Received: 22 May 2024 | Accepted: 16 October 2024

DOI: 10.1111/icad.12788

ORIGINAL ARTICLE

Insect Conservation and Diversity

Royal Entomological Society

Reaching new heights: Arboreal ant diversity in a North American temperate forest ecosystem

Michelle Kirchner[1,2,3] | **Clyde Sorenson**[2] | **Bonnie B. Blaimer**[4] | **Elsa Youngsteadt**[3,5]

[1]U.S. Department of Agriculture–Agricultural Research Service, Pollinating Insect–Biology, Management, Systematics Research Unit, Logan, Utah, USA

[2]Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, North Carolina, USA

[3]Department of Applied Ecology, North Carolina State University, Raleigh, North Carolina, USA

[4]Museum für Naturkunde, Leibniz Institute for Evolution and Biodiversity Science, Center for Integrative Biodiversity Discovery, Berlin, Germany

[5]Center for Geospatial Analytics, North Carolina State University, Raleigh, North Carolina, USA

Correspondence
Michelle Kirchner, U.S. Department of Agriculture–Agricultural Research Service, Pollinating Insect–Biology, Management, Systematics Research Unit, 1410 N. 800 E., Logan, UT, 84341, USA.
Email: michelle.kirchner@usda.gov

Funding information
North Carolina State University; North Carolina Wildlife Federation; North Carolina Entomological Society

Editor: Yves Basset and Associate Editor: Jerome Orivel

Abstract

1. Most canopy insect research takes place in tropical forests, where communities are highly vertically stratified. However, temperate forest canopies also provide critical resources to many species and are under intense pressure from global change drivers. The relative lack of knowledge regarding temperate canopy insect ecology impedes our forest management and conservation decisions such that we may be losing temperate canopy biodiversity before we know it exists.
2. We directly compared ant diversity and community composition on the ground and in the tree canopy of North American temperate deciduous forests for the first time. We also evaluated two canopy sampling methods—baits and hand collections.
3. We collected 34 ant species from 102 trees across seven sites. Ant diversity was greater on the ground than in the canopy, and species turnover created distinct communities across vertical strata. Only 12% of species were exclusively arboreal, but 47% were collected in both strata, indicating the canopy is an important resource for temperate ants, even if they are not restricted there.
4. Baiting and hand-collecting recovered similar species richness, but whether baits captured a subset of hand-collected species or a unique assemblage was site-dependent. Nevertheless, we suggest that these methods are most effective in conjunction.
5. Hand collection allowed us to document arboreal nests of 10 species, including the invasive needle ant, *Brachyponera chinensis*, which was previously thought to be strictly terrestrial.
6. Our results emphasise the importance of including the canopy in temperate forest ecology and conservation assessments.

KEYWORDS
arthropod, biodiversity, *Brachyponera chinensis*, canopy, Formicidae, sampling method, vertical stratification

INTRODUCTION

With its complex three-dimensional structure and unique microclimates, the forest canopy harbours diverse biota across more than a quarter of Earth's land area (Nakamura et al., 2017). For example, ~9% of extant vascular plants are canopy epiphytes and an estimated 20–25% of arthropods are arboreal specialists (Ozanne et al., 2003). While interest in canopy ecology has increased in recent decades

(Nakamura et al., 2017), most arboreal research has focused on tropical forests, where biodiversity is high, forest structure is complex, and numerous taxa exhibit remarkable vertical stratification (e.g., Basset et al., 2003; Scheffers et al., 2013; Shaw, 2004). However, temperate forest canopies also provide critical resources for a variety of taxa spanning multiple trophic levels, resulting in communities that are vertically stratified, albeit often to a lesser degree than in the tropics (Sallé et al., 2021; Ulyshen, 2011).

The relative dearth of canopy-oriented research in temperate forests leaves gaps in our understanding of forest ecology, including basic biodiversity, and ultimately limits our ability to make informed conservation and management decisions (McNellie et al., 2020). Temperate forests are among the most heavily disturbed biomes on the planet (Haddad et al., 2015; Hoekstra et al., 2005). Forecasts for further transformation by fragmentation, climate change and outbreaks of pests and pathogens are particularly severe in North American forests, making conservation an urgent concern (Chen et al., 2020; Millar & Stephenson, 2015; Seidl et al., 2017). These disturbances often result in reduced abundance and species richness and altered community composition in the canopy and on the ground (Haddad et al., 2015; Sallé et al., 2021) such that we may be losing temperate forest biodiversity before knowing it exists.

In tropical forests where arboreal research is better established, ants (Hymenoptera: Formicidae) are prominent and ecologically important members of the canopy fauna. Usually the most abundant arboreal animals, ants account for up to 86% of all arthropod biomass in tropical canopies (Davidson et al., 2003; Davidson & Patrell-Kim, 1996), and high species turnover across vertical strata results in distinct communities of arboreal and terrestrial ants (e.g., Almeida et al., 2023; Leahy et al., 2021; Ryder Wilkie et al., 2010; Yanoviak & Kaspari, 2000). Arboreal ants operate at multiple trophic levels to influence canopy community assembly and plant health. For example, many species function as cryptic herbivores by forming associations with their host plant or other arthropods (Blüthgen et al., 2003; Davidson et al., 2003; Styrsky & Eubanks, 2006), while others are keystone predators that control herbivore populations (Dejean et al., 2007; Floren et al., 2002). Despite their dominance and importance in tropical canopies, we know relatively little about the arboreal ant fauna of temperate forests. In temperate North America, we often know which species are foraging on tree trunks (Fowler et al., 2014; Frye & Frye, 2012; Mann et al., 2018; Tschinkel & Hess, 1999), but the ant fauna of the high canopy remains largely out of reach (but see Kaspari, 2000; Moreira, 2012). Most temperate arboreal ant work takes place in Europe, where arboreal ants are less diverse, less abundant and less vertically stratified than those in tropical canopies (Dolek et al., 2009; Floren et al., 2014; Jaffe et al., 2007; Seifert, 2008; Southwood et al., 1982). These differences are likely due to temperate seasonal variation and harsh winter conditions. The lack of basic biodiversity knowledge for a dominant group of forest arthropods ultimately hinders our understanding of myrmecology, forest ecology and patterns of diversity.

Accessing the forest canopy is notoriously challenging in both temperate and tropical forests (Nakamura et al., 2017). This fact, combined with long-standing impression that temperate seasonality attenuates canopy arthropod diversity (e.g., Dobzhansky, 1950; Floren et al., 2014; Jaffe et al., 2007; Seifert, 2008; Ulyshen, 2011), may help to explain the relative scarcity of temperate canopy ant research. To date, temperate canopy surveys have relied on pre-existing canopy crane infrastructure (Jaffe et al., 2007; Seifert, 2008), opportunistic sampling of felled or fallen trees (Mottl et al., 2020; Seifert, 2008) or non-specific canopy fogging (Dolek et al., 2009; Floren et al., 2014; Southwood et al., 1982). Many temperate studies have tried to adapt existing ground-based sampling methods for arboreal use, but arboreal pitfall traps have a very low capture rate in temperate forests (Kaspari, 2000; M Kirchner pers. obs.) and arboreal baits are usually placed 2 m above the ground, not directly in the canopy (Tschinkel & Hess, 1999; Fowler et al., 2014; Mann et al., 2018; but see Moreira, 2012). None of these methods allow for systematic sampling of the vertical stratification of arboreal fauna, direct comparison with epigaeic communities or the ability to distinguish between arboreal foragers and true specialists that also nest in the canopy. Tropical researchers have developed appropriate sampling procedures to address these issues (e.g., Leponce et al., 2021; Yanoviak & Kaspari, 2000; Yusah et al., 2012), but due to the structural and ecological differences between temperate and tropical forests, aspects of these methods do not translate in a temperate system. For example, arboreal baiting protocols developed in tropical systems often collect the baits after an hour or less (e.g., Yanoviak & Kaspari, 2000; Yusah et al., 2012), but time to discovery for temperate arboreal baits is regularly over an hour (M. Kirchner, pers. obs.).

