Urbanisation and invasion as drivers of insect diversity

by

Luke Edenborough

A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfilment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
August 8th, 2026

Keywords: urbanisation, Brachyponera chinensis, biodiversity, urban ecology

Approved by

Clint A. Penick, Chair, Assistant Professor, Department of Entomology and Plant Pathology
Arthur P. Appel, Dean Ag & Director AAES, Department of Entomology and Plant Pathology
Daniel A. Warner, Associate Professor, Department of Biological Sciences

Abstract

Urbanisation often reduces local biodiversity, yet cities can maintain high total species richness, a pattern underlying the "cities-as-bioarks" hypothesis that urban remnant habitats serve as refuges for native species. We surveyed ants across 60 sites in Atlanta, Georgia (USA), spanning streetscapes, manicured parks, and forested parks. Contrary to bioark predictions, native richness was lowest in forested parks and declined strongly with abundance of the invasive Asian needle ant, *Brachyponera chinensis*, indicating that habitat protection alone is insufficient without active invasive species management. Because sampling protocols developed for natural habitats may not capture urban ant diversity effectively, we also evaluated four common methods (pitfall trapping, leaf-litter extraction, hand collection, and baiting) at these sites and reviewed 86 urban ant studies published between 2001 and 2025. Hand collection and pitfall trapping captured the greatest diversity, yet pitfall traps and baiting dominate the literature while hand sampling remains underused.

Artificial Intelligence (AI) Use Disclosure Agreement

In preparation of this thesis, the following Artificial Intelligence (AI) tools were used: Anthropic's Claude, ChatGPT. These tools were primarily used to support the organisation of written sections, editing language for grammar and clarity, and assistance with code. The author acknowledges full responsibility for the intellectual content of this work and has ensured that all AI-assisted sections have been reviewed and revised for accuracy .

Digital Accessibility Disclosure Statement

In the preparation of this thesis / dissertation, the following digital accessibility tools were used to ensure this document complies with federal requirements: Microsoft Word, Adobe Acrobat. The author acknowledges full responsibility for the intellectual content of this work and has made a good faith effort to comply with digital accessibility requirements in publishing, wherein the nature of the content does not significantly change in order to do so. Furthermore, all content has been reviewed and revised to meet these requirements prior to final publication.

Acknowledgements

I would first like to thank my partner, Eva, for her continued and unrelenting support since the moment I met her. I would also like to thank my parents and my sister for making me the person I am today and helping me work towards my ambitions.

Thank you to my advisor, Dr. Penick, for bringing me into his lab and enabling me to pursue research I am truly interested in, and opening up many more opportunities in this field. Thank you to my committee, Dr. Appel and Dr. Warner, for their valuable contribution to study design, and their support throughout the process.

Finally, thank you to all my fellow graduate students in the Penick Lab, along with all of the undergraduate workers that helped me with data collection.

Table of Contents

Abstract 2
Artificial Intelligence (AI) Use Disclosure Agreement 3
Digital Accessibility Disclosure Statement 4
Acknowledgements 5
Table of contents 6
List of tables 8
List of figures 9
Chapter 1: Invasive species undermine the bioark hypotesis in a low-latitude urban biodiversity hotspot 10
Introduction 10
Methods 12
Ant Sampling 14
Statistical Analyses 15
Results 23
Community richness and abundance across habitats 23
Abiotic drivers: urban heat and environmental variables 23
Biotic drivers: invasive species 24
Discussion 37
Chapter 2: Counting urban ants: Evaluating best practices for sampling ant diversity in cities 43
Introduction 43
Methods 45
Ant sampling: comparative evaluation of methods 45
Literature review: variation in urban sampling design 48
Statistical analyses 49
Results 50
Sampling method performance 50
Variation in urban sampling design across studies 52
Discussion 58
Sampling method performance in urban environments 58
Mismatch between current practice and effective sampling 61
Alternative sampling approaches and their trade-offs 61
Spatial frameworks: gradients vs habitat mosaics 62
Logistical constraints 63

Toward a standardized protocol for urban ant sampling 65
References 68

List of Tables

Table 1. Site location and sampling methods.....17

Table 2. Occurrence data for all species found during hand collection, pitfall trapping, winkler extraction and baiting.....26

List of Figures

Fig. 1 Spatial distribution of impervious surface cover across Atlanta 21
Fig. 2 Sampling methods 22
Fig. 3 Ant species richness across habitat types 31
Fig. 4 Relationships between ant species richness and six abiotic and environmental variables 32
Fig. 5 Abiotic and biotic drivers of ant community composition 33
Fig. 6 Occurrences of the six most frequently detected ant species in Atlanta by habitat type 34
Fig. 7 Stacked bar plot showing the mean abundance of the three main invasive ant species 35
Fig. 8 Relationships between Brachyponera chinensis abundance and (a) native ant abundance and (b) native ant richness 36
Fig. 9 Comparison of four sampling methods 54
Fig. 10 Geographic and temporal overview of urban ant sampling studies 55
Fig. 11 Summary of habitat types and sampling methods reported across 83 urban ant studies (2001–2025) 56
Fig. 12 Proportion of urban ant sampling studies (N = 83) that reported obtaining permits or permission to conduct fieldwork. 57

Chapter 1: Invasive species undermine the bioark hypotesis in a low-latitude urban biodiversity hotspot

Introduction

Although biodiversity frequently declines with increasing urban intensity (Sanllorente et al. 2025), studies of urban insects show that total species richness at the city scale can remain high (Baldock et al. 2015; Savage et al. 2015). This paradox arises, in part, because cities are mosaics of habitats that differ in urban intensification and management (Sattler et al. 2011). Protected natural habitats within city bounds may function as “bioarks” that preserve native species within otherwise heavily altered landscapes (Brassard et al. 2021; Diamond et al. 2023). Most support for the cities-as-bioarks hypothesis comes from northern latitudes. Because most of the world’s biodiversity is concentrated at low latitudes, testing the bioark hypothesis in these regions is essential for understanding how urbanisation reshapes global diversity. Low-latitude areas may experience stronger pressures from urban heat and invasive species, potentially limiting their capacity to sustain native communities (Youngsteadt et al. 2017; Guo et al. 2021; Rajesh et al. 2022). Whether remnant natural habitats in low-latitude cities can function as bioarks under these combined pressures remains unresolved.

Urban climate represents one major pathway through which cities restructure insect communities. Urbanisation elevates temperatures and alters microclimatic conditions, often increasing thermal stress in exposed habitats (Angilletta Jr et al. 2007). However, the ecological consequences of urban warming may vary with latitude, altering the strength or direction of these effects. For example, in a global analysis of bird and plant diversity, Aronson *et al.*, (2014) found that the magnitude

of urban biodiversity loss depended strongly on regional climate and biogeography, and Leveau *et al.* (2017) demonstrated that urbanisation flattened latitudinal patterns of avian species richness across the southern Neotropics, with the largest urban-rural differences occurring at lower latitudes. Similar patterns have emerged in studies of insects and other arthropods, for example, Youngsteadt *et al.* (2016) found that urban warming increased insect abundances at high latitudes but had increasingly negative effects with decreasing latitude. In a study focused on ants, Penick *et al.* (2025) observed consistent declines in species richness with urban heat across cities, but there were potentially greater absolute losses in species-rich, low-latitude regions. These findings suggest that urban heat may relax thermal constraints in cooler cities while intensifying them in already warm regions where species operate closer to their physiological limits. Stronger negative effects of urban warming at low latitudes may therefore undermine the capacity of cities to function as bioarks, especially if warming extends into remnant habitats.

Biological invasion represents a second major pathway through which cities restructure insect communities. Invasive species are major drivers of biodiversity loss worldwide and impose substantial economic costs (Senator and Rozenberg 2017; Gruber et al. 2021; Angulo et al. 2022). Urban ecosystems, in particular, tend to support high densities of invasive ants (Gochnour et al. 2019), which thrive in disturbed habitats and can suppress native species through competition, predation, and resource monopolisation (Holway et al. 2002; Carpintero and Reyes-López 2008). In a large-scale analysis of urban ant diversity, Perez *et al.* (2022) found that urbanisation had stronger negative effects on diversity at low latitudes. Although that study did not directly test the role of invasive ants, it documented greater divergence between urban and non-urban communities in low-latitude cities, suggesting a shift

toward communities dominated by non-native species. These patterns suggest that low-latitude cities may experience stronger invasion pressure than their high-latitude counterparts, which may erode the bioark potential of low-latitude cities.

Here, we surveyed ant communities in Atlanta, Georgia (USA), to test whether protected remnant habitats can sustain high native insect diversity (the bioark hypothesis) or whether this potential declines at lower latitudes. Atlanta lies within the largest urban corridor in the southeastern United States (Terando et al. 2014) and hosts three prominent invasive ants: red imported fire ants (*Solenopsis invicta* Buren 1972), Argentine ants (*Linepithema humile* (Mayr, 1868)), and the more recently established Asian needle ant (*Brachyponera chinensis* (Emery, 1895)). Of these, *B. chinensis* is unique in its ability to colonise and dominate intact forest habitats, making it a potential driver of diversity loss even in low-disturbance environments (Guénard and Dunn 2010; Warren et al. 2015; Guénard et al. 2018; Kanes et al. 2025). Under the bioark hypothesis, we predicted that forested parks would support the highest native diversity. If urban climate effects dominate, we expected steeper declines in diversity with increasing urban heat. If invasion exerts stronger control, we predicted reduced native diversity in sites where invasive species abundance is high. Together, these predictions allow us to evaluate whether urban habitats in a low-latitude city function as bioarks and how their responses to climate and invasion differ from those observed at higher latitudes.

Methods

Study sites

We sampled ant communities at 60 sites in Atlanta, Georgia (USA) during June-August 2021 and June 2023 (**Table S1**; **Fig. 1**). We selected sites to represent three common urban habitat types that vary in disturbance: streetscapes (high disturbance), manicured parks (moderate disturbance), and forested parks (low disturbance). We defined streetscapes as highly fragmented areas under 5,000 m², such as traffic islands, roadside plantings, and greenspaces near buildings. We defined manicured parks as green spaces over 5,000 m² with mixed canopy cover and an understory dominated by mowed grass or planted woody vegetation. Finally, forested parks were forested areas over 5,000 m² categorised by minimal maintenance, dense canopy cover, and the ground being covered primarily in leaf litter. The dominant plants in forested parks were white oak (*Quercus alba* L.), loblolly pine (*Pinus taeda* L.), American beech (*Fagus grandifolia* Ehrh.), poison ivy (*Toxicodendron radicans* [L.] Kuntze), English ivy *(Hedera helix* L.), and Virginia creeper (*Parthenocissus quinquefolia* [L.] Planch.). In total, we sampled 18 streetscapes, 20 manicured parks, and 22 forested park sites across the city across all sampling methods.

To quantify environmental variation among sites, we ran two perpendicular transects (20 m each) that intersected at the centre of each site. At 5-meter intervals along each transect, we measured leaf litter depth and ground cover type by visual inspection (categories included: bare ground, mulch, leaf litter, fine vegetation, or coarse vegetation). Following methods from Penick *et al.*, (2025), we computed a vegetation complexity index (VCI) as the Shannon diversity index (*H*) of vegetation categories, where each vegetation type was treated as a ‘species’ and it’s abundance was the percentage of cover. At the centre of each site, we also recorded canopy cover using the ‘Percentage Cover’ iOS app (Mignanelli 2021) at each

cardinal direction, taking the average. We supplemented on-site data with NLCD and Landsat Collection in ArcGIS Pro to extract percent impervious surface and surface temperature with a resolution of 30 m (U.S. Geological Survey 2021; Dewitz 2023). Surface temperature values were extracted from all available cloud-free Landsat scenes collected between June 1 and August 31, 2023.

Ant Sampling

To capture the widest diversity of ant species across habitats, we used a combination of standard ant sampling methods (Gotelli *et al.*, 2011; **Fig. 2**) across two sampling periods. During June-August 2021, we conducted timed hand collection, baiting, and leaf litter extraction at all sites (**Table 1**). In June 2023, we conducted pitfall trapping at most previously sampled sites and included twelve additional sites not sampled in 2021 (**Table 1**).

In 2023, we deployed pitfall traps along a 10-meter transect at each site where ground conditions allowed. Plastic specimen cups (5 cm diameter) were filled halfway with water mixed with dish detergent to break surface tension, and then placed flush with the soil surface at 5 m intervals (**Fig. 2a**). The traps were retrieved after 48 hours, the contents were strained, and all remaining material was stored in 70% ethanol for later sorting and identification. For hand collection, we used forceps and aspirators (**Fig. 2b**) to collect ants across microhabitats (e.g., under rocks, logs, within leaf litter, and up tree trunks) within our 20 x 20 m plots for a cumulative total of one hour per site (sum of time each individual spent sampling). Specimens were preserved in 70% ethanol for later identification. For baiting, we provided a multi-bait buffet that included olive oil, 20% L-glutamine, 20% sucrose, 5% NaCl, and tap

water. We arranged the baits in a circular pattern spaced evenly apart (10 cm) at the center of our two transects and recorded the species and number of individuals feeding at each bait after one hour (**Fig. 2c**). We retained voucher specimens for any ants that could not be identified in the field. For leaf litter sampling, we collected a 1 m² quadrat from the area with the densest visible litter. We sifted the litter and placed it in a Winkler extractor (Ivanov et al. 2010; **Fig. 2d**) for 48 hours to collect ants and other arthropods into propylene glycol.

We identified all ants to species using the Mississippi Entomological Museum's dichotomous key to the ants of the southeastern United States (MacGown 2024). Voucher specimens were deposited in the Auburn University Museum of Natural History.

Statistical Analyses

All analyses were performed in R 4.4.1 (R Core Team 2025). To assess differences in species richness among habitat types, we computed sample-based species accumulation curves (random site accumulation, 999 permutations) using the 'vegan' package (Oksanen et al. 2025) based on pooled species counts from baiting, leaf litter extraction, and hand collection (pitfall traps were excluded as not all sites were sampled using this method). We also compared site-level richness among habitat types using the same sampling methods with a Kruskal-Wallis rank-sum test. To compare ant abundance among habitats, we calculated mean per-site pitfall abundance (averaged across all species records at each site) and fit a Poisson generalized linear model (GLM) with a log link function, and differences among

habitats were followed up with estimated marginal means (EMMs) and pairwise contrasts with Benjamini-Hochberg adjustment.

To evaluate the effects of urban heat and other environmental factors on community composition and species richness, we used a Jaccard distance-based redundancy analysis (db-RDA). Predictor variables were retained only after confirming acceptable collinearity (variance inflation factors < 10). Overall and marginal significance were assessed using ANOVA-like permutation tests (999 permutations). For variables with acceptable collinearity, we also tested bivariate relationships between site-level richness and six environmental predictors (percent canopy cover, percent impervious surface, percent leaf litter cover, leaf litter depth [cm], surface temperature [°C], and vegetation complexity) using separate linear models (LMs).

