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Urbanization reduces prey diversity and promotes dietary divergence in sympatric hornet species

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With 5 figures

Abstract: Urbanization is a major driver of ecological change, but its effects on predator foraging behavior and trophic structure remain underexplored. We investigated how fine-scale urbanization affects prey diversity and composition in two sympatric hornet species, *Vespa analis* Fabricius (Hymenoptera: Vespidae) and *V. simillima* Smith, in central Japan. Using DNA metabarcoding of larval gut contents and high-resolution land cover data, we quantified prey richness and community turnover across urban and nonurban colonies. We found that prey species richness declined significantly with increasing developed land area, particularly within a 500 m radius of the nest. This pattern was observed in both species, suggesting that local habitat structure strongly constrains dietary opportunities. Despite similar declines in prey species richness, the two species presented distinct trophic responses: *V. analis* presented greater dietary turnover between urban and nonurban sites, whereas *V. simillima* showed moderately smaller compositional shifts. Prey order composition revealed species-specific preferences. *V. analis* consumed more Coleoptera and Hymenoptera in urban areas, whereas *V. simillima* selectively foraged on Lepidoptera in nonurban environments. Indicator species analysis and nonmetric multidimensional scaling confirmed stronger compositional shifts in *V. analis*, indicating greater trophic plasticity. Notably, *V. simillima* DNA was frequently detected in *V. analis* gut, raising the possibility of intraguild interactions under urban pressure. Our results highlight how fine-scale urbanization drives trophic divergence in sympatric predators, shaped by prey accessibility, quality, and behavioral flexibility. These findings contribute to the development of trait-based frameworks of urban ecological filtering and predator coexistence, and they have broader implications for understanding trophic responses to landscape modification across diverse ecosystems and predator guilds.

Keywords: dietary analysis; DNA metabarcoding; prey diversity; *Vespa*; ecological niche

1 Introduction

Urbanization is a globally accelerating process that is transforming natural landscapes and generating novel ecosystems (Grimm et al. 2008). These changes frequently lead to habitat simplification, resource fragmentation, and trophic dynamic modifications, having particularly strong consequences for animal behavior and species interactions (Seto et al. 2012; McDonald et al. 2019; Alberti et al. 2020). Because urban environments alter both the availability and accessibility of trophic resources, understanding how animal consumers, particularly predators, respond to these shifts is a central goal in urban ecology and behavioral ecology.

Predatory insects play critical ecological roles as regulators of herbivore populations, competitors, and prey for higher-order consumers. However, their trophic ecology in urbanized environments remains understudied compared with that of pollinators or herbivores (Piano et al. 2020). Urbanization can reduce prey diversity and abundance, increase habitat homogeneity, and constrain foraging options. Under these conditions, cooccurring predators may experience heightened interspecific competition, potentially leading to behavioral divergence and trophic niche segregation (Corcos et al. 2019; Rocha & Fellowes 2020). Despite increasing interest in the ways that environmental filtering influences functional traits, the foraging behavior of sympatric predators across urban gradients remains poorly characterized.

Urban environments often act as ecological filters, selecting for species with particular functional traits, such as generalist diets, behavioral flexibility, or tolerance of human activity (Williams et al. 2009; Díaz et al. 2013). This filtering operates consistently across biogeographic regions and taxonomic groups (Piano et al. 2020). The mechanisms underlying urban ecological filtering, including habitat sim-

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plification, resource fragmentation, and altered disturbance regimes, are universal drivers of community assembly in human-modified landscapes. These characteristics make urban systems into valuable model systems for the understanding of broader patterns of environmental change. These trait-based processes can influence species coexistence and functional diversity, which has cascading effects on the trophic structure and ecological resilience. In predator communities, urban environments may selectively favor generalists or behaviorally flexible foragers while constraining more specialized or inflexible species. Therefore, investigating the ways that closely related predators differ in their trait-mediated responses to urban constraints provides insight into coexistence mechanisms and the assembly of predator guilds in changing landscapes. Dietary divergence is a key mechanism that facilitates coexistence among closely related or ecologically similar species. When prey resources are limited or spatially structured, differences in prey selection, foraging plasticity, or risk tolerance may reduce overlap and stabilize predator coexistence (MacArthur & Levins 1967; Chesson 2000). Such behavioral traits can be influenced by both evolutionary history and plastic responses to local conditions. Recent studies suggest that, in urban contexts, trait-mediated responses, such as increased diet breadth or altered prey selectivity, are critical for predator persistence (Straka et al. 2025). However, empirical evidence for species-specific trophic responses to urbanization in natural predator guilds remains limited.

