

Article

Do Pastures Diversified with Native Wildflowers Benefit Honeybees (*Apis mellifera*)?

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Abstract

Tall fescue-dominated pasturelands are widespread in the eastern United States and typically lack substantial plant diversity. Establishing native wildflowers into tall fescue pastures has the potential to benefit bee populations and boost pollinator ecosystem services. In this study, tall fescue pastures at five on-farm sites in Virginia, USA, were planted with wildflowers native to North America and paired with sites with conventional tall fescue pastures. Honeybee apiaries were established at the ten locations, and variables related to hive strength were measured over two years. The main study objectives were to: (1) compare metrics of hive strength between diversified and conventional pastures, (2) determine whether honeybees used native-sown wildflowers as a source of pollen, and (3) explore whether native-sown wildflowers were visited more by honeybees and other pollinators compared with nonnative, unsown forbs. Diversified pastures had many more plant species and blooms compared with conventional pastures, but this had little effect on hive parameters. Pollen DNA metabarcoding revealed that honeybee diets were similar regardless of whether hives were associated with diversified or conventional pastures. Honeybees foraged mostly on plants in the surrounding landscape—especially white clover (*Trifolium repens*) and less so on native wildflowers. Native-sown wildflowers received more visits from native pollinators, however. We hypothesize that the native-sown wildflowers had little impact on hive strength metrics because honeybees had access to abundant, white clover blooms and other flowering species in these landscapes. Native wildflowers that bloom in late summer/early autumn after white clover blooms diminish may be of greater value to honeybees in pasture settings.

Keywords: honeybees; native pollinators; wildflowers; pollen DNA metabarcoding; white clover



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1. Introduction

Between 1940 and 1960, tall fescue (*Schedonorus arundinaceus* Schreb.) became a dominant perennial cool-season grass in many pastures across the eastern United States [1]. Tall fescue plants are typically infected with a fungal endophyte (*Epichloë coenophiala*) that can improve drought and grazing tolerance due to the presence of ergot alkaloids produced by the fungus [2,3]. Some 14 million hectares of pastureland in the eastern US is dominated by tall fescue, and other common forage species such as orchardgrass (*Dactylis glomerata* L.), and Kentucky bluegrass (*Poa pratensis* L.), and white clover (*Trifolium repens*) [4].

Because of the competitiveness of tall fescue, pasturelands usually lack substantial forb diversity to provide sufficient food resources and habitat for insect pollinators, including honeybees [5,6]. In addition to its competitiveness, tall fescue may have allelopathic effects on native plant species [7,8], and this may contribute to low floral diversity and robust populations of pollinators.

Establishing native wildflowers into pasturelands could serve as a vital pollinator resource [9,10]. Studies have shown that adding native wildflowers to farmland can have an immediate and positive impact on pollinator diversity [11–13] and may also increase the frequency of pollinator visits to nearby crops [14]. Such responses may be a function of behavioral plasticity many pollinators exhibit that allows rapid adaptation to vegetation changes in the landscape [15,16].

This paper summarizes work related to a broader research effort that we have termed ‘Bee-friendly Beef’, which explored how the diversification of tall fescue pastures with native plants may affect cattle performance and pollinators. In this study, a mix of native grasses and wildflowers was planted into pastures at multiple locations in southwestern Virginia and compared with conventional tall fescue pastures that received no native seeding. We hypothesized that converting tall fescue-dominated pastures into meadow-like grasslands populated by native wildflowers would provide beneficial habitat to a diversity of pollinators—specifically honeybees. As a generalist pollinator, honeybees can be used as bioindicators to approximate the available floral resources of an ecosystem [17,18]. Additionally, measuring hive attributes such as colony growth and the sources of foraged nectar and pollen should provide insights into how well a pasture agroecosystem can support pollinators. As an extension of this idea, we measured honey production in apiaries located adjacent to these diversified pastures to assess the output a possible ecosystem service that would be provided by adding wildflowers to tall fescue pastures.

The specific objectives of this study were to: (1) compare the productivity of honeybee colonies in diversified and conventional pastures, (2) determine whether honeybees used native wildflowers sown into diversified pastures as a source of pollen, and (3) explore whether native-sown wildflowers were visited more by honeybees and other pollinators compared with non-native, unsown plants.

