Current Biology

Report

Declines of bees and butterflies over 15 years in a forested landscape

Highlights

- Declines in pollinators were detected over 15 years in southeastern US forests
- Bees that nest above ground exhibited stronger declines than ground-nesting taxa
- Possible causes include increasing minimum temperatures and an invasive ant

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In brief

Ulyshen and Horn report significant declines of bees and butterflies over a 15-year period in southeastern US forests. Their findings suggest that pollinator declines may be occurring even in relatively undisturbed habitats and that above-ground-nesting bees may be experiencing stronger declines than soil-nesting species.

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Report

Declines of bees and butterflies over 15 years in a forested landscape

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SUMMARY

Despite growing concerns about pollinator declines,[1–4] evidence that this is a widespread problem affecting entire communities remains limited.[5] There is a particular shortage of pollinator time series from relatively undisturbed natural habitats, such as forests, which are generally thought to provide refuge to biodiversity from anthropogenic stressors.[6] Here, we present the results from standardized pollinator sampling over 15 years (2007–2022) at three relatively undisturbed forested locations in the southeastern United States. We observed significant declines in the richness (39%) and abundance (62.5%) of bees as well as the abundance of butterflies (57.6%) over this time period. Unexpectedly, we detected much stronger declines in the richness and abundance of above-ground-nesting bees (81.1% and 85.3%, respectively) compared with below-ground-nesting bees. Even after dropping the first or last year of sampling, which happened to yield the greatest and lowest numbers of pollinators, respectively, we still detected many of the same negative trends. Our results suggest that sharp declines in pollinators may not be limited to areas experiencing direct anthropogenic disturbances. Possible drivers in our system include increasing mean annual minimum temperatures near our study sites as well as an invasive wood-nesting ant that has become increasingly widespread and abundant in the region over the course of this study.

RESULTS AND DISCUSSION

A variety of factors are implicated in pollinator declines, including the destruction and degradation of natural habitats, pesticide exposure, the introduction of pests and pathogens, and climate change.[7,8] A general expectation is that stronger declines will occur in anthropogenic habitats experiencing a greater number of these stressors compared with comparatively undisturbed, semi-natural habitats.[6] Although this prediction is largely supported by the existing literature,[6,9,10] a shortage of long-term monitoring efforts in semi-natural habitats, despite the recognized need for such research,[11,12] leaves the question largely unresolved. Here, we document changes in bee and butterfly communities over a 15-year period at three forested locations in the southeastern United States (Figure 1).

Patterns of pollinator decline

Total bee richness and abundance declined significantly (by 39% and 62.5%, respectively) over the 15 years of sampling (Figure 2; Table 1; Data S1A and S1B). The same was true for the richness and abundance of above-ground-nesting bees (by 81.1% and 85.3%, respectively) and for the abundance of below-ground-nesting bees (by 46%). The abundances of all three sub-groups of above-ground-nesting bees analyzed separately also declined significantly over time: *Ceratina* spp. by 97.2%, wood-nesting halictids by 78.2%, and cavity-nesting megachilids by 83.9% (Table 1). Butterfly abundance, but not richness, also declined significantly (by 57.6%) over the 15-year period.

Observed patterns of decline were largely unchanged when data from the first or last year of sampling were excluded from the analysis. After dropping data from 2007, for example, we still detected significant reductions in total bee abundance (by 57.8%), the richness and abundance of above-ground-nesting bees (by 67.8% and 83.5%, respectively), as well as the abundances of all three sub-groups of above-ground-nesting bees (Table 1). Similarly, when we dropped data from 2022, we still detected significant reductions in total bee richness (by 38.5%), the richness and abundance of above-ground-nesting bees (by 81.2% and 58.0%, respectively), and the abundance of cavity-nesting megachilids (by 90.4%) (Table 1). Finally, we still detected significant declines in butterfly abundance after excluding data from 2007 (by 48.0%) or 2022 (by 48.3%).

Compositional changes in pollinator communities

Our results indicate that some pollinator taxa have experienced stronger declines than others. Most notably, above-ground-nesting bees declined much more strongly in both richness and abundance (by 81.1% and 85.3%, respectively) than below-ground nesters (Figure 3). In fact, the richness of below-ground nesters did not change significantly over time and even the negative trend in abundance for this group disappeared when data from the first or last year of sampling were dropped (Table 1).

