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Article

Dwarf White Clover Supports Pollinators, Augments Nitrogen in Clover–Turfgrass Lawns, and Suppresses Root-Feeding Grubs in Monoculture but Not in Mixed Swards

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Abstract: The runoff or leaching of nitrogen fertilizers from monoculture turfgrass lawns contributes to water pollution, and such lawns are susceptible to insect pests and provide few resources for pollinators. One approach to creating more sustainable lawns is to incorporate white clover (*Trifolium repens* L.), a nitrogen-fixing legume, into grass seed mixtures or existing turfgrass swards. “Dutch” white clover (DWC), a ubiquitous landrace, forms non-uniform clumps when intermixed with turfgrasses, thus it is often considered to be a lawn weed. Recently, several dwarf varieties of white clover have been selected for their small leaf size and low growth habit, allowing them to tolerate low mowing heights and blend better with grasses. To date, there have been no studies published on the entomological aspects of dwarf clover in pure stands or intermixed with turfgrass. We established field plots with combinations of DWC, two cultivars of dwarf clover, and tall fescue (*Schedonorus arundinaceus* (Schreb.) Dumort.) in monoculture or mixed swards, and compared the invertebrate communities therein. Predatory arthropods and earthworm numbers were similar in all plot types. The clover monocultures were resistant to white grubs, but the grub densities in the clover–tall fescue dicultures were similar to those found in the pure tall fescue swards. Dwarf clovers and DWC were similarly attractive to bees and supported similar bee assemblages. The tall fescue foliar N content was elevated 17–27% in the dicultures with clovers.

Keywords: *Trifolium repens*; *Schedonorus arundinaceus*; pollinators; *Popillia japonica*; low-input lawns



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1. Introduction

Lawns are a pervasive feature of urban and suburban landscapes worldwide [1,2]. Traditionally defined as “ground covered with closely mowed vegetation, usually grasses” [3], lawns dominate residential lots, institutional and commercial landscapes, parks, cemeteries, playgrounds, street verges, and medians, occupying an estimated 39–54% of the total area devoted to urban development in the continental United States [4]. Traditional turfgrass lawns provide aesthetic, recreational, and human psychological and physical health benefits while providing ecosystem services; e.g., reducing soil erosion and surface runoff, dissipating heat through evaporative cooling, and reducing noise and glare compared with impervious surfaces [2,5–7]. Turfgrass lawns also sequester atmospheric carbon [4,8–10], although those benefits may be more than offset by the amounts of fossil fuels, water, nitrogen, and pesticides used in lawn maintenance [4,10–12]. Thus, there is growing interest in alternative lawns needing fewer resource inputs and providing greater ecosystem services than traditional all-turfgrass lawns [1,2].

Nitrogen (N) fertilization is a common cultural practice for establishing and maintaining turfgrass lawns. Nitrogen enhances sward density, color, and vigor, and is the nutrient required in the highest amount by the grass plant [13]. However, lawn fertilizers

that are applied off-target to impervious surfaces, such as driveways and sidewalks, or to dormant, dying, or dead turfgrass can be moved by irrigation or rainfall into bodies of water, contributing to non-point source pollution [14,15]. In addition, N applied in soluble forms (e.g., urea) can result in nitrates leaching into groundwater [16].

One way to reduce the need for N inputs is to incorporate white clover (*Trifolium repens* L.), a nitrogen-fixing legume, into grass seed mixtures or existing turfgrass swards. Productive mixtures of white clover and cool-season turfgrasses can often fix between 100 and 200 kg N ha⁻¹ year⁻¹ with positive effects on turfgrass growth, color, and visual appeal [17,18]. The typical recommended N fertilization rates for moderately maintained cool-season turfgrass lawns range from 98–195 kg N ha⁻¹ year⁻¹. Consequently, if white clover can supply just a portion of a lawn's annual N needs, the resulting reductions in fertilizer use could be substantial [19].

Trifolium repens, a perennial forb of Eurasian origin [20], was introduced into North America during the early colonial period, and has also been exported to other temperate regions of the world [20]. It grows naturally in pastures and along roadsides where climatic and soil conditions are suitable, and is intentionally seeded in mixed grass/clover pastures. "Dutch" white clover (DWC) is the vernacular term for a ubiquitous landrace found in many landscapes. Like all *T. repens*, it grows and spreads via stolons that root at the nodes [20]. A common component of lawn seed mixes before World War II, DWC fell out of favor with the introduction and marketing of broadleaf weed lawn herbicides in the 1950s. Furthermore, DWC tends to form taller, dark green clover-only patches that reduce lawn uniformity, and its flowers attract bees [21–23], which some people, e.g., persons with stinging allergies or families with small children, may consider to be a hazard.

Microclover[®] (DLF-Pickseed, Tangent, OR) is a dwarf variety of white clover (*T. repens* var. "Pipolina") selected for its small leaf size and low growth habit to allow it to tolerate low mowing heights and to blend in with cool-season turfgrasses without clumping or outcompeting the grass [19,24–26]. Used at a low rate in grass seed mixtures, or seeded into an existing turf stand, it supplies N to the grass and helps to prevent the establishment of weeds by increasing the canopy density [26]. Microclover[®] was bred to have fewer and smaller flowers than DWC, although it will flower more if mowed less frequently (L. Brillman, DLF-Pickseed, pers. comm.). *Trifolium repens* var. "Barbian", marketed as Turf Clover (Barenbrug USA, Tangent, OR), is another dwarf clover variety developed to blend more easily with turfgrasses than DWC.

In the United States where there are normative social pressures to maintain lawns as low-mowed turfgrass monocultures [27,28], spontaneous flowering forbs such as clover, the common dandelion (*Taraxacum officinale* F.H. Wigg.), and violets (*Viola* spp.) are traditionally viewed as undesirable weeds to be eliminated. Indeed, the US home and garden sector spent USD 450 million on lawn herbicides and plant growth regulators in 2012 [29]. That mindset seems to be changing, however, as the public becomes more aware of the threats to honey bee health [30], as well as steep declines in the populations of wild bees and other pollinators [31–34]. A reduction in floral resources (nectar and pollen) due to urbanization and other land-use changes is thought to be a major factor contributing to wild pollinator decline, especially in North America and Europe [32,35,36]. Urban ecologists are increasingly encouraging the conversion of traditional monoculture turfgrass lawns to more diverse habitats (e.g., [2,37]). Millions of citizens engage in ecological gardening and landscaping to support pollinators [38,39], and there is a growing public acceptance of flowering lawns in community parks [40] and residential yards [41–43].

