University of North Carolina Asheville
Journal of Undergraduate Research
Asheville, North Carolina
Spring 2026

Ecosystem Services from Native Wildflowers: Insect Visitation and Arbuscular Mycorrhizal Fungi

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Abstract

Urbanization is a complex socio-economic process that is occurring rapidly, with consequences for urban and adjacent ecosystems. One factor affecting plant and insect interactions in urban landscapes is habitat connectivity, which can be influenced by planting choices, fragmentation, and the presence of corridors. In cities, plantings' aesthetics and ease of management are typically prioritized, with effects on insect communities and soil health usually treated as secondary considerations. To examine the effects of planting choices on ecosystem services in urban landscapes, we created four spatial arrangements of ten native plant species from two different color groups. We observed these plantings over 20 days in summer 2025, noting the number and types of insects visiting flowers. We also quantified the abundance of Arbuscular Mycorrhizal Fungi (AMF) spores in soils around native plants and plant roots from seeds germinated under different planting arrangements. Total insect visitation was higher on yellow flower species than purple flowers, and visitor diversity differed by plant species. Planting patterns and spacing did not have a significant effect on insect abundance, richness, or their combined metric, diversity. This study can inform best practices for maximizing ecosystem services

in western North Carolina's urban landscapes by considering what will attract the most pollinators and increase beneficial relationships with AM Fungi.

Introduction

Urban Landscapes

In urban environments, vital plant and insect interactions face several obstacles that can affect their frequency and complexity, including habitat loss; habitat fragmentation; introduction of non-local species; and changes in climate, soils, and water cycles (Kowarik 2011). While most of these obstacles are caused by anthropogenic factors, structural connectivity is most affected by human constructions and landscape choices. Structural landscape connectivity focuses on how habitats are linked and arranged in space, while functional connectivity refers to how well organisms can move and disperse in these habitats (Correa Ayram et al. 2016). Urban landscape design typically focuses on structural connectivity, with decisions made by different communities, private property owners, and legislators. For example, in public rights-of-way such as roads, sidewalks, and paths, planting designs, including species choice, must be approved by local government and other parties such as roadside environmental engineers. Aspects like visibility and safety are considered by municipalities and garden clubs. Legislation also impacts these decisions, such as North Carolina's Senate Bill 391 which requires the use of native plants in NCDOT right-of-way replanting projects and mandates the development of an approved list of native species for these purposes. Asheville, North Carolina's Code of Ordinances Sec. 7-11-3 provides guidelines for tree and shrub planting, which affect structural connectivity. For example, it requires planting trees that reach only 35 ft at maturity if there are overhead utilities and specifies spacing so that no tree can be closer than 15 ft or farther than 65 ft from one another. These are examples of how urban construction layouts and available space to plant determine how effective landscape connectivity can be. Though North Carolina has state laws promoting native plant conservation and planting, additional factors affect landscape planting decisions. These include aesthetics for tourism and site conditions such as light, water availability, wind, soil composition, drainage, and hardiness zone ("Right Plant, Right Place"- A Plant Selection Guide for Managed Landscapes).

Plants and Insects in Urban Landscapes

Flying arthropods, including Diptera, Hymenoptera, and Lepidoptera, are important in urban landscapes as they provide ecosystem services including pollination, decomposition, and structuring food webs (Lagucki et al. 2017). Urban landscapes may not inherently affect all plants and arthropods negatively, but some features and design decisions do. For example, small spontaneous vegetative patches may provide food, refuge, and habitat for different species (Sirhoi et al. 2015). In contrast, parks and spaces with high cover of turf grass crowd out weedy species like dandelion and clover while compacting the soil, making it harder for nesting species to find habitat and reducing foraging weed flowers that support bees (Tonietto et al. 2011). Structures such as green roofs with high plant diversity and native plants have been found to support a higher level of bee richness, and because of the substrate commonly used in green roofs, such as sand and pebbles, they may provide suitable nesting substrates. A study in a British town also found a high abundance of solitary and eusocial bees in the urban core, in contrast to meadows and nature reserves, potentially connected to high floral diversity and landscape heterogeneity at the study site. This shows how urban sites that are structurally adapted may increase certain bee species (Sirhoi et al. 2015). In addition, because of decreases in plant and pollinator population diversity, some species experience reduced reproductive success. Generalist species often increase in abundance while specialists decline, and some pollinators might adopt generalist behaviors to adapt to altered landscapes (Xiao et al. 2016).

Though urban landscapes can be beneficial, and the right plant species can increase bee richness, beneficial pollinators such as bees have been found to decrease by 41% per 1 °C increase in temperature. Impervious surfaces contribute to the heat island effect, which raises temperatures by 1-7 °C compared to rural or suburban temperatures (US EPA 2014, Braman and Griffin 2022). Butterflies are more susceptible to temperature changes than bees. Specialist butterflies avoid urban habitats, and a surge of generalists occurs (Fang et al. 2023). Butterflies typically depend more on herbaceous than woody plants, making their maintenance crucial in urban landscapes (Subedi et al. 2021).

Along with affecting arthropod abundance and diversity, urbanization also affects plant phenology - the timing of plant life cycles. Urban heat islands affect rates of vegetative and reproductive development, and at extremes can cause plant sterility or seed abortion (Hatfield and Prueger 2015). Urbanization and subsequent habitat fragmentation alter edge-to-area ratios, creating microclimates with higher temperatures, greater light intensity, and other abiotic factors. These changes can shift plant phenology, shorten flowering periods for spring and fall flowers, cause plants to flower 1-2 weeks earlier in urban landscapes and reduce habitat connectivity (Sexton et al. 2023). In some cases, flowers located at the center of a habitat have longer flowering periods than those near the edge (Xiao et al. 2016).

Plant Cues for Insect Visitation

Landscape layout and connectivity are not the sole factors affecting insect visitation, as there are several plant cues that insects use. Honeybees (*Apis mellifera)* are among the most studied in the Order Hymenoptera. It has been shown that honeybees can easily remember floral cues and memorize colors, with some plants forming a concentric pattern referring to the central color of the flower, contrasting with the rest of the flower petals' color. Other aspects of flowers, such as their UV-absorbing and UV-reflecting parts, can create a contrast line for honeybees to orient themselves after landing (Hempel de Ibarra et al. 2022). In other orders such as Lepidoptera, flower visitation is driven by olfaction, chemoreceptors, and mechanoreceptors that detect volatile compounds (Chen et al. 2021). Other factors also determine flower visitation, such as floral density. More flower-visiting insects are sometimes found on single plants with many flowers, perhaps because they can be seen from farther away and provide higher nectar and pollen rewards. However, the relationship between flower number and visitation is not linear, and according to optimal foraging theory, insects may leave a patch once its rewards fall below those available in another patch (Akter et al. 2017).