2. Materials and Methods

2.1. Study Sites

This study was conducted across five counties in southwestern Virginia, US (36° N, 80° W) in 2021 and 2022. The region is primarily characterized by managed agricultural and grazing land, fragmented deciduous forests, and semi-natural grassland habitats among ridges and valleys. Ten beef cattle farms were selected for this study (Table 1). All sites were unimproved, permanent pastureland (average slope 5–10%) dominated by cool-season pasture grasses such as tall fescue (*Schedonorus arundinaceus* Schreb.), orchardgrass (*Dactylis glomerata* L.), and Kentucky bluegrass (*Poa pratensis* L.), as well as white clover (*Trifolium repens* L.), and weedy vegetation. Five farm sites were selected for native wildflower (WF) diversification and five sites served as controls with no wildflower inclusion. Because honeybees will forage several kilometers beyond their hives, the control and diversified sites had to be located on separate farms so should not be considered ‘paired’ in a strict sense. Fields ranged in size between 1.6 and 6.7 hectares and farm sites were separated by 15–50 km. Prior to planting, all sites were sprayed with Roundup herbicide (glyphosate, 2 qt/ac) (Bayer USA, Parsippany, NJ, USA) in fall 2019 and in spring 2020 to suppress existing vegetation. Sites then were no-till planted with a mixture of common native wildflower species and native grasses between 20 May and 8 June 2020 (Table 2). It should be noted that one site received a slightly different mixture of native wildflower species with

several species that were not included in the list below. No soil amendments were applied to sites to adjust fertility.

Table 1. Selected site characteristics of on-farm sites.

Treatment	Elevation (m)	Hectares	Dominant Soil	Planting Date
Diversified	310	1.8	Minnieville Loam	5 June 2020
Diversified	827	1.9	Chester-Glenelg Loam	6 June 2020
Diversified	810	2	Chester-Glenelg Loam	7 June 2020
Diversified	426	2.6	Braddock Fine Sandy Loam	8 June 2020
Diversified	1174	4.3	Tate Loam	20 May 2020
Conventional	772	6.7	Pigeonroost Loam	-
Conventional	765	4.3	Tate Loam	-
Conventional	555	4	Nicelytown Tilt Loam	-
Conventional	682	4	Frederick Gravelly Silt Loam	
Conventional	682	4	Frederick Gravelly Silt Loam	

Table 2. Primary native wildflower mixture sown in 2020. Recommended seeding rates and seeds per pound were based on information from Roundstone Native Seed LLC (Upton, KY, USA).

Scientific Name	Common Name	Mix Proportion	kg/ha Seeding Rate
<i>Andropogon gerardii</i>	Big Bluestem	0.32	3.59
<i>Schizachyrium scoparium</i>	Little Bluestem	0.20	2.24
<i>Sorghastrum nutans</i>	Indian Grass	0.30	3.36
<i>Coreopsis lanceolata</i>	Lance Leaved Coreopsis	0.02	0.22
<i>Linum perenne</i>	Blue Flax	0.02	0.22
<i>Tradescantia ohioensis</i>	Ohio Spiderwort	0.01	0.11
<i>Rudbeckia hirta</i>	Blackeyed Susan	0.01	0.11
<i>Echinacea purpurea</i>	Purple Coneflower	0.04	0.45
<i>Agastache foeniculum</i>	Lavender Hyssop	0.003	0.03
<i>Ratibida pinnata</i>	Gray-headed Coneflower	0.01	0.11
<i>Helianthus maximiliani</i>	Maximillian Sunflower	0.03	0.34
<i>Solidago rigida</i>	Rigid Goldenrod	0.01	0.11
<i>Gaillardia pulchella</i>	Indian Blanket	0.02	0.22

2.2. Hive Attributes

All honeybee hives were established via nucleus hives sourced from a local apiculturist in April 2021. At the time of establishment, queens were less than one year old and colonies were inspected to ensure disease-free status. Standard pinewood Langstroth hives (10-frame) with plasticell foundation were used in the study (Figure 1). A total of twenty hives were placed across ten apiaries with two hives per apiary. Five of the apiaries were established within conventional tall fescue pastures used for grazing, and five were established within wildflower-diversified pastures. Within each apiary, partner hives were placed ~1 m apart on 0.3 m cinder block foundations (Figure 1).

The following colony attributes were monitored in 2021 and 2022 through bimonthly inspections: (1) hive weight, (2) pre-eclosion population metrics (estimated numbers of eggs, larvae, capped brood), (3) stored resources (nectar, capped honey, pollen), and (4) quality of brood pattern. Management activities such as sugar syrup feeding, supering, and Formic Pro (NOD Apiary Products Ltd., Trenton, ON, Canada) miticide treatments were kept consistent across sites.