Trifolium repens produces copious amounts of pollen and nectar [36,44,45], and is highly attractive to wild bees and honey bees that forage in urban landscapes [21–23,46–49]. It is the most important nectar source in Britain [45], providing the highest portion of total nectar production by flowering plants in parks, cemeteries, and road verges, and more than half of the total nectar resources in lawns and other mowed amenity grasslands [36]. Due to its prolonged bloom period, *T. repens* can help to sustain pollinators during seasonal gaps in floral resource availability from other plants [46,49,50]. Clover lawns can serve as

stepping stones for the movement of bees and other pollinators between gardens, natural-area remnants, and other urban green spaces [21,37,51–53]. Such connectedness of floral resources is thought to be important for supporting species richness and an abundance of urban bees [54–57]. Clearly, if most homeowners, schools, and parks dedicated just a portion of their lawns to clover, the benefits to urban bee conservation could be substantial. Besides planting it, encouraging spontaneous *T. repens* by reducing lawn herbicide use and mowing less frequently are other ways to augment the food supply for urban bees [21,58,59].

White clover monostands are intolerant of heavy foot traffic, and are thus unsuitable for competitive sports fields or busy playgrounds [20,60]. Mixed clover–turfgrass swards, however, are relatively more resilient to foot traffic [18,20], and therefore suitable for use in home lawns, park lawns, medians, and other low-to-medium traffic sites [24,61]. White clover is relatively intolerant of shade, high heat and drought, and winter cold, which can cause canopy dieback and leave bare areas that can become muddy and require reseeding [20,61]. Attaining and maintaining a consistent percentage of clover to grass is difficult [20] as clover often disappears from mixed swards over several years [62], however it can be reseeded back in. It is also intolerant of many broadleaf herbicides, which can limit options for weed control, although some herbicides provide selective control of some broadleaf weeds in mixed clover–turfgrass swards [63,64]. Breeding for dwarf clover genotypes that are more resistant to environmental stress may improve their persistence [24].

There have been no previous studies focusing on the entomological aspects of dwarf clover versus DWC in lawns. A better understanding of the pests, natural enemies, and other invertebrates associated with each clover type alone or intermixed with grasses is needed to clarify if such lawns are a sustainable alternative to turf-only lawns. It is also important to determine if dwarf clovers and DWC support similar pollinator assemblages. Herein we: (1) compared communities of macroinvertebrates in DWC, two varieties of dwarf white clover, and tall fescue (*Schedonorus arundinaceus*) in monoculture or mixed swards; (2) documented the types of bees visiting each clover type; and (3) tested the hypothesis that incorporating clover into tall fescue provides agronomic benefits, including nitrogen augmentation and associational resistance to root-feeding white grubs (Coleoptera: Scarabaeidae).

2. Materials and Methods

2.1. Plot Establishment

The study was established in a stand of turf-type tall fescue, var. “Forest Green”, that had been seeded (293 kg/ha) on 9 September 2014 on a Maury silt loam soil (Fine-silty, mesic, typic Paleudalf; pH 6.5) at the University of Kentucky A.J. Powell Turfgrass Research Center, Lexington, KY. Before this study, the tall fescue had been maintained with 49–98 kg N ha⁻¹ y⁻¹ and mowed at 7.6 cm. Glyphosate (9.35 L/ha) was applied on 28 May 2015 to kill the tall fescue in the clover-only plots.

The methodology for establishing the clover followed the protocols of Sparks [19]. On 24 June 2015, we mowed (scalped) all plots to 2.54 cm, made one pass over all plots with a vertical mower (7.5 mm depth, 2.5 cm spacing) to create seed channels, and removed all clippings and debris before seeding. The three clover types were hand-seeded (24.4 kg/ha) on 25 June (Figure 1A). To ensure even coverage, each plot was divided into quadrats into which pre-weighed portions of seed were then sprinkled in two directions. There were seven treatments. Four of them were monocultures of either tall fescue (as above), DWC (Fayette Seed, Lexington, KY), Microclover® (*T. repens* var. “Pipolina”; DLF Pickseed, Halsey, OR), hereafter referred to as Microclover, or *T. repens* var. “Barbian” (Barenbrug USA, Tangent OR), hereafter referred to as Turf Clover. In addition, there were three diculture treatments: DWC + tall fescue, Microclover + tall fescue, and Turf Clover + tall fescue, created by over-seeding the respective clover type into the existing tall fescue. The plot size was 6.1 × 6.1 m, with five replications in a randomized complete design. Following seeding, the study site was raked with spring-tooth metal rakes flipped over to

increase seed-soil contact, and then covered with a lightweight spun-bond polyester fabric (Reemay, Avintiv, Old Hickory, TN), and irrigated with an overhead system to encourage clover establishment. The fabric was removed after germination. The plots were irrigated (two 10-min cycles per day) as needed during summer 2015, after which there was no supplemental irrigation or other inputs. The plots were mowed weekly at 12.7 cm except in a few cases when inclement weather prevented the scheduled pollinator sampling and mowing was delayed another 1–3 days to retain existing blooms (Figure 1B).