To test the effects of invasive species on community composition as well as native ant abundance and species richness, we conducted a Jaccard db-RDA with site-level abundances of three invasive ants as predictors (*Brachyponera chinensis*, *Solenopsis invicta*, and *Linepithema humile*). Because abundance estimates were derived from pitfall traps, these analyses were restricted to pitfall samples. Significance of overall and marginal effects was assessed using ANOVA-like permutation tests (999 permutations). We followed significant effects with Poisson GLMs (log link) to test the effects of invasive species abundance on native ant abundance and native species richness with habitat type included as an additional predictor.

Table 1. Site location and sampling methods

Site	Habitat type	Latitude	Longitude	Sampling method(s)
S01	Forested park	33.7875	-84.371944	Hand, bait, winkler
S02	Forested park	33.791111	-84.371944	Hand, bait, winkler
S03	Forested park	33.790414	-84.382421	Hand, bait, winkler, pitfall
S04	Manicured park	33.78857	-84.380784	Hand, bait, winkler
S05	Streetscape	33.788456	-84.383533	Hand, bait, winkler
S06	Streetscape	33.794966	-84.387935	Hand, bait, winkler, pitfall
S07	Manicured park	33.7825	-84.375556	Hand, bait, winkler
S08	Manicured park	33.7825	-84.375556	Hand, bait, winkler
S09	Forested park	33.782787	-84.375558	Hand, bait, winkler, pitfall
S11	Manicured park	33.769551	-84.352222	Hand, bait, winkler, pitfall
S12	Forested park	33.772462	-84.336777	Hand, bait, winkler
S13	Forested park	33.793338	-84.363592	Hand, bait, winkler
S14	Forested park	33.785469	-84.361223	Hand, bait, winkler, pitfall
S15	Forested park	33.7858	-84.363114	Hand, bait, winkler, pitfall
S16	Forested park	33.795475	-84.374626	Hand, bait, winkler, pitfall

Site	Habitat type	Latitude	Longitude	Sampling method(s)
S17	Streetscape	33.7925	-84.386944	Hand, bait, winkler, pitfall
S18	Streetscape	33.790278	-84.387222	Hand, bait, winkler, pitfall
S19	Forested park	33.792438	-84.377936	Hand, bait, winkler
S20	Streetscape	33.787796	-84.387486	Hand, bait, winkler
S21	Streetscape	33.754937	-84.388715	Hand, bait, winkler
S22	Manicured park	33.770658	-84.347666	Hand, bait, winkler, pitfall
S23	Manicured park	33.760184	-84.394471	Hand, bait, winkler, pitfall
S24	Manicured park	33.760184	-84.394471	Hand, bait, winkler, pitfall
S25	Streetscape	33.782778	-84.384167	Hand, bait, winkler
S26	Streetscape	33.782302	-84.383878	Hand, bait, winkler
S27	Manicured park	33.761365	-84.394063	Hand, bait, winkler
S28	Streetscape	33.755661	-84.388431	Hand, bait, winkler
S30	Streetscape	33.758475	-84.402273	Hand, bait, winkler, pitfall
S31	Streetscape	33.784041	-84.382932	Hand, bait, winkler
S32	Streetscape	33.767653	-84.354766	Hand, bait, winkler, pitfall
S33	Streetscape	33.759582	-84.389542	Hand, bait, winkler, pitfall

Site	Habitat type	Latitude	Longitude	Sampling method(s)
S35	Manicured park	33.769029	-84.358211	Hand, bait, winkler, pitfall
S37	Forested park	33.758045	-84.357692	Hand, bait, winkler, pitfall
S38	Forested park	33.757785	-84.357449	Hand, bait, winkler, pitfall
S39	Manicured park	33.784492	-84.378273	Hand, bait, winkler
S40	Manicured park	33.769838	-84.346813	Hand, bait, winkler
S41	Forested park	33.771101	-84.343492	Hand, bait, winkler
S42	Manicured park	33.758305	-84.35788	Hand, bait, winkler, pitfall
S43	Forested park	33.793736	-84.3763	Hand, bait, winkler, pitfall
S44	Forested park	33.793188	-84.376863	Hand, bait, winkler
S45	Forested park	33.768662	-84.356275	Hand, bait, winkler
S46	Forested park	33.7660605	-84.3376181	Hand, bait, winkler
S47	Manicured park	33.7660605	-84.3376181	Hand, bait, winkler
S48	Manicured park	33.7366051	-84.3722826	Hand, bait, winkler
S49	Manicured park	33.7393781	-84.3734571	Hand, bait, winkler
S51	Streetscape	33.755519	-84.359384	Hand, bait, winkler, pitfall
S52	Streetscape	33.758307	-84.393585	Hand, bait, winkler

Site	Habitat type	Latitude	Longitude	Sampling method(s)
S101	Streetscape	33.774225	-84.416938	Pitfall
S102	Streetscape	33.781395	-84.399355	Pitfall
S103	Manicured park	33.770191	-84.376930	Pitfall
S105	Manicured park	33.766500	-84.337191	Pitfall
S106	Forested park	33.738082	-84.371237	Pitfall
S107	Manicured park	33.736457	-84.369385	Pitfall
S108	Forested park	33.790225	-84.368503	Pitfall
S109	Forested park	33.790335	-84.366770	Pitfall
S110	Manicured park	33.789940	-84.368322	Pitfall
S111	Manicured park	33.789886	-84.366507	Pitfall
S112	Forested park	33.793308	-84.363614	Pitfall
S113	Streetscape	33.782159	-84.383785	Pitfall
S114	Forested park	33.773726	-84.338143	Pitfall

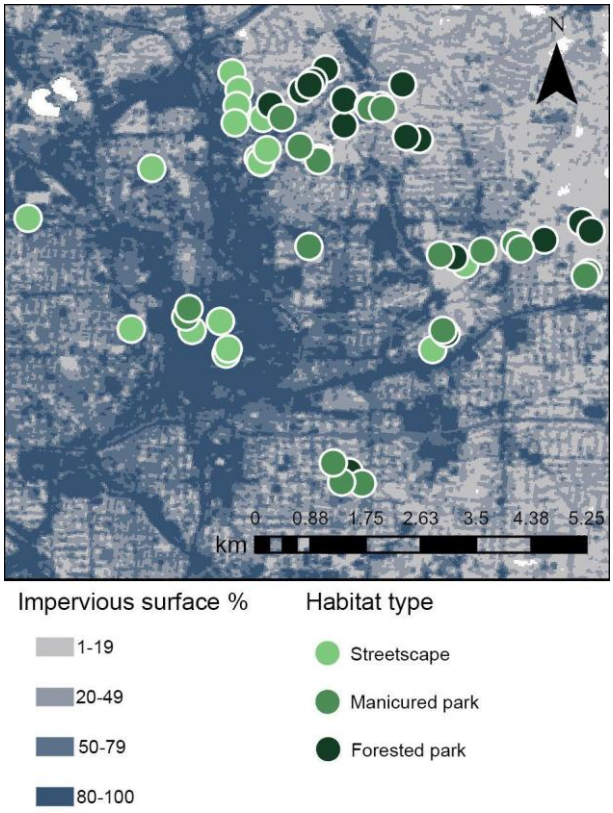

Fig. 1 Spatial distribution of impervious surface cover across Atlanta, Georgia (USA), to a resolution of 30 metres using Dewitz (2023). Darker shading indicates higher percentages of impervious surface. Sampling sites are shown and color-coded by habitat type.

Fig. 2 Sampling methods**.** (a) Pitfall trap filled with soapy water, (b) aspirator used for hand sampling, (c) liquid baits, and (d) Winkler bags used to extract ants from leaf litter samples.

Results

Community richness and abundance across habitats

We identified 54 ant species from 22 genera across all sampling methods and sites (**Table 2**). Contrary to expectations, species richness did not peak in the least disturbed habitat (forested parks) but instead was highest in habitats with intermediate urban stress (manicured parks; **Fig. 3a**); richness was 32 species in streetscapes, 33 in forested parks, and 40 in manicured parks. Mean site-level richness was highest in manicured parks, intermediate in streetscapes, and lowest in forested parks (**Fig. 3b**), though differences were not significant (Kruskal-Wallis, N = 47, df = 2, χ^2 = 5.35, p = 0.069). Ant abundance varied significantly among habitats based on pitfall samples (Poisson GLM, N = 34, df = 31, residual deviance = 124.25, $p < 0.0001$). Post hoc comparisons of estimated marginal means (EMMs) revealed that streetscapes supported higher mean per-site ant abundance than manicured parks (ratio = 2.22, SE = 0.37, z = 4.81, $p < 0.0001$) and forested parks (ratio = 1.70, SE = 0.27, z = 3.35, p = 0.001), while forested and manicured parks did not differ significantly (ratio = 1.31, z = 1.66, p = 0.097).

Abiotic drivers: urban heat and environmental variables

Ant community composition was significantly related to the environmental predictor set in a distance-based redundancy analysis (db-RDA) on Jaccard dissimilarities (ANOVA-like permutation test, pseudo-F = 2.03, p = 0.001; 999 permutations). Marginal permutation tests indicated that surface temperature (pseudo-F = 1.66, p = 0.039) and canopy cover (pseudo-F = 1.61, p = 0.042) were significant predictors of community composition, whereas leaf litter cover (F =

1.53, $p = 0.061$), leaf litter depth ($F = 1.37$, $p = 0.094$), and vegetation complexity ($F = 1.39$, $p = 0.099$) were marginally non-significant, and habitat category had no significant effect ($F = 0.86$, $p = 0.739$; **Fig. 5a**). Separate LMs of site-level species richness on each abiotic predictor showed that richness decreased significantly with increasing leaf litter cover ($\beta = -0.050$, $t = -2.93$, $p = 0.005$, $R^2 = 0.16$) and leaf litter depth ($\beta = -2.21$, $t = -3.46$, $p = 0.001$, $R^2 = 0.21$), but was not significantly related to surface temperature ($t = 0.31$, $p = 0.761$), canopy cover ($t = -1.01$, $p = 0.316$), impervious surface ($t = 0.51$, $p = 0.611$), or vegetation complexity ($t = 0.41$, $p = 0.683$; **Fig. 4**).

Biotic drivers: invasive species

Ant community composition varied significantly with invasive ant abundance in a db-RDA constrained by abundances of *Brachyponera chinensis, Solenopsis invicta,* and *Linepithema humile* (ANOVA-like permutation test: $F = 1.98$, $p = 0.001$, 999 permutations; $R^2 = 0.165$, adj. $R^2 = 0.082$; **Fig. 5b**). Marginal tests indicated significant effects of *B. chinensis* ($F = 2.99$, $p = 0.001$) and *L. humile* ($F = 1.50$, $p = 0.017$), while *S. invicta* had no significant effect ($p > 0.0552$). Of the six most common species, two were invasive (*B. chinensis, S. invicta*; **Fig. 6**), and of those *B. chinensis* had the highest abundance in forested parks and overall (**Fig. 7**). Results from GLMs showed that native ant abundance declined significantly with increasing *B. chinensis* abundance (Poisson GLM, $\beta = -0.0119 \pm 0.0010$ SE, $z = -11.48$, $p < 0.0001$; **Fig. 8a**). On the response scale, each additional *B. chinensis* individual corresponded to an ~1.2% decrease in expected native abundance ($\exp(\beta) = 0.988$), with an estimated baseline of ~50 native individuals at sites where *B. chinensis* was

absent (exp(intercept) = 49.9). The negative relationship between *B. chinensis* abundance and native abundance remained significant after including habitat as a covariate, although the effect size was reduced (Poisson GLM: β = −0.0042 ± 0.00126 SE, z = −3.36, p = 0.00078; **Fig. 8a**). After controlling for habitat type, *B. chinensis* abundance showed a marginal association with native richness (Poisson GLM: β = -0.0064 ± 0.0034 SE, z = -1.88, p = 0.0607; **Fig. 8b**).

Table 2. Occurrence data for all species found during hand collection, pitfall trapping, winkler extraction and baiting. There were 18 total streetscapes, 20 total manicured parks, and 22 total forested parks for a total of 60 total sites. Non-native species are indicated by an asterisk (*).

Species	Streetscape	Manicured Park	Forested Park	Total Sites Found
Aphaenogaster flemingi Smith	1	0	0	1
Aphaenogaster fulva Roger	0	1	1	2
Aphaenogaster rudis complex Wesson & Wesson	0	1	0	1
Brachymyrmex depilis Emery	0	1	1	2
Brachymyrmex obscurior Forel *	1	3	1	5
Brachymyrmex patagonicus Mayr *	10	6	1	17
Brachymyrmex sp.02	1	1	2	4
Brachyponera chinensis * (Emery)	12	17	21	50
Camponotus americanus Mayr	0	0	2	2
Camponotus castaneus Latreille	5	6	12	23
Camponotus chromaiodes Bolton	0	0	2	2

Species	Streetscape	Manicured Park	Forested Park	Total Sites Found
Camponotus nearcticus Emery	1	1	0	2
Camponotus pennsylvanicus (De Geer)	3	12	16	31
Camponotus snellingi Bolton	3	0	5	8
Crematogaster ashmeadi Mayr	5	8	6	19
Crematogaster lineolata (Say)	0	1	0	1
Crematogaster minutissima Mayr	1	0	1	2
Crematogaster missouriensis Emery	1	2	0	3
Crematogaster vermiculata Emery	1	0	1	2
Forelius mccooki (McCook)	0	1	0	1
Formica pallidefulva Latreille	4	10	12	26
Formica rubicunda Emery	0	2	3	5
Formica subsericea Say	1	3	3	7
Hypoponera punctatissima (Roger)	0	1	0	1

Species	Streetscape	Manicured Park	Forested Park	Total Sites Found
*				
Lasius americanus Emery	10	14	8	32
Lasius aphidicola (Walsh)	0	0	1	1
Lasius nearcticus Wheeler	0	1	0	1
Lasius neoniger Emery	5	16	12	33
Linepithema humile (Mayr) *	5	2	0	7
Monomorium carbonarium (Smith)	10	4	3	17
Monomorium viridum Brown	1	1	0	2
Myrmecina americana Emery	1	0	0	1
Nylanderia faisonensis (Forel)	0	4	0	4
Nylanderia wojciki (Trager)	15	14	7	36
Pheidole bicarinata Mayr	4	2	2	8
Pheidole dentata Mayr	1	3	0	4
Pheidole dentigula Smith	0	0	1	1
Pheidole megacephala (Fabricius)	0	1	0	1