Colony attributes were measured bimonthly from June to September in 2021 and May to September in 2022. In 2021, each colony's weight was recorded using SolutionBee® scales (SolutionBee LLC, Raleigh, NC, USA). In 2022, all hives were manually weighed using an analog scale suspended from a livestock tripod. In both years, colony weight (bees, comb, pollen and honey) was calculated by subtracting the weight of empty hive gear (i.e., bottom board, empty frames, boxes, lids) from the total weight. The weight of a

honeybee colony can be attributed to honey stores mostly and considered an indicator of nectar collection success [19]. Colonies that died over the 2021–2022 winter were replaced in spring 2022. Wildflower-diversified pasture sites lost seven colonies over that winter while eight were lost in conventional pastures.

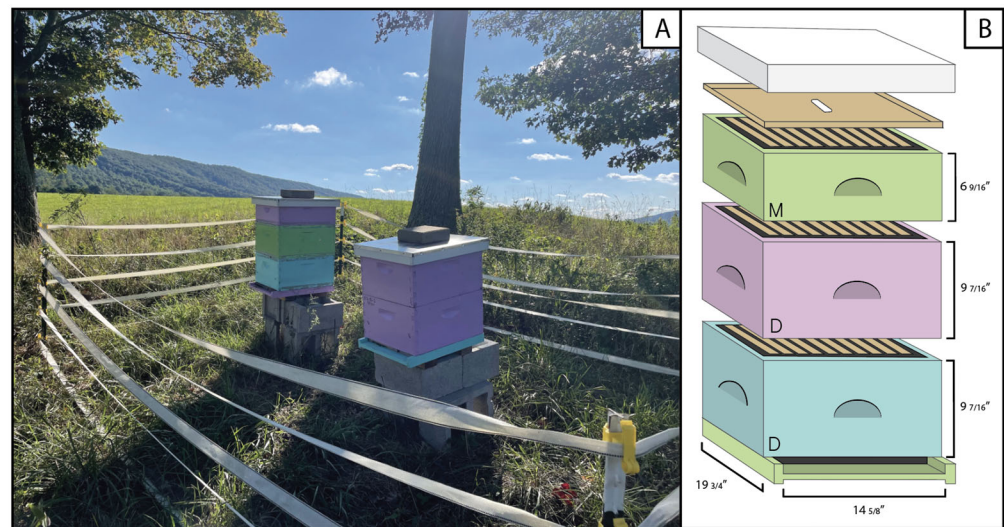


Figure 1. Apiary near a conventional pasture site (A) and dimensions of a 3-box hive (B).

During each bimonthly inspection, every frame in each colony was visually inspected and the area allocated to pre-eclosion population metrics (eggs, larvae, capped brood) and stored resource metrics (nectar, honey, pollen) was estimated to the nearest 5% for each frame, summed, and then converted to cm^2 using methods described in [20]. Brood pattern was rated on a scale of 0–3, from poor to excellent, respectively [21]. A brood pattern is considered excellent if all or most cells within an area of sealed brood are filled. Sparse or spotty brood patterns are those where $\geq 20\%$ of the cells within an area of sealed brood are empty. A spotty brood pattern indicates that either the queen is not laying enough eggs or that the developing larvae are not surviving to eclosion [22].

2.3. Pollen Analysis

Pollen was collected from hives and analyzed using DNA metabarcoding, which uses small regions of DNA to identify plant species [23,24]. To collect pollen, entrance traps were placed on each hive over three 24 h periods (25 May 2022, 27 July 2022, and 1 October 2022). These dates were chosen to capture the peak flowering windows of wildflower species in our sown mixture. Pollen was frozen at $-30\text{ }^\circ\text{C}$ until processing and samples were weighed prior to metabarcoding. This weight value served as a proxy of daily collected pollen mass per colony.

Pollen samples were prepared for amplification and sequencing using methods outlined in [25]. DNA was extracted using the QIAGEN DNeasy Plant Mini Kit (Qiagen USA, Valencia, CA, USA). The ITS2 barcoding locus was amplified using a 3-step PCR amplification process in which the product of each step was used as the primer for the next. Primers in the 3rd PCR step were modified to append sample indexing and lane hybridization oligos to the PRC2 amplicons. Gel electrophoresis confirmed library quality. Libraries were then purified using a SequalPrep Normalization Plate kit (Thermo Fisher Scientific, Waltham, MA, USA) and sequenced on a 15 million reads, 300 base-pair standard Illumina MiSeq Flow Cell at the Molecular and Cellular Imaging Center in Wooster, Ohio.

