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Evaluating Different Methods to Establish Biodiverse Swards of Native Grasses and Wildflowers for Pasturelands

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Abstract: Many cool-season pastures in the southeastern U.S. are dominated by a competitive cool-season grass, tall fescue (*Schedonorus arundinaceus*), and lack substantial plant diversity. Planting native warm-season grasses (NWSGs) and wildflowers (WFs) into these pastures could provide summer forage for cattle and more floral resources for pollinators. This paper summarizes field experiments designed to evaluate different spatiotemporal planting arrangements of NWSGs and WFs to improve their establishment success. The study was conducted from April 2021 to October 2023 in central Virginia (USA). Planting treatments included NWSG and WF mixtures planted: (1) together in the same space, (2) spatially separated in space (i.e., side by side), or (3) temporally separated where NWSGs and WFs were planted in difference sequences. Results showed few differences in forage mass, floral production, and botanical composition as well as stand density in 2021 and 2022. In 2023, NWSG abundance was greater where grasses were planted first or mixed with WFs. Similarly, the WF component was favored when they were planted before NWSGs. Overall, planting NWSG and WF mixes separately, either spatially or temporally, favors successful establishment and could offer more flexibility for using selective herbicides to suppress the heavy weed pressure that often accompanies these plantings.

Keywords: native warm-season grasses; wildflowers; pasture establishment; plant diversity; weed pressure

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1. Introduction

In the Southeast USA, pasturelands are largely dominated by the cool-season grass tall fescue (*Schedonorus arundinaceus*). Most tall fescue plants support a fungal endophyte that produces alkaloid compounds, which, in turn, makes them stress tolerant, competitive, and also toxic to livestock [1]. To manage the toxicity of tall fescue, native warm-season grasses (NWSGs) can be established to provide an alternative forage for cattle especially in summer when fescue toxicosis is the greatest problem [2]. Within existing tall fescue pasturelands, NWSG species help maintain animal performance when tall fescue growth slows and its anti-quality effects are elevated [3]. Common native grasses include big bluestem (*Andropogon gerardii*), little bluestem (*Schizachyrium scoparium*), indiangrass (*Sorghastrum nutans*), eastern gamagrass (*Tripsacum dactyloides*), and switchgrass (*Panicum virgatum*). These species were native to the tall grass prairies in North America [4].

Ecosystem services also can be generated from the incorporation of native wildflowers into pasturelands as the wildflowers provide habitat and food resources for insect pollinators [5,6]. Insect pollinators are in decline across many regions around the world because of grain agriculture intensification, land use change, and intensive pesticide regimes [7,8]. We hypothesized that establishing stands of NWSGs and WFs could improve the output of ecosystem services generated from low diversity, tall fescue pasturelands by providing more summer forage for cattle and greater flower resources for pollinators.

In a pasture production context, an ideal mixture of NWSGs–WFs should provide sufficient forage supply to sustain cattle and provide abundant blooms that will attract pollinators to supply them with food resources. A challenge is how best to plant these NWSG and WF mixtures to ensure a favorable balance between these components since they will provide different benefits to the pasture ecosystem. Previous attempts to plant NWSGs and WFs together have been challenging in terms of species selection as well as defining establishment success criteria [9–11]. Some of the challenges with establishing a favorable balance of NWSG and WF in pastures were seen in a recent study in Virginia [10]. A mixture of three NWSG species and eight WF species was planted into former TF pastures after vegetation suppression. While establishment was largely successful for most species, the composition was so heavily dominated by WF that NWSG forage biomass was insufficient to support cattle. The high abundance of WF occurred even though NWSG species made up 70% of the seed mix. Native warm-season grasses eventually accounted for less than 5% of the final plant community. Four previous experiments in Virginia also suggested that a seed mixture should be weighted towards NWSGs to avoid an overabundance of WFs [9]. Lack of establishment success in the pastures was likely caused by competition from both the WFs included in the mix and weeds present in the pasture soil seedbanks.

