Native Forb Establishment in Tall Fescue-dominated Cattle Pastures

David Nsame Bellangue

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J. Leighton Reid, Chair

Benjamin F. Tracy

Jacob N. Barney

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Abstract

Temperate grasslands and the services they provide are threatened with severe degradation from human-driven land use changes. Among the worst affected services is pollinator support with grassland degradation contributing to the global decline in insect abundance due to habitat loss and a lack of floral resources. This has prompted conservation organizations to support pollinator conservation on working landscapes by increasing floral resources, but gaps remain in the consistent establishment of native forbs in intensively managed agricultural areas. We evaluated factors that influence native forb establishment during seed-based enrichment planting of tall fescue-dominated cattle pastures in two separate experiments: one testing a range of site preparation treatments with different seasons of sowing and comparing their effects on tall fescue suppression and seeded native plant establishment and the other investigating the effects of seed rate and pre-seeding cold stratification on native forb establishment on separate plots. For the site preparation experiment, we observed a mean target plant density of 0.12 target plants per m^2 (SD = 0.247) The greatest target plant stem density (P < 0.0001) and species richness (P < 0.001) was in plots treated with a 2% glyphosate solution and sown with native seeds in early summer with the next five best treatments composed solely of fall sown replicates. For the seed and stratification experiment, we observed a mean target plant density of 88 target plants per m^2 (SD = 73.9). Higher seeding resulted in greater target plant abundance in plots (P < 0.0001) with a seed rate of 56 kg/ha^{-1} resulting in almost three times as

many target plants compared to 2.24 kg/ha^{-1}. Pre-seeding stratification resulted in an increase in target plant abundance ($P < 0.01$). Target species richness was consistent between treatment levels. Results suggest that native forb establishment can be enhance by eliminating pasture grasses prior to seeding and the use of high seeding rates sown in the fall or using stratified seed.

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General Audience Abstract

Temperate grasslands and the services they provide are threatened with severe degradation from human-driven land use changes. Among the worst affected services is pollinator support with grassland degradation contributing to the global decline in insect abundance due to habitat loss and a lack of floral resources. To reverse this decline, conservation groups are encouraging the use of native plants throughout the landscape especially on farms and ranches to provide more resources for insect pollinators. One exciting opportunity exists in planting wildflowers into tall fescue-dominated cattle pastures that occupy millions of hectares of land in the Southeastern United States to provide food for pollinating insects. However little information exists on how to successfully establish wildflowers as much expertise is based on work done in the tallgrass prairie region of the Midwest. This study's goal was to investigate what control wildflower establishment by evaluating the success of an existing establishment experiment testing several site preparation techniques and different sowing seasons. A separate experiment was set up looking at the effect different seed rate and cold moist stratification had on establishment success of wildflowers. For the site preparation experiment, establishment was low for all treatments with a mean target plant density of 0.12 target plant per m^2 ($SD = 0.247$). Summer sown 2% glyphosate had the highest wildflower richness and abundance at 0.35 target plants per m^2 ($SD = 0.247$) and fall sown treatments were found to have higher sown wildflower abundance and richness than summer sown treatments. For the seed and stratification

experiment, we observed a mean target plant density of 88 target plants per m^2 ($SD = 73.9$). Target plant abundance did change between treatment levels with the highest and second highest seed levels yielding nearly three times and twice as many sown wildflowers as the lowest treatment respectively. Stratification resulted in an increase in sown wildflower abundance and sown wildflower richness did not differ significantly between treatment levels. Results suggest that native forb establishment can be enhance by eliminating pasture grasses prior to seeding and the use of high seeding rates sown in the fall using stratified seed.

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Chapter 1: Literature Review

Grasslands in peril

Grasslands and savannas are among the world's most widely distributed ecosystems covering close to one-third of the earth's terrestrial surface and supporting the livelihoods of more than two billion people (Dengler et al. 2018). For millennia, grasslands have nurtured human communities and supported diverse assemblages of organisms. These ancient productive ecosystems provide numerous benefits to human communities such as provisioning services with direct economic impact in the form of high-quality forage essential for the production of wool, meat, milk, and leather (Sala & Paruelo, Jose 1997; Veldman, Buisson, et al. 2015). Grasslands also provide regulatory services with indirect economic value including soil formation, erosion control, water infiltration, genetic diversity for cultivated crops, and pollination. These services are critical in the maintenance of a healthy biotic community and, by extension, healthy human communities (Bengtsson et al. 2019; Hönigová et al. 2012; Smith et al. 2022).

However, grasslands – in particular temperate grasslands – are among the most threatened and least conserved biomes on Earth (Carbutt et al. 2017; Hoekstra et al. 2005). Top threats include habitat destruction and fragmentation due to agriculture and human settlement, invasive species, loss of large herbivores, fire suppression, and more recently climate change (Henwood 2010). Despite their importance to human sustenance and global biodiversity, grasslands are often overlooked and underappreciated in the eyes of the general public and policymakers. Even conservationists have long overlooked temperate grasslands and the diverse biota they contain in favor of a heavy focus on forests (Bond & Parr 2010; Noss et al. 2015). As of 2016, only 4.6% of temperate grasslands are conserved globally, the lowest of any terrestrial biome (Carbutt et al. 2017), with a risk of further degradation due to ill-conceived tree planting campaigns under the banner of afforestation and carbon capture (Dudley et al. 2020; Fleischman et al. 2020; Holl & Brancalion 2020; Veldman, Overbeck, et al. 2015).

North America exemplifies the trend of degradation and underappreciation with the continent's vast expanses of grasslands either severely degraded or almost destroyed following Euro-American settlement. Some systems such as the tallgrass prairies of Central North America were reduced by as much as 99% (Samson & Knopf 1994; Samson et al. 2004). Much of this was driven by the rapid expansion of agriculture, turning the region into a breadbasket for the United States and Canada. The speed and totality of the conversion of the tallgrass prairie is one of the most profound ecological events to occur on the continent with more than 90% converted to agriculture with 70 years of Euro-American settlement (Smith et al. 2010). The formerly widespread tallgrass taxa are now restricted to small remnants that have escaped conversion to agriculture or urban development. The decline of the tallgrass prairie did not go unnoticed and has prompted a surge of interest in restoration projects and native plant utilization in recent years to halt its decline and conserve the diversity in this ecosystem (Anderson 2009). This growing interest in grassland restoration has contributed immensely to the development of restoration ecology as a scientific field that began with the first concerted effort by researchers to recreate a tallgrass prairie at the University of Wisconsin-Madison in the 1930s (Allison 2002).

Southeastern grasslands

While much of the attention has been focused on the Great Plains grasslands, the less well-known but no less important grasslands of the Southeast deserve attention and restoration as well (Noss 2013). The grasslands of the Southeast have been stable for millions of years and were largely unaffected by geologic events such as glaciation or sea level rise that regularly disrupted the biological communities of the Great Plains. Owing to their age, substrate diversity and relative protection from geologic upheavals, Southeastern grasslands are among the most biodiverse ecosystems in North America with high levels of endemism and a greater floral species richness at a fine-scale in some areas than some tropical forests (Noss et al. 2021; Noss 2013). Like other grassland systems, they perform a variety of irreplaceable ecosystem services including CO_2 capture, erosion control, groundwater recharge, and pollinator support (Török et al. 2018). These important ecosystems have been severely degraded through habitat destruction and modification, overexploitation, and fire suppression (Noss 2013).

In Virginia and the Southeast, grasslands have been an integral part of the landscape for thousands of years supporting a rich assemblage of grassland-dependent species (Noss 2013). Numerous European explorers noted the open and park-like nature of the savannas, grasslands, and woodlands they encountered in the Piedmont during initial exploration of the Eastern North American (Barden 1997; Fowler & Konopik 2007). In many areas such as the Shenandoah Valley, the driest area in Virginia, explorers saw herds of bison and elk in large grassy clearings (Heus 2003). While climatic conditions, substrate composition and large herbivories allowed for the development of these grassland communities before the arrival of humans (Noss 2013), settlement of the area by Native peoples and their extensive use of fire to maintain an open landscape for hunting and agriculture expanded grassland habitats dramatically (Fowler & Konopik 2007). However, upon settlement of Virginia by Europeans, burning almost ceased, allowing close-canopy forests to predominate over much of the landscape not actively maintained for agriculture or urban development (Brennan & Kuvlesky 2005). This reduced the available habitat for grassland-dependent species to areas where chronic disturbance that kept the landscape open such as roadsides, powerline cuts, railroad rights-of-way, and recently harvested pine plantations (Davis et al. 2002; Fleming 2020). However, large areas of land in Virginia used for cattle pasture still retain an ecosystem structure similar to the grasslands that once existed.

Cattle pastures as a tool for pollinator conservation

Cattle pastures offer an opportunity for the conservation of grassland taxa (Hopkins & Holz 2006; Johnson et al. 2019; Ledvina et al. 2020). While these working grasslands are composed primarily of non-native cool season grasses such as tall fescue (*Lolium arundinaceum*) and orchardgrass (*Dactylis glomerata*), they have the potential to contribute to the conservation of Virginia's grassland communities. Cattle pastures have a grass-dominated vegetation structure open to sunlight and maintained by large herbivores. While there have been recent positive developments in the use of native warm-season grasses as forage for cattle, these systems must do more to support biodiversity, especially among insects that provide numerous ecosystem services critical to agricultural landscapes (Isaacs et al. 2009; Losey & Vaughan 2006; Öckinger & Smith 2007).

A thriving insect population is among the most essential factors in the maintenance of a healthy ecosystem and productive agricultural areas (Burghardt et al. 2008; Jones & Snyder 2018; Tallamy et al. 2021). Pollinator support is especially crucial at this time due to the dramatic and ongoing decline of insect populations globally (Forister et al. 2019). This crisis has been caused by several factors ranging from habitat loss to pesticide use and climate change (Potts et al. 2010). Declines in insect abundance is a serious matter as an estimated 87.5% of all flowering plants rely on animal visitation for reproduction and with the estimated benefit of pollinators for global agriculture valued at $200 billion (Buchmann & Nabhan 1996; Kearns et al. 1998). For beneficial insect populations to thrive, reintroducing native forbs back into the landscape is crucial given the global insect decline, especially in intensively managed agricultural environments (Potts et al. 2010). In response to the decline in pollinators, a number of organizations including the Xerces Society for Invertebrate Conservation and Native Plant Trust have encouraged the use of properly sourced native plant material on public and private lands, especially on working landscapes such as farms and ranches (Hopwood et al. 2016; Xerces Society for Invertebrate Conservation 2021).

Forbs provide resources that grasses are unable to including protein-rich pollen, nectar, and hosts for insect species that require plants other than grasses (Eierman 2020). Additionally, agricultural lands planted with a diverse mix of native forbs and grasses are more hospitable to insects (Garibaldi et al. 2021). This allows for the increased movement of insects across the landscape between isolated nature reserves and reduces the risk of extirpation across the landscape as it is easier for organisms to recolonize unoccupied habitat patches and maintain viable populations (Perfecto et al. 2019). However, while native warm-season grass propagation is relatively well-studied (Kedzierski 2013), much less is known about the propagation and establishment of native forbs particularly in the Southeast (Wright et al. 2015). Currently, agricultural grasslands are primarily dominated by non-native graminoids and legumes and are actively maintained to discourage non-leguminous forbs (Lüscher et al. 2014; Popp et al. 2000). This presents significant challenges in the wider utilization of native flora in agriculture.

Opportunity exists in Southeastern cattle pastures to increase the diversity of native forbs for the benefit of both pollinators and cattle. Several projects are underway in this region with the goal of developing strategies by which cattle-raising landowners in the fescue belt can seed their pastures with palatable and non-toxic native forbs that not only provide additional forage for cattle but pollinators as well. Such initiatives hold the promise of increased productivity within pasture systems to the benefit of both landowners and the environment (Bullock et al. 2007). Integrating native forbs into tall fescue pastures has been previously focused on improving animal performance such as mitigating fescue toxicosis (Roberts & Andrae 2004). Using native forbs to support pollinators across the growing season is a present goal. Such novel forage-livestock-pollinator systems sometimes called bee-friendly beef must maintain existing forage production and animal performance in addition to pollinator support (Wagner et al. 2021).

