Effects of late-summer prescribed fire on botanical composition, soil cover, and forage production in Caucasian bluestem-infested rangelands in the Kansas Smoky Hills

by

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Abstract

The invasive old-world bluestem species, yellow bluestem (*Bothriochloa ischaemum*) and Caucasian bluestem (*Bothriochloa bladhii*), have been displacing native grasses and forbs in the Great Plains since they were first introduced to the United States for soil conservation and as a forage source in the early 20th century. Targeted removal of old-world bluestems has proven difficult. Traditional, spring-season prescribed fire appears to promote growth and proliferation of the invasive species to a greater extent than native species; moreover, treatment with glyphosate is expensive and non-selective. After reports of successful yellow bluestem removal using late-summer prescribed fire, a similar experiment targeting Caucasian bluestem was conducted in a heavily invaded, mixed-grass pasture in Ellsworth County, Kansas. Eighteen 4,047 m^2 (i.e., 1 acre) plots were assigned randomly to one of three treatments: no burn (control), one burn (August 14, 2019), or two burns (August 14, 2019, and August 11, 2021). Beginning in 2019 and ending in 2023, annual measurements of soil cover, botanical composition, forage biomass, and Caucasian bluestem frequency were conducted in each plot. In plots burned once or twice, bare soil increased ($P < 0.01$) and Caucasian bluestem cover decreased ($P < 0.01$) the year following each fire, allowing for increases in forb cover ($P = 0.03$) and grass species richness ($P = 0.01$). While C_4 tallgrass and shortgrass covers were not affected by fire ($P \geq 0.13$), C_4 midgrasses, of which Caucasian bluestem is a member, decreased ($P = 0.04$) in response to fire. These results were interpreted to suggest that late-summer prescribed fire may provide targeted Caucasian bluestem control without inflicting harm on native vegetation; however, it is believed that fire must be regularly applied to maintain control over time.

Key words: *Bothriochloa bladhii*, Caucasian bluestem, invasive plants, mixed-grass prairie, old-world bluestem, prescribed fire

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Chapter 1 - Review of Literature

Introduction

Invasive plant species pose a major threat to native grasslands by decreasing richness and evenness of native species. They often have the ability to disrupt ecosystem function while also proving less useful by livestock due to poor nutritive and palatability characteristic. Specifically, traits associated with invasive exotics include greater biomass, maturation rate, ground cover, photosynthetic efficiency, nutrient (especially nitrogen) sequestration, and nitrogen-fixing symbioses, as well as root size and shape differing from that of natives (Ehrenfeld, 2004). The old-world bluestems *Bothriochloa bladhii* (Caucasian bluestem) and *Bothriochloa ischaemum* (yellow bluestem) are no exception to the rule and have been rapidly invading rangeland in Kansas, Oklahoma, and Texas since their introduction in the United States in the early 20th century (Eck and Sims, 1984; Robertson and Hickman, 2012).

According to the USDA Plant Database, Caucasian bluestem is now present in a total of 10 states and yellow bluestem is now present in 18. Because these plants demonstrate greater tolerance for drought, fire, and grazing than native grass species, they were an attractive addition to areas requiring soil stabilization and rehabilitation in the first half of the 20th century (Wied et al., 2020). Furthermore, elevated forage biomass production encouraged usage in pastures and hay meadows. Although they offered brief periods of acceptable forage quantity early during their respective growth cycles, they matured very quickly resulting in poor quality relatively early in the growing season (Teague et al., 1996; White and Dewald, 1996; Sanderson et al., 1999). Conflicting research documented similar nutrient profiles between native grasses and Caucasian bluestem at the end of the growing season; these researchers attributed grazing

avoidance to morphological aspects and buildup of dead stem material (Harmoney and Hickman, 2012).

White and Dewald (1996) determined that yellow bluestem had greater levels of crude protein and *in vitro* dry matter digestibility than Caucasian bluestem but that regrowth of both species was not able to meet generalized cattle nutrient requirements beyond mid-June. These authors recommended cutting it for hay or doubling the stocking rate in June, or if pasturage was needed for the latter growing season, rotating cattle through regrowth of other forages. Caucasian bluestem hay was found to have adequate nutrient quality if cut before or during the early boot stage of development (i.e., in mid-June) but decreased intake and digestibility were documented with hay harvested after this point (Burns, 2011). Grazing pastures at relatively aggressive stocking rates maintains plants in a vegetative state, which maintains nutrient concentration and improves animal performance to a degree, but this comes at the expense of root biomass (Teague et al., 1996).

Native grasses mature later and are more desirable for livestock grazing throughout the growing season compared with old-world bluestems. Most grazing systems would benefit from removal of old-world bluestems; however, this is difficult to achieve because they share C_4 metabolism with many native grasses of the Southern Plains and tend to exhibit greater resilience to adverse environmental conditions. It becomes necessary to investigate and exploit the physiological characteristic specific to old-world bluestems in order to realize targeted removal. This process begins by determining how these invasive species are able to gain a foothold in novel environments and what agronomic traits promote their rampant spread and their displacement of native species. Relating these traits to specific characteristics of old-world bluestems may contribute to sustainable control practices.

Theories of exotic dominance

Numerous hypotheses have been formulated to determine why plants introduced to a novel environment often exhibit improved growth and reproductive traits compared to the same species in their native regions. Several of these are summarized in the following paragraphs. Due to the relatively small number of experiments involving old-world bluestem directly, examples of more highly studied species are used to support each hypothesis in relation to old-world bluestems.

Enemy-Release Hypothesis

The Enemy-Release Hypothesis is based on three points: (1) certain enemies found in natural environments regulate the plant population so that no one species becomes dominant over the others; (2) these enemies are adapted to affect a suite of native species and have lesser effects on exotics; and (3) escape from enemies allows exotic populations to grow and proliferate beyond usual capabilities. Enemies may consist of both vertebrate and invertebrate herbivores, fungi, bacteria, and viruses. Assuming specialist enemies attack specific plants while generalist enemies target all species in a community, additional assumptions of the hypothesis include three specifications. First, exotic species' specialist enemies will not be present in the new environment. Second, specialists will rarely be able to switch hosts. Finally, generalists' impacts will still be greater for the native plants than the invasives. Not only will a species in a new environment be faced with fewer enemies, but also relatively more pressure will fall upon the native species, resulting in reduced interspecific plant competition (Keane and Crawley, 2002).

Klironomos (2002) reported that plants which multiplied rapidly in an environment (i.e., invasive species) accumulated species-specific pathogens at a rate lower than non-invasive plants. This was also associated with decreased negative feedback responses related to growth

and reproduction. Wilson et al. (2011) further supported this hypothesis by determining that introduction of an insect enemy of an invasive plant successfully controlled spread and growth of the invader.

Evolution of Increased Competitive Ability Hypothesis

Similar to the Enemy-Release Hypothesis, the Evolution of Increased Competitive Ability relates escape from natural predators to the success of invasive plants in their non-native environments. Unlike Enemy Release, invasive plants are suspected to adapt over time to spend less energy in defense and to allocate more energy for growth and reproduction instead of merely escaping the direct effects of enemies. Due to lack of plant defense activity, enemies would experience greater performance if re-introduced to previously protected host plants (Blossey and Nötzold, 1995).

Blossey and Nötzold (1995) conducted an experiment using the Eurasian plant *L. salicaria*, an invasive species in North American wetlands, and natural-predator insects which, in the native Eurasian environment, caused damage to aboveground and belowground tissues. Even when grown in the same location, seeds from non-native Ithaca, New York compared to native Lucelle, Switzerland produced plants with greater biomass and height. In further support of the hypothesis, natural root-feeding herbivores experienced increased survival rates and larval weight on plants from New York, suggesting the plants' lack of expression of defense mechanisms resulted in less inhibition of the herbivore. In contrast, there were no differences in survival or pupal weights among leaf-feeding enemies. The authors hypothesized that, because foliage is more easily replaced than roots, plants in both environments may put less energy into leaf defense while prioritizing root health.

It is difficult to attribute results to either evolutionary traits or environmental factors. For instance, Colautti et al. (2010) found environment (i.e., climate) to have a major effect on *L. salicaria*, with reproductive biomass and fecundity varying greatly between locations differing in latitude. In another experiment to account for confounding effects of climate and herbivory escape, Uesugi and Kessler (2013) compared competitive ability of *S. altissima* grown in the same location and originating from the same gene pool, with or without 12-year treatment of insecticide to eradicate common insect enemies of the plant. Competitive ability was compared between plants grown alone, plants grown with conspecifics, and plants grown with *Poa pratensis.* Logic for these treatments included the following: *Poa pratensis* is known for competing heavily with *S. altissima* and insect herbivory results in stem mortality of *S. altissima*, thereby allowing more sunlight to the understory plants, including *P. pratensis*.

Effects on growth and survival were greater with competition from *P. pratensis* than from competition with *S. altissima* conspecifics, suggesting greater evolutionary selection for inter-specific competitive ability. Furthermore, herbivore-suppressed plants produced more foliage with inter-specific competition. Reproduction by herbivore-affected plants was also decreased in the presence of *P. pratensis*. Interestingly, reproductive tissue production did not differ between herbivore-treated and non-treated plants, suggesting domination occurred because of rapid, early growth associated with shading out other plants that could otherwise present competition (Uesugi and Kessler, 2013).

Theory of Fluctuating Resource Availability

Under the Theory of Fluctuating Resource Availability, invasion susceptibility of an area is expected to increase in response to increased availability of resources, specifically, if resources available are greater than those required by plants in order to optimize growth. This occurs either

when resource use by plants declines – such as occurs during overgrazing – or when resource increase occurs at a rate exceeding plant use – such as occurs in a particularly wet year. This theory attempts to dethrone other theories that assume certain fixed characteristics affect invasion, such as community diversity, but rather states that propensity to invade fluctuates over time due to the circumstances (Davis et al., 2001). Hobbs and Atkins (1988) found that soil disturbance in the form of road grading - an example of reduction of plants resulting in an increase in available nutrients - allowed introduced annual species in Australia to establish themselves while the same species were found to a much lower extent in non-disturbed areas.

Providing fertilizer, an example of resource addition, promoted growth of the newly established invasives. In dry areas where water was a limiting resource, years with abundant rainfall led to increased invasive capabilities. For example, *Bromus mollis*, the major non-native invader of northern California grasslands, increased in population during abnormally high-moisture years and subsequently decreased following drought (Hobbs and Mooney, 1991).

Framework of Vacant Niches Theory

The Framework of Vacant Niches Theory involves changes to environment which allow nonnative species to invade. Native species predominate until some sort of disturbance, such as human infrastructure establishment, changes the environment. When disturbance occurs, if the native species is still best suited for the environment, it will remain. Conversely, if a non-native finds the environment similar to its native environment, it can establish and outcompete the native (Moles et al., 2008). Simply removing the invasive species will not allow native species to return; the disturbance must somehow be reversed (Moles et al., 2008). Natural and human disturbances to the landscape allowed vegetative species to establish themselves where they were previously not found in Hawai‘i and New Zealand (Vitousek et al., 1987 and McGlone, 1989,

respectively). Fauna in New Zealand were also influenced, either directly by human means or indirectly through changed vegetation (McGlone, 1989).

Enhanced Mutualism Hypothesis and arbuscular mycorrhizal fungi/soil nitrifying bacteria

While it is relatively easy to attribute invasive tendencies to environmental changes, it is difficult to observe changes caused by underground organisms. With the billions of microorganisms found in a single gram of soil, including fungi, protozoa, bacteria, and nematodes, it is challenging but important to determine how some of the major organisms which interact with plants influence, or are influenced by, invasion. Clearly, not every interaction between plant and microbe is well understood or even discovered as of yet, but the major mutualistic organisms and their roles in invasion are discussed below.

The Enhanced Mutualism Theory states that species can form more and better mutualistic interactions in a new environment than in their native ranges (Lekberg and Callaway, 2022). A given plant attracts a variety of soil biota, some of which are beneficial and some inhibitory. While beneficial biota promote the growth of the already-established plant, inhibitory biota reduce the likelihood of further establishment of the same plant in the same vicinity. Thus, plant diversity is promoted (Bever et al., 1997; Bever, 2003; Bever et al., 2010). For this reason, farmers rotate crops, as accumulation of species-specific pathogens inhibits the growth of a repeated crop (Bever et al., 1997).

In reviewing multiple experiments, Reinhart and Callaway (2006) determined that negative plant-soil biota feedbacks dominate in natural systems, such that species diversity is encouraged by managing frequency of individual species. In contrast, invasive species appeared to promote the growth and reproduction of their own kind through positive soil-biota feedbacks

because their specialist enemies are not present in non-native soils. Normally, a beneficial attribute for maintenance of diversity, negative feedback becomes lethal to populations of native species as they inhibit their own establishment while invasives promote their own, eventually leading to complete takeover by invasive species.

One major component of soil biota, arbuscular mycorrhizal fungi (AMF), comes to the forefront of plant-biota relationships. A relatively recent discovery, AMF penetrate the root cortex zones of plants and extend hyphae beyond the root zone (Vives-Peris et al., 2020). Hyphal exudates recruit soil nutrients, primarily nitrogen and phosphorus, and exchange these for photosynthetic carbon from plant root exudates at host-microbe interfaces known as arbuscules. Unlike the root rhizosphere, the hyphosphere contains enzymes that mineralize nutrients, providing a plant-usable source to the roots (Zhang et al., 2022). Further benefits of AMF to the plant include limiting uptake of toxic heavy metals from the soil and protection from pathogens (Newsham et al., 1995). Different plant species release distinct exudates that recruit specific types of AMF; the fungi, in turn, recruit specific organisms within the hyphosphere using exudates of their own (Zhou et al., 2020). Since biota are responsible for nutrient acquisition for the plant host, plants' various nutrient needs can be met by the biota their exudates recruit.

Because plants rely most heavily on nutrient uptake during their early stages of development due to rapid growth rates, plants generally have the highest colonization rates when they are immature. Seedlings planted in perennial communities where AMF are already present become colonized within days of establishment so that nutrients become accessible immediately (van der Heijden et al., 2015).

Invasive species can potentially use relationships with AMF to their advantage. On average, invasives have greater growth and AMF colonization than native species and benefit

from these relationships more so than natives (Yu et al., 2022). Marler et al. (1999) determined that the native *F. idahoensis* and the invasive *C. maculosa* were both infected with AMF; however, the invasive tendencies of *C. maculosa* were facilitated in the presence of AMF. When grown in soil containing AMF, growth of the invasive was inhibited when planted with conspecifics but enhanced with heterospecifics. When the two species were started from seed, AMF presence greatly inhibited *F. idahoensis* growth.

Hyphal networks have been suggested to transfer phosphorus (Chiariello et al., 1982) and carbon (Francis and Read, 1984; Simard et al., 1997) between plants. Some have attempted to attribute invasive advantages to parasitic relationships where fungal hyphae transfer nutrients from native to invasive plants; however, substantial direct evidence is lacking linking nutrient advantages found in invasive plants to the AMF network (Zabinski et al., 2002; Carey et al., 2004; Bever et al., 2010).

Nutrient alterations

Much research has pointed to the occurrence of altered nutrient cycling, particularly that of carbon and nitrogen, in environments invaded by foreign species. Likely due to changing relationships with biota, nutrient shifts in an environment promote the invasive properties of the exotic. In a meta-analysis of 94 plant-invasion studies, Liao et al. (2007) discovered nutrient cycling tendencies to be very similar among invasive plants regardless of the environment. Increased carbon and nitrogen concentrations were found in shoots, roots, litter, soils, and soil microbes of invasive plants compared to natives. Furthermore, nutrient use efficiency was greater among the invasives, as they were associated with greater rates of mineralization and nitrification. This corresponded with a larger amount of plant-available nitrogen in the form of ammonium and nitrate, as well as greater above-ground net primary production. Smaller C:N

ratios and lignin:N ratios were also present, resulting in more rapid litter decomposition and, ultimately, greater nutrient cycling.

In another analysis of multiple invasives, Allison and Vitousek (2004) suggested the combination of increased litter (i.e., due to greater biomass), greater nutrient concentration in the litter, and more rapid litter decomposition rates could vastly increase rates of nutrient cycling in the vicinity of invasive plants and foster further growth and spread. Invasive exudates may also select for a different abundance and composition of ammonium-oxidizing bacteria, the presence of which is directly correlated with gross nitrification rates in the soil (Hawkes et al., 2005).

Baruch and Goldstein (1999) compared the efficiency of nutrient use among 34 native and 30 invasive species in Hawai‘i. The invasives were found to have a greater specific leaf area (SLA), directly correlating to increased rates of photosynthesis and improved carbon allocation and assimilation. Elevated SLA and carbon assimilation were also directly linked to increased plant growth. Nitrogen was found in large concentrations in the foliage where it would either contribute to leaf resiliency (i.e., direct use of nitrogen by plant tissues) or construction of photosynthetic proteins. These authors indicated that, in general, these two factors were negatively correlated and that plants generally forfeited one for the other; however, they also suggested these species may enjoy the benefits of both to some extent with the already greater amount of nitrogen contained in invasives. Lastly, construction cost, indicative of efficiency of plant tissue synthesis, was also less among invasive plants than among natives (Baruch and Goldstein, 1999; Allison and Vitousek, 2004).

Increased photosynthetic capability would increase carbon production in plants. This would result in greater nutrient content in the plant itself and also increases in carbon exudates that would enhance activity of nitrogen fixing bacteria and AMF. The expected result would be

enhanced mineralization of soil organic matter and, ultimately, greater nitrogen and phosphorus uptake by the plant (Liao et al., 2007; Thirkell et al., 2016). Aboveground and belowground tissue growth would be promoted under nutrient-rich conditions (generated by both photosynthesis and microbe acquisition). Increased root biomass would further contribute to elevated nutrient concentrations in invasive plants compared to natives, leading to a positive feedback loop (Liao et al., 2007; Baptist et al., 2015; Shahzad et al., 2015).

In an experiment with a native *Stipa pulchra* and invasive *Avena fatua*, *S. pulchra* performance was diminished in the presence of the invasive or when grown in soil cultured with *A. fatua* soil communities (Larios and Suding, 2014). This same reduction in performance occurred when *S. pulchra* was grown in soils containing large amounts of nitrogen. Changes in soil nitrogen concentration have been shown to alter root exudates and fungal community structure, meaning that the altered nutrient cycling associated with invasive plants could influence the microbial community (Wang et al., 2022). Bardgett et al. (1999) discovered that nutrient changes in the soil altered microbial communities but suggested this occurred indirectly by first changing the plant community in the area.

Novel Weapons Hypothesis and chemical alteration of soil biota

The Novel Weapons Hypothesis holds that plants in their native place possess defensive mechanisms to which their neighboring plants have adapted, whereas in a non-native location these same plants can use these "weapons" to their advantage. Researchers compared this to the "guns, germs, and steel" used by European invaders to conquer indigenous peoples. The defensive mechanisms may either be used against plants directly or against mycorrhizae (Callaway and Ridendour, 2004). Defense occurs through the release of allelochemicals into the soil or atmosphere which can negatively impact cell structures, cell division and growth, the

antioxidant system, membrane permeability, growth regulators and enzymes, respiration, photosynthesis, metabolism, or uptake of water and nutrients (Cheng and Cheng, 2015).

Non-mycorrhizal plants have been shown to invade an area by killing the AMF communities relied upon by the native species (Zubek et al., 2016). This tactic is used by the highly invasive garlic mustard *Alliaria petiolata*. The plant greatly reduced fungal presence, diversity, and spore reproductive capability. Vigor of plants with high dependency on mycorrhizal relationships was significantly reduced when plants were grown in soils that had been invaded by *A. petiolata*, whereas plants with no mycorrhizal dependency showed no response (Stinson et al., 2006; Callaway et al., 2008). These effects were not observed when the plant was grown in its native Europe. Furthermore, leaf extracts that could contain allelochemicals suppressed AMF in six different locations in North America, while no AMF suppression was documented in six different locations in Europe. This suggested that long-term exposure of European soil biota to *A. petiolata* resulted in resistance to the chemicals it produced (Callaway et al., 2008).

Vaughn and Berhow (1999) discovered *A. petiolata* contained the phytochemicals allyl isothiocynate (AITC) and benzyl isothiocyanate (BzITC) and hypothesized these could be responsible for growth inhibition of other plants, either by direct harm to the plant or mycorrhizal inhibition. In response to this discovery, Stinson et al. (2006) further tested the effects of root extracts from *A. petiolata* on mycorrhizal plants. They found the same effect whether plants were grown with *A. petiolata* or treated only with the extracts from *A. Petiolata*. In fact, BzITC curbed germination of *Glomus etunicatum*, a species of AMF. Roberts and Anderson (2001) found that *A. petiolata* leachate greatly reduced germination of *Gigaspora rosea* azygospores and inhibited colonization of tomato plants by the fungus, as well as germination of the seeds. Root length of

non-colonized tomato plants was significantly stunted compared to the control. Root length of sorghum plants was also stunted in the presence of *A. petiolata*.

Invaders forming mycorrhizal relationships have also been shown to disrupt the AMF community. Plants within the same functional group tend to rely on similar AMF communities. Conversely, plants originating from different functional groups are generally associated with contrasting AMF communities than the native species and can invade by altering the native AMF (Aslani et al., 2019).

Callaway and Ridendour (2004) conducted an experiment involving the knapweed *Centaurea diffusa* which is native to Europe and Asia but invasive in North America. Though the plant has non-combative relationships with other plants in its native Caucasus Mountain foothills, it has negative effects on the neighboring grasses in the Palouse prairie in eastern Oregon. These regions are comparable in terms of species of similar size and phylogeny. Among North-American plant species, *C. diffusa* was found to reduce growth to a much greater extent than among Caucasian species. Addition of activated carbon to exudates, which would absorb allelopathic molecules, strongly inhibited negative effects on North American plants, though no change was noted with addition of activated carbon among the Caucasian species. Upon further investigation, it was found that acquisition of a radioactive isotope of phosphorus by the North American plants was inhibited when grown in association with *C. diffusa*, suggesting an allelopathic root exudate may have been responsible for chelation of the phosphorus.

Similarly, Vivanco et al. (2004) reported 80-100% mortality rates two weeks after administration of *C. diffusa* root exudates among several North American plants. Seed germination was also drastically decreased. Application of the exudates to other *C. diffusa* plants did not result in plant mortality or reduced germination. In a bioassay, the only part of the root

exudate found to be phytotoxic was 8-hydroxyquinoline, a product not previously reported in a natural setting and used commercially for its metal-chelating, antifungal, and antibacterial properties (Merck Index, 1996). Vivanco et al. (2004) also found the substance to have inhibitory effects on several plant pathogens, suggesting its usefulness to *C. diffusa* could extend to its antimicrobial activity. The concentration of this chemical in North American soils was more than three times the concentration found in similar Eurasian soils. The authors attributed the greater concentration in North American soils to either greater release by plants, higher densities of plants exuding it, or less microbial breakdown in the soil.