Weed competition within native grassland plantings can be substantial especially during the first year [12,13], and optimizing the establishment of NWSG-WF mixtures might require separating the grass and wildflower component in space or time. Spatial separations of grass and forb components have been used in Florida, USA, to establish rhizoma peanut (*Arachis glabrata*) and bahiagrass (*Paspalum notatum*), as well as in tallgrass prairie restorations [14,15]. Temporal separation can be considered as planting one component across the entire unit area and then overseeding different species later. Temporal separation of plantings could also improve establishment by allowing the NWSG or WF component to serve as a companion or nurse crop to the corresponding species planted later [16,17]. Alternatively, planting NWSGs and WFs together might be more optimal in some cases, as grasses could suppress weeds in the establishment year [12].

The goal of this study was to evaluate different spatiotemporal planting methods for NWSG–WF mixtures to learn which might produce a favorable balance of NWSG and WF that could potentially benefit both livestock and pollinators in a biodiverse pasture.

2. Materials and Methods

2.1. Study Sites

Two sites within the Commonwealth of Virginia (USA) were selected for the experiment. Each site was soil tested in early spring 2021 prior to planting and samples were submitted to the Virginia Tech Soil Testing Lab. Samples were collected to 10 cm depth across the experimental site. Soil pH, P, K, micronutrients, and organic matter levels were assessed at the start of the project in spring 2021 (Table 1). These sites included the Kentland Farm (hereafter Kentland; 37° N, 80° W) in the New River Valley and the Shenandoah Valley Agricultural Research and Education Center (hereafter SVAREC; 37° N, 79° W) in the Shenandoah Valley. Both locations lie within the Great Valley subprovince of the Valley and Ridge Province. The Kentland site was previously used for industrial hemp (*Cannabis sativa*) with regular a cool-season cover crop mixture of a small grain, crimson clover (*Trifolium incarnatum*) and a Brassica (*Brassica* spp.) for several seasons.

Table 1. Soil fertility indices at two study locations before experiment began.

Location	pH	Buffer pH	P_2O_5 (mg kg^{-1})	K_2O (mg kg^{-1})	Organic Matter (%)
Kentland	6.6	6.4	27.5	66.0	3.7
SVAREC	6.4	6.2	20.5	36.5	7.2

The SVAREC site had been a permanent tall fescue pasture for 40+ yrs. The Kentland site soil consisted of Unison and Braddock loams. The SVAREC plots covered in equal parts eroded Weikert–Berks channery silt loams and eroded Bookwood silt loam. The fields had not received any fertilizers or soil amendments in the past 5 yrs. No adjustments were made to soil fertility for the duration of the experiment.

2.2. Experimental Design

The experiment was replicated at two sites with site preparation beginning in March 2021. All plots first received a glyphosate herbicide application at a rate of 4.7 L ha^{-1}, conventional tillage with a rotary tiller, and then a subsequent glyphosate spray at the same rate. Treatments were replicated in four plots for a total of 16 plots at each field site. Plot dimensions were 3 m $\times$ 12.2 m at Kentland; at SVAREC, plots were 3 m $\times$ 6.1 m.

Four planting treatments were implemented such that NWSG and WF components were planted as follows: (1) simultaneously in the same space (Together), (2) simultaneously, but spatially-separated (Spatial), (3) planted together in the same space, but temporally-separated with NWSG planted before WF (Grass), and (4) similar to treatment #3 but with WF planted before NWSG (Wild). Treatments 1 and 2 were planted in early June 2021. The spatial planting separations were done lengthwise down the respective plot. For temporal separations, one component was planted in early June 2021 and the subsequent component was planted at the end of the growing season in November 2021. It should be noted that planting native plant seeds in late fall can be an effective establishment technique in this region because cold winter temperatures can help break seed dormancy to allow for more plants to emerge in spring. Seed was hand broadcast into plots using cracked corn as a carrier on 1 June 2021 at SVAREC and 2 June 2021 at Kentland. The NWSG and WF components of each temporal separation were sown on 8 November 2021 at Kentland and 10 November 2021 at SVAREC.