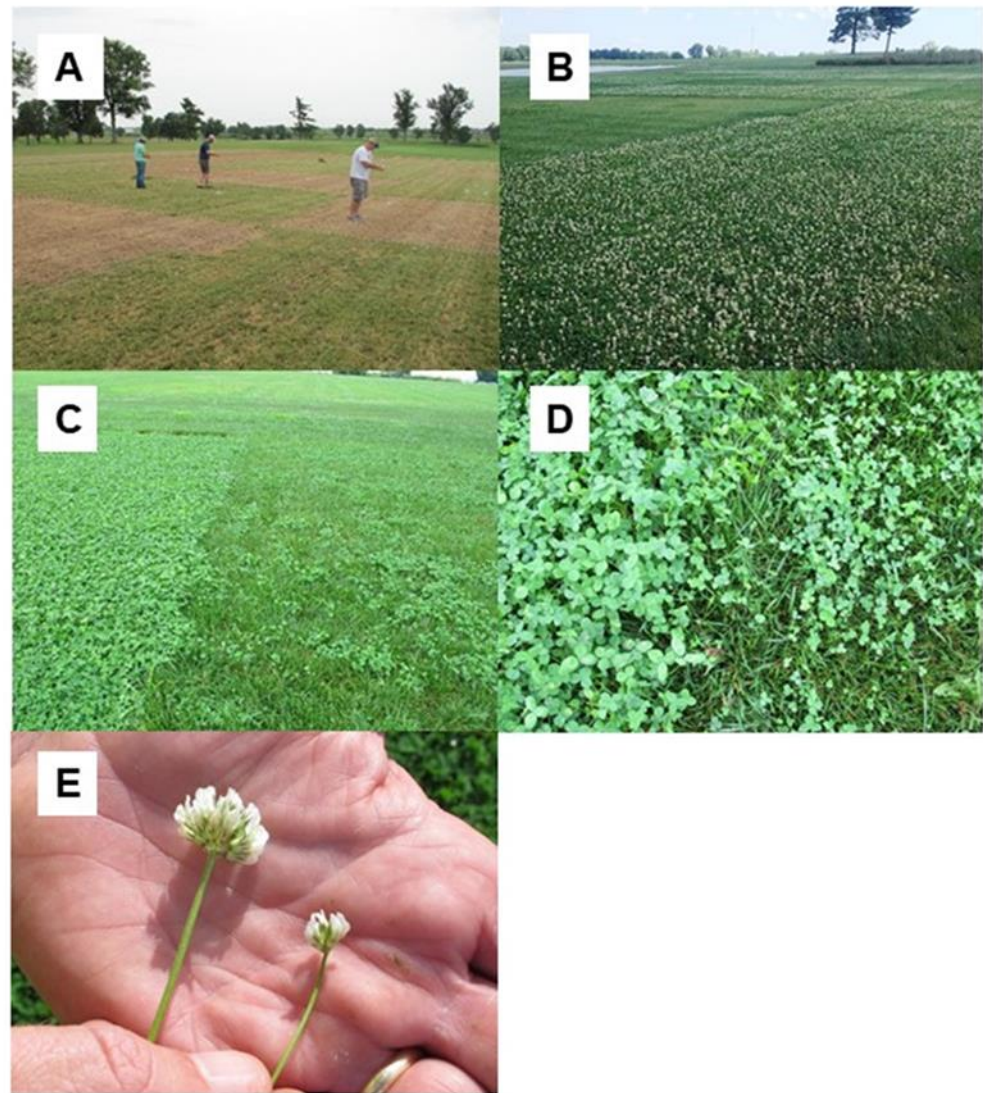


Figure 1. (A) Seeding the field plots, June 2015. (B) Cover monoculture plot in bloom, June 2016. (C) Interface of monoculture clover and clover–tall fescue diculture plot. (D,E) Differences in “Dutch” white clover and Microclover leaf and flower sizes.

2.2. Floral Coverage

Clover flower counts were taken on each plot on three dates, 11 September 2015 (11 weeks after planting), and on 31 May and 20 June 2016, all bright sunny days, to assess clover establishment and compare the bloom coverage between the clover types in the monoculture, or in the dicultures with tall fescue. The plots were divided in half with string and two observers standing on opposite edges of the plot visually counted all flowers on their half-plot; then those half-plot counts were pooled.

2.3. Bee Visitation and Bee Assemblages on the Three Clover Types

We compared bee visitation and assemblages by direct counts and by collecting samples from each monoculture clover plot. “Snapshot” counts [47] were taken on five dates between 31 May and 30 June 2016 by walking slowly around the perimeter of each plot for 30 s and counting all the bees that alighted and remained on a clover flower for ≥ 3 s. The counts were taken between 11:00 a.m. and 3:00 p.m. on days with favorable weather for pollinator activity, i.e., low wind (< 15 km/h) and temperatures of 18–25 °C. Bee assemblages were sampled in both June and August 2016 by hand-netting individual bees observed foraging on the clover. Sampling continued over several days during each period until approximately 40 bees had been collected from each plot (40×5 replicates = 200 bees per clover type per period; approximately 400 total bees per clover type). The bees were killed and preserved in 95% ethyl alcohol, washed with water and dish soap, then dried using a fan-powered dryer and pinned. Initial identification was made using the Discover Life keys [65], which include high-quality reference images along with reprinted taxonomic descriptions from the primary literature. Identification was then verified by consulting the original published keys and taxonomic literature [66–69]. Reference specimens were deposited in the University of Kentucky Department of Entomology Insect Collection.

2.4. Macroinvertebrate Communities in Monocultures and Dicultures of Clover and Tall Fescue

We compared the macroinvertebrate communities among the seven plot types by vacuum sampling for canopy-dwelling taxa, pitfall trapping for active epigeal species, and soil sampling for earthworms and root-feeding white grubs (Scarabaeidae). Vacuum samples were taken on three dates: 24 September 2015, 1 June 2016, and 14 July 2016; pitfall sampling was done twice, from 23–26 May and from 13–18 August 2016; and earthworms and white grubs were sampled on 20 September 2016 near the end of the second growing season after the plots were established. At that time, the grubs of the predominant species present were mainly third instars:

- Vacuum sampling was performed with a gas-powered leaf blower (Troy-Bilt, Cleveland, OH), reversed for suction with a soft mesh paint strainer clamped inside the intake tube to catch arthropods and organic matter. Sampling was done between 11:00 and 15:00 on dry, sunny days. A sample consisted of two parallel transects across a given plot, walking slowly while lightly dragging and guiding the 14-cm diameter opening of the intake tube through the plant canopy. The paint strainers with enclosed arthropods from a given plot were transferred to sealed paper bags and frozen until the invertebrates were sorted under a binocular microscope. The predominant taxa of insects and spiders were identified to family or, in some cases, to feeding guild (e.g., predatory Hemiptera (mostly Geochoridae and Nabidae), predatory Coleoptera (mainly Staphylinidae and Carabidae), parasitic wasps (including Braconidae, Ichneumonidae, and others), or small Diptera (mostly Chloropidae).
- The activity density of epigeal invertebrates was assessed with pitfall traps made from a pair of nested plastic cups (473 mL (16 oz), 9.53 cm (3.75 inch) top diameter; Solo, Lake Forest, IL) set into the ground level with the soil surface. This allowed the inner cup to be lifted and refilled without disturbing the trap site. There were five traps per plot, a central trap and one halfway along a diagonal from the center toward each corner. Ethylene glycol (2.5 cm (1 inch)) was added to the cups to kill and preserve the captured invertebrates. The captures from each 3-d sample period were pooled within the plots and stored in 90% ethyl alcohol, with specimens sorted and identified as described above.
- Endogeic earthworms (mainly *Apporectodea* spp.) were sampled by digging two pits (20×20 cm, 21 cm deep), approximately 2 m apart within each plot and by hand-sorting to recover all specimens, which were counted and collectively weighed [70]. We sampled grub populations by using a gasoline-powered sod cutter to cut a 1.22 m long strip (46 cm wide, 8 cm deep) lengthwise through the center of each plot. Each

strip was broken apart and examined for grubs, which were identified by their rastral patterns [71] and weighed.

2.5. Foliage-Feeding Caterpillar Assays

The black cutworm (BCW), *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae), a native species, feeds on various cool-season turf and pasture grasses, as well as small grains and some garden crops [71]. We used it as a model to compare the suitability of each of the clover types and tall fescue for supporting the growth and survival of generalist grass-feeding caterpillars.

Clippings were collected in August 2016 from at least six locations within three monoculture field plots of each clover type or tall fescue, consolidated, and brought to the lab in coolers. Petri dishes (9.0 cm diameter), eight replicates for each clover type or tall fescue, were provisioned with clippings and a dental wick moistened with distilled water. Ten neonate (<1 d old) larvae, eclosed overnight from eggs, were added to each dish. The dishes with larvae were held in a growth chamber at 25 °C and for a 14:10 L:D photoperiod. The dental wick was remoistened as needed to maintain foliage turgidity. The larvae were not food-limited. The dishes were inspected after 1 week to determine the number, instar, and mean weight of survivors. Late-instar BCW can be cannibalistic, so 30 randomly selected 1-week survivors (3–4 from each dish) from each plant type were then transferred to individual 30 mL cups with fresh clippings corresponding to the larva's original clover or tall fescue treatment. We placed the cups with the larvae in a growth chamber, as above, checked them daily, and added additional foliage ad libitum as needed. Larval survival, instar, and weight were determined at the end of the second week.