Sterilizing soils to remove microbial communities had a much greater effect on suppression by *C. diffusa* growth in North America than Eurasia, suggesting greater adaptation of microbes to 8-hydroxyquinilone in Eurasian soils. Vivanco et al. (2004) suggested microbes in Eurasian soils may even use the chemical as a carbon source. Curiously, other invasive knapweeds were found to use structurally different chemicals to achieve an inhibitory effect on neighboring plants. Specifically, the highly invasive *Centaurea maculosa* was found to exude (-)-catechin (Weir et al., 2003) while 7,8-benzoflavone was the phytotoxin found in root exudates of *Acroptilon repens* (Stermitz et al., 2003).

Ranked as one of the worst plant invaders in the world, *Imperata cylindrica* (cogongrass) alters populations of fungi in the soil of forest ecosystems, affecting native plants directly through their association with mycorrhizae and indirectly through changed nutrient cycling. The invasive plant was able to absorb nutrients at a much faster rate than native species due to changed mycorrhizae. Chemicals from multiple different classes that were found in various parts of the plant (i.e., leachate, exudate, essential oil, rhizome extract, or leaf extract) were isolated

and found to inhibit the growth or reproduction of several plant species, likely due to their negative effects on soil biota (Kato-Noguchi, 2022).

Due to the extremely small number of AMF species relative to the vast number of plant species around the world, it is clear that specificity for AMF species is rare. Though some fungi have species-specific relationships, generalist fungi interact with a large number of plants. In fact, fungal hyphae extend between many different plant species and connect these plants in a common mycorrhizal network so that each plant reacts with multiple fungal varieties and most fungi interact with multiple plants. This lends plants and fungi the opportunity to associate with preferred partners to form the most beneficial mutualisms (Smith and Read, 2008; Fellbaum et al., 2014; van der Heijden et al., 2015). Invasive plants that belong to different functional groups than native plants may be more successful if they change or disrupt the native AMF communities. Conversely, invasive plants that belong to the same functional groups as native plants tend to have shared mycorrhizal preferences with the natives and their invasion success may be due to leveraging relationships with the AMF already present in the native soil (Aslani et al., 2019; Sun et al., 2022).

While in some cases, invasives may tap into the common mycorrhizal network largely unnoticed and without altering native AMF composition. In others, their presence renders severe implications to the AMF communities through direct alteration of abiotic soil characteristics, through release of allelochemicals, or through shading of native species. The latter is an indirect effect; however, it decreases photosynthetic capacity of native plants and disrupts their capability to provide associated AMF with carbon exudates (Aslani et al., 2019). Such invasions resulted in decreased AMF abundance and species richness, such that benefits received from AMF by invasive plants exceeded those received by natives (Zubek et al., 2016).

Invasive plants can selectively choose fungal partners with which to associate. In the same manner, fungi can choose to associate with a new host and also place sanctions on hosts providing relatively less nutrient delivery (Zubek et al., 2016; Kiers and Denison, 2024). Greater photosynthetic efficiency in invasive plants results in greater carbon exudation compared to natives. In many experiments, AMF rewarded plants releasing the most carbon with the greatest transfer of nitrogen or phosphorous in both artificial (Bücking and Shachar-Hill, 2004; Lekberg et al., 2010; Hammer et al., 2011; Kiers et al., 2011; Fellbaum et al., 2012) and natural settings (Fellbaum et al., 2014; Zheng et al., 2015; Awaydul et al., 2023).

Carbon allocations had a greater effect on reciprocal nutrient delivery than did dependence of plant species on AMF (Awaydul et al., 2023). Nitrogen uptake to the extraradical mycelium (ERM) of AMF was stimulated with carbon allocation to fungi; nitrogen was subsequently transported to the intraradical mycelium (IRM), where it was then transferred across the mycorrhizal-host interface (Fellbaum et al., 2012).

Phosphorous was also gathered and stored in the form of long-chain polyP in the ERM. Carbon exudation stimulated its breakdown into short-chain polyP that was then transferred to the IRM and, eventually, to the host for use in plant growth (Bücking and Shachar-Hill, 2004; Fellbaum et al., 2012). Furthermore, plants with hindered photosynthetic abilities had reduced expression of the gene coding for mycorrhiza-inducible plant phosphorous transporters, ultimately leading to arbuscule degradation and decreased colonization by AMF (Fellbaum et al., 2014). Phosphorous and nitrogen were found in greater concentrations in roots and shoots of unshaded plants (i.e., those most competitive for photosynthesis) compared to shaded plants. Nutrient uptake nor site of nutrient deposition were dependent on the type of fungi with which the plant was associated (Fellbaum et al., 2014).

While AMF can discriminate between high- and low-quality hosts, plant hosts can also discriminate between fungi by rewarding preferred fungal varieties (i.e., those that transfer more resources to the host) with exudates and penalizing less beneficial partners (i.e., those using more of the host-provided resources for their own needs) with lesser carbon sharing. The result is a loss of nutrient transporters and, ultimately, arbuscular degradation (Kiers et al., 2011; Fellbaum et al., 2014; Kiers and Denison, 2024).

This method of selecting fungal partners may help explain the variations observed between types of fungi colonizing individual plants. Interestingly, the ability to discriminate between fungal partners with varying levels of favorability to the plant host decreased as levels of carbon exudation decreased; nutrient exchange with beneficial partners was diminished but that with less beneficial partners was unchanged (Kiers et al., 2011; Zheng et al., 2015). This may be due, in part, to beneficial partners foraging for nutrients for the favorable hosts while sequestering acquired carbon from poor hosts (Lekberg et al., 2010).

Preferential choosing of fungal species by invasive plants does not, in and of itself, result in loss of fungal association with native species (Aslani et al., 2019). Interestingly, when common mycorrhizal networks (CMN) connected two shaded plants, shaded plants received more resources than they did when connected to an unshaded plant by the CMN. This clearly demonstrated fungal preference for the better host. Conversely, while arbuscule numbers declined with decreased resource delivery from photosynthetically-inhibited plants (i.e., suggesting diminished phosphorous transfer), colonization rates to low-quality hosts were not reduced (Lekberg et al., 2010; Hammer et al., 2011). This may have occurred because, under shifting circumstances, a poor host became a relatively good host. Shading, defoliation, or senescence of a good host may reduce the benefits of association in favor of the previously

poorer host. Overall, colonization of poor hosts may serve as a strategy for fungi to mitigate risk (Lekberg et al., 2010).

Invasive plants alter the fungal communities based on the species they preferentially recruit, both for themselves and for native species. Species of AMF have been observed to avoid one another, such that multiple fungi in close proximity had lesser individual colonization rates than when grown alone (Cano and Bago, 2005; Bennett and Bever, 2009). Individual AMF species cannot dominate in a system where fungal diversity is promoted (i.e., in an area associated with great plant species diversity).

Conversely, as invasive plants spread throughout an area, the fungi they associate with also spread. Non-preferred fungi will retreat along with the native plants with which they are associated (Bever, 2002; Cano and Bago, 2005). When native plants are replaced by invasive plants, it is likely that fungal communities will shift from specialist to generalist in nature because invasive species of similar phylogeny to native species can utilize the generalist fungi already present (Hawkes et al., 2006). Invasion results, therefore, in decreased AMF species richness, dominance of a select few AMF species, disrupted structure, degraded composition, and diminished viability of the plant community (van der Heiden et al., 1998).

Bever (2003) described effects of invasion on soil fungi as a positive feedback loop, where presence of an invasive plant alters soil community composition, which then alters the growth rates of both the host plant and the competing plants nearby. Van der Heijden et al. (1998) reported that increased AMF species richness induced greater plant species diversity while also increasing above- and belowground biomass. Coincident were increased hyphae length in the soil, decreased soil phosphorous content, and increased plant phosphorous content.

In contrast, when there was no presence of AMF or when only a few species were present, plant species diversity and efficiency of soil nutrient utilization were relatively poor.

Sun et al. (2022) studied the AMF characteristics of seven invasive plant species belonging to the Asteraceae family in comparison with those of seven native species belonging to the same family. Invasive and native plants alike had similar biomass and AMF colonization rates when grown in monocultures; however, when grown in co-culture, biomass and colonization increased for invasive species but decreased for natives. These benefits were only observed when AMF colonization was present. If AMF were absent, invasives did not receive advantages in resource acquisition or growth. When grown in monocultures, native and invasive plants all had similar root concentrations of myristic acid, a fatty acid used as a carbon and energy source for AMF. When grown in competition, invasive plants had greater myristic acid concentrations than natives. Concurrently, expression of two fatty acid transporters were evaluated in one of the invasive species, *A. artemisiifolia*, compared to one of the native species. Competition increased gene expression in the invasive but decreased it in the native. These authors acknowledged that more investigation is required to understand why invasive species synthesize more myristic acid when grown in competition. They also recognized that a positive feedback cycle was likely to ensue. Greater AMF colonization among the invasive plants, encouraged by greater carbon delivery and myristic acid transport, would result in increased nutrient delivery, photosynthetic capacity, and biomass of invasive plants.

In related research, Zeng et al. (2022) isolated multiple sesquiterpenoids from *A. artemisiifolia* that were found to inhibit both above- and belowground growth of wheat seedlings. Additional research is needed to determine if related phytochemicals were partially responsible for the suppression of the non-invasive asters in the work of Sun et al. (2022) and if

exudation of chemicals beneficial to the invasive species and chemicals harmful to native species are coupled in order to facilitate rapid invasion.

As summarized by Scognamiglio et al. (2013), many different chemical compounds are responsible for allelopathy in plants that are not necessarily structurally related. Allelochemicals are released by different methods, such as exudation or leaching, and may have positive or negative effects on neighboring plants. Yu et al. (2022) evaluated root exudates and AMF from 19 invasive plants and compared them with non-invasive confamilials. On average, biomass and AMF colonization were greater among the invasives. Quercetin and strigolactones were identified in root exudates. These compounds were found in greater concentrations in invasive compared to non-invasive plants. They have been associated with AMF germination and growth. Quercetin was shown to specifically affect *Gigaspora* fungal species (Tsai and Phillips, 1991; Poulin et al., 1993; Scervino et al., 2005; Steinkellner et al., 2007).

In multiple experiments summarized by Yu et al. (2022), invasive-plant root exudates were found to be associated with AMF colonization to a greater degree than non-invasive root exudates. Though levels of quercetin in invasive root exudates far exceeded those in non-invasive plants early and midway through the growing season, levels did not differ at the end of the growing season. It was hypothesized that invasives may establish relationships with AMF relatively early in the growth period, thereby giving them an advantage over natives. This suggested that the need to establish fungal relationships through chemical exudation may be time-dependent. As AMF communities have also been found to vary greatly with species-specific root exudates, and exudates in this experiment differed between invasive and natives, the authors also acknowledged that native and invasive plants may have preferentially recruited different AMF (Yu et al., 2022).

Tian et al. (2021) studied the plant *Triadica sebifera* in environments where it was native and in environments where it had invaded. Biomass and AMF colonization of *T. sebifera* were greater in invaded environments, which was attributed to greater quercetin secretion and its positive influence on AMF spore germination. Interestingly, when plants from native populations were inoculated with invasive exudates, AMF populations did not differ from those seen in the invasives. The authors speculated that escape from enemies may be related to greater exudate release by invasives compared with natives. As outlined in the Enemy Release Hypothesis and Evolution of Increased Competitive Ability, plants in new environments that have escaped specialist enemies found in their native environments may be able to reallocate energy previously used for defense into systems that help the plants establish and flourish. Furthermore, exudates are associated with specific biotic communities. Some of the attracted biota are pathogenic rather than mutualistic; however, in a new location, some of these pathogenic biota may not exist. This would allow the plant to release larger amounts of exudate (i.e., thereby increasing AMF recruitment) without incurring the harmful effects it may have encountered in its native environment (Zhou et al., 2020; Tian et al., 2021). Positive feedback from AMF communities is experienced if negative feedback from pathogens does not offset it (Klironomos, 2002).

Uesugi and Kessler (2013) supported the Evolution of Increased Competitive Ability hypothesis. In doing so, they pointed to results which indicated that insecticide treatment or movement to a novel, enemy-free environment both allowed plants to develop much higher concentrations of polyacetylene compounds, known to act as allelochemicals. In both cases, effects of native-environment enemies would be minimized. By applying *cis*-dehydromatricaria ester, they documented inhibition of seed germination and seedling mass of *P. pratensis*, a major

competitor of the invasive plant. The chemical did not harm the invasive species itself but improved its growth by inhibiting pathogenic fungi growth on the seeds. In a later experiment (Uesugi and Kessler, 2016), these authors found that greater plant production of polyacetylenes was linked to lesser production of anti-herbivory diterpene acids. Authors hypothesized that escape from herbivory decreased the need for plants to produce their own defense mechanisms and allowed them to allocate more energy to competition with other plants.

Application to old-world bluestems

Old-world bluestems, including Caucasian and yellow bluestem, exhibit many of the properties observed in other invasive species. Invasion is associated with decreased native species richness, evenness, and cover (Reed et al., 2005; Gabbard and Fowler, 2007; Robertson and Hickman, 2012). Native plant cover and old-world bluestem cover are negatively related, so native plant cover decreases as old-world bluestem invades a rangeland (Robertson and Hickman, 2012). Because it can inhabit almost any area, only to the exclusion of areas under dense woody canopies, dispersal is limited by the speed with which old-world bluestems spread rather than availability of suitable habitat. As such, it is often most dense near roads or areas where seed dispersal is facilitated by accidental transportation and proceeds subsequently to spread outward from those locations (Gabbard and Fowler, 2007).

Big bluestem (*Andropogon gerardii*) and little bluestem (*Schizachrium scoparium*) were both affected by old-world bluestems and experienced reduced aboveground and belowground growth parameters, including inhibited root production (Schmidt et al., 2008; Wilson et al., 2012). Conversely, Caucasian bluestem was not negatively affected when grown with the native bluestems and growth of yellow bluestem was even promoted in the presence of little bluestem (Schmidt et al., 2008).

Caucasian bluestem also demonstrated intraspecific competition and had greater above- and belowground biomass compared with native species as long as conspecifics weren't present (Schmidt et al., 2008; Wilson et al., 2012). Interestingly, sideoats grama was competitive with Caucasian bluestem and could potentially inhibit its establishment and spread (Schmidt et al., 2008).

Native seed richness and evenness were reduced as yellow bluestem seed concentration in the seedbank increased to the point that old-world bluestem seeds were the most abundant. Yellow bluestem seed density was positively correlated with its own aboveground cover (Robertson and Hickman, 2012). In the long term, viability of native seeds in the seedbank declines for various reasons and invasion of an exotic species may decrease native seed proportions (Fourie, 2008). Due to the finite ability of seeds to survive in the seedbank, Holmes and Cowling (1997) noted a lag in the decline of native seed bank diversity following invasion-induced reduction of the aboveground plant community. Therefore, it may be difficult to recover natural plant populations in rangeland following a long period of old-world bluestem dominance.

Both old-world bluestems are grazing- and drought-resistant but cessation of grazing did not decrease yellow bluestem presence (Eck and Sims, 1984; Gabbard and Fowler, 2007). Mowing had variable effects on yellow bluestem; cutting in hot months had produced only minor effects on tiller count of yellow bluestem and little bluestem, whereas cutting in colder months tended to increase yellow bluestem tiller count (Havill et al., 2015). Invasive plant seeds withstand wide ranges of soil temperatures and levels of moisture and may germinate under poor conditions (Yuan and Wen, 2018). In fact, some experiments reported that root exudate amounts and organic acid concentrations increased in response to stressful conditions (Pramanik et al., 2000; Henry et al., 2007; Gabbard and Fowler, 2007).

It has been well-documented that positive feedback mechanisms govern nutrient availability in plant-soil-arbuscular mycorrhizal fungi (AMF) relationships. Prescribed fire, an essential component of tallgrass prairie maintenance, decreases soil nitrogen concentrations, nitrogen mineralization rates (i.e., associated with water availability, which declines with burning), and plant tissue nitrogen concentrations (Blair, 1997). In this nutrient-limiting environment, plants grow slowly, use the available nutrients efficiently, and deposit low-quality (i.e., high carbon, low nitrogen) litter which breaks down slowly and promotes modest rates of nutrient cycling (Hobbie, 1992; Ojima et al., 1994). In contrast, increased soil temperature and photosynthetically active radiation resulting from burning increased photosynthesis and nitrogen use efficiency. Plant nitrogen requirements per unit carbon decreased, allowing plants to adapt to less nitrogen-rich conditions (Ojima et al., 1994).

Over time, tallgrass prairie dominants such as big bluestem and little bluestem become highly competitive in these nutrient-poor conditions (Wedin and Tilman, 1990). Part of this adaptation is likely related to increased dependence on relationships with AMF. Grasses in low-fertility areas have been found to rely much more on relationships with the soil biological community than those in nutrient-rich soils, due to the dependence on AMF to obtain nutrients (Bardgett et al., 1999; Hammer et al., 2011; van der Heijden et al., 2015). Indeed, big bluestem was highly dependent on mycorrhizal relationships when phosphorous availability was poor and mycorrhizal-associated plants produced more biomass in these conditions (Noyd et al., 1995).

Experiments conducted in the tallgrass prairie ecosystems have shown C_4 grasses to be obligately mycotrophic with dependencies on mycorrhizal relationships exceeding 99% (Hetrick et al., 1990). A large variety of AMF species are present with various richness, evenness, and spore densities associated with different plant species. Different fungi likely benefit the host in

unique ways. Some species of fungi appear to be host-specific while others are generalist in nature and occur more frequently (Eom et al., 2000). With greater plant diversity, mycorrhizal colonization of individual plants increased, likely due to the need to attain resources when neighboring species were highly competitive (Jastrow and Miller, 1993).

Several reported that AMF presence was positively correlated to growth and reproduction of C_4 grasses, while the opposite was true for C_3 grasses. This is logical because suppression of C_4 plants when mycorrhizal abundance was low indirectly increased competitive ability of C_3 species (Hetrick et al., 1990; Hartnett and Wilson, 1999; Wilson et al., 2011).

Bentivenga and Hetrick (1991) found mycorrhizal, burned, big-bluestem pastures had the greatest plant growth rates while non-mycorrhizal, non-burned pastures had the least. No differences were present between non-mycorrhizal, burned pastures and mycorrhizal, non-burned pastures. This suggested that lack of mycorrhizal activity eliminated the benefits associated with burning and that the positive effects of fire can likely be attributed to the role of mycorrhizae in the soil.

Hartnett et al. (1994) determined that suppressing AMF decreased flowering stem density of C_4 grasses in burned but not non-burned sites. Correspondingly, AMF presence increased flowering in burned but not non-burned prairie. Burning was also found to increase root colonization, likely linked to the enhanced live root carbon concentrations found in plants after prescribed fire treatment (Bentivenga and Hetrick, 1991; Ojima et al., 1994). Suppression of fungi in burned sites advantaged C_3 grasses, which exhibited greater flowering and stem densities (Hartnett et al., 1994). Wilson and Hartnett (1998) also documented increases in C_3 abundance and biomass with reduced mycorrhizal activity.

Assuredly, one of the primary benefits of fire is promoting mycorrhizal populations among C_4 grasses and keeping native and exotic C_3 plant populations to a minimum. Because old-world bluestems belong to the same C_4 functional group as native grasses, fire provides the same beneficial effects to both. In fact, winter fire application on the eastern Edwards Plateau in central Texas did not reduce old-world bluestem cover but, instead, tended to increase it (Gabbard and Fowler, 2007; Novak et al., 2021). In central Arizona, a February burn resulted in less total forage biomass but boosted yellow bluestem biomass by 16% (Pase, 1971). At the Konza Prairie Research site in eastern Kansas, spring burning increased the productivity of Caucasian bluestem and resulted in greater concentrations of foliar nitrogen compared to big bluestem (Reed et al., 2005). Accordingly, fires conducted in central Texas in October, November, and December had neutral to positive effects on yellow bluestem populations. Neutral effects were associated with cooler temperatures and limited effects on resource availability to dormant grasses, whereas positive effects were attributed to removal of litter that would allow light to penetrate to the soil thereby promoting seed germination at the beginning of the following growing season (Havill et al., 2015).

Like the native C_4 grasses, old-world bluestems were found to adapt to and become efficient in nitrogen-poor conditions associated with burning. The high concentration of nitrogen found in the invasive plant combined with the loss of nitrogen from burning may reduce the nitrogen pool to the point that natives can no longer survive (Reed et al., 2005).

Typical prairie topsoil consists of a dense mat in the top 5 cm of soil composed of rhizomes and roots which prevent erosion (Weaver, 1969). Caucasian bluestem presence was shown to induce soil heterogeneity with roots being highly concentrated under bunches (Reed et al., 2005). Soil under bunches was also associated with greater concentrations of carbon and

nitrogen, including microbial biomass nitrogen, and greater rates of decomposition than soil between bunches. Further nutrient losses could occur due to erosion and leaching of soil in the sparsely populated areas between bunches (Reed et al., 2005).

Greer et al. (2014) suggested allelopathy may be responsible for old-world bluestem dominance. Germination, above- and belowground biomass production, and survival of big and little bluestems in close proximity were decreased with application of yellow bluestem leachate or litter. The authors noted the differences may have been due to alterations in soil biotic communities, as opposed to direct effects to the plant.

This conclusion was challenged by DaSilva et al. (2022) who conducted a very similar experiment but increased the number of native plant species and used sterilized leachate and soil, as opposed to raw leachate, to detect direct chemical effects of allelopathy. Though the authors acknowledged that different environments and genetics may have influenced their results, they determined allelopathic effects to be minimal. In fact, sterilized yellow bluestem leachate was only effective in reducing germination on one day of the experiment. Interestingly, there was a slight negative effect of native species leachate on old-world bluestem germination and some autotoxicity in yellow bluestem. Unlike the Greer et al. (2014) experiment, application of yellow bluestem leachate did not kill any native seedlings and had insignificant effects on seedling growth. With these results, the authors demonstrated direct allelopathy did not likely occur and hypothesized that yellow bluestem dominance could be linked to pathogen-mediated effects. It should be noted that the authors' use of sterilized soil in the experiment would negate effects of allelochemicals on soil microbes, including AMF.

As established previously, native grasses rely heavily on mycorrhizal relationships. Caucasian bluestem also relies greatly on mycorrhizae (Wilson and Hartnett, 1998), although

colonization of native roots was found to be greater than that of invasive roots when plants were grown with conspecifics. When grown in competition with Caucasian bluestem, native root colonization was drastically reduced. Conversely, Caucasian bluestem colonization remained unchanged when grown in competition with natives, suggesting the old-world bluestem altered microbial characteristics.