Species used in the experiment consisted of three NWSGs and 10 WF species. These species were chosen based on establishment success in preliminary experiments and to ensure WF blooms would be present over the course of the growing season. The mixture sown was a 4:1 ratio of grasses to forbs based on the number of seeds sown per unit area. The grasses included: big bluestem (*Andropogon gerardii*), little bluestem (*Schizachyrium scoparium*), and indiangrass (*Sorghastrum nutans*). The wildflower forbs included: lance leaved coreopsis (*Coreopsis lanceolata*), blue flax (*Linum perenne*), Ohio spiderwort (*Tradescantia ohiensis*), black-eyed Susan (*Rudbeckia hirta*), purple coneflower (*Echinacea purpurea*), lavender hyssop (*Agastache foeniculum*), grey-headed coneflower (*Ratibida pinnata*), Maximillian sunflower (*Helianthus maximilliani*), rigid goldenrod (*Solidago rigida*), and Indian blanket (*Gaillardia pulchella*). The resulting planting rate was 9 kg ha^{-1} and 1.7 kg ha^{-1} for the NWSG and WF mixtures, respectively.

2.3. Measurements

Stand density in each plot was recorded in two, 0.25 m^{-2} quadrats. The following categories were used to assess stands where establishment of >20 plants m^{-2} was considered successful, 10 to 20 plants m^{-2} was considered adequate, and <10 plants m^{-2} unsuccessful [18].

Forage sampling began once stands surpassed a 20 cm canopy height. Forage mass samples were taken from two randomly placed 0.25 m^2 quadrats within each plot a month following planting (July 2021) and at the end of the season (September 2021). Forage mass consisted of all sown and unsown species material above ground level within the quadrat. Subsequent harvests occurred in May 2022, June 2022, September 2022, May 2023, June 2023, and September 2023. The material within quadrats was cut to ground level and dried at 60 °C for ~72 hr. In all of these samples, floral resources for pollinators were estimated in each quadrat prior to forage harvest. Bloom density was quantified for the sown species mixture as well as red (*Trifolium pratense*) and white (*Trifolium repens*) clover using methods described by [19]. At the end of the season, the plots were mowed to 20 cm.

Plant species composition was assessed visually using a modified Daubenmire cover class method in September 2021, June 2022, and June 2023 [20]. Cover categories were reported as % ground cover. Single species cover analysis included the thirteen sown NWSGs and WFs. Cover categories combined to include NWSG cover, WF cover, and weed cover. Weed cover included all unsown species observed, including red and white clovers.

2.4. Data Analysis

This experiment was analyzed as a randomized complete block design of four treatments replicated four times at two locations. The four planting arrangements were the treatment terms in all models. Preliminary analyses indicated no significant treatment × site interactions ($p > 0.05$) so the plot data from two locations were combined for subsequent analyses. Stand density of sown species, forage mass, and bloom density were analyzed using mixed-model ANOVAs where treatment was considered the sole fixed effect and random effects included block, and block × treatment in each respective year. Forage mass was assessed within the growing season for 2021, 2022, and 2023. The model was adjusted to include a repeated measure of sampling time over the course of a season. Tukey's HSDs were used for post hoc comparison among treatments when necessary. Species richness was analyzed as an aggregate of all sown and unsown species observed in the plots. Species composition results were assessed within each year as single species contributed differently to cover categories over the duration of the experiment. Analyses were done in SAS by means of PROC GLIMMIX (SAS v9.4; SAS Institute, Cary, NC, USA).

3. Results

3.1. Stand Density and Forage Mass

Stand density of sown species averaged 15.3 ± 4.7 plants m^{-2} in May 2022 and did not differ among treatments. Overall, 67% of all plots had 10 or more plants m^{-2}, which was considered adequately or successfully established. The remaining plots were deemed unsuccessful.

Mean forage mass in 2021 and 2022 did not differ among treatments ($p > 0.05$). Forage mass was affected by the sampling period in both years ($p < 0.0001$) and peaked in September (Figure 1). A significant treatment × sampling period interaction was noted for forage mass in 2023 ($F_{6, 106.7} = 2.84$, $p = 0.0133$). Forage mass was similar among treatments in May and June, but, in September, the treatments where grasses were sown first or together with WFs accumulated more forage (Figure 1).