To address these knowledge gaps, we assessed ant species diversity and community composition on the ground and in the tree canopy of North American temperate deciduous forests for the first time. We present two viable canopy sampling methods adapted for temperate forests—a novel bait design and a hand collection protocol—and compare their effectiveness for temperate canopy ant research. We expected temperate ant diversity in North America to reflect patterns seen in Europe, where ants are more diverse on the ground than in the canopy and vertical stratification is less pronounced than in tropical forests (Floren et al., 2014). We also expected hand-collecting to capture more canopy species than baiting, as is true in tropical canopies (Antoniazzi et al., 2020). Finally, we predicted that systematic sampling of tree canopies in North America would provide new occurrence records or natural history observations for rarely observed species. Collectively, the results from this study will begin to clarify the importance of the canopy in maintaining ant diversity in temperate forests and provide valuable insight for future studies in the temperate canopy.

MATERIALS AND METHODS

Study sites

We sampled from seven sites of secondary, mixed hardwood forest in state parks, game lands or private property in the Piedmont ecoregion of central North Carolina (NC), USA (Figure 1a). This region has mean monthly temperatures ranging from −2.8°C in January to 31.7°C in

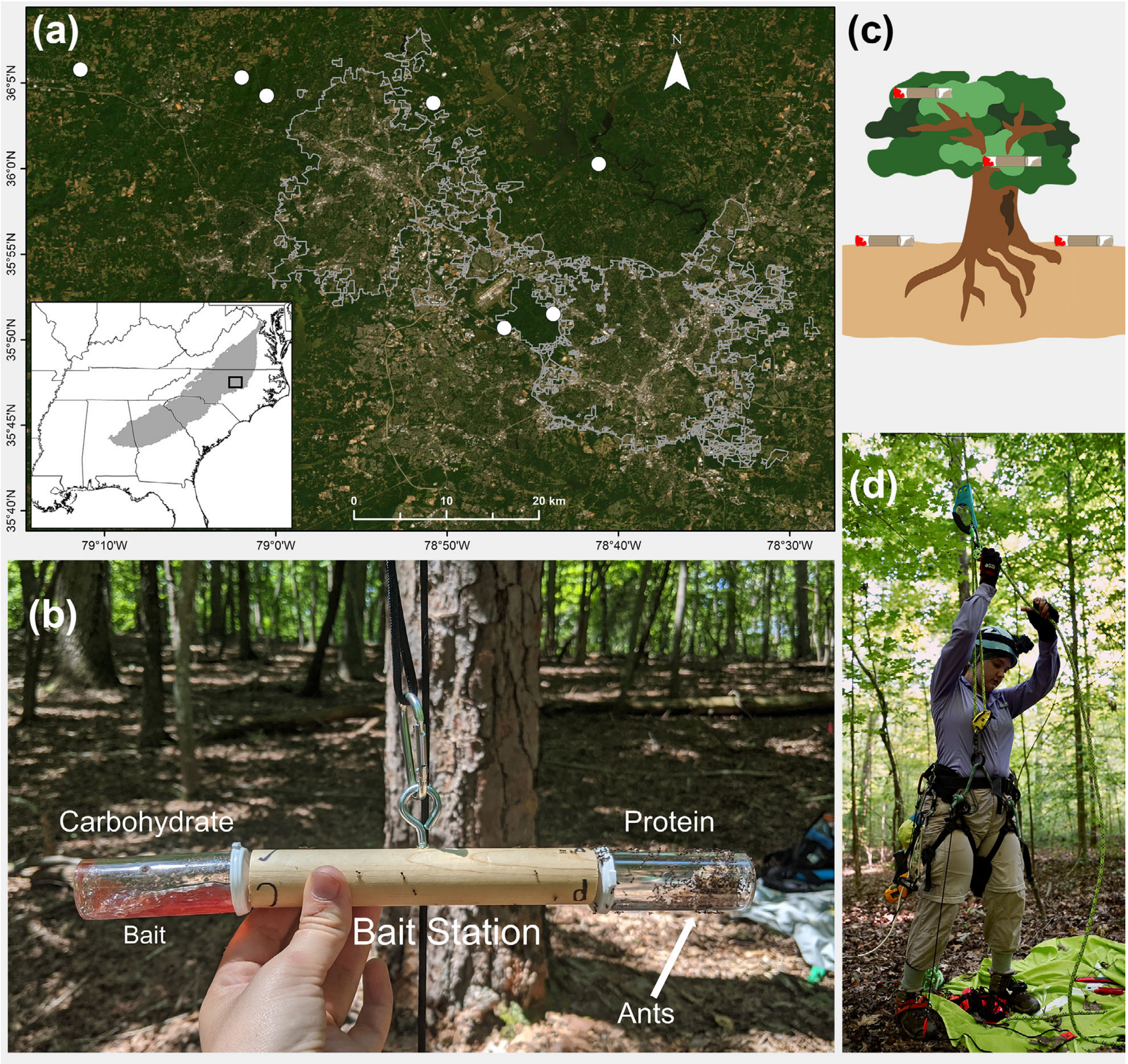

FIGURE 1 (a) Map of seven study sites in central North Carolina. Grey outline indicates Raleigh and Durham city limits. Inset: Black box is study extent. Grey shaded area shows extent of Piedmont ecoregion. (b) Photograph of bait station design. (c) Cartoon of bait station placement at each tree. (d) Photograph of M Kirchner preparing to climb a tree using SRT setup. Photo credit: M Kirchner (bait station), M Jewell (climbing). Illustration credit: J Schlauch.

July (Griffith et al., 2002). Sites were separated by at least 3 km, and trees within a site were at least 20 m apart. At each site, we selected 6–21 healthy, mature trees split across the four most common genera in the region: oak (*Quercus* L.), maple (*Acer* L.), sweetgum (*Liquidambar* L.) and pine (*Pinus* L.). All sampling was done with permits from NC State Parks, NC Wildlife Resources Commission, or owner permission.

Ant sampling

Each tree was sampled on one dry day in the summer (June–August) of 2020, 2021 or 2022, using a bait station or hand collection. In 2020, we sampled by baiting only, with paired baits in the canopy and on the ground (48 trees at four sites). In 2021, we again sampled with arboreal and terrestrial baits (42 trees at 7 sites), and then we resampled a subset of 15 trees with arboreal hand collections at least 1 week after the initial baiting. In 2022, we hand-collected ants in the canopy and on the ground simultaneously (12 trees at five sites). Due to constraints on minimum tree size and architecture for canopy access, we only hand-collected in oak canopies. For this study, we considered a tree's canopy to encompass everything from the first major branch to the outermost leaves (Erwin, 2013).

At each baited tree, we placed four bait stations between 9:00 and 11:30 am, two in the canopy (mean height: 16.9 m, standard deviation (SD): 4.4 m, range: 4.9–31.1 m) and two on the ground (5 m N and S of the base of the tree) (Figure 1b,c). Each bait station presented ants with ~6 cm^3 of a carbohydrate-rich (strawberry preserves, Smucker's®) and a protein-rich food (tuna, StarKist® chunk light in water, drained). Details of bait construction are published elsewhere (Kirchner 2024). Briefly, each bait station consisted of two perforated 9-dram styrene vials, one on each end of a 15-cm pine dowel outfitted with an eye screw. We used a Big Shot™ slingshot to place a cord over a branch, attached the bait station to a cord, and elevated it until it was in direct contact with the anchoring branch. After 4 and 8 hr., we collected all ants on each bait into 70% ethanol (See Text S1 for detailed protocol). For arboreal hand collections, we used single-rope climbing techniques (SRT) to access the canopy (mean height: 16.5 m, SD: 2.6 m, range: 12–24 m) (Figure 1d). Using the climbing rope as a vertical transect, we collected arboreally foraging ants and searched for arboreal ant nests by peeling bark and breaking dead branches for 2 hr. Terrestrial hand collections occurred simultaneously to canopy collections in a 10 m circle around the base of the tree for 2 hr. We searched for terrestrial ant nests by turning leaf litter, flipping rocks, breaking open dead logs and following foragers (see Text S2 for detailed protocol). Foraging ants and at least 10 representatives from each nest (arboreal and ground) were collected into 70% ethanol. Ants were identified to species using genus-specific keys (Table 1). Vouchers are deposited in the NC State University Insect Museum.