When non-sterile, live soil from native prairie was applied to sterilized soil from invaded areas, native grass biomass increased, but not to the same extent as when non-sterile, live soil from native prairie was applied to sterilized soil from native prairie. The sterilization process removed biota but not chemicals present in the soil (Wilson et al., 2012). Because old-world bluestems rely heavily on mycorrhizal relationships, killing all AMF through allelochemicals would be detrimental to the invasive plants, as well as the natives. Old-world bluestems belong to the same functional group as native grasses; therefore, it is likely they can utilize generalist fungi present in the soil. These generalist species may be present in their native ranges.

Greater concentrations of carbon and nitrogen, microbial biomass nitrogen, and rates of decomposition beneath Caucasian bluestem bunches suggest elevated microbial activity. Caucasian bluestem bunches may employ fungi recruitment methods using carbon exudates, which they can release in maximal amounts because they don't have to expend energy on defense in an environment novel to them. Mycorrhizae could preferentially establish relationships with these invasive species. Specialist fungi, like those described by Eom et al. (2000), which would not have been present in the native ranges of old-world bluestems, may not be adapted to the chemicals released by the invasive species and may find them toxic. Dominance of Caucasian bluestem would then lead to dominance of the AMF communities associating with these species and declining presence of non-associated fungi.

This may explain why native colonization by AMF was greatly reduced, although not completely eliminated, in the presence of Caucasian bluestem while colonization was unchanged for the invasive species in competition with the natives. In concurrence, native grass growth was improved when grown in sterilized native or invasive soils when native soil inoculum was added, but the effect was much less substantial in invaded soils. Invasive species, such as those studied by Yu et al. (2022) and Tian et al. (2021), produced chemicals that could attract beneficial fungi or kill non-beneficial fungi using allelopathy. Further research is needed to determine what chemicals might be produced by Caucasian bluestem that would have attractive or lethal effects on soil fungi.

Further evidence suggesting allelopathic effects on AMF include results from the experiments conducted by Schmidt et al. (2008) and Wilson et al. (2012). Generally, plants grown in close proximity to each other would be expected to reduce aboveground biomass of neighbor plants, due to competition for soil resources. This was found to be the case with native big and little bluestem. Not only did both yellow and Caucasian bluestems reduce aboveground biomass of the native species, but they also reduced belowground biomass, with old-world bluestem above- and belowground growth actually increasing in the presence of native grasses. This may suggest effects on the method of resource acquisition more than simply resource competition itself.

Old-world bluestems grow rapidly and reach reproductive maturity earlier in the growing season than native grasses (Wilson and Hartnett, 1998; Harmoney and Hickman, 2004). Interestingly, Harmoney and Hickman (2004) discovered that rates of Caucasian bluestem growth and maturation declined toward the end of the growing season. This would have been coincidental with elevated levels of secondary metabolites in root exudates of invasives in the

early and middle, but not late, parts of the growing season, as noted by van der Heijden et al. (2015). This suggested that chemicals produced by Caucasian bluestem may play a part in inducing AMF relationships during periods of rapid growth and, therefore, during plant nutrient acquisition and growth (Yu et al., 2022). Furthermore, DaSilva et al. (2022) noted an unexpected growth of mold in yellow bluestem leachate which did not occur in little bluestem leachate. It is possible that secondary metabolites, such as those that induce spore germination, are present in old-world bluestem leachate and exudate.

In summary, escape from native pathogens may allow old-world bluestems to release maximum amounts of root exudates in invaded ranges without incurring the harmful effects from pathogenic soil biota. Not only would greater root exudation stimulate AMF to acquire more nutrients, but also the presence of chemical compounds in the exudates may serve to recruit the generalist AMF already present in the native soil while harming the specialist fungi. In this manner, nutrient acquisition would be augmented to the detriment of native species that also rely on the same nutrient pool. Once invasives have an advantage in growth, they can continue to deposit significant amounts of carbon into the soil to benefit associated AMF. Correspondingly, suppressed growth of natives would restrict the amount of carbon they could allocate to soil biota, resulting in further loss of specialist fungi associated with the native plant species and overall declines in AMF species richness. With large concentrations of soil nutrients and a lack of herbivory, old-world bluestems grow rapidly and efficiently, allocating the nutrients to their own growth instead of to defensive compounds. Their robust growth, as well as the large amount of litter produced, would effectively shade out competing plants, resulting in a continuous, positive feedback loop and eventual formation of an old-world bluestem monoculture.

Old-World Bluestem Control

Herbicide application

Few methods have been successful in decreasing invasive old-world bluestem abundance in a targeted manner. Herbicide application produced varied results based on type of herbicide used, timing of application, and frequency of application (Harmoney et al., 2010; Ruffner and Barnes, 2012). Sequential glyphosate applications, or one high-dose application in normal or wet years, reduced old-world bluestem frequencies the following year (Harmoney et al., 2010). Combining herbicide with disking reduced cover of the invasives but at the expense of the native plants as well (Ruffner and Barnes, 2012). Three annual imazapyr treatments nearly eliminated Caucasian bluestem cover without negatively affecting cover of native tall and midgrasses; however, weedy forbs (i.e., marestail and western ragweed), which were a minor component before the experiment began, took over the treated area and, together, exceeded 40% of total vegetative cover. Following treatment with dicamba and reseeding of native grasses, the landscape more closely resembled native composition, though old-world bluestem cover increased following cessation of imazapyr treatment (Harmoney, 2022).

Unfortunately, several problematic issues arise with herbicide treatment. First, it can be extremely costly and time-consuming, especially if repeated treatment is required. Second, effectiveness of treatment in old-world bluestem plots decreased over time (Harmoney et al., 2007; Grichar and Foster, 2019). Third, many herbicides are not selective and negatively impact native vegetation as well as old-world bluestems (Ruffner and Barnes, 2012; Duell et al., 2023). Finally, though it was previously believed that glyphosate was inactivated upon contact with soil, recent research has documented residual effects of glyphosate application as well as inhibition of seed germination and decreased AMF abundance, which would help eliminate the invasive but

make reestablishment of natives difficult (Helander et al., 2018; Helander et al., 2019; Maggi et al., 2020; Duell et al., 2023). Conversely, if seeds are not killed by the herbicide, control becomes temporary as the vast amount of old-world bluestem seeds contained in the seedbank can quickly germinate and perpetuate invasion (Ruffner and Barnes, 2012).

Growing-season prescribed fire

Though spring and winter fires were unsuccessful in controlling old-world bluestem populations, fires conducted during the growing season may be more successful. Native grasses, as well as their seeds, were found to be more heat tolerant than yellow bluestem grasses and seeds (Ruckman et al., 2012a; Havill et al., 2015). This may be the result of grass adaptation to traditional grazing and burning practices in America and Europe.

While time-restricted grazing of livestock using fences has been practiced in Europe for many years, wild herds of buffalo grazed the grasslands of North America which likely contributed to greater heterogeneity in forage biomass (Mack and Thompson, 1982). Higgins (1984) reported that the vast majority of lightning-strike fires in the Northern Great Plains were ignited in July or August. High ambient temperatures coupled with considerable forage biomass would have resulted in relatively hot fires to which the native plant species would have adapted. In fact, Novak et al. (2021) found that prescribed fire conducted in summer promoted grassland composition that more closely resembled the suspected composition of old-growth grasslands compared with any other fire season. The average rhizome depth of 3.2 centimeters for grasses and forbs in the tallgrass prairie may be an adaptation allowing native species to better protect themselves from heat (Benson et al., 2004).

In contrast to North American environments, a more intensive style of grazing in Europe would have resulted in less forage biomass accumulation so that, even if fires occurred, the heat

would have been less intense. Grasses originating from Europe, like old-world bluestems, may not have developed ways to insulate their rhizomes from heat. Indeed, Havill et al. (2015) found yellow bluestem to be the most sensitive to fire temperatures at a depth of one centimeter below the soil surface. Conversely, little bluestem, a bunchgrass with similar physical structure, was not sensitive to heat until a depth of two centimeters was reached in the soil.

Along with elevated sensitivity to heat, yellow bluestem plants were found to be most vulnerable to fire shortly before flowering. At this point, plants re-direct energy usage from growth to reproduction and decrease their nutrient allocation to roots for both root growth and exudation to AMF, while simultaneously increasing nutrient allocation for flowering. Therefore, removal of aboveground biomass during this time would prove injurious to the plant, as lack of belowground nutrient storage would make recovery difficult (Ruckman et al., 2012b).

Because native grasses mature more slowly than old-world bluestems, they would still be at a point in their life cycles where relationships with AMF would be well-established and ample resources in belowground biomass would allow them to quickly overcome the loss of nutrients in aboveground biomass. This point in reproductivity coincides with a time in summer where forage biomass is large and ambient temperatures are elevated, both of which contribute to fire heat and intensity. To further increase heat intensity, pastures may be rested from grazing pre-burn to allow for accumulation of biomass (Twidwell et al., 2009; Rissi et al., 2017; Reemts et al., 2021). Utilizing backfires for the majority of a targeted area would result in a slower burn that could potentially elevate heat intensity as well.

Multiple experiments have been conducted to evaluate the effects of growing-season fires on old-world bluestem presence with varying results. Novak et al. (2021) discovered that, following winter and fall prescribed fire, cover of yellow bluestem was 51% and 23%,

respectively. Conversely, 6% cover was documented following summer prescribed fire. Furthermore, as old-world bluestems have been found to establish in any location except under woody canopies (Gabbard and Fowler, 2007), sites treated with winter or fall prescribed fire or that were protected from prescribed fire had elevated cover of woody-stemmed plants and yellow bluestem cover, whereas sites treated with summer fire had no such relationship. Both woody canopy cover and yellow bluestem cover remained low, which contributed to the increase in species richness observed in summer fire sites.

Havill et al. (2015) reported that controlled burns conducted in October, November, or January either had no effects on yellow bluestem or promoted it. Conversely, controlled burns in June, July, or August reduced the presence of yellow bluestem even though all treatments were cut to the same height (3 to 4 cm above ground level) before fire was applied. The authors noted that average temperatures were 100°C higher inside yellow bluestem bunches compared to little bluestem bunches, suggesting a lack of heat-intensity adaptation in yellow bluestem. Peak surface temperatures during the cold-season burns were an average of 80°C lower than the warm-season burns. All fires were conducted during a drought year and the dry conditions combined with the hot warm-season fires reduced tiller densities by 88%. Related effects on little bluestem were less drastic, with a reduction in tiller densities of 51%. Authors reported that plants investing in relatively early reproductive growth suffered most during warm-season fires, which targeted yellow bluestem. In the year following growing-season fire treatment, yellow bluestem growth remained suppressed (Havill et al., 2015).

While some of the success in the previous experiment was attributed to drought conditions concurrent with warm-season fire, drought conditions leading up to a fire may be less beneficial if forage biomass accumulation is reduced. Twidwell et al. (2012) conducted a

prescribed fire in June in the coastal prairies of Texas following five months of drought when forage biomass was less than 2,500 kg/ha at the time of the burn. Shortly after the fire, a large amount of precipitation was received at the experimental site which may have minimized tissue damage caused by fire. In addition, use of headfires in that experiment may have decreased the duration and, therefore, the intensity of heat. The authors reported populations of yellow bluestem were not affected by fire treatment.

Simmons et al. (2007) compared the effects of fire timing and moisture conditions in Texas using early growing-season fire (mid- or late-August) or late growing-season fire (late October or early November) at each of two locations – a Blackland Prairie representative site and an Edwards Plateau representative site. Less grazing rest, as well as lower rainfall over the course of the year at the Edwards Plateau site, contributed to lower fuel loads at that location; however, neither site exceeded 2,500 kg/ha in forage biomass. Reemts et al. (2019) had subsequently suggested that this biomass level was needed to achieve desirable fire intensity.

All fires decreased canopy cover of yellow bluestem but the effect was more modest at the Edwards Plateau site with the late-season burn, likely due to abundant rainfall occurring before and after the fire event. The earlier burn at Edwards Plateau and both treatments at the Blackland Prairie site had greater reductions in yellow bluestem cover than the late burn at Edwards Plateau, even with varying amounts of forage biomass and precipitation accumulation throughout the year. Elevated biomass levels at the Blackland Prairie site and drier conditions surrounding fire treatment at the Edwards Plateau site were both suspected to increase the negative effects on yellow bluestem populations, suggesting they may have offset each other. No native species were negatively affected by fire treatments.

Perhaps the most promising results in yellow bluestem removal were documented by Reemts et al. (2019). Two different study sites were represented – Fort Hood and Onion Creek, Both sites were heavily infested with yellow bluestem at the outset of the experiment. The Onion Creek site had relatively recent grazing history and much less forage biomass (approximately 2,873 kg/ha) than the Fort Hood site (7,600 kg/ha). The latter had not been grazed for a decade prior to the experiment. Burning in September 2006 reduced yellow bluestem frequency from 74% to 32% at the Onion Creek site and from 74% to 9% at the Fort Hood site compared to adjacent non-burned controls. The beneficial effects of fire lasted longer at Fort Hood, where fuel loads were more significant. No negative effects on native plant species composition were detected, while forb frequency increased in burned plots.

Research at the Ford Hood site continued with an experiment determining the long-term effects of fire treatment. After 12 years, yellow bluestem cover increased somewhat, up to 21%, in fire-treated plots but was still less than in non-burned control plots. Native graminoid cover did not differ between burned and non-burned treatments. Reduced soil cover following removal of litter and reduced biomass of dominant species following fire application allowed germination of forbs, with annual forbs dramatically increasing in cover the year after treatment. As open spaces were filled by native species reestablishment and litter, annual forb cover gradually declined and was not different from pre-treatment levels by 2018. Rhizomatous, perennial forbs were able to displace annuals and their cover continued to increase through 2018 (Reemts et al., 2021).

Restoration following old-world bluestem removal

In an area long-dominated by yellow bluestem where native plants are not able to grow, the native seed concentration in the seedbank declines and reestablishment may prove difficult.

In two experiments, native grass cover remained low for 12 years after prescribed fire, suggesting lack of seed presence (Novak et al., 2021; Reemts et al., 2021). Following eight years of glyphosate treatment that removed Caucasian bluestem along with most other vegetation and AMF, Duell et al. (2023) attempted to reconstruct the native landscape. Seeds supplemented to the rangeland alone, in whole native soil, or in sterile native soil all germinated; however, seedling survival was greatest in plots receiving whole soil. This suggested inclusion of native AMF populations was crucial for native-plant reestablishment. Improved end-of-season plant species richness and evenness and reduced reinvasion of Caucasian bluestem were also observed with whole soil addition.

Whole soil addition was costlier and more labor-intensive than simply planting seed. As previously mentioned, fire positively affected arbuscular mycorrhizal fungi colonization; moreover, AMF may survive in the soil for some time without a host. In perennial plant communities with AMF already present, seedlings became colonized quickly and accessibility to nutrients could, therefore, be rapid (Zubek et al., 2013; van der Heijden et al., 2015). Removal of old-world bluestem by summer fire, as opposed to herbicide which can negatively affect AMF populations, may allow native seeds to flourish without the addition of soil. Unfortunately, AMF species richness likely declines with old-world bluestem invasion so that some species that benefit native flora may need to be re-introduced.

Conclusions

Old-world bluestems are spreading rapidly in many areas of the United States resulting in a decrease in native species richness and diversity. Because they are a member of the C_4 grass functional group (i.e., warm-season grasses), they have critically similar attributes to native grasses: adaptation to spring season fires and association with arbuscular mycorrhizal fungi

(AMF). Unlike the native species, old-world bluestems lack environmental predators and can, therefore, allocate less energy to defense mechanisms and more to biomass production and release of root exudates. Root exudates serve to recruit soil biota that form symbiotic relationships with the plant to promote nutrient exchange. They may also have negative effects on specialist, native AMF. Greater exudation may result in preference for old-world bluestems by soil biota and effectively contribute to greater growth rates than native grasses during the early growing season. Correspondingly, AMF colonization is drastically less among native species in the presence of old-world bluestems. When priority turns from growth to reproduction, relationships with AMF may no longer be as essential but later-maturing native plants are still at a disadvantage due to shading from invasives. The combination of greater access to AMF and shading likely facilitates greater spread of the invasive plant over time. By comparison, fewer native species are able to reach reproductive maturity, resulting in greater relative deposition of old-world bluestem seed in the seedbank.

Based on these principles, an efficient control method would be one that selectively eradicates old-world bluestem plants and seeds while leaving soil biotic communities intact. Eradication efforts targeted against old-world bluestems are best conducted when the grass is in its pre-flowering stage, a time in which native species are still growing and less phenologically mature.

Likely due to less fire adaptation compared to native plant species, old-world bluestem plants and seeds are highly vulnerable to destruction via heat. Late-summer fires are hotter than traditional spring fires due to greater ambient temperatures; they also coincide with the plant's most susceptible life-cycle point. These conditions may have contributed to successful control of yellow bluestem.

Following a protracted period of invasion, there may be a dearth of native seeds in the seedbank. Removal of old-world bluestem and native plant species with glyphosate may require whole-soil amendment to allow reestablishment of native species. Conversely, survival of fire-adapted AMF communities following a burning event may allow for successful seeding of native grasses without the added labor and expense of amending the landscape with whole soil.

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Chapter 2 - Effects of late-summer prescribed fire on botanical composition, soil cover, and forage production in Caucasian bluestem-infested rangelands in the Kansas Smoky Hills

Abstract

The invasive old-world bluestem species, yellow bluestem (*Bothriochloa ischaemum*) and Caucasian bluestem (*Bothriochloa bladhii*), have been displacing native grasses and forbs in the Great Plains since they were first introduced to the United States for soil conservation and as a forage source in the early 20th century. Targeted removal of old-world bluestems has proven difficult. Traditional, spring-season prescribed fire appears to promote growth and proliferation of the invasive species to a greater extent than native species; moreover, treatment with glyphosate is expensive and non-selective. After reports of successful yellow bluestem removal using late-summer prescribed fire, a similar experiment targeting Caucasian bluestem was conducted in a heavily invaded, mixed-grass pasture in Ellsworth County, Kansas. Eighteen 4,047 m^2 (i.e., 1 acre) plots were assigned randomly to one of three treatments: no burn (control), one burn (August 14, 2019), or two burns (August 14, 2019, and August 11, 2021). Beginning in 2019 and ending in 2023, annual measurements of soil cover, botanical composition, forage biomass, and Caucasian bluestem frequency were conducted in each plot. In plots burned once or twice, bare soil increased ($P < 0.01$) and Caucasian bluestem cover decreased ($P < 0.01$) the year following each fire, allowing for increases in forb cover ($P = 0.03$) and grass species richness ($P = 0.01$). While C_4 tallgrass and shortgrass covers were not affected by fire ($P \geq 0.13$), C_4 midgrasses, of which Caucasian bluestem is a member, decreased ($P = 0.04$) in response to fire. These results were interpreted to suggest that late-summer prescribed fire may provide targeted

Caucasian bluestem control without inflicting harm on native vegetation; however, it is believed that fire must be regularly applied to maintain control over time.

Key words: *Bothriochloa bladhii*, Caucasian bluestem, invasive plants, mixed-grass prairie, old-world bluestem, prescribed fire

Introduction

The invasive old-world bluestem species yellow (*Bothriochloa ischaemum*) and Caucasian bluestem (*Bothriochloa bladhii*) have been invading rangelands in the United States since their introduction in the early 20th century for soil conservation and for their forage production characteristics (Eck and Sims, 1984; Sanderson et al., 1999; Robertson and Hickman, 2012). Although very productive, these species reach reproductive maturity more quickly, and therefore lose palatability more quickly, than native grasses. Quality is sharply reduced relatively early in the growing season, making them undesirable for grazing and haying (Teague et al., 1996; White and Dewald, 1996). Physiological similarity to native warm-season grasses, and resilience to drought, spring fire, and grazing has created a situation where targeted removal of old-world bluestems has been difficult (Wied et al., 2020).

Old-world bluestems appear to be well-adapted to traditional spring fires (Reed et al., 2005). Conversely, they also appear to have greater sensitivity to heat than native species at or following reproductive maturity (Ruckman et al., 2012; Havill et al., 2015). Some summer-season prescribed burning has been effective in targeting yellow bluestem. Factors that augment the suppressive effects of summer fire on yellow bluestem include elevated biomass and elevated ambient temperatures. These contribute to hotter fires (Simmons et al., 2007; Havill et al., 2015; Reemts et al., 2019). Prescribed fire conducted in late summer resulted in a decrease in yellow bluestem frequency from 74% to 9% when rest from grazing had resulted in significant biomass

accumulation and when precipitation was modest immediately preceding and following application of fire (Reemts et al., 2019). In light of these recent developments in successful yellow bluestem removal, late-summer prescribed fire was evaluated in our experiment for its potential to reduce presence of the phenotypically-related Caucasian bluestem in heavily invaded mixed-grass, native pastures in Ellsworth County, Kansas.

Materials and Methods

Experimental Design and Location

Our five-year experiment was conducted near Kanopolis, Kansas, on a privately owned pasture heavily infested with Caucasian bluestem. Soils were primarily of the Harney-Wells complex (3 to 7% slope) making up 73.3% of the total area. Remaining soils were Crete silt loam (0 to 1% slope). Grazing cows with calves were allowed to freely access the research site during the growing seasons involved with our experiment.

Eighteen plots, each with an area of 4,047 m^2 (i.e., 1 acre) and separated by 10-m fire breaks, were arranged in 9 x 2 block fashion, with each of the 9 blocks of 2 plots assigned one of three treatments: no burn (n = 6 plots), burned once in 2019 (n = 6 plots), and burned twice in 2019 and 2021 (n = 6 plots). Fifty-m transects were established in each plot, on a southwest-to-northeast gradient. Orange survey stakes (Forestry Suppliers Inc., Jackson, MS) were used to mark the endpoints of each transect and the corners of the plots; marker coordinates were recorded using GPS (Garmin, Olathe, KS).

Prescribed-Fire Application

The first fire was applied 14 August 2019. The high temperature was 29.3°C, winds were from the south with speeds ranging from 7.7 to 32.8 km/h, and relative humidity at mid-day was

67.1%. On the day of the second fire, applied 11 August 2021, the high temperature was 39.9°C, winds were again southerly (12.4 to 27.8 km/h), and relative humidity at mid-day was 49.0%.

Soil Cover, Botanical Composition, Caucasian Bluestem Frequency, and Forage Biomass

Soil cover and botanical composition were measured pre-treatment in 2019 and then annually thereafter in the months of June and July; within year, these measurements always preceded fire application. A modified step-point technique (Owensby, 1973) was used at 50 randomly selected points along transects at approximately 1-m intervals. Soil cover (i.e., bare ground, litter, or live plant matter) and the nearest plant species within a 180° arc in front of each point were recorded using the method described by Farney et al. (2017). If the nearest plant to the point was a grass, the nearest non-grass species was also recorded to better estimate forb and shrub composition.