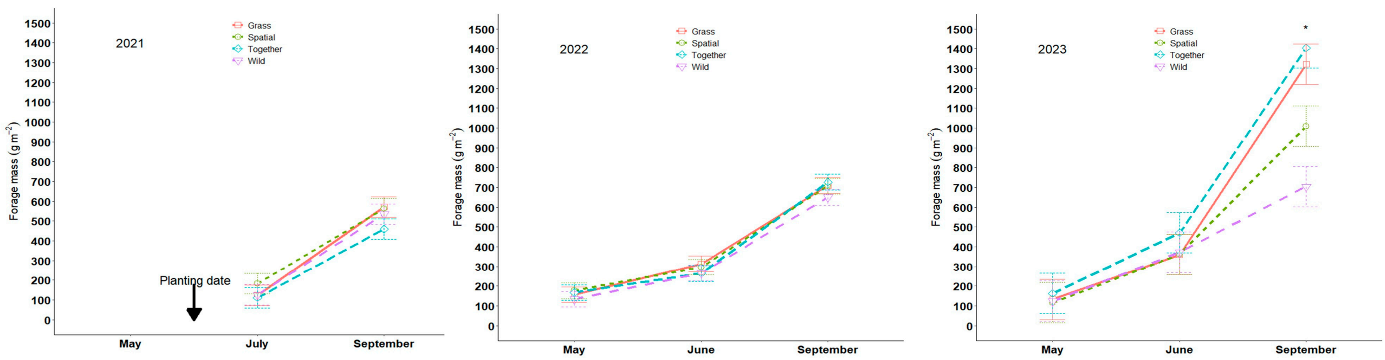

Figure 1. Mean forage mass in the four planting treatments 2021–2023. Means are plotted ± 1 SE. An asterisk (*) denotes a significant difference among treatments at $p = 0.05$. Legend: Grass: NWSGs planted before WFs, Spatial: NWSGs and WFs planted separately, Together: NWSG/WF seed mixed together, and Wild: wildflowers planted before NWSGs.

3.2. Species Composition

The percent cover of NWSGs was not different among treatments in 2021 ($F_{3, 9} = 0.64$, $p = 0.6093$) or 2022 ($F_{3, 9} = 1.72$, $p = 0.2327$) (Figure 2A,B). Treatment differences for NWSG cover did appear in 2023, however ($F_{3,9}$ = 6.66, p = 0.0116). Cover of NWSG was lower in the Wild treatment (where WFs were planted first) than in treatments Grass and Together (Figure 2C). Wildflower cover also did not differ among treatments in 2021 ($F_{3,9}$ = 0.26, p = 0.8550) (Figure 2A), but a weak treatment effect was noted in 2022 ($F_{3,9}$ = 3.16, p = 0.0785) (Figure 2B). In 2023, there was little evidence that WF cover differed among treatments ($F_{3,9}$ = 2.04, p = 0.1784) (Figure 2C). Sown species cover remained relatively low throughout the experiment. In particular, Indiangrass cover increased each year and in 2023 was higher in treatments where NWSG/WF were sown together compared with the Wild treatment ($F_{3,9}$ = 4.98, p = 0.0263) (Table 2). By 2023, NWSG cover was more than 60% greater than WF cover in the Grass, Together and Spatial treatments in 2023. In contrast, WF cover was approximately 24% and NWSG cover 4% when WFs were planted first (Figure 2C).