Non-metric multidimensional scaling (NMDS) on bee data yielded a three-dimensional solution with a final stress of 6.33. The ordination suggests that bee community composition varied among years (Figure S1), and PERMANOVA supports

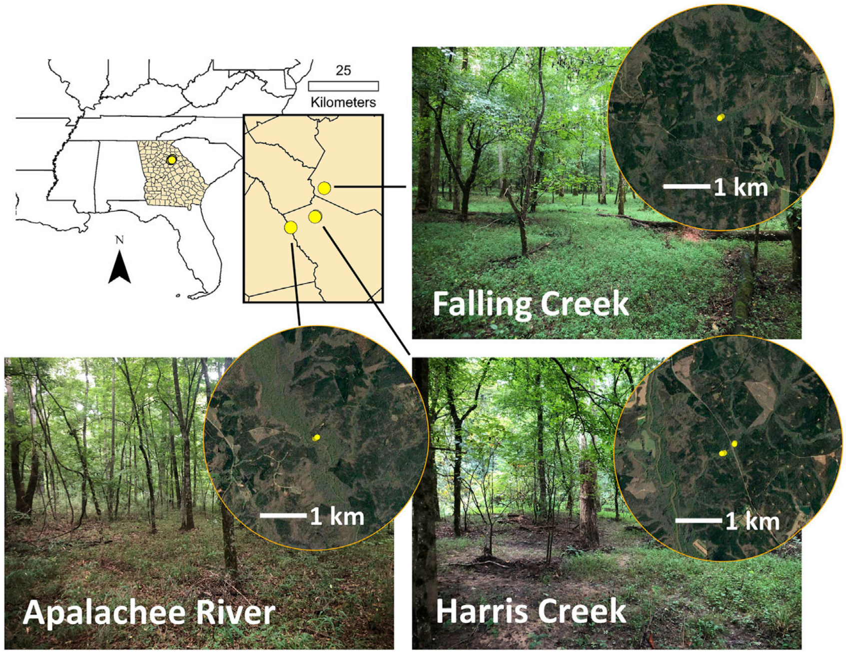

Figure 1. Locations of the three study areas in northeast Georgia, USA

The dark and light green areas visible in the satellite images largely consist of conifer- and hardwood-dominated forests, respectively. See key resources table for coordinates of sampling locations.

this conclusion ($F = 2.45$, $p < 0.001$). However, pairwise comparisons between years were all non-significant (Table S1). NMDS for butterflies failed to yield a useful ordination but, based on PERMANOVA, butterfly communities also differed among years ($F = 2.27$, $p = 0.01$). However, as for bees, all pairwise comparisons of butterfly communities between years were non-significant (Table S1). Based on indicator species analysis, twelve species of bee and one species of butterfly were significantly associated with one or more years (Table S2). The number of indicator species associated with each year declined over time from nine in 2007 to zero in 2022. Among the twelve indicator bee taxa, seven were above-ground-nesting species (Table S2). All but two of these were associated with 2012 or earlier.

Taken together, these results suggest that bee and butterfly communities have both changed compositionally over the course of this study and that observed changes in bee communities can be largely, but not entirely, attributed to declines of above-ground-nesting bee species (Table S2).

Changing weather patterns

Weather data collected near our study sites revealed a significant increase in mean annual minimum temperature over the past 20 years (estimate: 0.0779, $t = 3.86$, $p = 0.001$; Figure S2). This amounts to about a 1.2°C increase in mean minimum temperature over the 15-year period in which pollinators were sampled in this study. By contrast, we found no significant change in mean annual maximum temperature (estimate: 0.0087, $t = 0.31$, $p = 0.76$), annual rainfall (estimate: 0.684, $t = 0.58$, $p = 0.57$), or temperature anomalies (estimate: -0.0374, $t = -0.61$, $p = 0.55$) (Figure S2).