Species	Streetscape	Manicured Park	Forested Park	Total Sites Found
Pheidole morrisii Forel	0	1	0	1
Pheidole tysoni Forel	7	12	8	27
Prenolepis imparis (Say)	0	0	1	1
Pseudomyrmex ejectus (Smith)	0	1	0	1
Solenopsis invicta Buren *	13	8	5	26
Solenopsis molesta (Say)	12	7	5	24
Stigmatomma pallipes (Haldeman)	1	0	0	1
Strumigenys archboldi (Deyrup & Cover)	0	0	1	1
Strumigenys hexamera (Brown) *	1	0	0	1
Strumigenys louisianae Roger	1	2	3	6
Strumigenys membranifera Emery *	3	2	1	6
Strumigenys ornata Mayr	0	0	1	1

Species	Streetscape	Manicured Park	Forested Park	Total Sites Found
Strumigenys silvestrii (Emery) *	0	1	0	1
Tapinoma sessile (Say)	7	9	6	22
Temnothorax schaumii (Roger)	0	2	0	2
Trachymyrmex septentrionalis (McCook)	0	1	0	1

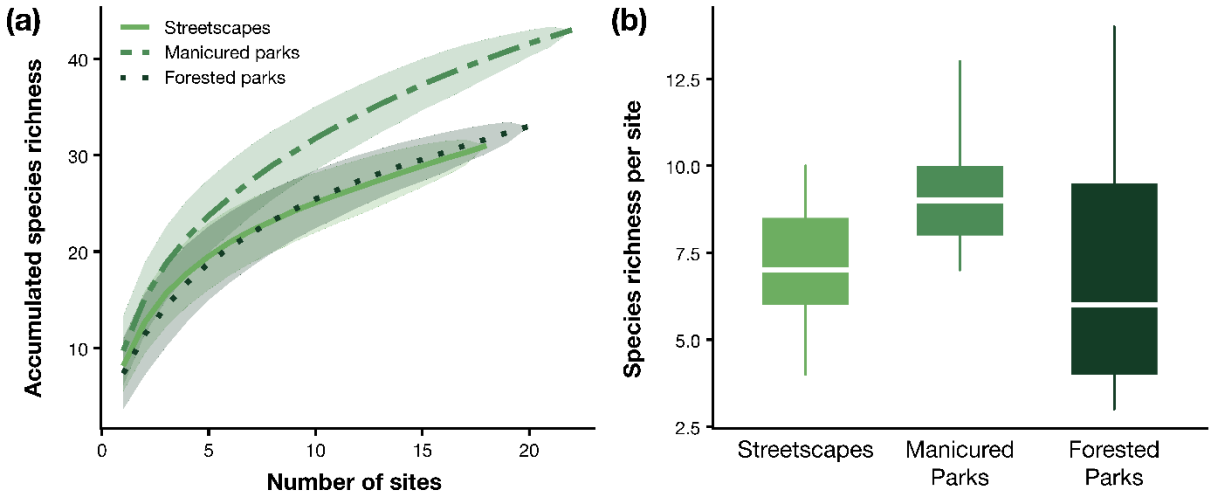

Fig. 3 Ant species richness across habitat types. (a) Species accumulation curves comparing ant species richness among habitat types. Shaded areas represent 95% confidence intervals. (b) Boxplots showing site-level species richness across habitat types (Median and interquartile range), though differences among habitat types were not statistically significant (Kruskal-Wallis, p = 0.069).

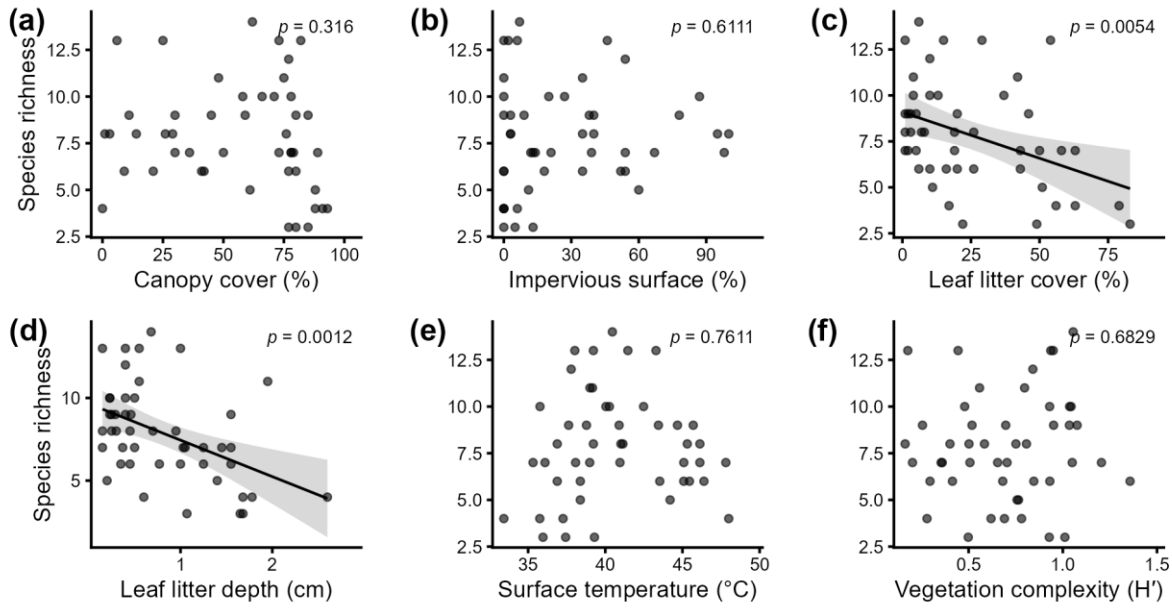

Fig. 4 Relationships between ant species richness and six abiotic and environmental variables across all sites. **(a)** Canopy cover, **(b)** impervious surface cover, **(c)** leaf litter cover, **(d)** leaf litter depth, **(e)** maximum surface temperature, and **(f)** vegetation complexity (Shannon index, H′). Each point represents one site. Linear regression lines with 95% confidence intervals are shown for significant relationships ($p < 0.05$). *p*-values are from linear models.

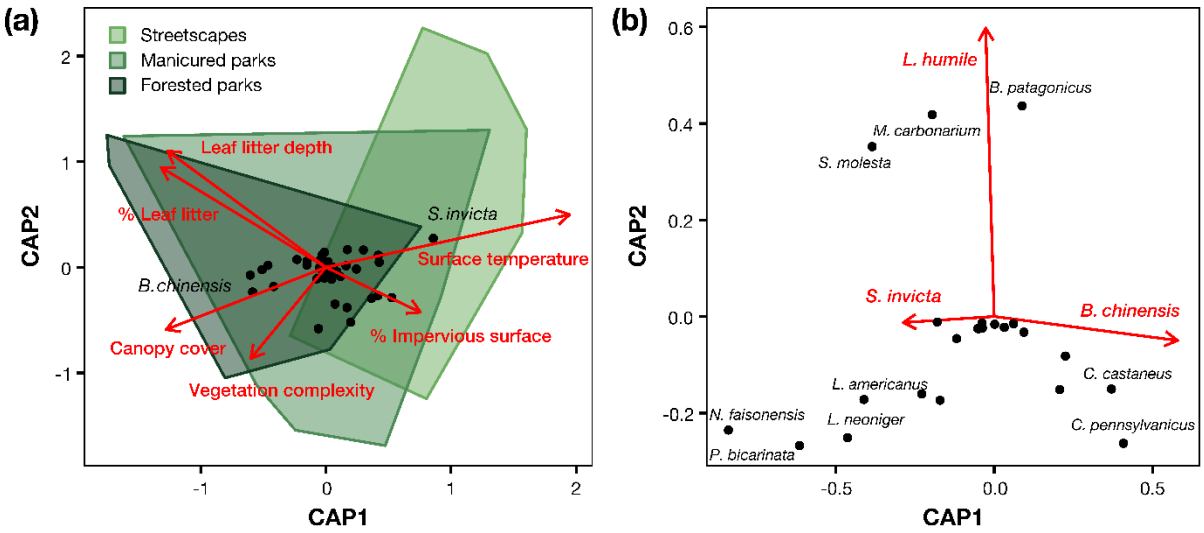

Fig. 5 Abiotic and biotic drivers of ant community composition. (a) Jaccard distance-based redundancy analysis (db-RDA) of species composition constrained by environmental predictors; convex hulls indicate habitat types. (b) Jaccard db-RDA of pitfall-trap communities constrained by abundances of Brachyponera chinensis, Solenopsis invicta, and Linepithema humile. CAP1 and CAP2 are the first and second constrained axes of the db-RDA, which are the linear combinations of predictor variables that capture the largest fractions of variation in ant community composition. Predictors are shown in red.

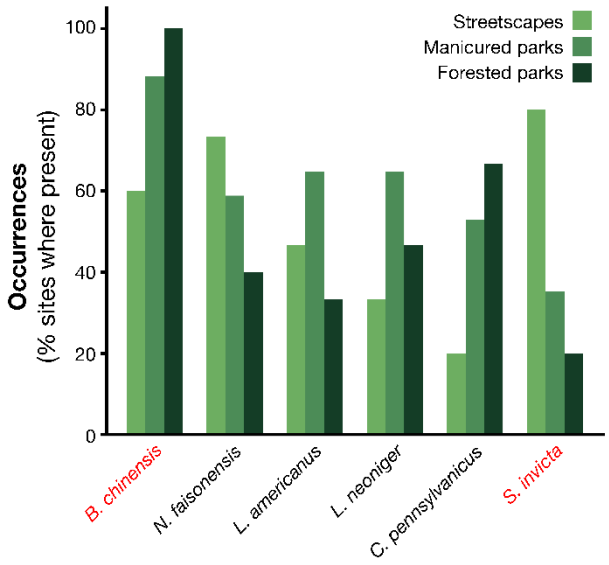

Fig. 6 Occurrences of the six most frequently detected ant species in Atlanta by habitat type, ordered from most to least common (left to right). Invasive species are indicated in red text.

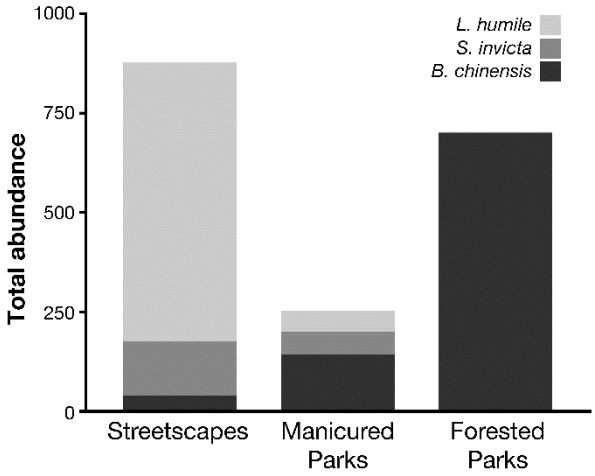

Fig. 7 Stacked bar plot showing the mean abundance of the three main invasive ant species (Brachyponera chinensis, Solenopsis invicta, and Linepithema humile), grouped by habitat type (streetscapes, manicured parks, and forested parks).

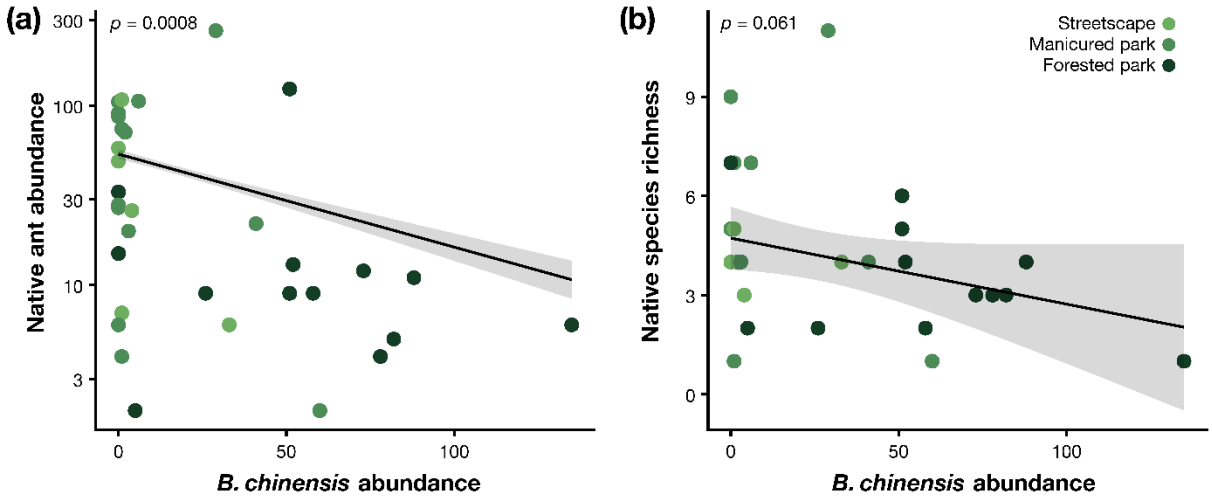

Fig. 8 Relationships between Brachyponera chinensis abundance and (a) native ant abundance and (b) native ant richness. Plots show best fit line with 95% confidence intervals, and habitat type is indicated by colour. P-values are taken from Poisson GLMs with habitat type included as a predictor.

Discussion

Although previous studies suggest that cities function as bioarks for insect diversity, our results indicate that this capacity may be reduced due to invasion. Consistent with the global latitude-diversity gradient (Wallace 1878; Perez et al. 2022; Griffith et al. 2025; Penick et al. 2025), overall ant species richness was higher in Atlanta (55 species) than in comparably sampled northern cities, such as New York City, New York (USA), (40 species; Penick, 2021). However, within Atlanta, species richness was lowest in relatively protected forested parks. This pattern contrasts with findings from northern cities, where intact forest habitats typically support the highest native richness (Sattler et al. 2011; Savage et al. 2015; Fenoglio et al. 2020; Sanllorente et al. 2025). This decline in species richness was not related to common abiotic urban stressors, such as urban heat or impervious surface cover. Instead, native diversity and abundance declined with increasing abundance of the invasive Asian needle ant (*Brachyponera chinensis)*. This result aligns with broader patterns showing greater divergence between urban and non-urban ant communities at lower latitudes (Perez et al. 2022) and suggests that biological invasion may limit the ability of remnant habitats to function as bioarks in low-latitude cities, even where regional species pools are large.