Using the NCBI plant reference database for the ITS2 locus, sequences were identified to the genus and species levels using the MetaClassifier [26] pipeline via the Owens cluster at the Ohio Supercomputer Center. Paired-end reads were converted to FASTA format

using Seqtk [27]. Paired-end reads were then aligned to NCBI plant reference database sequences (<https://plants.sc.egov.usda.gov/>, assessed 15 December 2022) using VSEARCH with a minimum query coverage of 80% and a minimum identity of 92.5%. The median proportions were then calculated for each marker. Following sequence alignment, all taxa detected at 0.1% proportional abundance or greater were included in analyses to avoid low-abundance false positives. Relative to other detected taxon, proportional abundance reflects the quantity of a given taxa present within a sample. A total of 53 samples were adequately sequenced. Detected genera were classified as either native or exotic to contiguous United States or present in sown seed mix.

2.4. In-Pasture Floral Resources

Percent cover of sown wildflowers, weedy species, and bare ground was visually estimated in 1-m² square quadrats in pastures adjacent to the apiaries in June, July, and August of 2022 using a modified Daubenmire method [28]. Thirty quadrats haphazardly located in each field were sampled. Within these quadrats, blooming species were identified and counted to determine the diversity of active floral resources in each pasture. Comprehensive data on plant species composition and bloom density were not collected in 2021 so we report data from only 2022.

2.5. Pollinator Counts

To assess pollinator visitation to wildflowers, each diversified site was sampled for one day each month June–August in 2021 and 2022. At each visit, the site was sampled every hour from 9:00 a.m.–3:00 p.m. for a total of seven sampling events per site per day. Sampling days were selected when the weather was mostly sunny with little wind, so that conditions would be favorable for insect pollinator activity. The sites were not sampled in August 2021 because no blooms were present after a period of prolonged drought.

Pollinators were counted using a modified version of the “snapshot method” [29]. This method was developed to quickly and non-destructively count the number and type of pollinators visiting a plant. During the sampling period, ten plants of each species currently in bloom in the field were visited each hour by the same observer. For the most part, the plants sampled were from the native wildflower seed mix; however, non-sown species that were already present in the soil seed bank, such as red and white clover, daisies, and fleabane, were also sampled when prevalent. Plants located at the field margins and those that were excessively aged were avoided. To reduce the possibility of sampling the same pollinator twice, the observer walked several steps away before sampling another plant. At each visit, the number and type of all the pollinators present on the plant during a period of three seconds were counted.

The snapshot method is optimized for rapid sampling that does not require the destruction of pollinators through trapping or netting. Because it is performed quickly before the insects fly away, it is not possible to make specific identifications in most cases. Thus, the pollinators were binned into the following categories for ease of accurate rapid identification: honeybees (*Apis mellifera*), bumble bees (*Bombus* spp.), small native bees (*Lasioglossum* and *Halictus* spp.), large native bees (*Xylocopa* spp.), flies (*Milesia* spp.), wasps (*Polistes* spp.), butterflies and moths (Lepidoptera), and beetles (*Popillia* spp.).

2.6. Statistical Analysis

ANOVA models were used to evaluate the relative and interactive effects of pasture treatment (diversified; conventional) and sample period (month) regarding colony weight, pre-eclosion population metrics, stored resource metrics, and brood pattern. To account for the reestablishment of lost hives, we treated each year separately in the analysis. For the analyses, data from the two hives at each site were averaged, and sites were treated as

replicates. ANOVA models also were used to evaluate the relative and interactive effects of site treatment and month on pollen weight. Differences were considered statistically significant if $p < 0.05$. It should be noted that one of the conventional sites was dropped from the study in 2021 when we discovered the apiary was located adjacent to many flowering cherry trees (*Prunus* spp.) that were attracting honeybees. One diversified site also was excluded from the 2022 analysis after the apiary was severely damaged by a storm.

To evaluate potential differences in pollinator visitation to native versus unsown wildflowers, a two-way ANOVA was used with site and wildflower type (sown or unsown) were the independent variables. All statistical analyses were performed in RStudio IDE release 2022.07.0.

3. Results

3.1. Hive Attributes

In 2021, hive weight was not affected by pasture diversification treatment ($p = 0.45$, $df = 1, 27$), month ($p = 0.77$), or the interactive term ($p = 0.54$). Hive weight data from 2022 showed the same trend ($p > 0.05$).

In both 2021 and 2022, few consistent trends were apparent in the colony attributes measured (Table 3). The area allotted to eggs, larvae and capped brood was greater in hives from diversified sites in 2021, but those differences disappeared in 2022. Colony attributes were affected by the month sampled especially in 2021 ($p < 0.05$). In that year, the area allocated to eggs, larvae, capped brood and nectar were highest in May, while area allocated to honey was highest in August. In 2002, the area allocated to eggs, nectar and pollen was highest in May. We detected no interactions between treatment and month in either year $p > 0.05$.

Table 3. Measured hive attributes and associated p -values from 2021 and 2022.