Vegetation growth-form composition was summarized as described by Hickman et al. (2004). Of the warm-season grasses, basal plant cover composition was dominated by Caucasian bluestem (32.4%), little bluestem (13.6%), big bluestem (5.3%), and tall dropseed (5.3%). Extensive lists of the graminoids, forbs and shrubs identified while sampling are listed in appendix tables 1, 2, and 3, respectively. The common names, scientific names, and taxonomic authorities were those published by Haddock (2005).

Caucasian bluestem aerial frequency was measured at 1-m intervals along permanent transects in each fire-management plot (50, 30-cm^2 frames / transect). Within the frame, presence of Caucasian bluestem was noted (e.g., yes or no). In addition, visual obstruction within each frame was noted using the technique of Robel et al. (1970). Three 0.25-m^2 clipping frames were placed randomly along permanent transects in each plot and clipped to a height of 1 cm above

the soil surface. Litter and standing dead material were removed; the remaining material was weighed, dried in a forced-air oven (50ºC; 96 h), and re-weighed to estimate standing forage biomass (kg DM · ha^{-1}).

Statistical Analyses

Data were analyzed with SAS (PROC MIXED; SAS 9.4, SAS Inst. Inc., Cary, NC) using a mixed statistical model with fixed effects for treatment, year, and treatment x year and a random effect for plot within treatment. When protected by a significant *F*-test ($P \leq 0.05$), least-square means of treatment main effects or treatment × year effects were separated using the method of Least Significant Difference. Treatment means for the highest-order significant interaction were reported unless otherwise noted in the text. Select within-year treatment effects were discussed when relevant to interpreting our data.

Results and Discussion

Soil Cover

Litter cover on our experimental site decreased dramatically (Figure 1 - treatment × time; $P < 0.01$) the year following each prescribed fire application. Before the experiment began, litter had accumulated to a great extent (an average of 74.9% pre-treatment soil cover) because fire had been absent for an undetermined length of time. Biomass production of old-world bluestem plants, compared to native plants, resulted in significant amounts of litter deposition. By the end of our experiment (i.e., year 5) litter cover in non-burned plots was 88.7% of total soil cover. In contrast, litter cover averaged 64% in plots burned once and 36% in plots burned twice at that time.

The benefits of litter removal by fire may have been two-fold. Excessive litter accumulation on the soil surface likely reduced sunlight contact with bare soil and understory

plants, thus limiting photosynthetic activity; moreover, allelopathic compounds contained in old-world bluestem litter may have had negative effects on establishment and growth of native plant species (Greer et al., 2014; Heim et al., 2020).

Consistent with other research, decreased litter was associated with increased bare soil (Figure 2 – treatment × time; $P < 0.01$). Dry plant-deposited litter is readily consumed by fire and is a particularly good fuel source during the growing season when standing biomass contains elevated moisture levels. Re-deposition of litter is rapid when plant growth resumes following fire (Harmoney, 2020).

Pre-treatment differences were present in total basal plant cover (Figure 3 - treatment × time; $P < 0.01$) in 2019; however, no differences ($P \geq 0.10$) in basal plant cover between treatments were documented in any year following treatment. During the pre-treatment measurement, basal plant covers were atypically high for the region at 16.1% compared to 7% recorded at a nearby area (Harmoney, 2020). As the reintroduction of fire asserted its effect, basal plant cover decreased and ranged from 7.8% to 11.8% in all years following initial fire treatment.

Forage Biomass

Prairie parameters in non-equilibrium systems have been found to be driven primarily by abiotic factors, most notably precipitation (Vetter, 2005). Biomass is affected by fire but influenced even more by the weather each year (Vermeire and Russell, 2018). In our experiment, biomass varied from year to year and also decreased in response to each fire (Figure 4 - treatment × time; $P < 0.01$). Other experiments also documented decreased biomass in response to fire. This response is likely due to loss of litter and more exposed soil which may contribute to greater water evaporation and runoff (Harmoney, 2020; Lautenbach et al., 2021; Parker et al.,

2022). Before treatment, biomass did not differ between treatments and averaged 2,358 kg/ha. In 2021, when only one prescribed burn had taken place, biomass was recorded to be 3,424 kg/ha and 2,947 kg/ha in non-burned plots and burned plots, respectively. Reemts et al. (2019) suggested that a minimum standing forage biomass of 2,500 kg/ha was needed to create sufficient fire and heat intensity to suppress old-world bluestem presence. Forage biomass was slightly above this value at the time of the second fire. In addition, it was much less than the 7,600 kg/ha value recorded at the burn site of Reemts et al. (2019) that yielded the most significant reductions in old-world bluestem cover. Interestingly, individual plots at the beginning of our experiment with relatively large forage biomass levels had comparatively less biomass at the end of our measurements. In the final year of the experiment, biomass declined to values of 2,829 kg/ha, 2,552 kg/ha, and 1,390 kg/ha in non-burned plots, plots burned once, and plots burned twice, respectively. It should be noted that litter reductions associated with fire generally encourage grazing, which took place throughout the duration of the experiment and may have been associated with biomass reductions.

Reductions in biomass following application of prescribed fire corresponded closely to visual obstruction height measurements (Figure 5). Visual-obstruction height was also lower in burned plots compared to non-burned plots in each year following fire (treatment × time; $P \leq 0.01$); plots burned in 2019 and 2021 had lower ($P < 0.01$) visual-obstruction heights than non-burned plots and plots burned in 2019 only during the last two years of our experiment. Less overall biomass and shorter visual-obstruction height in plots burned twice compared with non-burned plots were linked temporally to changes in cover and frequency of Caucasian bluestem plants in 2022 (i.e., year 4).

Graminoid cover, composition, and species richness

Total basal cover composition of grasses was less (Figure 6 - treatment main effect; P = 0.05) in burned plots compared with non-burned plots in years 2 and 3; however, fire effects on total grass cover composition appeared less pronounced in years 4 and 5. Before the first fire treatment, total basal grass cover composition was 90.8%. This far exceeded the average measurement of 64.5% taken in nearby mixed-grass pastures that had been rested from fire for three or more years (Lautenbach et al., 2021). Plant diversity in prairies is typically promoted by a negative feedback system in which certain species attract specific pathogens which inhibit the germination of that same species in the immediate vicinity; however, pathogens targeting invasive species are not present in a novel environment. Positive feedback loops may be found in invaded areas as fewer obstacles stand in the way of rhizomes and seeds emanating from established invasive plants giving rise to new plants of the same species in the vicinity (Klironomos, 2002; Reinhart and Callaway, 2006). At the start of our experiment, a near-monoculture of Caucasian bluestem had formed, leaving little space for native forbs and grasses.

Effects of fire on total C_4 grass cover composition appeared to be temporally dependent (Figure 7 - treatment × time; P = 0.01). In the year following the first prescribed fire (i.e., year 2), total C_4 grass cover composition was less ($P < 0.01$) in burned plots than in non-burned plots; thereafter, total C_4 grass cover composition was similar between treatments. We speculated that the heat generated by burning the large amount litter (Figure1) in year 1 may have suppressed growth of all C_4 grasses the following year.

Basal cover composition of C_4 tall and shortgrasses were not affected ($P \geq 0.13$) by treatment and averaged 14.0% and 0.71%, respectively, over the course of our experiment (data not shown). Basal cover composition of C_4 midgrasses, of which old-world bluestems are a sub-

category, decreased (Figure 8 - treatment × time; $P = 0.04$) in plots burned twice compared to non-burned plots in years 3, 4, and 5 of the experiment. Plots burned a single time had C_4 midgrass cover composition similar to non-burned plots throughout the experiment. Notably, C_4 midgrass basal cover composition appeared to increase in all treatments from year 1 (approximately 10 to 20% of total basal cover) to year 5 (approximately 45 to 60% of total basal cover).

During pre-treatment measurements, basal cover composition of Caucasian bluestem was not different ($P > 0.25$) between treatments and averaged 32.4% (data not shown). Following pre-treatment measurements, Caucasian bluestem basal cover composition began to trend upward (Figure 9 – treatment x time; $P < 0.01$) in non-burned plots, such that it was always numerically greater in non-burned plots compared with plots burned twice, but not in plots burned once. Aerial frequency of Caucasian bluestem was not different (Figure 10 – treatment main effect; $P = 0.25$) between treatments; however, in the year following the second fire (i.e., year 4), average frequency of Caucasian bluestem was approximately two standard-error values less in plots burned twice than in non-burned plots and plots burned once.

Relationships with arbuscular mycorrhizal fungi (AMF) are essential for most warm-season grasses, including Caucasian bluestem (Hetrick et al., 1990; Wilson and Hartnett, 1998). Arbuscular mycorrhizal fungi can survive in the soil for some time without a host plant (Zubek et al., 2013), and their presence favors establishment of C_4 grasses over C_3 grasses and forbs (Hetrick et al., 1990; Hartnett and Wilson, 1999; Wilson et al., 2011); therefore, even if basal cover of C_4 grasses or a subgroup of C_4 grasses decreases with fire, AMF-facilitated reestablishment may easily and quickly occur. This may be the case with Caucasian bluestem, as its cover and frequency declined in response to fire, but treatments did not differ in cover or

frequency at the end of the experiment. The lowest Caucasian bluestem frequency in any treatment group throughout the duration of the experiment was 49% in plots burned twice during year 4 (i.e., the year following second fire treatment). Because the plant spreads rapidly outward from its inhabited area (Gabbard and Fowler, 2007; Reemts et al., 2021), it would not take long for repopulation to occur with such high prevalence; therefore, we interpreted our data on Caucasian bluestem basal cover and frequency to indicate that repeated fire had subtle suppressive effects on the plant.

We characterized cover responses to fire among C_4 native grasses as largely neutral, similar to reports by Simmons et al. (2007) and Reemts et al. (2019), in spite of perceived trends in Caucasian bluestem abatement. We conjectured three possible reasons for this response. First, allelopathic compounds remaining in the soil subsequent to diminished old-world bluestem cover may have prevented native-species seed germination (Schmidt et al., 2008; Wilson et al., 2012; Greer et al., 2014). Second, the well-established invasive plant would have been the primary contributor to the seedbank prior to our experiment, whereas native seed concentrations correspondingly declined. Native plants may require a relatively long interval to reestablish seed dominance in the soil of an invaded area. Third, vegetative reproduction of native plants may have been suppressed if Caucasian bluestem altered the composition of soil biota, particularly the AMF upon which native plants rely.

The latter of these speculations may be the most likely. Reemts et al. (2019) reported that native grasses failed to reestablish dominance 12 years following targeted removal of yellow bluestem. Throughout their experiment, yellow bluestem presence remained low subsequent to fire treatment. Allelopathic chemicals associated with the initial, heavy concentrations of yellow bluestem should not have had great suppressive effects on native-seed germination after a certain

point. Post-treatment inoculation of invaded areas with native-soil biota may be necessary for regeneration of desirable plant species to take place (Duell et al., 2023).

The C_3 grasses showed temporal increases (Figure 11 - treatment × time; $P < 0.01$) in basal cover composition in years following fire application (i.e., years 2 and 4); however, plots had no differences ($P \geq 0.32$) in the final year of the experiment. Short-term changes were likely due to removal of litter and increased soil exposure. No effects ($P \geq 0.27$) of treatment was present on cover of introduced C_3 grass species, such as Kentucky bluegrass or tall fescue (data not shown).

Studies recording effects of dormant season fire have documented returns to pre-fire levels of visual obstruction, litter depth, bare ground, and grass cover within two years of a fire event (Parker et al., 2022; Harmoney, 2020). On the other hand, dry litter, high temperatures, and elevated biomass contribute to more extreme fires in the summer that delays recovery of these variables (Vermeire and Russell, 2018). This proved to be the case in our experiment and may have been amplified further due to the vast buildup of litter before the first fire.

Grass species richness increased (Figure 12 - treatment main effect; $P = 0.01$) in burned plots compared with non-burned plots, even though overall total basal cover composition of C_4 grasses decreased (Figure7). New grass species over the course of the experiment primarily included the native warm-season perennial grasses blue grama, fall witchgrass, and sand dropseed, as well as the introduced cool-season perennial smooth bromegrass. Increasing richness of C_4 grasses shows promise, as it may suggest enough suppression of the dominating Caucasian bluestem to allow other native species to establish and connect with the mycorrhizal network. Furthermore, if Caucasian bluestem altered the composition of AMF in the soil, increasing native grass species richness suggests some native plant species may still be capable

of utilizing the mycorrhizal network in its altered state. The presence of new grass species may also suggest the seedbank is not fully replete of native grass seeds, a circumstance that may occur with long-term invasion (Holmes and Cowling, 1997; Fourie, 2008).

Forb cover, composition, and species richness

Before fire, total basal cover composition of forbs was 8.9%. In accordance with atypically high grass basal cover composition, forb basal cover composition was unusually low, as nearby mixed-grass prairie pastures with three or more years of fire exclusion averaged 18.5% basal forb cover composition (Lautenbach et al., 2021). Total basal cover composition of forbs was greater (Figure 13 - treatment main effect; $P = 0.03$) in plots burned twice compared with non-burned plots and was estimated to average 14.1% in plots burned twice and 8.7% in non-burned plots. Basal cover composition of perennial forbs and native forbs followed almost identical trends (Figures 14 and 15 - treatment main effect; $P \leq 0.06$). Annual forb cover composition was greater (Figure 16 - treatment main effect; $P = 0.02$) in both burn treatments compared with the non-burned treatment. The first fire increased introduced forb cover composition (Figure 17 - treatment × time; $P = 0.02$) and nectar-producing forb cover composition (Figure 18 - treatment × time; $P < 0.01$), such that cover was greater in burned plots compared with non-burned plots in year 2. Treatment differences disappeared after that point. Leguminous forb cover composition did not differ ($P = 0.80$; data not shown) between treatments. Along with increased forb cover composition in burned plots during year 2 came an increase in forb species richness (Figure 19 - treatment × time; $P < 0.01$); this also declined to pre-treatment levels and did not differ meaningfully for the remainder of the experiment. Forb species that were not present before the first fire but appeared at some point during the experiment included ashy goldenrod, blackeyed susan, buffalobur, common groundcherry,

common milkweed, common ragweed, fringeleaf ruellia, glandular croton, grooved flax, hairy aster, hemp dogbane, Johnny-jump-up, low milkvetch, plains pricklypear, prairie pepper-grass, prairie trefoil, prickly lettuce, purple prairieclover, rough false pennyroyal , snow-on-the-mountain, spotted spurge, sweetclovers, tall goldenrod, Texas croton, Venus looking glass, violet wood sorrel, and whorled polygala. Several forb species, namely American burnweed, corn speedwell, daisy fleabane, giant ragweed, green milkweed, long-bearded hawkweed, pitcher sage, showy evening primrose, sulphur cinquefoil, violet lespedeza, and Virgnia groundcherry, were present before initial treatment and proceeded to decrease over the course of the experiment until they were no longer present by the end. Few common factors (i.e., leguminous or not, nectar-producing or not, introduced or native) appeared among species that increased or decreased in richness; however, in accordance with the substantial increase in annual forbs in year 2, a comparatively greater proportion of forbs increasing in richness were annual or biennial compared to this ratio in total forb species recorded in the experiment (i.e., 51.9% vs. 33.8%).

Litter accumulation has been documented to decrease forb species abundance and richness. This has been attributed primarily to shading, but other factors, such as mechanical resistance and harboring of pathogens, may further contribute. Removal of litter with fire or other methods has immediate positive effects on forb populations, especially annual forbs which have no previous establishment but rather rely on the suitability of each year's conditions to grow (Foster and Gross, 1998; Tix and Charvat, 2005; Heim et al., 2020). Old-world bluestem invasion decreases the diversity, evenness, and cover of native species, including forbs (Reed et al., 2005; Gabbard and Fowler, 2007; Robertson and Hickman, 2012). Therefore, less C_4 grass basal cover, more exposed soil, and a slight weakening of Caucasian bluestem presence may have combined to allow the increase in forb cover and richness in year 2 of our experiment.

Similar to our experiment, other researchers also documented substantial increases followed by subsequent decreases in forb cover and diversity following summer fire. They attributed this observation to fire suppression of dominant grasses, which allowed the more shallow-rooted forbs to germinate and grow (Twidwell et al., 2012; Reemts et al., 2019).

The greater increase in forb cover following the first fire compared to the second may have been due to direct effects of fire-induced germination. It has been demonstrated that smoke and heat can induce germination by scarifying dormant seeds of some forb species (Auld and Bradstock, 1996; Tix and Charvat, 2005; Ren and Bai, 2016; Hodges et al., 2019). Because no fire was present for a long period of time before our experiment took place, substantial buildup of forb seeds may have occurred in the seedbank.

All forb groups had the highest cover numerically in year 2 of the experiment. Declining levels in the following years can be attributed to redeposition of litter and increased basal cover of C_4 grasses, both of which would allow less sunlight to permeate to the soil.

Shrub cover, composition, and species richness

Total basal cover composition of shrubs decreased (Figure 20 - treatment main effect; $P < 0.01$) in both burn treatments compared with the non-burned treatment. The response was immediate and lasted throughout the experiment. Average cover composition estimates in non-burned plots, plots burned once, and plots burned twice were 0.58%, 0.27%, and 0.10%, respectively. Shrub species richness declined (Figure 21 - treatment main effect; $P < 0.04$) in burned plots compared to non-burned controls. The two shrub species recorded in our study site, buckbrush and Chickasaw plum, were both increaser shrubs (i.e., those that proliferate in the presence of disturbances like grazing).

Total species richness

Total combined richness of grass, forb, and shrub species increased (Figure 22 - treatment × time; $P = 0.01$) with burning, most notably in year 2 in correspondence with the increase in forb species richness ($P = 0.62$). Increased total species richness in burned plots suggests improved species diversity which is correlated with prairie health.

Implications

Late-summer fire, especially when repeated over time, appeared to suppress the dominance of invasive Caucasian bluestem in mixed-grass rangeland in Ellsworth County, Kansas. The strongest evidence for this conjecture included temporal decreases in Caucasian bluestem basal cover composition and frequency, greater forb cover composition, and increased species richness among native grasses. Timing of fire in our experiment was similar to that described by Reemts et al. (2019), who reported clear reductions in yellow bluestem dominance. Results of our experiment was not as dramatic as those reported in that study. Though phenotypically very similar, yellow bluestem and Caucasian bluestem may have subtle differences in physical structure or growth characteristics which allow the latter to be more resilient to fire.

Heat intensity resulting from fire is another factor that may have contributed to differences between these experiments. Reemts et al. (2019) reported greater fuel accumulations during the late growing season than we observed. Frequent grazing by domestic herbivores on our research site likely caused this condition. Increased dryness of fuel would also contribute to more intense fire temperatures (Simmons et al., 2007; Twidwell et al., 2012). In the Reemts et al. (2019) experiment, a total of 35 mm of rain fell in the 20 days before and after their most successful fire, whereas 302 mm fell in the 20 days before and after their least successful fire. In

our experiment, 24 mm precipitation occurred in the 20 days preceding the first fire and 89.2 mm in the 20 days after. Total precipitation 20 days before and after the second fire was 22.6 mm and 21.1 mm, respectively. In addition, ambient temperature was 29.3°C the day of our first fire and 39.9°C the day of our second fire. While precipitation events immediately before and after our first fire treatment and ambient heat may have had some depressive effects on fuel dryness and fire intensity, precipitation totals around our second fire were similar to those identified by Reemts et al. (2019) as favorable to fire intensity.

Factors contributing most to heat intensity would have been greater litter concentration in the case of our first fire and greater biomass, less precipitation, and greater ambient temperature in the case of our second fire. In future research, it would be interesting to combine these factors with rest from grazing to produce hotter, more intense fires.

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Figures

Figure 1. Effects of late-summer prescribed fire on litter cover (% of total ground cover) in Caucasian bluestem (*Bothriochloa bladhii*)-infested, mixed-grass prairie in the Kansas Smoky Hills. Prescribed fires were applied on 14 August 2019 (single-burn treatment) or 14 August 2019 and 11 August 2021 (double-burn treatment). These treatments were compared with a non-burned control. Measurements were conducted in July from 2019 to 2023 (Years 1 to 5, respectively). No differences ($P > 0.19$) were detected between treatments before fires were applied (Year 1; 2019). Late-summer prescribed fire resulted in less (treatment × time - $P < 0.01$) litter cover in burned treatments compared with non-burned controls in each year following initial treatment (Years 2 to 5). In each year following the second fire treatment (Years 4 and 5), litter cover was less in the double-burn treatment than in the single-burn treatment. Treatments differ ($P \leq 0.05$) from one another when standard-errors bars (multiple-means comparison SEM = 6.58) do not overlap.

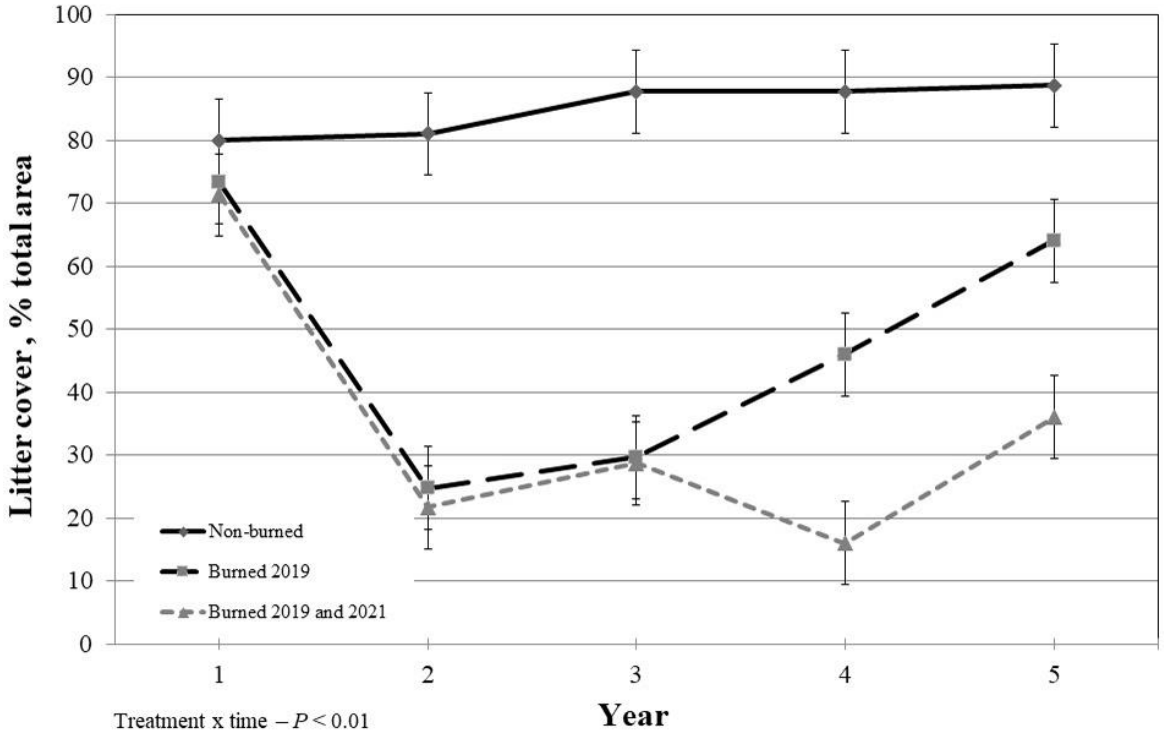


Figure 2. Effects of late-summer prescribed fire on bare soil (% of total ground cover) in Caucasian bluestem (*Bothriochloa bladhii*)-infested, mixed-grass prairie in the Kansas Smoky Hills. Prescribed fires were applied on 14 August 2019 (single-burn treatment) or 14 August 2019 and 11 August 2021 (double-burn treatment). These treatments were compared with a non-burned control. Measurements were conducted in July from 2019 to 2023 (Years 1 to 5, respectively). No differences ($P > 0.73$) were detected between treatments before fires were applied (Year 1; 2019). Late-summer prescribed fire resulted in more (treatment × time - $P < 0.01$) exposed soil in burned treatments compared with non-burned controls in each year following initial treatment (Years 2 to 5). In each year following the second fire treatment (Years 4 and 5), bare soil was greater in the double-burn treatment than in the single-burn treatment. Treatments differ ($P \leq 0.05$) from one another when standard-errors bars (multiple-means comparison SEM = 6.60) do not overlap.