Obstacles to successful seed-based restorations

Reliable strategies applicable to the Southeast for consistent native forb establishment into cool-season cattle pastures are needed to increase the conservation benefit of rural lands. One of the largest obstacles to conserving biodiversity on private land is the need to compromise with landowner's interests in such a way as to protect livelihoods (Farrier 1985; Knight 1999; Norton 2000). While this may not be a critical issue in regions with large amounts of public land like the Appalachian Mountains, this advocacy is advantageous in regions such as the Piedmont of Virginia where public land is scarce and private land predominates (Weakley et al. 2020). For these wildflower-pasture grass systems to succeed, researchers must develop practical and reliable methods of seeding native forbs into existing tall fescue pastures as currently little technical guidance exists applicable to the Southeast (Wagner et al. 2021).

In order to restore or enrich pastureland via seeding, several factors must be taken into consideration if the planting is to be successful including microsite availability, seeding rate, seed conditioning, and diversity of seed mix (Kildisheva et al. 2020; Williams et al. 2018). Microsites must be available to provide seeds with the ideal conditions for germination and survival. Pasture vegetation can inhibit this by reducing seed-soil contact and limiting resources through competition (Frances et al. 2010; Millikin et al. 2016; Tognetti & Chaneton 2012;

Wilson & Gerry 1995) Tall fescue – one of the most widely planted pasture grasses in the Southeast – is known to form a dense root mass that excludes native vegetation and once established, is extremely difficult to weaken or kill (Watling 2016). This obstacle can be overcome by either eliminating or severely suppressing pasture vegetation before planting seed.

In addition to microsite availability, an adequate seeding rate must be achieved for successful establishment; if the seeding rate is exceedingly high, target species will compete against each other and will have reduced fitness, resulting in the waste of very costly seed. If the seeding rate is exceedingly low, target plants risk being overwhelmed by proliferation of non-target vegetation (Natural Resources Conservation Service 2009). The seeding rate also needs to match the weed pressure and conditions of the site based on climatic and edaphic variables. Sites with higher soil organic matter and annual precipitation have been shown to have higher weed pressures, making higher seed rates necessary to establish dominant native cover quickly (Helzer et al. 2010; Kleiman 2016). Often with grassland plantings carried out in the Midwest, higher seeding rates improve the density and richness of sown plants in the resulting stand (Barr et al. 2017; Gardner 2011). Diversity can also play a key role in the success of a restoration. Piper (2014) found that planting a diverse mix of forbs and grasses in the Midwest hastens the establishment of a grassland-like community and increases persistence in the face of invasion by non-target species.

Likewise, the rapid germination of sown seed allows target species to stay ahead of annual and biennial weeds that are common in the first few years of a restoration's life and maximize the use of scarce seed resources (Elzenga et al. 2019). Dormancy in seeds can hinder this effort, especially when certain species require scarification/stratification (Kildisheva et al. 2020). In temperate North America, many seeds have developed dormancy mechanisms that can only be broken with a period of moist cold stratification making this a significant hurdle in establishing these species from seed (Baskin & Baskin 2020). Restorations that do not take dormancy into account can fail or result in reduced diversity by pushing the plant community toward species with non-dormant seeds (Kildisheva et al. 2020). Practically, North American restorationists will sow in late fall through early spring to give the seeds ample exposure to cold moist conditions (Helzer et al. 2010). Another strategy is to stratify seeds in a refrigerator and sow in late spring to early summer to accomplish several goals: 1) limit predation and pathogen risks in the seedbed and 2) allow for rapid germination of sown seeds that, combined with proper management, could give target species a competitive edge of non-planted vegetation.

Even after successful germination and survival in the first few years of a planting's life, the restoration can still fail if target species are outcompeted by non-native plants (Schramm 1976). Thus, it is essential to have follow-up maintenance of restoration plots including reintroducing disturbance regimes such as fire, mowing, or well-timed grazing (Burton et al. 1988). For particularly persistent invasive plants, spot application of herbicide or manual removal may be necessary (Solecki 2005). If desired, additional seeding can be carried out to increase the diversity of the planting to mimic a remnant grassland community more closely (Smith et al. 2010).

Grassland research for the Southeast is particularly needed since much of the work around site preparation, seeding rates and pre-seeding stratification comes from the Central Plains Grasslands (Williams et al. 2018) and little work has been carried out in other grassland systems of North America such as the Southeastern grasslands (Noss 2013). This work is important as grassland restoration and pasture enrichment projects have a tendency to result in highly unpredictable outcomes (Brudvig 2017; Stuble et al. 2017). The unpredictability of restoration outcomes is a major roadblock to greater implementation of restoration projects, especially for landowners whose livelihoods depend on agriculture. A cattle producer is much less likely to expend time and resources to sow expensive native forbs into pastures unless there is a reasonably high chance of getting adequate establishment.

The goal of this research project is addressing these potential concerns to make interseeding of native forbs more attractive to rural cattle owners. We seek to increase the knowledge base on the factors that contribute to the successful establishment of native forb interseeding into tall fescue-dominated cattle pastures. Most research and technical guidance for interseeding forbs originates from the Great Plains region and may not be the most appropriate for the Southeast's diverse climate, soils, and agricultural weed communities. This project seeks to develops recommendations to assist landowners in increasing the ecological integrity of their property as well as encourage further research dealing with the wider utilization of Southeastern flora.

Chapter 2: Native Forb Establishment in Tall Fescue-dominated Cattle Pastures

In preparation for submission to Native Plants

Running Head: Native Forb Establishment in Tall Fescue-dominated Cattle Pastures

Authors: David Bellangue[1], Benjamin F. Tracy[1], Michael Flessner[1], Jacob N. Barney[1], & J. Leighton Reid[1]

Affiliation: [1]School of Plant and Environmental Sciences, Virginia Tech, Blacksburg, VA

Keyword: direct seeding, enrichment planting, native plants, pollinator decline, seeding rate, stratification

Author Contributions: BFT & MF designed the initial site preparation experiment that DB collected data from to evaluate establishment success. DB designed, implemented, and analyzed the seed and stratification experiment with input from JNB and JLR. DB wrote the first draft of this manuscript; all authors edited the manuscript except MF.

Abstract

Temperate grasslands and the services they provide are threatened with severe degradation from human-driven land use changes. Among the worst affected services is pollinator support with grassland degradation contributing to the global decline in insect abundance due to habitat loss and a lack of floral resources. This has prompted conservation organizations to support pollinator conservation on working landscapes by increasing floral resources, but gaps remain in the consistent establishment of native forbs in intensively managed agricultural areas. We evaluated factors that influence native forb establishment during seed-based enrichment of tall fescue-dominated cattle pastures in two separate experiments: one testing a range of site preparation treatments with different seasons of sowing and comparing their effects on tall fescue suppression and seeded native plant establishment and the other investigating the effects of seed rate and pre-seeding cold stratification on native forb establishment on separate plots. For the site preparation experiment, we observed a mean target plant density of 0.12 target plants per m^2 (*SD* = 0.247) The greatest target plant stem density ($P<$ 0.0001) and species richness ($P < 0.001$) was in plots treated with a 2% glyphosate solution and sown with native seeds in early summer with the next five best treatments composed solely of fall sown replicates. For the seed and stratification experiment, we observed a mean target plant density of 88 target plants per m^2 (*SD* = 73.9). Higher seeding resulted in greater target plant abundance in plots ($P < 0.0001$) with a seed rate of 56 kg/ha^{-1} resulting in almost three times as many target plants compared to 2.24 kg/ha^{-1}. Pre-seeding stratification resulted in an increase in target plant abundance for 2022 plots ($P < 0.01$). Target species richness was consistent between treatment levels. Results suggest that native forb establishment can be enhance by eliminating pasture grasses prior to seeding and the use of high seeding rates sown in the fall or using stratified seed.

Introduction

Grasslands and savannas are among the world's most widely distributed ecosystems covering close to one third of the earth's terrestrial surface and supporting the livelihoods of more than two billion people (Dengler et al. 2018). Around the world, grasslands nurture human communities, support diverse assemblages of organisms, and provide numerous ecosystem services such as soil formation, water infiltration, and pollination (Bengtsson et al. 2019; Dengler et al. 2018; Hönigová et al. 2012). However, grasslands – particularly temperate grasslands – are among the most threatened and least conserved biomes on Earth (Carbutt et al. 2017; Hoekstra et al. 2005). Temperate grasslands are at risk from habitat destruction and fragmentation due to agriculture and human settlement as well as invasive species, loss of large herbivores, fire suppression, and more recently, climate change (Henwood 2010). Despite their importance to human sustenance and global biodiversity, grasslands are often overlooked and underappreciated in the eyes of conservationists, the general public, and policy makers (Bond & Parr 2010; Holl & Brancalion 2020; Noss et al. 2015).

The majority of temperate grasslands under private ownership. This presents challenges in balancing the need to conserve grassland taxa and landowners' needs to maintain their livelihoods (Farrier 1995; Knight 1999). With a biome as fragmented as temperate grasslands with very few large intact areas, it is imperative to conserve grassland taxa wherever possible including within agricultural lands (Packard & Mutel 2005). Grassland remnants often exist as small patches surrounded by intensively managed agricultural land that isolates remnants from each other and accelerates species loss (Packard & Mutel 2005). Thus, to reverse the decline of grassland taxa, it is critical to increase the connectivity between grassland remnant, allowing for greater gene transfer between remnants (Carbutt et al. 2017). By finding ways to make the landscape surrounding remnants more hospitable to grassland taxa, greater connectivity can be restored between remnants that allows for greater mobility for grassland taxa and reduces the extinction risk across the landscape (Perfecto et al. 2019).

North America exemplifies the trend of degradation and underappreciation with the continent's vast expanses of grasslands almost completely destroyed following Euro-American settlement (Samson & Knopf 1994). While much attention has been focused on the Great Plains grasslands and their restoration, the less well known but no less important grasslands of the Southeast, with high levels of plant endemism, deserve attention and restoration as well (Noss 2013). Grassland research for this region is particularly needed since most research about site preparation and seeding rates comes from the Central Plains Grasslands (Williams et al. 2018). With the degradation of grassland ecosystems, the ecosystem services offered by these systems are also diminished and among the hardest hit, especially in agricultural areas, is pollinator support (Forister et al. 2019; Potts et al. 2016; Vanbergen et al. 2013). There is growing evidence of insect and pollinator declines globally especially in intensively managed agricultural lands. For example, some well-studied taxa such as North American bumblebees have seen a 96% reduction in abundance and range contraction of up to 86% in some cases (Cameron et al. 2011). Ironically, agricultural areas depend most heavily on insect pollination for the production of food and the maintenance of livelihoods (Isaacs et al. 2009; Öckinger & Smith 2007).

In response to the decline in pollinators, some organizations such as the Xerces Society have encouraged the use of properly sourced native plant material in public and private lands especially on working landscapes such as farms and ranches (Vaughan et al. 2015; Xerces Society for Invertebrate Conservation 2021). The tall fescue-dominated cattle pastures of the

Southeastern US, if planted with native forbs, has potential to assist with pollinator conservation. By layering ecosystem services, pastureland can provide forage for cattle and floral resources for pollinators for the benefit of landowners and the environment (Bardgett et al. 2021). Currently, agricultural grasslands are primarily dominated by non-native graminoids and legumes and are actively maintained to discourage non-leguminous forbs (Lüscher et al. 2014; Popp et al. 2000; Rhodes & Phillips 2012). The focus on non-native forbs presents significant challenges in the wider utilization of native flora in agriculture, especially since much less is known about the propagation and establishment of native forbs especially for the Southeast. This lack of propagation and establishment information is a critical stumbling block as pasture enrichment projects utilizing native forbs tend to result in highly unpredictable outcomes (Brudvig et al. 2017; Stuble et al. 2017). The unpredictability of restoration outcomes is a major roadblock for greater implementation of restoration projects especially to landowners whose livelihoods depend on agriculture.

Several methods have been developed to increase native plant diversity in pastures including sod removal (Řehounková et al. 2021), plug planting (Henderson 2010), and reduced grazing pressure to express a native seed and bud bank (Smith 2010). However, especially with larger areas, enrichment via interseeding is often the most cost-effective way to increase native plant diversity. When interseeding pastureland with native forbs, several factors must be taken into consideration if the planting is to be successful including microsite availability, seeding rate, and seed conditioning (Williams et al. 2018). Microsites must be available to provide seeds with the ideal conditions for germination and survival. Pasture vegetation can inhibit this by reducing seed-soil contact and limiting resources through competition (Frances et al. 2010; Millikin et al. 2016; Tognetti & Chaneton 2012; Wilson & Gerry 1995). Tall fescue (*Lolium arundinaceum*) –

one of the most widely planted pasture grasses in the Southeast – forms a dense root mass that excludes native vegetation and is difficult to remove or weaken once established necessitating its removal or disruption before seeding (Watling 2016).