Coincident with the above assay, we harvested additional foliage from each replicate plot of the three monoculture clover types and tall fescue, froze and lyophilized the samples, and ground them in a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA). Subsamples (25 mg) were combusted and analyzed for percentage nitrogen, carbon, fiber, and lignin using a Flash EA1112 elemental analyzer (Thermo Fisher Scientific, Waltham, MA, USA).

2.6. Nitrogen Benefits of Clover to Tall Fescue

Tall fescue leaf blades were harvested from 10 spots within each clover–tall fescue diculture plot on 29 August 2016. The clippings were cleaned of any extraneous clover foliage, frozen, lyophilized, ground, and analyzed for percentage nitrogen and carbon as described above.

2.7. Data Analyses

We used separate two-way analyses of variance (ANOVA) for a randomized complete block design to test for differences in dependent variables (clover blooms, bee counts, abundance of invertebrate taxa in vacuum and pitfall samples, numbers and mass of earthworms and white grubs, caterpillar growth, and foliage parameters) among all plot types, or among just the three types of clover monocultures. For some variables, we used single degree of freedom contrasts to compare means from clover monocultures versus dicultures, and clover monocultures or dicultures versus tall fescue, or compared means via Fisher's least significant difference (LSD), when the overall F-statistic was significant. The species richness and diversity of the bees (inverse Simpson Index of Diversity 1-D [72]) were compared among the clover types by two-way ANOVA, with the proportionate representation of the bee families compared using chi-square analyses.

3. Results

3.1. Floral Coverage

All clover types germinated and provided near-total plot coverage in the monoculture plots by mid-September 2015, <2 months after planting. By then, those plots had begun to sparsely bloom, but the clovers that were overseeded into tall fescue (diculture plots) did not produce flowers until the following spring (Table 1). The monocultures of all

clover types bloomed profusely in May and June 2016 (Table 1). There were fewer total flowers in the plots where the clovers were incorporated into tall fescue, with no significant differences among clover types (Table 1).

Table 1. Flower counts on clover monocultures and clover + tall fescue (TF) dicultures, and combined “snapshot” counts of total bees observed foraging on monoculture clover plots. Data are means (\pm SE) for five replicates.

Plot Type ^a	Composition	September 2015 ^b		Spring 2016		Bee Counts per Plot ^c
		Flowers per Plot	Flowers per Plot		Bee Counts per Plot ^c	
			31 May	20 June		
Monoculture	Dutch white clover	35.0 \pm 10.9 b	946 \pm 148 a	3261 \pm 269 a	14.8 \pm 1.2	
Monoculture	Microclover	268.0 \pm 35.6 a	820 \pm 45 ab	2136 \pm 206 c	11.4 \pm 2.0	
Monoculture	Turf Clover	46.2 \pm 15.7 b	509 \pm 37 b	2908 \pm 167 b	11.2 \pm 1.7	
Diculture	Dutch white clover + TF	0	139 \pm 59	440 \pm 130	NA	
Diculture	Microclover + TF	0	244 \pm 43	498 \pm 75	NA	
Diculture	Turf Clover + TF	0	169 \pm 42	510 \pm 64	NA	
Monoculture	TF	0	0	16 \pm 10 ^d	NA	
	$F_{6,24}$ (p) all plot types		30.5 (<0.01)	98.5 (<0.01)		
	$F_{2,8}$ (p) clover monocultures	53.8 (<0.001)	4.79 (0.04)	31.2 (<0.01)	1.49 (0.28)	
	$F_{2,8}$ (p) clover dicultures		3.23 (0.09)	0.54 (0.60)		

^a Five replicates of 6.1 \times 6.1 m plots, seeded on 25 June 2015. ^b Within columns, means not followed by the same letter are significantly different ($p < 0.05$; Fisher’s least significant difference (LSD) test). Separate LSD tests were done on clover counts from monoculture and diculture plots, with mean separation tests applied only in cases where the ANOVA F -statistic indicated significant differences among the three clover types. ^c Based on 30-s “snapshot” counts taken on five dates between 31 May and 30 June on monoculture clover plots only; NA = not applicable. ^d Spontaneous clover plants of unknown origin.

3.2. Bee Visitation and Assemblages on the Three Clover Types

“Snapshot” counts taken on the monoculture clover plots from late May to late June suggested that all clover types are similarly attractive to bees (Table 1). The assemblages of bees visiting those plots were also similar, with four families, 14 genera, and 16 species represented amongst the 1373 total bees sampled (Figure 2, Table 2). Bee species richness and diversity were also similar in all clover types (Table 2). Assemblages in June were dominated by apid bees, particularly honey bees (*Apis mellifera* L.), and bumble bees (Bombini); whereas Bombini (mainly *Bombus impatiens* Cresson and *Bombus griseocollis* DeGeer) and Halictidae predominated in August (Figure 3, Table 2). Species richness was generally higher in August than in June. Various Lepidoptera, including Hesperidae (skippers), Pieridae (sulfurs and whites; e.g., *Colias philodice* Godart, *Pieris rapae* (L.)), Lycaenidae (blues and hairstreaks), Nymphalidae (e.g., *Phycodes tharos* (Drury), *Chlosyne nycteis* (Doubleday), *Boloria belona* (F.), *Euptoieta claudia* (Cramer)), and others were also observed landing and nectaring on the clover blooms.

3.3. Macroinvertebrate Communities in Monocultures and Dicultures of Clover and Tall Fescue

Grass flies (Chloropidae), leafhoppers (Cicadellidae), froghoppers (Cercopidae), aphids (Aphididae), flea beetles (Chrysomelidae), and slugs (Gastropoda) were the predominant taxa of plant-feeding invertebrates in the vacuum and pitfall samples (Table 3). Leafhoppers and froghoppers were significantly more abundant in the clover–tall fescue dicultures than in monocultures of clover or tall fescue (Table 3), but none of the other taxa differed in abundance between the plot types (ANOVA, $p > 0.05$). Other herbivorous arthropods, e.g., crickets (Gryllidae), grasshoppers (Acrididae), weevils (Curculionidae), and various caterpillars also were present, but in numbers too low for meaningful analysis.

The taxa of predominantly predatory arthropods captured by vacuum sampling and in the pitfall traps included Formicidae (ants), Araneae (spiders), Staphylinidae (rove beetles), Carabidae (ground beetles), Geocoridae (big-eyed bugs), Nabidae (damselfly bugs), Anthorcoridae (minute pirate bugs), and Coccinellidae (lady beetles) (Table 4). The predator communities were similar in all plot types (Table 4). The samples also contained small parasitic Hymenoptera (e.g., Chalcidoidea, Platygastroidea, Cynipoidea, Ichneumonoidea,

and probably others), with no significant difference in their collective abundance between plot types (data not shown).