Possible causes of observed pollinator declines

One possible explanation for the sharp declines in above-ground-nesting bees is that these taxa may be less buffered from temperature extremes than below-ground nesters, similar to the idea that above-ground nesters are more vulnerable to fire.[13] This seems plausible, given that elevated temperatures are known to negatively affect the health—and potentially the survival and reproductive success—of nesting bees.[14] Interestingly, among above-ground-nesting bees, we observed particularly dramatic declines in *Ceratina* abundance. These bees nest in pithy stems or twigs of a relatively small diameter,[15] and may therefore be especially exposed to temperature fluctuations. Previous studies have implicated climate change, particularly extremes in dormant-season conditions, in the declines of pollinators in other systems. For example, Graham et al.[8] concluded that early warm temperatures followed by a late frost were responsible for sharp reductions in bee numbers in Michigan. However, this was largely attributed to the loss of flowers from frost, and population trajectories were not found to differ between above- and below-ground nesters in that study.[8] Increasing temperatures are also considered an important driver behind declining butterfly numbers[16] and may have contributed to the declines in butterfly abundance observed in this study.

Another possible explanation for stronger declines in above-ground-nesting bees is that these taxa are more exposed to invasive ants. One species in particular, *Brachyponera chinensis* (Emery) (i.e., the Asian needle ant), has become increasingly well established and abundant in our study region over the past decade.[17] Although information on when *B. chinensis* arrived at our study sites is limited, we know from pitfall trap data that the species was present at the Falling Creek site and other locations within or near Oconee National Forest in 2011.[18] Although *B. chinensis* was not captured at Harris Creek or Apalachee River in 2011, we can confirm that the species was at all three locations by 2022 (S.H., unpublished data). Unlike most other invasive ants in the region, *B. chinensis* commonly occurs in undisturbed forests, while also readily utilizing a variety of anthropogenic habitats.[17] Because *B. chinensis* prefers to nest in dead wood and is known to significantly reduce the diversity of native ants and other invertebrates,[19,20] these ants may pose a considerable threat to female bees attempting to nest in dead wood or other above-ground plant cavities. Furthermore, herbaceous insects are thought to be included among the invertebrates preyed upon by this species,[20] thus the decline in butterfly abundance observed here may also be at least in part related to this invasion.

Conclusions

The results from this study indicate that sharp declines in pollinators are not limited to areas experiencing direct

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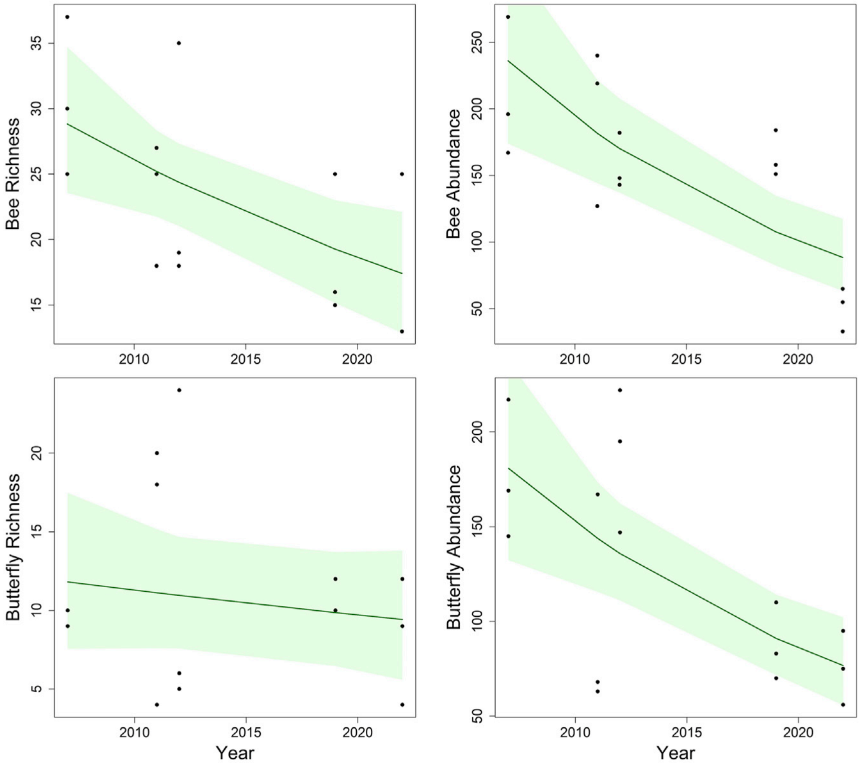

Figure 2. Observed (black dots) and expected richness and abundance of bees and butterflies as predicted by generalized linear mixed models (GLMMs) (green line) with bootstrapped 95% confidence intervals (green shading)

See also Data S1.

anthropogenic disturbances. While the loss and degradation of semi-natural habitats, pesticide use, and other activities are no doubt contributing to insect declines, our findings suggest more pervasive stressors, such as climate change or invasive species, may be negatively affecting pollinator populations even within relatively undisturbed interior forest habitats.