Plant Relationships with Arbuscular Mycorrhizal Fungi in Urban Landscapes

Native plants in urban landscapes can engage in symbiotic, mutualistic relationships with arbuscular mycorrhizal fungi (AMF). AMF penetrate the cortex of the plant and can increase uptake of water and nutrients such as phosphorus and nitrogen; this can reduce the negative effects of abiotic stressors like salinity, drought, and temperature by increasing photosynthetic rates and plant biomass (Samanta et al. 2025). AMF might be especially important in urban landscapes, where waste disposal, sewage, heavy metals, and soil compaction negatively influence soil health and create abiotic stress (Li et al. 2022, Tu et al. 2025).

Plant Interactions with AMF

The establishment of symbiotic relationships between the plant and AM fungi is not consistent. Some studies have found that the seed stage of a plant is more receptive to microbial colonization, while older plants retain more microbial communities prior to inoculum, and other studies have shown that bacteria may colonize faster than fungi (Aleklett et al., 2022). This is important, as colonization may be affected by phenological aspects of plants, such as a possible window of colonization, auxin production that increases AM fungi establishment, and environmental conditions (Samanta et al. 2025). Additionally, plant communities may filter out AM fungi; this can pertain to non-native species, which may alter soil physicochemical properties, biodiversity, and the stability of native plant communities (Shen et al., 2024). Non-native plant species, of growing concern in urban areas, are guiding government sectors such as the N.C. Forest Service to encourage people to plant native plants and provide eradication services (N.C. Forest Service – Invasive Plants | NC Agriculture). These invasive plants may share mycorrhizae with native plants, which can redirect greater carbon to invasive species or inhibit mycorrhizae for native plants (Fahey and Flory 2021).

While many abiotic and biotic factors affect the establishment of beneficial AM fungi with plants, there are suggestions for increasing these relationships. For example, a study utilizing commercially sold AM fungi versus native AM fungi from the study site showed that plants flowered more frequently and had greater biomass with native AM fungi (Middleton et al., 2015). Polycultures have also been suggested to positively affect AM fungi communities by providing a larger pool of hosts (Guzman et al., 2021). However, much of the work on AM fungi colonization has focused on agriculture and commercial products, while urban native plant interactions, particularly the potential to increase beneficial AM fungi through polycultures or monocultures, have not been extensively studied.

Objectives

Because urban landscape decisions have become increasingly important for maintaining biodiversity as well as creating aesthetically pleasing spaces, they were the focus of this work. The study aimed to observe whether pollinators show a preference among four spatial patterns of flowering plants commonly found in such environments. The goal was to determine whether plants' floral color and spatial arrangement influence pollinator visitation, thereby informing future research and potentially supporting pollinator-friendly landscape design. In addition, the study examined the effect of native wildflower monoculture and polyculture on AMF colonization density.

Methodology

Patterns and Plant Species

Circular and row planting patterns were chosen for this study because they are easy to establish, and are examples of small patches of herbaceous vegetation, commonly observed in urban environments (Fig.1).The patterns used were Circle Clustered, Circle Spaced, Row Clustered, and Row Spaced, with "Clustered" referring to plants grouped by color, and “Spaced” referring to plants mixed in color (Fig. 2). The species selected for these observations were flowering herbaceous plants native to western North Carolina and included six species with purple/pink flowers and six with yellow flowers (Table 1). Potted plants were sourced from Carolina Native Nursery in Yancey County, NC, except for *Asclepias syriaca*, *Euthamia caroliniana*, and *Helianthus angustifolius*, which were moved from UNC Asheville campus gardens into pots.

Figure 1. Small patches of land available for planting on the UNC Asheville campus, with pictures A and B depicting circular patterns and pictures C and D depicting row patterns.

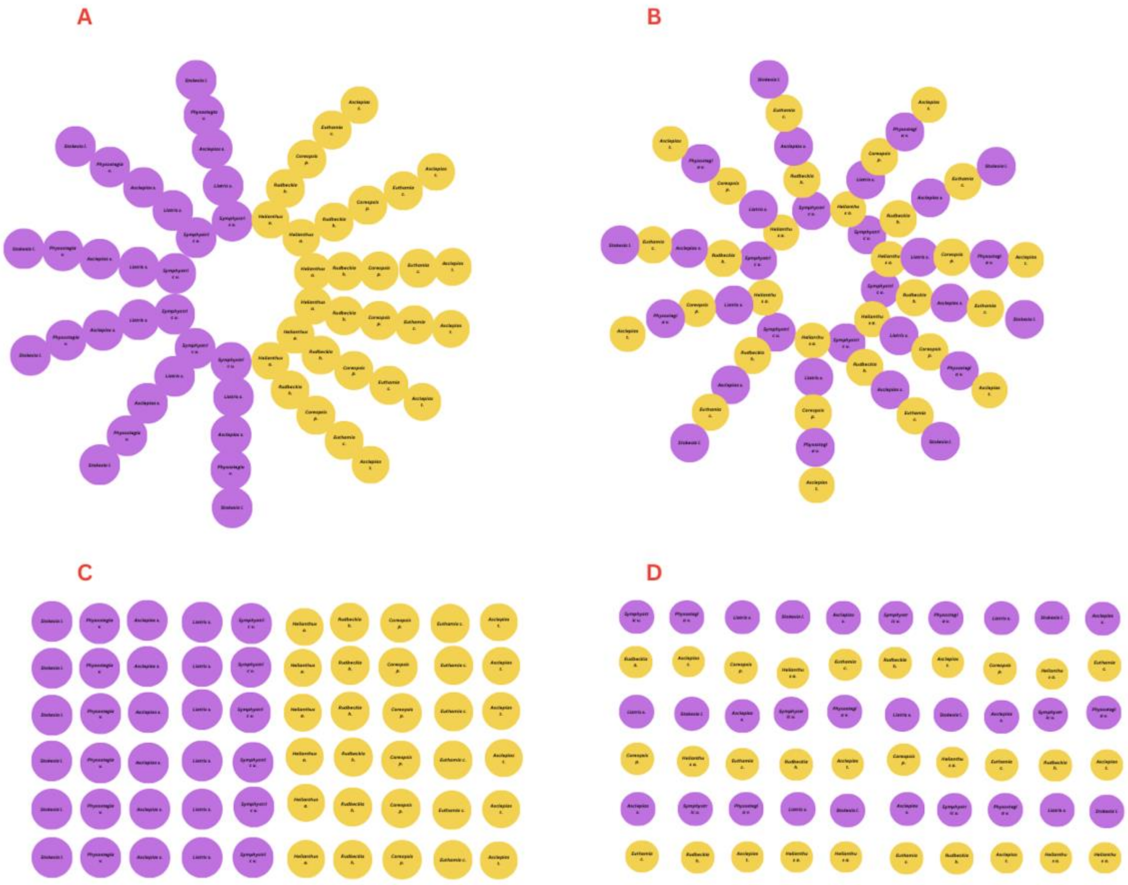


Figure 2. Planting patterns utilized in these experiments. (A) Circle Clustered, (B) Circle Spaced, (C) Row Clustered, and (D) Row Spaced. Purple and yellow depict floral colors.