Hornets (*Vespa* spp.) are apex insect predators, characterized by substantial dietary generalism and flexible foraging strategies. In East Asia, sympatric species such as *Vespa analis* and *V. simillima* are commonly found at nonurban forest edges and, increasingly, within urban areas. Prior studies have suggested behavioral differences between them, including dominance over sugary resources, territoriality, and potential dietary breadth, with *V. analis* typically exhibiting stronger competitive dominance than *V. simillima* (Matsuura & Yamane 1990; Ono 1995). However, their prey use during the larval stage, a key trophic phase that drives colony success, has rarely been studied in urban contexts. In particular, it remains unclear whether these species shift their foraging strategies in response to prey limitations or anthropogenic environmental pressures.

DNA metabarcoding has emerged as a powerful tool for assessing predator diets with high resolution, particularly in complex food webs where visual identification is difficult or incomplete (Clare 2014; Takahashi et al. 2016; Lefort et al. 2020). This approach enables the accurate characterization of prey communities across spatial gradients and diverse landscape contexts and has been successfully applied to the study of trophic plasticity in social wasps, including *Vespa, Vespula*, and *Polistes* species (Schmack et al. 2021; Wilson et al. 2023; Saga & Fujioka 2025; Xie et al. 2025). The methodology shows broad transferability to other predator guilds across various habitat modification scenarios.

In this study, we examined how urbanization influences the dietary richness and composition of two sympatric hornet species across urban and nonurban habitats in central Japan. Using DNA metabarcoding of larval gut contents, we addressed two key questions: (1) How does fine-scale urban land cover influence prey richness and composition? (2) Do the two species exhibit divergent trophic responses to urbanization? We predicted that *V. simillima*, known for its generalist diet, would exhibit greater dietary flexibility in urban areas, whereas *V. analis*, which is considered competitively dominant, would maintain more consistent prey preferences across environments.

Our study adopted a novel method that combines high-resolution DNA metabarcoding of predator diets with fine-scale land-use data analysis. This approach enabled us to directly link prey community structure to specific urban landscape features at ecologically relevant spatial scales, providing unprecedented insight into how habitat modification shapes trophic interactions in sympatric predators. Unlike previous studies that have examined either urban effects on insect diversity or predator diets separately, our integrated framework provides a mechanistic understanding of how environmental filtering translates into dietary shifts.

2 Materials and methods

2.1 Sample collection and land-use classification

Larvae of *V. analis* and *V. simillima* were collected between August 3 and September 29, 2023, from nests in the cities of Kobe and Itami, Hyogo Prefecture, Japan (Fig. 1). We sampled five nests of *V. analis* and four nests of *V. simillima* in urban areas and four nests of each species in nonurban, suburban, or forest-edge areas. From each nest, three final-instar worker larvae were sampled for gut content analysis (n = 51 larvae in total). The land-use metrics for each nest location were calculated using QGIS version 3.22.8 and the High Resolution Land-Use and Land-Cover Map (JAXA, 2022), which has a spatial resolution of 10 m. Developed land was defined as the sum of built-up area and road area. We calculated the proportion of developed land within a 500 m radius of each nest centroid. Colonies were classified as urban or nonurban on the basis of their geographic location. The urban colonies (n = 9) were located in the densely developed southern areas of the city of Kobe, whereas the nonurban colonies (n = 8) were located in the less developed northern mountainous regions (Fig. 1). This classification resulted in urban sites having 62.8–96.4% developed land and nonurban sites having 0.0001–51.3% developed land. All colonies of the same species were separated by >1 km, ensuring adequate independence of dietary samples within the species. Specifically, *V. analis* colonies were all separated by >1.5 km, whereas *V. simillima* colonies included one pair at 1 km separation and all others at >1.5 km. This separation