Data analysis

Data analyses were performed in *R* 4.3.2 and *RStudio* 2023.12.0 + 369 (Posit team, 2024; R Core Team, 2023).

Strata comparison

To compare diversity and community composition between vertical strata, we excluded hand collections from 2021, because the arboreal hand collections at those trees were not paired with simultaneous terrestrial hand collections. All other trees were simultaneously sampled in the canopy and on the ground. We pooled all samples, regardless of collection method, year or tree species, into each combination of site and stratum ($N = 7$ sites × 2 strata = 14) and recorded each ant species' presence or absence per site-stratum combination. Previous analyses indicated that neither ant diversity nor community composition differed between tree genera (Figure S1). Each pooled sample represented 1–3 hand-collected trees and 6–18 baited trees per site. Although trees per sample differed among sites, they were equal for paired canopy/ground samples within each site. To assess differences in diversity between strata (arboreal/terrestrial), we performed a sample-based rarefaction and extrapolation and obtained estimates of sample coverage (a measure of sample completeness) using *iNEXT* 3.0.0 (Chao et al., 2014; Hsieh et al., 2016). Then, after removing singleton species (those that occurred only at one site-stratum combination), we tested for changes in community composition in *vegan* 2.6–4 (Oksanen et al., 2022). We conducted permutational multivariate analysis of variance (PERMANOVA) using Jaccard dissimilarity indices. We included the additive effects of stratum and site as predictors in "adonis2," performed 999 permutations and assessed the marginal effects of each term. Then we tested for multivariate homogeneity of dispersion around each stratum's median (PERMDISP2) with "betadisper." We visualised shifts in community composition with two-dimensional non-metric multidimensional scaling (NMDS) on the species occurrence Jaccard distance matrix using "metaMDS." Finally, to clarify the role of species replacement (turnover) versus loss (nestedness) in separating the canopy and ground communities, we computed species turnover and nestedness between the canopy and ground for each site using "beta. pair" in *betapart* 1.6 (Baselga et al., 2023).

Methods comparison

Because all arboreal hand collections were performed in oak trees, we used only samples from oaks to compare the effectiveness of baits and hand collection in the canopy. Thus, we compared the diversity and community composition of ants collected in baits in oak canopies in 2020 and 2021 to ants collected by hand from oak canopies in 2021 and 2022. We pooled all samples within each site by collection method ($N = 7$ sites x 2 methods = 14) and recorded each ant species' presence or absence. Each pooled sample represented 2–5 hand-collected trees and 3–7 baited trees; 2–3 hand-collected trees per site were also baited. Then, as described above, we performed a sample-based rarefaction and extrapolation. To assess whether baiting and hand-collecting detect different components of the arboreal community, we removed singleton species and performed PERMANOVA on the Jaccard dissimilarity matrix with additive effects of collection method and site. Then we conducted the PERMDISP2 routine to assess homogeneity of dispersion for the two collection methods, visualised the community differences with two-dimensional NMDS and calculated species turnover and nestedness between collection methods. To understand how the two methods compared directly on a per-tree basis, we repeated this process using only the 15 trees which were both baited and hand-collected.

RESULTS

Across our seven sites, we collected ants from a total of 102 individual trees. Seventy-five trees were only baited, 12 trees were only hand-collected and 15 trees were both baited and hand-collected (Table 2). We collected 34 ant species from 13 genera and 4 subfamilies (Table 1, Figure 2), representing ~20% of the 164 species known from the North Carolina Piedmont (Guénard et al., 2012). Ants showed no preference for carbohydrates or protein in the canopy or on the ground (see Kirchner et al. in revision for detailed analysis). We

TABLE 1 Occurrence of ant species in arboreal and terrestrial baits and hand collections.

		Arboreal			Terrestrial		
Taxonomic references	**Species**	**Bait**	**HC**	**Nest (height)**	**Bait**	**HC**	**Nest**
(DeMarco, 2015)	*Aphaenogaster fulva* Roger 1863	–	–	–	3	–	–
	Aphaenogaster lamellidens Mayr 1886	–	–	–	3	–	–
	Aphaenogaster mariae Forel 1886	3	2	1 (15–19 m)	–	–	–
	Aphaenogaster rudis s.l.	–	–	–	5	3	3
	Aphaenogaster tennesseensis (Mayr 1862)	1	1	1 (15 m)	1	2	1
(Fisher & Cover, 2007)	*Brachyponera chinensis* (Emery 1895)	1	7	7 (9.5–18 m)	7	4	4
(MacGown, 2022)	*Camponotus americanus* Mayr 1862	2	2	–	1	1	1
	Camponotus caryae (Fitch 1855)	–	5	3 (9.5–20 m)	–	–	–
	Camponotus castaneus (Latreille 1802)	3	6	–	5	5	4
	Camponotus chromaiodes Bolton 1995	4	5	1 (15 m)	6	4	3
	Camponotus nearcticus Emery 1893	7	7	6 (8–17 m)	3	1	–
	Camponotus pennsylvanicus (De Geer 1773)	6	5	2 (10–16 m)	6	2	–
	Camponotus snellingi Bolton 1995	4	1	–	–	2	–
	Camponotus subbarbatus Emery 1893	2	–	–	3	1	–
(MacGown, 2022)	*Colobopsis impressa* Roger 1863	1	1	–	–	–	–
	Colobopsis obliqua (Smith 1930)	1	1	–	–	–	–
(MacGown, 2022; Ward & Blaimer, 2022)	*Crematogaster ashmeadi* Mayr 1886	7	5	4 (13–18 m)	3	–	–
	Crematogaster lineolata (Say 1836)	–	–	–	2	–	–
	Crematogaster vermiculata Emery 1895	–	1	1 (9–13 m)	1	–	–
(Creighton, 1950; Francoeur, 1973; Snelling & Buren, 1985; Trager et al., 2007)	*Formica pallidefulva* Latreille 1802	2	3	–	5	2	–
	Formica pergandei Emery 1893	–	–	–	1	–	–
	Formica subintegra Wheeler 1908	–	–	–	–	1	1
	Formica subsericea Say 1836	6	5	–	7	5	5
(AntWiki, 2018)	*Lasius americanus* Emery 1893	–	–	–	2	1	1
(AntWiki, 2019)	*Monomorium minimum* (Buckley 1867)	–	–	–	–	1	1
(Kallal & LaPolla, 2012; MacGown, 2022)	*Nylanderia arenivaga* (Wheeler 1905)	–	–	–	3	–	–
	Nylanderia faisonensis (Forel 1922)	–	–	–	5	2	2
	Nylanderia vividula (Nylander 1846)/ *Nylanderia terricola* (Buckley 1866)	–	–	–	4	–	–
(Fisher & Cover, 2007)	*Prenolepis imparis* (Say 1836)	–	–	–	–	1	1
(MacGown, 2022; Pacheco & Mackay, 2013)	*Solenopsis invicta* Buren 1972	1	–	–	1	–	–
	Solenopsis molesta s.l.	–	–	–	2	1	1
(AntWiki, 2014)	*Tapinoma sessile* (Say 1836)	–	–	–	2	–	–
(MacGown, 2022)	*Temnothorax curvispinosus* (Mayr 1866)	4	2	–	5	1	1
	Temnothorax schaumii (Roger 1863)	5	5	1 (15.5 m)	–	1	–

Note: Values are the number of sites from which the ant species was collected within a given stratum using each sampling method (max = 7). The "Nest" column indicates the number of sites at which the ant species was found nesting in each stratum via hand-collecting. Height ranges for arboreal nests are included below the number of sites. Species lacking an authority are species complexes. HC = hand collection.

TABLE 2 Total number of trees sampled by each collecting method at each site.

Genus	Method	Total	ER1	ER2	FL1	FL2	MB1	UM1	UM2
Quercus	HC	12	1	3	0	3	0	3	2
	Bait	19	4	4	1	1	4	4	1
	Both	15	2	2	2	2	3	2	2
Acer	Bait	34	6	6	3	3	7	6	3
Pinus	Bait	9	3	3	0	0	0	3	0
Liquidambar	Bait	13	3	3	0	0	4	3	0
Total	HC	12	1	3	0	3	0	3	2
Total	Bait	75	16	16	4	4	15	16	4
Total	Both	15	2	2	2	2	3	2	2

Note: Values are the number of trees in each genus sampled using each collection method across all years. Columns to the right of the solid line are the values for each site. Rows below the solid line are the values summed across all tree genera. HC = hand collection. ER1, ER2, FL1, FL2, MB1, UM1, and UM2 are arbitrary abbreviations for each site.

