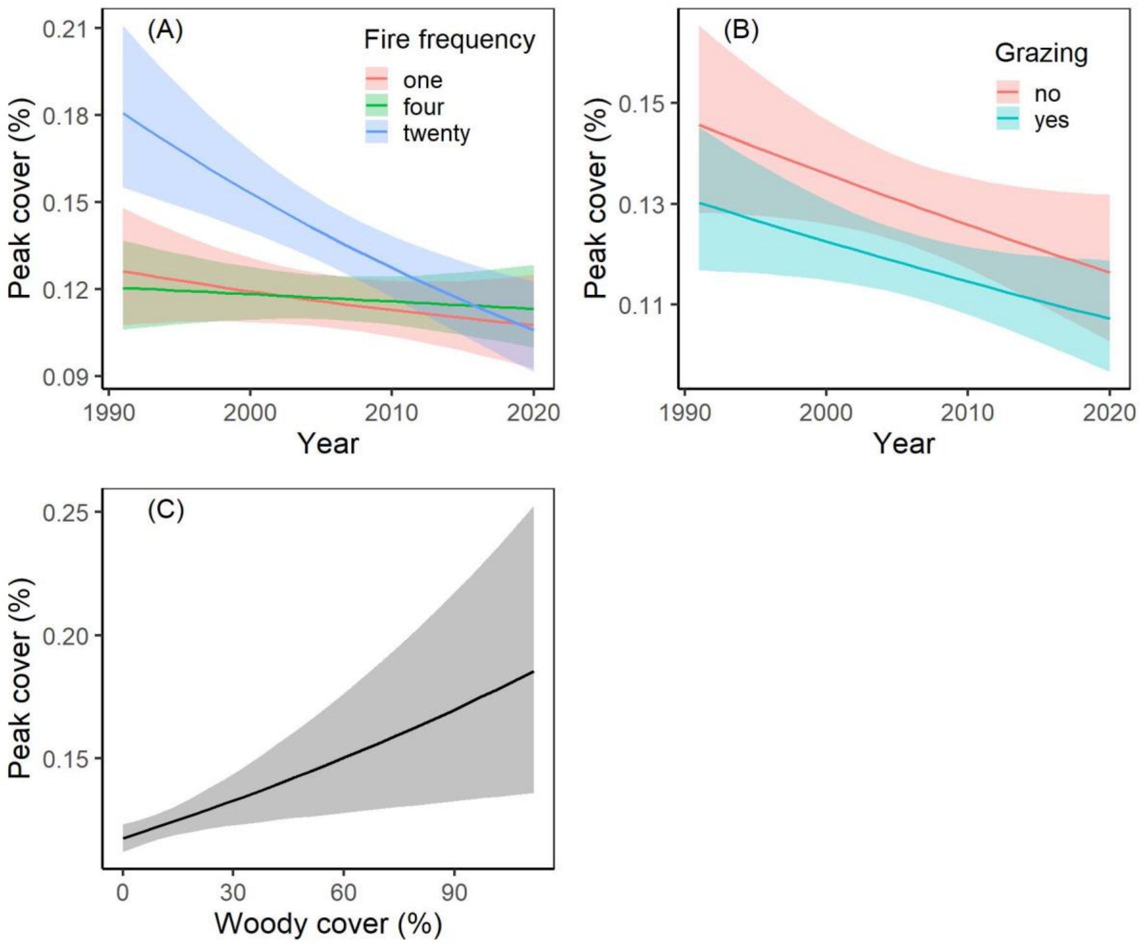

Figure 4.4. The long-term effects of (A) fire frequency, (B) bison grazing, and (C) woody cover on abundance of *Asclepias syriaca*. For fire frequency (A), the red line and ribbon represents annual fire frequency, the green line and ribbon represent four-year (intermediate) fire frequency, and the blue line and ribbon represent 20-year fire frequency. For bison grazing (B), the red line and ribbon represent non-grazed watersheds, while the blue line represents grazed watersheds. For all lines in panels A-C, ribbons represent 95% confidence intervals.

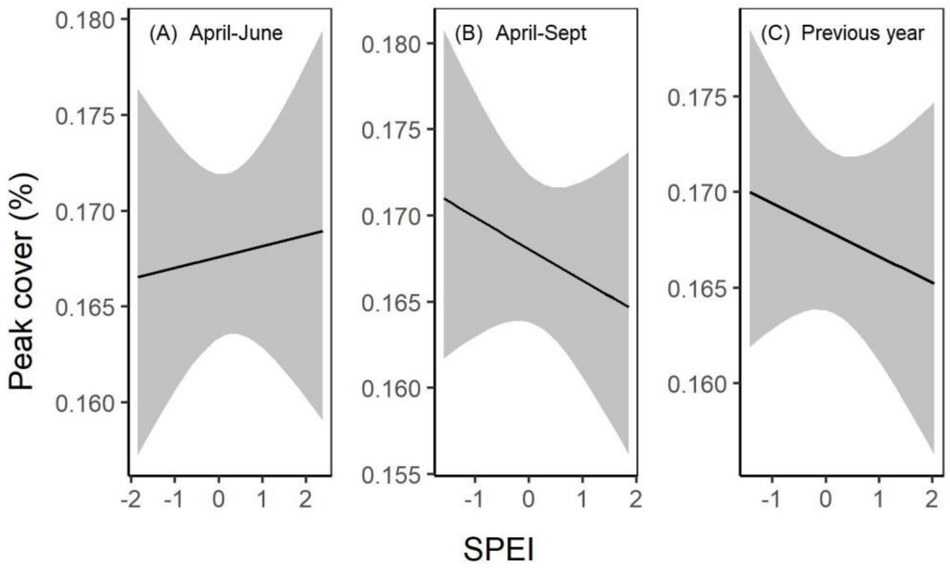

Figure 4.5. *Asclepias syriaca* abundances in relation to drought across early growing season (A), growing season (B), and the previous year (C).