An adequate seeding rate must be achieved for successful establishment and maximum utilization of scarce seed resources (Elzenga et al. 2019). Sites with higher soil organic matter, fertility, and annual precipitation have been shown to have higher weed pressures, making higher seed rates necessary to establish dominant native cover quickly (Kleiman 2016). Often with grassland plantings carried out in the Midwest, higher seeding rates improve the density and richness of sown plants in the resulting stand (Barr et al. 2017; Gardner 2011). Likewise, the rapid germination of sown seed allows target species to stay ahead of annual and biennial weeds that are common in the first few years of a restoration. Seed dormancy can hinder this effort, especially when certain species require stratification or scarification (Kildisheva et al. 2020). For plantings in temperate North America, many taxa have developed dormancy mechanisms that can only be broken with a period of moist cold stratification experienced between late fall and early spring (Baskin & Baskin 2020; Helzer et al. 2010). Restorations that do not take dormancy into account can fail or result in reduced diversity by pushing the plant community towards species with non-dormant seeds (Kildisheva et al. 2020). Alternatively, some restorationists stratify seeds artificially and sow in late spring to early summer to limit predation and pathogen risks in the seedbed and allow for rapid germination of sown seeds that, combined with proper management, should give target species a competitive edge of non-planted vegetation (Williams 2010).

To address the lack of information about how best to establish native forbs in fescue-dominated grazing systems in the Southeast, we evaluated factors that influence native forb establishment during enrichment interseeding of tall fescue-dominated pastures in two separate experiments. First, we established native forbs through a range of site preparation treatments and sowing seasons and compared their effects on tall fescue suppression and seeded native plant establishment hereafter referred to as the site preparation experiment. We hypothesized that native plant establishment in cattle pastures is limited by competition from non-native plants primarily from cool season pasture grasses based on their ability to monopolize site resources and therefore treatments that are better able to disrupt pasture grass cover should facilitate greater target forb cover. Additionally, we hypothesized that native plant establishment is limited by a lack of seed-soil contact as the exotic grasses would decrease the number of microsites available for the establishment of the sown forb seed. Since strip tillage would work to break up the grass root masses and compacted soil, we expected to observe greater target forb cover in areas where bare soil was exposed through strip tilling. Lastly, we expected native plant establishment to be delayed by seed dormancy due to sowing the forb mix in early summer without pre-seeding stratification.

Few target plants established from the site preparation experiment, prompting a second experiment to better understand the effects of seeding rate and pre-stratification. We hypothesized that native forb establishment success would be positively influenced by increasing seeding rate given the abundance of ruderal plant species in intensely managed agricultural land. We expected higher seeding rates to give a competitive advantage to the target species allowing for greater target plant cover. We also hypothesized that pre-seeding stratification would improve native plant establishment and allow for a greater diversity of species to establish more rapidly from an early summer sowing.

Methods

Study area

The two study sites used were located within the Ridge and Valley Province of Virginia in the Shenandoah Valley as well as in Southwest Virginia in the New River Valley. The study area falls within the humid subtropical (Cfa) Köppen climate classification characterized by warm to hot summers and mild winters with precipitation evenly distributed throughout the year (Peel et al. 2007). Annual average precipitation varies from 850 to 1270 mm historically supporting broadleaf deciduous forests as well as woodlands, savannas, and open grasslands (Lafon et al. 2017). Much of the study area has been heavily fragmented and modified especially through fire suppression by five centuries of Euro-American settlement with the area today forming a matrix of secondary forest, urban development, and agricultural land (Fleming 2020). Plots for both experiments were set up at two locations managed by Virginia Tech: the Kentland Research Farm in Blacksburg, Montgomery Co. VA (37°11'45.0"N, 80°34'46.2"W), and the Shenandoah Valley Agricultural Research and Extension Center (SVAREC) in Raphine, VA on the border between Augusta Co. and Rockbridge Co. (37°55'50.8"N, 79°12'43.3"W).

The Kentland farm area for the site preparation and seed and stratification experiments sits on top of a river terrace near the New River at 543 m for the site preparation experiment summer sown and 527 m for the seed and stratification with the area has been used for agriculture since the early 1800s. The soils consist of Unison and Braddock series which are very deep and well-draining soils found on stream terraces, alluvial fans, and footslopes (Soil Survey Staff 2022). The soil is overlaid on Elbrook Formation bedrock composed of dolostone and limestone with lesser shale and siltstone (Horton et al. 2017). The fall sown site preparation experiment sits at 643 m in elevation with soils consisting of Berks-Groseclose complex that are very deep and well drained formed from weathering of limestone, shale, siltstone, and sandstone. The site is also overlaying bedrock composed of Elbrook Formation.

SVAREC has been under cultivation for the past 200 years and the site was most recently maintained as pasture dominated by tall fescue (*Lolium arundinaceum*), orchardgrass (*Dactylis glomerata*), and Kentucky bluegrass (*Poa pratensis*) sod for the last 40 years. The site for the site preparation experiment sits at 536 m in elevation. The soils consist of Frederick silt loam that are very deep and well-drained formed from predominately weathered dolomitic limestone. The soil overlays bedrock made of Conococheague formations. The site for the seed stratification experiment sits 510 m above sea level on the floodplain of the McCormick Mill tributary of Marl Creek. The soil consists predominantly of fluvaquents derived from limestone and dolostone supplied from Conococheague formations underneath (Horton et al. 2017; Soil Survey Staff 2022).

Site preparation experiment

To evaluate the effectiveness of a range of site preparation treatments and different seasons of sowing for the establishing native forbs, we tested eight establishment treatments: planted and unmanipulated, strip tillage, and six herbicides (Table 1). Establishment treatments were applied in 3 × 18-m strips (55 m^2) that were replicated four times per site with replicates grouped into two blocks covering approximately 0.4 hectare in a 6.8-hectare pasture. These blocks were separated by a 9-m buffer strip. Planting season was also a factor with two blocks sown in June 2020 and another planting sown in November 2020. Control plots were seeded but otherwise unmanipulated; tillage plots were plowed twice with a disk plow to a depth of 15 cm to disrupt cool season grass root masses to open space for native forb establishment and seeded;

and herbicide plots had herbicide applied before seeding to weaken the pasture grass sod in order to promote wildflower establishment except with the 2% glyphosate treatment that was intended to fully kill the pasture grasses before seeding. The less severe herbicide treatments were used to investigate the coexistence of pasture grasses and native forbs to maintain forage production for cattle.

Table 1: Treatments used prior to seeding with native forbs in site preparation experiment to weaken pasture grasses in fescue-dominated cattle pastures

Treatment (Active Ingredient)	Application
Control	Seeded and unmanipulated
Pastora (Nicosulfuron & Metsulfuron methyl)	0.110 L/ha + 1% crop oil concentrate
Strip till	Disk plowed twice to 15 cm prior to seeding
1% Glyphosate	0.292 L/ha
Plateau (Imazapic)	0.146 L/ha + 1% methylated seed oil
Cimarron (Metsulfuron methyl & Chlorsulfuron)	0.015 L/ha + 0.25% nonionic surfactant
Select Max (Clethodim)	1.169 L/ha + 0.25% nonionic surfactant + ammonium sulfate at 3.4 kg/ha
2% Glyphosate	4.7 L/ha

Native forb seed was sourced from Ernst Conservation Seed (Meadsville, PA) and were selected based on their palatability and non-toxic nature to cattle as well as commercial seed availability (Table 2). Native forb seeding rate was designed to deliver five seeds per m^2 for each species used. Grazing rotation remained unchanged to see if forb establishment was possible with normal grazing regimes to limit loss of forage production from the establishment period. Cattle were allowed to graze the paddock in one-week periods in May, July, and August 2020 and February, May, June, and July 2021 with individual cattle numbers varying from 48 to 67 in the 6.8-ha pasture where experimental plots were established.

The summer sown plots had treatments applied in early June 2020 with herbicide treatments applied using a tractor mounted sprayer using a 6-nozzle hand-held boom with 11002XR TeeJet nozzles on 46-cm spacing calibrated to deliver 140 liters per hectare. Plots were sown with native forb seed mix in mid-June using a dew drop seed drill (Little Sioux Prairie Co. Spencer, IA) pulled by an ATV with a target seed rate of 7.6 kg/ha^{-1} with 6.8 kg of cracked corn as a carrier material. The fall sowing was planted in mid-November 2020. Due to difficulty in sourcing *Silphium terebinthinaceum* for the fall planting, this species was dropped from the fall seed mix resulting in a lower seeding rate (4.8 kg/ha^{-1}) with 8.1 kg of cracked corn as a carrier Seeding rates for all other species in the fall seed mix remained unchanged. Herbicide application for the fall planting was carried out the April, 2021.

Table 2: Forb species sown into tall fescue-dominated cattle pastures in central and southwest Virginia

Species	Family	Germination Requirements*	Seeds/kg	Seeding Rate (kg per ha)
Agastache foeniculum	Lamiaceae	Nondormant	3,080,000	0.03
Chamaecrista fasciculata	Fabaceae	Cold Moist Stratification	143,000	0.78
Coreopsis lanceolata	Asteraceae	Nondormant	486,200	0.22
Dalea candida	Fabaceae	Nondormant	611,600	0.22

Dalea purpurea	Fabaceae	Nondormant	660,000	0.11
Desmanthus illinoiensis	Fabaceae	Nondormant	187,000	0.56
Desmodium canadense	Fabaceae	Nondormant	159,500	0.11
Desmodium paniculatum	Fabaceae	Nondormant	440,000	0.22
Echinacea purpurea	Asteraceae	Nondormant	254,460	0.44
Gaillardia pulchella	Asteraceae	Nondormant	506,000	0.22
Helianthus maximiliani	Asteraceae	Nondormant	431,992	0.22
Heliopsis helianthoides	Asteraceae	Cold Dry Stratification	224,400	0.44
Lespedeza capitata	Fabaceae	Cold Moist Stratification	605,000	0.22
Lespedeza virginica	Fabaceae	Cold Moist Stratification	352,000	0.33
Ratibida columnifera	Asteraceae	Moist Cold Stratification	1,478,400	0.11
Ratibida pinnata	Asteraceae	Nondormant	978,942	0.11
Rudbeckia hirta	Asteraceae	Cold Moist Stratification	3,466,672	0.033
Silphium perfoliatum	Asteraceae	Cold Moist Stratification	220,000	0.44
Silphium terebinthinaceum	Asteraceae	Cold Moist Stratification	37,400	2.9
Solidago canadensis	Asteraceae	Cold Moist Stratification	10,120,000	0.011
Total				7.72

*Germination requirements derived from Prairie Moon Nursery Winona, MN

Seed rate and stratification experiment

To elucidate the influence of seeding rate and pre-seeding stratification on native forb establishment, our second experiment tested 4 seeding rates (2.24, 11.2, 28, 56 kg/ha^{-1}) using either stratified or non-stratified seeds. Native forb seed was purchased from Ernst Conservation Seeds (Meadville, PA). We used a nested block design with thee replicates per site. The area within each 6 m × 3.8 m plot was divided into eight 1-m^2 subplots, two for each seeding rate that were located next to each other separated by a 10-cm buffer (Fig. 2), following the method of Groves & Brudvig (2019). The experimental sites were prepared with a 2% glyphosate application, tilled using a 1.5-m wide tractor mounted Land Pride rototiller, and sprayed again prior to planting with two-week periods in between each activity to kill the aboveground vegetation, loosen soil, and break up grass root masses. The stratified seeds were mixed with moist peat moss and refrigerated four weeks prior to sowing. Seeds were sown between June 13-15th in 2021 and 2022.