Urban heat is widely recognized as one of the most consistent stressors in urban ecosystems (Youngsteadt et al. 2017; Fenoglio et al. 2020; Polidori et al. 2023; Cabon et al. 2024; Dietzel et al. 2025; Penick et al. 2025). Across taxa, urban warming has been shown to alter phenology, abundance, and species turnover, and global analyses suggest that thermal stress should intensify toward lower latitudes

where organisms operate closer to upper thermal limits (Deutsch et al. 2008; Diamond et al. 2012; Sunday et al. 2014). Based on these patterns, we predicted that urban heat would be a primary driver of species richness declines in Atlanta. However, surface temperature was not a significant predictor of native richness or composition. We cannot rule out temperature effects entirely, as work from Raleigh, North Carolina (USA), suggests urban heat can structure southeastern ant communities (Penick et al. 2025), but in our system, abiotic gradients were overshadowed by invasion pressure. Outside of temperature, species richness declined with increasing leaf litter cover and depth, factors typically associated with higher ant diversity under non-invaded conditions (e.g., Lassau & Hochuli, 2004; Queiroz *et al.*, 2013; Silva *et al.*, 2011). This counterintuitive relationship suggests that abiotic habitat features alone cannot explain the observed diversity pattern. Instead, leaf-litter-rich forests were also the habitats most heavily invaded by *B. chinensis*, indicating that biotic pressure may have overridden expected habitat effects.

Instead of abiotic stress, declines in richness and native abundance were strongly associated with *B. chinensis*, the most common ant in Atlanta based on species occurrence. Although first documented in the Atlanta metro area in 1932, *B. chinensis* recently expanded in range and abundance and only became prominent in Atlanta between 1991 and 2010 (Guénard et al. 2018). Unlike many urban invaders, *B. chinensis* readily persists in relatively undisturbed forests, including protected areas, such as Great Smoky Mountains National Park (Guénard and Dunn 2010; Kanes et al. 2025). This ecological trait likely explains its disproportionate impact on forested parks in Atlanta. Based on patterns from New York City, where forested parks support ~23% more species than manicured parks (Savage et al. 2015), we

would have expected ~49 species in Atlanta's forested parks. Instead, we observed only 33 species, a ~33% reduction. These losses are consistent with previous studies documenting ~32% declines in richness and up to 70% reductions in native abundance in sites heavily invaded by *B. chinensis* (Guénard and Dunn 2010). Patterns in native abundance followed similar trends in Atlanta, with an 80% decline in native ant abundance across the *B. chinensis* invasion gradient. These patterns suggest that *B. chinensis* exerts a substantial suppressive effect on native ant communities both in and outside of urban areas.

In contrast to *B. chinensis*, the two most economically damaging invasive ants in the southeastern United States, the red imported fire ant (*Solenopsis invicta*) and the Argentine ant (*Linepithema humile)*, had comparatively limited effects on native diversity in this study. Both species rank among the world's 100 worst invasive alien species as determined by the International Union for Conservation of Nature (IUCN) (Lowe et al. 2000). Both species impose substantial economic costs due to damage and cost of management, with *S. invicta* alone estimated to have caused approximately $3 billion USD in damage in the United States since 1937 (Angulo et al. 2022). Yet neither species was particularly dominant across sites in Atlanta. We found that *S. invicta* was only the sixth most common ant species in Atlanta based on species occurrences, and was largely restricted to streetscapes and manicured parks; *L. humile* was rare based on species occurrences, but locally abundant in in the relatively few sites it was collected. While *S. invicta* and *L. humile* remain costly urban pests, particularly in highly disturbed habitats near structures, the forest-adapted *B. chinensis* exerted far stronger effects on native ant communities within remnant parks. These results underscore an important distinction between economic prominence and biodiversity impact, as relatively few resources have been deployed

towards control of *B. chinensis* compared to these other invaders (Buczkowski 2023).

Overall, our results suggest that the capacity of cities to function as protected habitats for insects may be reduced by invasive species, particularly at lower latitudes. If the patterns observed in Atlanta are representative, low-latitude cities may experience greater biodiversity declines associated with stronger invasion pressure. This interpretation is consistent with global analyses showing greater losses of ant diversity and increased divergence between urban and non-urban communities at lower latitudes (Perez et al. 2022), patterns attributed to higher dominance of non-native species, though not directly tested. In Atlanta, relatively intact forested parks did not provide refuge from invasion. This suggests that habitat protection alone may be insufficient to conserve native biodiversity in warmer regions where invasion pressure is high. Notably, Atlanta remains within a temperate region, underscoring the need for comparative studies in lower-latitude and tropical cities, where these dynamics may be even more pronounced and where the bioark hypothesis remains largely untested.

These findings have practical implications for urban conservation. Cities are major hubs for species introductions (Francis and Chadwick 2015), and future urban expansion is projected to occur disproportionately in biodiverse regions (Seto et al. 2012; Potgieter et al. 2024). Yet most urban invasive ant management focuses on nuisance control around structures, where chemical baits are the primary treatment strategy (Dimarco et al. 2017). While chemical controls can be effective, they may generate non-target effects (Hoffmann et al. 2023; Amaral et al. 2024). Adjusting bait grain size, placement, timing, and formulation can further reduce collateral effects on

native ants (Vogt et al. 2005; Hoffmann et al. 2023). Species-specific approaches may be particularly important for managing *B. chinensis*. Buczkowski (2016) demonstrated that termite-based “Trojan horse” baiting can selectively suppress *B. chinensis* with reduced non-target effects, although scaling such approaches may be challenging. More recently, Buczkowski (2023) showed that incorporating termite cuticular extracts into baits improves acceptance by *B. chinensis*, potentially increasing treatment efficacy. Seasonal timing may also matter, as bait acceptance by *B. chinensis* peaks between July and September (Corsetti 2024). However, additional investment in effective control strategies for *B. chinensis* is needed to develop scalable methods at local eradication.

More broadly, our findings indicate that invasive insects can exert strong pressure even within relatively protected park habitats. Urban forests are often fragmented and subject to edge effects that facilitate invasion (Holway 2005). Indeed, *B. chinensis* appears to perform particularly well in fragmented forests (Campbell et al. 2019). Cities may therefore act as incubators for forest-adapted invaders, not merely hubs for disturbance-associated species. The recent establishment of the ManhattAnt, *Lasius emarginatus* (Olivier, 1792), in New York City illustrates this broader pattern, as they were also first detected in urban parks before spreading into highly urban areas (Kennett et al. 2024). Cities are thus simultaneously potential bioarks and frontlines of biological invasion (Lin et al. 2025). In low-latitude regions where biodiversity is highest, preserving insect diversity will require not only protecting remnant habitats but actively managing invasive species within them. Without coordinated invasion control, remnant urban forests may fail to serve as refuges where they are most needed. Whether cities ultimately function as

bioarks or sources of biodiversity loss will depend on our ability to manage invasion within these protected habitats.

Chapter 2: Counting urban ants: Evaluating best practices for sampling ant diversity in cities

Introduction

Cities have emerged as a critical frontier for ant ecology (Santos 2016; Perfecto and Philpott 2023; Penick and Edenborough 2026 Apr 15). Urban environments can support both extraordinarily high ant abundance and diversity: for example, there are estimated to be roughly 2,000 ants for every human resident in New York City (Penick 2021), while Macao—the most densely populated region on the planet with more than 20,000 people per square kilometre—hosts over 150 ant species, more than are found in most European countries (Brassard et al. 2021). Because ant communities respond predictably to environmental conditions, they have long been used as bioindicators of ecological change, particularly to assess ecosystem responses to disturbance and land use (Andersen 1997; King et al. 1998). Changes in ant species richness and composition have been used to track habitat degradation (Andersen et al. 2002), monitor ecological restoration (Gerhardt 2002; Andersen and Majer 2004), and infer broader shifts in soil communities. Despite their growing importance as indicators of urban ecological change, there remains little consensus on how best to sample ant communities in cities.

The current standard for sampling ground-dwelling ant communities is the Ants of the Leaf Litter (ALL) protocol, which combines pitfall trapping, leaf-litter extraction, and hand collection along a 200 m transect (Bestelmeyer et al. 2000 Jan 1; Fisher et al. 2000). This protocol has been widely validated across temperate and subtropical natural ecosystems and underpins most rigorous ground-dwelling ant inventories (King and Porter 2005; Wiezik et al. 2015). However, the physical and

spatial requirements needed for the ALL protocol are frequently unmet in urban environments: leaf litter is often sparse or absent, impervious surfaces limit the placement of pitfall traps, and highly fragmented habitat patches make long transects impractical. As a result, urban ecologists often modify or abandon elements of the ALL protocol by omitting certain methods (most commonly pitfall trapping or litter sampling) or substituting simpler alternatives, such as baiting (Curry et al. 2024; Eydoux et al. 2024; Penick et al. 2025). This has led to wide variation in methodological approaches across studies, which complicates comparisons and limits our ability to synthesize patterns of urban biodiversity.

In addition to methodological differences, the spatial framework used to sample cities can strongly influence ecological inference. Studies of vertebrates often treat urbanisation as a continuous gradient, using metrics such as impervious surface cover to quantify environmental change (Melles et al. 2003; Riem et al. 2012; Demartín et al. 2024). However, ants and many other ground-dwelling arthropods experience cities at much finer spatial scales. Most species forage only meters from their nest, meaning that colonies separated by short distances may occupy fundamentally different environments; for example, an ant colony in New York City's Central Park experiences a vastly different habitat than one located just two blocks west on Broadway. To account for this, many urban insect studies adopt a habitat mosaic approach, categorizing sites into discrete habitat types that vary in disturbance or management intensity (Tommasi et al. 2004; Savage et al. 2015; Boeing et al. 2023). Yet these classifications differ widely among studies in both definition and scope, leading to inconsistent representations of urban biodiversity. This lack of standardization raises a key question: whether urban ant communities

are better understood along continuous gradients, within discrete habitat mosaics, or through some combination of both.

Here, we evaluated the performance of four commonly used sampling methods in urban habitats (pitfall trapping, leaf litter extraction, hand collection, and baiting) across 60 urban sites in Atlanta, Georgia (USA). We quantified each method's contribution to total species richness and its unique contribution to the community. We then conducted a systematic review of urban ant studies published between 2001 and 2025 to characterize prevailing practices in sampling method choice and habitat sampling frameworks. We also assessed how consistently studies report permitting and access requirements as well as other practical constraints unique to urban fieldwork, such as safety. By linking empirical comparisons with patterns in the literature, our goal is to identify methodological gaps and provide an evidence-based foundation for more standardized and comparable approaches to sampling ant communities in urban environments.

Methods

Ant sampling: comparative evaluation of methods

We sampled ant communities from 60 sites across Atlanta, Georgia (USA) between June–August 2021 and June 2023. Sites were selected to represent a habitat mosaic of common urban land-use types that vary in disturbance intensity, following the framework described in Edenborough et al. (2026). Specifically, sites were categorized into three discrete habitat types: streetscapes (high disturbance), manicured parks (moderate disturbance), and forested parks (low disturbance). At each site, sampling was conducted within a standardized plot centred on two

intersecting 20 m transects, reduced from 200 m from the ALL protocol to allow sampling within smaller and more fragmented urban habitat patches.

To evaluate sampling performance in urban habitats, we compared four commonly used methods: pitfall trapping, leaf-litter extraction, timed hand collection, and baiting. Methods were applied using standardized protocols to allow comparison of their relative and complementary contributions to species detection as described below. Once collected, all ants were identified to species using the Mississippi Entomological Museum's dichotomous key to the ants of the southeastern United States (MacGown 2024), and voucher specimens were retained for verification and deposited in the Auburn University Museum of Natural History.

Pitfall trapping: Pitfall traps were placed along one transect at 5 m intervals. At each point, a 5 cm diameter plastic specimen cup was installed flush with the ground surface and filled halfway with water containing 2–3 drops of dish detergent to reduce surface tension. Traps remained in place for 48 hours before collection, after which contents were strained and transferred to 70% ethanol for preservation and identification. We used soapy water instead of propylene glycol to reduce trap visibility in high-traffic environments. Relative to the ALL protocol, which typically deploys a greater number of traps spaced at wider intervals along a longer transect, our design reduced both transect length (20 m vs. 200 m) and spacing (5 m vs. 10 m) to accommodate spatial constraints in urban habitats.

Leaf-litter extraction: Leaf-litter sampling was conducted by collecting a single 1 m² quadrat of litter from each site and extracting arthropods using Winkler extractors for 48 hours (Fisher et al. 2000; Delsinne and Arias-Penna 2012), with propylene glycol as a temporary preservative. In contrast to the ALL protocol, which

typically includes multiple litter samples per site, we limited sampling to one quadrat per site due to the patchy and often limited availability of leaf litter in urban environments. Sampling was targeted to areas with the greatest visible litter accumulation. In habitats with limited litter (e.g., streetscapes), available material was pooled from the surrounding surface, including mulch and organic debris, to maximize recovery of litter-associated species.

Timed hand collection: Hand collection was conducted by trained researchers within the transect area and standardized to one hour of cumulative search time per site (i.e., the summed time across all collectors). Collectors systematically searched a range of microhabitats, including beneath rocks and logs, within leaf litter, and on vegetation, using aspirators and forceps. Although hand collection is included in the ALL protocol, it is not always time-standardized; here, we imposed a fixed sampling duration to enable direct comparison among sites.

Baiting: Baiting consisted of a multi-resource array designed to attract ants with different nutritional preferences. Baits (olive oil, 20% L-glutamine solution, 20% sucrose solution, 5% NaCl solution, and water) were arranged in a circular pattern with openings facing inward and spaced <20 cm apart. Species identity and the number of individuals at each bait were recorded after one hour, and voucher specimens were collected when necessary. In contrast to many baiting protocols that use high-reward food items (e.g., peanut butter, tuna in oil, or pecan sandies) to maximize recruitment, our approach prioritized diversity of resource types to quantify foraging preferences, even if this reduced recruitment intensity at individual baits.

Literature review: variation in urban sampling design

We conducted a systematic review of urban ant community studies published between 2001 and 2025 using Web of Science (search string: TS = (urban AND ants AND (community OR communities) AND (richness OR diversity))). Results were manually screened to retain only studies that sampled ant communities in urban or peri-urban environments; studies focused solely on single species, pest management, solely agricultural habitat, or non-urban landscapes were excluded.

For each study, we recorded three key aspects of sampling design: (1) the sampling methods, (2) the spatial framework used to characterize urban environments, and (3) the habitat types sampled. Sampling methods were classified as baiting, pitfall trapping, leaf-litter extraction, hand collection, vegetation beating, or other. Spatial frameworks were classified as either gradient-based (continuous measures of urbanisation) or mosaic-based (discrete habitat categories). For the latter, we categorized habitat types into the following classes: remnant habitat, manicured park, streetscape, residential, agricultural, community garden, gradient-based sampling, or other.