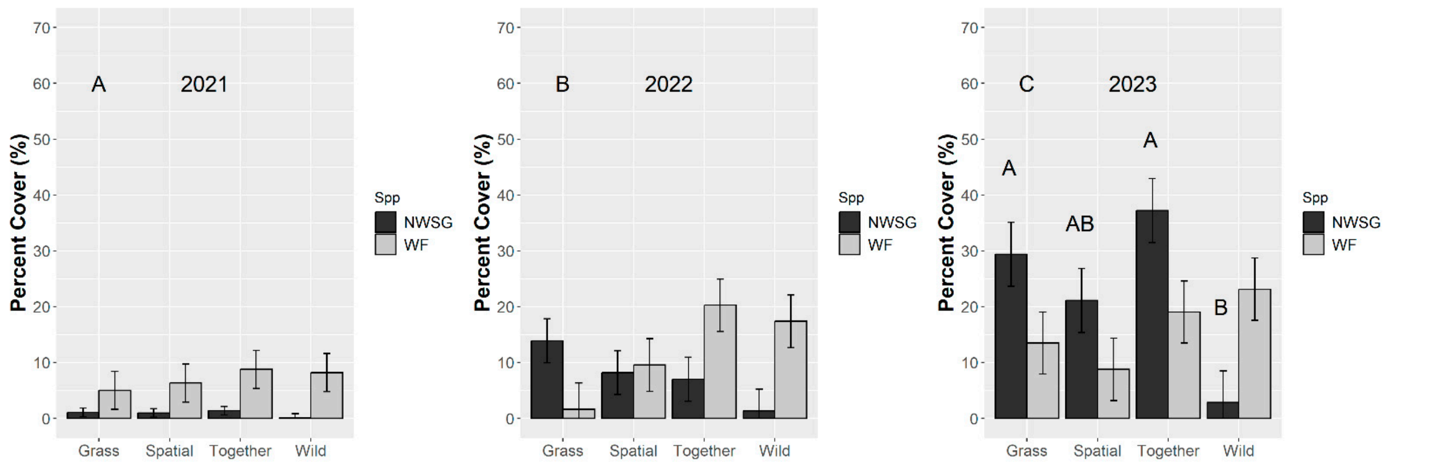

Figure 2. Percent ground cover of native warm-season grasses (NWSGs) and wildflowers (WFs) across treatments in 2021 (**A**), 2022 (**B**), 2023 (**C**). Means did not differ among treatments (p = 0.05) in 2021 and 2022. Means are plotted ± 1 SE. The x-axis labels are 'Grass': NWSGs planted before WFs, 'Spatial': NWSGs and WFs planted separately, 'Together': NWSG/WF seed mixed together, and 'Wild': wildflowers planted before NWSGs. Same letters indicate no statistical difference in NWSG cover among treatments (p < 0.05).

Table 2. Percent cover composition of sown species averaged across treatments. Means are presented ± 1 SE. Treatment means with superscripts of the same letter do not differ at p = 0.05.

Species	2021	2022	2023
Big bluestem (*Andropogon gerardii*)	0.39 (±0.53)	2.05 (±1.57)	6.02 (±3.68)
Little bluestem (*Schizachyrium scoparium*)	0.00 (±0.00)	1.86 (±1.15)	3.42 (±1.66)
Indiangrass (*Sorghastrum nutans*)	0.46 (±0.41)	3.69 (±1.94)	Together: 24.38 (±4.29) ^{A} Grass and Spatial: 13.50 (±4.29) ^{AB} Wild: 1.38 (±4.29) ^{B}
Lanceleaf coreopsis (*Coreopsis lanceolata*)	0.06 (±0.16)	0.49 (±0.68)	0.19 (±0.34)
Perennial blue flax (*Linum perenne*)	0.00 (±0.00)	1.27 (±1.50)	0.33 (±0.46)
Ohio spiderwort (*Tradescantia ohiensis*)	0.00 (±0.00)	1.66 (±1.10)	4.38 (±2.83)
Black-eyed Susan (*Rudbeckia hirta*)	0.88 (±0.95)	2.44 (±1.81)	3.69 (±2.84)

Table 2. *Cont.*

Species	2021	2022	2023
Purple coneflower (*Echinacea purpurea*)	0.56 (±0.71)	1.25 (±0.89)	1.42 (±1.14)
Anise hyssop (*Agastache foeniculum*)	0.33 (±0.38)	0.16 (±0.24)	0.00 (±0.00)
Grey-headed coneflower (*Ratibida pinnata*)	0.14 (±0.22)	1.72 (±1.39)	5.64 (±3.04)
Maximilian sunflower (*Helianthus maximilliani*)	0.83 (±0.60)	0.00 (±0.00)	0.00 (±0.00)
Rigid goldenrod (*Solidago rigida*)	0.00 (±0.00)	0.03 (±0.04)	0.39 (±0.56)
Annual gaillardia (*Gaillardia pulchella*)	4.23 (±2.74)	2.73 (±2.93)	0.08 (±0.16)