Table 1. Plant common name, scientific name, color, and minimum plant to space in meters obtained from NC Extension Gardener Toolbox.

Common Name	Scientific name	Color	Space Between Plants (m)
Black-Eye Susan	*Asclepias tuberosa*	Yellow	0.31
Butterfly Weed	*Rudbeckia hirta*	Yellow	0.91
Common Milkweed	*Asclepias syriaca*	Pink	0.31
Gayfeather	*Liatris spicata*	Purple	0.31
Goldenrod	*Euthamia caroliniana*	Yellow	0.31
New England Aster	*Symphyotrichum novae-angliae*	Purple	0.31
Obedient Plant	*Physostegia virginiana*	Purple	0.31
Stokes Aster	*Stokesia laevis*	Purple	0.31
Sunflower	*Helianthus angustifolius*	Yellow	0.31
Tickseed	*Coreopsis pubescens*	Yellow	0.31

Observations

Potted plants were placed according to each pattern, with 0.4 m between pots. This spacing was determined using the minimum planting distance listed in the North Carolina Extension Gardener Toolbox for each species, which was then averaged (Fig. 3). All patterns were observed on the same day for 20 minutes each to reduce weather as a variable and to ensure that all observations occurred on sunny days between 0700 and 1500. Plants were observed 5.6 m away from a pollinator garden on the UNC Asheville campus, and patterns were established in random orders on each day. Plants were repotted when they showed signs of stress and were watered daily in the morning unless rainfall occurred the previous night.

During observations, insects were identified to closest taxonomic level (at least Family; more often genus and specific epithet) using a pictorial key or were captured with centrifuge tubes and placed on ice. Insects captured were released after identification. Visitors were not counted if they visited another flower on the same pot but were counted if they visited multiple plant species.

Figure 3. Set up for circle and square shapes, using meter tapes. A is a circle with a diameter of 4 m and a radius of 2 m. B is a 2 m x 3.6 m square.

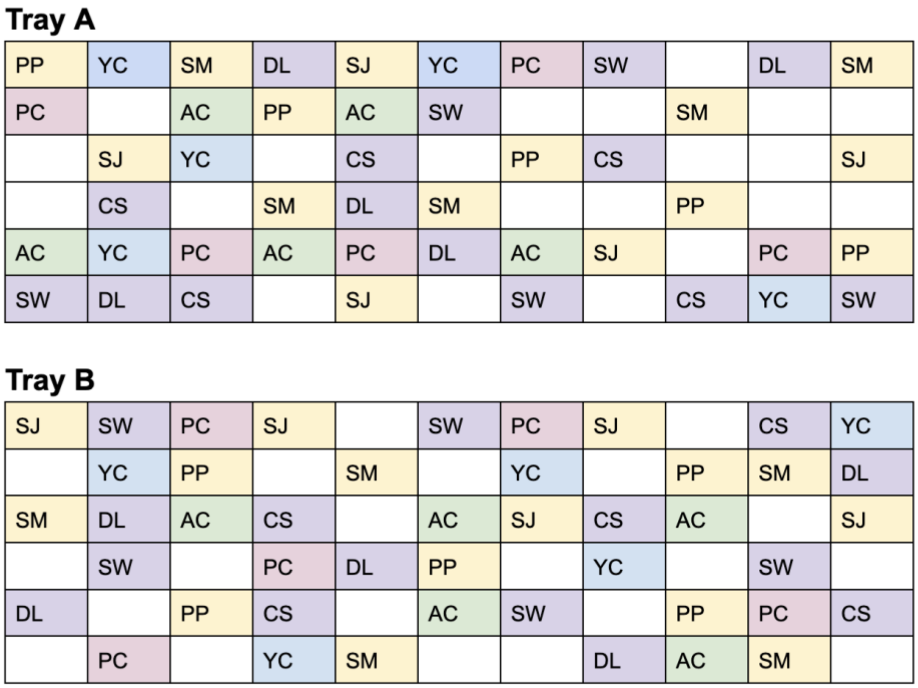
Seed Layout for AM Fungi Colonization

To observe AM fungi colonization density in monocultures and polycultures of wildflowers, seeds of three yellow species and three purple/pink species of native herbaceous angiosperms were sourced from Sow True Seeds in Buncombe County, NC (Table 2). Seeds were sterilized following a protocol that was adapted for this experiment to remove surface microorganisms (Parnell et al. 2024). In this process, seeds were placed in their respective categories in microcentrifuge tubes, submerged in 70% ethanol for 3 minutes, and the ethanol was removed using a micropipette. Seeds were then treated with a 50% bleach solution (50% bleach, 50% water) for 3 minutes, after which the solution was carefully removed with a micropipette, ensuring seeds were not removed during the process. Seeds were subsequently rinsed three times with distilled water and allowed to dry.

Seeds' planting arrangements were randomized, then seeds were placed at appropriate depths in plastic seedling trays filled with native soil. Planting combinations included monocultures of individual species, combined yellow species, combined purple species, and a mixture of all species (Fig. 4), with 6 seeds in each cell and 10 replicates per treatment. Seeds were allowed to grow at 20-25 °C with ambient sunlight for 45 days to obtain sufficiently mature roots for AM fungi colonization (Fig. 5).

Table 2. Plant common name, scientific name, and color of seeds used for AMF experiment.

Common Name	Scientific name	Color
Cleome Spider Flower	*Cleome hassleriana*	Pink
Delphinium Larkspur	*Delphinium consolida*	Purple
St. John's Wort	*Hypericum perforatum*	Yellow
Sweet Alyssum Painted Carpet	*Lobularia maritima*	Purple
Sunflower Maximilian	*Helianthus maximiliani*	Yellow
Partridge Pea	*Chamaecrista fasciculata*	Yellow



Tray A

PP	YC	SM	DL	SJ	YC	PC	SW		DL	SM
PC		AC	PP	AC	SW			SM		
	SJ	YC		CS		PP	CS			SJ
	CS		SM	DL	SM			PP		
AC	YC	PC	AC	PC	DL	AC	SJ		PC	PP
SW	DL	CS		SJ		SW		CS	YC	SW

Tray B

SJ	SW	PC	SJ		SW	PC	SJ		CS	YC
	YC	PP		SM		YC		PP	SM	DL
SM	DL	AC	CS		AC	SJ	CS	AC		SJ
	SW		PC	DL	PP		YC		SW	
DL		PP	CS		AC	SW		PP	PC	CS
	PC		YC	SM			DL	AC	SM	

Figure 4. Plastic trays with experimental treatments. Yellow species include PP (Partridge pea, *Chamaecrista fasciculata*), SM (Maximilian sunflower, *Helianthus maximiliani*), and SJ (St. John's wort, *Hypericum perforatum*). Purple species include SW (Sweet alyssum, *Lobularia maritima*), CS (Cleome/Spider flower, *Cleome hassleriana*), and DL (Delphinium/Larkspur, *Delphinium consolida*). Treatments consisted of monocultures (individual yellow or purple species) and polycultures: YC (all yellow species), PC (all purple species), and AC (all species combined). All treatments contained six seeds of each species per cell.