Remnant habitats were defined as fragments of relatively undisturbed natural habitat embedded within the urban matrix, which could include forests, grasslands, or deserts. Manicured parks were actively managed green spaces with mowed lawns, planted vegetation, and/or moderate human disturbance. Streetscapes represented highly urbanised environments characterized by high impervious surface cover and frequent human activity. Residential habitats included areas within or immediately surrounding residential housing, typically neighbourhoods with single family homes. Agricultural sites included actively or recently managed farmland, and

community gardens consisted of small, intensively managed plots typically used by multiple individuals. The “other” category included habitat types not captured by these classifications.

Finally, we recorded whether each study explicitly reported obtaining sampling permits or landowner permission either in the main text or acknowledgments.

Statistical analyses

All analyses were conducted in R version 4.5.2 (R Core Team 2025). To compare total species richness detected by each sampling method, we generated species accumulation curves separately for each method using randomized site-order resampling (1000 permutations across all sites; 999 permutations within streetscapes; Oksanen et al. 2025)). For each method, cumulative richness was summarized as the mean across permutations, with uncertainty expressed as 95% confidence bands.

To quantify each method’s marginal contribution to total species richness, we calculated Shapley values from site × species presence–absence data. Because not all sampling methods were applied at all sites, comparisons among methods were restricted to the subset of sites where all methods were conducted (N = 21). For each of B = 999 bootstrap resamples of sites (sampled with replacement), detections were pooled within each method, and the average marginal number of new species contributed by each method was calculated across all 24 possible method-addition orders. We summarized uncertainty using bootstrap means and 95% percentile intervals and visualized the bootstrap distributions using beeswarm plots. The “best” method was defined as the method with the highest mean Shapley value across

bootstrap replicates. For all other methods, we calculated bootstrap differences relative to the best method and summarized these differences using means and 95% percentile intervals. We also report the proportion of bootstrap replicates in which differences were ≤0 or ≥0 and assessed deviations from zero using two-sided bootstrap sign tests.

To compare per-site detection among sampling methods, we calculated mean species richness per site separately for each method, using all sites where that method was applied (baiting and Winkler: $N = 47$; pitfall: $N = 34$; hand: $N = 49$). To assess the extent to which methods captured unique components of the community, we also calculated the total number of species detected only by each method. In addition, to formally test whether methods differed in the number of species they captured uniquely at the same site, we calculated, for each of the 21 sites where all four methods were applied, the number of species detected only by that method at that site (i.e., not detected by any of the other three methods at the same site). Pairwise differences among methods in the per-site count of method-unique species were tested using Wilcoxon rank-sum tests (rstatix; Kassambara 2019), with Benjamini–Hochberg false discovery rate correction applied to p-values across all six pairwise comparisons.

Results

Sampling method performance

To evaluate how sampling methods differed in their ability to capture ant diversity, we compared species accumulation patterns, marginal contributions to total richness, and per-site detection. Species accumulation curves revealed clear

differences among methods (**Fig. 9A**). Hand collection and pitfall trapping detected the greatest number of species (39 and 34 species, respectively) and continued to accumulate new species across sites, suggesting incomplete sampling at the scale of this study. In contrast, Winkler extraction reached an intermediate richness (23 species), while baiting detected substantially fewer species (16 species). Mean species richness per site followed the same pattern, with hand collection and pitfall trapping recording the highest pre-site richness and baiting the lowest (**Fig. 9C**).

Consistent with these patterns, sampling methods differed in their marginal contributions to the pooled species inventory when overlap among methods was accounted for using Shapley values (**Fig. 9B**). Hand sampling had the highest mean Shapley contribution (mean = 13.6 species; 95% bootstrap CI = 9.58–17.0; B=999). Pitfall traps were similar to hand collection (mean = 12.4; 95% CI = 8.5–15.5) and did not differ from Hand in bootstrap comparisons (mean difference = −1.25 species; 95% CI = −7.51 to 4.50; p = 0.7830). In contrast, Winkler sampling contributed fewer additional species than hand collection (mean = 8.22; 95% CI = 4.58–11.3; mean difference = −5.40; 95% CI = −9.84 to −0.667; p = 0.026), and baiting contributed substantially fewer than hand collection (mean = 3.49; 95% CI = 2.33–4.33; mean difference = −10.1; 95% CI = −13.2 to −6.83; p < 0.001).

To assess whether methods captured distinct components of the community, we compared the number of species detected exclusively by each method (**Fig. 9D**). Pairwise Wilcoxon rank-sum tests on the number of method-unique species per site (BH-adjusted; N = 21 sites per method) showed significant differences between baiting and pitfall (W = 36, p < 0.0001) and between baiting and hand collection (W = 33, p < 0.0001). Winkler also differed significantly from pitfall (W = 76, p = 0.0004)

and from hand sampling (W = 77, p = 0.0004). In contrast, baiting and Winkler did not differ (W = 157, p = 0.0820), and pitfall and hand sampling did not differ significantly (W = 236, p = 0.6920). All p-values were BH-adjusted to account for multiple comparisons.

Variation in urban sampling design across studies

To evaluate how urban ant studies vary in sampling design and geographic scope, we summarized patterns across 83 studies spanning 25 years (2001–2025). Studies were conducted across all six continents where ants occur (**Fig. 10A**). The number of studies increased steadily over time, particularly after 2010, with approximately 6–7 studies published per year in recent decades (**Fig. 10B**). Cities spanned a wide range of populations, ranging from 2,756 to 13,010,112, with a median city size of 272,773 (**Fig. 10C**).

Studies differed in how they characterized urban environments. Defining a mosaic-based design as one in which two or more discrete habitat types were sampled, 43.5% of studies (N = 37) used a mosaic-based approach, while 56.5% (N = 48) sampled only a single habitat type; only 5% (N = 4) explicitly sampled along continuous urban gradients (**Fig. 11B**). Across all studies, remnant habitats (e.g. forest fragments, grasslands) were the most frequently sampled habitat type (51%), followed by manicured parks (45%), streetscapes (28%), residential (19%), agricultural land (14%), community gardens (9%), and urban gradients (5%; **Fig. 11A**). Habitats defined as 'other' included green rooftops, bromeliad flowers, and one study that did not define habitat type. Among studies that sampled multiple habitats

(N = 37), most sampled only two (48.6%, N = 18), with the remaining 51.4% (N = 19) sampling three or more.

Sampling approaches also varied widely across studies. Passive trapping methods, pitfall traps (64%, *N* = 54) and baits (44%, *N* = 37), were the most commonly employed across the literature, whereas more labour-intensive or specialised techniques such as leaf-litter extraction (11%, *N* = 9), beat sampling (5%, *N* = 4), and pan traps (2%, *N* = 2) were used by only a small minority of studies (**Fig. 11C**). Pitfall traps were the dominant collection method, used in 64% of studies, followed by baits (44%) and hand collection (33%), leaf litter extraction (11%), beat sampling (5%), and other methods (2%; **Fig. 11D**). 'Other' methods used were pan traps (bees were the primary focus organism) and targeted collection of entire nest sites.

Reporting of sampling permissions was inconsistent across studies. Only 38% of studies (*N* = 32) explicitly reported obtaining permits or permission to sample, while the remaining 62% (*N* = 53) made no statement regarding permission (**Fig. 12**).

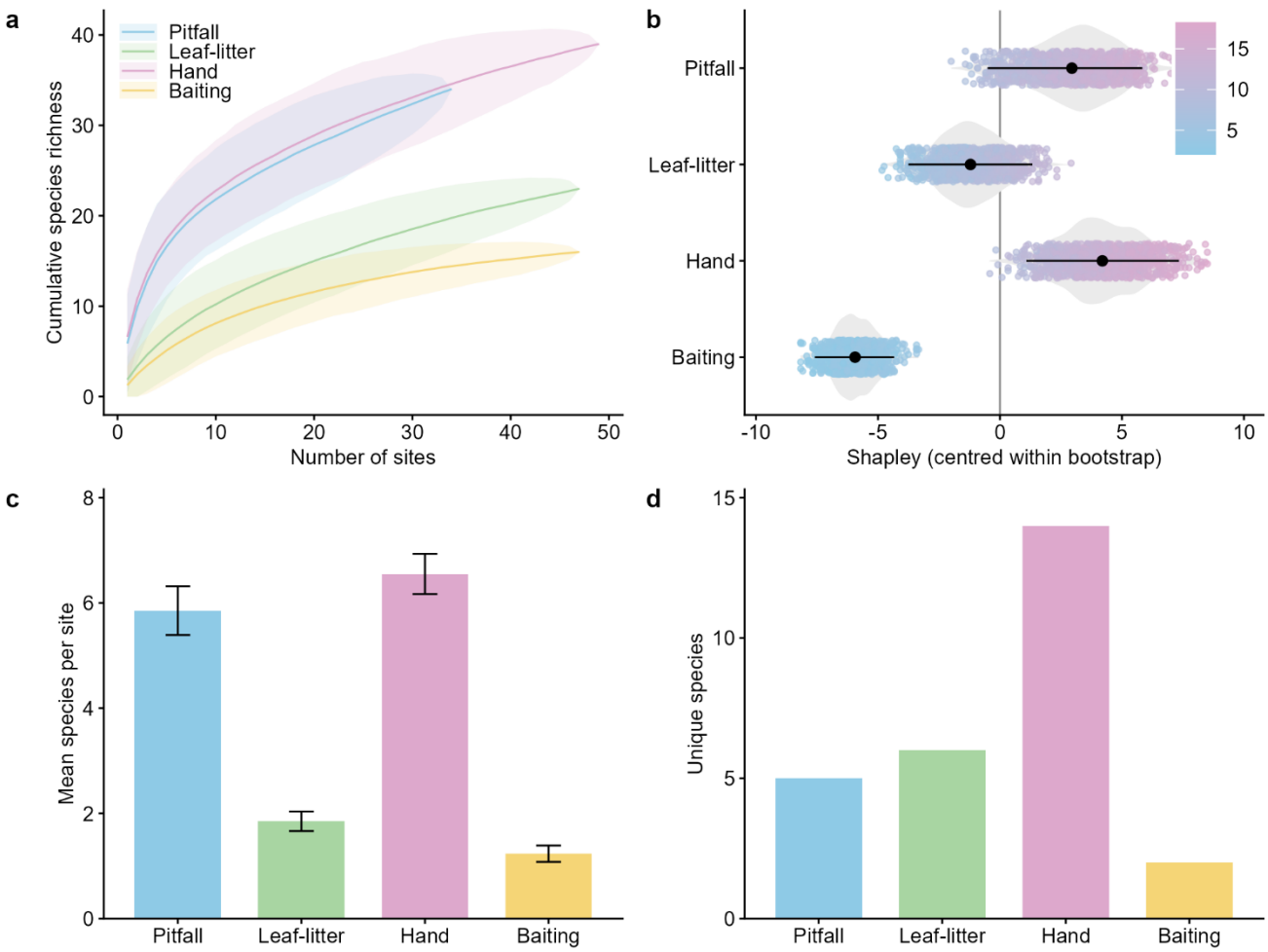

Fig. 9 Comparison of four sampling methods (baiting, Winkler extraction, pitfall trapping, hand collection) for ant diversity across 60 sites in Atlanta, Georgia, USA. (a) Species accumulation curves with 95% confidence intervals based on 1,000 random permutations of site order. (b) Shapley value analysis showing each method's marginal contribution to total species richness (centred within 999 bootstrap replicates); points are coloured by raw Shapley value, horizontal bars indicate 95% bootstrap confidence intervals, and black dots mark the mean (c) Mean species richness per site (± SE) for each method. (d) Number of unique species detected exclusively by each method across all sites.

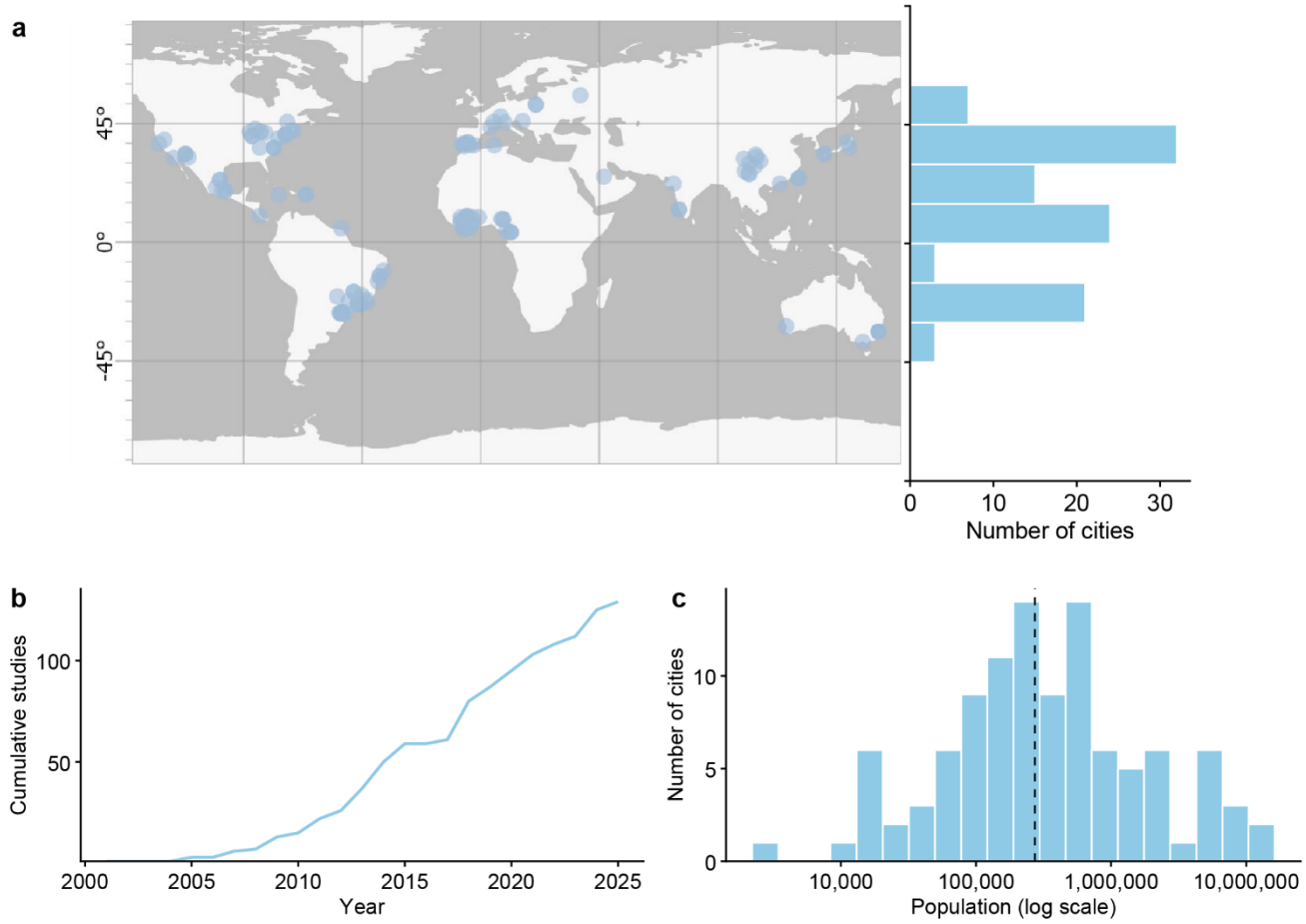

Fig. 10 Geographic and temporal overview of urban ant sampling studies (*N* = 83, 2001–2025). (a) World map showing the locations of study cities, with a horizontal histogram summarising the number of cities sampled per latitudinal band (grouped per 15°). (b) Cumulative number of published studies over time. (c) Distribution of study city populations on a logarithmic scale, with the dashed line indicating the median population (272,773).