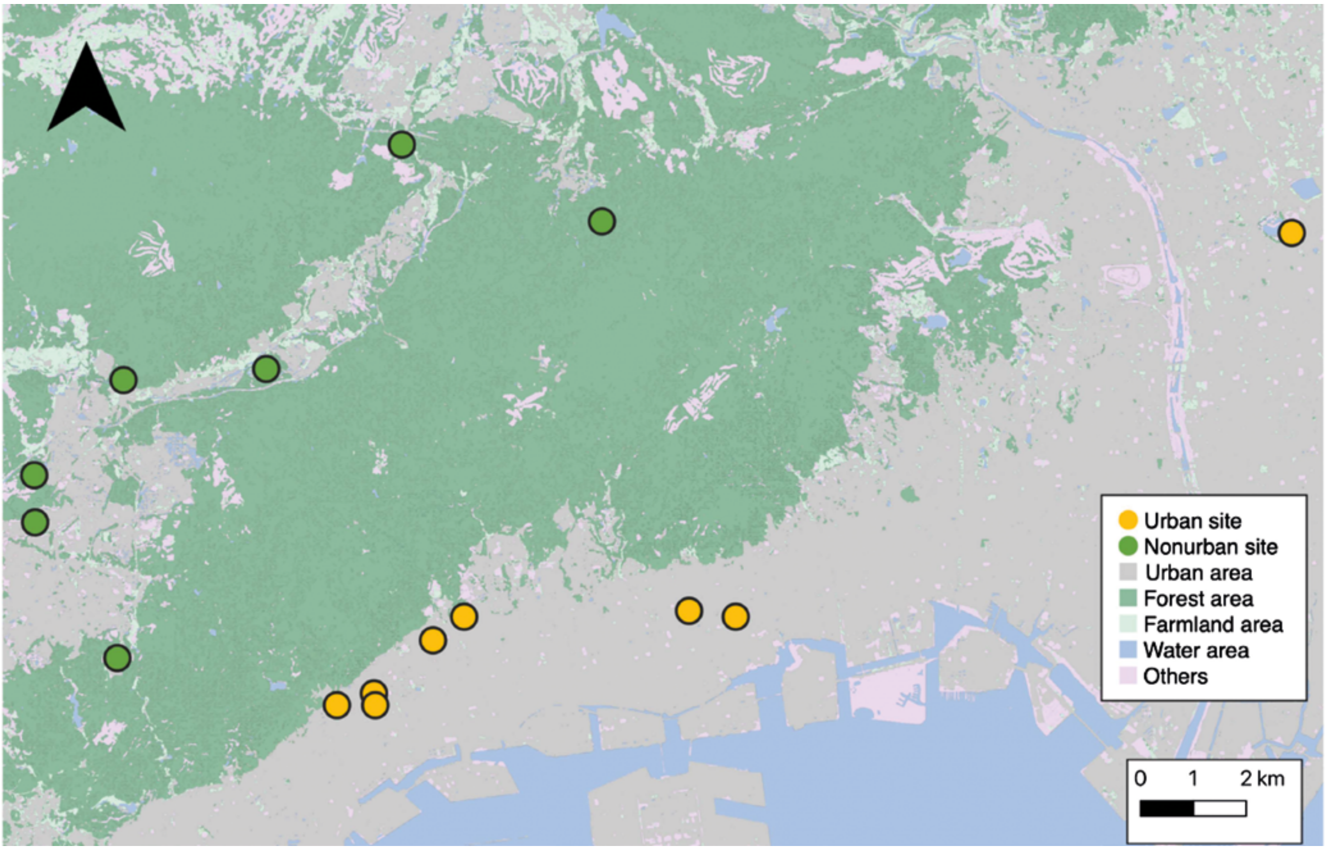

Fig. 1. Map of the study sites in Kobe and Itami, Hyogo Prefecture, Japan. Circles indicate the nest locations of *V. analis* and *V. simillima* colonies sampled between August and September 2023. Background colors represent land-use categories based on the High Resolution Land-Use and Land-Cover Map (JAXA, 2022): green = forest/vegetation, gray = developed areas (built-up and roads), and blue = water bodies. The 500 m buffer zones used for urbanization analysis are shown around each nest location.

strategy is well supported by previous findings that 99.2% of *V. analis* and 92.3% of *V. simillima* foraging occurs within 1.5 km of the nest, with 80% of *V. simillima* foraging occurring within 1 km (Matsuura & Yamane 1990). While one *V. simillima* colony and one *V. analis* colony were located within 500 m of each other, this does not affect the independence of our analyses, as we compared dietary patterns within species across habitats and between species within the same habitat type.