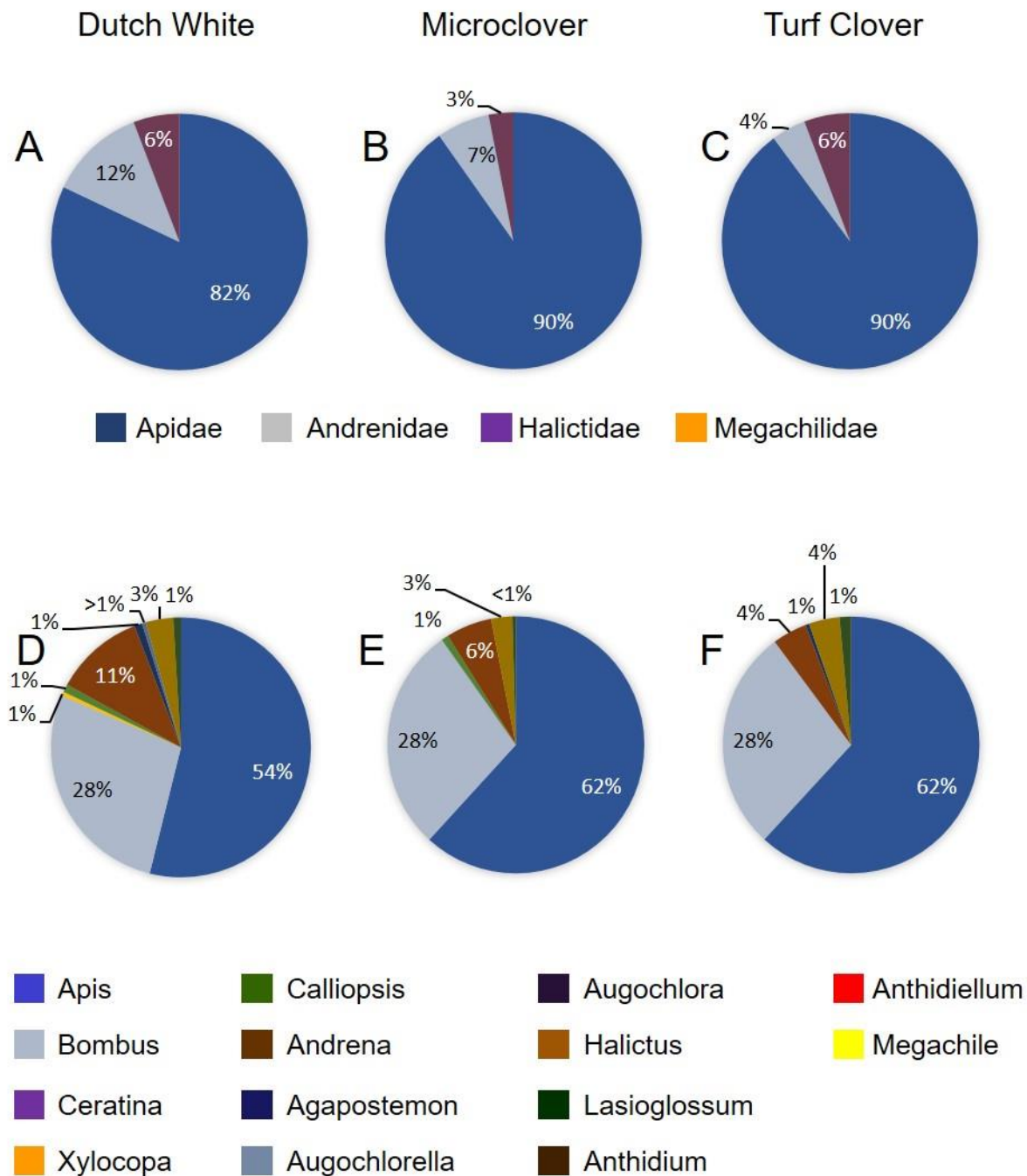


Figure 2. Bee assemblages collected in June 2016 from blooms of three phenotypes of *Trifolium repens* in replicated clover lawn plots. Top and bottom rows show relative proportions of bee families and genera, respectively, in samples from Turf Clover (charts A,D); Dutch white clover (B,E) and Microclover (C,F).

The numbers and biomass of epigeal earthworms, mainly *Aporrectodea* spp., did not differ significantly among the plot types (Table 5). In contrast, white grubs were 82–93% less abundant in the clover monocultures compared with the dicultures of the same clover types intermixed with tall fescue, or in tall fescue alone. The white grub population consisted of 92% *Popillia japonica* Newman (Japanese beetle; >97% third instars and the remainder late second instars), 6% *Cyclocephala* spp. (masked chafers, all third instars), and 2% *Phyllophaga* spp. (May beetles, second and a few third instars). The numbers and mean weight of *P. japonica* were significantly lower in the monoculture clovers than in the clover–tall fescue dicultures or tall fescue alone (Table 5). There was a slight, statistically significant reduction in mass per *P. japonica* grub in the diculture versus tall fescue plots, but their numbers were not significantly reduced in the diculture plots compared with tall fescue alone (Table 5). The numbers of *Cyclocephala* spp. and *Phyllophaga* spp. grubs were too low for meaningful analysis.

3.4. Foliage-Feeding Caterpillar Assays

The black cutworms grew quickly and survived well on all clover types. By the end of Week 2, their weights were 3–5-fold higher and, developmentally, they were approximately a full instar ahead of cohorts reared on tall fescue (Table 6). The clover foliage was higher in nitrogen and lower in fiber compared with tall fescue. Foliar nitrogen in DWC, Microclover, and Turf Clover averaged 5.5 ± 0.07 , 5.4 ± 0.05 , and $5.3 \pm 0.02\%$, respectively, compared with $3.6 \pm 0.09\%$ for tall fescue from monoculture plots ($F_{3,12} = 96.2$, $p < 0.001$). Fiber averaged 22.3 ± 1.0 , 25.2 ± 1.8 , and $23.3 \pm 0.6\%$, respectively, compared with $29.7 \pm 0.8\%$ for tall fescue ($F_{3,12} = 7.19$, $p < 0.001$).

Table 2. Bee assemblages netted from blooms of three phenotypes of white clover (*Trifolium repens*) in replicated clover lawn plots in Lexington, Kentucky (2016).