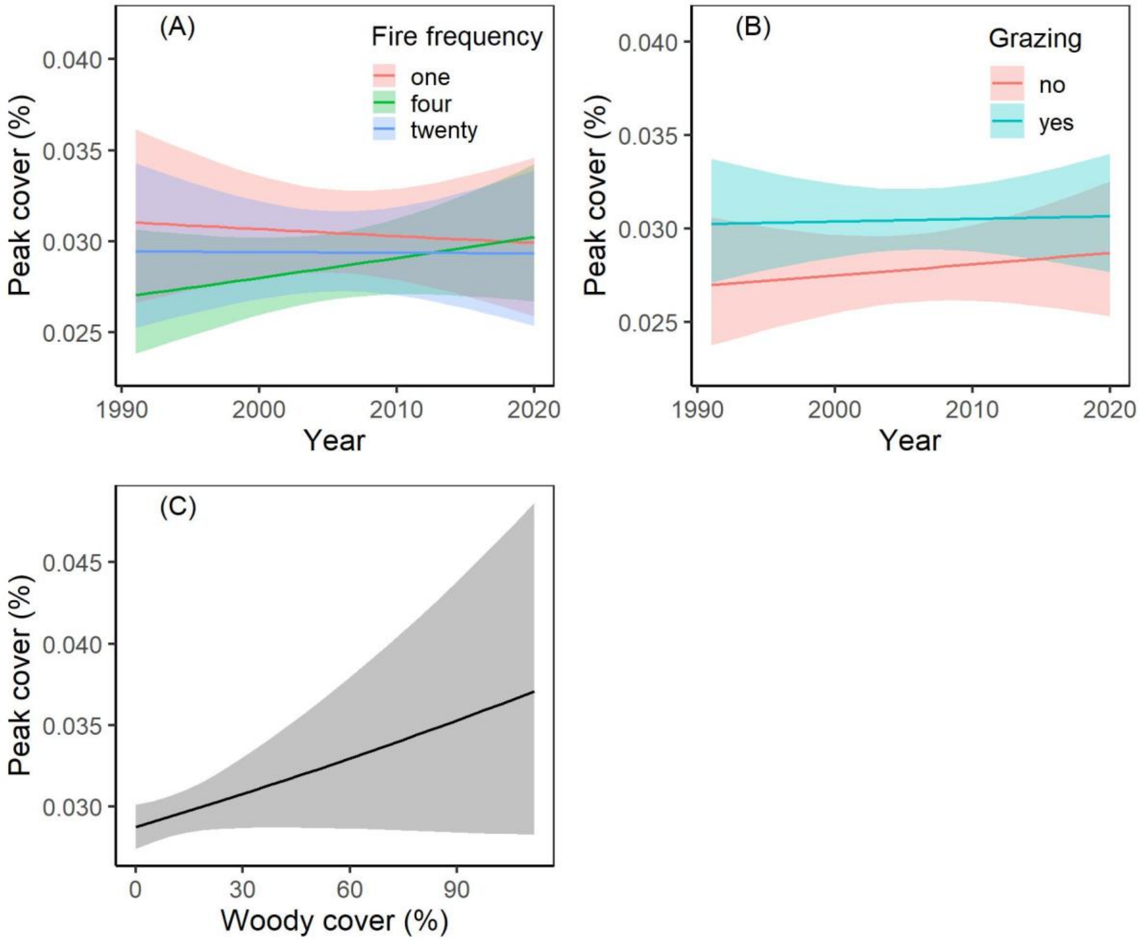

Figure 4.6. The long-term effects of (A) fire frequency, (B) bison grazing, and (C) woody cover on abundance of *Asclepias tuberosa*. For fire frequency (A), the red line and ribbon represents annual fire frequency, the green line and ribbon represent four-year (intermediate) fire frequency, and the blue line and ribbon represent 20-year fire frequency. For bison grazing (B), the red line and ribbon represent non-grazed watersheds, while the blue line represents grazed watersheds. For all lines in panels A-C, ribbons represent 95% confidence intervals.

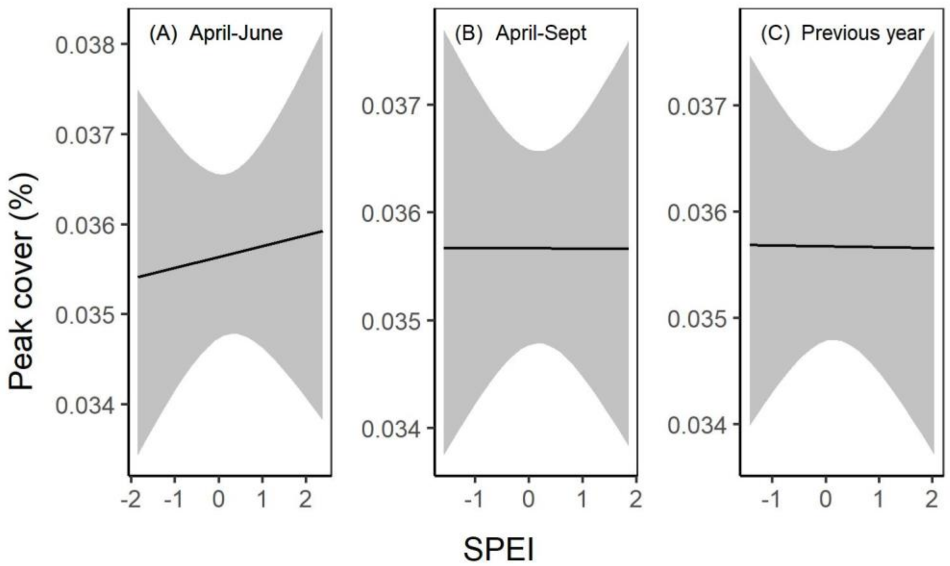

Figure 4.7. *Asclepias tuberosa* abundances in relation to drought across early growing season (A), growing season (B), and the previous year (C).

REFERENCES

Allred BW, Fuhlendorf SD, Smeins FE, Taylor CA (2012) Herbivore species and grazing intensity regulate community composition and an encroaching woody plant in semi-arid rangeland. Basic and Applied Ecology 13: 149–158.