As evidence that insect populations are declining continues to grow, so too do examples of perceived declines weakening or disappearing when analyzed differently or after incorporating longer time series.[5,8] Insect populations naturally exhibit dramatic inter-annual variability and there is always a risk of misinterpreting normal fluctuations as evidence for alarming declines in diversity or abundance. Although future sampling will be needed to confirm the patterns reported here, the fact that our findings remained largely unchanged after dropping the first or last year of sampling from the analysis lends considerable support to the conclusion that both bees and butterflies have experienced significant declines over the past 15 years within our study area.

Table 1. Effects of time (year scaled) on various pollinator response variables based on GLMMs

	All years			Excluding 2007			Excluding 2022		
Response	Estimate	z	p	Estimate	z	p	Estimate	z	p
Bee richness	−0.19	−2.63	<0.01	−0.12	−1.35	0.18	−0.18	−2.53	0.01
Bee abundance	−0.37	−3.62	<0.001	−0.38	−3.06	<0.01	−0.09	−1.58	0.12
AG bee richness	−0.63	−4.89	<0.001	−0.50	−3.11	<0.01	−0.63	−4.70	<0.001
AG bee abundance	−0.73	−5.71	<0.001	−0.79	−6.06	<0.001	−0.33	−3.59	<0.001
Ceratina spp.	−1.35	−4.56	<0.001	−1.55	−6.90	<0.001	–	–	–
Wood-nesting halictids	−0.58	−2.95	<0.01	−0.63	−2.45	0.01	−0.13	−1.33	0.18
Cavity-nesting megachilids	−0.69	−3.17	<0.01	−0.76	−3.61	<0.001	−0.88	−2.80	<0.01
BG bee richness	−0.08	−0.80	0.42	−0.03	−0.28	0.78	−0.01	−0.13	0.90
BG bee abundance	−0.23	−2.22	0.03	−0.20	−1.55	0.12	<0.01	0.07	0.94
Butterfly richness	−0.09	−0.67	0.51	−0.16	−1.1	0.27	0.01	0.05	0.96
Butterfly abundance	−0.33	−3.50	<0.001	−0.29	−2.52	0.01	−0.25	−2.20	0.03

Results are shown for all years combined (left) and after excluding 2007 (middle) or 2022 (right) from the analysis. The abbreviations “AG” and “BG” refer to above-ground- and below-ground-nesting bees, respectively. Note that the model for *Ceratina* abundance failed to converge when 2022 data were excluded.

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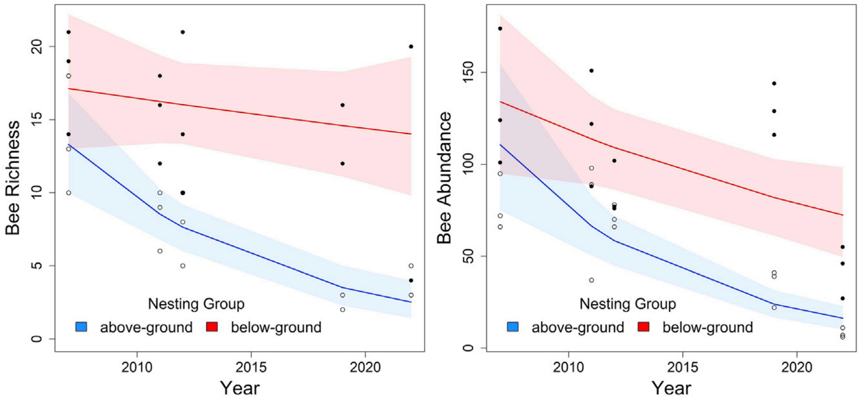

Figure 3. Observed and expected richness and abundance of above- and below-ground-nesting bees (open and closed dots, respectively) as predicted by GLMMs with bootstrapped 95% confidence intervals

See also Data S1.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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- EXPERIMENTAL MODEL AND SUBJECT DETAILS
- METHOD DETAILS
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2023.02.030.