	2021			2022		
	Diversified	Conventional	p -Value	Diversified	Conventional	p -Value
Eggs (cm ²)	4606	3603	0.004	2736	3647	0.05
Larvae (cm ²)	4820	4066	0.06	2521	3258	0.78
Capped Brood (cm ²)	11,956	9929	0.06	6478	7812	0.91
Honey (cm ²)	21,925	24,706	0.26	28,041	26,087	0.91
Nectar (cm ²)	10,758	8373	0.07	8395	7919	0.75
Pollen (cm ²)	8539	9060	0.59	5209	5426	0.73

3.2. Pollen Analysis

Pollen collected from all sites and sampling periods averaged 5.6 g and tended to be highest in samples collected in May. The weight of pollen samples averaged 6.6 g and 4.6 g among diversified and conventional sites, respectively. Pollen weight data showed an interaction between month and pasture treatment ($p = 0.0244$). Pollen weight declined from May to September in diversified pastures but remained constant across sample periods in the conventional sites.

3.3. Species-Level eDNA Indices

A total of 210 and 199 unique species were detected in pollen samples from the diversified and conventional sites, respectively. At the species level, *Trifolium repens* (white clover) was the most abundant species detected representing 40% and 36% of the pollen collected in diversified and conventional pastures, respectively (Table 4). *Plantago lanceolata* (ribwort plantain), and *Toxicodendron radicans* (eastern poison ivy) were also abundant in pollen samples collected from diversified pastures. Within conventional pastures, abundant species detected were *Symphotrichum novae-angliae* (New England aster), and *Solidago juncea* (early goldenrod) (Table 4). Proportional species abundance was not significantly ($p > 0.05$) associated with pasture treatment in any month ($p > 0.05$). Of the 24 species detected greater than 5% of pollen samples, 11 were present in pastures during pollen collection (Table 4). Only three native wildflower species that were planted in 2020 were detected in pollen samples (Table 4). *Gaillardia pulchella* was the most abundant of the sown species and was also detected from a hive at one of the conventional sites.

Table 4. Proportion of wildflower species detected in >5% of pollen samples from May, July and October 2022. Species in bold were present in the on-farm pastures that were surveyed in 2022. * indicates wildflower species that were planted at study sites, TR = proportion less than 0.001.

Genus Species	Diversified	Conventional
<i>Cichorium intybus</i>	TR	TR
<i>Brassica juncea</i>	-	0.045
<i>Daucus carota</i>	TR	0.003
<i>Centaurea nigra</i>	0.034	-
<i>Coreopsis lanceolata</i> *	0.001	TR
<i>Gaillardia pulchella</i> *	0.022	0.002
<i>Erigeron annuus</i>	TR	TR
<i>Erigeron philadelphicus</i>	TR	TR
<i>Hypericum nitidum</i>	-	0.040
<i>Nyssa sylvatica</i>	-	0.051
<i>Plantago lanceolata</i>	0.109	0.055
<i>Plantago major</i>	0.039	-
<i>Rosa rubus</i>	0.053	0.059
<i>Rubus perrobustus</i>	-	0.048
<i>Solanum carolinense</i>	TR	0.001
<i>Rudbeckia hirta</i> *	0.001	TR
<i>Solidago canadensis</i>	0.030	0.000
<i>Solidago juncea</i>	0.042	0.117
<i>Symphotrichum novae-angliae</i>	0.078	0.143
<i>Taraxacum officinale</i>	TR	TR
<i>Toxicodendron radicans</i>	0.104	-
<i>Trifolium pratense</i>	0.030	-
<i>Trifolium repens</i>	0.408	0.368
<i>Verbascum thapsus</i>	0.052	0.065

3.4. Pasture Floral Resources

In 2022, sown wildflower species accounted for 32% ground cover, while unsown, weedy species already present in each location's seed bank averaged 58% cover in diversified pastures. An average of 40.2% of all blooms in diversified pastures were sown species and 59.7% were unsown species. *Rudbeckia hirta* was the dominant sown wildflower species, representing an average of 16.5% of total blooms counted (31.05% of sown species), followed by *Coreopsis lanceolata* (11.86% of total; 22.27% of sown) and *Ratibida pinnata* (7.4% of total; 13.88% of sown). *Trifolium repens* was the dominant unsown wildflower species,

representing an average of 15.7% of total blooms counted, followed by *Trifolium pratense* (13.8%) and *Erigeron* spp. (9.0%)

A total of 4472 blooms were recorded in 2022, and mean bloom density was highest in July across all pastures (Table 5). We identified 29 species in bloom across all sites—12 of which were native wildflowers that had been sown in 2020. Diversified pastures had approximately 2× more species in bloom (24) than conventional pastures (11) and had more blooms overall averaging 32/m² compared with 7/m² in conventional pastures.