Apidae	Turf Clover		Dutch White		Microclover	
	June	August	June	August	June	August
<i>Apis mellifera</i> L.	128	10	111	10	139	10
<i>Bombus impatiens</i> Cresson	45	120	39	145	45	148
<i>Bombus griseocollis</i> DeGeer	13	1	18	0	19	0
<i>Ceratina dupla</i> Say	0	0	0	0	0	1
<i>Xylocopa virginica</i> (L.)	0	0	1	0	0	0
Andrenidae						
<i>Calliopsis andreniformis</i> Smith	0	31	2	38	2	13
<i>Andrena</i> sp.	9	0	23	0	13	0
Halictidae						
<i>Agapostemon virescens</i> (Fab.)	1	3	2	2	0	3
<i>Augochlorella aurata</i> (Smith)	0	4	1	4	0	1
<i>Augochlora pura</i> Say	0	21	0	31	0	39
<i>Halictus rubicundus</i> (Christ)	5	8	4	5	5	2
<i>Halictus confusus</i> Smith	3	3	3	3	1	5
<i>Lasioglossum</i> sp.	3	17	2	22	1	25
Megachilidae						
<i>Anthidium manicatum</i> (L.)	0	3	0	1	0	2
<i>Anthidiellum notatum</i> (Latreille)	0	2	0	1	0	1
<i>Megachile brevis</i> Say	0	0	0	1	0	0
Total bees sampled	207	223	205	263	225	250
Mean (SE) species richness per plot ^a	6.0 (0.3)	8.4 (0.5)	6.6 (0.8)	7.4 (0.7)	5.4 (0.5)	7.2 (0.5)
Mean (SE) species diversity ^b	2.25 (0.16)	3.06 (0.12)	2.95 (0.40)	2.90 (0.20)	2.30 (0.16)	2.63 (0.28)

^a Comparison among clover types: $F_{2,8} = 1.23$, $p = 0.34$ (June); $F_{2,8} = 3.65$, $p = 0.08$ (August). ^b Comparison among clover types: $F_{2,8} = 2.0$, $p = 0.20$ (June); $F_{2,8} = 0.83$, $p = 0.47$ (August).

3.5. Nitrogen Benefits of Clover to Tall Fescue

Tall fescue foliage harvested from plants in the tall fescue–clover dicultures had a significantly higher nitrogen content than did foliage from plots with tall fescue only ($F_{3,12} = 33.4$, $p < 0.001$). The percentage nitrogen for tall fescue grown in dicultures with DWC, Microclover, or Turf Clover averaged 4.1 ± 0.02 , 4.2 ± 0.04 , 4.3 ± 0.06 , respectively, compared with 3.5 ± 0.09 for tall fescue in monoculture. The C/N ratio was higher for tall fescue from monoculture (12.1 ± 0.3) as opposed to the aforementioned three diculture treatments (10.5 ± 0.1 , 10.2 ± 1.0 , and 10.1 ± 1.0 , respectively; $F_{3,12} = 29.7$, $p < 0.001$).

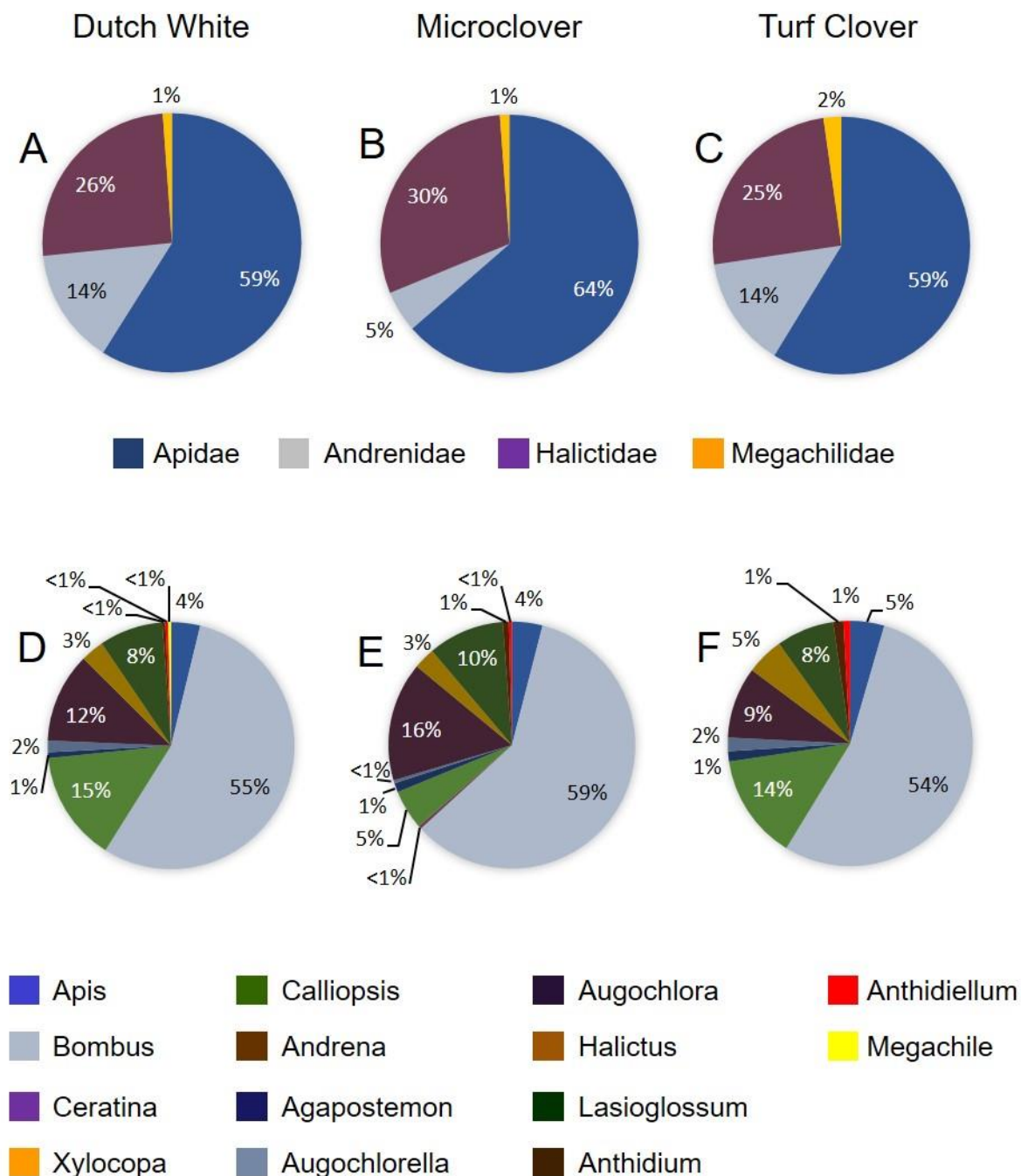


Figure 3. Bee assemblages collected in August 2016 from blooms of three phenotypes of *Trifolium repens* in replicated clover lawn plots. Top and bottom rows show relative proportions of bee families and genera, respectively, in samples from Turf Clover (charts A,D), Dutch white clover (B,E), and Microclover (C,F).

Table 3. Relative abundance of the six predominant taxa of herbaceous invertebrates sampled by combined pitfall and vacuum sampling pooled over five intervals in 2015 and 2016 in three types of clover lawn monocultures (M), clover–tall fescue dicultures (D), or tall fescue (TF) turf.