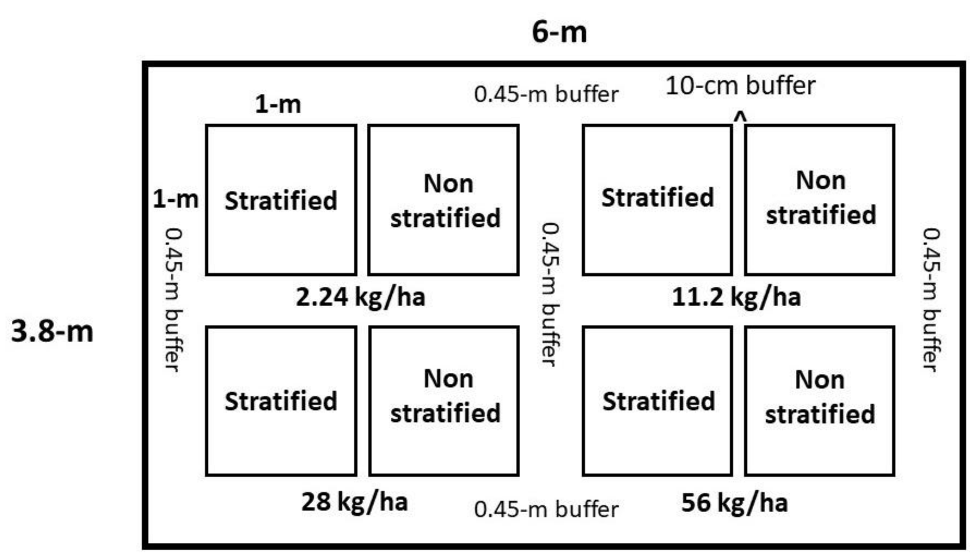

Fig. 1. Experimental layout for one replication of the seed rate and stratification plot. Seeding rate plots split into m² plots with one half sown with stratified seed and the other half sown with non-stratified seed at indicated seeding rate separated by a 10 cm buffer. A 0.45 m buffer surrounds the plots on all sides.

Seeds were mixed with sand and hand broadcast over the plots with non-stratified seed also mixed with peat moss at time of sowing. Plots were then raked to incorporate seeds into the soil. When vegetation growth reached 37.5 cm, vegetation was cut back to 11±4 cm to prevent weed growth from overtaking target seedlings. At the end of the 2021 growing season, we observed that *Heliopsis helianthoides* was aggressively dominating the experimental plots. This was especially evident at the SVAREC where *H. helianthoides* almost completely dominated the sown target vegetation to the detriment of other target plants especially the target grasses used in the seed mix. Due to the possibility that expected trends were masked by the proliferation of *H. helianthoides*, we decided to treat the 2021 experiment as a pilot study and replicate the experiment in 2022. New plots were established adjacent to the 2021 plots with minor adjustments to the seed mix including a reduced amount of *H. helianthoides* and a slight increase in the 3 warm-season grasses used. Further, the 2022 Kentland plots had only one round of herbicide application due to issues with equipment availability. These plots were tilled too close to planting time to make a second herbicide spray feasible.

The seed mix contained 16 species: 13 forbs and 3 grasses with a ratio of 40% grass to 60% forbs on a Pure Live Seed (PLS) basis (Table 4). Species choice reflected a wide range of taxa for deriving broader conclusions with species falling into three broad functional groups: grasses, forbs, and legumes. Species were also selected based on being native to Eastern North America and being heliophytic.

Table 4: Species list for seed rate and stratification experiment

Species	**Family**	**Dormancy**	**Seeds/kg**
Bouteloua curtipendula	Poaceae	Nondormant	349,800
Coreopsis lanceolata	Asteraceae	Nondormant	486,200
Dalea purpurea	Fabaceae	Nondormant	660,000
Desmanthus illinoensis	Fabaceae	Nondormant	187,000
Eryngium yuccifolium	Apiaceae	Cold Moist Stratification	391,600
Heliopsis helianthoides	Asteraceae	Cold Dry Stratification	224,400
Lespedeza virginica	Fabaceae	Cold Moist Stratification	352,000
Pycnanthemum virginianum	Lamiaceae	Nondormant	8,518,400
Rudbeckia hirta	Asteraceae	Cold Moist Stratification	3,466,672
Schizachyrium scoparium	Poaceae	Cold Dry Stratification	530,200
Senna hebecarpa	Fabaceae	Cold Moist Stratification	44,000
Solidago juncea	Asteraceae	Cold Moist Stratification	5,583,600
Symphyotrichum lateriflorum	Asteraceae	Nondormant	1,760,000
Tradescantia ohiensis	Commelinaceae	Cold Moist Stratification	281,600
Tridens flavus	Poaceae	Cold Moist Stratification	1,023,000
Zizia aurea	Apiaceae	Cold Moist Stratification	378,400

Data collection

Data collection for the site preparation experiment occurred between September and November of 2021 and data was collected on the seed and stratification experiment in from September to November 2022. For the site preparation experiment, we measured vegetation by laying a transect along the length of each treatment plot and placing a 1-m² quadrat at 3.0, 7.6, and 12 m. We recorded vegetation that fell within and consisted of estimated percent cover for grasses and non-target forbs. Stem count of each target species and target species identity were recorded for the entire treatment plot. For the seed rate and stratification experiment, the quadrat was laid over the subplots and data recorded on the vegetation that fell within. Data consisted of percent cover of target species, stem count of target species broken down by species, and percent cover of non-target vegetation. Target plant establishment was considered successful at a density of between two to three target plants per m^2. This benchmark was derived from Morgan et al. (1995) recommendation that put a minimum density for a successful planting at five target forbs per m^2 and this was reduced to match the agricultural priorities of this project.

Analysis

All statistical analyses were performed using R Statistical Software with R Studio (R Version 4.2.0 R Core Team 2022; RStudio Version 2022.07.1.544 RStudio Team 2022). For the site preparation experiment, we used generalized linear mixed effects regression to evaluate the effects of the site preparation treatments (8 levels) and season sown (fall, summer) on target forb density and richness. Mixed effect models were used to account for the random effects from site location and block with block effects nested within site location. We used the following R packages to complete the analysis for site preparation experiment: lme4 was used to run the linear regressions (Bates et al. 2015); lmerTest was used to generate *p* values for the linear regression output (Kuznetsova et al. 2017); and AICcmodavg was used to generate AICc numbers and rank models based on the lowest AICc (Mazerolle 2020). We modeled target species stem abundance and richness using a Poisson distribution as the data did not conform to a Gaussian distribution.

A series of models were constructed in increasing complexity and compared using Akaike Information Criterion (AICc) numbers, corrected for small sample size to select the most parsimonious model for investigation (Burnham & Anderson 1998). Models compared include null [intercept only], establishment treatment, season sown, establishment treatment + season sown, and establishment treatment × season sown (Table S1). By comparing AICc number, we found that establishment treatment × season sown was the most prudent model indicating an interaction between establishment treatment and season sown. Similar results were obtained for the model selection for target species richness (Table S2). After model selection, we ran Tukey's HSD tests to determine pairwise differences between treatment levels. Percent cover of grasses were analyzed using linear mixed effect regression models due to its approximate conformity to a Gaussian distribution and similar model was run for non-target vegetation that is presented in the supplemental materials. Model performance was reported using R^2 and calculated using the 'performance' package (Lüdecke et al. 2021).

For the seed and stratification experiment, we used linear mixed effect regression to evaluate the effect seeding rate and pre-seeding stratification had on target plants per m^2 and richness because these data consisted of larger numbers and approximated a normal distribution. A similar method for model construction was performed with models including null [intercept only], seed rate, stratification, seed rate + stratification, and seed rate × stratification. From the AICc numbers, the model seed rate + stratification was found to be the most prudent. After model selection, we ran Tukey's HSD tests on target species richness to determine pairwise differences between treatment levels. Inkscape was used in the final preparation of all graphs presented (Inkscape 2022).

Results

Site preparation experiment

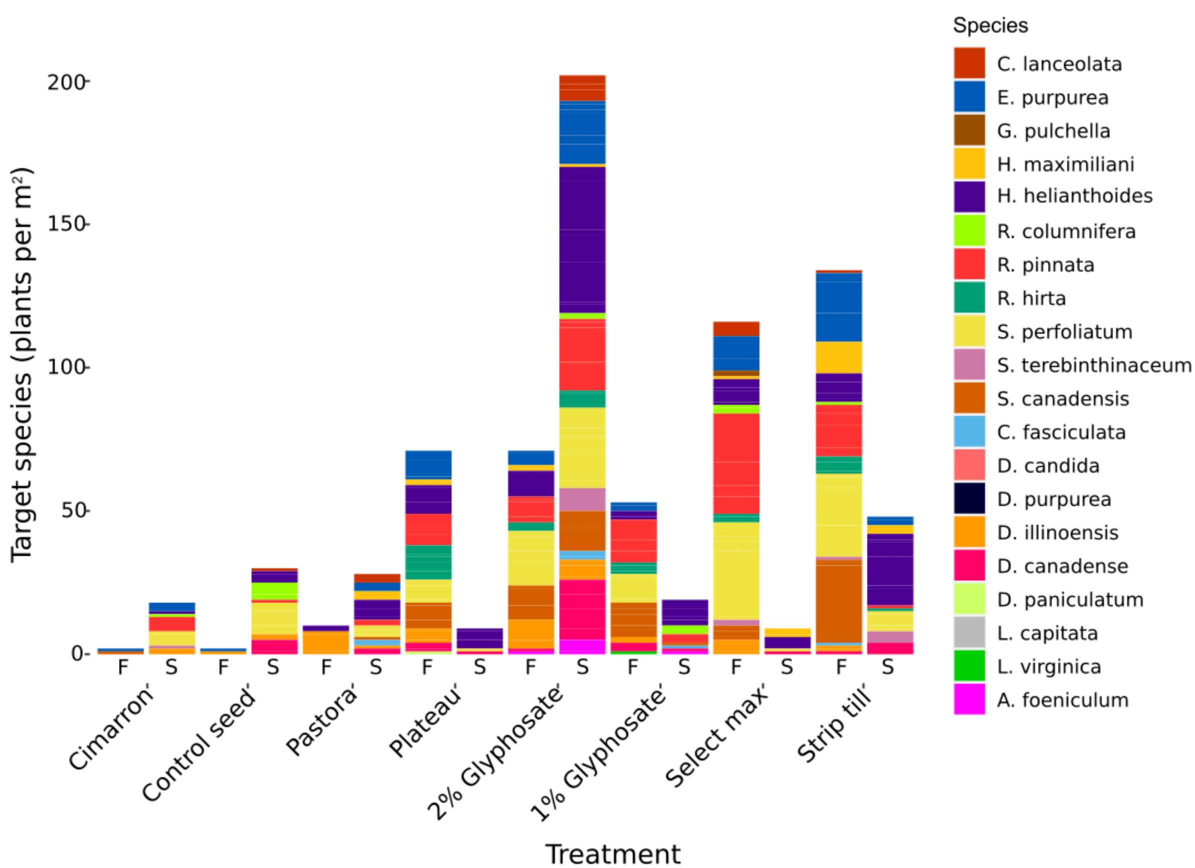

Fig. 2: Sum of target plants per m^2 broken down by individual species and compared by site preparation and season of sowing. F = fall sown, S = summer sown

We observed a mean target plant density of 0.12 target plants per m^2 (*SD* = 0.247). Of the 20 target forbs sown, less than half were observed during sampling with the most observed target forbs including *Silphium perfoliatum* (19.1%), *Heliopsis helianthoides* (18.4%), *Ratibida pinnata* (15.2%), *Solidago canadensis* (10.2%), and *Echinacea purpurea* (10.6%) that accounted for 73.5% of all observed target plants (Fig. 2).