Figure 5. Plastic seedling trays showing planting categories, including monocultures and polycultures of yellow and purple/pink wildflower species.

Soil Inoculum and AMF Count

Soil inoculum was collected from a garden at UNC Asheville (Bee Hotel site) and used directly for planting. Additionally, soil was collected from a nearby mulched area to compare inoculum from a managed wildflower site and a mulched site (Fig. 6). To observe and quantify AM fungi in soil samples, a wet sieving protocol was adapted (Boyno et al. 2023). Three 10 g replicates were prepared from each of the two Bee Hotel soil sources used in this study. Soil samples (10 g) were mixed with 100 mL of distilled water and passed through 63 μm and 100 μm meshes to separate larger particles and spores. The filtrate was transferred to centrifuge tubes and centrifuged at 4000 rpm for 5 min. The supernatant was then passed through a 63 μm mesh and combined with a 55% sucrose solution, followed by centrifugation at maximum speed for 2 min. The supernatant was discarded, as no spores were observed in it, and the pellet was examined at 100X total magnification.

Figure 6. Soil sources for the experiment at the Bee Hotel, UNC Asheville. (A) Maintained wet meadow garden. (B) Adjacent mulched area.

Root Preparation for AMF Density Quantification

AMF staining followed a modified protocol from the University of California Riverside (Center for Conservation Biology 2006). Harvested plants were preserved in Farmer's solution prior to root processing. Roots were then soaked in 1 M HCL for 3 minutes and rinsed with distilled water. Roots were submerged in centrifuge tubes with Cotton Blue staining solution for 24 hours. Lastly, the roots were then rinsed with distilled water and transferred to a 1:1:1 solution of acetic acid: glycerol: distilled water and left for 24 hours and rinsed. Samples were observed at 100X total magnification, and AM fungi colonization density was quantified with a clicker to count arbuscules in roots.

Data analysis

As data were counts, we used Kruskal-Wallis tests to compare insect diversity among the four planting patterns and to examine the effects of plant species, plant color, shape, and spacing on insect abundance, richness, and to analyze the differences of the Shannon diversity. This test was also used for comparing fungal spores in our two soil samples, and to examine the effect of planting treatment and plant species on arbuscule count in stained roots. Analyses were conducted in R version 4.5.1 (R Core Team, 2025).

Results

Abundance

There were 1,209 insect visitations across all plant species and observation days, including 13 Lepidoptera species, 17 Hymenoptera species, 11 Diptera species, and 1 Coleoptera species. There was no significant effect of planting shape or spacing on insect abundance. However, plant color had a significant effect, with yellow flowers showing higher visiting insect abundance (N = 789, $\chi2 = 11.20$, p = 0.0008).

The number of insect visitors differed by taxon ($\chi2 = 203.09$, p = 2.2 e-16). The species with the most visits was Black-eyed Susan (*Rudbeckia hirta*) with 640 visits, followed by Gayfeather (*Liatris spicata*) with 305 visits and Tickseed (*Coreopsis pubescens*) with 128 visits. Obedient Plant (*Physostegia virginiana*), Butterfly Weed (*Asclepias tuberosa*), New England Aster (*Symphyotrichum novae-angliae*), Stokes Aster (*Stokesia laevis*), Goldenrod (*Euthamia caroliniana*), Common Milkweed (*Asclepias syriaca*), and Sunflower (*Helianthus angustifolius*) each had fewer than 50 visits (Fig. 7).