From the 17 hornet colonies sampled, three nests (including one in Itami) were collected from public property where exact nest coordinates were available, and these were used as buffer centroids for land-use analysis. For the remaining 14 colonies, which were located on private residential property, the municipal authority overseeing wasp control in Kobe requested that we not use specific addresses. In these cases, we generated buffers centered on the geographic centroid of the corresponding postal code area. In Japan, postal code districts correspond to official administrative boundaries (cho/machi level) as established by municipal governments. In Kobe, these districts range in area from 0.001 to 17.80 km^2 (median: 0.18 km^2, 25th–75th percentile: 0.07–0.57 km^2). Although the postal code areas vary in size, none of the sampled nests were located near the outer boundaries of their respective postal zones, ensuring that the centroid-based buffers captured the relevant local environment. This approach was applied consistently across all spatial scales (500 m, 1,000 m, and 3,000 m) and was used to balance spatial accuracy with ethical and legal compliance.

2.2 DNA extraction and amplification

We followed the protocol of Takahashi et al. (2016) to extract undigested gut contents from larval samples. Approximately 2 mm^3 of tissue was collected from the anterior portion of the gut. DNA was extracted using the NucleoSpin Tissue kit (MACHEREY-NAGEL, Germany) according to the manufacturer's protocol.

The first PCR used the universal primers 1st-IntF (Leray et al. 2013) and 1st-HCOmR (Folmer et al. 1994) to amplify a region of the mitochondrial COI gene. Blocking primers specific to *V. analis* (5'-GGA TGA ACT TTA TAT CCC CCT CTC TCA TCA ATT ACT GGA CAT AAT AGA C-3'/3SpC3/) and *V. simillima* (5'-TCC TCC TTT ATA TCA ATT ACT GGA CAT AAT TCA CCT TCC GTA G-3'/3SpC3/) were designed using Primer-BLAST (NCBI) to reduce host DNA amplification. The optimal blocking primer concentrations (0.2–0.5 μM) were determined using gradient PCR. All reactions were performed using TaKaRa Ex Taq Hot Start polymerase (Takara Bio Inc., Japan). The PCR conditions were as follows: initial denaturation at 94 °C for 2 min; 35 cycles of 94 °C for 30 s, 67 °C for 15 s, 52 °C for 30 s, and 72 °C for 30 s; and final extension at 72 °C for 5 min.

A second PCR with indexed primers was conducted using KOD FX Neo polymerase (TOYOBO, Japan) in 20 μL volumes (1.0 U/μL). The PCR conditions were as follows: 94 °C for 2 min; 12 cycles of 98 °C for 10 s, 60 °C for 30 s, and 68 °C for 30 s; and a final extension at 68 °C for 2 min. The PCR products were purified using VAHTS DNA Clean Beads (Vazyme) at a 1:1 volume ratio. Negative controls (no-template) were included in the first PCR only. Electrophoresis of the first PCR products confirmed the absence of amplification in the negative controls. Only samples that showed successful amplification in the first PCR were submitted for sequencing.

2.3 Sequencing and bioinformatics

Sequencing was performed on an Illumina MiSeq platform using the MiSeq Reagent Kit v3 (2×300 bp). The raw reads were processed using QIIME2 (v2020.8) with the DADA2 plugin for denoising and chimera removal. Representative sequences and OTUs were generated. Taxonomic identification was performed using BLASTN (v2.13.0) against the NCBI GenBank database.

OTUs with fewer than 10 reads were discarded to minimize false positives. Read counts were normalized using the rrarefy function in the vegan package (R) on the basis of rarefaction. Sequences with ≥97% similarity to a GenBank reference were assigned to species when bit scores were unambiguous. When multiple species were matched at similar similarity levels, the most specific taxonomic level supported by the bit score was assigned. Sequences with <97% similarity or from humans, mites, or the host species were excluded as contaminants.

2.4 Statistical analyses

2.4.1 Urbanization and prey richness

We constructed separate linear models (LMs) to assess the effects of urbanization at three spatial scales (500 m 1000 m, and 3000 m) on prey species richness per colony. Because the urbanization metrics at different scales are likely to be spatially correlated, we did not include multiple scales in the same model. Instead, we compared the AIC, R^2, and adjusted R^2 values across the three single-scale models to determine which spatial scale best explained the variation in prey richness. The 500 m model had the best performance and was therefore used in all subsequent analyses (see Results and Table S1).