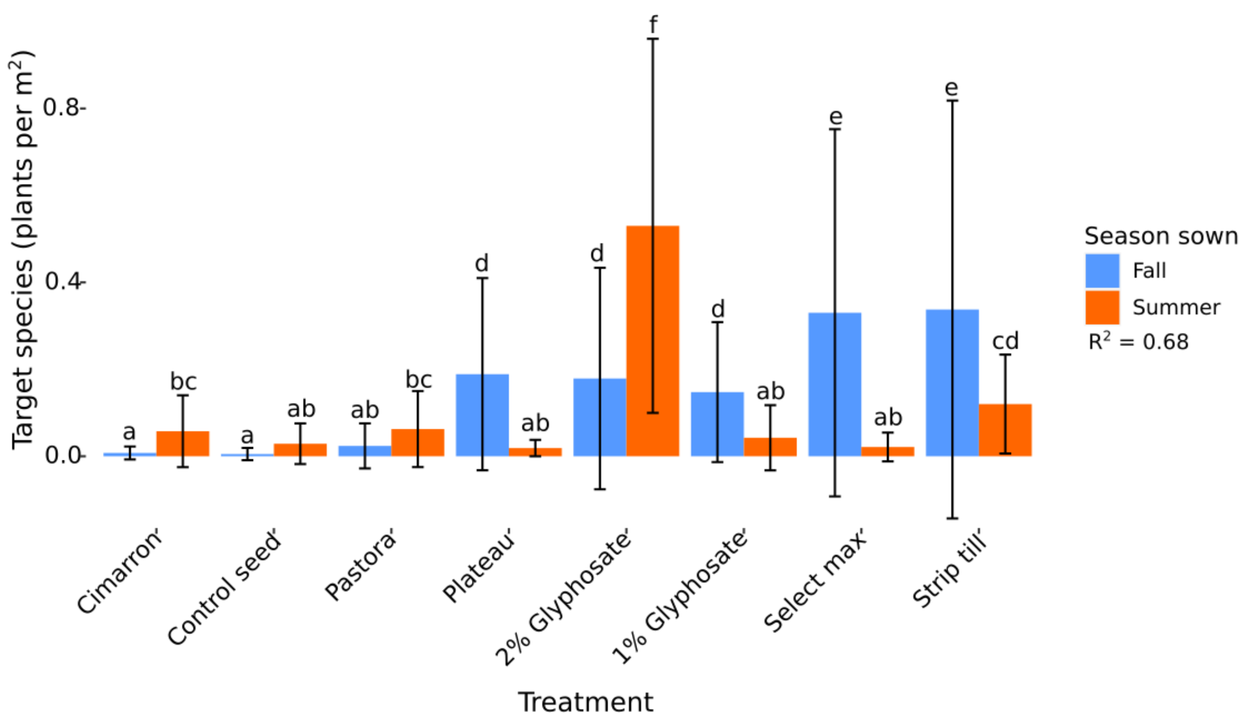

Fig 3. Mean target plants per m^2 compared by treatment and season sown. Letters denote groups that differ with $P < 0.05$ in a Tukey's HSD test and error bars created using standard deviation of data.

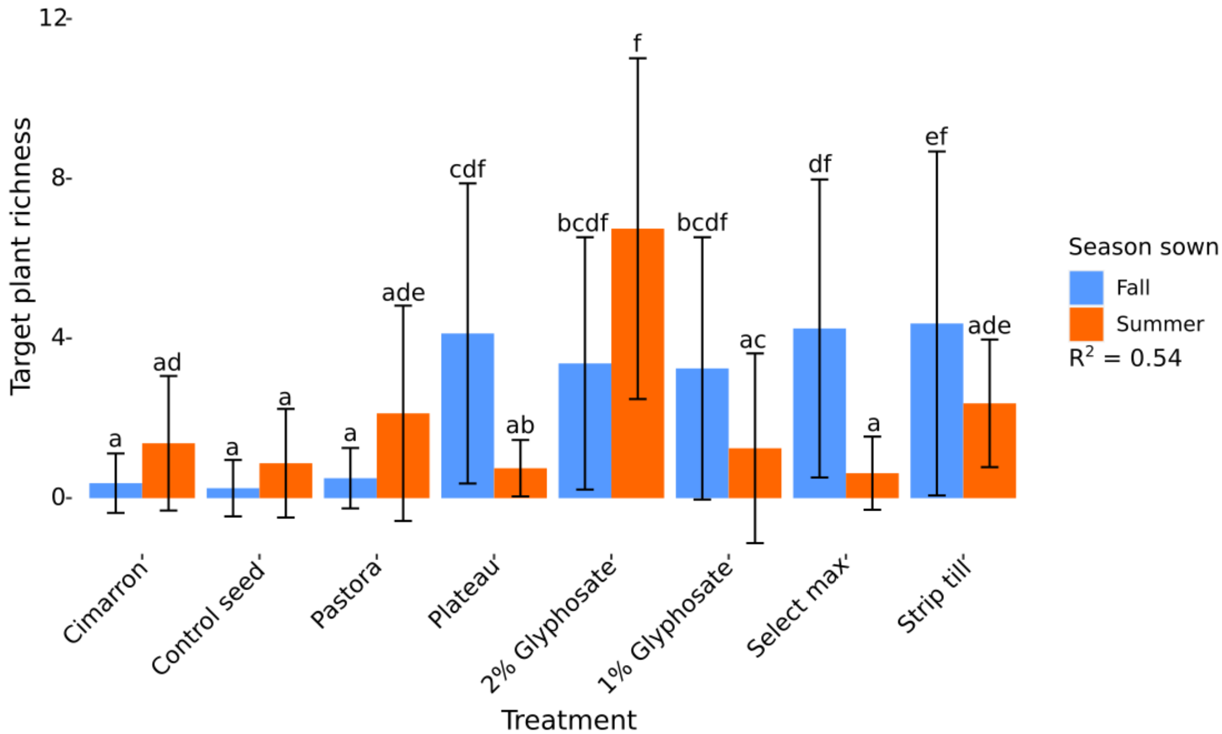

Fig 4. Target plant richness compared by treatment and season sown. Letters denote groups that differ with P < 0.05 in a Tukey's HSD test and error bars created using standard deviation of data.

There were significant differences in target plant density and richness between treatment levels shown in the results of the Tukey's HSD tests (Fig. 3 & 4). The most effective treatment was the summer sown 2% glyphosate as there was very strong evidence that summer sown 2% glyphosate treatment had both higher mean target plant density at 0.35 target plants per m^2 (SD = 0.386) ($P < 0.0001$) and richness of target plants ($P = 0.0002$) than the control treatment (Table 5, Fig. 3). Further, there is very strong evidence that the 2% glyphosate treatment was the most effective at reducing pasture grass competition ($P < 0.0001$) (Fig. 5). Several fall-sown treatment strips had intermediate target plant richness and stem count. These included strips prepared with Plateau, 2% Roundup, 1% Roundup, Select Max, and strip tilling (Figs. 3 & 4).

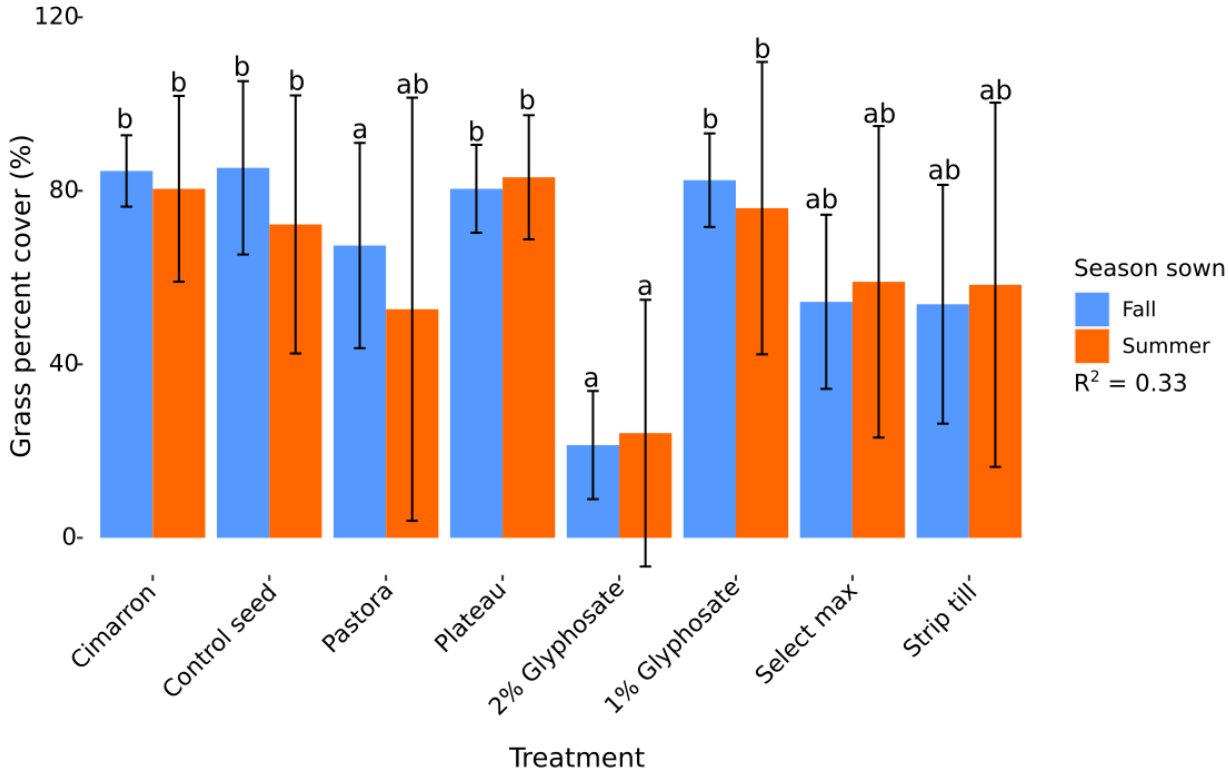

Fig. 5: Pasture grass percent cover comparison across treatments and season of sowing. Letters denote groups that differ with $P < 0.05$ in a Tukey's HSD test with error bars created using standard deviation of data.

Table 5: Parameter estimates for the associations between site preparation treatment, season of sowing, target plant density, and richness

Model	Parameters	Estimate	SE	Z	*P**
Plant abundance [plants per m^2]					
	Intercept [Control, Fall]	-0.405	0.645	-0.445	0.655
	Treatment [Cimarron]	-1.297	0.911	-2.011	**0.044**
	Treatment [Pastora]	1.203	0.656	1.832	0.066
	Treatment [Plateau]	3.319	0.586	5.661	**<0.0001**

	Treatment [2% glyphosate]	3.270	0.586	5.572	**<0.0001**
	Treatment [1% glyphosate]	3.075	0.589	5.219	**<0.0001**
	Treatment [Select max]	3.877	0.582	6.662	**<0.0001**
	Treatment [strip tillage]	3.898	0.581	6.699	**<0.0001**
	Season [Summer]	2.119	0.609	3.477	**0.0005**
	Season [Summer] × Treatment [Cimarron]	0.821	0.353	2.328	**0.0199**
	Season [Summer] × Treatment [Pastora]	-1.092	0.712	-1.531	0.125
	Season [Summer] × Treatment [Plateau]	-4.459	0.712	-6.255	**<0.0001**
	Season [Summer] × Treatment [2% glyphosate]	-1.034	0.623	-1.659	0.0971
	Season [Summer] × Treatment [1% glyphosate]	-3.349	0.662	-5.053	**<0.0001**
	Season [Summer] × Treatment [Select max]	-4.899	0.699	-7.004	**<0.0001**
	Season [Summer] × Treatment [strip till]	-3.146	0.630	-4.993	**<0.0001**
Plant richness					
	Intercept [Control, Fall]	-0.405	0.907	-0.447	0.655
	Treatment [Cimarron]	-1.149	0.612	-1.875	0.060
	Treatment [Pastora]	0.287	0.759	0.379	0.704
	Treatment [Plateau]	2.397	0.599	3.998	**<0.0001**
	Treatment [2% glyphosate]	2.197	0.605	3.630	**0.0002**
	Treatment [1% glyphosate]	2.159	0.606	3.561	**0.0003**
	Treatment [Select max]	2.427	0.599	4.053	**<0.0001**
	Treatment [strip tillage]	2.456	0.598	4.106	**<0.0001**
	Season [Summer]	1.299	0.647	2.006	**0.044**

Season [Summer] × Treatment [Cimarron]	0.150	0.368	0.408	0.6833
Season [Summer] × Treatment [Pastora]	0.147	0.851	0.173	0.862
Season [Summer] × Treatment [Plateau]	-3.004	0.783	-3.832	**0.0001**
Season [Summer] × Treatment [2% glyphosate]	-0.606	0.688	-0.880	0.378
Season [Summer] × Treatment [1% glyphosate]	-2.254	0.746	-3.022	**0.0025**
Season [Summer] × Treatment [Select max]	-3.216	0.804	-4.000	**<0.0001**
Season [Summer] × Treatment [strip till]	-1.910	0.707	-2.702	**0.0069**