Weeds dominated plots especially in 2021 and 2022 where ground cover ranged between 65 and 80% (Figure 3). Weed cover did not differ among treatments in 2021 ($F_{3,9}$ = 1.22, p = 0.3590) or 2022 ($F_{3,9}$ = 0.57, p = 0.6488). However, weed cover was marginally lower in treatments where NWSGs and WFs were sown together in 2023 ($F_{3,9}$ = 3.78, p = 0.0527).

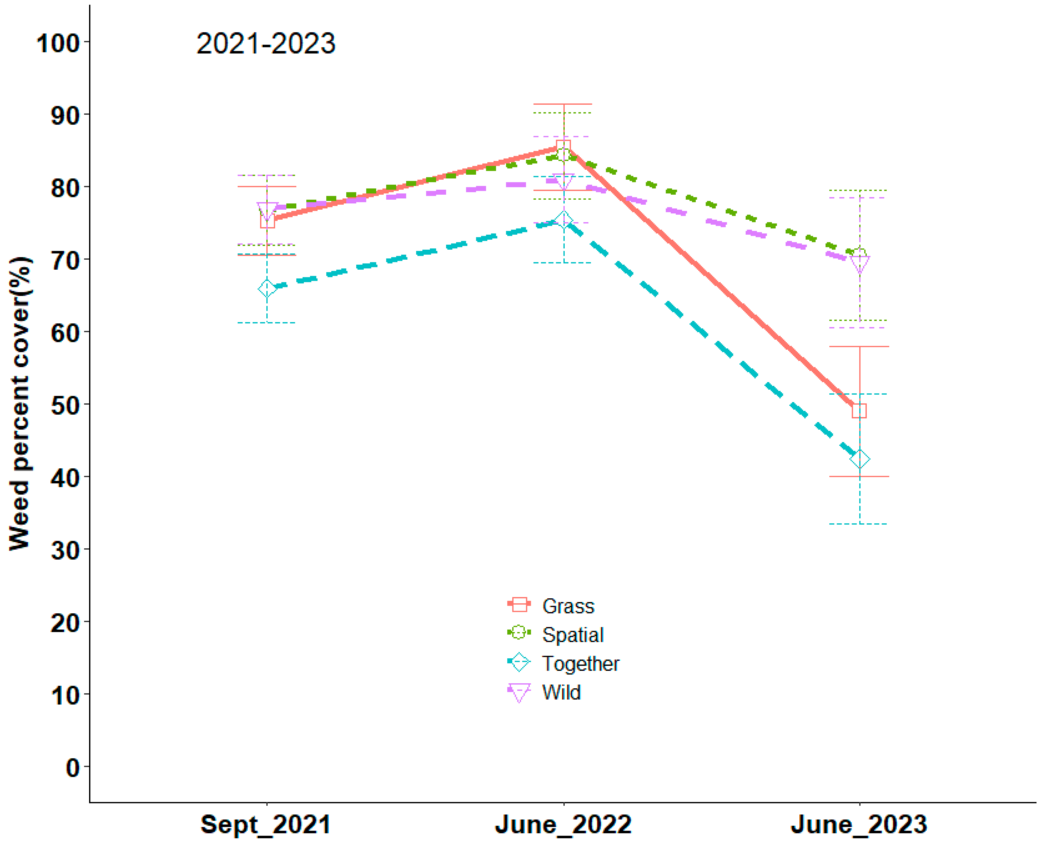

Figure 3. Mean weed cover among the four planting treatments of NWSG and WF components. Means are plotted ± 1 SE.

3.3. Bloom Density

Bloom density within each of the WF species showed no evidence of differing among treatments over the three seasons of the experiment ($p > 0.05$). Except for annual gaillardia, bloom density was especially low in 2021. Ohio spiderwort, back-eyed Susan and grey-headed cone flower produced the most blooms by 2023 (Table 3).