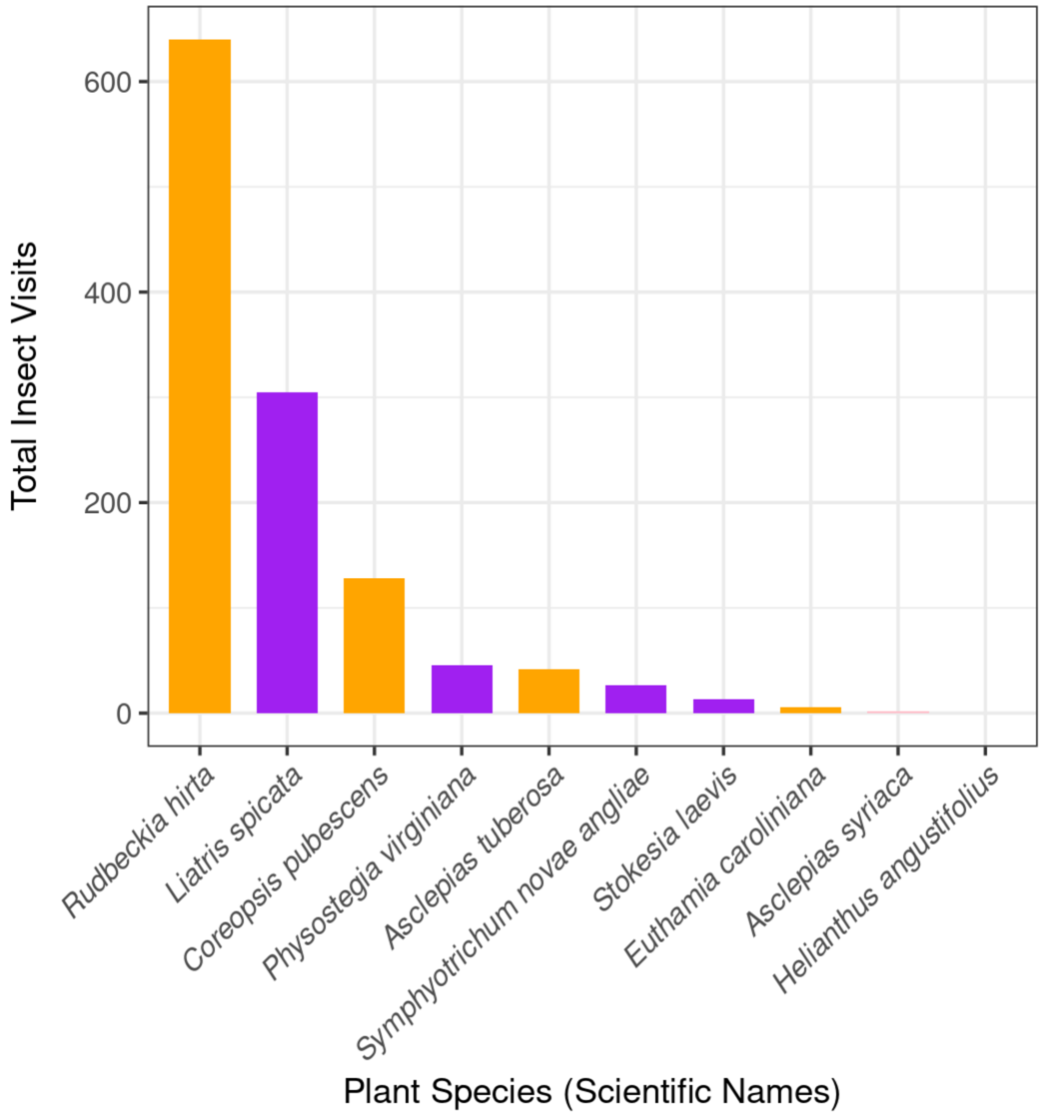


Figure 7. Total number of insect visitations on all plant species over 20 days. Sunflower (*Helianthus angustifolius*) was an exception due to few mature flowers.

Insect Richness

Insect richness was not related to planting shape, planting spacing, or overall planting pattern. Plant color (N = 789, χ2 = 10.57, p = 0.0011) and plant species (χ2 = 198.53, p = 2.2 e-16) had a significant effect on insect richness (Fig. 8). The order with the highest number of observed visitations was Hymenoptera, followed by Diptera. Within Hymenoptera, the most frequently observed group was Halictidae with 443 visits, followed by Augochlora with 112 visits, the Western Honeybee (*Apis mellifera*) with 97 visits, Lasioglossum with 59 visits, and the Gold-Marked Thread-Waisted Wasp (*Eremnophila aureonotata*) with 44 visits. The remaining species each had fewer than 30 visits (Fig. 9).

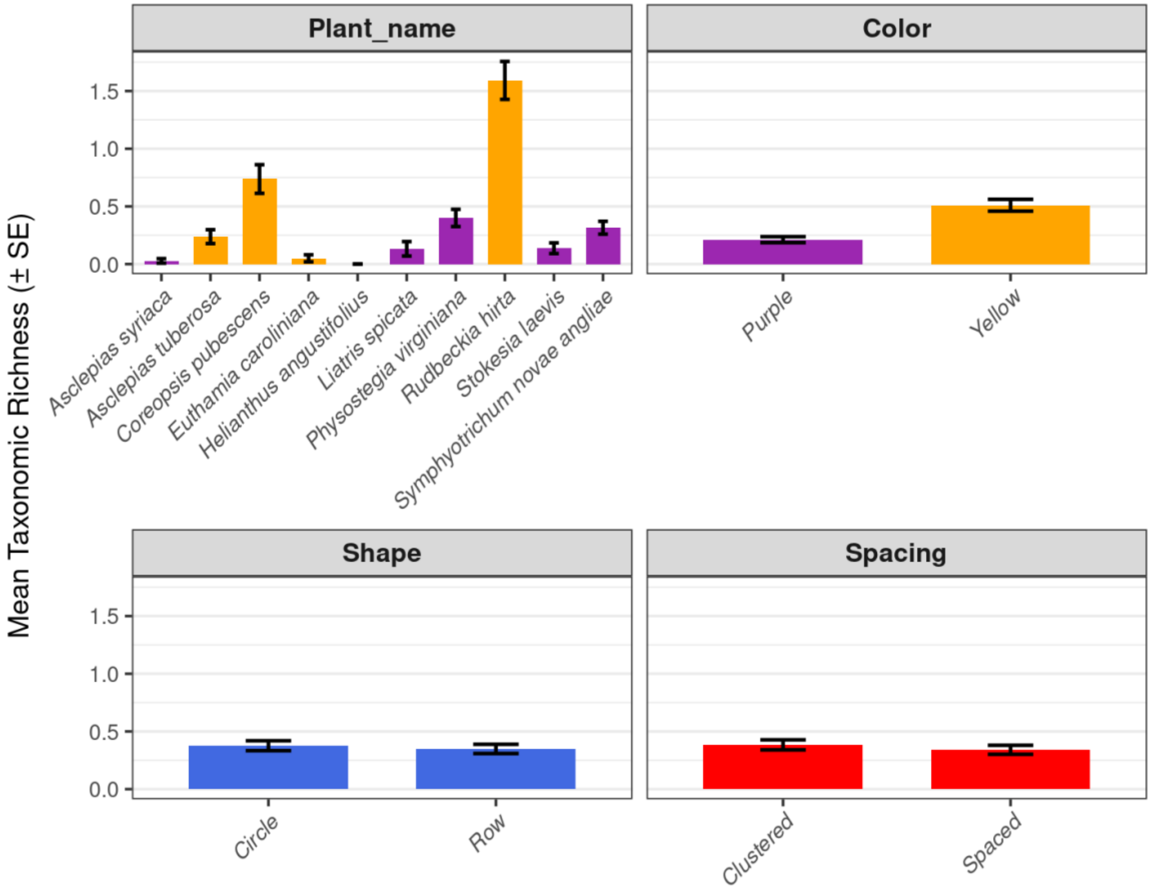


Figure 8. Mean ± SE Taxonomic Richness across plant species, color, shape, and spacing.