Agrawal AA (2005) Natural selection on common milkweed (*Asclepias syriaca*) by a community of specialized insect herbivores. Evolutionary Ecology Research 7: 651–667.

Agrawal AA, Erwin AC, Cook SC (2008) Natural selection on and predicted responses of ecophysiological traits of swamp milkweed (*Asclepias incarnata*). Journal of Ecology 96: 536–542.

Agrawal AA, Fishbein M, Halitschke R, Hastings AP, Rabosky DL, Rasmann S (2009) Evidence for adaptive radiation from a phylogenetic study of plant defenses. Proceedings of the National Academy of Sciences 106: 18067–18072.

Allison VJ, Miller RM (2005) Using fatty acids to quantify arbuscular mycorrhizal fungi. In Basic research and applications of mycorrhizae. Edited by G. Podila and A. Varma. I.K. International Pvt. Ltd., New Delhi, India. pp. 141–161.

Alofs KM, Fowler NL (2013) Loss of native herbaceous species due to woody plant encroachment facilitates the establishment of an invasive grass. Ecology 34: 751–760.

Anacker BL, Klironomos JN, Maherali H, Reinhart KO, Strauss SY (2014) Phylogenetic conservatism in plant-soil feedback and its implications for plant abundance. Ecology Letters 17: 1613–1621.

Anderson RC, Hetrick BAD, Wilson GWT (1994) Mycorrhizal dependence of *Andropogon gerardii* and *Schizachyrium scoparium* in two prairie soils. American Midland Naturalist 132: 366–376.

Arany AM, De Jong TJ, Kim HK, Van Dam NM, Choi YH, Verpoorte R, Van der Meijden E (2008) Glucosinolates and other metabolites in the leaves of *Arabidopsis thaliana* from natural populations and their effects on a generalist and a specialist herbivore. Chemoecology 18: 65–71.

Augé RM (2001) Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza 11: 3–42.

Augustine D, Davidson A, Dickinson K, Van Pelt B (2021) Thinking like a grassland: Challenges and opportunities for biodiversity conservation in the Great Plains of North America. Rangeland Ecology and Management 78: 281–295.

Barnett KL, Johnson SN, Power SA (2018) Drought negates growth stimulation due to root herbivory in pasture grasses. Oecologia 188: 777–789.

Bartomeus I, Ascher JS, Gibbs J, Danforth BN, Wagner DL, Hedtke SM, Winfree R (2013) Historical changes in northeastern US bee pollinators related to shared ecological traits. Proceedings of the National Academy of Sciences 110: 4656–4660.

Bauer JT, Koziol L, Bever JD (2018) Ecology of floristic quality assessment: testing for correlations between coefficients of conservatism, species traits and mycorrhizal responsiveness. AoB Plants 10: plx073.

Baum KA, Sharber WV (2012) Fire creates host plant patches for monarch butterflies. Biology Letters 8: 968–971.

Bennett JM, Steets JA, Burns JH, Burkle LA, Vamosi JC, Wolowski M, Arceo-Gómez G, Burd M, Durka W, Ellis AG, Freitas L, Li J, Rodger JG, Valentin Ş, Xia J, Knight TM, Ashman T-L (2020) Land use and pollinator dependency drives global patterns of pollen limitation in the Anthropocene. Nature Communications 11: 1–6.

Bever JD (1994) Feedback between plants and their soil communities in an old field community. Ecology 75: 1965–1977.

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

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

Bever JD, Mangan S, Alexander H (2015) Pathogens maintain plant diversity. Annual Review of Ecology and Systematics 46: 305–325.

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

Blair JM (1997) Fire, N availability, and plant responses in grasslands: A test of the transient maxima hypothesis. Ecology 78: 2359–2368.

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37: 911–917.

Blomberg SP, Garland T, Ives AR (2003) Testing for phylogenetic signal in comparative data: Behavioral traits are more labile. Evolution 57: 717–745.

Booth MG, Hoeksema JD (2010) Mycorrhizal networks counteract competitive effects of canopy trees on seedling survival. Ecology 91: 2294–2302.

Bowles TM, Jackson LE, Cavagnaro TR (2018) Mycorrhizal fungi enhance plant nutrient acquisition and modulate nitrogen loss with variable water regimes. Global Change Biology 24: e171–e182.

Boyer LR, Brain P, Xu X, Jeffries P (2015) Inoculation of drought-stressed strawberry with a mixed inoculum of two arbuscular mycorrhizal fungi: effects on population dynamics of fungal species in roots and consequential plant tolerance to water deficiency. Mycorrhiza 25: 215–227.

Bradford JB, Schlaepfer DR, Lauenroth WK, Palmquist KA (2020) Robust ecological drought projections for drylands in the 21st century. Global Change Biology 26: 3906–3919.

Briggs JM, Knapp AK, Blair JM, Heisler JL, Hoch GA, Lett MS, McCarron JK (2005) An ecosystem in transition: causes and consequences of the conversion of mesic grassland to shrubland. BioScience 55: 243–254.

Brookshire ENJ, Weaver T (2015) Long-term decline in grassland productivity driven by increasing dryness. Nature Communications 6: 1–7.

Brower LP, Taylor OR, Williams EH, Slayback DA, Zubieta RR, Ramirez MI (2011) Decline of monarch butterflies overwintering in Mexico: is the migratory phenomenon at risk? Insect Conservation and Diversity 5: 95–100.

Brundrett MC (2002) Coevolution of roots and mycorrhizas of land plants. New Phytologist 154: 275–304.

Brundrett MC, Tedersoo L (2018) Evolutionary history of mycorrhizal symbioses and global host plant diversity. New Phytologist 220: 1108–1115.

Burns JH, Strauss SY (2011) More closely related species are more ecologically similar in an experimental test. Proceedings of the National Academy of Sciences 108: 5302–5307.