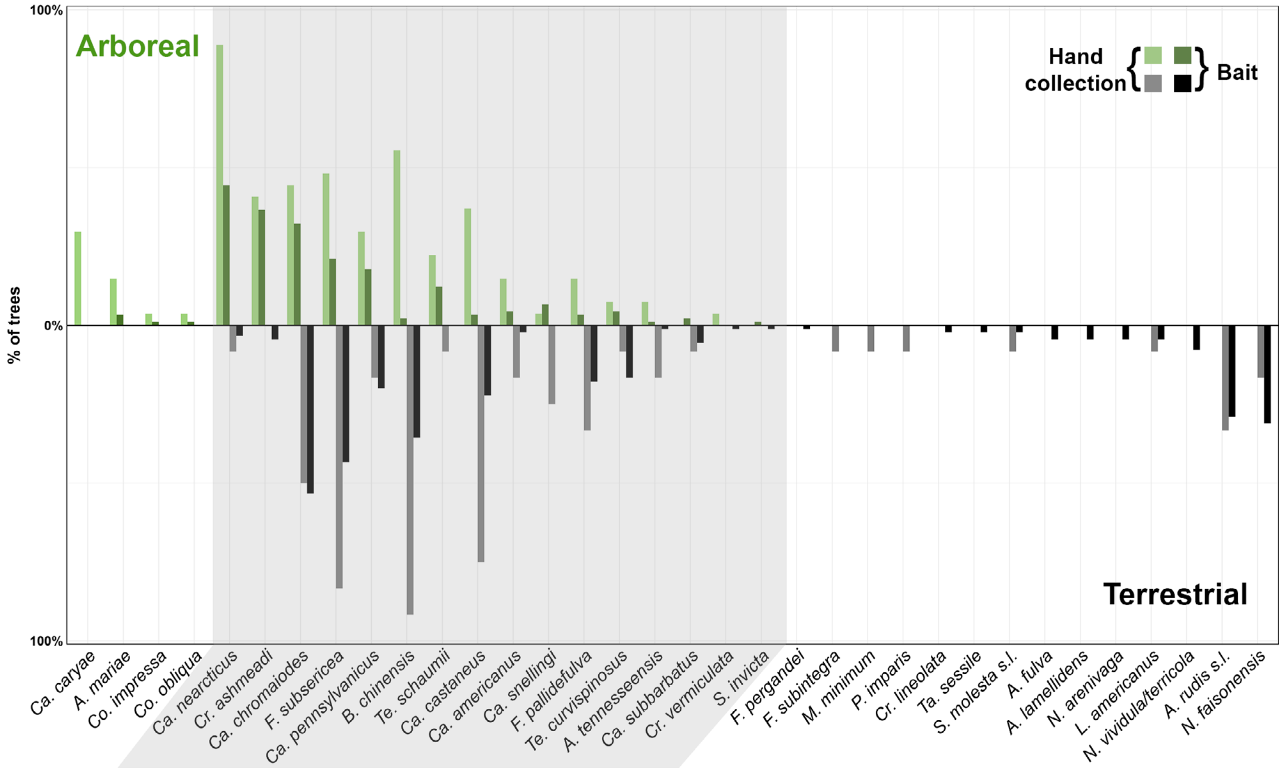

FIGURE 2 Relative frequency of each ant species in each stratum (canopy/ground) and collection method (hand collection/bait) across all years and trees. Grey box indicates species that occurred in both strata. Genus abbreviations are as follows: A = *Aphaenogaster*, B = *Brachyponera*, Ca = *Camponotus*, Cr = *Crematogaster*, Co = *Colobopsis*, F = *Formica*, L = *Lasius*, M = *Monomorium*, N = *Nylanderia*, P = *Prenolepis*, S = *Solenopsis*, Ta = *Tapinoma*, Te = *Temnothorax*.

observed 7 species nesting exclusively in the canopy, 11 species nesting exclusively on the ground and 3 species nesting in both strata. (For the remaining 13 taxa, no nests were observed.) In the canopy, most ants were nesting in dead branches, rotten branch stumps or under bark. Other arboreal nest locations included abandoned squirrel nests and empty tussock moth cocoons (Lepidoptera: Erebidae: Lymantriinae). At a given tree, we observed at most three species nesting in the same canopy but observed up to seven species nesting on the surrounding ground. We also detected, for the first time, arboreal nesting and foraging of the invasive needle ant, *Brachyponera chinensis* (Emery). This species occurred in arboreal samples from all seven sites, with arboreal foraging activity detected at 2 of the 90 baited trees and 14 arboreal nests observed in 8 of the 27 hand-collected trees.

Strata comparison

Across all sampling methods, we collected more ant species on the ground (30) than in the canopy (20), and Chao2 asymptotic estimators of species richness suggested that species richness was greater in the terrestrial stratum (arboreal: 22.7, terrestrial: 36.9) (Figure 3a). Other measures of diversity followed the same pattern (Figure S2). Because sample coverage was high in both strata (arboreal: 0.94, terrestrial: 0.93), and neither the rarefaction curves nor asymptotic estimators shared overlapping confidence intervals, we conclude that ant diversity was greater on the ground than in the canopy. While only 12% of species were uniquely arboreal, nearly half of the observed species (47%) were collected in both strata, indicating that much of the ant community uses resources in the canopy. Ant community composition shifted between vertical strata (PERMANOVA: $F_{(1,6)} = 5.32$, $R^2 = 0.258$, $p = 0.001$) with only a marginal effect of site (PERMANOVA: $F_{(6,6)} = 1.55$, $R^2 = 0.452$, $p = 0.059$). Community variances did not differ between strata (PERMDISP2: $F_{(1,12)} = 0.694$, $p = 0.421$), so we attribute the significant PERMANOVA to differences in the average community composition as represented by the community centroids (Figure 3b). Changes in community composition between the canopy and ground were largely driven by species turnover, not species loss (average turnover: 0.45, average nestedness: 0.19).

Methods comparison

We collected 18 ant species from oak canopies. Hand-collecting captured all species, while baiting captured 13. Chao2 asymptotic estimators of species richness agree that hand collection captures more species (21.6) than baiting (16.4), but with significant overlap of the 95% confidence intervals (Figure 4a). Other measures of diversity follow the same pattern (Figure S3). So, despite high sample coverage from both methods (hand collection: 0.94, baiting: 0.91), we cannot conclude that arboreal hand collection captures higher ant diversity

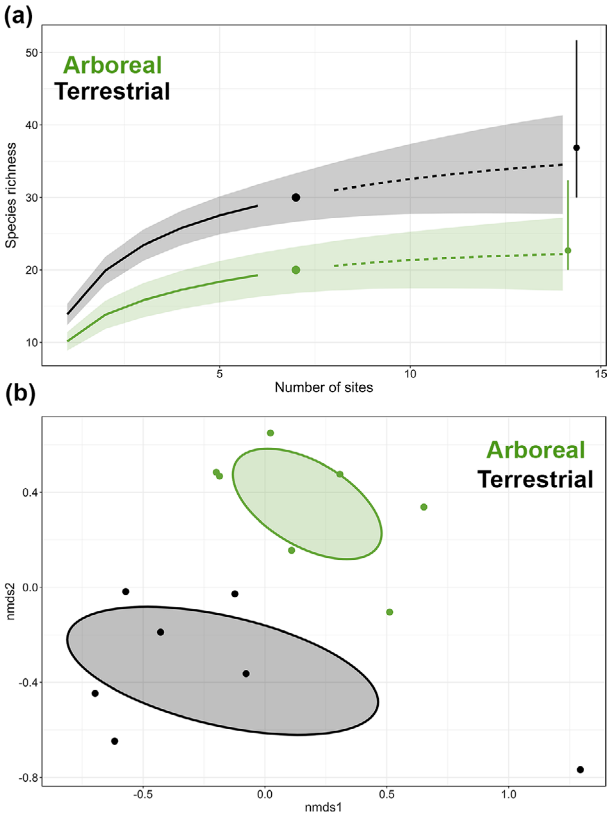

FIGURE 3 Terrestrial and arboreal ant communities differed in richness and composition. (a) Sample-based rarefaction (solid curves) and extrapolation curves (dashed curves) of ant species richness across vertical strata (canopy/ground). Shaded regions represent 95% confidence intervals. Observed species richness is denoted by a circle. To the right of each curve is the Chao2 asymptotic estimator and its 95% confidence interval. (b) Nonmetric multidimensional scaling (NMDS) plot of ant community composition across vertical strata. Shaded ellipses represent 95% confidence regions of group centroids. Stress = 0.09.