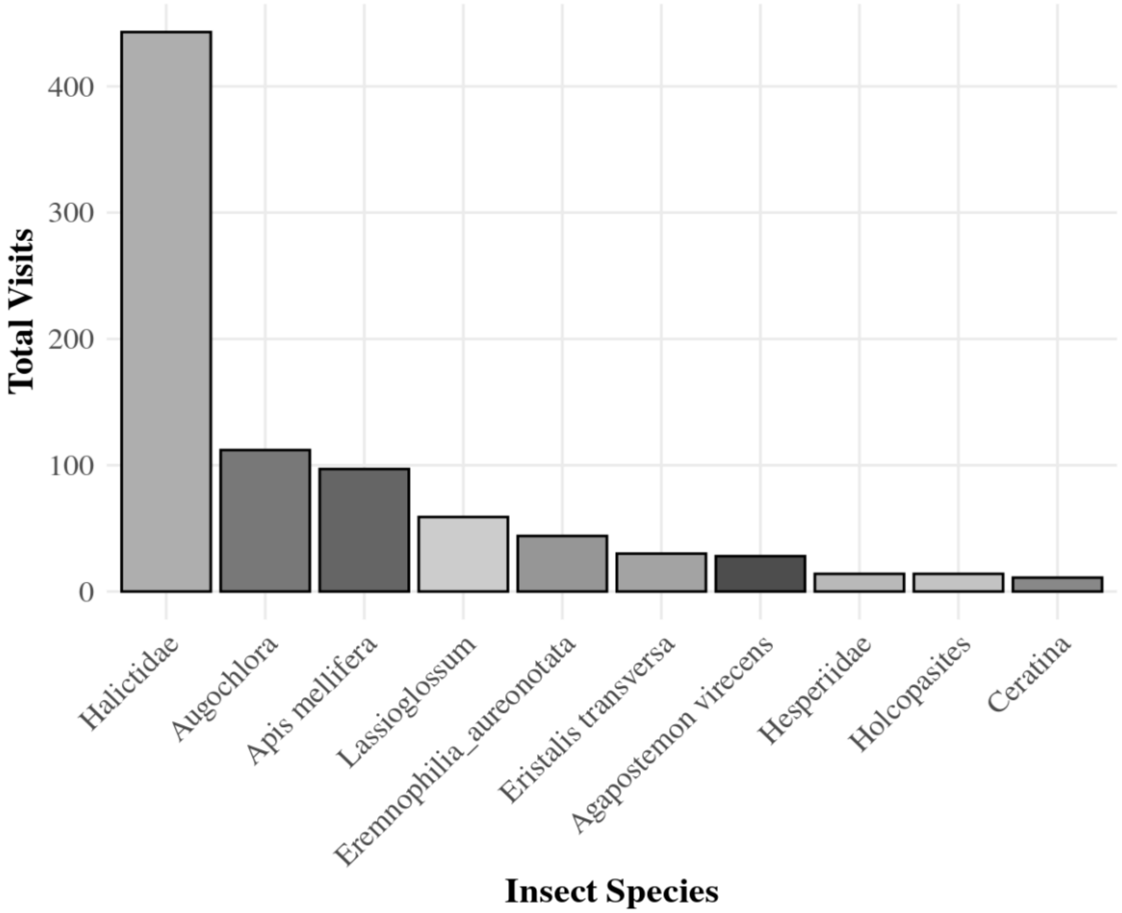


Figure 9. Total number of visits for most frequently observed insects across all plants for the study's duration.

Shannon Index

In order to measure biodiversity of floral visitors across planting patterns, the Shannon Index was calculated for all planting patterns and days. These indices were then used in a Kruskal-Wallis test to measure the effect of plant species, plant color, shape, and spacing on the Shannon Index. Plant shape and plant spacing did not have a significant effect on the Shannon Index ($P > 0.05$ for all). The Shannon Index varied by plant species ($\chi2 = 178.44$, $p = 2.2$ e-06), and the index was highest for yellow-flowered species ($N = 789$, $\chi2 = 23.78$, $p = 1.08$ e-06) (Fig. 10).

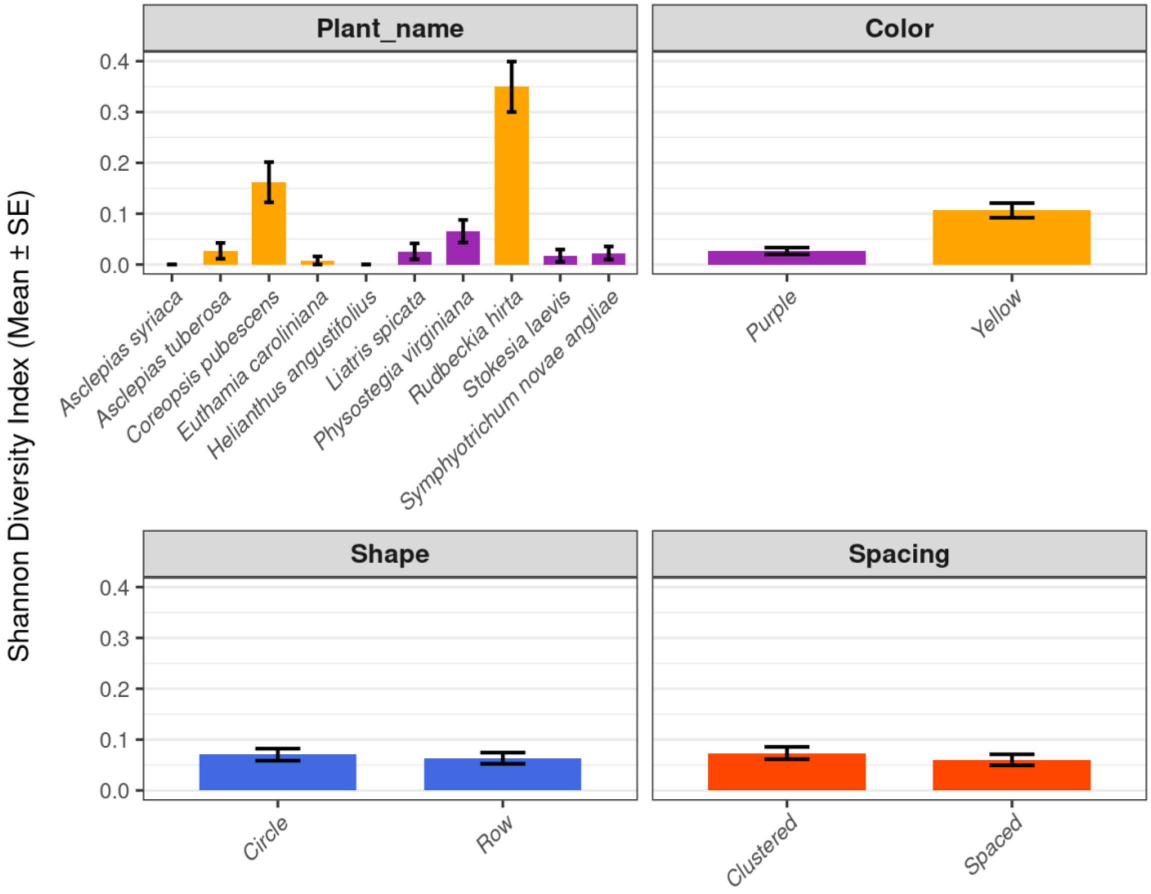


Figure 10. Mean ± SE Shannon Diversity Index across plant species, color, shape, and spacing.

AMF Spore Density in Soils

Fungal spore count for a site with a commonly maintained wet meadow garden, and an adjacent are which is maintained by turf grass and mulch was counted, and the Kruskal-Wallis test was used to measure the effect of different vegetated areas in spore count. Fungal Spore count from beneath the commonly maintained wet meadow garden was higher than the adjacent unplanted area ($\chi 2 = 19.41$, $p = 1.06$ e-05) (Fig. 11).