Table 5. Mean bloom density (per m²) of species in diversified and conventional pastures in 2022. * indicates native wildflower species that were planted in 2020.

Genus Species	Conventional				Diversified			
	June	July	August	Mean	June	July	August	Mean
<i>Agastache foeniculum</i> *	-	-	-	-	0	1.29	0.47	0.59
<i>Coreopsis lanceolata</i> *	-	-	-	-	2.28	0.27	0.53	1.03
<i>Coreopsis tinctoria</i> *	-	-	-	-	0	0.53	0	0.18
<i>Desmodium canadense</i> *	-	-	-	-	0	2.2	1.87	1.36
<i>Echinacea purpurea</i> *	-	-	-	-	0.33	0.39	1.04	0.59
<i>Gaillardia pulchella</i> *	-	-	-	-	1.01	0.53	1.33	0.96
<i>Helianthus maximiliani</i> *	-	-	-	-	0	0.59	0.63	0.4
<i>Heliopsis helianthoides</i> *	-	-	-	-	0	1.55	1.04	0.86
<i>Linum perenne</i> *	-	-	-	-	2.13	0	0.27	0.8
<i>Monarda fistulosis</i> *	-	-	-	-	0	2.83	3.6	2.14
<i>Ratibida pinnata</i> *	-	-	-	-	0	1.88	2.05	1.31
<i>Rudbeckia hirta</i> *	-	-	-	-	0.61	2.66	1.72	1.66
<i>Bellis perennis</i>	1	0	0	0.33	1.92	0	0	0.64
<i>Calystegia sepium</i>	-	-	-	-	0	0	0.4	0.13
<i>Chamaecrista fasciculata</i>	-	-	-	-	0	3.07	1.47	1.51
<i>Cichorium intybus</i>	1.17	1.33	0.33	0.94	0	0	0.67	0.22
<i>Daucus carota</i>	0	0	0.67	0.22	0	0	0.88	0.29
<i>Desmodium paniculatum</i>	-	-	-	-	0	0	3.29	1.1
<i>Diodia virginiana</i>	-	-	-	-	0	0	3.73	1.24
<i>Erigeron</i> spp.	-	-	-	-	1.04	24	5.42	10.15
<i>Plantago lanceolata</i>	0.58	1.28	0.56	0.81	-	-	-	-
<i>Plantago major</i>	0	0	1.08	0.36	-	-	-	-
<i>Polygonum</i> spp.	0	0.5	1.78	0.76	-	-	-	-
<i>Solanum carolinense</i>	0.33	0.92	0.56	0.6	0	0	0.27	0.09

Table 5. Cont.

Genus Species	Conventional				Diversified			
	June	July	August	Mean	June	July	August	Mean
<i>Sisymbrium officinale</i>	0	4.67	0	1.56	-	-	-	-
<i>Taraxacum officinale</i>	0.33	0	0	0.11	-	-	-	-
<i>Trifolium pratense</i>	0.41	0.62	0.44	0.49	1.49	1.05	0.48	1.01
<i>Trifolium repens</i>	0.76	1.7	0.8	1.09	1.73	1.65	0.37	1.25
<i>Verbena urticifolia</i>	-	-	-	-	0	0	8	2.67
Totals				7.3				32.2

3.5. Pollinator Observations

The total number of pollinators of each type was summarized in Figure 2. Small native bees and bumble bees were the most abundant pollinator types observed, with the exception of pollinating flies (e.g., *Milesia* spp.) being very abundant ($n = 720$) in June 2021. Few honeybees (*Apis mellifera* L.) were observed in 2021 or 2022.