* Significant results highlighted in boldface type

Seed & stratification experiment

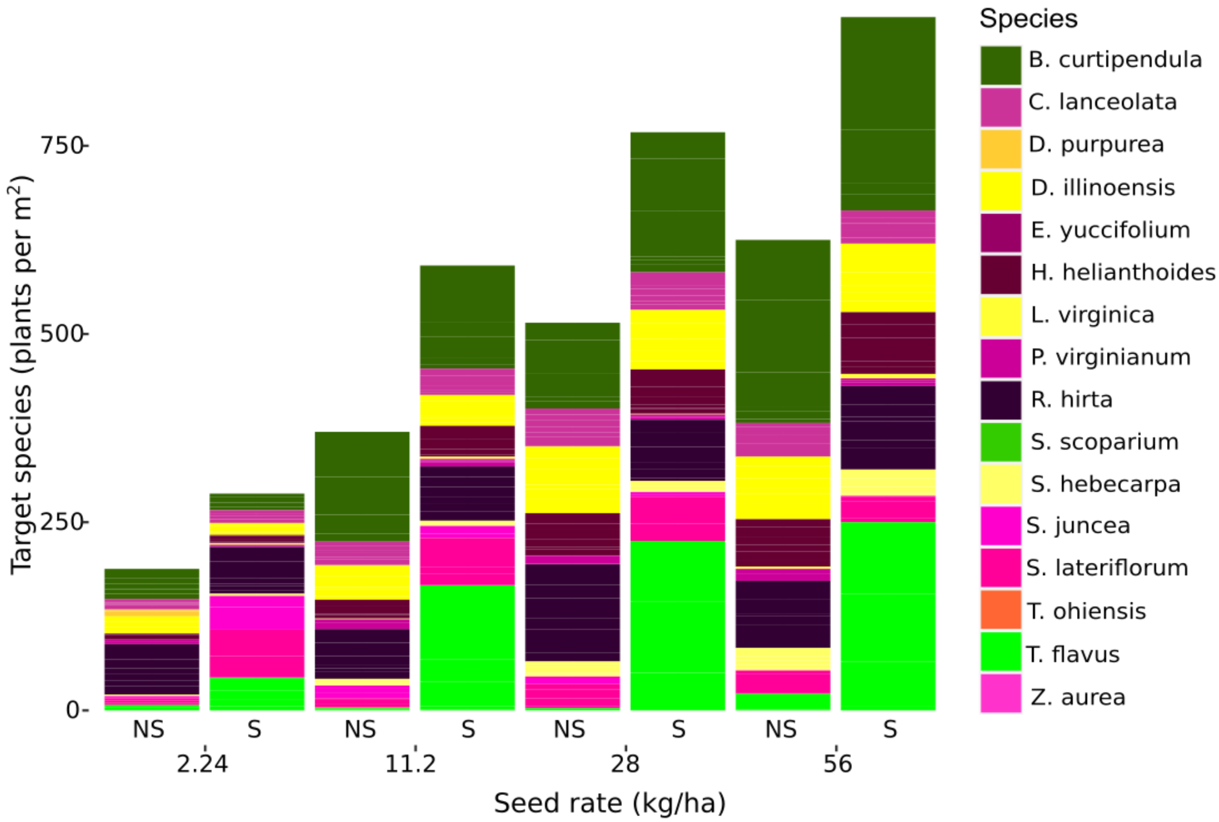

Fig. 4: Sum of target plants per m^2 broken down by target species observed compared by seed rate and pre-seeding stratification. NS = non-stratified, S = stratified.

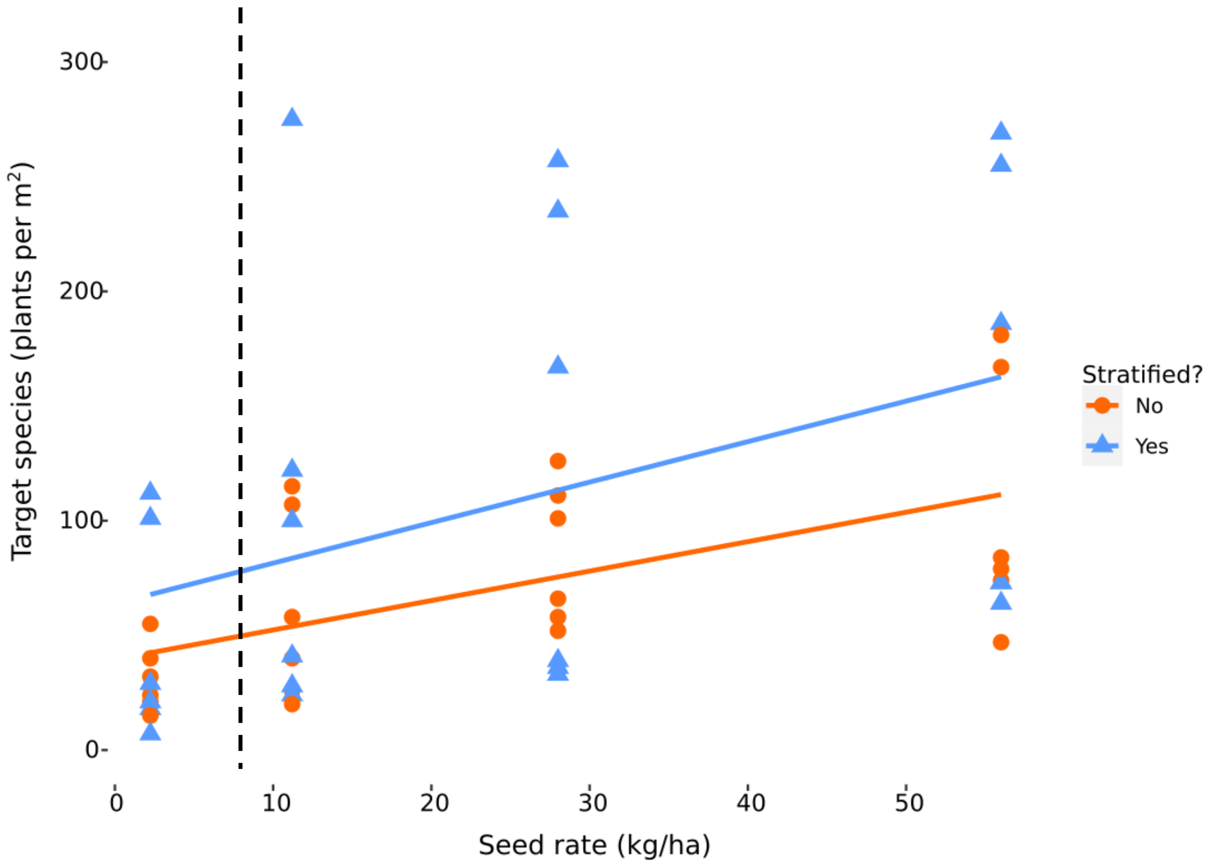

Fig. 5: Mean target plant density per m^2 compared by seed rate and pre-seeding stratification (R^2 = 0.22; seed rate: P = 0.00000499, stratification: P = 0.00409) Black dashed line indicates summer seeding rate used for the site preparation experiment at 7.62 kg/ha^{-1}.

We observed a mean target plant density of 88 target plants per m^2 (SD = 73.9) with the most common observed target species included *Heliopsis helianthoides*, *Rudbeckia hirta*, *Coreopsis lanceolata*, *Tridens flavus*, *Desmanthus illinoiensis*, *Bouteloua curtpiendula*, and *Senna hebecarpa* (Fig. 4). There were significant differences in target plant density between treatment levels. There was strong evidence of a positive effect of seed rate on target plant density (P < 0.0001) (Fig. 5, Table 6). Compared to 2.24 kg/ha^{-1}, a seed rate of 56 kg/ha^{-1} resulted in almost three times as many target plants while a seed rate of 28 and 11.2 kg/ha^{-1} resulted in twice and one and half times as many target plants respectively. There was also strong evidence that pre-seeding stratification had a positive effect on target plant density (*P* < 0.00409) (Fig. 5).

Table 6: Parameter estimates for the associations between seed rate, season of sowing, and target plant density and richness

Model	Parameters	Estimates	SE	df	t value	*P**
Plant abundance [plants per m^2]						
	Treatment [Seed rate]	1.524	0.289	40	1.357	< **0.0001**
	Stratification [Y]	36.08	11.85	40	3.046	**0.0040**
Plant richness						
	Treatment [Seed rate]	0.004	0.009	40	0.415	0.681
	Stratification [Y]	0.041	0.408	40	0.102	0.919

*Significant results highlighted in boldface type

Target plant richness was found to be uniform across all treatments from results of the Tukey's HSD test with a mean of seven species observed in each subplot (*SD* = 1.83) (Fig. S5). There was more variability in the number of species found for the 2022 plots, but the Tukey's HSD test and linear model found there was no evidence of significant differences in richness between treatment levels (Table 7).

Discussion

In this study, we investigated the factors that influence establishment success of native forbs seeded into tall fescue-dominated cattle pastures through two experiments. The first manipulated a number of site preparation techniques and sowing season to select the technique that resulted in the high target plant abundance and richness while second experiment manipulated seed rate and pre-seeding stratification to determine the optimum seeding rate for establishment success and what effect stratification would have on target plant abundance and richness. In the site preparation experiment, we observed a mean target plant density of 0.12 target plants per m^2 (*SD* = 0.247) and that summer sown 2% glyphosate treatment gave the highest target plant abundance and richness. Fall sown plots had higher target plant abundance and richness than summer sown plots. In the seed and stratification experiment, we observed a mean target density of 88 target plants per m^2 (*SD* = 73.9) Target plant abundance was significantly different between treatment levels with 56 kg/ha^{-1} yielding almost three times as many plants compared with 2.24 kg/ha^{-1} with evidence indicating that stratification positively influenced target plant abundance. Lastly, richness was not significantly different between treatment levels with a mean target plant richness of 7 species per plot (*SD* = 1.83).

Controlling cool-season pasture grasses

The first step of any seed-based restoration or enrichment project is the disruption of existing vegetation to facilitate the establishment of sown species (Smith et al. 2010). If not disrupted sufficiently, existing vegetation, particularly non-native cool season pasture grasses, compete directly with sown species and result in reduced vigor of the latter (Williams et al. 2018). We found that 2% glyphosate was better at controlling pre-existing vegetation than other treatments that took a more conciliatory route of weakening the pasture grasses. Summer sown 2% glyphosate had greater target plant richness and higher target plant abundance than any other treatment tested. Glyphosate has been shown to be an effective tool for eliminating or suppressing a wide range of vegetation, including cool-season pasture grasses in preparation for the sowing of native plant seeds (Gardner 2011; Smith et al. 2010). The reasoning behind weaker treatments was to find a strategy that would severely weaken the pasture grasses for target forb establishment while avoiding total suppression to maintain forage production for cattle. Because summer sown 2% glyphosate had the highest target richness and abundance, this suggests that the less severe treatments were not able to suppress the cool-season pasture grasses enough to connote an advantage to the sown forbs resulting in low numbers of sown forbs due to grass competition. While it is also possible to direct seed native forbs into established pasture grass sod without major disruption to the existing plant community, our results are consistent with the interpretation that native forb establishment can be much slower and can result in a lower density of sown forb compared to other methods (Smith et al. 2010). This maybe an acceptable compromise for landowners whose livelihoods depend on forage production for economic survival.

Fall sowing improved establishment

Given that between 50-90% of wild plants globally produce seed that is dormant upon maturity (Kildisheva et al. 2020), managing dormancy is one of the key concerns when implementing seed-based restorations or enhancements. Given the high cost of native forb seed, it is imperative that seed be used efficiently to ensure adequate establishment with limited waste (Kildisheva et al. 2020). In the site preparation experiment, fall sown plantings had higher mean target plant richness and abundance than summer sown plantings even with a lower seeding rate of 4.1 kg/ha^{-1} compared to the summer with 7.62 kg/ha^{-1}. Even though summer sown 2% glyphosate was the most effective treatment, the next five best treatments including Plateau, 2% glyphosate, 1% glyphosate, Select max, and strip tillage were all fall sown. In North America, many forb seeds require exposure to cold damp conditions for several months in order to germinate (Baskin & Baskin 2004) leading many practitioners and researchers to sow in the fall for natural stratification over winter and spring (Smith et al. 2010).