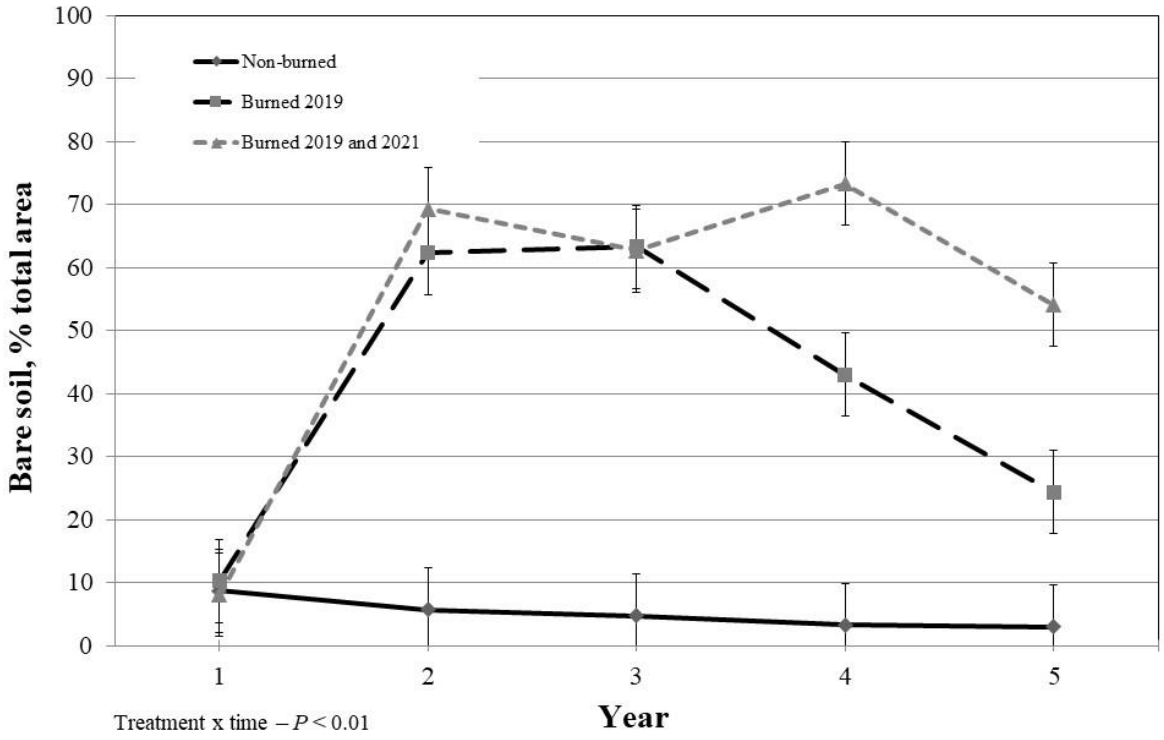


Figure 3. Effects of late-summer prescribed fire on basal-plant cover (% of total ground cover) in Caucasian bluestem (*Bothriochloa bladhii*)-infested, mixed-grass prairie in the Kansas Smoky Hills. Prescribed fires were applied on 14 August 2019 (single-burn treatment) or 14 August 2019 and 11 August 2021 (double-burn treatment). These treatments were compared with a non-burned control. Measurements were conducted in July from 2019 to 2023 (Years 1 to 5, respectively). Prior to fire application in Year 1, basal-plant cover was different ($P \leq 0.01$) between treatments. Thereafter, treatments did not differ ($P \geq 0.10$) in basal-plant cover. Treatments differ ($P \leq 0.05$) from one another when standard-errors bars (multiple-means comparison SEM = 1.99) do not overlap.

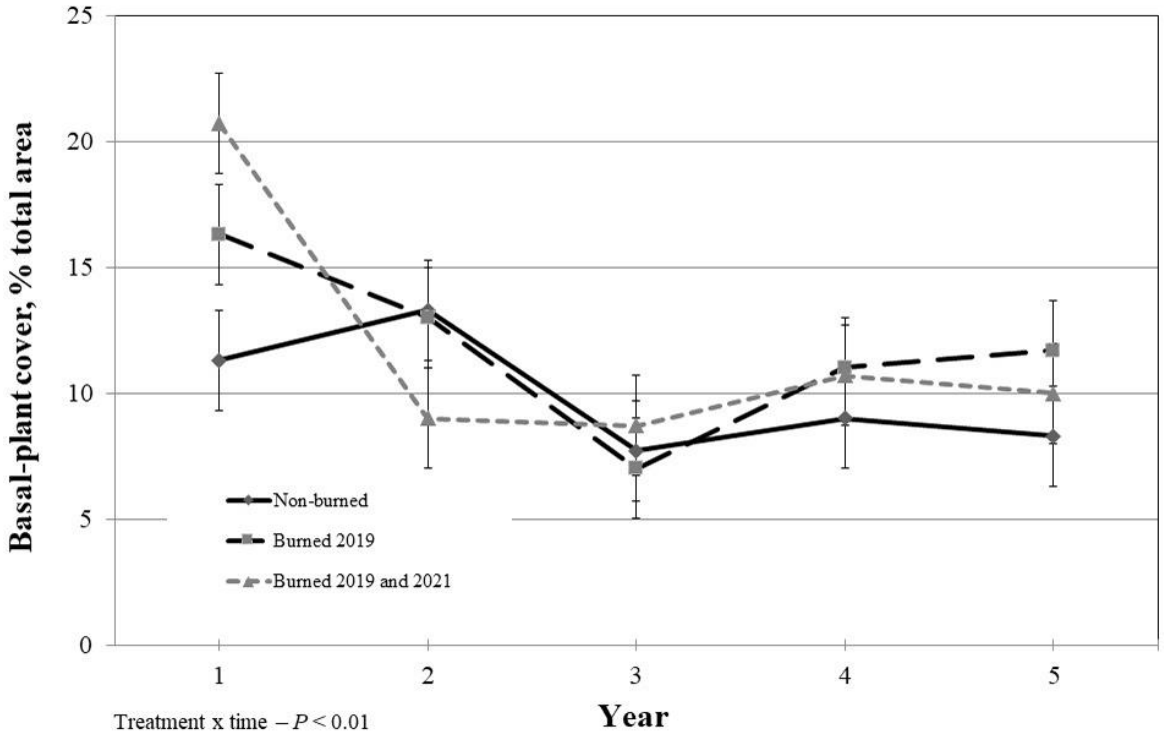


Figure 4. Effects of late-summer prescribed fire on forage biomass (kg DM/ha) in Caucasian bluestem (*Bothriochloa bladhii*)-infested, mixed-grass prairie in the Kansas Smoky Hills. Prescribed fires were applied on 14 August 2019 (single-burn treatment) or 14 August 2019 and 11 August 2021 (double-burn treatment). These treatments were compared with a non-burned control. Measurements were conducted in July from 2019 to 2023 (Years 1 to 5, respectively). No differences ($P > 0.13$) were detected between treatments before fires were applied (Year 1; 2019). Late-summer prescribed fire resulted in less (treatment × time - $P < 0.01$) forage biomass in burned treatments in each year following fire treatment (i.e., Years 2 and 4) compared with non-burned controls. Treatments differ ($P \leq 0.05$) from one another when standard-errors bars (multiple-means comparison SEM = 340.10) do not overlap.

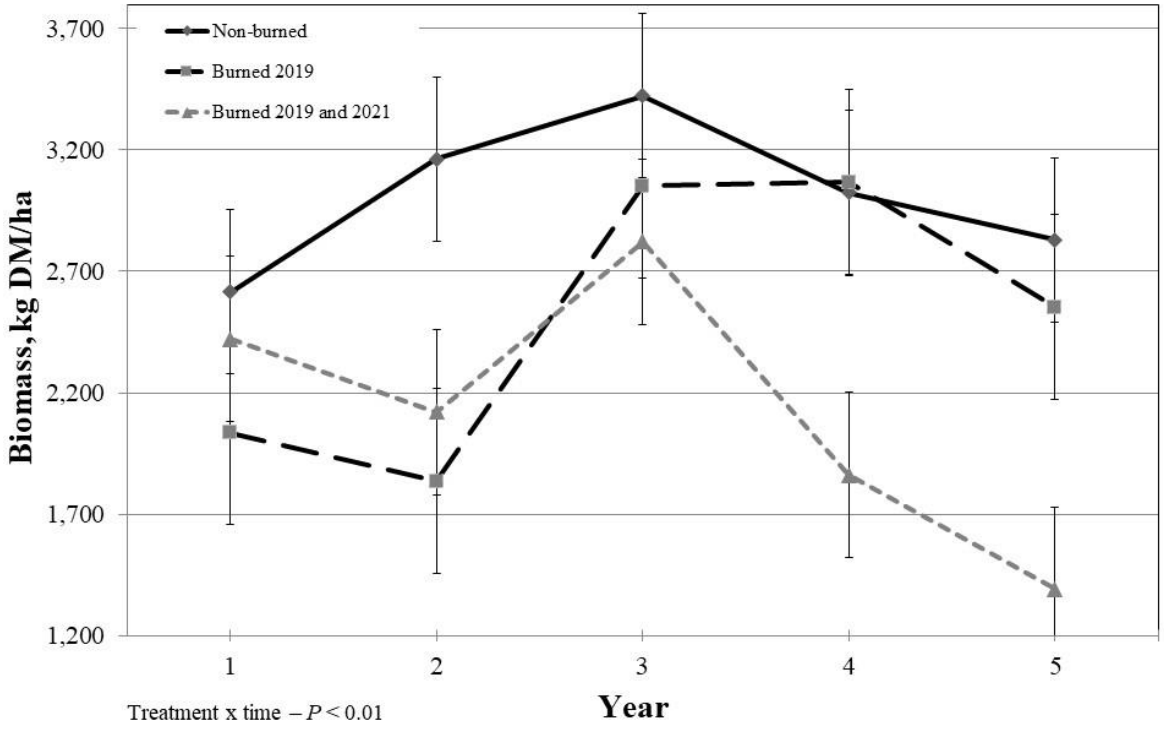


Figure 5. Effects of late-summer prescribed fire on forage visual-obstruction height (dm from the soil surface) in Caucasian bluestem (*Bothriochloa bladhii*)-infested, mixed-grass prairie in the Kansas Smoky Hills. Prescribed fires were applied on 14 August 2019 (single-burn treatment) or 14 August 2019 and 11 August 2021 (double-burn treatment). These treatments were compared with a non-burned control. Measurements were conducted in July from 2019 to 2023 (Years 1 to 5, respectively). No differences ($P > 0.41$) were detected between treatments before fires were applied (Year 1; 2019). Late-summer prescribed fire resulted in shorter (treatment × time - $P < 0.01$) forage visual-obstruction heights in burned treatments compared with non-burned controls in each year following initial treatment (Years 2 to 5; measure using a Robel pole). In each year following the second fire treatment (Years 4 and 5), forage visual-obstruction height was less in the double-burn treatment than in the single-burn treatment. Treatments differ ($P \leq 0.05$) from one another when standard-errors bars (multiple-means comparison SEM = 0.13) do not overlap.

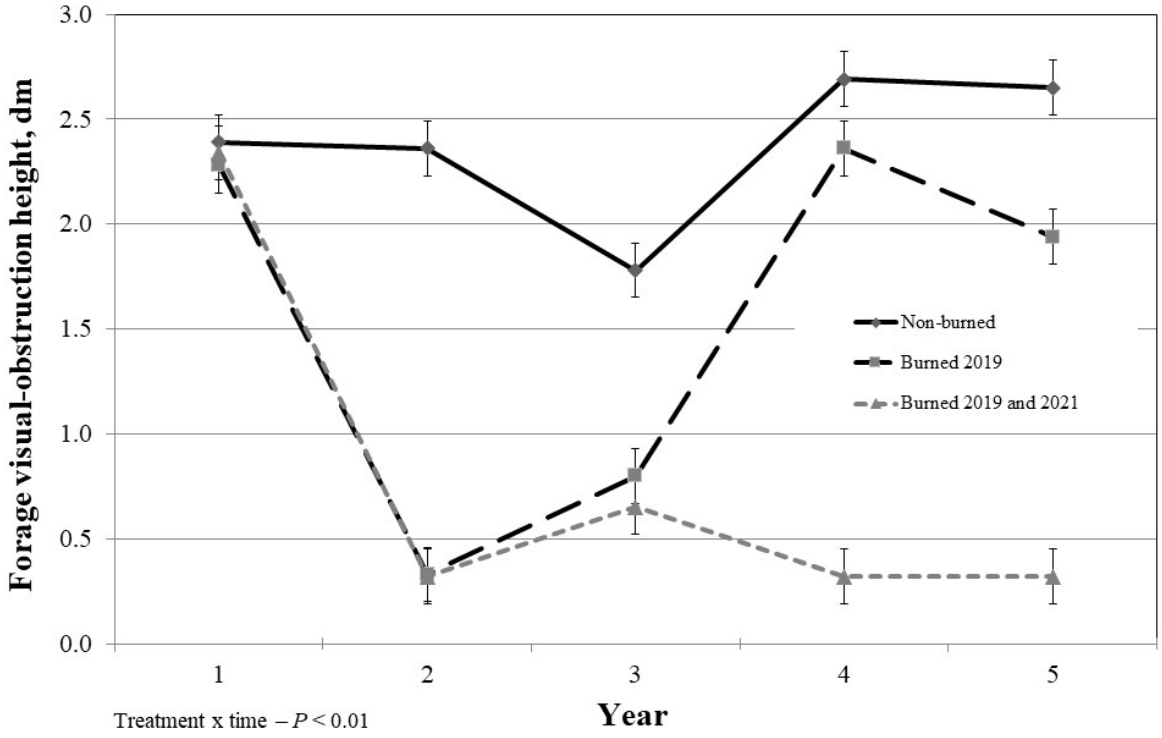


Figure 6. Effects of late-summer prescribed fire on total grass basal cover composition (% of total basal ground cover) in Caucasian bluestem (*Bothriochloa bladhii*)-infested, mixed-grass prairie in the Kansas Smoky Hills. Prescribed fires were applied on 14 August 2019 (single-burn treatment) or 14 August 2019 and 11 August 2021 (double-burn treatment). These treatments were compared with a non-burned control. Measurements were conducted in July from 2019 to 2023 (Years 1 to 5, respectively). No differences ($P > 0.54$) were detected between treatments before fires were applied (Year 1; 2019). Late-summer prescribed fires resulted in less (treatment main effect - $P = 0.05$) total grass cover in the double-burn treatment compared with non-burned controls (multiple-means comparison SEM = 1.85).

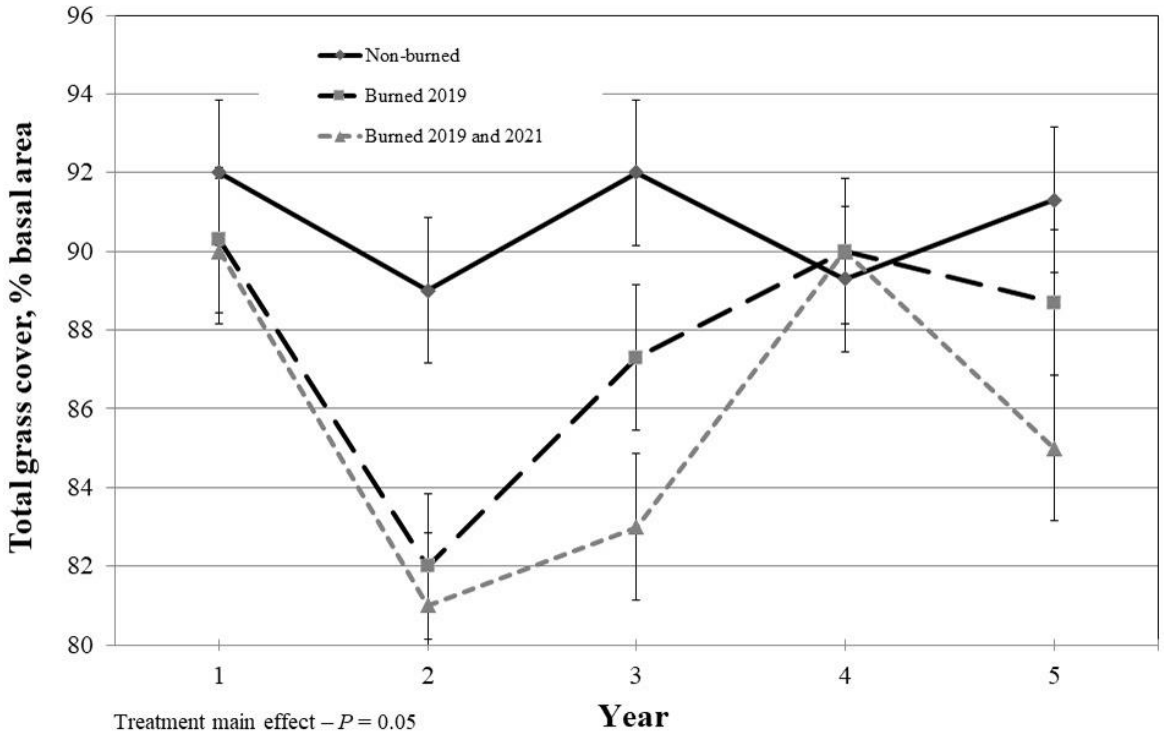


Figure 7. Effects of late-summer prescribed fire on total C_4-grass cover composition (% of total basal ground cover) in Caucasian bluestem (*Bothriochloa bladhii*)-infested, mixed-grass prairie in the Kansas Smoky Hills. Prescribed fires were applied on 14 August 2019 (single-burn treatment) or 14 August 2019 and 11 August 2021 (double-burn treatment). These treatments were compared with a non-burned control. Measurements were conducted in July from 2019 to 2023 (Years 1 to 5, respectively). No differences ($P > 0.61$) were detected between treatments before fires were applied (Year 1; 2019). Late-summer prescribed fire resulted in less (treatment × time - $P < 0.01$) total C_4-grass basal cover in burned treatments compared with non-burned controls. Following the first prescribed fire (i.e., year 2), total C_4 grass cover was less ($P < 0.01$) in burned plots than in non-burned plots; thereafter, total C_4 grass cover was similar between treatments. Treatments differ ($P \leq 0.05$) from one another when standard-errors bars (multiple-means comparison SEM = 6.56) do not overlap.

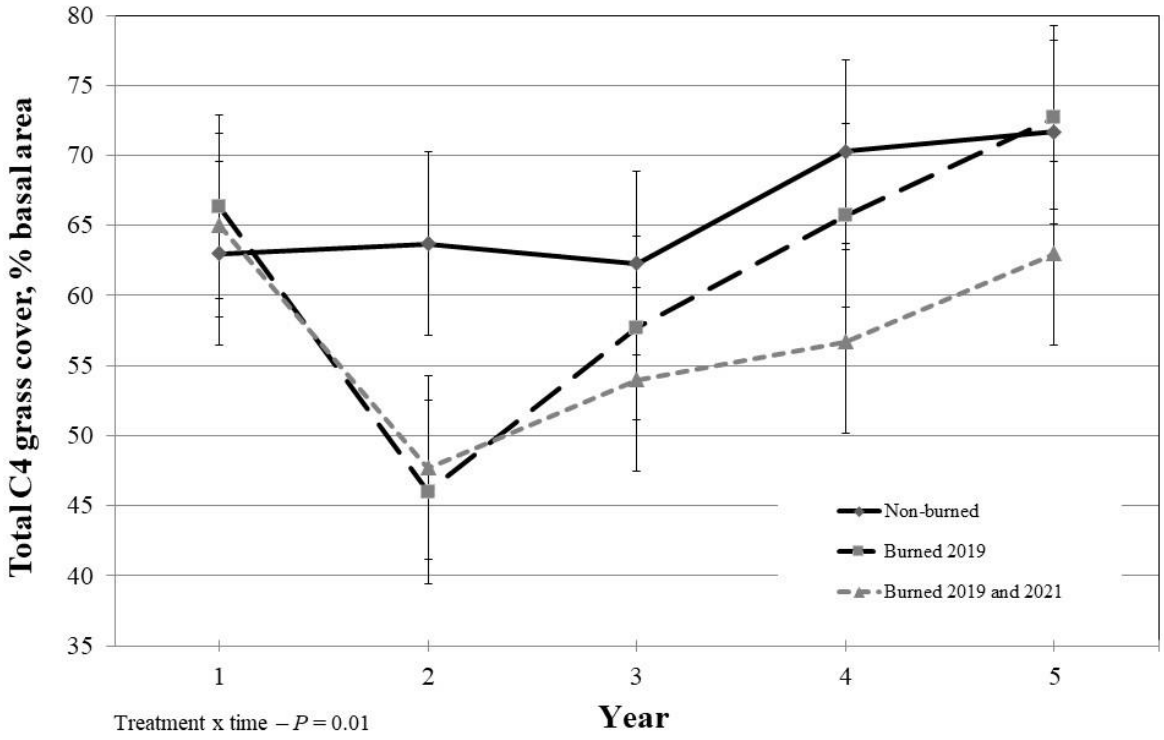


Figure 8. Effects of late-summer prescribed fire on C_4-midgrass cover composition (% of total basal ground cover) in Caucasian bluestem (*Bothriochloa bladhii*)-infested, mixed-grass prairie in the Kansas Smoky Hills. Prescribed fires were applied on 14 August 2019 (single-burn treatment) or 14 August 2019 and 11 August 2021 (double-burn treatment). These treatments were compared with a non-burned control. Measurements were conducted in July from 2019 to 2023 (Years 1 to 5, respectively). No differences ($P > 0.19$) were detected between treatments before fires were applied (Year 1; 2019). Basal cover of C_4 midgrasses was less (Figure 8 - treatment × time; $P = 0.04$) in the double-burn treatment compared with the non-burned treatment in Years 3, 4, and 5 of the experiment. Treatments differ ($P \leq 0.05$) from one another when standard-errors bars (multiple-means comparison SEM = 6.16) do not overlap.