Table 3. Species-level mean flower density (blooms m^{-2}) averaged across treatments. Means are presented ± 1 SE.

Species	2021	2022	2023
Lanceleaf coreopsis (*Coreopsis lanceolata*)	0.00 (±0.00)	0.31 (±0.42)	0.10 (±0.21)
Perennial blue flax (*Linum perenne*)	0.00 (±0.00)	0.85 (±0.88)	0.58 (±0.76)
Ohio spiderwort (*Tradescantia ohiensis*)	0.00 (±0.00)	1.31 (±1.36)	5.58 (±8.60)
Black-eyed Susan (*Rudbeckia hirta*)	0.81 (±0.83)	5.40 (±4.01)	8.48 (±4.65)
Purple coneflower (*Echinacea purpurea*)	0.00 (±0.00)	0.21 (±0.25)	0.35 (±0.44)
Anise hyssop (*Agastache foeniculum*)	1.06 (±1.59)	0.71 (±1.41)	0.00 (±0.00)
Grey-headed coneflower (*Ratibida pinnata*)	0.00 (±0.00)	0.58 (±0.83)	10.83 (±12.36)
Maximilian sunflower (*Helianthus maximilliani*)	0.06 (±0.13)	0.04 (±0.08)	0.00 (±0.00)
Rigid goldenrod (*Solidago rigida*)	0.00 (±0.00)	0.00 (±0.00)	1.56 (±3.11)
Annual gaillardia (*Gaillardia pulchella*)	3.13 (±2.84)	2.79 (±2.54)	0.00 (±0.00)
White clover (*Trifolium repens*)	0.00 (±0.00)	0.21 (±0.50)	0.00 (±0.00)
Red clover (*Trifolium pratensis*)	0.00 (±0.00)	0.00 (±0.00)	0.00 (±0.00)

4. Discussion

The main objective of this study was to evaluate different planting strategies to establish NWSG and WF stands that could improve the output of ecosystem services in low diversity pasturelands such as those dominated by tall fescue in the southeast US. In this case, we were interested in developing stands that would have a favorable balance of NWSGs to serve as forage for cattle and WFs to improve food resources for pollinators. In a previous study [10], similar species of NWSGs and WFs were mixed together and planted into pastures. Despite a high ratio of NWSG to WF in the mix, the subsequent stands that developed were heavily weighted towards WFs at the expense of NWSGs, which constituted less than 5% of the resultant stands. While such a scenario might benefit pollinators, this situation is not satisfactory to supply forage for beef cattle. We wanted to learn whether different planting arrangements of NWSGs and WFs, either planted separately in strips or at different times during the growing season, might produce a more balanced species composition that could benefit both cattle and pollinators.

Despite heavy weed pressure initially, stand establishment was deemed either fully successful or adequate in 67% of plots with an establishment failure rate in line with other recent studies [21]. By the end of the experiment in 2023, the NWSG and WF components had segregated as we had anticipated. For example, grass cover was approximately 60% higher in plots where NWSGs and WFs were planted together or when grasses were planted first. The seeding ratio of NWSG/WF was 4:1, so we expected more grass cover in these treatments. Similarly, wildflower establishment benefitted from being temporally separated from NWSG planting. By 2023, cover of WFs was greater than NWSGs when WFs were planted first in the spring. These results support [14], who found that WF establishment improved when WFs were planted separated from NWSGs. Our results suggest temporal and/or spatial separation of NWSGs and WFs should lead to a more favorable balance of plant diversity in native plant stands as has been shown in other studies [22].

It should be noted that planting NWSGs and WFs together also resulted in good overall establishment of both components. This finding was a bit surprising as NWSGs have been shown to suppress native WF establishment in many restoration plantings [23]. The subsequent balance of NWSG to WF when sown together largely reflected the 4:1 seed ratio of NWSG/WF that was planted, and this balance would be desirable if a biodiverse stand was sown to provide grass forage for cattle. In a related experiment, [9] evaluated imazapic applications in two planting treatments where NWSGs and WFs were combined within the plot, or NWSGs and WFs were planted in spatially separated adjacent strips within the plot. In the first year, more NWSG and WF plants (n = 13 and 33, respectively) established when planted together compared with being spatially separated (mean 7 and 17, respectively). Possibly, a higher density of sown seeds and the potential that grasses and forbs may complement each other during establishment might be one reason for the favorable establishment in the mixed plots.