To test species-specific responses, we fitted a linear model with fixed effects for species, urban cover at 500 m (urban 500 m), and their interactions. Slope comparisons were performed using the emtrends function from the emmeans package to estimate marginal trends and confidence intervals.

2.4.2 Species-specific prey preferences

To compare prey order frequencies between *V. analis* and *V. simillima*, we used chi-square tests on 2×N contingency tables. Standardized residuals >|1.96| were considered to be significant contributors. Furthermore, we further evaluated differences in three focal orders (Coleoptera, Hymenoptera, Lepidoptera) using 2×4 tables (species × environment). Holm's sequential Bonferroni correction adjusted for multiple comparisons was used. Fisher's exact test was used on a 2×2 table of species and habitat-specific unique prey to test for differences in foraging behavior in urban environments.

2.4.3 Community composition

We used nonmetric multidimensional scaling (NMDS; Bray–Curtis dissimilarity) to visualize the prey community structure at the larval level. PERMANOVAs (adonis2 function, vegan) with 999 permutations were used to test the effects of species, the environment, and their interactions. Pairwise PERMANOVAs were used to test within-species differences. To compare the magnitude of urban–nonurban shifts, we calculated Euclidean distances between group centroids and tested differences via bootstrap resampling (999 iterations) and two-sample t tests.

Indicator species analysis was conducted using multiple combinations from the indicspecies package, grouping samples into four combinations (species × environment). Taxa with significant IndVal values ($P < 0.05$, 999 permutations) were considered group-specific indicators.

3 Results

3.1 Prey richness and taxonomic coverage

A total of 153 prey species (11 orders, 62 families) were identified from *V. analis* colonies, and 181 species (21 orders, 78 families) were identified from *V. simillima* colonies. This finding indicates a broader prey spectrum in *V. simillima* colonies.

3.2 Fine-scale urbanization best predicts prey richness

Among the three spatial scales tested, the 500-m model had the lowest AIC (130.39) and the highest adjusted R^2 (0.646), indicating that it best explained the variation in prey species richness (see Table S1). This model also revealed a significant negative relationship between urbanization and prey richness ($\beta = -40.898$, $P < 0.001$) and was used for subsequent analyses.

A multiple linear model including species, the 500 m urbanization metric and their interaction explained a substantial proportion of the variation in prey richness (adjusted $R^2 = 0.66$, $P < 0.001$; Fig. 2). The trend analysis revealed that prey richness significantly decreased with increasing urbanization in both *V. analis* (slope = −34.1, 95% CI = [−57.4, −10.7]) and *V. simillima* (slope = −42.3, 95% CI = [−64.6, −20.0]). Post hoc comparisons revealed significant differences between urban and nonurban environments for both species but no significant difference between species within each habitat type (Fig. 3). These results suggest that even a 25% increase in developed land area could lead to a decline of 8–11 prey species, indicating ecologically meaningful effects at realistic levels of urbanization.

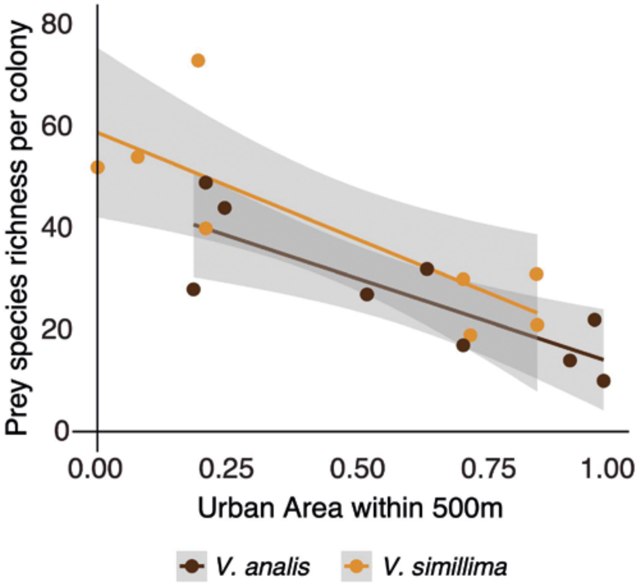

Fig. 2. Relationship between prey species richness and the proportion of developed land area within a 500 m radius of hornet nests. Points represent individual colonies of *Vespa analis* and *V. simillima*. Regression lines show values fitted from linear models with 95% confidence intervals (shaded areas). Both species showed significant negative relationships between urbanization and prey richness, with similar slopes but different intercepts.