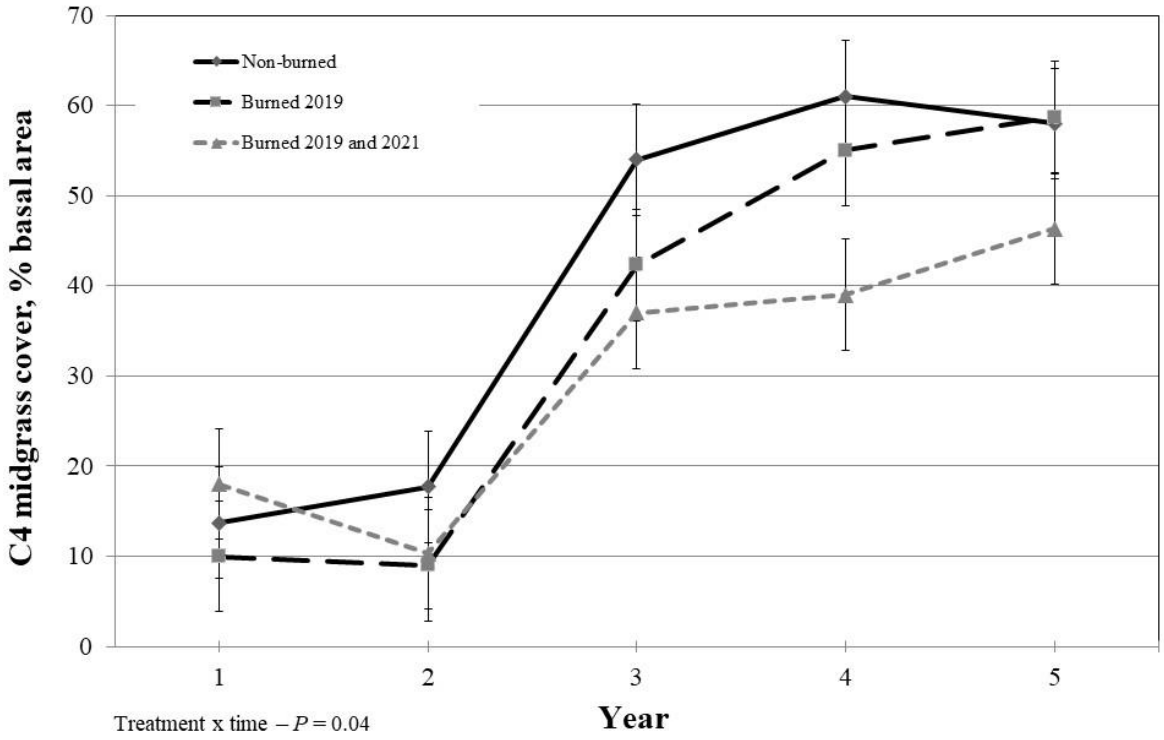


Figure 9. Effects of late-summer prescribed fire on Caucasian bluestem basal cover composition (% of total basal ground cover) in Caucasian bluestem (*Bothriochloa bladhii*)-infested, mixed-grass prairie in the Kansas Smoky Hills. Prescribed fires were applied on 14 August 2019 (single-burn treatment) or 14 August 2019 and 11 August 2021 (double-burn treatment). These treatments were compared with a non-burned control. Measurements were conducted in July from 2019 to 2023 (Years 1 to 5, respectively). No differences ($P > 0.25$) were detected between treatments before fires were applied (Year 1; 2019). Late-summer prescribed fire resulted in temporally decreased (treatment × time - $P < 0.01$) Caucasian bluestem basal cover in the double-burn treatment compared with non-burned controls in Year 4. Treatments differ ($P \leq 0.05$) from one another when standard-errors bars (multiple-means comparison SEM = 9.52) do not overlap.

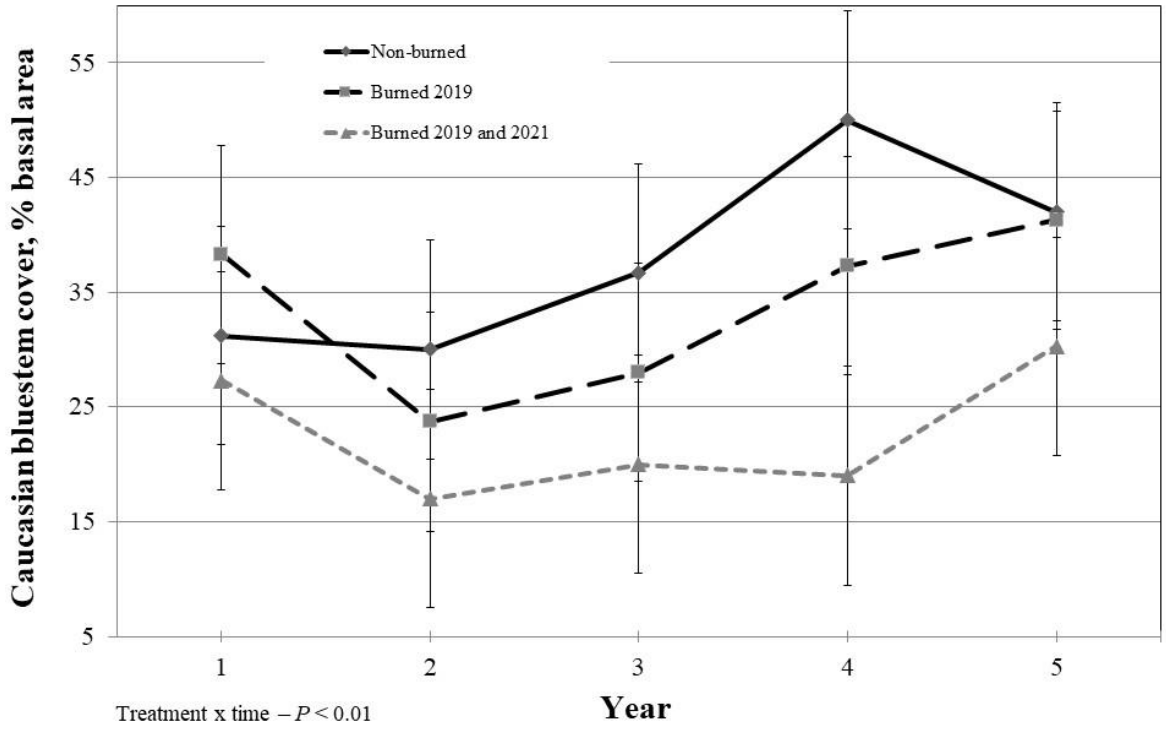


Figure 10. Effects of late-summer prescribed fire on Caucasian bluestem frequency (% presence in 30 × 30-cm plots) in Caucasian bluestem (*Bothriochloa bladhii*)-infested, mixed-grass prairie in the Kansas Smoky Hills. Prescribed fires were applied on 14 August 2019 (single-burn treatment) or 14 August 2019 and 11 August 2021 (double-burn treatment). These treatments were compared with a non-burned control. Measurements were conducted in July from 2019 to 2023 (Years 1 to 5, respectively). No differences ($P > 0.30$) were detected between treatments before fires were applied (Year 1; 2019). Late-summer prescribed fire had no consistent effects (treatment main effect – $P = 0.25$) on Caucasian bluestem frequency (multiple-means comparison SEM = 9.83).

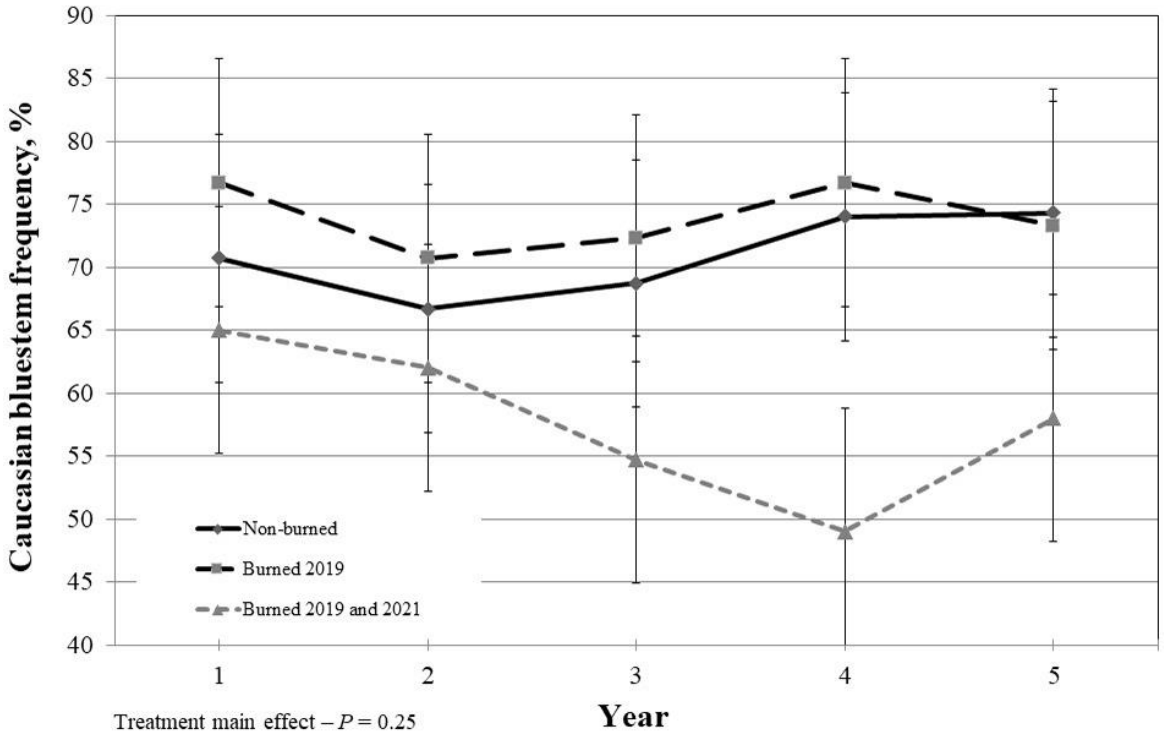


Figure 11. Effects of late-summer prescribed fire on C_3-grass basal cover composition (% of total basal ground cover) in Caucasian bluestem (*Bothriochloa bladhii*)-infested, mixed-grass prairie in the Kansas Smoky Hills. Prescribed fires were applied on 14 August 2019 (single-burn treatment) or 14 August 2019 and 11 August 2021 (double-burn treatment). These treatments were compared with a non-burned control. Measurements were conducted in July from 2019 to 2023 (Years 1 to 5, respectively). No differences ($P > 0.41$) were detected between treatments before fires were applied (Year 1; 2019). Late-summer prescribed fire temporally increased (treatment × time - $P < 0.01$) C_3-grass basal cover following application of fire compared with non-burned controls; however, there were no differences ($P > 0.32$) between treatments in Year 5. Treatments differ ($P \leq 0.05$) from one another when standard-errors bars (multiple-means comparison SEM = 9.52) do not overlap.

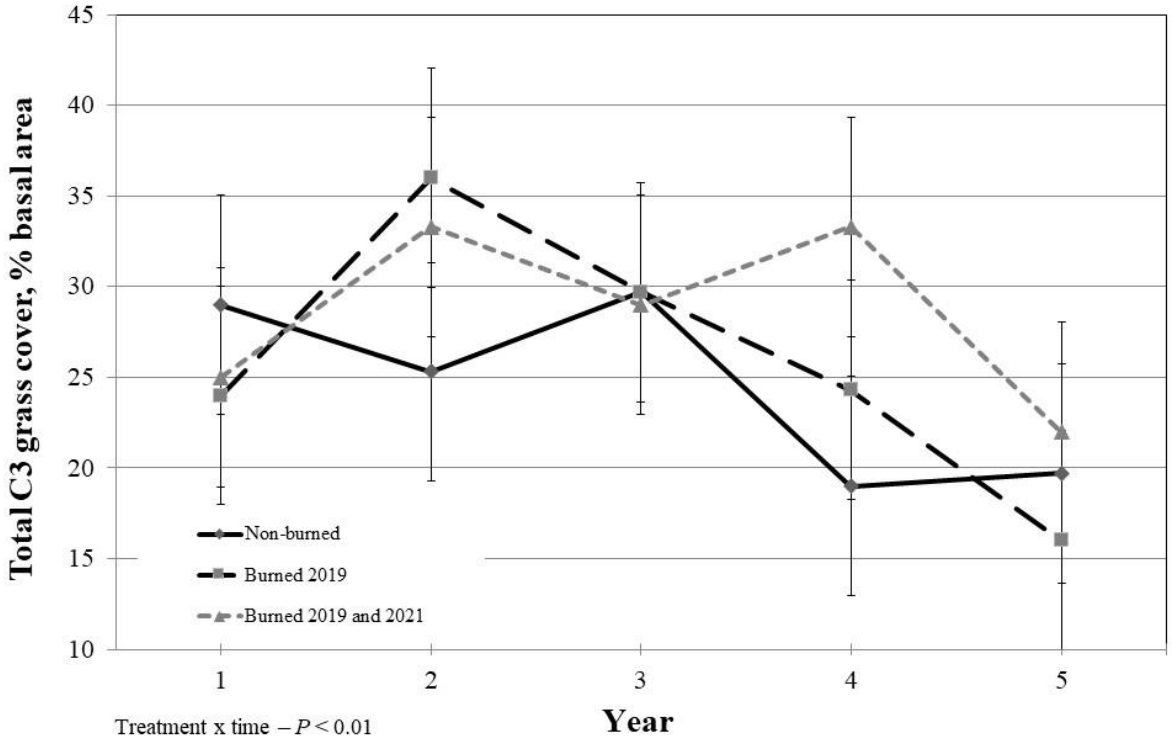


Figure 12. Effects of late-summer prescribed fire on grass-species richness (number of species present) in Caucasian bluestem (*Bothriochloa bladhii*)-infested, mixed-grass prairie in the Kansas Smoky Hills. Prescribed fires were applied on 14 August 2019 (single-burn treatment) or 14 August 2019 and 11 August 2021 (double-burn treatment). These treatments were compared with a non-burned control. Measurements were conducted in July from 2019 to 2023 (Years 1 to 5, respectively). No differences ($P > 0.67$) were detected between treatments before fires were applied (Year 1; 2019). Late-summer prescribed fire resulted in greater (treatment main effect - $P < 0.01$) grass-species richness in burned treatments compared with non-burned controls. Treatments differ ($P \leq 0.05$) from one another when standard-errors bars (multiple-means comparison SEM = 0.46) do not overlap.

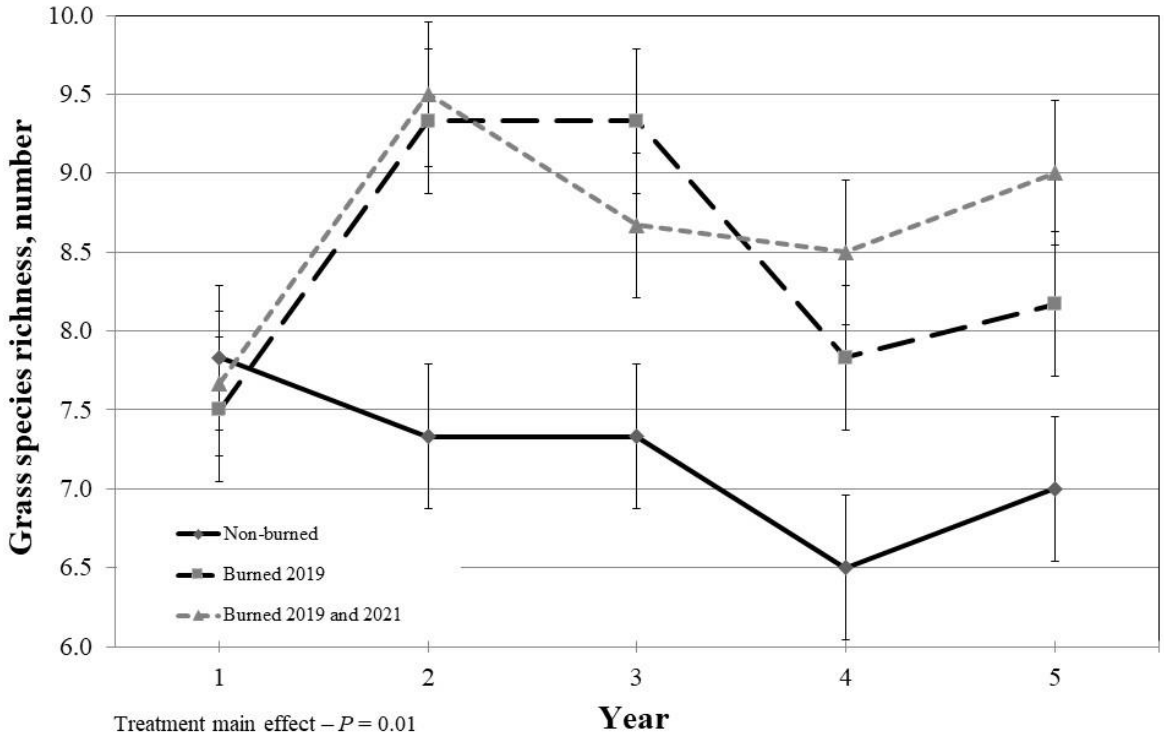


Figure 13. Effects of late-summer prescribed fire on forb basal cover composition (% of total basal ground cover) in Caucasian bluestem (*Bothriochloa bladhii*)-infested, mixed-grass prairie in the Kansas Smoky Hills. Prescribed fires were applied on 14 August 2019 (single-burn treatment) or 14 August 2019 and 11 August 2021 (double-burn treatment). These treatments were compared with a non-burned control. Measurements were conducted in July from 2019 to 2023 (Years 1 to 5, respectively). No differences ($P > 0.52$) were detected between treatments before fires were applied (Year 1; 2019). Late-summer prescribed fire resulted in increased (treatment main effect - $P = 0.03$) forb basal cover in burn treatments compared with non-burned controls. Treatments differ ($P \leq 0.05$) from one another when standard-errors bars (multiple-means comparison SEM = 1.83) do not overlap.

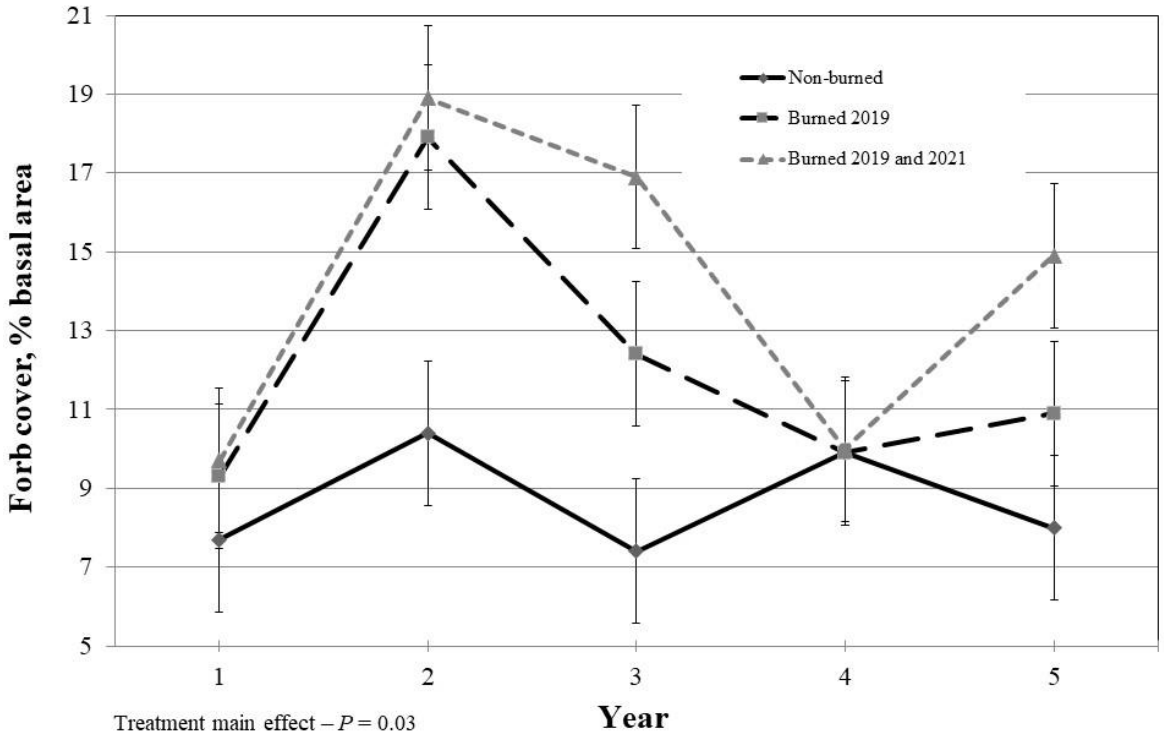


Figure 14. Effects of late-summer prescribed fire on perennial forb basal cover composition (% of total basal ground cover) in Caucasian bluestem (*Bothriochloa bladhii*)-infested, mixed-grass prairie in the Kansas Smoky Hills. Prescribed fires were applied on 14 August 2019 (single-burn treatment) or 14 August 2019 and 11 August 2021 (double-burn treatment). These treatments were compared with a non-burned control. Measurements were conducted in July from 2019 to 2023 (Years 1 to 5, respectively). No differences ($P > 0.47$) were detected between treatments before fires were applied (Year 1; 2019). Late-summer prescribed fire tended to increase (treatment main effect - $P = 0.06$) perennial forb basal cover in burn treatments compared with non-burned controls. Treatments differ ($P \leq 0.05$) from one another when standard-errors bars (multiple-means comparison SEM = 1.82) do not overlap.