Butler M, King AA (2004) Phylogenetic comparative analysis: a modeling approach for adaptive evolution. The American Naturalist 164: 683–695.

Cady SM, O’Connell TJ, Loss SR, Jaffe NE, Davis CA (2019) Species-specific and temporal scale-dependent responses of birds to drought. Global Change Biology 25: 2691–2702.

Carroll AB, Pallardy SG, Galen C (2001) Drought stress, plant water status, and floral trait expression in fireweed, *Epilobium angustifolium* (Onagraceae). American Journal of Botany 88: 438–446.

Chagnon P-L, Bradley RL, Maherali H, Klironomos JN (2013) A trait-based framework to understand life history of mycorrhizal fungi. Trends in Plant Science 18: 484–491.

Cobb AB, Duell EB, Haase KB, Miller RM, We YQ, Wilson GWT (2021) Utilizing mycorrhizal responsiveness to guide selective breeding for agricultural sustainability. Plants People Planet 3: 578–587.

Cole CT, Morrow CJ, Barker HL, Rubert-Nason KF, Riehl JFL, Köllner TG, Lackus ND, Lindroth RL (2021) Growing up aspen: ontogeny and trade-offs shape growth, defence and reproduction in a foundation species. Annals of Botany 127: 505–517.

Collins CD, Bever JD, Hersh MH (2020) Community context for mechanisms of disease dilution: insight from linking epidemiology and plant–soil feedback theory. Annals of the New York Academy of Sciences 1469: 65–85.

Collins SL, Calabrese LB (2012) Effects of fire, grazing and topographic variation on vegetation structure in tallgrass prairie. Journal of Vegetation Science 23: 563–575.

Collins SC, Nippert JB, Blair JM, Briggs JM, Blackmore P, Ratajczak Z (2021) Fire frequency, state change and hysteresis in tallgrass prairie. Ecology Letters 24: 636–647.

Connor EW, Hawkes CV (2018) Effects of extreme changes in precipitation on the physiology of C4 grasses. Oecologia 188: 355–365.

Copeland SM, Harrison SP, Latimer AM, Damschen EI, Eskelinen AM, Fernandez Going B, Spasojevic MJ, Anacker BL, Thorne JH (2016) Ecological effects of extreme drought on Californian herbaceous plant communities. Ecological Monographs 86: 295–311.

Coppedge BR, Leslie Jr. DM, Shaw JH (1998) Botanical composition of bison diets on tallgrass prairie in Oklahoma. Journal of Range Management 51: 379–382.

Cortois R, Schröder-Gerogi T, Weigelt A, van der Putten WH, De Deyn GB (2016) Plant-soil feedbacks: role of plant functional group and plant traits. Journal of Ecology 104: 1608–1617.

Couture JJ, Serbin SP, Townsend PA (2015) Elevated temperature and periodic water stress alter growth and quality of common milkweed (*Asclepias syriaca*) and monarch (*Danaus plexippus*) larval performance. Arthropod-Plant Interactions 9: 149–161.

Crawford KM, Bauer JT, Comita LS, Eppinga MB, Johnson DJ, Mangan SA, Queenborough SA, Strand AE, Suding KN, Umbanhowar J, Bever JD (2019) When and where plant-soil feedback may promote plant coexistence: a meta-analysis. Ecology Letters 22: 1274–1284.

Crawford KM, Hawkes CV (2020) Soil precipitation legacies influence intraspecific plant-soil feedback. Ecology 101: e03142.

de Nijs EA, Hicks LC, Leizeaga A, Tietema A, Rousk J (2019) Soil moisture dependences and responses to drying-rewetting: The legacy of 18 years of drought. Global Change Biology 25: 1005–1015.

Denton EM, Dietrich JD, Smith MD, Knapp AK (2017) Drought timing differentially affects above- and belowground productivity in a mesic grassland. Plant Ecology 218: 317–328.

Duell EB, Wilson GWT, Hickman KR (2016) Above- and below-ground responses of native and invasive prairie grasses to future climate scenarios. Botany 94: 471–479.

Duell EB, Zaiger KL, Bever JD, Wilson GWT (2019) Climate affects plant-soil feedback of native and invasive grasses: Negative feedbacks in stable but not in variable environments. Frontiers in Ecology and Evolution 7: 419.

Duell EB, Londe DW, Hickman KR, Greer MJ, Wilson GWT (2021) Superior performance of invasive grasses over native counterparts will remain problematic under warmer and drier conditions. Plant Ecology 1–14.

Elson A, Hartnett DC (2017) Bison increase the growth and reproduction of forbs in tallgrass prairie. The American Midland Naturalist 178: 245–259.

Felton AJ, Knapp AK, Smith MD (2021) Precipitation–productivity relationships and the duration of precipitation anomalies: An underappreciated dimension of climate change. Global Change Biology 27: 1127–1140.

Fishbein M, Chuba D, Ellison C, Mason-Gamer RJ, Lynch SP (2011) Phylogenetic relationships of *Asclepias* (Apocynaceae) inferred from non-coding chloroplast DNA sequences. Systematic Botany 36: 1008–1023.

Fishbein M, Straub SCK, Boutte J, Hansen K, Cronn RC, Liston A (2018) Evolution at the tips: *Asclepias* phylogenomics and new perspectives on leaf surfaces. American Journal of Botany 105: 514–524.

Freeman CC, Hulbert LC (1985) An annotated list of the vascular flora of Konza Prairie Research Natural Area, Kansas. Transactions of the Kansas Academy of Sciences 88: 84–115.

Gange AC, Ayres RL (1999) On the relation between arbuscular mycorrhizal colonization and plant 'benefit'. Oikos 87: 615–621.

Gardener MC, Gillman MP (2001) Analyzing variability in nectar and amino acids: composition is less variable than concentration. Journal of Chemical Ecology 27: 2545–2558.

Gibson DJ (2009) Grasses and grassland ecology. Oxford University Press. Oxford, UK.

Gilbert GS, Webb CO (2007) Phylogenetic signal in plant pathogen-host range. Proceedings of the National Academy of Sciences. 104: 4979–4983.