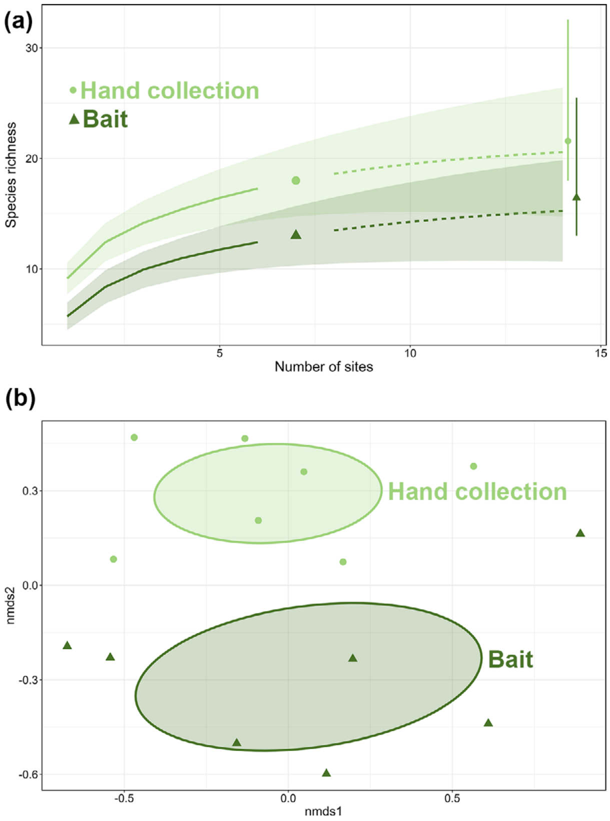

FIGURE 4 Hand-collecting and baiting recovered similar diversity but sampled distinct subsets of the arboreal ant community.
(a) Sample-based rarefaction (solid lines) and extrapolation curves (dashed lines) of ant species richness across canopy sampling methods (hand collection/bait). Shaded regions represent 95% confidence intervals. Observed species richness is denoted by a circle (hand collection) or triangle (bait). To the right of each curve is the Chao2 asymptotic estimator and its 95% confidence interval. (b) Nonmetric multidimensional scaling (NMDS) plot of ant community composition across sampling methods. Shaded ellipses represent 95% confidence regions of group centroids. Stress = 0.16.

than arboreal baiting. However, hand-collecting did capture a different subset of the arboreal community than baiting did (PERMANOVA: $F_{(1,6)} = 6.47$, $R^2 = 0.193$, $p = 0.001$), and site also influenced community composition (PERMANOVA: $F_{(6,6)} = 3.51$, $R^2 = 0.628$, $p = 0.001$). Community variances did not differ between methods (PERMDISP2: $F_{(1,12)} = 2.83$, $p = 0.118$), so we attribute the significant PERMANOVA to differences in the community centroids (Figure 4b). The contribution of species turnover and nestedness to community differences between collection methods differed by site, such that, on average, beta diversity was split equally between the two processes (average turnover: 0.26, average nestedness: 0.25). Results were qualitatively similar on a per-tree basis, but the bait-sampled species were more clearly nested within the hand-collected species (average turnover: 0.23, average nestedness: 0.43) (Figure S4).

DISCUSSION

To our knowledge, this research represents the first systematic survey of canopy ant diversity in North American temperate forests. As expected, ant diversity was greater on the ground than in the canopy, and a minority of species (12%) were true arboreal specialists that nested and foraged only in the canopy. We compared the effectiveness of two viable canopy sampling methods and found them to be equal with respect to species richness. Although baiting tended to capture a subset of those species detected by hand collection, some species were more *likely* to be captured at baits, such that the two methods were complementary and are best used in conjunction.

Consistent with results from European canopies and other temperate arthropod taxa (Floren et al., 2014; Jaffe et al., 2007; Sallé et al., 2021; Ulyshen, 2011), North American ants were more diverse on the forest floor than in the canopy (Figure 3a). However, the canopy community is not merely a subset of epigaeic species foraging arboreally, as species turnover contributed to distinct communities in each stratum (Figure 3b) and 12% of species were strictly arboreal. But while we do see vertical stratification and arboreal specialisation in this temperate ant community, it is not on the same scale as tropical forests, where up to half of the community nests and forages only in the canopy (Floren et al., 2014; Jaffe et al., 2007; Ryder Wilkie et al., 2010). A seasonal temperate climate, reduced structural complexity and altered resource availability in temperate forests likely contribute to this pattern (Seifert, 2008; Ulyshen, 2011). For example, in tropical forests, extensive canopy connectivity promotes high arboreal ant diversity by allowing species to forage between trees without leaving the canopy (Adams et al., 2019). In temperate systems, trees are typically more isolated and lack connecting lianas, so instead of foraging horizontally across the canopy, temperate arboreal ants are more restricted to a single tree canopy, limiting their access to resources. This could explain why we observed arboreal species also foraging on the ground, for example, *Camponotus nearcticus* Emery and *Crematogaster ashmeadi* Mayr (Figure 2, Table 1). Indeed, we observed nearly half of the species in both strata, and previous work in this system showed that 24% of forest ant biomass was in the canopy (M Kirchner unpublished data). This indicates that many ground-dwelling species venture into the canopy to forage on the abundance of insect-produced honeydew, for example, *Camponotus castaneus* (Latreille) and *Formica subsericea* Say and some species can nest in both the canopy and on the ground, for example, *Aphaenogaster tennesseensis* (Mayr) and *Brachyponera chinensis* (Table 1). Our results add to a growing body of work that points toward the significance of temperate forest canopies as a resource for arthropods, even if they are not specialised there (Sallé et al., 2021; Ulyshen, 2011).

When compared with previous North American studies that used trunk-based baiting or hand-collecting methods to assess arboreal ant diversity (e.g., Fowler et al., 2014), our study exhibits higher species turnover between arboreal and terrestrial communities, likely because we collected from the canopy community in situ. Nevertheless, species lists from trunk-based studies overlap broadly with the species list reported here, with a few notable exceptions. While some of the arboreally nesting species we collected in the canopy were also common among other surveys, for example, *Camponotus nearcticus* Emery and *Crematogaster ashmeadi* Mayr, others were conspicuously absent. For example, of the four exclusively arboreal species recorded in this study, three were not collected in previous trunk surveys in nearby states (*Camponotus caryae* (Fitch), *Colobopsis impressa* Roger and *Colobopsis obliqua* (Smith)) (Fowler et al., 2014; Frye & Frye, 2012; Kaspari, 2000; Mann et al., 2018; Tschinkel & Hess, 1999). The fourth species, *Aphaenogaster mariae* Forel, was collected from trunks in one study by hand (Frye & Frye, 2012) and is known to occasionally venture from the canopy to forage (Kirchner et al., 2023; MacGown et al., 2012). This suggests that trunk-based methods may be sufficient for the commonest arboreal species and those who nest and forage in both strata but are likely to miss the rarer arboreal specialists.

Contrary to our predictions, hand-collecting and baiting captured a similar number of ant species in the canopy (Figure 4a). This contrasts with results from surveys on the temperate forest floor and in Neotropical canopies, where hand-collecting yields higher species richness than baiting (e.g., Antoniazzi et al., 2020; Ellison et al., 2007). Still, we consider these two sampling methods complementary, as they collect different assemblages within the canopy ant community (Figure 4b). Whether baiting collected a nested subset of the arboreal community or experienced species replacement compared with hand-collecting was site-dependent in this study, further supporting the idea that the two methods are complementary. As a whole, our results agree with the conclusions of Antoniazzi et al. (2020), such that baiting and hand-collecting together provide a more robust sample of the canopy community than either one alone.