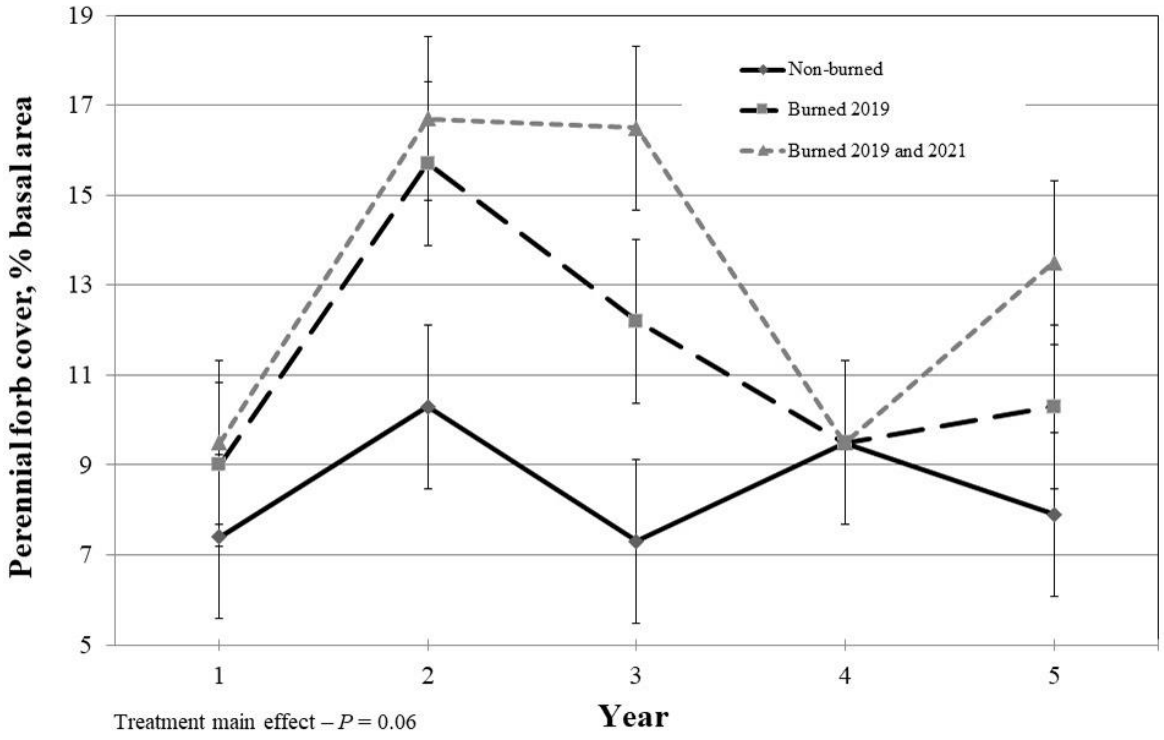


Figure 15. Effects of late-summer prescribed fire on native forb basal cover composition (% of total basal ground cover) in Caucasian bluestem (*Bothriochloa bladhii*)-infested, mixed-grass prairie in the Kansas Smoky Hills. Prescribed fires were applied on 14 August 2019 (single-burn treatment) or 14 August 2019 and 11 August 2021 (double-burn treatment). These treatments were compared with a non-burned control. Measurements were conducted in July from 2019 to 2023 (Years 1 to 5, respectively). No differences ($P > 0.50$) were detected between treatments before fires were applied (Year 1; 2019). Late-summer prescribed fire tended to increase (treatment main effect - $P = 0.06$) native forb basal cover in burn treatments compared with non-burned controls. Treatments differ ($P \leq 0.05$) from one another when standard-errors bars (multiple-means comparison SEM = 1.82) do not overlap.

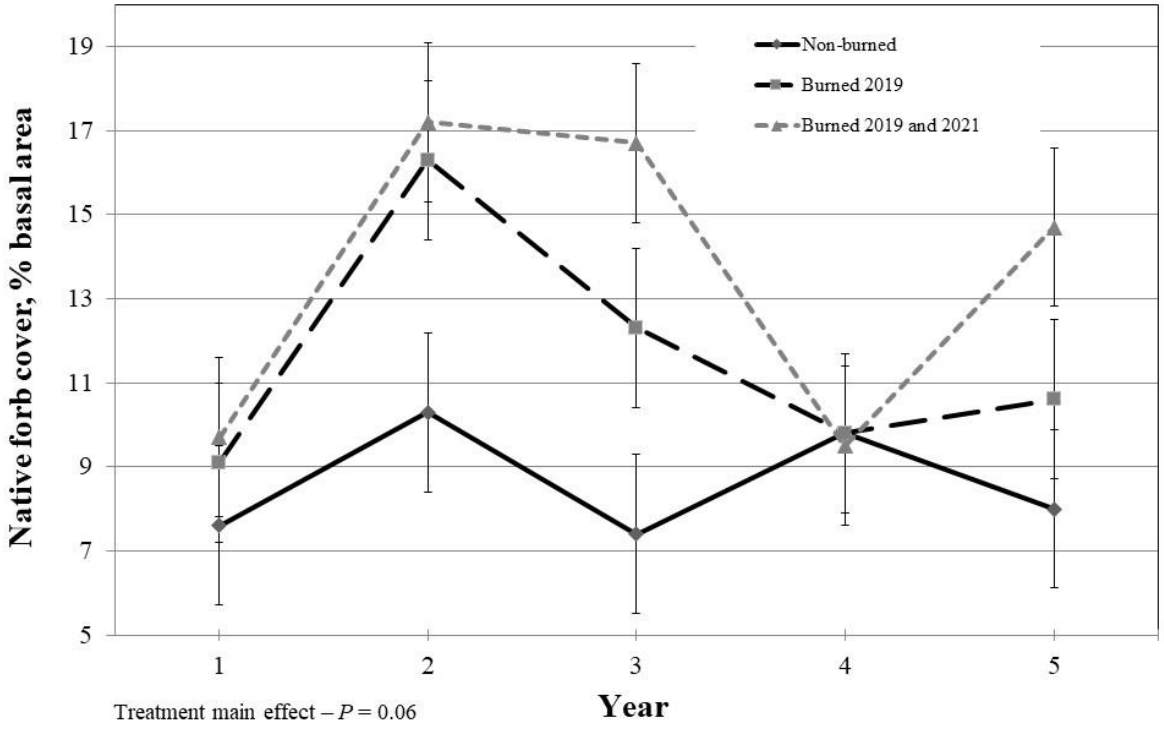


Figure 16. Effects of late-summer prescribed fire on annual forb basal cover composition (% of total basal ground cover) in Caucasian bluestem (*Bothriochloa bladhii*)-infested, mixed-grass prairie in the Kansas Smoky Hills. Prescribed fires were applied on 14 August 2019 (single-burn treatment) or 14 August 2019 and 11 August 2021 (double-burn treatment). These treatments were compared with a non-burned control. Measurements were conducted in July from 2019 to 2023 (Years 1 to 5, respectively). No differences ($P > 0.86$) were detected between treatments before fires were applied (Year 1; 2019). Late-summer prescribed fire increased (treatment main effect - $P = 0.02$) annual forb basal cover in burn treatments compared with non-burned controls, with pronounced effects following each fire treatment. Treatments differ ($P \leq 0.05$) from one another when standard-errors bars (multiple-means comparison SEM = 0.24) do not overlap.

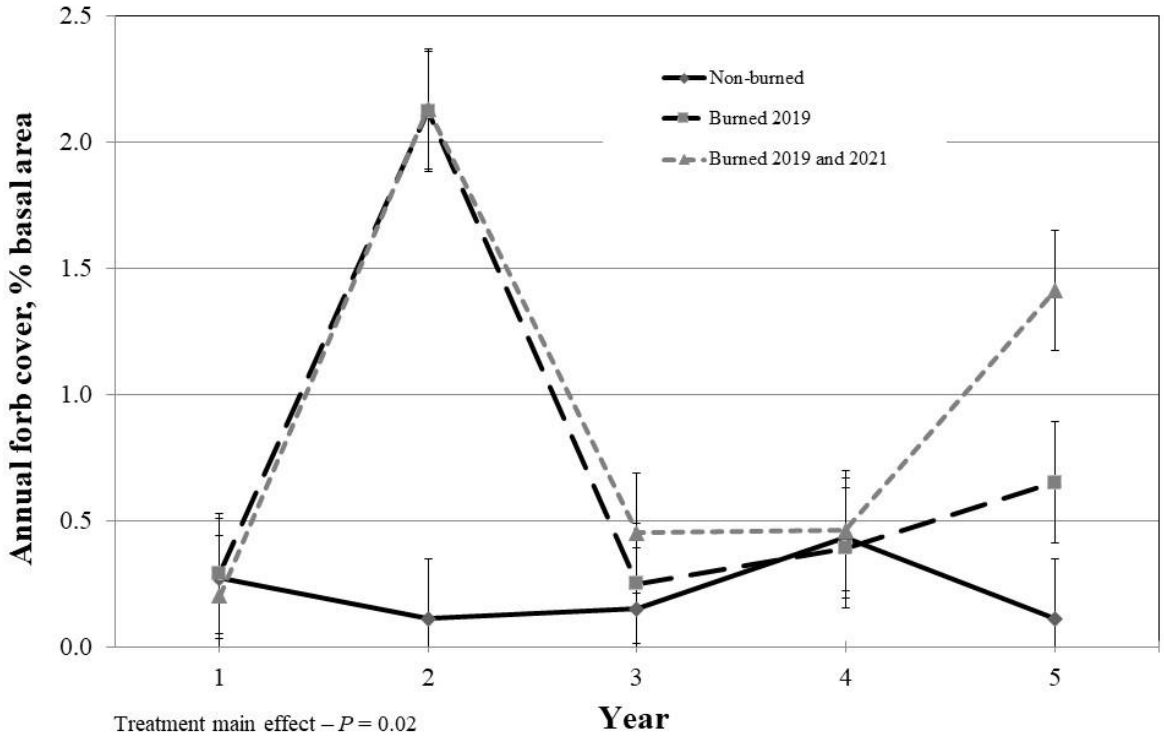


Figure 17. Effects of late-summer prescribed fire on introduced forb basal cover composition (% of total basal ground cover) in Caucasian bluestem (*Bothriochloa bladhii*)-infested, mixed-grass prairie in the Kansas Smoky Hills. Prescribed fires were applied on 14 August 2019 (single-burn treatment) or 14 August 2019 and 11 August 2021 (double-burn treatment). These treatments were compared with a non-burned control. Measurements were conducted in July from 2019 to 2023 (Years 1 to 5, respectively). No differences ($P > 0.61$) were detected between treatments before fires were applied (Year 1; 2019). Late-summer prescribed fire increased (treatment main × time - $P = 0.02$) introduced forb basal cover following fire in Year 2 only compared with non-burned controls; there were no differences ($P > 0.28$) between treatments thereafter. Treatments differ ($P \leq 0.05$) from one another when standard-errors bars (multiple-means comparison SEM = 0.39) do not overlap.

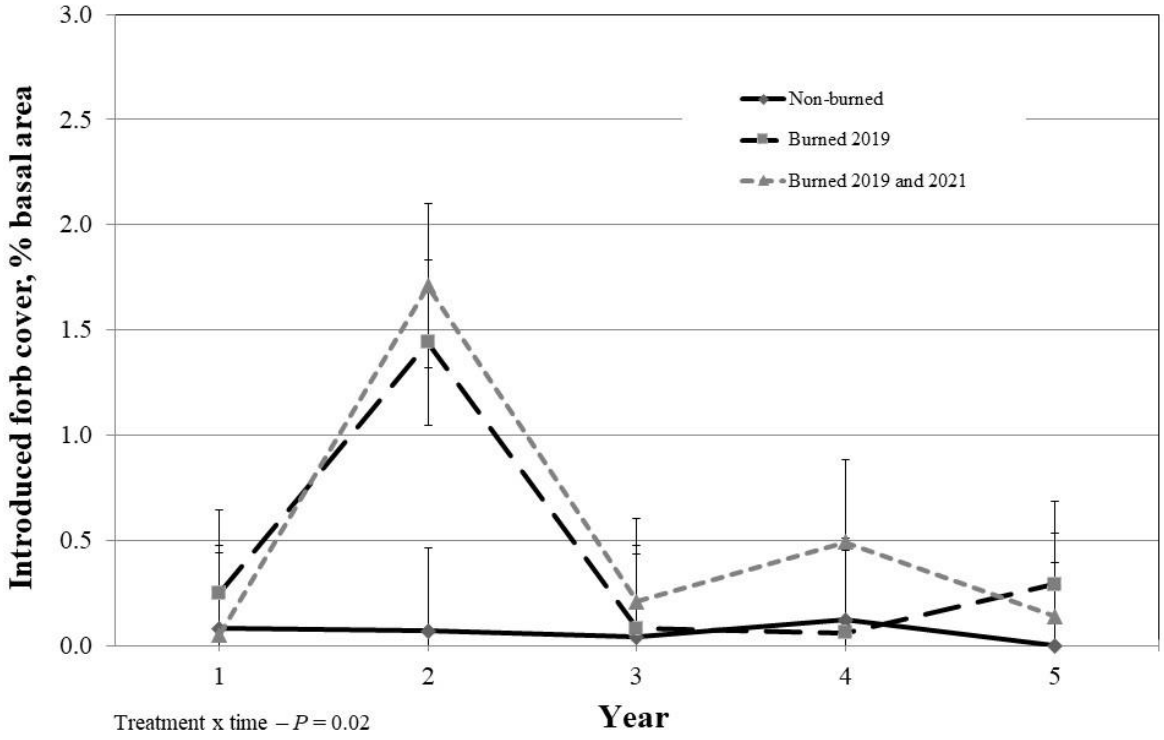


Figure 18. Effects of late-summer prescribed fire on nectar-producing forb basal cover composition (% of total basal ground cover) in Caucasian bluestem (*Bothriochloa bladhii*)-infested, mixed-grass prairie in the Kansas Smoky Hills. Prescribed fires were applied on 14 August 2019 (single-burn treatment) or 14 August 2019 and 11 August 2021 (double-burn treatment). These treatments were compared with a non-burned control. Measurements were conducted in July from 2019 to 2023 (Years 1 to 5, respectively). No differences ($P > 0.28$) were detected between treatments before fires were applied (Year 1; 2019). Late-summer prescribed fire increased (treatment main × time - $P < 0.01$) nectar-producing forb basal cover following fire in Year 1 only compared with non-burned controls; there were no differences ($P > 0.48$) between treatments thereafter. Treatments differ ($P \leq 0.05$) from one another when standard-errors bars (multiple-means comparison SEM = 1.06) do not overlap.

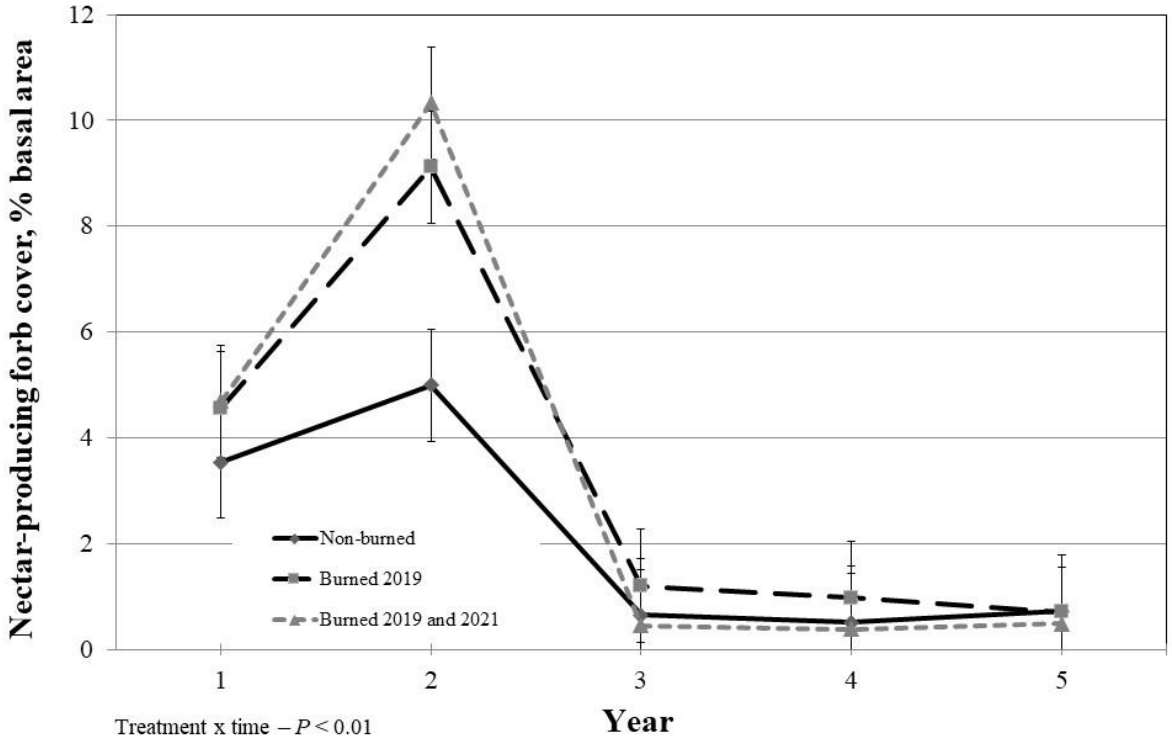


Figure 19. Effects of late-summer prescribed fire on forb species richness (number of species present) in Caucasian bluestem (*Bothriochloa bladhii*)-infested, mixed-grass prairie in the Kansas Smoky Hills. Prescribed fires were applied on 14 August 2019 (single-burn treatment) or 14 August 2019 and 11 August 2021 (double-burn treatment). These treatments were compared with a non-burned control. Measurements were conducted in July from 2019 to 2023 (Years 1 to 5, respectively). No differences ($P > 0.10$) were detected between treatments before fires were applied (Year 1; 2019). Late-summer prescribed fire increased (treatment main × time - $P < 0.01$) forb species richness in Year 2 compared with non-burned controls; the single-burn treatment had greater ($P < 0.01$) forb species richness than non-burned controls in Year 5. Treatments differ ($P \leq 0.05$) from one another when standard-errors bars (multiple-means comparison SEM = 1.53) do not overlap.

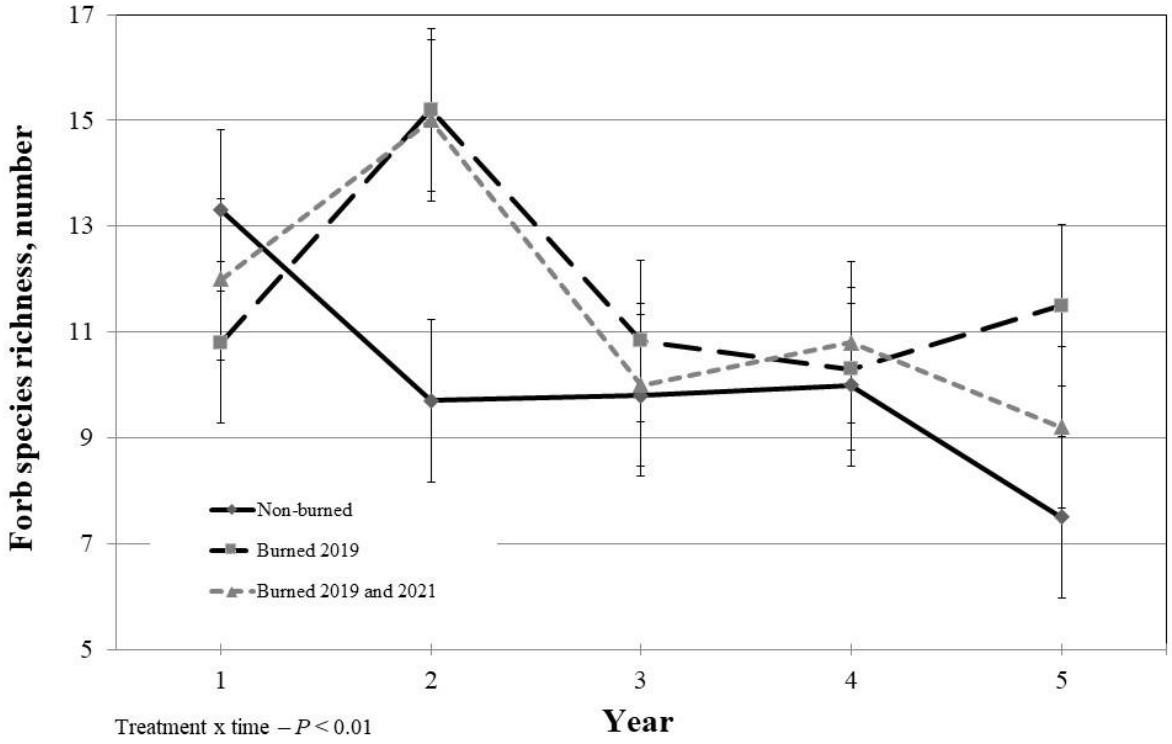


Figure 20. Effects of late-summer prescribed fire on shrub basal cover composition (% of total basal ground cover) in Caucasian bluestem (*Bothriochloa bladhii*)-infested, mixed-grass prairie in the Kansas Smoky Hills. Prescribed fires were applied on 14 August 2019 (single-burn treatment) or 14 August 2019 and 11 August 2021 (double-burn treatment). These treatments were compared with a non-burned control. Measurements were conducted in July from 2019 to 2023 (Years 1 to 5, respectively). No differences ($P > 0.67$) were detected between treatments before fires were applied (Year 1; 2019). Late-summer prescribed fire resulted in less (treatment main effect - $P < 0.01$) shrub basal cover in burn treatments compared with non-burned controls. Treatments differ ($P \leq 0.05$) from one another when standard-errors bars (multiple-means comparison SEM = 0.13) do not overlap.