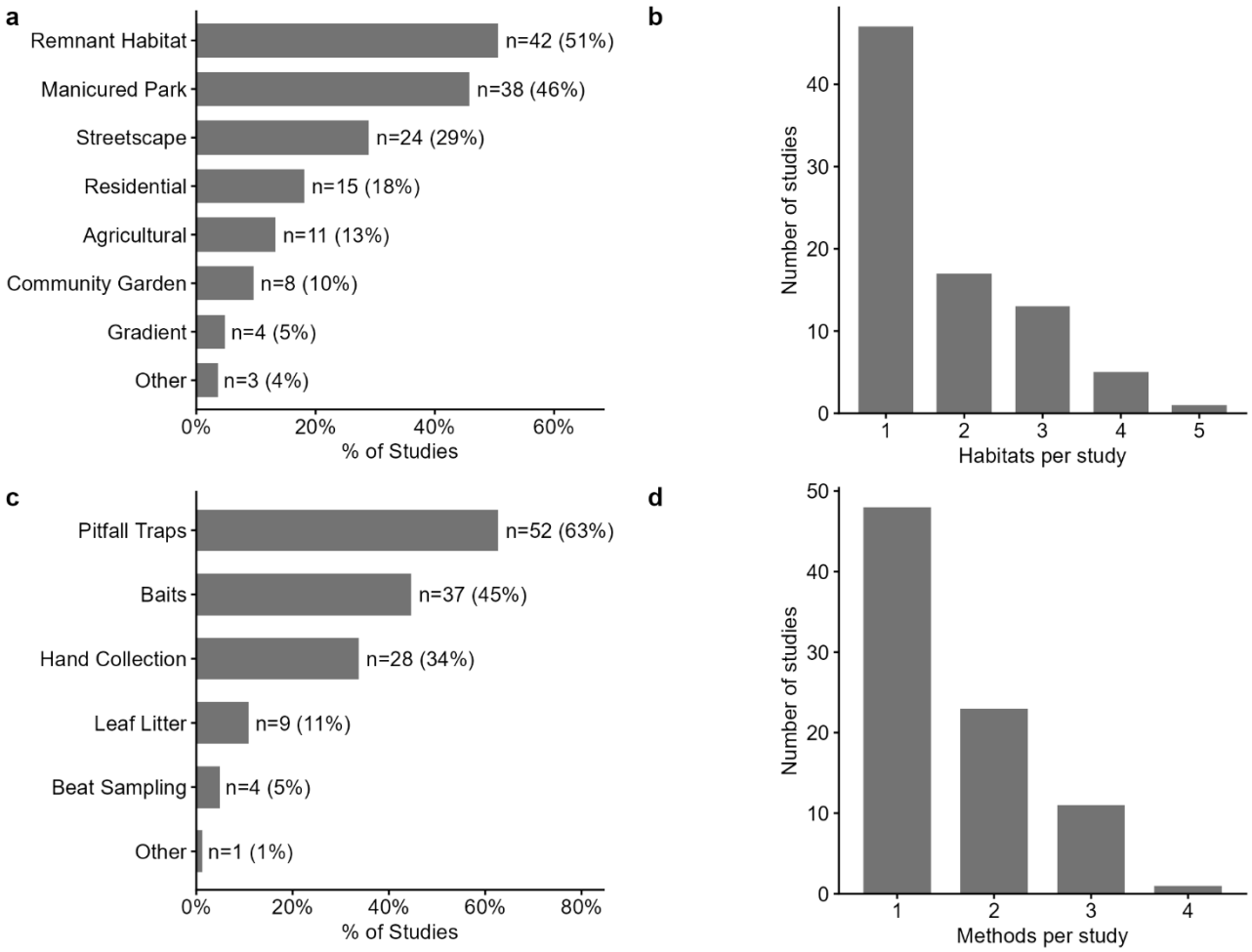

Fig. 11 Summary of habitat types and sampling methods reported across 83 urban ant studies (2001–2025)**.** (a) Proportion of studies conducted in each habitat type. (b) Distribution of the number of habitat types surveyed per study. (c) Proportion of studies employing each sampling method. (d) Distribution of the number of sampling methods used per study.

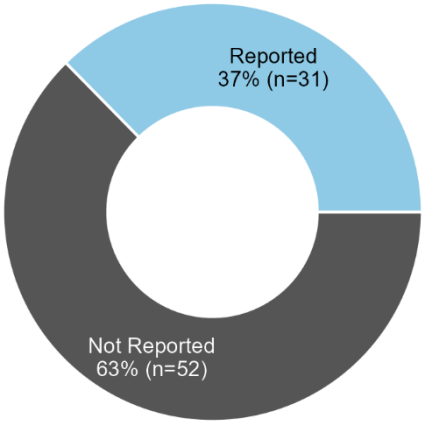

Fig. 12 Proportion of urban ant sampling studies (N = 83) that reported obtaining permits or permission to conduct fieldwork. The majority of studies (62%, n = 53) did not report obtaining any permits or permission.

Discussion

Ants have emerged as a model system for understanding how urbanisation shapes biodiversity (Buczkowski and Richmond 2012; Savage et al. 2015; Penick and Edenborough 2026 Apr 15), yet the methods used to sample them have not kept pace with the unique constraints of urban environments. Standardized approaches developed for natural habitats, such as the ALL protocol, assume conditions that are often absent in cities (Bestelmeyer et al. 2000 Jan 1; Fisher et al. 2000). The challenge of adapting biodiversity protocols to novel urban conditions is shared across taxa and reflects a wider methodological gap in urban ecology (McKinney 2008; Aronson et al. 2014). Consistent with this mismatch, we found that leaf-litter extraction, a cornerstone of forest sampling, captured significantly fewer species than either pitfall trapping or hand collection in urban habitats. Instead, hand collection performed best, despite being used relatively infrequently in urban studies. Across 83 studies, pitfall traps and baiting were the most commonly used methods (Lessard and Buddle 2005; Dattilo et al. 2011; Salyer et al. 2014; Curry et al. 2024), even though baiting performed poorly in our comparisons. Moreover, most studies relied on a single sampling method and a narrow set of habitat types, whereas our results show that combining methods is essential for capturing the full diversity of urban ant communities. These findings highlight a disconnect between current practice and effective sampling design, while also pointing to a clear path forward.

Sampling method performance in urban environments

Across Atlanta, hand collection and pitfall trapping were the most effective methods for sampling ant diversity, whereas leaf-litter extraction and baiting

contributed substantially fewer additional species. These results are consistent with predictions by Gotelli et al. (2011), who suggested that hand collection should perform particularly well in disturbed, rocky, or leaf-litter-poor habitats. They also align with King & Porter (2005), who showed that the relative performance of methods in the ALL protocol depends strongly on habitat structure, with leaf-litter sampling performing best where leaf litter is abundant and litter-specialist faunas are well developed. In our study, leaf litter was often sparse and patchy, and in some cases had to be collected from reduced or aggregated areas, likely contributing to the lower yield. We only collected one leaf litter sample per site compared to five pitfall samples, so there could be a marginal benefit to expanding leaf litter collections, especially in habitats where leaf litter is abundant.

Hand collection emerged as the most consistently effective approach, both in total richness and marginal contribution, highlighting its value as a primary method for urban surveys. Intensive hand sampling has previously been used to recover unexpectedly high ant diversity from heavily disturbed urban habitats, including Manhattan street medians and New York City greenspaces (Pećarević et al. 2010; Savage et al. 2015). Unlike passive methods, hand collection allows researchers to target a wide range of microhabitats, including vegetation, artificial structures, and small patches of organic material that are common in cities but poorly sampled by other techniques. Hand collection is also accessible to non-experts, who can be trained to produce samples comparable to those of expert myrmecologists (Lucky et al. 2014). Therefore, if only a single method can be used, our results suggest that standardized, timed hand sampling provides the most complete representation of urban ant communities.

Baiting, in contrast, captured relatively few additional species and contributed the least to overall richness despite being the second most common study method. This is consistent with the well-established selectivity of baiting toward dominant, rapidly recruiting species, which can monopolize resources and exclude other taxa (Delabie et al. 2021). As a result, baiting tends to under-sample cryptic, slow-moving, or subordinate species and provides a shallow view of community composition. Nonetheless, baiting remains useful for specific applications, such as assessing dominance hierarchies, competitive interactions, or the activity of behaviourally dominant species. In this context, baiting should be viewed not as a general survey tool, but as a targeted method suited to particular ecological questions rather than comprehensive biodiversity assessments.

Overall, our results support a mixed method approach, as no single method captured all species, and complementarity among methods remained high even after accounting for overlap using Shapley values. This is consistent with findings from intensively sampled tropical and temperate sites, where multi-method inventories show that no single technique approaches asymptotic ant richness (Longino et al. 2002; Gotelli et al. 2011 May 1). Each method detected species not recorded by the others, reinforcing that multi-method approaches are essential for fully characterizing urban assemblages. At a minimum, we recommend combining hand collection with pitfall trapping, which together captured the majority of species across sites. Additional methods are useful for gap filling, such as leaf litter collection when leaf litter is present, as this remains the best method to capture cryptic ant species that are unlikely to be found during routine hand collection.

Mismatch between current practice and effective sampling

Despite clear differences in method performance, our review shows that urban ant studies have not converged on a consistent or effective sampling strategy. Pitfall traps and baiting dominate the literature, while hand collection—our most effective method—is used relatively infrequently. This mismatch likely reflects a combination of tradition, logistical convenience, and the legacy of protocols developed for natural habitats. Pitfall traps are relatively easy to deploy and standardize, and baiting provides rapid assessments of conspicuous species, but both methods alone provide an incomplete view of community composition.

More broadly, most studies relied on only one or two sampling methods, limiting their ability to detect complementary subsets of the community. As a result, differences among studies may reflect methodological variation as much as ecological differences, complicating efforts to synthesize patterns across cities or regions. This lack of standardization represents a major barrier to developing a general understanding of urban biodiversity, particularly for taxa like ants that are highly sensitive to local environmental conditions.

Alternative sampling approaches and their trade-offs

Not all studies aim for standardized comparisons, and alternative sampling approaches can be highly effective for documenting maximum species richness. Intensive, multi-method surveys—such as those used to document the exceptionally high diversity of ants in Macao (Brassard et al. 2021)—demonstrate that combining diverse techniques can reveal far more species than any standardized protocol alone. For example, a study of a 1.3 km² university campus in Raleigh, North

Carolina, combined hand collection, baiting, pitfall traps, and Winkler extractors to record 89 ant species (representing 79% of the regional species pool), whereas a contemporaneous standardised survey of the same city recovered only 53% of those species (Menke et al. 2011; Guenard et al. 2015). Similarly, approaches that emphasize extended hand sampling within defined areas or targeted searches across microhabitats can overcome some of the limitations of pitfall trapping and litter extraction in highly urbanised settings.

However, these approaches come with trade-offs. Methods optimized for maximizing species detection often lack standardization, making comparisons across studies difficult. Conversely, highly standardized methods may sacrifice completeness for comparability. Our results suggest that urban ant ecology would benefit from approaches that explicitly balance these competing goals, combining standardized core methods with flexible, habitat-specific additions.

Spatial frameworks: gradients vs habitat mosaics

In line with expectations for organisms operating at fine spatial scales, mosaic-based study designs (sampling two or more discrete habitat types) were used roughly nine times as often as designs based on continuous urban gradients (Tommasi et al. 2004; Savage et al. 2015; Boeing et al. 2023). This likely reflects the biology of ants, whose colonies experience the environment at the scale of meters rather than kilometres. As a result, adjacent habitats, such as a park lawn and a nearby sidewalk, can support fundamentally different communities despite being embedded within the same broader urban matrix. This preference for mosaics over gradients for study designs is consistent with a broader critique that the urban-rural

gradient framework (McDonnell and Pickett 1990) oversimplifies the heterogeneity of the habitats that the organisms experience within cities (Cadenasso et al. 2007; McDonnell and Hahs 2008).

While mosaic-based approaches are well suited to capturing this heterogeneity, the lack of consistent definitions for habitat categories limits comparability across studies. Terms such as “park,” “green space,” or “remnant habitat” are often used inconsistently, obscuring meaningful ecological differences. Developing shared definitions based on measurable attributes—such as disturbance intensity, vegetation structure, and patch size—will be essential for enabling cross-city comparisons and for integrating mosaic and gradient approaches into a unified framework. We propose a framework in which discrete habitat categories are defined by a combination of patch size and land-use type: streetscapes as managed greenspaces ≤ 5,000 m², manicured parks as managed greenspaces > 5,000 m², and remnant habitat as patches of intact natural vegetation. These categorical designations should be paired with quantitative environmental metrics — such as impervious-surface cover measured within a fixed-radius buffer of each sampling site (e.g., 100 m), vegetation structure, and ground-cover composition — so that studies retain the comparability of categorical habitat designations while simultaneously capturing the continuous environmental gradients that drive ant community variation and enable quantitative cross-city comparisons.

Logistical constraints

Urban sampling is shaped not only by ecological considerations but also by the regulatory and social contexts in which fieldwork takes place. Our review found

that relatively few studies explicitly reported obtaining permits or permissions, despite the fact that urban sampling often requires coordination with local governments, park managers, or private landowners. In our experience, permitting processes vary widely, from formal applications and multi-step approvals to informal arrangements, such as a single email granting access. In some cases, clear permitting pathways are not available at all. The under-reporting of these constraints reflects a more general pattern in urban ecology, in which the practical and regulatory aspects of fieldwork are rarely documented in the published literature (Magle et al. 2012).

Public perception also influences which methods can be used. Techniques that involve leaving equipment in place, particularly pitfall traps, can draw attention or concern from passersby. We occasionally encountered negative reactions to traps or baits in visible locations, especially when using brightly coloured preservatives, such as fluorescent pink propylene glycol. To minimize disturbance and reduce visibility, we instead used soapy water and placed traps more discreetly. These adjustments illustrate how methodological choices in urban ecology are often shaped as much by human factors as by ecological ones.