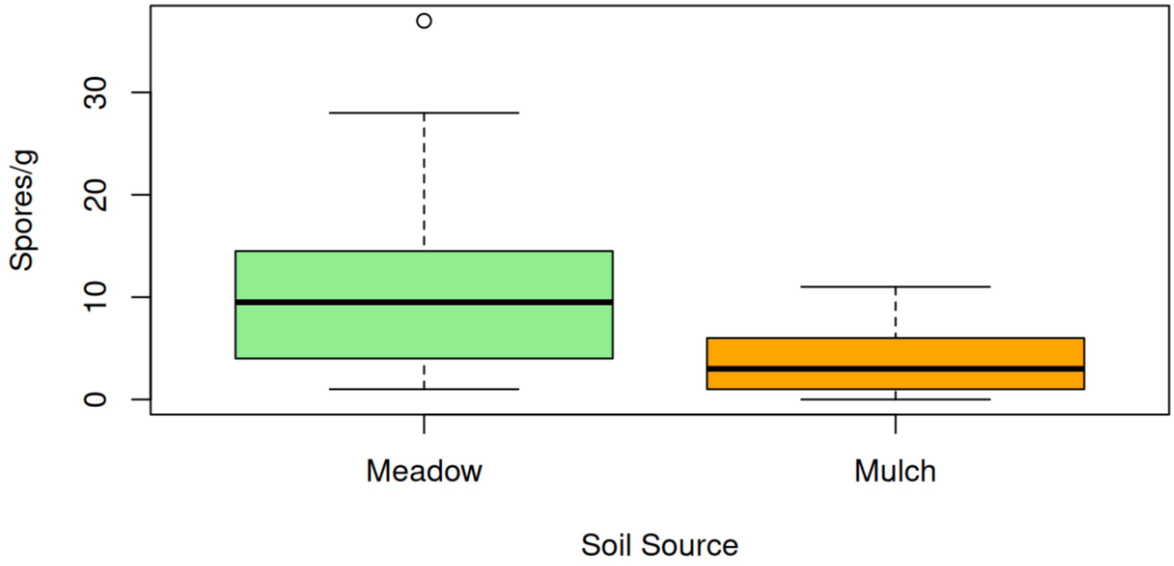


Figure 11. Spore/g per soil site.

Planting treatment effect on AMF Arbuscule count in stained roots.

Planting treatments (monocultures vs. polycultures) had no significant effect on arbuscule count ($\chi^2 = 5.88$, $p = 0.55$). Similarly, plant species had no significant effect on arbuscule count ($\chi^2 = 4.29$, $p = 0.37$) (Fig. 12).

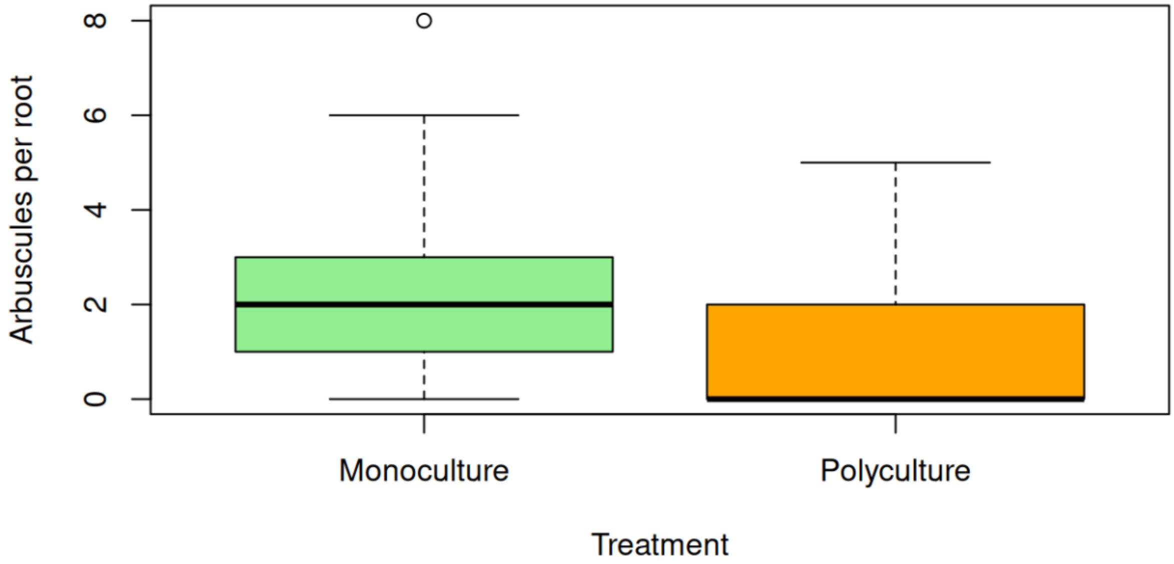


Figure 12. Arbuscule count in roots for the treatment groups (Monocultures versus Polycultures).

Discussion

In this study, only plant species and flower color significantly affected insect abundance, richness, and biodiversity. These trends were seen across all four planting patterns. In contrast, planting shape and spacing did not significantly influence insect visitors. There was, however, a trend for clusters and circles to have more insect abundance, richness, and biodiversity than spaced or row patterns.

Insect identification revealed that the majority of visitors were Sweat Bees (*Halictidae*), Western Honeybees (*Apis mellifera*), and Gold-Marked Thread-Waisted Wasps (*Eremnophila aureonotata*), along with other taxa such as Skipper Butterflies (*Hesperiidae*, order *Lepidoptera*). These groups contain many generalist species, a pattern commonly observed in urban landscapes (Xiao et al. 2016). Only in rare cases did we observe specialist species, such as Monarch butterflies (*Danaus plexippus*) visiting Butterfly Weed or Common Milkweed.

Plant species also had a significant effect on insect visitation, with Black-eyed Susan (*Rudbeckia hirta*) receiving the highest number of visits. This species belongs to the Asteraceae family, which contains many radially symmetric floral inflorescences, forms known to attract a broad range of generalist pollinators (Watson et al. 2022). Black-eyed Susan was also a yellow-flowering species, consistent with the trend observed in this study of greater insect visitation to yellow flowers. Similar findings have been reported elsewhere; for instance, a study in Nepal found that most butterflies preferred yellow flowers (Subedi et al. 2021).

Although color significantly influenced insect visitation, insects also display foraging plasticity based on nectar and pollen rewards. Thus, visitation rates might have depended on an insect's ability to learn and retain information about rewarding plants (Reverté et al. 2016). Furthermore, other studies have identified flower abundance as a key predictor of insect visitation, emphasizing the importance of maintaining continuous floral availability throughout both summer and fall (Tatarko et al. 2025).