While planting non-stratified seed in late spring may not necessarily lead to planting failure, it may reduce the number of nondormant seeds available for rapid germination and allow for greater mortality from pathogens and predation while seeds wait for appropriate dormancy-breaking conditions (Hemsath 2007; Smith et al. 2010). Additionally, while summer sown plots had herbicide treatment applied in early June 2020, fall sown plots were sprayed in early April 2021. This may have contributed to higher target plant richness and abundance due to enhanced tall fescue control as it has been shown that spring applications of glyphosate after thatch removal are better at reducing tall fescue cover over spraying at other times of the year (Barnes 2004; Madison et al. 2001). This combination of increased germination from natural stratification and enhance cool season grass control allowed for greater establishment in the fall sown plantings.

Potential causes of low native forb establishment

Seed-based planting outcomes are subject to high variability (Brudvig et al. 2017) and a successful outcome can look different for different groups and priorities. Conservationists who strive to restore lost grassland ecosystems would likely use Morgan et al. (1995) benchmark which defines successful establishment at least five target plants per m^2 while cattle producers may consider a planting successful at a lower benchmark such as two – three target plants per m^2. While summer sown 2% glyphosate had the highest target plant abundance and richness, it had a mean target plant density of 0.35 target stems per m^2 (SD = 0.247) – likely too low to be self-sustaining in the face of non-target species competition. Further, 2% glyphosate treatment strips were often dominated by ruderal species both native and non-native that took advantage of reduction of grass cover and competed directly with target forbs present (Fig. S3). 2% glyphosate treatment strips were revisited in 2022 to address the potential concern that establishment-year trends were not representative of second- or third-year trends in regard to an increase in target plant abundance as the planting matures. However, target plant abundance was not found to significantly higher than in the second growing season year 2021 (Fig. S1 and S2).

Several factors could account for the inadequate establishment in the site preparation experiment including inadequate site preparation, lower seed rate, and failure to mow planting during first growing season. Due to the aggressive nature of many non-native cool season pasture grasses, it is often necessary to severely disrupt or eliminate these grasses prior to seeding for native planting to succeed (Coon et al. 2021). The results of site preparation experiment support the notion that non-native cool season grasses and native forbs are incompatible with each other as summer sown 2% glyphosate, the treatment that reduce pasture grass cover the most, had the highest mean target plant abundance and richness. For the best establishment of native forbs, dominate cool season pasture grasses need to be eliminated or severely disrupted prior to seeding. Even though the 2% glyphosate had the desired effect of eliminating or severely reducing the existing plant community, the treatment still resulted in an insufficient density of sown forbs. This insufficient establishment could be attributed to an inadequate seed rate. Higher seeding rates may be necessary given the abundant weed seed bank (Renne & Tracy 2007) and high nutrient availability in pasture systems (Bellows 2001).

Lastly, mowing was not utilized to control non-target vegetation post seeding. The first growing season of a new planting is the most vulnerable stage where target seedlings can be easily overtaken by non-target vegetation. This results in a closed canopy that reduces light levels, stunting target seedlings and increasing mortality among target plants if no management is taken (Smith et al. 2010). Mowing reduces canopy height, improves light penetration, and can accelerate target plant establishment (Meissen et al. 2020; Williams et al. 2007). By not maintaining a low canopy height through mowing, target forbs could have been stunted by non-target vegetation released from pasture grass competition resulting in low numbers of established forbs even in treatments where pasture grasses were eliminated.

Effect of high seed rates

In the site preparation experiment, it was observed that target forb establishment was below acceptable levels and we proposed that poor establishment could be tied to an insufficient seeding rate and sowing of non-stratified seed in the summer and developed the seed and stratification experiment in investigate these factors. We predicted that target plant establishment would be positively influenced by higher seeding rates and pre-seeding stratification. As predicted, the higher seed rates resulted in greater and more consistent abundance of target plants. When it comes to seed-based restorations, practitioners often select for higher seeding rates to ensure success. Rowe (2010) found that in a survey of 20 tallgrass prairie managers, 50% used seeding rates at or greater than 11.2 kg/ha^{-1}. Given the potential high levels of seed mortality from predation in prairie restorations (Linabury et al. 2019; Pellish et al. 2018), higher seed rates allow for greater losses while still maintaining sufficient a number of seeds for adequate germination and establishment while also buffering against stochastic events (Groves et al. 2020; Jaksetic et al. 2018). Further, intense site preparation techniques employed and management of canopy cover during the establishment period may have contributed to higher target plant abundance. Our results are consistent with the understanding that higher seeding rates along with thorough site preparation and non-target vegetation management results in higher target plant abundance.

A more surprising result was the establishment observed at the lowest seeding level of the seed and stratification experiment. Given that 7.62 kg/ha failed to establish a sufficient number of plants in the site preparation experiment, 2.24 kg/ha was expected to fail from insufficient seed numbers. However, this level was able to establish a sufficient number of target plants despite often heavy competition by non-target vegetation. This could a result of site preparation used to prepare the seed bed and management of non-target vegetation during establishment. Plots were prepared with two applications of 2% glyphosate and tillage to eliminate existing vegetation and prepare seed bed for sowing. Further during establishment year, vegetation was routinely cut back to maintain a height of 11±4 cm to prevent excessive weed competition with sown species. These two management decisions may have allowed for the increased target plant establishment in the lowest seeding rate that won't be viable when interseeding into a weakened cool season grass sod. Without elimination of the existing vegetation, the aggressiveness of the cool-season grasses could result in insufficient target plant establishment from lowest seeding rate.

Pre-seeding stratification

For plantings sown during late spring and early summer, species that require cold moist stratification to break dormancy would have to wait until winter before being able to germinate in the planting's second spring. This exposes dormant seeds to seed predation which can happen rapidly in the field (Pellish et al. 2018). Artificial stratification can be used to mitigate this and can allow for prompt germination for species that require natural cold stratification (Schramm 1976; Smith et al. 2010). Stratification was found to positively effect target plant abundance with some species like *Tridens flavus* being much more abundant in stratified plots compared to unstratified plots. However, the four-week stratification period may not have been long enough to break the dormancy of some plants as several species including *Zizia aurea* and *Eryngium yuccifolium* were observed in greater abundance after plots had undergone a period of natural stratification. This suggests that a longer artificial stratification period of 60 – 90 days may better for satisfying the dormancy requirements of some forbs. Further, some non-dormant species such as *Dalea purpurea* began germinating while being cold stratified and this may have led to increased mortality at planting time. For planting in late spring or early summer, artificial stratification can be beneficial by allowing for more rapid germination of sown species.

Recommendations

From the results of this study, we produced several recommendations to inform landowners when interseeding tall fescue-dominated cattle pastures with native forbs. They are:

1. Even if the ultimate land management objective is to create a pasture that mixes native warm season grasses and forbs with cool-season forage grasses, completely eliminating cool-season grass turf with glyphosate prior to seeding native plants is much more effective at facilitating target plant establishment than weakening cool-season grasses with other site preparation techniques.
2. For many native plant species, sowing in fall or using an artificial cold stratification and sowing in early summer result in greater native plant establishment.
3. Greater seeding rates produced greater seedling establishment, but even relatively low seeding rates may be compensated through intensive site preparation and post-seeding vegetation management.

Future directions

Several challenges remain to developing cost-effective methods for establishing native forbs in fescue-dominated Southeastern grazing systems. Aside from several native warm season grasses and forbs, many native forbs and regional ecotypes lack commercial seed sources and the remainder can be prohibitively expensive for large-scale use. Further, to increase the ecological value to the local landscape, landowners should be encouraged to use local genotype seed most appropriate for use in the Southeast to preserve unique genetic diversity (Bischoff et al. 2010). However, native seed production in the Southeast is limited in scope and production capacity making it difficult to locate large quantities of local genotype seed for restoration and enrichment plantings (White et al. 2018). Improving the production capability of the Southeast native plant industry is crucial in to increasing the availability of native plant material for restoration, lower the cost of local ecotype native seed, and increase awareness of native plant to a wider audience (McCormick et al. 2021).

Despite better understanding as a result of this study, we still lack a method to guarantee consistent forb establishment. More research is needed to refine site preparation techniques that can result is the establishment of a sufficient density of target plants without killing off the cool season pasture grasses. After seeding, a minimum density for stand success should be determines as a definitive metric is lacking for our region and would be beneficial for landowners evaluating native forb establishment. Lastly, research projects should be carried out at larger scale especially in regard to the seeding rate and stratification which utilized microplots in this study and carried out across the tall fescue belt to generate management strategies applicable for the Southeastern fescue region.

Conclusion

Understanding the mechanisms that influence the establishment success of native forbs interseeded into tall fescue-dominated cattle pastures is a critical front in the struggle to stem biodiversity loss. Further, through using local genotype native plant material, such interseeding projects can not only increase resources availability for insect pollinators but also conserve grassland flora that was much more extensive throughout the Southeast in the past. Our objectives in this study were to investigate site preparation techniques and sowing season to facilitate the establishment of native forbs into tall fescue-dominated cattle pastures during active grazing and evaluate the effect seed rate and pre-seeding stratification had on target plants.

For the site preparation experiment, we found that summer sown 2% glyphosate treatment resulted in the highest target plant abundance and richness. Despite this, the next five best treatment all consisted of fall sown replicates that had on average higher target plant abundance and richness than summer sown replicates. Our results support the notion that heavy pasture grass competition can reduce the speed and magnitude of native forb establishment into cool season pasture grasses and that glyphosate is an appropriate tool for reducing pasture grass competition. However, the summer sown 2% glyphosate plots had a mean target density of only 0.35 plants per m^2 ($SD = 0.386$) and plots were often overrun with non-target vegetation, suggesting insufficient forb establishment for planting to be considered successful.

For the seed rate and stratification experiment, we observed a mean target plant density 88 target plants per m^2 ($SD = 73.9$). The experiment was replicated in 2022 due to plots being dominated by *Heliopsis helianthiodes*. Target plant abundance was found to be different between treatment levels. Seed rate was found to be significant with a seed rate of 56 kg/ha^{-1} resulting in almost three times as many target plants compared to 2.24 kg/ha^{-1}. Stratification positively influenced target plant abundance. Richness was not found to change significantly between treatment levels.

From this study, we recommend that landowners 1) elimination of cool season pasture grasses prior to seeding; 2) sow in the fall or use stratified seed to increase germination percentage and speed; and 3) greater seeding rates produced greater seedling establishment though low seeding rates can be compensated with intense site preparation and post-seeding vegetation management. Further research is needed to refine site preparation techniques, determine minimum target plant density for success, and replicate research trials on a larger scale across the tall fescue belt of the Southeast for applicable management strategies for the region.