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AUTHOR CONTRIBUTIONS

M.U. conceived of the study, identified bees, analyzed the data, and wrote the first draft of the manuscript. S.H. performed the field work and provided edits to the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

INCLUSION AND DIVERSITY

We support inclusive, diverse, and equitable conduct of research.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Sampling sites		
Sampling took place at 5 locations in each of three forested areas (Apalachee River, Falling Creek, and Harris Creek) on Oconee National Forest in Greene and Oglethorpe counties, Georgia, USA. Coordinates are provided below	N/A	N/A
N 33.65753, W 083.37302	N/A	Apalachee River 1
N 33.65756, W 083.37287	N/A	Apalachee River 2
N 33.65765, W 083.37273	N/A	Apalachee River 3
N 33.65773, W 083.37263	N/A	Apalachee River 4
N 33.65784, W 083.37246	N/A	Apalachee River 5
N 33.78417, W 083.24256	N/A	Falling Creek 1
N 33.78416, W 083.24267	N/A	Falling Creek 2
N 33.78403, W 083.24285	N/A	Falling Creek 3
N 33.78400, W 083.24303	N/A	Falling Creek 4
N 33.78381, W 083.24316	N/A	Falling Creek 5
N 33.69366, W 083.27602	N/A	Harris Creek 1
N 33.69346, W 083.27608	N/A	Harris Creek 2
N 33.69164, W 083.27863	N/A	Harris Creek 3
N 33.69152, W 083.27887	N/A	Harris Creek 4
N 33.69152, W 083.27924	N/A	Harris Creek 5
Deposited data		
Analyzed pollinator data	This paper	Data S1A
Weather data	http://www.georgiaweather.net/index.php?variable=HI&site=WATHORT, watkinsville horticulture farm	From weather station: “Watkinsville Horticulture Farm”
Software and algorithms		
R 4.2.1.	R Core Team.[21]	N/A
PC-ORD	McCune and Mefford[22]	N/A
Other		
Blue and yellow plastic food bowls, 530 ml capacity	Solo	N/A

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Michael Ulyshen (michael.d.ulyshen@usda.gov).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- Data used in the analyses are provided in Data S1A.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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EXPERIMENTAL MODEL AND SUBJECT DETAILS

The data used in this analysis originated from a long-term field study aimed at documenting the responses of pollinators to the experimental removal of an invasive shrub (Chinese privet, *Ligustrum sinense* Lour.) from riparian forests in northeastern Georgia, USA.[23] Three of the locations sampled in that study served as undisturbed reference plots with no history of privet invasion. These locations and the landscapes surrounding them remained largely undisturbed over the course of the study, thus providing a special opportunity to test temporal trends in forest pollinator communities in the absence of many anthropogenic stressors. All three locations were situated in Oconee National Forest (in Greene and Oglethorpe counties) and were separated from one another by at least 10 km. We refer to them here by their adjacent streams: Apalachee River, Harris Creek, and Falling Creek. The locations consisted of mature riparian forests that were either 79 (Harris and Falling Creeks) or 64 (Apalachee River) years old in 2007, at the beginning of this study. The forests consisted of mixed hardwoods typical of the region with dominant tree genera including *Quercus*, *Acer*, *Fraxinus*, *Ulmus*, *Celtis*, *Platanus*, and *Populus*. Wild bees and butterflies were sampled at these locations during five seasons over a 15-year period (2007-2022).