Plot Type	Herbivore Type ^a					
	Leaf-Hoppers	Frog-Hoppers	Flea Beetles	Aphids	Grass Flies	Slugs
Dutch white clover (M)	166 ± 21	49 ± 16	56 ± 10	52 ± 23	736 ± 72	18 ± 2
Microclover (M)	183 ± 26	32 ± 6	64 ± 17	54 ± 19	635 ± 74	15 ± 2
Turf Clover (M)	171 ± 17	33 ± 4	73 ± 18	41 ± 19	576 ± 53	12 ± 2
Dutch white + TF (D)	303 ± 62	106 ± 13	57 ± 7	25 ± 11	536 ± 160	18 ± 3
Micro + TF (D)	289 ± 33	101 ± 13	92 ± 31	41 ± 16	534 ± 79	16 ± 4
Turf Clo + TF (D)	271 ± 34	91 ± 28	64 ± 9	25 ± 12	491 ± 114	17 ± 2
Tall fescue	213 ± 42	53 ± 18	57 ± 11	38 ± 11	502 ± 135	21 ± 5
$F_{7,24}$ (p) ^b	3.7 (<0.01)	3.8 (<0.01)	0.54 (0.78)	0.44 (0.85)	0.70 (0.65)	1.0 (0.15)
M vs. TF	ns	ns	ns	ns	ns	1.9 (0.07)
M vs. D	4.6 (<0.01)	4.6 (<0.01)	ns	ns	ns	ns
D vs. TF	2.1 (<0.05)	2.5 (<0.05)	ns	ns	ns	ns

^a Leafhoppers = Cicadellidae, froghoppers = Cercopidae, flea beetles = Chrysomelidae, aphids = Aphididae, grass flies = Chloropidae, slugs = Gastropoda. Data are means (± SE) for five replicates. ^b Two-way analysis of variance; Contrasts (1 df).

Table 4. Relative abundance of the six predominant taxa of predatory arthropods sampled by combined pitfall and vacuum sampling pooled over five intervals in 2015 and 2016 showing similar communities in three types of clover lawn monocultures (M), clover–tall fescue dicultures (D), or tall fescue (TF) turf.

Plot Type	Predator Type ^a					
	Spiders	Rove Beetles	Ground Beetles	Ants	Predatory Bugs	Lady Beetles
Dutch white clover (M)	185 ± 15	64 ± 6	18 ± 3	115 ± 22	34 ± 3	8 ± 2
Microclover (M)	191 ± 18	81 ± 13	22 ± 5	120 ± 9	34 ± 11	9 ± 3
Turf Clover (M)	221 ± 22	77 ± 15	17 ± 4	112 ± 17	32 ± 7	12 ± 6
Dutch white + TF (D)	192 ± 20	87 ± 12	11 ± 2	13 ± 20	17 ± 3	4 ± 2
Micro + TF (D)	191 ± 10	87 ± 21	18 ± 3	101 ± 6	22 ± 7	10 ± 4
Turf Clo + TF (D)	176 ± 15	99 ± 13	15 ± 2	96 ± 18	17 ± 4	7 ± 3
Tall fescue	182 ± 11	85 ± 13	18 ± 3	102 ± 16	16 ± 4	10 ± 12
$F_{7,24}$ (p)	0.77 (0.6)	0.62 (0.71)	1.87 (0.13)	0.28 (0.94)	1.64 (0.18)	0.58 (0.74)

^a Spiders = Araneae, rove beetles = Staphylinidae, ground beetles = Carabidae, ants = Formicidae, predatory bugs = combined Nabidae, Geocoridae, and Anthocoridae, lady beetles = Coccinellidae. Data are means (± SE) for the five replicates.

Table 5. Abundance and biomass of earthworms (mainly *Aporrectodea* spp.) and Japanese beetle (*Popillia japonica*) grubs in samples of turf and soil from three types of clover lawn monocultures (M), clover–tall fescue dicultures (D), or tall fescue (TF) turf. Data are means (±SE) for the five replicates.

Plot Type	Earthworms ^a		<i>P. japonica</i> Grubs ^b	
	Number	Mass (g) per Worm	Number	Mass (mg) per Grub
Dutch white clover (M)	43 ± 7	234 ± 38	2.0 ± 1.0	127 ± 15
Microclover (M)	56 ± 5	145 ± 14	6.2 ± 1.1	134 ± 10
Turf Clover (M)	54 ± 12	172 ± 15	4.6 ± 1.3	124 ± 7
Dutch white + TF (D)	34 ± 11	241 ± 43	33.8 ± 5.1	176 ± 11
Micro + TF (D)	40 ± 6	187 ± 23	36.2 ± 6.8	179 ± 4
Turf Clo + TF (D)	38 ± 7	163 ± 22	38.5 ± 5.6	162 ± 6
Tall fescue	28 ± 5	219 ± 36	38.4 ± 9.4	191 ± 5
$F_{6,24}$ (p)	1.7 (0.18)	1.6 (0.18)	10.8 (<0.001)	12.1 (<0.001)
M vs. TF (t_1, p)	ns	ns	5.7 (<0.001)	7.0 (<0.001)
M vs. D (t_1, p)	ns	ns	7.3 (<0.001)	7.0 (<0.001)
D vs. TF (t_1, p)	ns	ns	0.4 (0.68)	2.2 (0.04)

^a Earthworms hand-sorted from two 20 × 20 × 21 cm deep samples per plot. ^b Grubs in 122 × 46 × 8 cm deep strips cut lengthwise through the center of each plot.

Table 6. Growth and survival of black cutworms (*Agrotis ipsilon*) reared on foliage of one of three white clover phenotypes or tall fescue for 2 weeks.

Foliage Type	Week 1 ^a			Week 2 ^b	
	% Survival	Instar Attained	Wt (mg) Attained	Instar Attained	Wt (mg) Attained
Dutch white	98.8 ± 1.2	1.8 ± 0.1	13.6 ± 1.7 ab	3.1 ± 0.1 ab	163 ± 9 a
Microclover	95.0 ± 2.6	1.5 ± 0.1	7.5 ± 1.3 b	2.9 ± 0.1 b	91 ± 12 b
Turf Clover	88.8 ± 3.5	1.6 ± 0.1	11.7 ± 2.5 a	3.3 ± 0.1 a	144 ± 15 a
Tall fescue	93.8 ± 2.6	1.3 ± 0.1	5.7 ± 0.5 c	2.2 ± 0.1 c	31 ± 3 c
<i>F</i> (<i>p</i>) ^c	2.49 (0.09)	2.54 (0.08)	4.23 (0.02)	20.2 (<0.01)	33.5 (0.01)
Clovers vs. TF (<i>t</i> ₁ , <i>p</i>)	Ns	2.19 (0.04)	2.56 (0.02)	7.1 (<0.01)	9.0 (<0.01)

Data are means ± SE. ^a Based on 8 cohorts of 10 neonates (replicates) per foliage type. ^b Based on 30 randomly selected individuals from Week 1 survivors. ^c *F*_{3,28} for Week 1 (cohorts), *F*_{3,112} for Week 2 (individuals). Within columns, means without letters or followed by the same letter do not significantly differ (LSD, *p* > 0.05).