Hand-collecting directly in the canopy has its logistical challenges, because it involves specialised skills, equipment and training to safely climb trees (Anderson et al., 2015) and expends significant effort on each individual tree, which may limit sample size. Hand collection can also suffer from observer bias, as more experienced collectors are often more effective (Bestelmeyer et al., 2000). Baiting, on the other hand, is logistically simpler and requires no previous experience, but it may sample only a subset of the community. Trophic generalists and aggressive and numerically dominant species are more likely to recruit to baits, which can lead to competitive exclusion of subdominant, rare or specialised species (Antoniazzi et al., 2020; Bestelmeyer et al., 2000; Davidson, 1998). Consequently, baiting accumulates species more slowly per tree sampled when compared with hand-collecting (Figure S4). However, the ability to sample more trees in the same number of person-hours effectively counterbalances this difference between the methods. We recognise that neither of these methods is completely exhaustive as described, but we nevertheless achieved high sample coverage for both methods, and our species richness estimates approached the asymptotic estimators. While thorough sampling from felled trees (as in Mottl et al., 2020) could provide a more complete inventory of arboreally nesting ants, the methods presented here are less destructive, allow for repeat sampling of the same nests, and provide insight into the context in which these organisms live. Ultimately, if the goal is to detect arboreal specialists, locate arboreal nests or study natural history in the canopy, hand-collecting in situ remains the most effective method, despite the logistical downsides. In this study, hand-collecting in oak canopies captured five ant species that did not occur at baits, including three rarely collected arboreal species: *Camponotus caryae*, *Colobopsis obliqua* and *Crematogaster vermiculata* Emery. Furthermore, from hand collections, we were able to document the arboreal nesting habits of 10 species, including the invasive needle ant, *Brachyponera chinensis*, which was previously considered to be a poor climber restricted to the ground (Guénard & Dunn, 2010).

Future conservation research should focus on interactions between arboreal species and *B. chinensis*, availability of arboreal nesting resources to native species, and cascading ecological effects of *B. chinensis* invasion. First recorded in the United States in 1932 (Smith, 1934), *Brachyponera chinensis* has rapidly spread across the southeastern United States, invading disturbed habitats and mature, intact forests (Guénard & Dunn, 2010). Throughout its invasive range, this species outcompetes and displaces native ants, disrupting key ecosystem services (Guénard & Dunn, 2010; Warren et al., 2015), and these effects are expected to intensify with future climate change and habitat fragmentation (Warren et al., 2020, 2023). Therefore, the ability of *B. chinensis* to also nest and forage in the forest canopy raises conservation concerns for arboreally active arthropods, as well. For example, on the ground, *B. chinensis* is a voracious predator that kills competing ants and dominates nesting and food resources (Bednar et al., 2013; Warren et al., 2020, 2023). Because nesting space is a major driver of canopy diversity and is already limited in temperate canopies (Adams et al., 2023; Seifert, 2008), the ecological repercussions of *B. chinensis* invasion may be more pronounced in the canopy. In this study, we found *B. chinensis* nests at all seven sites and in nearly 30% of hand-collected trees, indicating that the species is already well established as a member of the arboreal community, but the effects of its presence remain unclear.

More broadly, the abundance and diversity of ants foraging in the temperate canopy also warrants attention in conservation and forest management research. For example, ants can suppress or support populations of plant pests (Anjos et al., 2022; Rosumek et al., 2009) and can be essential to conservation or restoration of myrmecophilous insects, such as lycaenid butterflies (Hill et al., 2022; Thomas et al., 2020). In temperate ecosystems, these kinds of interactions are best known in agricultural or herbaceous systems, but our results reinforce their relevance in forest canopies as well, where understanding the structure and function of arboreal communities may be relevant for rapid management responses to pest outbreaks or species invasions.

CONCLUSION

We present evidence that ant communities in temperate North American forests are vertically stratified, with species turnover contributing to distinct communities on the ground and in the canopy. While we do not see extensive arboreal specialisation as in the tropics, some species nest and forage exclusively in the canopy, and nearly half of the community uses the canopy as a resource. This underscores the importance of understanding canopy ecology and conservation in temperate systems, because even species typically regarded as ground-dwelling will be affected by shifts in canopy climate, health and structure as temperate forests face global change pressures. We hope the methods presented in this paper will help fellow researchers to document and manage temperate canopy biodiversity before it disappears. Future work should seek to understand the relative importance and patterns of canopy use by stratum-generalist species, explore the functional and phylogenetic diversity of the arboreal community, assess the effects of *Brachyponera chinensis* in the canopy, and incorporate canopy arthropod communities into forest health projections.

AUTHOR CONTRIBUTIONS

Michelle Kirchner: Conceptualization; methodology; formal analysis; investigation; visualization; data curation; writing – original draft; writing – review and editing. **Clyde Sorenson:** Conceptualization; methodology; supervision; resources; writing – review and editing; funding acquisition. **Bonnie B. Blaimer:** Conceptualization; methodology; writing – review and editing; resources. **Elsa Youngsteadt:** Conceptualization; methodology; supervision; resources; funding acquisition; writing – review and editing.

ACKNOWLEDGEMENTS

Lucie Ciccone, Kayla Beckwith and E Redick were invaluable assistance in the field. Sarah Ferriter, Lucie Ciccone, Kirsten Keleher and Kayla Beckwith picked thousands of ants out of tuna and jam and assisted with specimen curation. Peter Willadsen showed off his Big Shot™ skills, James Trager consulted on *Formica* identifications and Keary Dosier generously lent his drill press. Thank you to the Brandt family and Piedmont Tree Climbing for private land access, tree climbing instruction, and genuine hospitality. Ed Corey (NC State Parks), Kim Radewicz (NC State Parks), Zac Cowan (NC State Parks), Scott Letchworth (NC State Parks) and Chris Baranski (NC Wildlife

Resources Commission) facilitated research permissions and site access. Two anonymous reviewers and members of the Irwin/Youngsteadt lab group provided valuable feedback on earlier drafts of this manuscript.

FUNDING INFORMATION

MK was supported by Research Assistantships with North Carolina State University Department of Entomology and Plant Pathology and Department of Applied Ecology. The research was also supported by a North Carolina Wildlife Federation Scholarship Grant and a North Carolina Entomological Society Student Development Award to MK. The funders played no role in the research or publication process. There was no additional external funding received for this study.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Dryad at https://doi.org/10.5061/dryad.2v6wwpzwn.

ORCID

Michelle Kirchner https://orcid.org/0000-0001-8541-5077

REFERENCES

Adams, B.J., Gora, E.M., Donaldson-Matasci, M.C., Robinson, E.J.H. & Powell, S. (2023) Competition and habitat availability interact to structure arboreal ant communities across scales of ecological organization. *Proceedings of the Royal Society B: Biological Sciences*, 290, 20231290.

Adams, B.J., Schnitzer, S.A. & Yanoviak, S.P. (2019) Connectivity explains local ant community structure in a neotropical forest canopy: a large-scale experimental approach. *Ecology*, 100, e02673.

Almeida, R.P.S., Teresa, F.B., Camarota, F., Izzo, T.J., Silva, R.R., Andrade-Silva, J. et al. (2023) The role of morphological traits in predicting the functional ecology of arboreal and ground ants in the Cerrado–Amazon transition. *Oecologia*, 201, 199–212.

Anderson, D.L., Koomjian, W., French, B., Altenhoff, S.R. & Luce, J. (2015) Review of rope-based access methods for the forest canopy: safe and unsafe practices in published information sources and a summary of current methods. *Methods in Ecology and Evolution*, 6, 865–872.

Anjos, D.V., Tena, A., Viana-Junior, A.B., Carvalho, R.L., Torezan-Silingardi, H., Del-Claro, K. et al. (2022) The effects of ants on pest control: a meta-analysis. *Proceedings of the Royal Society B: Biological Sciences*, 289, 20221316.

Antoniazzi, R., Ahuatzin, D., Pelayo-Martínez, J., Ortiz-Lozada, L., Leponce, M. & Dáttilo, W. (2020) On the effectiveness of hand collection to complement baits when studying ant vertical stratification in tropical rainforests. *Sociobiology*, 67, 213–222.