3.3 Species-specific differences in prey order composition

The prey order composition by species and habitat is summarized in Fig. 4, highlighting distinct foraging patterns. A chi-square test revealed significant differences in prey order proportions between *V. analis* and *V. simillima* ($\chi^2 = 23.97$, df = 6, $P < 0.001$). The standardized residuals indicated that *V. analis* consumed Coleoptera (+3.22) and Hymenoptera (+2.34) more frequently than expected, whereas *V. simillima* consumed Coleoptera and Hymenoptera less frequently than expected (standardized residuals: –3.22 and –2.34, respectively). Conversely, *V. simillima* consumed Lepidoptera more frequently than expected (+2.56), whereas *V. analis* consumed Lepidoptera less frequently than expected (–2.56).

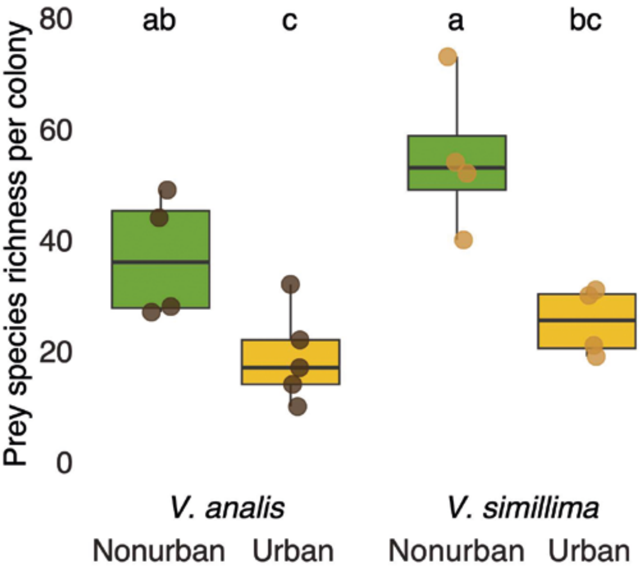

Fig. 3. Comparison of prey species richness between hornet species and habitat types. Box plots show median values (bold horizontal lines), interquartile ranges (boxes), and individual colony values (points) for *V. analis* and *V. simillima* in nonurban and urban environments. Different letters indicate significant differences among groups on the basis of post hoc comparisons ($P < 0.05$). Both species presented significant reductions in prey richness in urban habitats compared with nonurban habitats, with no significant difference between species within each habitat type.

Chi-square tests for individual orders confirmed significant differences (Coleoptera: $P = 0.010$; Hymenoptera: $P < 0.001$; Lepidoptera: $P = 0.016$), and all the differences remained significant after Holm's correction. For example, *V. analis* was overrepresented in Coleoptera in both habitats, whereas *V. simillima* was underrepresented in nonurban areas. *V. analis* in urban environments showed strong overrepresentation of Hymenoptera (+4.15), suggesting urban adaptation to this prey group.

3.4 Unique prey species by species and habitat

Fisher's exact test revealed significant differences in the number of prey species uniquely consumed by each species across habitats ($P = 0.016$, odds ratio = 2.02, 95% CI = 1.11–3.68). *V. analis* consumed 41 unique species in urban colonies and 64 in nonurban colonies, whereas *V. simillima* consumed 32 unique species at urban sites and 101 in nonurban areas. This suggests greater urban trophic flexibility or specialization in *V. analis*.

3.5 Community composition and indicator taxa

Non-metric multidimensional scaling (NMDS; Bray–Curtis dissimilarities) revealed distinct clustering by species and habitat (Fig. 5). The final two-dimensional solution had a stress value of 0.221, indicating an acceptable fit for interpretation.

PERMANOVA revealed a significant species × habitat interaction effect on prey composition ($F_{1,50} = 27.94$, $R^2 = 0.64$, $P < 0.001$). Within-species analyses revealed significant differences between urban and nonurban colonies in both *V. analis* ($F_{1,25} = 28.58$, $R^2 = 0.53$, $P < 0.001$) and *V. simillima* ($F_{1,25} = 35.55$, $R^2 = 0.62$, $P < 0.001$). PERMANOVA within habitats revealed significant species differences only in urban colonies ($F_{1,25} = 36.89$, $R^2 = 0.60$, $P < 0.001$), not in nonurban colonies ($P = 0.189$).