Gutbrodt B, Mody K, Dorn S (2011) Drought changes plant chemistry and cause contrasting responses in lepidopteran herbivores. Oikos 120: 1732–1740.

Hahn PG, Maron JL (2018) Plant water stress and previous herbivore damage affect insect performance. Ecological Entomology 43: 47–54.

Halpern SL, Adler LS, Wink M (2010) Leaf herbivory and drought stress affect floral attractive and defensive traits in *Nicotiana quadrivalvis*. Oecologia 163: 961–971.

Hart MM, Antunes PM, Chaudhary VB, Abbott LK (2018) Fungal inoculants in the field: Is the reward greater than the risk? Functional Ecology 32: 126–135.

Hartnett DC, Collins SL (2019) PVC02 plant species composition on selected watersheds at Konza Prairie ver 1. Environmental Data Initiative.

Hetrick BAD, Wilson GWT, Todd TC (1990) Differential responses of C_3 and C_4 grasses to mycorrhizal symbiosis, phosphorus fertilization, and soil microorganisms. Canadian Journal of Botany 68: 461–476.

Hetrick BAD, Wilson GWT, Todd TC (1996) Mycorrhizal response in wheat cultivars: relationship to phosphorus. Canadian Journal of Botany 74: 19–25.

Hoeksema JD, Bever JD, Chakraborty S, Chaudhary VB, Gardes M, Gehring CA, Hart MM, Housworth EA, Kaonongbua W, Klironomos JN, Lajeunesse MJ, Meadow J, Milligan BG, Piculell BJ, Pringle A, Rúa MA, Umbanhowar J, Viechtbauer W, Wang YW, Wilson GWT, Zee PC (2018) Evolutionary history of plant hosts and fungal symbionts predicts the strength of mycorrhizal mutualism. Communications Biology 1: 1–10.

Hoeksema JD, Chaudhary VB, Gehring CA, Johnson NC, Karst J, Koide RT, Pringle A, Zabinski C, Bever JD, Moore JC, Wilson GWT, Klironomos JN, Umbanhowar J (2010) A meta-analysis of the context-dependency in plant response to inoculation with mycorrhizal fungi. Ecology Letters 13: 394–407.

Jacobson MM, Tucker EM, Mathiasson ME, Rehan SM (2018) Decline of bumble bees in northeastern North America, with special focus on *Bombus terricola*. Biological Conservation 217: 437–445.

Ji B, Gehring CA, Wilson GW, Miller RM, Flores-Rentería L, Johnson NC (2013) Patterns of diversity and adaptation in Glomeromycota from three prairie grasslands. Molecular Ecology 22: 2573–2587.

Jin L, Wang Q, Wang Q, Wang X, Gange AC (2017) Mycorrhizal-induced growth depression in plants. Symbiosis 72: 81–88.

Johnson BE, Cushman JH (2006) Influence of a large herbivore reintroduction on plant invasions and community composition in a California grassland. Conservation Biology 21: 515–526.

Johnson NC, Graham JH, Smith FA (1997) Functioning of mycorrhizal associations along the mutualism-parasitism continuum. New Phytologist 135: 575–585.

Johnson NC, Wilson GWT, Bowker MA, Wilson JA, Miller RM (2010) Resource limitation is a driver of local adaptation in mycorrhizal symbioses. Proceedings of the National Academy of Sciences 107: 2093–2098.

Jung SC, Martinez-Medina A, Lopez-Raez JA, Pozo MJ (2012) Mycorrhiza-induced resistance and priming of plant defenses. Journal of Chemical Ecology 38: 651–664.

Kaisermann A, de Vries FT, Griffiths RI, Bardgett RD (2017) Legacy effects of drought on plant soil feedbacks and plant-plant interactions. New Phytologist 215: 1413–1424.

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS (2017) ModelFinder: Fast Model Selection for Accurate Phylogenetic Estimates. Nature Methods 14: 587–589.

Kamilar JM, Cooper N (2013) Phylogenetic signal in primate behavior, ecology and life history. Philosophical Transactions of the Royal Society B 368: 20120341.

Karban R, Pezzola E (2017) Effects of a multi-year drought on a drought-adapted shrub, *Artemisia tridentata*. Plant Ecology 218: 547–554.

Kempel A, Rindisbacher A, Fischer, Allan E (2018) Plant soil feedback strength in relation to large-scale plant rarity and phylogenetic relatedness. Ecology 99: 597–606.

Kleine S, Müller C (2014) Drought stress and leaf herbivory affect root terpenoid concentrations and growth of *Tanacetum vulgare*. Journal of Chemical Ecology 40: 1115–1125.

Knapp AK, Briggs JM, Hartnett DC, Collins SL, eds. (1998) Grassland dynamics: long term ecological research in tallgrass prairie. Oxford University Press, New York, New York, USA.

Knapp AK, Briggs JM, Collins SL, Archer SR, Bret-Harte MS, Ewers BE, Peters DP, Young DR, Shaver GR, Pendall E, Cleary MB (2008) Shrub encroachment in North American grasslands: shifts in growth form dominance alters control of ecosystem carbon inputs. Global Change Biology 14: 615–623.

Knapp AK, Carroll CJW, Denton EM, La Pierre KJ, Collins SL, Smith MD (2015) Differential sensitivity to regional-scale drought in six central US grasslands. Oecologia 177: 949–957.

Knapp AK, Ciais P, Smith MD (2016) Reconciling inconsistencies in precipitation-productivity relationships: implications for climate change. New Phytologist 214: 41–47.

Koerner S, Collins S (2013) Small-scale patch structure in North American and South African grasslands responds differently to fire and grazing. Landscape Ecology 28: 1293–1306.

Köhl L, Van Der Heijden MGA (2016) Arbuscular mycorrhizal fungal species differ in their effect on nutrient leaching. Soil Biology and Biochemistry 94: 191–199.