Heavy weed pressure in our experimental plots likely impacted our results. Weeds dominated plots especially in 2021 and 2022 where ground cover ranged between 65 and 80%. The weed competition came primarily from summer annual weeds like crabgrass (*Digitaria* spp.), foxtail (*Setaria* spp.), barnyardgrass (*Echnichoa crus-galli*), pigweed (*Amaranthus* spp.), as well as pokeweed (*Phytolacca americana*) consistent with previous observations [9,10]. In different contexts, selective herbicides could be used to control grass or broadleaf weeds when NWSGs or WFs are planted separately, either temporally or spatially. For consistency, we did not attempt to manage weeds with herbicides in this experiment, but several strategies could be employed to reduce this weed pressure. For example, broadleaf or grass-selective herbicides could be used to control weeds in spatially separated plantings. Although not strictly selective, imazapic is an effective herbicide for establishing NWSG stands, particularly because it controls annual grasses without harming several important NWSG species that are resistant to the herbicide effects [24–26]. A NWSG stand could be established using a glyphosate burndown with imazapic as a pre-emergent herbicide, and then some broadleaf herbicide may be applied until a stand's weed competition is deemed low enough to overseed with WF species. Wildflowers respond variably to imazapic applications though, and using this herbicide produced mixed results depending on the species and ecotypes selected [9,13,27]. In addition to selective herbicides, weed control in native grass–wildflower stands might involve use of cover crops, smother crops, nurse crops, or mechanical defoliation, for example [28]. Regardless of method, controlling weeds prior to NWSG–WF establishment is crucial. The single application of glyphosate we used to suppress vegetation before experiment establishment was probably insufficient to reduce weed pressure during establishment phase.

Forage mass (mean ~550 g m^{-2}) in the first season matched or exceeded that seen in reported complex restoration and agronomic seed mixtures [14,29]. In all treatments, forage mass usually peaked in September. By 2023, treatments where grasses were sown first or together with wildflowers had highest forage yield at the end of the growing season compared with other treatments. The differences in forage production were mostly tied to the presence of Indiangrass. Indiangrass was more competitive at our sites compared with the other NWSG species, which was surprising as other studies have found Indiangrass to be a weak competitor in mixtures with NWSGs like big bluestem and switchgrass [30]. Although we did not document mycorrhizal interactions in this experiment, productive growth of Indiangrass has been shown to be dependent on mycorrhizal infection [31,32]. Possibly, differential mycorrhizal infection at our sites might have benefitted Indiangrass over the other species during the course of the experiment.

Although wildflowers established across all treatments, their abundance remained low and that might have explained the lack of treatment difference seen in bloom density. Of the sown species, grey-headed coneflower, black-eyed Susan and Ohio spiderwort produced the most blooms by 2023. Since these were mostly summer-flowering species, such a bloom distribution was not ideal, especially given previous research suggesting pollinators' need for both early spring and late summer/fall floral resources [33]. Planting strategies like those investigated by [22] where early, mid, and late season-flowering WFs are planted in small monocultures might encourage a more favorable seasonal distribution of flower production that would benefit more pollinators.

In closing, we found that mixing NWSG and WF seed together at planting could lead to successful establishment. Using either spatial or temporal separation planting of NWSG and WF components might produce more consistent results, however, especially if selective herbicides are used to control aggressive weed pressure during the establishment phase. Such planting methods may offer better control over the establishment process if a specific balance of NWSG to WF species is needed to suit specific conservation or livestock production goals. Lastly, it should be noted that the experimental scale and time span of this study were relatively limited and insufficient to fully reveal the long-term stability of the establishment effects we documented. Future studies should be undertaken that include larger-scale, longer-term comparative experiments, also evaluating the cost-effectiveness of different establishment methods at these larger spatial scales.

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