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Supplemental Materials

Table S1: Model selection for target stem analysis for site preparation experiment

Model	K	AICc	Δ AICc	AICc Wt	Cum. Wt	LL
Total Target Stem ~ treatment × Season + random effects	**17**	**994.32**	**0.000**	**1**	**1**	**-477.57**
Total Target Stems ~ treatment +Season + random effects	10	1364.5	370.1	0	1	-671.35
Total Target Stems ~ treatment + random effects	9	1384.1	389.8	0	1	-682.34
Total Target Stems ~ Season + random effects	3	1996.2	1001.9	0	1	-995.01
Total Target Stems ~ 1 + random effects	2	2032.4	1038.1	0	1	-1014.2

Table S2: model selection for target species richness for site preparation experiment

Model	K	AICc	Δ AICc	AICcWt	Cum. Wt	LL
Target plant richness ~ Treatment × season + random effects	**17**	**505.59**	**0.00**	**1.00**	**1.00**	**-233.20**
Target plant richness ~ Treatment +season + random effects	10	567.78	62.18	0.00	1.00	-273.01
Target plant richness ~ Treatment + random effects	9	569.17	63.58	0.00	1.00	-274.87
Target plant richness ~ Season + random effects	3	660.49	154.90	0.00	1.00	-327.16
Target plant richness ~ 1 + random effects	2	665.36	159.76	0.00	1.00	-330.63

Table S3: Model selection for target stem count for seed and stratification experiment establishment year 2021

Model	K	AICc	Δ AICc	AICcWt	Cum. Wt	LL
Target stem count ~ Seed rate + stratification + random effects	**5**	**398.85**	**0.00**	**0.70**	**0.70**	**-193.71**
Target stem count ~ Seed rate + random effects	4	401.26	2.41	0.21	0.9	-196.16
Target plant Richness ~ Seed Rate × Stratification + random effects	6	402.82	3.97	0.10	1.0	-194.39
Target stem count ~ Stratification + random effects	4	418.01	19.16	0.00	1.0	-204.54
Target stem count ~ 1 + random effects	3	420.98	22.12	0.00	1.0	-207.22

Table S4: Model selection for target stem count for seed and stratification experiment establishment year 2022

Model	K	AICc	Δ AICc	AICcWt	Cum. Wt	LL
Target stem count ~ Seed rate + stratification + random effects	**5**	**503.28**	**0.00**	**0.64**	**0.64**	**-245.93**
Target stem count ~ Seed Rate × Stratification + random effects	6	504.46	1.18	0.36	1.00	-245.21
Target plant Richness ~ Seed rate + random effects	4	516.11	12.82	0.00	1.00	-194.39
Target stem count ~ Stratification + random effects	4	521.75	18.47	0.00	1.00	-204.54
Target stem count ~ 1 + random effects	3	532.02	28.74	0.00	1.00	-207.22

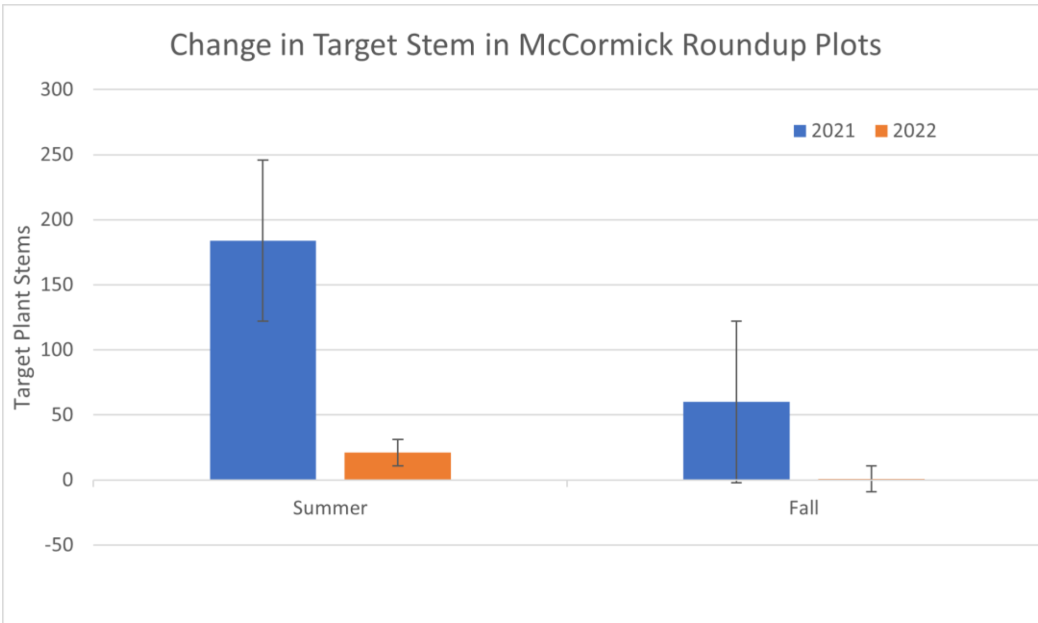

Fig. S1: Change in target plant abundance between 2021 and 2022 at SVAREC. The significant drop at McCormick site was attributed to cattle grazing at the time of sampling but it is doubtful that even without cattle grazing that significant numbers of target forbs would have been present. Error bars calculated using standard deviation of the data

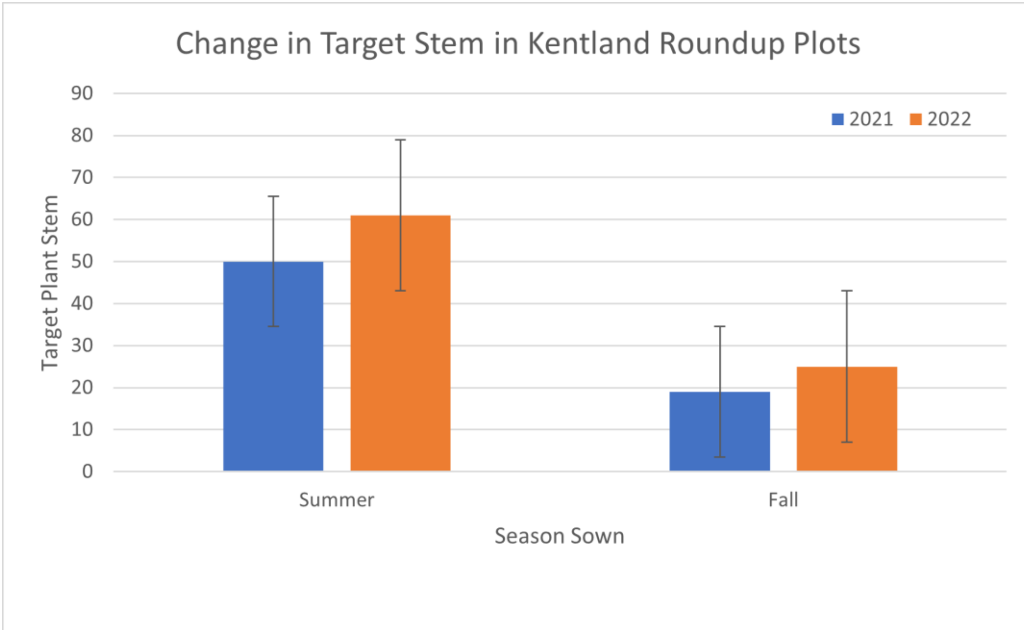

Fig S2. Change in target stem count between 2021 and 2022 at the Kentland Roundup plots. Error bars calculated using standard deviation of the data

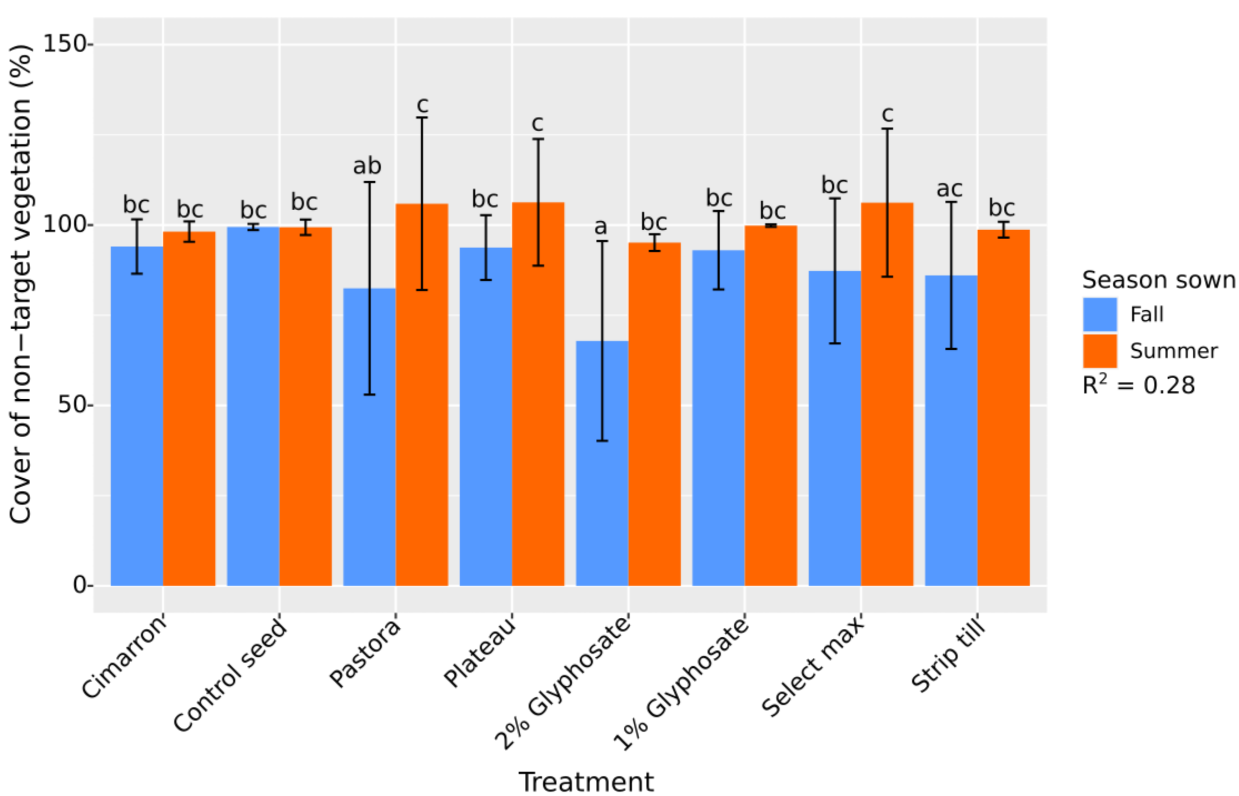

Fig. S3: Non-target plant percent cover compared across all treatments in the site preparation experiment. Letters denote groups that differ with P < 0.05 in a Tukey's HSD test. Error bars calculated from the standard deviation of the data.

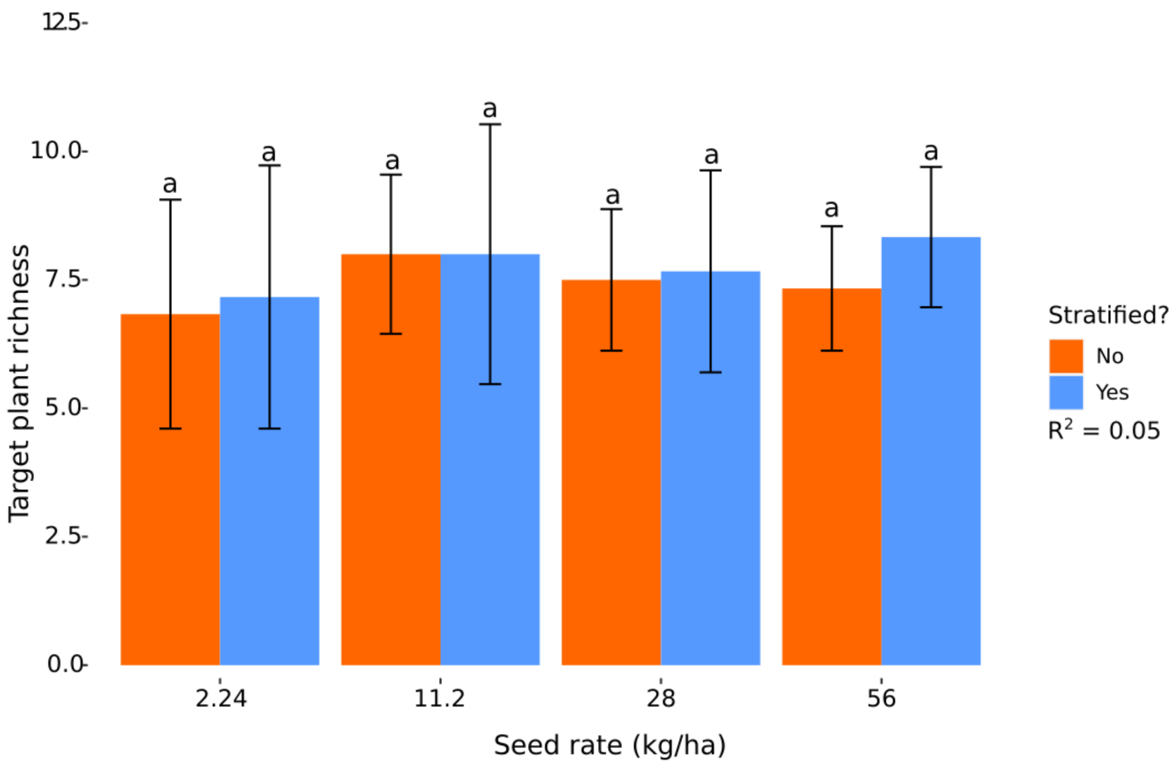

Fig. S4. Target plant richness for plots established in June 2022. Letters denote groups that differ with P < 0.05 in a Tukey's HSD test and error bars created from standard deviation of the data.