AntWiki. (2014) Key to US Tapinoma species. AntWiki. Available from: https://antwiki.org/wiki/Key_to_US_Tapinoma_species Accessed 2022.

AntWiki. (2018) Key to North American Lasius Species. AntWiki. Available from: https://antwiki.org/wiki/Key_to_North_American_Lasius_Species Accessed 2022.

AntWiki. (2019) Key to US Monomorium species. AntWiki. Available from: https://antwiki.org/wiki/Key_to_US_Monomorium_species Accessed 2022.

Baselga, A., Orme, D., Villeger, S., Bortoli, J.D., Leprieur, F., Logez, M. et al. (2023) betapart: partitioning beta diversity into turnover and nestedness components.

Basset, Y., Hammond, Y., Holloway, J.D. & Miller, S.E. (2003) Vertical stratification of arthropod assemblages. In: Basset, Y., Novotny, V., Miller, S.E. & Kitching, R.L. (Eds.) *Arthropods of tropical forests: spatio-temporal dynamics and resource use in the canopy*. Cambridge, UK: Cambridge University Press, pp. 17–27.

Bednar, D.M., Shik, J.Z. & Silverman, J. (2013) Prey handling performance facilitates competitive dominance of an invasive over native keystone ant. *Behavioral Ecology*, 24, 1312–1319.

Bestelmeyer, B.T., Agosti, D., Alonso, L., Brandão, C.R.F., Brown, W.L., Jr., Delabie, J.H.C. et al. (2000) Field techniques for the study of ground-dwelling ants. In: Agosti, D., Majer, J.D., Alonso, L.E. & Schultz, T.R. (Eds.) *Ants: standard methods for measuring and monitoring biodiversity, biological diversity handbook series*. Washington: Smithsonian Institution Press, pp. 122–144.

Blüthgen, N., Gebauer, G. & Fiedler, K. (2003) Disentangling a rainforest food web using stable isotopes: dietary diversity in a species-rich ant community. *Oecologia*, 137, 426–435.

Chao, A., Gotelli, N.J., Hsieh, T.C., Sander, E.L., Ma, K.H., Colwell, R.K. et al. (2014) Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in species diversity studies. *Ecological Monographs*, 84, 45–67.

Chen, G., Li, X., Liu, X., Chen, Y., Liang, X., Leng, J. et al. (2020) Global projections of future urban land expansion under shared socioeconomic pathways. *Nature Communications*, 11, 537.

Creighton, W.S. (1950) The ants of North America. *Bulletin of the Museum of Comparative Zoology at Harvard College*, 104, 584.

Davidson, D. & Patrell-Kim, L. (1996) Tropical arboreal ants: why so abundant? In: Gibson, A.C. (Ed.) *Neotropical biodiversity and conservation*. Los Angeles, Los Angeles, CA: Mildred E. Mathias Botanical Garden, University of California, pp. 127–140.

Davidson, D.W. (1998) Resource discovery versus resource domination in ants: a functional mechanism for breaking the trade-off. *Ecological Entomology*, 23, 484–490.

Davidson, D.W., Cook, S.C., Snelling, R.R. & Chua, T.H. (2003) Explaining the abundance of ants in lowland tropical rainforest canopies. *Science*, 300, 969–972.

Dejean, A., Corbara, B., Orivel, J. & Leponce, M. (2007) Rainforest canopy ants: the implications of territoriality and predatory behavior. *Functional Ecosystems and Communities*, 1, 105–120.

DeMarco, B.B. (2015) *Phylogeny of North American Aphaenogaster species (Hymenoptera:Formicidae) reconstructed with morphological and DNA data* (Ph.D. dissertation).

Dobzhansky, T. (1950) Evolution in the tropics. *American Scientist*, 38, 208–221.

Dolek, M., Freese-Hager, A., Bussler, H., Floren, A., Liegl, A. & Schmidl, J. (2009) Ants on oaks: effects of forest structure on species composition. *Journal of Insect Conservation*, 13, 367–375.

Ellison, A.M., Record, S., Arguello, A. & Gotelli, N.J. (2007) Rapid inventory of the ant assemblage in a temperate hardwood forest: species composition and assessment of sampling methods. *Environmental Entomology*, 36, 766–775.

Erwin, T.L. (2013) Forest canopies, animal diversity. In: Levin, S.A. (Ed.) *Encyclopedia of biodiversity*. Waltham: Academic Press, pp. 511–515.

Fisher, B.L. & Cover, S.P. (2007) *Ants of North America: a guide to the genera*. Berkeley: University of California Press.

Floren, A., Biun, A. & Linsenmair, E.K. (2002) Arboreal ants as key predators in tropical lowland rainforest trees. *Oecologia*, 131, 137–144.

Floren, A., Wetzel, W. & Staab, M. (2014) The contribution of canopy species to overall ant diversity (Hymenoptera: Formicidae) in temperate and tropical ecosystems. *Myrmecological News*, 19, 65–74.

Fowler, D., Lessard, J.-P. & Sanders, N.J. (2014) Niche filtering rather than partitioning shapes the structure of temperate forest ant communities. *Journal of Animal Ecology*, 83, 943–952.

Francoeur, A. (1973) Révision taxonomique des espèces néarctiques du groupe fusca, genre Formica (Formicidae, Hymenoptera). *Mémoires de la Société entomologique du Québec*, 3, 1–316.

Frye, J.A. & Frye, C.T. (2012) Associations of ants (Hymenoptera: Formicidae) on oaks and pines in inland dune and ridge woodlands in Worcester County, Maryland. *The Maryland Entomologist*, 5, 37–46.

Griffith, G.E., Omernik, J.M., Comstock, J.A., Schafale, M.P., McNab, W.H., Lenat, D.R. et al. (2002) Ecoregions of North Carolina and South Carolina.

Guénard, B. & Dunn, R.R. (2010) A new (old), invasive ant in the hardwood forests of eastern North America and its potentially widespread impacts. *PLoS One*, 5, e11614.

Guénard, B., Mccaffrey, K.A., Lucky, A. & Dunn, R.R. (2012) Ants of North Carolina: an updated list (Hymenoptera: Formicidae). *Zootaxa*, 3552, 1–36.

Haddad, N.M., Brudvig, L.A., Clobert, J., Davies, K.F., Gonzalez, A., Holt, R. D. et al. (2015) Habitat fragmentation and its lasting impact on Earth's ecosystems. *Science Advances*, 1, e1500052.

Hill, G.M., Trager, M.D., Lucky, A. & Daniels, J.C. (2022) Protective benefits of tending ants to a critically endangered butterfly. *Journal of Insect Science*, 22, 9.

Hoekstra, J.M., Boucher, T.M., Ricketts, T.H. & Roberts, C. (2005) Confronting a biome crisis: global disparities of habitat loss and protection. *Ecology Letters*, 8, 23–29.

Hsieh, T.C., Ma, K.H. & Chao, A. (2016) iNEXT: an R package for rarefaction and extrapolation of species diversity (Hill numbers). *Methods in Ecology and Evolution*, 7, 1451–1456.

Jaffe, K., Horchler, P., Verhaagh, M., Gomez, C., Sievert, R., Jaffe, R. et al. (2007) Comparing the ant fauna in a tropical and a temperate forest canopy. *Ecotropicos*, 20, 74–81.

Kallal, R.J. & LaPolla, J.S. (2012) Monograph of *Nylanderia* (Hymenoptera: Formicidae) of the world, part II: *Nylanderia* in the Nearctic. *Zootaxa*, 3508, 1.

Kaspari, M. (2000) Do imported fire ants impact canopy arthropods? Evidence from simple arboreal pitfall traps. *The Southwestern Naturalist*, 45, 118.

Kirchner, M., Bertone, M., Blaimer, B.B. & Youngsteadt, E. (2023) Colony structure and redescription of males in the rarely collected arboreal ant, *Aphaenogaster mariae* Forel (Hymenoptera: Formicidae). *Proceedings of the Entomological Society of Washington*, 125, 77–88.

Kirchner, M. (2024) *Arboreal Ant Diversity and Ecology in a North American Temperate Forest Ecosystem* (Ph.D. dissertation).