Different colors have also been found to attract different assemblages of insects. For example, bees have been associated with purple flowers, while wasps and dipterans are more commonly associated with yellow flowers in other studies (Reverté et al. 2016). This pattern was observed in the field, as bumble bees were repeatedly seen visiting Obedient Plant (*Physostegia virginiana*), and many Gold-Marked Thread-Waisted Wasps were observed on Black-eyed Susan. However, this was not a strictly linear relationship, as many Halictidae species also visited Black-eyed Susan. Additionally, other factors can influence visitation rates, including the history and geography of a location (Sirohi et al. 2015).

The plant species used in this part of the study were watered consistently, minimizing stressors such as afternoon heat. However, indicators of plant health, such as reproductive success and photosynthetic rate, were not measured, so phenological changes could not be recorded. Though studies examining urban plant populations have found phenological changes. For example, a study examining flower phenology and synchrony in urban and rural areas found higher outcross pollen limitation in urban populations of an herb, and that flowering synchrony differed among populations in drainage habitats in urban areas (Fujiwara et al. 2025). This is of particular concern for food resources for insects and the reproductive success of plants found in secluded urban areas.

Though there is often species-level specificity between plants and insects, this relationship is less likely between plants and AM fungi, as most terrestrial plants form associations with AM fungi (> 80%) (Davidson et al. 2020). Similarly, in this study, the plant species used in monocultures or polycultures did not influence arbuscule count within the roots, though other types of selectivity may occur. For example, small-scale studies have shown that AM fungal community richness and composition can be more strongly associated with plant growth habit (grass vs. woody), generalist vs. specialist plants, shade tolerance vs. intolerance, photosynthetic pathways, and plant survival strategies (competitor, tolerant, or ruderal) (Davidson et al. 2020). A study also found that C4 plants had higher AM fungal richness, while C3 plants had greater phylogenetic diversity, this pattern may have occurred in our study, as all plants used were C3 species belonging to different families, but would need quantification (Davidson et al. 2020).

There was a slight trend toward more arbuscules in monocultures, which contradicts the general competition prediction that intraspecific competition is more intense than interspecific competition (Shen et al. 2024). However, treatments likely did not experience strong stressors such as limited water or light. Even though this small-scale study found no difference in arbuscule density between monocultures and polycultures, other studies have shown that adding more than one crop to monocultures increased total soil Carbon by 3.6% and soil Nitrogen by 5.3%, with organic matter also higher in prairie soil than in turfgrass. Therefore, additional studies examining polyculture effects are necessary, as AM fungi depend on carbon provided by plants (Yi Chou et al. 2024).

Previous studies have found that seeds are more receptive to microbial colonization from soil inoculum compared to older plants, which is an important consideration for planting decisions, particularly regarding soil conditions (Aleklett et al. 2022). For example, one study found that a plant flowered more frequently when grown in native inoculum, and several late-successional plants benefited most from native AM fungi (Middleton et. al 2015). This is important because urban areas often remove native plants as they die back in late fall, and such disturbances may disrupt established plant-fungal relationships.

Our study also found that in the two closely spaced sites with different vegetation, spore abundance differed: more spores were present in the commonly maintained wet meadow gardens than in mulched turfgrass. This highlights how management decisions and anthropogenic disturbances can influence both spore density and the types of fungal spores present. Although our study did not identify spore families, other studies in a city in Argentina found *Glomeraceae*, a ruderal fungal group with rapid growth and high spore production, to be dominant due to constant disturbance, demonstrating how disturbance and management influence spore communities (Build et al. 2021).

While one of our sites had continuous planting and was recently mowed, other studies have shown that spore density can double in protected areas compared to polluted urban sites, though the study in Argentina found no significant differences among its sites (Buil et al. 2021). Another study comparing turfgrass and unmanaged prairie found that turfgrass had similar bacterial diversity, but lower fungal diversity compared to unmanaged prairie (Yi Chou et al. 2024). Therefore, spore density likely depends on multiple factors beyond vegetation alone, and further studies on urban spore dynamics are needed.

Because urban landscapes often have degraded soils, AM fungal spores and hyphae play an important role in soil restoration. They produce glomalin-related soil proteins (GRSP), which are not easily degradable and act as a binding agents to promote soil aggregation. This improves soil structure, enhances organic carbon accumulation, and increases water infiltration, key factors for plant survival in urban environments (Yinong Li et al. 2022). Additionally, GRSP may increase the absorption and sequestration of heavy metals, which is important due to polluted soils negatively affecting plant establishment and their beneficial relationships with AM fungi (Tu et al. 2025).

Conclusion

In this study, we observed that plant species and flower color were the primary factors influencing insect visitation. In contrast, plant shape (row vs. circle) and plant spacing (spaced vs. clustered) did not have a significant effect. Therefore, municipalities, land managers, and homeowners may choose whichever planting pattern they prefer for their pollinator gardens but should place greater consideration on plant species selection and flower color when designing for pollinator attraction. Additionally, greater consideration of denser vegetation may reduce temperatures by decreasing impervious surfaces. This can lower the frequency of high-temperature conditions experienced in urban landscapes and increase connectivity between habitats, thereby lessening stressors on plants and insects. While plant and soil health depend on multiple factors, including soil conditions, ecological connectivity, and interactions with other organisms such as fungi and insects, this highlights the importance of thoughtful and informed planting decisions. Given the design of this study, future research could extend these findings by planting directly in the ground to observe longer-term trends and by examining plant phenology and additional ecosystem services, such as erosion control, shade provision, and other landscape benefits. AM fungi density could be assessed using molecular techniques to quantify abundance and identify the fungi present, while increasing sample sizes across urban landscapes more accurately. This would further improve understanding of how landscape choices affect ecosystem services, and the important relationships plants form with their habitats.

Acknowledgements

I sincerely thank the McCullough Institute at UNC Asheville, the McCullough Fellowship, and the McCullough family, which gave financial and logistical support for this project's design, execution, and dissemination. Dr. Casey King, Environmental Science faculty and Director of UNC Asheville's McCullough Institute, and my McCullough cohort students provided invaluable feedback over this project's duration. I also thank Dr. Jen Rhode Ward from the Biology department at UNC Asheville for continuous support in guidance in all steps of this project. Ms. Copland Rudolph and Mr. Gregory Hall from Asheville City Schools Foundation advised on outreach portions of the project through their In Real Life (IRL) Program, and Ms. Maria Campozano provided invaluable support in observation days. Comments from Dr. R. Graham Reynolds improved the manuscript. I am grateful to the students at Asheville City Schools and the Buncombe County Soil and Water Conservation District for their participation in afterschool camps. Thank you to Ms. Rose Wall and Ms. Jen Knight, coordinators of Buncombe County Soil and Water Conservation and Environmental Educators. Finally, thanks are due to Ms. Melissa Acker, Ms. Bethany Beliveau, Mr. Ray Magalski, and the entire crew of UNC Asheville Grounds Crew, who gave important advice along the way.

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