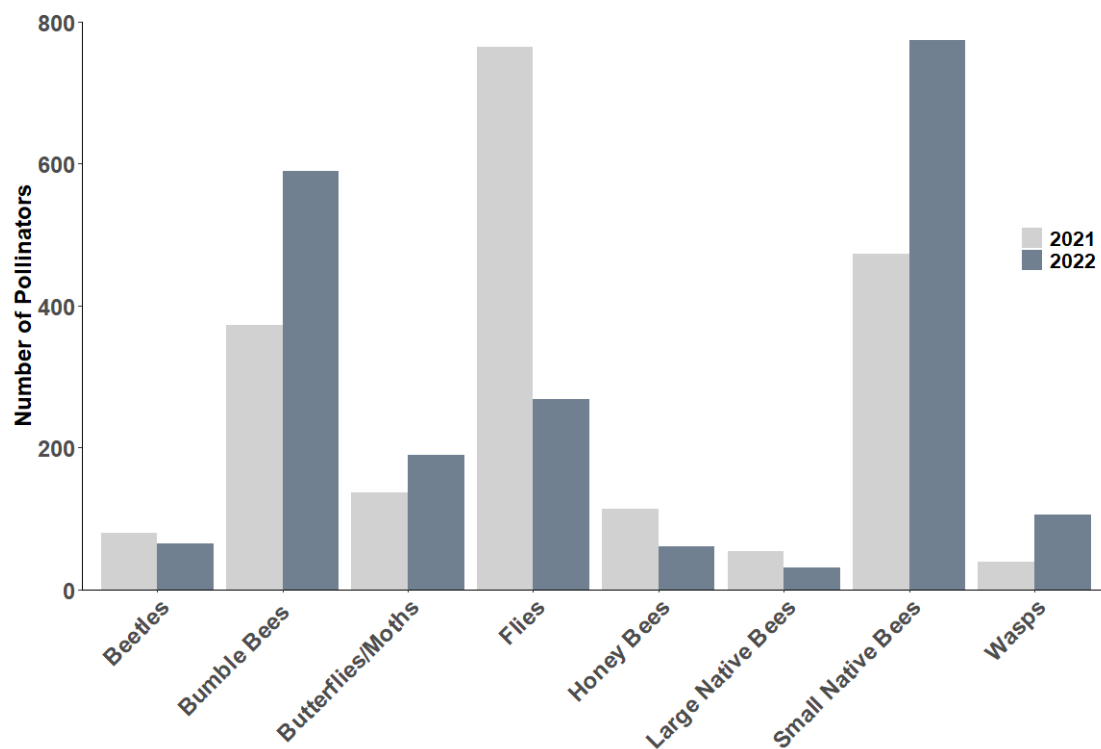


Figure 2. Total number of pollinator types observed in 2021 and 2022.

When the total number of flower species in bloom were categorized into sown (native) and not sown (non-native), ANOVA results indicated that native-sown plant species attracted significantly more pollinators (p -value = 0.0247) than the unsown weedy species already present in the seed bank (Figure 3). The number of pollinators also was affected by site (p -value = 0.05) but the interaction between the site and the number of flower species in bloom was not statistically significant (p -value = 0.26).

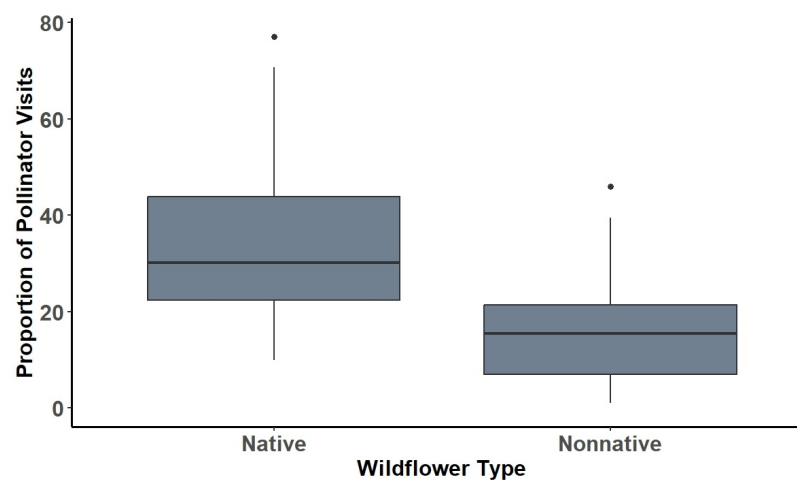


Figure 3. Box plot of pollinator visits recorded on native (sown) or nonnative (unsown) flowers. Plant species composition differed across sites so we standardized the data by dividing the total number of pollinator visits by the total number of plant species (native or nonnative) recorded at each site. Values are means combined from 2021 and 2022. Dots beyond the whiskers represent outlier data points.

4. Discussion

The results from this study suggested that diversification of pastures with wildflowers had little impact on honeybee hive productivity. The findings were surprising given the greater floral resources that were available to bees at diversified sites. Analysis of pollen collected across the sites may point to a possible explanation. Rather than foraging on nearby wildflowers, honeybees instead collected most pollen from plants in the surrounding landscape, particularly white clover (*Trifolium repens*), which is ubiquitous in pastures. Preferential foraging on white clover blooms across the landscape may partially explain why hive metrics were similar despite a high diversity of native floral resources available from the wildflower plantings.