Koyama A, Maherali H, Antunes PM (2019) Plant geographic origin and phylogeny as potential drivers of community structure in root-inhabiting fungi. Journal of Ecology 107: 1720–1736.

Koziol L, Bever JD (2015) Mycorrhizal response trades off with plant growth rate and increases with plant successional status. Ecology 96: 1768–1774.

Koziol L, Bever JD (2016) AMF, phylogeny, and succession: specificity of response to mycorrhizal fungi increases for late-successional plants. Ecosphere 7: e01555.

Koziol L, Bever JD (2017) The missing link to grassland restoration: arbuscular mycorrhizal fungi inoculation increases plant diversity and accelerates succession. Journal of Applied Ecology 54: 1301–1309.

Koziol L, Schultz PA, House GL, Bauer JT, Middleton EL, Bever JD (2018) The plant microbiome and native plant restoration: the example of native mycorrhizal fungi. BioScience 68: 996–1006.

Kulmatiski A, Beard KH, Stevens JR, Cobbold SM (2008) Plant-soil feedbacks: a meta-analytical review. Ecology Letters. 11: 980–992.

Larsen J, Bødker L (2001) Interactions between pea root-inhabiting fungi examined using signature fatty acids. New Phytologist 149: 487–493.

Leizeaga A, Hicks LC, Manoharan L, Hawkes CV, Rousk J (2021) Drought legacy affects microbial community trait distributions related to moisture along a savannah grassland precipitation gradient. Journal of Ecology 109: 3195–3210.

Lenth RV (2021) emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.6.3.

Liu X, Liang M, Etienne RS, Wang Y, Staehelin C, Yu S (2012) Experimental evidence for a phylogenetic Janzen-Connell effect in a subtropical forest. Ecology Letters 15: 111–118.

Lugo MA, Reinhart KO, Menoyo E, Crespo EM, Urcelay C (2014) Plant functional traits and phylogenetic relatedness explain variation in associations with root fungal endophytes in an extreme arid environment. Mycorrhiza 25: 85–95.

Luo W, Zuo X, Griffin-Nolan RJ, Xu C, Sardans J, Yu Q, Wang Z, Han X, Peñuelas J (2020) Chronic and intense droughts differentially influence grassland carbon-nutrient dynamics along a natural aridity gradient. Plant and Soil 1–12.

Mack KML, Eppinga MB, Bever JD (2019) Plant–soil feedbacks promote coexistence and resilience in multi-species communities. PLoS One 14: e0211572.

Mackie KA, Zeiter M, Bloor JM, Stampfli A (2019) Plant functional groups mediate drought resistance and recovery in a multisite grassland experiment. Journal of Ecology 107: 937–949.

Malik RJ, Ali JG, Bever JD (2018) Mycorrhizal composition influences plant anatomical defenses and impacts herbivore growth and survival in a life-stage dependent manner. Pedobiologia 66: 29–35.

Mangan SA, Herre EA, Bever JD (2010a) Specificity between neotropical tree seedlings and their fungal mutualists leads to plant–soil feedback. Ecology 91: 2594–2603.

Mangan SA, Schnitzer SA, Herre EA, Mack KML, Valencia MC, Sanchez EI, Bever JD (2010b) Negative plant-soil feedback predicts tree-species relative abundance in a tropical forest. Nature 7307: 752–755.

McGonigle TO, 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 Phytologist 115: 495–501.

Mehrabi Z, Tuck SL (2014) Relatedness is a poor predictor of negative plant–soil feedbacks. New Phytologist 205: 1071–1075.

Meiners SJ, Phipps KK, Pendergrast IV, TH, Canam T, Carson WP (2017) Soil microbial communities alter leaf chemistry and influence allelopathic potential among coexisting plant species. Oecologia 183: 1155–1165.

Metz J, Ribbers K, Tielbörger K, Müller C (2014) Long- and medium-term effects of aridity on the chemical defence of a widespread Brassicaceae in the Mediterranean. Environmental and Experimental Botany 105: 39–45.

Middleton EL, Bever JD (2012) Inoculation with native soil community advances succession in a grassland restoration. Restoration Ecology 20: 218–226.

Montesinos-Navarro A, Valiente-Banuet A, Verdũ (2019) Plant facilitation through mycorrhizal symbiosis is stronger between distantly related species. New Phytologist 224: 928–935.

Moora M, Öpik M, Davison J, Jairus T, Vasar M, Zobel M, Eckstein RL (2016) AM fungal communities inhabiting the roots of submerged aquatic *plant Lobelia dortmanna* are diverse and include a high proportion of novel taxa. Mycorrhiza 26: 735–745.

Mozjes A, Kalapos T (2015) Plant-derived smoke enhances germination of invasive common milkweed (*Asclepias syriaca* L.). Polish Journal of Ecology 63: 280–285.

Münkemüller T, Lavergne S, Bzeznik B, Dray S, Jombart T, Schiffers K, Thuiller W (2012) How to measure and test phylogenetic signal. Methods in Ecology and Evolution 3: 743–756.

Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. Molecular Biology and Evolution 32: 268–274.

Nippert JB, Fay PA, Carlisle JD, Knapp AK, Smith MD (2009) Ecophysiological responses of two dominant grasses to altered temperature and precipitation regimes. Acta Oecologica 35: 400–408.

Ochoa-Huaso R, Collins SL, Delgado-Baquerizo M, Hamonts K, Pockman WT, Sinsabaugh RL, Smith MD, Knapp AK, Power SA (2018) Drought consistently alters the composition of soil fungal and bacterial communities in grasslands from two continents. Global Change Biology 24: 2818–2827.

Olsson PA (1999) Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soil. FEMS Microbiology Ecology 29: 303–310.

Pagel M (1999) Inferring the historical patterns of biological evolution. Nature 401: 877 884.

Paradis E, Schliep K (2019) ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics 35: 526–528.

Parker IM, Saunders M, Bontrager M, Weitz AP, Hendricks R, Margarey R, Suiter K, Gilbert GS (2015) Phylogenetic structure and host abundance drive disease pressure in communities. Nature 520: 542–544.