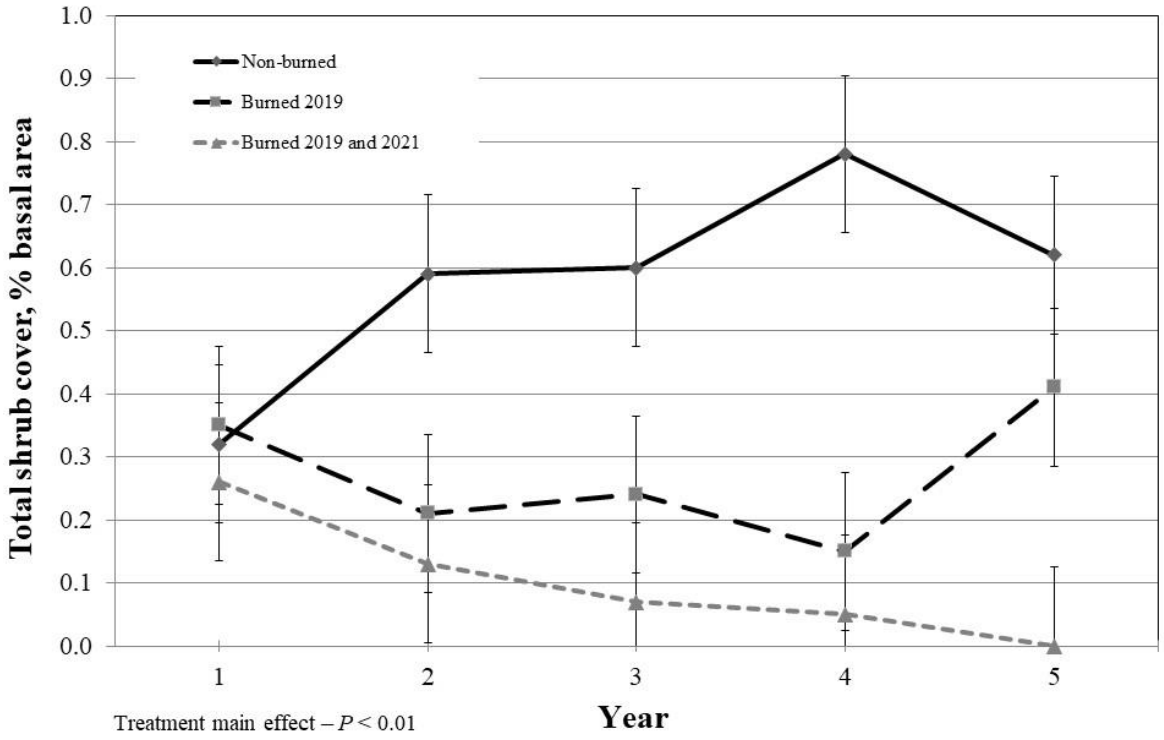


Figure 21. Effects of late-summer prescribed fire on shrub species richness (number of species present) in Caucasian bluestem (*Bothriochloa bladhii*)-infested, mixed-grass prairie in the Kansas Smoky Hills. Prescribed fires were applied on 14 August 2019 (single-burn treatment) or 14 August 2019 and 11 August 2021 (double-burn treatment). These treatments were compared with a non-burned control. Measurements were conducted in July from 2019 to 2023 (Years 1 to 5, respectively). No differences ($P > 0.55$) were detected between treatments before fires were applied (Year 1; 2019). Late-summer prescribed fire decreased (treatment main effect - $P < 0.01$) shrub species richness in burn treatments compared with non-burned controls. Treatments differ ($P \leq 0.05$) from one another when standard-errors bars (multiple-means comparison SEM = 0.19) do not overlap.

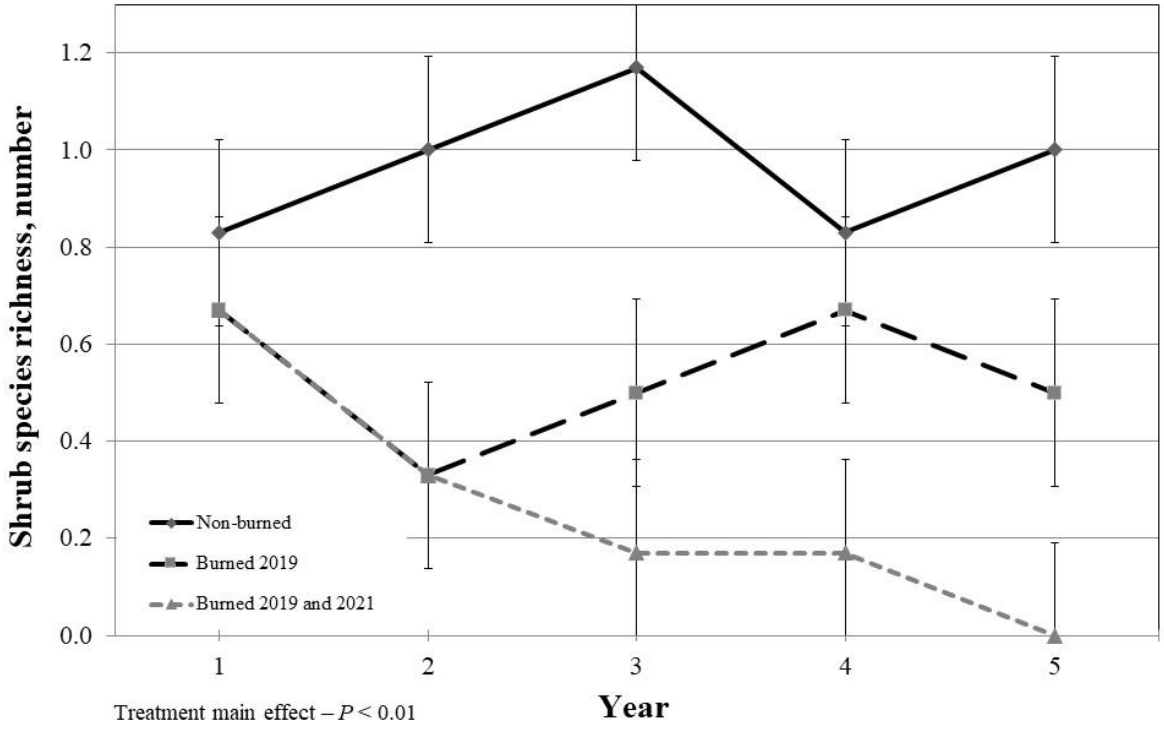
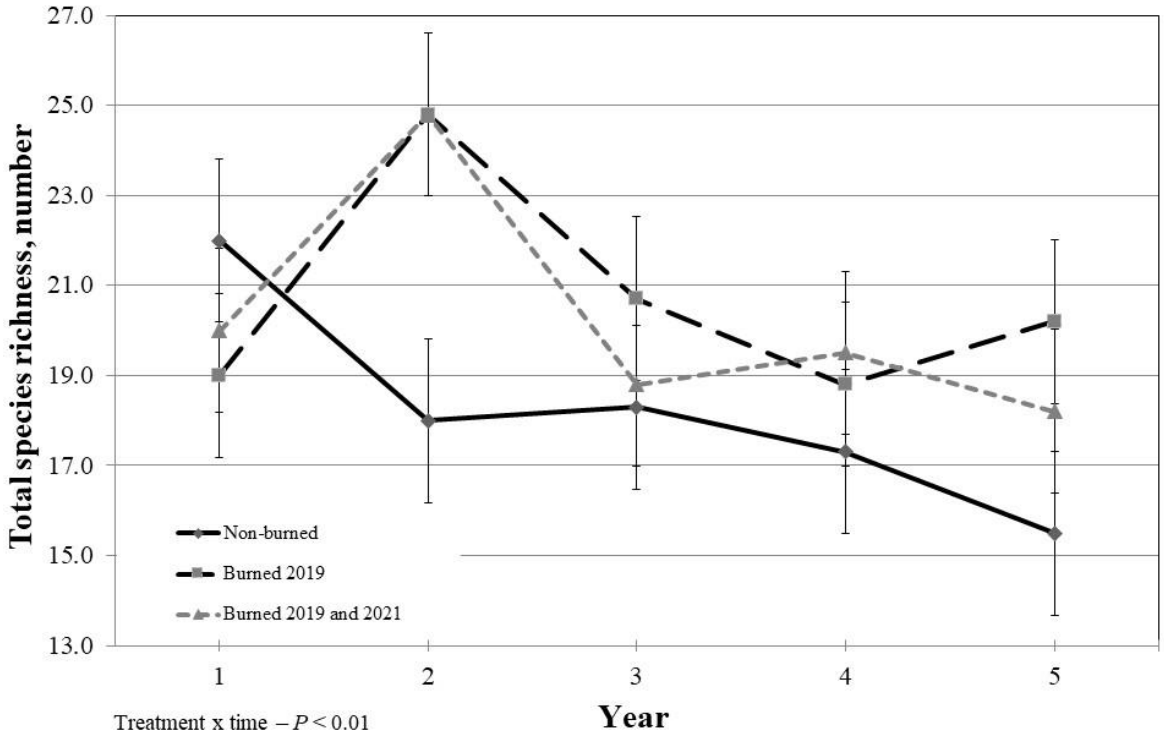


Figure 22. Effects of late-summer prescribed fire on total species richness (number of species present) in Caucasian bluestem (*Bothriochloa bladhii*)-infested, mixed-grass prairie in the Kansas Smoky Hills. Prescribed fires were applied on 14 August 2019 (single-burn treatment) or 14 August 2019 and 11 August 2021 (double-burn treatment). These treatments were compared with a non-burned control. Measurements were conducted in July from 2019 to 2023 (Years 1 to 5, respectively). No differences ($P > 0.10$) were detected between treatments before fires were applied (Year 1; 2019). Late-summer prescribed fire resulted in greater (treatment × time - $P < 0.01$) total species richness following the first fire treatment (i.e., Year 2); no treatment differences ($P > 0.20$) were detected in Years 3 and 4 but total species richness was greater ($P = 0.01$) in the single-burn treatment compared to the non-burned control in Year 5. Treatments differ ($P \leq 0.05$) from one another when standard-errors bars (multiple-means comparison SEM = 1.82) do not overlap.



Appendix 1

Tables

Appendix Table 0.1 Graminoid species encountered in Caucasian bluestem (*Bothriochloa bladhii*)-infested, mixed-grass prairie in the Kansas Smoky Hills in the 2019, 2020, 2021, 2022, and 2023 growing seasons.

Common Name	Scientific Name	Classification	Metabolism	Status	Growth Form
Big bluestem	*Andropogon gerardii*	Perennial	C4	Native	Tall
Blue grama	*Bouteloua gracilis*	Perennial	C4	Native	Short
Buffalograss	*Buchloe dactyloides*	Perennial	C4	Native	Short
Canada wildrye	*Elymus canadensis*	Perennial	C3	Native	n.a.
Caucasian bluestem	*Bothriochloa bladhii*	Perennial	C4	Introduced	Mid
Fall witchgrass	*Digitaria cognata*	Perennial	C4	Native	n.a.
Green Foxtail	*Setaria viridis*	Annual	C4	Introduced	n.a.
Indian grass	*Sorghastrum nutans*	Perennial	C4	Native	Tall
Japanese brome	*Bromus japonicus*	Annual	C4	Introduced	n.a.
Kentucky bluegrass	*Poa pratensis*	Perennial	C3	Introduced	n.a.
Little bluestem	*Schizachyrium scoparium*	Perennial	C4	Native	Mid
Paspalum	*Paspalum setaceum*	Perennial	C4	Native	n.a.
Plains muhly	*Muhlenbergia cuspidata*	Perennial	C4	Native	Mid
Purple lovegrass	*Eragrostis spectabilis*	Perennial	C4	Native	n.a.
Purpletop	*Tridens flavus*	Perennial	C4	Native	Tall
Sand dropseed	*Sporobolus cryptandrus*	Perennial	C4	Native	Tall
Scribner panicum	*Dichanthelium oligosanthes*	Perennial	C3	Native	n.a.
Sedges	*Carex spp.*	Perennial	C3	Native	n.a.
Side-oats grama	*Bouteloua curtipendula*	Perennial	C4	Native	Mid
Smooth bromegrass	*Bromus inermis*	Perennial	C3	Introduced	n.a.
Switchgrass	*Panicum virgatum*	Perennial	C4	Native	Tall
Tall dropseed	*Sporobolus compositus*	Perennial	C4	Native	Mid
Western wheatgrass	*Pascopyrum smithii*	Perennial	C3	Native	n.a.
Witchgrass	*Panicum capillare*	Annual	C4	Native	n.a.
Yellow bluestem	*Bothriochloa ischaemum*	Perennial	C4	Introduced	Mid

Appendix Table 0.2 Forb species encountered in Caucasian bluestem (*Bothriochloa bladhii*)-infested, mixed-grass prairie in the Kansas Smoky Hills in the 2019, 2020, 2021, 2022, and 2023 growing seasons.

Common Name	Scientific Name	Growth	Status
American burnweed	*Erechtites hieracifolius*	Annual	Native
American vetch	*Vicia americana*	Perennial	Native
Aromatic aster	*Symphyotrichum oblongifolium*	Perennial	Native
Ashy goldenrod	*Solidago mollis*	Perennial	Native
Baldwin's ironweed	*Vernonia baldwinii*	Perennial	Native
Black medic	*Medicago lupulina*	Annual	Introduced
Blackeyed susan	*Rudbeckia hirta*	Perennial	Native
Blue wildindigo	*Baptisia australis*	Perennial	Native
Buffalobur	*Solanum rostratum*	Annual	Native
Butterfly milkweed	*Asclepias tuberosa*	Perennial	Native
Carolina cranesbill	*Geranium carolinianum*	Annual	Native
Common blue violet	*Viola sororia*	Perennial	Native
Common groundcherry	*Physalis longifolia*	Perennial	Native
Common milkweed	*Asclepias syriaca*	Perennial	Native
Common ragweed	*Ambrosia artemisiifolia*	Annual	Native
Common St. Johnswort	*Hypericum perforatum*	Perennial	Introduced
Corn speedwell	*Veronica arvensis*	Annual	Introduced
Daisy fleabane	*Erigeron strigosus*	Annual	Native
Dotted gayfeather	*Liatris punctata*	Perennial	Native
False boneset	*Brickellia eupatorioides*	Perennial	Native
Field bindweed	*Convolvulus arvensis*	Perennial	Introduced
Field pussytoes	*Antennaria neglecta*	Perennial	Native
Fringed puccoon	*Lithospermum incisum*	Perennial	Native
Fringeleaf ruellia	*Ruellia humilis*	Perennial	Native
Giant ragweed	*Ambrosia trifida*	Annual	Native
Glandular croton	*Croton glandulosus*	Annual	Native
Goat's beard	*Tragopogon dubius*	Biennial	Native
Green milkweed	*Asclepias viridiflora*	Perennial	Native
Grey-green wood sorrel	*Oxalis dillenii*	Perennial	Native
Grooved flax	*Linum sulcatum*	Annual	Native
Hairy aster	*Symphyotrichum pilosum*	Perennial	Native
Heath aster	*Symphyotrichum ericoides*	Perennial	Native
Hemp dogbane	*Apocynum cannabinum*	Perennial	Native
Illinois tickclover	*Desmodium illinoense*	Perennial	Native
Johnny-jump-up	*Viola bicolor*	Annual	Native
Long-bearded hawkweed	*Hieracium longipilum*	Perennial	Native
Louisiana sagewort	*Artemisia ludoviciana*	Perennial	Native
Low milkvetch	*Astragalus lotiflorus*	Perennial	Native
Marestail	*Conyza canadensis*	Annual	Native

Table 2 continued

Common Name	Scientific Name	Growth	Status
Missouri goldenrod	*Solidago missouriensis*	Perennial	Native
Narrowleaf milkweed	*Asclepias stenophylla*	Perennial	Native
Oneseed croton	*Croton monanthogynus*	Annual	Native
Pitcher sage	*Salvia azurea*	Perennial	Native
Plains pricklypear	*Opuntia polyacantha*	Perennial	Native
Plain-seeded plantain	*Plantago virginica*	Annual	Native
Prairie coneflower	*Ratibida columnifera*	Perennial	native
Prairie groundsel	*Senecio plattensis*	Perennial	Native
Prairie pepper-grass	*Lepidium densiflorum*	Annual	Native
Prairie trefoil	*Lotus unifoliolatus*	Annual	Introduced
Prickly lettuce	*Lactuca serriola*	Biennial	Introduced
Purple poppymallow	*Callirhoe involucrata*	Perennial	Native
Purple prairieclover	*Dalea purpurea*	Perennial	Native
Red river scaleseed	*Spermolepis inermis*	Annual	Native
Rough false pennyroyal	*Hedeoma hispida*	Annual	Native
Serrateleaf evening primrose	*Catylophus serrulatus*	Perennial	Native
Showy evening primrose	*Oenothera speciosa*	Perennial	Native
Slick-seed bean	*Strophostyles leiosperma*	Annual	Native
Snow-on-the-mountain	*Euphorbia marginata*	Annual	Native
Spider milkweed	*Asclepias viridis*	Perennial	Native
Spotted spurge	*Chamaesyce maculata*	Annual	Native
Stiff goldenrod	*Solidago rigida*	Perennial	Native
Sulphur cinquefoil	*Potentilla recta*	Perennial	Introduced
Sweetclovers	*Melilotus spp.*	Perennial	Introduced
Tall goldenrod	*Solidago altissima*	Perennial	Native
Tall thistle	*Cirsium altissimum*	Annual	Native
Texas croton	*Croton texensis*	Annual	Native
Tube penstemon	*Penstemon tubaeflorus*	Perennial	Native
Venus looking glass	*Triodanis perfoliata*	Annual	Native
Violet lespedeza	*Lespedeza violacea*	Perennial	Native
Violet wood sorrel	*Oxalis violacea*	Perennial	Native
Virginia groundcherry	*Physalis virginiana*	Perennial	Native
Viscid euthamia	*Euthamia gymnospermoides*	Perennial	Native
Wavyleaf thistle	*Cirsium undulatum*	Perennial	Native
Western ragweed	*Ambrosia psilostachya*	Perennial	Native
Western yarrow	*Achillea millefolium*	Perennial	Native
Whorled milkweed	*Asclepias verticillata*	Perennial	Native
Whorled polygala	*Polygala verticillata*	Annual	Native
Wild alfalfa	*Psoralidium tenuiflorum*	Perennial	Native
Woolly verbena	*Verbena stricta*	Perennial	Native

Appendix Table 0.3 Shrub species encountered in Caucasian bluestem (*Bothriochloa bladhii*)-infested, mixed-grass prairie in the Kansas Smoky Hills in the 2019, 2020, 2021, 2022, and 2023 growing seasons.

Common Name	Scientific Name	Growth	Status
Buckbrush	*Symphoricarpos orbilculatus*	Perennial	Native
Chickasaw plum	*Prunus angustifolia*	Perennial	Native

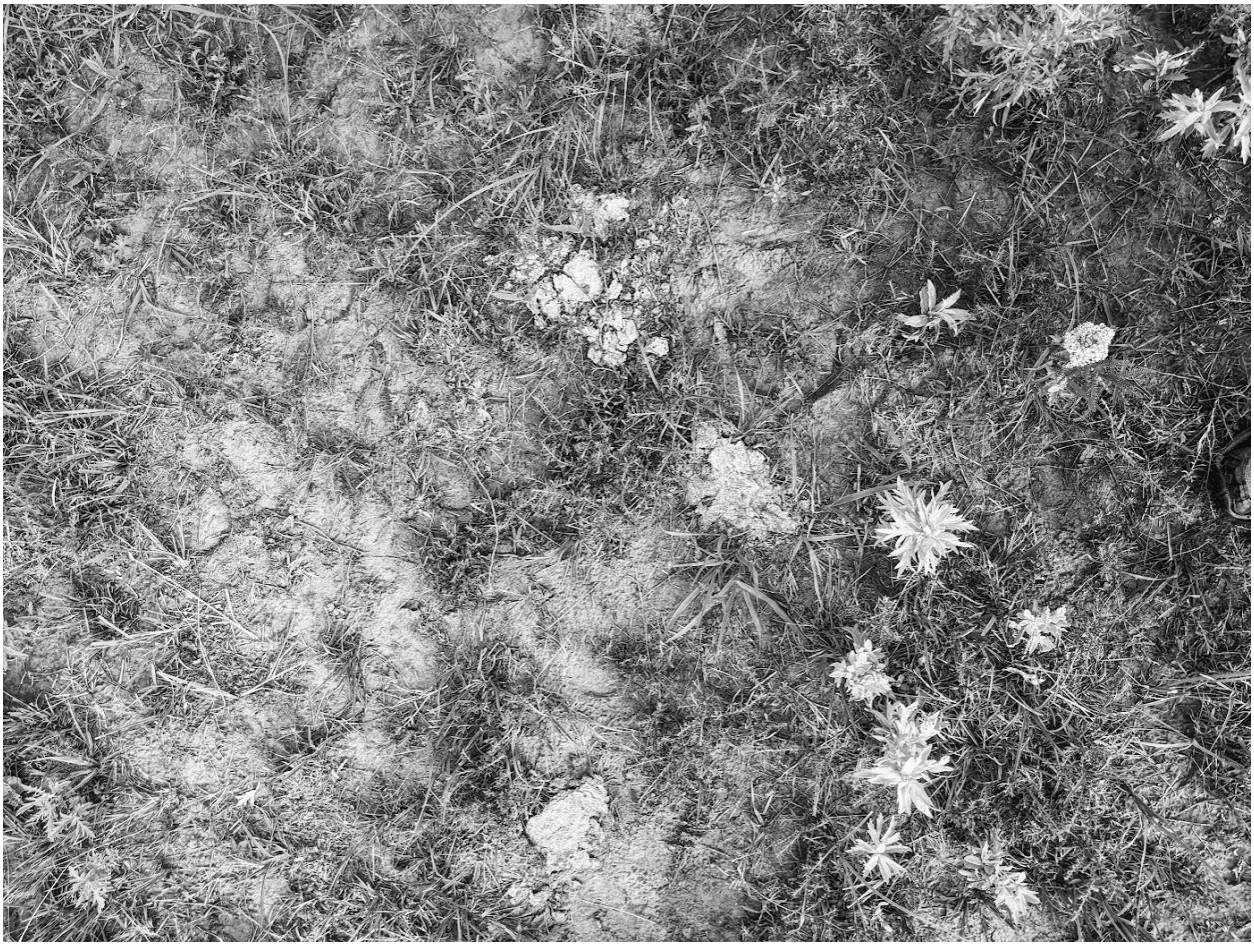
Figures

Appendix Figure 0.1. A distinct fire line is visible between non-burned (right side) and twice-burned (left side; prescribed fire applied in 2019 and 2021) plots in summer 2023 of a five-year experiment in the mixed-grass prairie of the Kansas Smoky Hills. Increasing species diversity is evident with fire treatment while a thick stand of Caucasian bluestem is visible with no fire treatment.

Appendix Figure 0.2. In summer 2023, a near monoculture of Caucasian bluestem is present in a non-burned plot of a five-year experiment in the mixed-grass prairie of the Kansas Smoky Hills.

Appendix Figure 0.3. In summer 2023, improved species diversity and richness were discernable in a twice-burned plot of a five-year experiment in the mixed-grass prairie of the Kansas Smoky Hills.

Appendix Figure 0.4. In summer 2023, improved species diversity and richness as well as increased extent of bare soil were discernable in a twice-burned plot of a five-year experiment in the mixed-grass prairie of the Kansas Smoky Hills.



Appendix Figure 0.5. In summer 2023, improved species diversity and richness were discernable in a twice-burned plot of a five-year experiment in the mixed-grass prairie of the Kansas Smoky Hills.