4. Discussion

Reconciliation ecology, “the science of inventing, establishing, and maintaining new habitats to conserve species diversity in places where people live, work, and play” [73], seeks ways to modify urban landscapes to support native biota without compromising societal utilization. Even in communities where there are normative social pressures to maintain residential and commercial front lawns as turfgrass monocultures, other sites, including backyards, institutional grounds, community parks, cemeteries, and street verges and medians, offer opportunities to integrate low-growing flowers into mowed turfgrass without compromising aesthetics or use for informal games and sports, picnicking, dog-walking, or other recreational activities [2,40]. However, movement to clover lawns to support pollinators and reduce the need for nitrogen fertilizer and other lawn chemicals will require changes in the aesthetic expectations of homeowners, turfgrass managers, and the general public. Using dwarf varieties of *T. repens* that blend into the turfgrass sward may encourage a greater acceptance of clover lawns [19].

This study indicates that dwarf white clover supplies nitrogen to tall fescue to a similar degree as Dutch white clover. The elevation in foliar N for grass in association with clover ranged from 17–23%, depending on the clover type, which is comparable to increases that were obtained in tall fescue turf by applying 150–200 kg N/ha per year [74]. All three clover types bloomed profusely in the monocultures and produced similar numbers of flowers when incorporated into existing turf-type tall fescue. Despite their generally smaller bloom size, both dwarf clover varieties were visited by similar types of bees as those foraging on DWC. The monocultures of all the clover types had very few white grubs compared with the plots of tall fescue alone, although a similar reduction in grub numbers did not occur in the clover–tall fescue dicultures. Except for somewhat higher populations of leafhoppers and froghoppers, which caused no obvious aesthetic damage, the invertebrate communities in all three clover–tall fescue dicultures were similar to those found in plots of tall fescue alone.

Nitrogen is a limiting element in the diet of most plant-feeding insects [75], with a higher nitrogen content commonly associated with increased performance [74,75]. Black cutworms grew more quickly on all clover types than on tall fescue, likely due to the clovers’ higher foliar nitrogen and lower fiber content. While it is also possible that by augmenting the foliar nitrogen in the associated turfgrasses, the clover in the mixed stands could confer associational susceptibility [76], causing the grass to become nutritionally more suitable for graminivorous pests, the same caveat applies when grasses are fertilized with other nitrogen sources [74]. In this study, however, none of the field plots sustained noticeable damage from cutworms or other grass-feeding caterpillars (e.g., sod webworms (Crambidae) or armyworms (*Spodoptera*, *Mythimna* spp.)) that feed on the same suite of cool-season lawn grasses [71].

We collected 17 different bee species foraging on *T. repens* in our monoculture clover plots, compared with 31 and 56 species of bees found in previous surveys of bees visiting

spontaneous white clover in urban and suburban park lawns and other lawn sites in Kentucky and Minnesota, respectively [21,43]. The lower species richness in this study doubtless reflects the fact that the bees were collected from replicated plots at a single site, a turfgrass research facility; whereas in the previous surveys, bees foraging on *T. repens* were sampled across 16–18 established lawn sites surrounded by relatively more diverse vegetation. In the Minnesota study, the number of bee species collected on clover at a given site ranged from 5–17 in a given year, suggesting that dwarf clovers, too, would recruit additional bee species if planted more widely. Notably, in each of the three aforementioned studies, $\geq 90\%$ of the non-*Apis* bee species that were sampled from *T. repens* are native to the United States, underscoring that this non-native flowering legume hosts a range of polylectic wild urban bees. Although *A. mellifera* and *Bombus* spp. (especially *B. impatiens*) were numerically dominant in all three studies, we found *Bombus* spp., all native, were even more abundant than *A. mellifera* in late summer. White clover blooms and provides floral resources for much of the growing season, a characteristic that is particularly valuable to pollinators such as *A. mellifera* and *Bombus* spp. that have season-long foraging activity [77]. A caveat to our study is that we did not measure the quantity of nectar and pollen provided by the dwarf clovers versus DWC. It is possible that the dwarf clovers' value to bees could be somewhat reduced if foraging workers must visit more blooms to obtain equivalent floral rewards.

Low-maintenance turfgrass lawns typically harbor numerous species of predatory invertebrates, mostly generalist feeders, that collectively help to regulate populations of herbivorous invertebrates, including pests [78,79]. The epigeal and arboreal predators we found inhabiting the dwarf and DWC monocultures and clover–tall fescue dicultures, including spiders (mostly Linyphiidae, Erigonidae, and Lycosidae), ants (mostly *Lasius* and *Solenopsis* spp.), ground beetles, rove beetles, lady beetles, and predatory Hemiptera, were typical of those found in turf and pasture grass in the eastern United States [80–82]. Our samples also contained small parasitic wasps, which were not identified because their trophic relationships in cool-season turfgrasses are largely unknown [71,78]. Herbivores and earthworms in the clover plots also were similar to those inhabiting turf-type tall fescue lawns in Kentucky [74,82]. Overall, we saw no indication that incorporating dwarf clovers into tall fescue would significantly change the invertebrate community of lawns.

We found very low numbers of *P. japonica* grubs in all clover monocultures compared with the tall fescue and mixed clover–tall fescue plots. Larval *P. japonica* feed on the roots of a wide range of plants, including all cool-season turfgrasses [83], but according to Fleming [84], they “do not thrive” in plantings of *T. repens* or other *Trifolium* species. Studies with another generalist root herbivore, *Melolontha melolontha* (L.) (Scarabaeidae), suggested that *T. repens* resistance to its grubs is more likely due to a high root lignin content resulting in reduced feeding, as opposed to chemical repellence [85]. Many *T. repens* genotypes contain foliar cyanogenic glucosides [86], which deter the feeding and oviposition of some non-adapted insect herbivores [87]. However, adult *P. japonica* feed on the foliage of many cyanogenic plants, including *T. repens* [84], therefore it seems unlikely that those chemicals account for *T. repens* resistance to the grubs. White clover roots also contain flavonoids [88], but whether they play any role in resistance to root-feeding scarab grubs is unknown.

Importantly, *P. japonica* grub populations were not reduced in any of our clover–tall fescue dicultures compared with tall fescue alone, suggesting that incorporating dwarf or DWC into turf-type tall fescue will not provide associational resistance [76] to the grass itself, at least not at the clover density we attained by over-seeding it into existing tall fescue (e.g., Figure 1C,D). It is possible that by starting with a seed mix containing a relatively high proportion of clover, or by reseeding additional clover into a mixed sward, one could establish mixed clover–tall fescue lawns with enhanced resistance to *P. japonica*. Further research on how clover monocultures and various ratios of clover to turfgrass affect white grub species other than *P. japonica*, and other lawn insect pests, is warranted. Regardless of the agronomic challenges of obtaining and maintaining a consistent, optimal percentage of clover to grass to match the aesthetic, recreational, and other use requirements for

particular sites, the benefits of dwarf clover for supporting pollinators and reducing the need for inorganic or synthetic organic fertilizers justify their more widespread use in low-input lawns.

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