Urban fieldwork also introduces safety considerations that are largely absent from studies in more remote or protected habitats. Sampling frequently occurs near roads, sidewalks, and other high-traffic areas, increasing risks associated with vehicle traffic and limited visibility. These hazards parallel those documented across other field-based sciences; in geological fieldwork, vehicles and environmental exposure have been identified as the leading causes of fieldwork-related fatalities (Cantine 2021). Interactions with members of the public, while often positive, can

also introduce unpredictability, particularly when researchers are working with unfamiliar equipment in shared spaces. To mitigate these risks, we adopt practices from applied field sciences, including wearing high-visibility clothing (e.g., safety vests identifying us as researchers or entomologists), clearly labelling equipment, and selecting sampling locations with both ecological and safety considerations in mind (Dyson et al. 2019). As urban ecology continues to expand, incorporating standardized safety practices and more consistent reporting of logistical constraints will be important for ensuring both researcher safety and the reproducibility of urban field studies. More broadly, the systematic under-reporting of logistical, regulatory, and ethical aspects of fieldwork has been highlighted as a barrier to reproducibility and best practice across the biological sciences (Costello et al. 2016).

Toward a standardized protocol for urban ant sampling

Taken together, our results suggest that a standardized protocol for sampling urban ant communities should prioritize a combination of complementary methods while remaining flexible to the constraints of urban environments. Coordinated frameworks of this kind have already proven valuable in other urban taxa for detecting general biodiversity patterns under urbanisation (Aronson et al. 2014). Based on our findings, we recommend a mixed-method approach centred on timed hand collection and pitfall trapping, which together captured the majority of species across sites. Leaf-litter extraction should be used selectively in habitats where sufficient litter is present, rather than applied uniformly as in forest-based protocols. In contrast, baiting appears to contribute relatively little to overall species richness

and may be best reserved for studies focused on dominant or behaviourally active species rather than community-wide inventories.

In addition to method selection, we advocate for a habitat mosaic framework that samples discrete habitat types within cities using consistent and clearly defined categories based on disturbance level and patch size. These categorical approaches should be complemented with quantitative environmental measurements—such as impervious surface cover, ground cover composition, and vegetation structure—to facilitate comparisons across studies and cities.

Urban ant research has expanded rapidly over the past two decades, reflecting growing recognition of cities as important systems for studying biodiversity under global change. Our review revealed a steady increase in studies since 2010, with research now spanning all six continents where ants occur. This continued growth presents an opportunity to improve both methodological consistency and geographic coverage across urban ant ecology. Studying ants in urban systems can provide important insights across both basic and applied ecology. Because many ant invasions originate in urban habitats, cities offer opportunities to detect new invasions at early stages of establishment (Yang and Gratton 2014; Kennett et al. 2024). Urban systems also facilitate studies of functional ecology, including dietary (Penick et al. 2015), physiological (Diamond et al. 2017; Penick et al. 2017; Nascimento et al. 2022), and community interactions (Alder and Silverman 2005; Gaber et al. 2024), while allowing researchers to examine the roles ants play in ecosystem services, such as refuse removal and competition with other urban pests (Youngsteadt et al. 2015; Perfecto and Philpott 2023). With increasing attention to cities as epicentres of ecological change (Vörösmarty et al. 2025), urban ant

communities are well positioned to serve as a globally relevant model system for studying how biodiversity responds to rapid environmental transformation.

References

Alder P, Silverman J. 2005. Effects of interspecific competition between two urban ant species, *Linepithema humile* and *Monomorium minimum*, on toxic bait performance. Journal of economic entomology. 98(2):493–501.

Amaral KD, Marinho CGS, Della Lucia TMC. 2024. Non-target ants and bioinsecticides: A short review. Current Opinion in Environmental Science & Health. 42:100586. doi:10.1016/j.coesh.2024.100586.

Andersen AN. 1997. Using ants as bioindicators: multiscale issues in ant community ecology. Conservation Ecology. 1(1). [accessed 2025 Jan 15]. https://www.jstor.org/stable/26271646.

Andersen AN, Hoffmann BD, Müller WJ, Griffiths AD. 2002. Using ants as bioindicators in land management: simplifying assessment of ant community responses. Journal of Applied Ecology. 39(1):8–17. doi:10.1046/j.1365-2664.2002.00704.x.

Andersen AN, Majer JD. 2004. Ants show the way Down Under: invertebrates as bioindicators in land management. Frontiers in Ecology and the Environment. 2(6):291–298. doi:10.1890/1540-9295(2004)002[0292:ASTWDU]2.0.CO;2.

Angilletta Jr MJ, Wilson RS, Niehaus AC, Sears MW, Navas CA, Ribeiro PL. 2007. Urban Physiology: City Ants Possess High Heat Tolerance. PLOS ONE. 2(2):e258. doi:10.1371/journal.pone.0000258.

Angulo E, Hoffmann BD, Ballesteros-Mejia L, Taheri A, Balzani P, Bang A, Renault D, Cordonnier M, Bellard C, Diagne C, et al. 2022. Economic costs of invasive alien ants worldwide. Biol Invasions. 24(7):2041–2060. doi:10.1007/s10530-022-02791-w.

Aronson MFJ, La Sorte FA, Nilon CH, Katti M, Goddard MA, Lepczyk CA, Warren PS, Williams NSG, Cilliers S, Clarkson B, et al. 2014. A global analysis of the impacts of urbanization on bird and plant diversity reveals key anthropogenic drivers. Proceedings of the Royal Society B: Biological Sciences. 281(1780):20133330. doi:10.1098/rspb.2013.3330.

Baldock KCR, Goddard MA, Hicks DM, Kunin WE, Mitschunas N, Osgathorpe LM, Potts SG, Robertson KM, Scott AV, Stone GN, et al. 2015. Where is the UK's pollinator biodiversity? The importance of urban areas for flower-visiting insects. Proceedings of the Royal Society B: Biological Sciences. 282(1803):20142849. doi:10.1098/rspb.2014.2849.

Bestelmeyer B, Agosti D, Alonso L, Brandao CR, Brown WLJ, Delabie J, Silvestre R. 2000 Jan 1. Field techniques for the study of ground-dwelling ants: an overview, description and evaluation. Ants: standard methods for measuring and monitoring biodiversity.

Boeing J, Cuper K, Menke SB. 2023. Ant species richness in the urban mosaic: size is more important than location. Urban Ecosyst. 26(2):605–615. doi:10.1007/s11252-022-01308-6.

Brassard F, Leong C-M, Chan H-H, Guénard B. 2021. High diversity in urban areas: how comprehensive sampling reveals high ant species richness within one of the most urbanized regions of the world. Diversity. 13(8):358. doi:10.3390/d13080358.

Buczkowski G. 2016. The Trojan horse approach for managing invasive ants: a study with Asian needle ants, *Pachycondyla chinensis*. Biological Invasions. 18(2):507–515. doi:10.1007/s10530-015-1023-z.

Buczkowski G. 2023. Termite cuticular extracts improve acceptance of bait for controlling invasive Asian needle ants, *Brachyponera chinensis*. Pest Management Science. 79(10):4004–4010. doi:10.1002/ps.7601.

Buczkowski G, Richmond DS. 2012. The effect of urbanization on ant abundance and diversity: a temporal examination of factors affecting biodiversity. PLOS ONE. 7(8):e41729. doi:10.1371/journal.pone.0041729.

Cabon V, Quénol H, Dubreuil V, Ridel A, Bergerot B. 2024. Urban heat island and reduced habitat complexity explain spider community composition by excluding large and heat-sensitive species. Land. 13(1):83. doi:10.3390/land13010083.

Cadenasso ML, Pickett STA, Schwarz K. 2007. Spatial heterogeneity in urban ecosystems: reconceptualizing land cover and a framework for classification. Frontiers in Ecology and the Environment. 5(2):80–88. doi:10.1890/1540-9295(2007)5[80:SHIUER]2.0.CO;2.

Campbell JW, Grodsky SM, Halbritter DA, Vigueira PA, Vigueira CC, Keller O, Greenberg CH. 2019. Asian needle ant (*Brachyponera chinensis*) and woodland ant responses to repeated applications of fuel reduction methods. Ecosphere. 10(1):e02547. doi:10.1002/ecs2.2547.

Cantine MD. 2021. Dying to know: Death during geological fieldwork. The Sedimentary Record. 19(3).

Carpintero S, Reyes-López J. 2008. The role of competitive dominance in the invasive ability of the Argentine ant (*Linepithema humile*). Biol Invasions. 10(1):25–35. doi:10.1007/s10530-007-9103-3.

Corsetti KA. 2024. Bait acceptance and seasonal activity of the Asian needle ant, *Brachyponera* (=*Pachycondyla*) *chinensis* (Emery), an emerging medically important species, in central Georgia [M.S.]. [United States -- Georgia]: University of Georgia. [accessed 2026 Apr 21]. https://www.proquest.com/docview/3152412420/abstract/AD51C0B69723470CPQ/1.

Costello MJ, Beard KH, Corlett RT, Cumming GS, Devictor V, Loyola R, Maas B, Miller-Rushing AJ, Pakeman R, Primack RB. 2016. Field work ethics in biological research. Biological Conservation. 203(8):268–271.

Curry AKL, Nelsen JA, Stevens II DR, Mathis KA. 2024. The effects of land use change on ant communities in New England. Insect Conservation and Diversity. 17(2):189–195. doi:10.1111/icad.12696.

Dattilo W, Sibinel N [UNESP, de Faria Falcao JC, Nunes RV. 2011. Ant fauna in a urban remnant of atlantic forest in the municipality of marilia, state of sao paulo, brazil. Biosci J. 27(3):494–504.

Delsinne TD, Arias-Penna TM. 2012. Influence of leaf litter moisture on the efficiency of the Winkler method for extracting ants. J Insect Sci. 12(1):57. doi:10.1673/031.012.5701.

Demartín RP, Ghirardi R, López JA. 2024. Amphibian diversity across an urban gradient in southern South America. Front Ecol Evol. 12. doi:10.3389/fevo.2024.1461147. [accessed 2026 May 12]. https://www.frontiersin.org/journals/ecology-and-evolution/articles/10.3389/fevo.2024.1461147/full.

Deutsch CA, Tewksbury JJ, Huey RB, Sheldon KS, Ghalambor CK, Haak DC, Martin PR. 2008. Impacts of climate warming on terrestrial ectotherms across latitude. Proceedings of the National Academy of Sciences. 105(18):6668–6672. doi:10.1073/pnas.0709472105.

Dewitz J. 2023. National Land Cover Database (NLCD) 2021 Products. doi:10.5066/P9JZ7AO3.

Diamond SE, Bellino G, Deme GG. 2023. Urban insect bioarks of the 21st century. Current Opinion in Insect Science. 57:101028. doi:10.1016/j.cois.2023.101028.

Diamond SE, Chick L, Perez ABE, Strickler SA, Martin RA. 2017. Rapid evolution of ant thermal tolerance across an urban-rural temperature cline. Biological Journal of the Linnean Society. 121(2):248–257.

Diamond SE, Nichols LM, McCoy N, Hirsch C, Pelini SL, Sanders NJ, Ellison AM, Gotelli NJ, Dunn RR. 2012. A physiological trait-based approach to predicting the responses of species to experimental climate warming. Ecology. 93(11):2305–2312. doi:10.1890/11-2296.1.

Dietzel A, Moretti M, Perrelet K, Cook LM. 2025. Urban heat exacerbates climatic risks to urban biodiversity. npj Urban Sustain. 6(1):4. doi:10.1038/s42949-025-00309-6.

Dimarco RD, Masciocchi M, Corley JC. 2017. Managing nuisance social insects in urban environments: an overview. International Journal of Pest Management. 63(3):251–265. doi:10.1080/09670874.2017.1329566.

Dyson K, Ziter C, Fuentes TL, Patterson MS. 2019. Conducting urban ecology research on private property: advice for new urban ecologists. J Urban Ecol. 5(1):juz001. doi:10.1093/jue/juz001.

Edenborough L, Hellenbrand JP, Kennett S, Rojas SC, Penick C. 2026. Invasive species undermine the bioark hypothesis in a low-latitude urban biodiversity hotspot. :2026.05.24.727550. doi:10.64898/2026.05.24.727550. [accessed 2026 May 27]. https://www.biorxiv.org/content/10.64898/2026.05.24.727550v1.

Eydoux L, Jay Robert P, Kaufmann B, Hedde M, Vergnes A. 2024. Soil unsealing in Mediterranean schoolyards: what factors drive ant communities? Urban Ecosyst. 27(6):2543–2555. doi:10.1007/s11252-024-01608-z.

Fenoglio MS, Rossetti MR, Videla M. 2020. Negative effects of urbanization on terrestrial arthropod communities: A meta-analysis. Global Ecology and Biogeography. 29(8):1412–1429. doi:10.1111/geb.13107.

Fisher BL, Malsch AKF, Gadagkar R, Delabie JHC, Heraldo L. Vasconcelos, Jonathan D. Majer. 2000. Applying the ALL protocol. Ants: standard methods for measuring and monitoring biodiversity. 9:280.

Francis RA, Chadwick MA. 2015. Urban invasions: non-native and invasive species in cities. Geography. 100(3):144–151. doi:10.1080/00167487.2015.12093969.

Gaber H, Ruland F, Jeschke JM, Bernard-Verdier M. 2024. Behavioural changes in the city: The common black garden ant defends aphids more aggressively in urban environments. Ecology and Evolution. 14(7):e11639. doi:10.1002/ece3.11639.

Gerhardt A. 2002. Bioindicator species and their use in biomonitoring. Environmental monitoring. 1:77–123.

Gochnour BM, Suiter DR, Booher D. 2019. Ant (hymenoptera: formicidae) fauna of the marine port of Savannah, Garden City, Georgia (USA). Journal of Entomological Science. 54(4):417–429. doi:10.18474/JES18-132.

Gotelli NJ, Ellison AM, Dunn RR, Sanders NJ. 2011 May 1. Counting ants (Hymenoptera: Formicidae): Biodiversity sampling and statistical analysis for myrmecologists. College of Arts and Sciences Faculty Publications. https://scholarworks.uvm.edu/casfac/95.

Griffith J, Sunday JM, Hargreaves AL. 2025. Urbanization weakens the latitudinal diversity gradient in birds. :2025.08.04.668553. doi:10.1101/2025.08.04.668553. [accessed 2026 Feb 6]. https://www.biorxiv.org/content/10.1101/2025.08.04.668553v1.

Gruber MAM, Janssen-May S, Santoro D, Cooling M, Wylie R. 2021. Predicting socio-economic and biodiversity impacts of invasive species: Red Imported Fire Ant in the developing western Pacific. Ecological Management & Restoration. 22(1):89–99. doi:10.1111/emr.12457.