Leahy, L., Scheffers, B.R., Andersen, A.N., Hirsch, B.T. & Williams, S.E. (2021) Vertical niche and elevation range size in tropical ants: implications for climate resilience. *Diversity and Distributions*, 27, 485–496.

Leponce, M., Dejean, A., Mottl, O. & Klimes, P. (2021) Rapid assessment of the three-dimensional distribution of dominant arboreal ants in tropical forests. *Insect Conservation and Diversity*, 14, 426–438.

MacGown, J.A. (2022) Ants (Formicidae) of the Southeastern United States – Keys to subfamilies, genera, and species in or possible in the southeastern United States. Missipippi Entomological Museum. https://mississippientomologicalmuseum.org.msstate.edu/Researchtaxapages/Formicidaepages/Identification.Keys.htm [access ed in 2022].

MacGown, J.A., Hill, J.G., Brown, R.L., Schiefer, T.L. & Lewis, J.G. (2012) Ant diversity at Noxubee National Wildlife Refuge in Oktibbeha, Noxubee, and Winston counties, Mississippi. *Mississippi Agricultural and Forestry Station Bulletin*, 1197, 1–30.

Mann, H.R., Rowe, E., Selfridge, J. & Price, D.L. (2018) Leaf litter and arboreal ants (Hymenoptera: Formicidae) in a mid-Atlantic forest. *Northeastern Naturalist*, 25, 341–354.

McNellie, M.J., Oliver, I., Dorrough, J., Ferrier, S., Newell, G. & Gibbons, P. (2020) Reference state and benchmark concepts for better biodiversity conservation in contemporary ecosystems. *Global Change Biology*, 26, 6702–6714.

Millar, C.I. & Stephenson, N.L. (2015) Temperate forest health in an era of emerging megadisturbance. *Science*, 349, 823–826.

Moreira, P.F. (2012) *Canopy ants of Central Arkansas and the Atlantic forest of Brazil: community patterns* (Master's Thesis).

Mottl, O., Fibich, P., Klimes, P., Volf, M., Tropek, R., Anderson-Teixeira, K. et al. (2020) Spatial covariance of herbivorous and predatory guilds of forest canopy arthropods along a latitudinal gradient. *Ecology Letters*, 23, 1499–1510.

Nakamura, A., Kitching, R.L., Cao, M., Creedy, T.J., Fayle, T.M., Freiberg, M. et al. (2017) Forests and their canopies: achievements and horizons in canopy science. *Trends in Ecology & Evolution*, 32, 438–451.

Oksanen, J., Simpson, G.L., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R. et al. (2022) vegan: community ecology package.

Ozanne, C.M.P., Anhuf, D., Boulter, S.L., Keller, M., Kitching, R.L., Körner, C. et al. (2003) Biodiversity meets the atmosphere: a global view of forest canopies. *Science*, 301, 183–186.

Pacheco, J.A. & Mackay, W.P. (2013) *The systematics and biology of the New World thief ants of the genus Solenopsis (Hymenoptera: Formicidae)*. Lewiston, New York, USA: The Edwin Mellen Press.

Posit team. (2024) RStudio: integrated development environment for R.

R Core Team. (2023) R: a language and environment for statistical computing.

Rosumek, F.B., Silveira, F.A.O., de S Neves, F., de U Barbosa, N.P., Diniz, L., Oki, Y. et al. (2009) Ants on plants: a meta-analysis of the role of ants as plant biotic defenses. *Oecologia*, 160, 537–549.

Ryder Wilkie, K.T., Mertl, A.L. & Traniello, J.F.A. (2010) Species diversity and distribution patterns of the ants of Amazonian Ecuador. *PLoS One*, 5, e13146.

Sallé, A., Cours, J., Le Souchu, E., Lopez-Vaamonde, C., Pincebourde, S. & Bouget, C. (2021) Climate change alters temperate forest canopies and indirectly reshapes arthropod communities. *Frontiers in Forests and Global Change*, 4, 710854.

Scheffers, B.R., Phillips, B.L., Laurance, W.F., Sodhi, N.S., Diesmos, A. & Williams, S.E. (2013) Increasing arboreality with altitude: a novel biogeographic dimension. *Proceedings of the Royal Society B: Biological Sciences*, 280, 20131581.

Seidl, R., Thom, D., Kautz, M., Martin-Benito, D., Peltoniemi, M., Vacchiano, G. et al. (2017) Forest disturbances under climate change. *Nature Climate Change*, 7, 395–402.

Seifert, B. (2008) The ants of central European tree canopies (Hymenoptera: Formicidae) – an underestimated population? In: Floren, A. & Schmidl, J. (Eds.) *Canopy arthropod research in Europe: basic and applied studies from the high frontier*. Nuremberg, Germany: Bioform Entomology, pp. 157–173.

Shaw, D.C. (2004) Vertical organization of canopy biota. In: *Forest canopies, physiological ecology*. Burlington, MA, USA: Elsevier Academic Press, pp. 73–101.

Smith, M.R. (1934) Ponerine ants of the genus *Euponera* in the United States. *Annals of the Entomological Society of America*, 27, 557–564.

Snelling, R.R. & Buren, W.F. (1985) Description of a new species of slave-making ant in the *Formica sanguinea* group (Hymenoptera: Formicidae). *The Great Lakes Entomologist*, 18, 11.

Southwood, T.R.E., Moran, V.C. & Kennedy, C.E.J. (1982) The richness, abundance and biomass of the arthropod communities on trees. *The Journal of Animal Ecology*, 51, 635.

Styrsky, J.D. & Eubanks, M.D. (2006) Ecological consequences of interactions between ants and honeydew-producing insects. *Proceedings of the Royal Society B: Biological Sciences*, 274, 151–164.

Thomas, C.C., Tillberg, C.V. & Schultz, C.B. (2020) Facultative mutualism increases survival of an endangered ant-tended butterfly. *Journal of Insect Conservation*, 24, 385–395.

Trager, J.C., MacGown, J.A. & Trager, M.D. (2007) Revision of the Nearctic endemic *Formica pallidefulva* group. *Memoirs of the American Entomological Institute*, Advances in ant systematics (Hymenoptera: Formicidae): homage to E. O. Wilson – 50 years of contributions, 80, 610–636.

Tschinkel, W.R. & Hess, C.A. (1999) Arboreal ant community of a pine forest in northern Florida. *Annals of the Entomological Society of America*, 92, 63–70.

Ulyshen, M.D. (2011) Arthropod vertical stratification in temperate deciduous forests: implications for conservation-oriented management. *Forest Ecology and Management*, 261, 1479–1489.

Ward, P.S. & Blaimer, B.B. (2022) Taxonomy in the phylogenomic era: species boundaries and phylogenetic relationships among north American ants of the *Crematogaster scutellaris* group (Formicidae: Hymenoptera). *Zoological Journal of the Linnean Society*, 194, 893–937.

Warren, R.J., Candeias, M., Lafferty, A. & Chick, L.D. (2020) Regional-scale environmental resistance to non-native ant invasion. *Biological Invasions*, 22, 813–825.

Warren, R.J., Frankson, P.T. & Mohan, J.E. (2023) Global change drivers synergize with the negative impacts of non-native invasive ants on native seed-dispersing ants. *Biological Invasions*, 25, 773–786.

Warren, R.J., McMillan, A., King, J.R., Chick, L. & Bradford, M.A. (2015) Forest invader replaces predation but not dispersal services by a keystone species. *Biological Invasions*, 17, 3153–3162.

Yanoviak, S.P. & Kaspari, M. (2000) Community structure and the habitat templet: ants in the tropical forest canopy and litter. *Oikos*, 89, 259–266.

Yusah, K.M., Fayle, T.M., Harris, G. & Foster, W.A. (2012) Optimizing diversity assessment protocols for high canopy ants in tropical rain forest. *Biotropica*, 44, 73–81.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Data S1. Supporting Information.

How to cite this article: Kirchner, M., Sorenson, C., Blaimer, B. B. & Youngsteadt, E. (2024) Reaching new heights: Arboreal ant diversity in a North American temperate forest ecosystem. *Insect Conservation and Diversity*, 1–12. Available from: https://doi.org/10.1111/icad.12788