METHOD DETAILS

Pollinators were sampled using blue and yellow pan traps (i.e., Solo colored plastic food bowls) filled with soapy water (530 ml capacity). Although pan traps are known to be more effective at sampling some taxa than others,[24] and may under-sample pollinators when flowers are abundant,[25] they provide a highly effective and standardized method for sampling bees and other pollinators. One advantage of the large pan traps used in this study is that they are effective at sampling butterflies in addition to bees.[23] At each location we established a linear transect consisting of five sampling points with a between-point spacing of ~30-50 m (see key resources table for coordinates). At each sampling point, one blue and one yellow bowl was suspended about 30 cm above the ground on a wire stand and separated from one another by 2 m. We operated the traps for a total of seven, seven-day periods (March, April, May, June, July, August, and October) in 2007, 2011, 2012, 2019, and 2022 during periods of mostly-clear weather. Due to the capacity of the pan traps and to the shady forest conditions within which we sampled, the loss of samples from evaporation was not an issue. All samples from each location and sampling period were combined and stored in ethanol. They were then pinned and dried prior to identification. We used printed[26–30] and online (https://www.discoverlife.org) keys along with an established reference collection to identify all bees and butterflies to species. Where possible, bees were categorized as below-ground and above-ground nesters based on published life history information.[15,26,31] Over the course of the study, 5.4% of all deployed bowls were disturbed (primarily by vertebrates), thus introducing some variability in sampling effort among locations and years. These losses were recorded (Data S1A) and dealt with in the analysis (see below).

QUANTIFICATION AND STATISTICAL ANALYSIS

Unless otherwise stated, all analyses were conducted in R 4.2.1.[30] Data were pooled by location and year prior to analysis (Data S1A). Due to disturbed bowls and potential differences in stand structure over the course of the study (e.g., tree falls, etc.), it is probable that pollinators were sampled more completely at some locations or times than others. To account for this, we standardized species richness (q=0) by sample coverage using the estimateD function in the R package iNext.[32,33] Unlike traditional sample-based rarefaction, coverage-based richness estimates are much less prone to underestimating the degree to which species richness differs among communities.[34] In our analysis, we standardized by the lowest observed coverage for each taxon which was 0.92 for bees, 0.93 for butterflies, 0.92 for ground-nesting bees, and 0.93 for above-ground-nesting bees. However, data for bees from Harris Creek in 2022 were dropped due to unusually low coverage to avoid inaccurate richness estimates.[34] We also adjusted abundance data prior to analysis by dividing by the total number of bowls collected and then multiplying this by the total number of bowls deployed (n=70) per location and year. In addition to the four groups mentioned above, we calculated the combined abundances of the following three sub-groups of above-ground-bees: *Ceratina* spp., wood-nesting halictids (e.g., *Augochlora pura*, *Lasioglossum coeruleum*, *L. cressonii*, *L. oblongum* and *L. subviridatum*), and cavity-nesting megachilids (*Hoplitis producta*, *Megachile campanulae*, and *Osmia* spp.). All richness and abundance values were rounded to the nearest whole number prior to analysis. We used the lme4 package to model the richness and/or abundance of each taxon separately. We first assumed the Poisson distribution but, because overdispersion was an issue for all but one (above-ground-nesting bee richness) of the eleven response variables, we used negative binomial generalized linear models (GLMMs) in all other cases. Year was the only fixed effect in the models while location was included as a random intercept term. The random effect variances were estimated to be zero (i.e., singular fits, likely due to only three locations being sampled in this study) in many cases but we retained the random term in all models. Finally, to test for potential false baseline effects,[35] we repeated the analyses after dropping data from 2007, the first year of sampling, when pollinator numbers happened to be the highest over the entire study. We also repeated the analyses after dropping data from 2022 when particularly few pollinators were collected relative to previous years.

To better understand how the composition of pollinator communities changed over time, we conducted non-metric multidimensional scaling (NMDS) (Figure S1) followed by PERMANOVA (Table S1) in PC-ORD.[22] Data for bees and butterflies were analyzed separately. To test which species were strongly associated with one or more years of sampling, we conducted indicator species

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analysis (Table S2) using the function multipatt (multilevel pattern analysis) in the package indicspecies.[36] This analysis yields indicator values ranging from 0 (no association) to 1 (complete association).

Because climate change is often implicated in pollinator declines, we also investigated trends in local climate data over our study period. To do this, daily temperature and precipitation data from a 20-year period (2003-2022) were obtained from a weather station located in Watkinsville, Georgia (UGA Horticulture Farm) approximately 30 km from our study sites. From these data we calculated the mean annual maximum and minimum temperatures and total precipitation. We also calculated the total number of annual temperature anomalies which were defined as observations exceeding 1.5 standard deviations above the mean maximum or minimum temperatures from 2003-2021. We performed linear regression to explore the relationships between these climate metrics and time (Figure S2). To satisfy the normality assumption, data on temperature anomalies were square-root transformed prior to analysis.