Pennell MW, Eastman JM, Slater GJ, Brown JW, Uyeda JC, FitzJohn RG, Alfaro ME, Harmon LJ (2014) geiger v2.0: an expanded suite of methods for fitting macroevolutionary models to phylogenetic trees. Bioinformatics 30: 2216–2218.

Phillips BB, Shaw RF, Holland MJ, Fry EL, Bardgett RD, Bullock JM, Osborne JL (2018) Drought reduces floral resources for pollinators. Global Change Biology 24: 3226–3235.

Pierce NA, Archer SR, Bestelmeyer BT, James DK (2019) Grass-shrub competition in arid lands: an overlooked driver in grassland-shrubland state transition? Ecosystems 22: 619–628.

Pineda A, Pangesti N, Soler R, Van Dam NM, Van Loon JJA, Dicke M (2016) Negative impact of drought stress on a generalist leaf chewer and a phloem feeder is associated with, but not explained by an increase in herbivore-induced indole glucosinolates. Environmental and Experimental Botany 123: 88–97.

Pleasants JM, Oberhauser KS (2013) Milkweed loss in agricultural fields because of herbicide use: effects on the monarch butterfly population. Insect Conservation and Diversity 6: 135–144.

Pocius VM, Debinski DM, Pleasants JM, Bidne KG, Hellmich RL, Brower LP (2017) Milkweed matters: Monarch butterfly (Lepidoptera: Nymphalidae) survival and development on nine Midwestern milkweed species. Environmental Entomology 46: 1098–1105.

Power EF, Stabler D, Borland AM, Barnes J, Wright GA (2018) Analysis of nectar from low volume flowers: A comparison of collection methods for free amino acids. Methods in Ecology and Evolution 9: 734–743.

Püschel D, Janoušková M, Hujslová M, Slavíková R, Gryndlerová H, Jansa J (2016) Plant- fungus competition for nitrogen erases mycorrhizal growth benefits of *Andropogon gerardii* under limited nitrogen supply. Ecology and Evolution 6: 4332–4346.

R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rasmann S, Bennett A, Biere A, Karley A, Guerrieri E (2017) Root symbionts: Powerful drivers of plant above- and belowground indirect defenses. Insect Science 24: 947–960.

Ratajczak Z, Nippert JB, Hartman JC, Ocheltree TW (2011). Positive feedbacks amplify rates of woody encroachment in mesic tallgrass prairie. Ecosphere 2: 1–14.

Ratajczak Z, Nippert JB, Collins SL (2012) Woody encroachment decreases diversity across North American grasslands and savannas. Ecology 93: 697–703.

Ratajczak Z, Nippert JB, Briggs JM, Blair JM (2014) Fire dynamics distinguish grasslands, shrublands and woodlands as alternative attractors in the Central Great Plains of North America. Journal of Ecology 102: 1374–1385.

Reinhart KO, Wilson GWT, Rinella MJ (2012) Predicting plant responses to mycorrhizae: integrating evolutionary history and plant traits. Ecology Letters 15: 689–695.

Revell LJ (2012) phytools: An R package for phylogenetic comparative biology (and other things). Methods in Ecology and Evolution 3: 217–223.

Richards LA, Weaver LR (1943) Fifteen-atmosphere percentage as related to the permanent wilting percentage. Soil Science 56: 331–340.

Richardson L, Gallagher MK, Hayes TE, Galliant AS, Kiefer G, Manion K, Jenkins M, Diersen G, Wagenius S (2021) Competition for pollination and isolation from mates differently impact four stages of pollination in a model grassland perennial. Journal of Ecology 109: 1356–1369.

Ricono A, Dixon R, Eaton I, Brightbill CM, Yaziji Y, Puzey JR, Dalgleish HJ (2018) Long- and short-term responses of *Asclepias* species differ in respect to fire, grazing, and nutrient addition. American Journal of Botany 105: 2008–2017.

Rillig M (2004) Arbuscular mycorrhizae and terrestrial ecosystem processes. Ecology Letters 7: 740–754.

Ruiz-Sánchez M, Aroca R, Muńoz Y, Polón R, Ruiz-Lozano JM (2010) The arbuscular mycorrhizal symbiosis enhances the photosynthetic efficiency and the antioxidative response of rice plants subjected to drought stress. Journal of Plant Physiology 167: 862–869.

Schenk HJ, Jackson RB (2002) Rooting depths, lateral root spreads and below ground/above ground allometries of plants in water-limited ecosystems. Journal of Ecology 90: 480–494.

Schlesinger WH, Reynolds JF, Cunningham GL, Huenneke LF, Jarrell WM, Virginia RA, Whitford WG (1990) Biological feedbacks in global desertification. Science 247: 1043–1048.

Scymanczik S, Lehmann ML, Wiemken A, Boller T, Courty P (2018) Effects of two contrasted arbuscular mycorrhizal fungal isolates on nutrient uptake by *Sorghum bicolor* under drought. Mycorrhiza 28: 779–785.

Sikes BA, Cottenie K, Klironomos JN (2009) Plant and fungal identity determines pathogen protection of plant roots by arbuscular mycorrhizas. Journal of Ecology 97: 1274–1280.

Senior JK, Potts BM, O'Reilly-Wapstra JM, Bissett A, Wooliver RC, Bailey JK, Glen M, Schweitzer JA (2018) Phylogenetic trait conservatism predicts patterns of plant-soil feedback. Ecosphere 9: e02409.

Smith SA, O'Meara BC (2012) Divergence time estimation using penalized likelihood for large phylogenies. Bioinformatics 28: 2689–2690

Smith SE, Read DJ (2008) Mycorrhizal symbiosis. New York, NY, USA. Academic Press.

Snyder AE, Harmon-Threatt AN (2019) Reduced water-availability lowers the strength of negative plant–soil feedbacks of two Asclepias species. Oecologia 190: 425–432.

Stein C, Mangan SA (2020) Soil biota increase the likelihood for coexistence among competing plant species. Ecology 101: e03147.