Bootstrap analysis of the NMDS group centroids revealed a greater compositional shift across environments in *V. analis* (mean $\Delta = 1.137$, SD = 0.114) than in *V. simillima* (mean $\Delta = 0.959$, SD = 0.088) ($t = 39.112$, $P < 0.001$).

Indicator species analysis revealed that prey taxa were strongly associated with each species–habitat group (IndVal > 0.25, $P < 0.05$). For example, *Truljalia hibinonis* Matsumura (Orthoptera: Gryllidae) and *Sphinx constricta* Butler (Lepidoptera: Sphingidae) were indicators of *V. simillima* colonies in nonurban areas, whereas *Apis mellifera* Limmeaus (Hymenoptera: Apidae) and *Graphium sarpedon* Linnaeus (Lepidoptera: Papilionidae) were indicators of urban *V. simillima* colonies. *Polistes jokahamae* Radoszkowski (Hymenoptera: Vespidae) and *Apis cerana* Fabricius (Hyemenoptera: Apidae) were significant indicators of urban *V. analis* colonies, whereas *Meimuna opalifera* Walker (Hemiptera: Cicadidae) and *Vespa crabro* Linnaeus (Hyemenoptera Vespidae) were associated with nonurban *V. analis* colonies. A full list of indicator taxa is available in Supplementary Table S2.

4 Discussion

Urbanization is a dominant driver of biodiversity loss, but its effects on trophic dynamics and predator foraging strategies remain incompletely understood. Our study revealed that fine-scale urbanization significantly reduces prey richness and alters prey composition in two sympatric hornet species, *V. analis* and *V. simillima*, but in contrasting ways. Using DNA metabarcoding of larval gut contents, we demonstrated that urban land cover within 500 m of hornet nests is a strong predictor of dietary diversity and that species-specific trophic divergence is intensified in urban environments. These findings contribute to emerging trait-based frameworks for understanding species' responses to environmental filtering in urban ecosystems.

4.1 Fine-scale urbanization reduces prey richness in both species

The superior performance of the 500 m model supports the idea that urbanization within a relatively small radius most strongly influences prey richness for wasp colonies. This fine-scale effect likely reflects the foraging range of workers and highlights the ecological relevance of local habitat conditions. Our decision to adopt a single-scale model also avoids potential multicollinearity among spatial predictors.

Prey richness declined with increasing developed land area at the 500 m scale, suggesting that local foraging environments near hornet nests strongly constrain access to dietary resources (Fig. 2). This aligns with previous findings showing that fine-grained habitat heterogeneity drives arthropod availability in urban areas (Piano et al. 2020). The relationship between prey richness and urbanization was best explained by a linear model at the 500 m scale, suggesting a consistent decline in prey diversity with increasing habitat modification rather than a threshold response. Although *V. simillima* is considered a generalist with behavioral flexibility (Ono 1995), it does not demonstrate greater resistance to urban prey loss than *V. analis* does, challenging the assumption that dietary breadth inherently buffers generalist predators from environmental stress.

These results underscore the ecological importance of spatial scale when evaluating the impacts of urbanization on hornet foraging behavior. While many landscape-scale studies rely on buffer zones of 1,000 m or more, our findings demonstrate that the proportion of developed land area within just 500 m of the nest was the strongest predictor for prey richness. This finding supports ecological theory, suggesting that fine-scale habitat features often exert stronger influences on central-place foragers than broader landscape-level factors do (Turner et al. 2001; McGarigal & Cushman 2002).

Our results also align with previous behavioral observations on the foraging distances of hornets. For example, *Vespa mandarinia* workers have been reported to fly up to 8 km from the nest (Matsuura & Sakagami 1973), but the majority of foraging activity occurs within a 1–2 km radius. Similarly, Matsuura & Yamane (1990) reported that other hornets tend to forage predominantly within 1 km of their nests. In *Vespa velutina*, radio-frequency identification (RFID) tracking data revealed that most individuals forage within a 500–800 m radius, with return rates dropping steeply beyond 2,000 m (Poidatz et al. 2018). While these studies assessed movement capacity and behavior, our study is novel in that the prey community actually consumed is most strongly shaped by environmental conditions within 500 m of the nest. This provides a complementary and biologically grounded perspective on the foraging range, emphasizing realized ecological interactions rather than potential movement limits.