Guenard B, Cardinal-De Casas A, Dunn RR. 2015. High diversity in an urban habitat: are some animal assemblages resilient to long-term anthropogenic change? Urban Ecosyst. 18(2):449–463. doi:10.1007/s11252-014-0406-8.

Guénard B, Dunn RR. 2010. A new (old), invasive ant in the hardwood forests of eastern north america and its potentially widespread impacts. PLOS ONE. 5(7):e11614. doi:10.1371/journal.pone.0011614.

Guénard B, Wetterer JK, MacGown JA. 2018. Global and temporal spread of a taxonomically challenging invasive ant, *Brachyponera chinensis* (Hymenoptera: Formicidae). flen. 101(4):649–656. doi:10.1653/024.101.0402.

Guo Q, Cade BS, Dawson W, Essl F, Kreft H, Pergl J, van Kleunen M, Weigelt P, Winter M, Pyšek P. 2021. Latitudinal patterns of alien plant invasions. Journal of Biogeography. 48(2):253–262. doi:10.1111/jbi.13943.

Guzman-Hernandez I. 2019. Oil preference in ants and arthropod diversity in urban environments.

Hoffmann BD, Pettit M, Antonio J, Chassain J, Ferrieu E, Gutierrez A, Holley P, Lavóréi T, Moreno J, Querat S. 2023. Efficacy, non-target impacts, and other considerations of unregistered fipronil-laced baits being used in multiple invasive ant eradication programs. Management of Biological Invasions. 14(3):437–457.

Holway DA. 2005. Edge effects of an invasive species across a natural ecological boundary. Biological Conservation. 121(4):561–567. doi:https://doi.org/10.1016/j.biocon.2004.06.005.

Holway DA, Lach L, Suarez AV, Tsutsui ND, Case TJ. 2002. The causes and consequences of ant invasions. Annual Review of Ecology, Evolution, and Systematics. 33(Volume 33, 2002):181–233. doi:10.1146/annurev.ecolsys.33.010802.150444.

Kanes D, Malagon D, Camper B, Hewitt A, Dunn S, Purcell E, Bewick S. 2025. Species distribution models reveal varying degrees of refugia from the invasive Asian needle ant for native ants versus ant-plant seed dispersal mutualisms. Ecol Evol. 15(1):e70750. doi:10.1002/ece3.70750.

Kassambara A. 2019. rstatix: Pipe-friendly framework for basic statistical tests. doi:10.32614/cran.package.rstatix.

Kennett SM, Seifert B, Dunn RR, Pierson TW, Penick CA. 2024. The ManhattAnt: identification, distribution, and colony structure of a new pest in New York City, *Lasius emarginatus*. Biological Invasions. 26(8):2759–2772.

King JR, Andersen AN, Cutter AD. 1998. Ants as bioindicators of habitat disturbance: validation of the functional group model for Australia's humid tropics. Biodiversity & Conservation. 7(12):1627–1638.

King JR, Porter SD. 2005. Evaluation of sampling methods and species richness estimators for ants in upland ecosystems in Florida. Environ Entomol. 34(6):1566–1578. doi:10.1603/0046-225X-34.6.1566.

Lassau SA, Hochuli DF. 2004. Effects of habitat complexity on ant assemblages. Ecography. 27(2):157–164.

Lessard JP, Buddle CM. 2005. The effects of urbanization on ant assemblages (Hymenoptera: Formicidae) associated with the Molson Nature Reserve, Quebec. Can Entomol. 137(2):215–225. doi:10.4039/N04-055.

Leveau LM, Leveau CM, Villegas M, Cursach JA, Suazo CG. 2017. Bird communities along urbanization gradients: a comparative analysis among three neotropical cities. Ornitología Neotropical. 28:77–87. doi:10.58843/ornneo.v28i0.125.

Lin W-J, Hsu P-W, Vargo EL, Yang C-CS. 2025. Microbial, genetic, and urban drivers of ant invasions. Current Opinion in Insect Science. 72:101417. doi:10.1016/j.cois.2025.101417.

Longino JT, Coddington J, Colwell RK. 2002. The ant fauna of a tropical rain forest: estimating species richness three different ways. Ecology. 83(3):689–702. doi:10.1890/0012-9658(2002)083[0689:TAFOAT]2.0.CO;2.

Lowe S, Browne M, Boudjelas S, De Poorter M. 2000. 100 of the world's worst invasive alien species: a selection from the global invasive species database. Invasive Species Specialist Group Auckland.

Lucky A, Savage AM, Nichols LM, Castracani C, Shell L, Grasso DA, Mori A, Dunn RR. 2014. Ecologists, educators, and writers collaborate with the public to assess backyard diversity in The School of Ants Project. Ecosphere. 5(7):1–23.

MacGown J. 2024. Ants (Formicidae) of the southeastern United States. [accessed 2025 Apr 2]. https://mississippientomologicalmuseum.org.msstate.edu/Researchtaxapages/Formicidaepages/Identification.Keys.htm.

Magle SB, Hunt VM, Vernon M, Crooks KR. 2012. Urban wildlife research: Past, present, and future. Biological Conservation. 155:23–32. doi:10.1016/j.biocon.2012.06.018.

McDonnell MJ, Hahs AK. 2008. The use of gradient analysis studies in advancing our understanding of the ecology of urbanizing landscapes: current status and future directions. Landscape Ecol. 23(10):1143–1155. doi:10.1007/s10980-008-9253-4.

McDonnell MJ, Pickett STA. 1990. Ecosystem structure and function along urban-rural gradients: an unexploited opportunity for ecology. Ecology. 71(4):1232–1237. doi:10.2307/1938259.

McKinney ML. 2008. Effects of urbanization on species richness: A review of plants and animals. Urban Ecosyst. 11(2):161–176. doi:10.1007/s11252-007-0045-4.

Melles S, Glenn S, Martin K. 2003. Urban bird diversity and landscape complexity: species–environment associations along a multiscale habitat gradient. Conservation Ecology. 7(1). [accessed 2026 May 12]. https://www.jstor.org/stable/26271915.

Menke SB, Guenard B, Sexton JO, Weiser MD, Dunn RR, Silverman J. 2011. Urban areas may serve as habitat and corridors for dry-adapted, heat tolerant species; an example from ants. Urban Ecosyst. 14(2):135–163. doi:10.1007/s11252-010-0150-7.

Mignanelli M. 2021. Percentage Cover. percentagecover.com.

Nascimento G, Câmara T, Arnan X. 2022. Critical thermal limits in ants and their implications under climate change. Biological Reviews. 97(4):1287–1305.

Oksanen J, Simpson GL, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Solymos P, Stevens MHH, Szoecs E, et al. 2025. vegan: Community Ecology Package. [accessed 2025 June 27]. https://cran.r-project.org/web/packages/vegan/index.html.

Pećarević M, Danoff-Burg J, Dunn RR. 2010. Biodiversity on Broadway - enigmatic diversity of the societies of ants (Formicidae) on the streets of New York City. PLOS ONE. 5(10):e13222. doi:10.1371/journal.pone.0013222.

Penick CA. 2021. Urban social insects. In: Encyclopedia of social insects. Springer. p. 983–988.

Penick CA, Diamond SE, Sanders NJ, Dunn RR. 2017. Beyond thermal limits: comprehensive metrics of performance identify key axes of thermal adaptation in ants. Functional Ecology. 31(5):1091–1100.

Penick CA, Edenborough L. 2026 Apr 15. Pathways to city dominance: The urban ant syndrome. Current Opinion in Insect Science.:101529. doi:10.1016/j.cois.2026.101529.

Penick CA, Peagler Z, Buttram L, Dunn RR, Frank SD, Youngsteadt E. 2025. Urban heat and latitude: contrasting effects on ant diversity across cities. Urban Ecosystems. 28(4):139.

Penick CA, Savage AM, Dunn RR. 2015. Stable isotopes reveal links between human food inputs and urban ant diets. Proceedings of the Royal Society B: Biological Sciences. 282(1806):20142608. doi:10.1098/rspb.2014.2608.

Perez A, Chick L, Menke S, Lessard J-P, Sanders N, Del Toro I, Meldgaard NS, Diamond S. 2022. Urbanisation dampens the latitude-diversity cline in ants. Insect Conservation and Diversity. 15(6):763–771. doi:10.1111/icad.12598.

Perfecto I, Philpott SM. 2023. Ants (Hymenoptera: Formicidae) and ecosystem functions and services in urban areas: a reflection on a diverse literature. Myrmecological News. 33. [accessed 2026 Apr 29]. https://www.biotaxa.org/mn/article/view/80927.

Polidori C, Ferrari A, Ronchetti F, Tommasi N, Nalini E. 2023. Warming up through buildings and roads: what we know and should know about the urban heat island effect on bees. Front Bee Sci. 1. doi:10.3389/frbee.2023.1269600. [accessed 2026 Mar 20]. https://www.frontiersin.org/journals/bee-science/articles/10.3389/frbee.2023.1269600/full.

Potgieter LJ, Li D, Baiser B, Kühn I, Aronson MFJ, Carboni M, Celesti-Grapow L, Matos ACL de, Lososová Z, Montaño-Centellas FA, et al. 2024. Cities shape the diversity and spread of nonnative species. Annual Review of Ecology, Evolution, and

Systematics. 55(Volume 55, 2024):157–180. doi:10.1146/annurev-ecolsys-102722-012749.

Queiroz ACM de, Ribas CR, França FM. 2013. Microhabitat characteristics that regulate ant richness patterns: the importance of leaf litter for epigaeic ants. Sociobiology. 60(4):367–373. doi:10.13102/sociobiology.v60i4.367-373.

R Core Team. 2025. R: a language and environment for statistical computing. https://www.R-project.org/.

Rajesh TP, Manoj K, Prashanth Ballullaya U, Shibil VK, Asha G, Varma S, Mohan P, Sinu PA. 2022. Urban tropical forest islets as hotspots of ants in general and invasive ants in particular. Sci Rep. 12(1):12003. doi:10.1038/s41598-022-16243-x.

Riem JG, Blair RB, Pennington DN, Solomon NG. 2012. Estimating mammalian species diversity across an urban gradient. amid. 168(2):315–332. doi:10.1674/0003-0031-168.2.315.

Salyer A, Bennett GW, Buczkowski GA. 2014. Odorous house ants (*Tapinoma sessile*) as back-seat drivers of localized ant decline in urban habitats. PLOS ONE. 9(12):e113878. doi:10.1371/journal.pone.0113878.

Sanllorente O, Blanco-Urdillo E, Sánchez-Tójar A, Diego Ibáñez-Álamo J. 2025. A systematic review and meta-analysis on urban arthropod diversity. Insect Conservation and Diversity. 18(4):447–464. doi:10.1111/icad.12831.

Santos MN. 2016. Research on urban ants: approaches and gaps. Insect Soc. 63(3):359–371. doi:10.1007/s00040-016-0483-1.

Sattler T, Obrist MK, Duelli P, Moretti M. 2011. Urban arthropod communities: Added value or just a blend of surrounding biodiversity? Landscape and Urban Planning. 103(3–4):347. doi:10.1016/J.LANDURBPLAN.2011.08.008.

Savage AM, Hackett B, Guenard B, Youngsteadt EK, Dunn RR. 2015. Fine-scale heterogeneity across Manhattan's urban habitat mosaic is associated with variation in ant composition and richness. Insect Conserv Divers. 8(3):216–228. doi:10.1111/icad.12098.

Senator SA, Rozenberg AG. 2017. Assessment of economic and environmental impact of invasive plant species. Biol Bull Rev. 7(4):273–278. doi:10.1134/S2079086417040089.

Seto KC, Güneralp B, Hutyra LR. 2012. Global forecasts of urban expansion to 2030 and direct impacts on biodiversity and carbon pools. Proceedings of the National Academy of Sciences. 109(40):16083–16088. doi:10.1073/pnas.1211658109.

Silva PSD, Bieber AGD, Corrêa MM, Leal IR. 2011. Do leaf-litter attributes affect the richness of leaf-litter ants? Neotrop entomol. 40:542–547. doi:https://doi.org/10.1590/S1519-566X2011000500004.

Sunday JM, Bates AE, Kearney MR, Colwell RK, Dulvy NK, Longino JT, Huey RB. 2014. Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. Proceedings of the National Academy of Sciences. 111(15):5610–5615.

Terando AJ, Costanza J, Belyea C, Dunn RR, McKerrow A, Collazo JA. 2014. The southern megalopolis: using the past to predict the future of urban sprawl in the southeast U.S. PLOS ONE. 9(7):e102261. doi:10.1371/journal.pone.0102261.

Tommasi D, Miro A, Higo HA, Winston ML. 2004. Bee diversity and abundance in an urban setting. The Canadian Entomologist. 136(6):851–869. doi:10.4039/n04-010.

U.S. Geological Survey. 2021. Landsat Collectoin 2 Level-2 Science Products. Fact Sheet Report No.: 2021–3055.

Vogt JT, Reed JT, Brown RL. 2005. Timing bait applications for control of imported fire ants (Hymenoptera: Formicidae) in Mississippi: Efficacy and effects on non-target ants. International journal of pest management. 51(2):121–130.

Vörösmarty CJ, Trujillo M, Casaccia P, Cak A, Gardner KH, Greenfield DI, Groffman PM, Reinmann A, Solecki W, Serreze M. 2025. A climate BioStress sentinel system: identifying climate impacts from the genome to the urbanized biosphere. Cell Reports Sustainability. 2(11).

Wallace AR. 1878. Tropical nature, and other essays. Macmillan and Company.

Warren RJ, McMillan A, King JR, Chick L, Bradford MA. 2015. Forest invader replaces predation but not dispersal services by a keystone species. Biol Invasions. 17(11):3153–3162. doi:10.1007/s10530-015-0942-z.

Wiezik M, Svitok M, Wieziková A, Dovčiak M. 2015. Identifying shifts in leaf-litter ant assemblages (Hymenoptera: Formicidae) across ecosystem boundaries using multiple sampling methods. PLOS ONE. 10(7):e0134502. doi:10.1371/journal.pone.0134502.

Yang LH, Gratton C. 2014. Insects as drivers of ecosystem processes. Current Opinion in Insect Science. 2:26–32. doi:10.1016/j.cois.2014.06.004.

Youngsteadt E, Ernst AF, Dunn RR, Frank SD. 2017. Responses of arthropod populations to warming depend on latitude: evidence from urban heat islands. Global Change Biology. 23(4):1436–1447. doi:10.1111/gcb.13550.

Youngsteadt E, Henderson RC, Savage AM, Ernst AF, Dunn RR, Frank SD. 2015. Habitat and species identity, not diversity, predict the extent of refuse consumption by urban arthropods. Global change biology. 21(3):1103–1115.