Sylvia DM, Wilson DO, Graham JH, Maddox JJ, Mlllner P, Morton JB, Skipper HD, Wright SF, Jarstfer AG (1993) Evaluation of vesicular-arbuscular mycorrhizal fungi in diverse plants and soils. Soil Biology and Biochemistry 25: 705–713.

Tao L, Ahmad A, de Roode JC, Hunter MD (2016) Arbuscular mycorrhizal fungi affect plant tolerance and chemical defences to herbivory through different mechanisms. Journal of Ecology 104: 561–571.

Tedersoo L, Mett M, Ishida TA, Bahram M (2013) Phylogenetic relationships among host plants explain differences in fungal species richness and community composition in ectomycorrhizal symbiosis. New Phytologist 199: 822–831.

Thomson DM (2016) Local bumble bee decline linked to recovery of honeybees, drought effects on floral resources. Ecology Letters 19: 1247–1255.

Towne EG, Kemp KE (2008) Long-term response patterns of tallgrass prairie to frequent summer burning. Rangeland Ecology and Management 61: 509–520.

Tunlid A, White DC (1992) Biochemical analysis of biomass, community structure, nutritional status, and metabolic activity of microbial communities in soil. In Soil biochemistry. Edited by G. Strotzky and J.M. Bollag. Marcel Dekker Inc., New York, N.Y. pp. 229–262.

Van Der Heijden MGA (2003) Arbuscular mycorrhizal fungi as determinant of plant diversity: in search of underlying mechanisms and general principles. In: Mycorrhizal Ecology (eds Van Der Heijden, M.G.A. & Sanders, I.R.). Springer, Berlin, Germany, pp. 243–266.

Van der Heijden MG, Martin FM, Selosse MA, Sanders IR (2015) Mycorrhizal ecology and evolution: the past, the present, and the future. New Phytologist 205: 1406–1423.

Vanette RL, Hunter MD, Rasmann S (2013) Arbuscular mycorrhizal fungi alter above- and below-ground chemical defense expression differentially among *Asclepias* species. Frontiers in Plant Science 4: 1–9.

Vanette RL, Rasmann S (2012) Arbuscular mycorrhizal fungi mediate below-ground plant herbivore interactions: a phylogenetic study. Functional Ecology 26: 1033–1042.

Veen GF, Blair JM, Smith MD, Collins SL (2008) Influence of grazing and fire frequency on small-scale plant community structure and resource availability in native tallgrass prairie. Oikos 117: 859–866.

Vicente-Serrano SM, Beguería S, López-Moreno JI (2010) A multiscalar drought index sensitive to global warming: The standardized precipitation evapotranspiration index. Journal of Climate 23: 1696–1718.

Wandrag EM, Bates SE, Barrett LG, Catford JA, Thrall PH, van der Putten WH, Duncan RP (2020) Phylogenetic signals and predictability in plant–soil feedbacks. New Phytologist 228: 1440–1449.

Wang B, Qiu Y-L (2006) Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza 16: 299–363.

Wang GZ, Li HG, Christie P, Zhang FS, Zhang JL, Bever JD (2017) Plant-soil feedback contributes to intercropping overyielding by reducing the negative effect of take-all wheat and compensating growth of faba bean. Plant and Soil 415: 1–12.

Wang Y, Meng B, Zhong S, Wang D, Ma J, Sun W (2018) Aboveground biomass and root/shoot ratio regulated drought susceptibility of ecosystem carbon exchange in a meadow steppe. Plant and Soil 432: 259–272.

Wang X-X, Hoffland E, Mommer L, Feng G, Kuyper TW (2019) Maize varieties can strengthen positive plant-soil feedback through beneficial arbuscular mycorrhizal fungal mutualists. Mycorrhiza 29: 251–261.

Waser NM, Price MV (2016) Drought, pollen and nectar availability, and pollination success. Ecology 97: 1400–1409.

Wehner J, Antunes PM, Powell JR, Mazukatow J, Rillig MC (2010) Plant pathogen protection by arbuscular mycorrhizas: a role for fungal diversity? Pedobiologia 53: 197–201.

Welti EAR, Joern A (2018) Fire and grazing modulate the structure and resistance of plant-floral visitor networks in a tallgrass prairie. Oecologia 186: 517–528.

Wilbur HM (1976) Life history evolution in seven milkweeds of the genus Asclepias. Journal of Ecology 64: 223–240.

Wilcox BP, Birt A, Fuhlendorf SD, Archer SR (2018) Emerging frameworks for understanding and mitigating woody plant encroachment in grassy biomes. Current Opinion in Environmental Sustainability 32: 46–52.

Wilmer H, Augustine DJ, Derner JD, Milchunas DG (2020) Assessing the rate and reversibility of large herbivore effects on community composition in a semi-arid grassland ecosystem. Journal of Vegetation Science 32:e12934.

Wilschut RA, van der Putten WH, Garbeva P, Harkes P, Konings W, Kulkarni P, Martens H, Geisen S (2019) Root traits and belowground herbivores relate to plant–soil feedback variation among congeners. Nature Communications 10: 1–9.

Wilson GWT, Hartnett DC (1998) Interspecific variation in plant responses to mycorrhizal colonization in tallgrass prairie. American Journal of Botany 85: 1732–1738.

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

Winter SL, Allred BW, Hickman KR, Fuhlendorf SD (2015) Tallgrass prairie vegetation response to spring fires and bison grazing. The Southwestern Naturalist 60: 30–35.

Wood S (2017) Generalized Additive Models: An Introduction with R. 2nd edn. Boca Raton, FL: Chapman and Hall/CRC.

Wyatt R, Broyles SB, Derda GS (1992) Environmental influences on nectar production in milkweeds (*Asclepias syriaca* and *A. exaltata*). American Journal of Botany. 79: 636–642.

Zaya DN, Pearse IS, Spyreas G (2017) Long-term trends in Midwestern milkweed abundances and their relevance to monarch butterfly declines. BioScience 67: 343–356.

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