Floral resource quality is well known to positively affect honeybee colony success [30,31]. Hive weight should correlate with honey production, which is a good proxy to estimate colony success as honey serves as a major food source for the colony over winter. Previous research has shown that colony weight may not vary significantly in response to slight differences in surrounding land types [25,32] unless hives occur in more florally deprived landscapes such as urban environments [26]. We predicted that hive weight would be greater in pastures diversified with wildflowers since bees had close access to rich floral resources during the summer. In fact, diversified sites had roughly 4× more flowering species and blooms compared with conventional pastures. Despite these differences in floral resources, hives from conventional sites were as productive as those from diversified locations. Pre-eclosion population and stored resource metrics across hives also support this notion. A factor that may have contributed to the lack of hive differences between diversified and conventional sites might be related to the fact that many hives had to be replaced due to losses incurred over the 2021–2022 winter. Establishing new colonies probably contributed to additional variation among the hives due to factors such as increased disease load, introduction of new queens, and other factors. If that was the case, this added variation from introduction of the new colonies may have limited our ability to detect treatment effects from the wildflower enhancement.

Pollen analysis suggested that honeybee diets were similar across sites, and this probably reflected a wide foraging radius and selection of similar plants in the landscape. Landscape composition within a 3–5 km radius around each apiary showed a similar distribution of forests, pastures, wetlands and cultivated fields around sites [33]. While

research has shown that a greater abundance of diverse blooms will provide more robust nutritional resources for honeybee colonies [34–36] surrounding land cover may not influence honeybee colony success to a large extent [37]. Moreover, our study sites diversified with wildflowers ranging between 1.8 and 4.3 hectares, and it is known that honeybees may forage up to several kilometers from a hive [38,39]. In contrast, recent work in Iowa found that comparatively small areas of pollinator habitat in prairie strips embedded within a row crop-dominated landscape positively affected hive productivity and honeybee health [40]. This study was conducted in a simpler landscape matrix dominated by corn and soybean fields, however. In a more complex landscape like Southwestern Virginia with abundant pasturelands, wetlands, and forested areas with flowering woody plants, it seems plausible that patches of native wildflowers in these converted pastures may not have been large enough to attract honeybees in substantial numbers. Another possibility is that our pollen collection dates were too infrequent to capture the full spectrum of plants foraged by honeybees during the summer of 2022.

Honeybees from our hives may have preferentially foraged from plants with protein-rich pollen such as white clover. Honeybees collect pollen and nectar for their individual nutrient requirements and developing larvae in the hive. While pollen provides protein, lipids, amino acids, sterols, and other micronutrients necessary to sustain colonies [23], bees typically avoid collecting pollen that is low in protein [41] especially after initial foraging experiences [42]. Honeybees also are known to demonstrate high floral patch fidelity [43]. This behavior might cause honeybees to seek out specific patches with protein-rich food sources that have been established in florally diversified systems. For example, Ref. [44] found that honeybees collected a greater proportion of pollen from just four plants, despite the availability of many other flower species. As in our study, the principal flower pollen collected in this study was white clover, which also had the highest amino acid content. Similarly, a study in Michigan, USA, found that the weight gain of honeybee colonies was more strongly correlated with grassland/pasture landscapes that likely supported more nonnative plants such as white clover [45].

While honeybees often favor nonnative plants like clover, it should be noted that honeybees will also forage on pollen and nectar from perennial flowering plants native to North America [40,46,47]. The study by [47] may have relevance to our findings as they found that honeybees collected more pollen from nonnative plants in early summer (June and July) but relied more on native plants for pollen later in the summer (August and September). The later blooming native wildflowers in our seed mixes did not establish well. The absence of late flowering wildflowers in our pastures may help explain the lack of native-sown species in the pollen collections and why hives associated with wildflower plantings were not more productive.

Lastly, our results provide evidence that although honeybees did not benefit substantially from native wildflower species in pastures, those species may have attracted more native pollinating insects compared with weedy, unsown species that emerged from the soil seed bank. In fact, each category of pollinator, except for nonnative honeybees, showed a statistically significant preference for one or more species of native wildflower. One caveat to note is that pollinator observations were carried out at three points during the growing seasons. Admittedly, the limited frequency of sampling events could have underestimated the scope of pollinator visitation at the sites. Nonetheless, our findings still support other studies demonstrating that native pollinators tend to have stronger preferences for native wildflowers compared with nonnative species [48–50].

5. Conclusions

Native and nonnative plants have the potential to be a source of forage for honeybee colonies located near grazed pasturelands. Native plants may be a more valuable source of food for bees later in the season when blooms from nonnative species like white clover begin to decline. Most of the native wildflowers that established in our renovated fields flowered in mid-summer, which tends to be a time when there is a dearth of flowering plants for most native pollinators in our region. If honeybee production is a management goal for wildflower-enhanced pastures, successful establishment of late-summer or early-fall flowering wildflowers (e.g., asters and goldenrod species) should be a priority.

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