4.2 Contrasting prey preferences and foraging strategies

The two hornet species differed markedly in prey order composition and unique prey usage, particularly in urban environments (Fig. 4). *V. analis* showed elevated consumption of Coleoptera and Hymenoptera, especially in urban areas, whereas *V. simillima* preferentially consumed Lepidoptera in nonurban habitats. These patterns deviate from expectations on the basis of dominance hierarchies; although *V. analis* has been described as competitively superior (Matsuura 2004), its diet in urban settings has shifted toward harder-bodied, potentially lower-quality prey. *V. simillima* retained a bias toward soft-bodied prey (especially Lepidoptera) across environ-

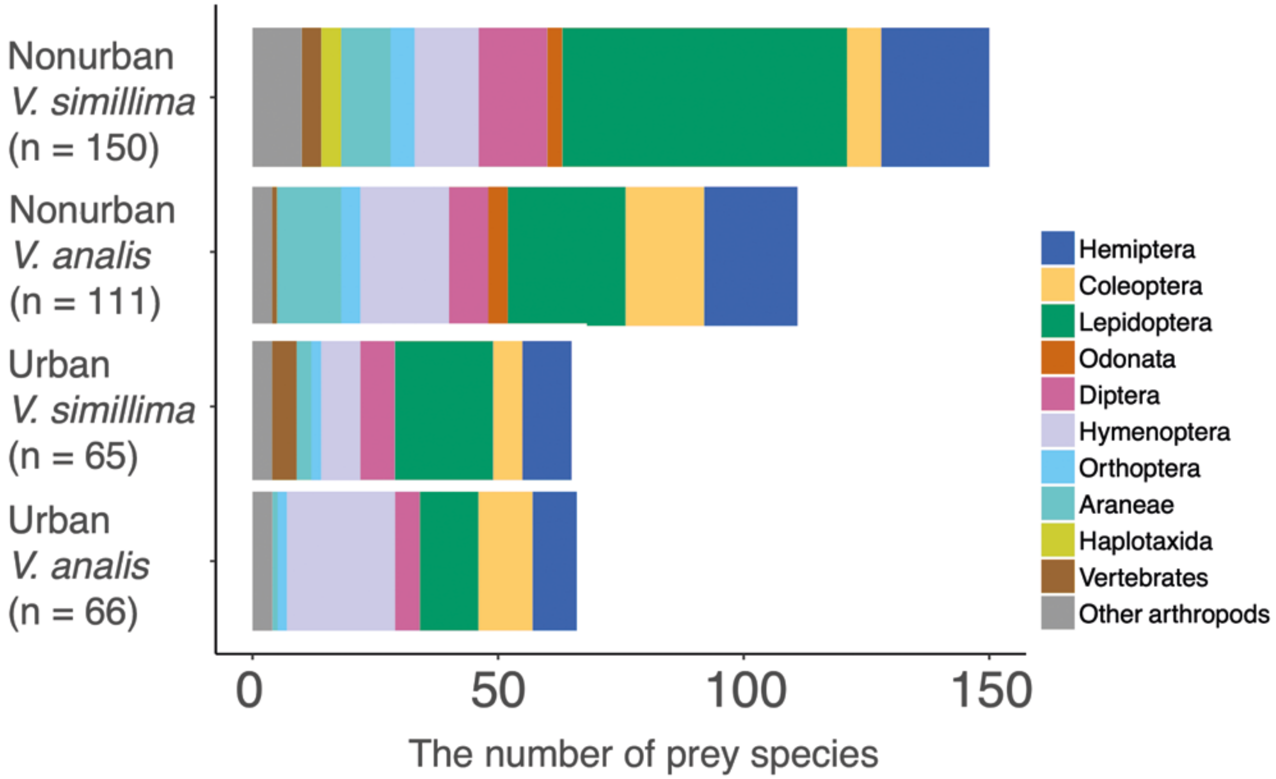

Fig. 4. Prey order composition by species and habitat type. Stacked bar charts show relative proportions of different prey orders consumed by *V. analis* and *V. simillima* in urban and nonurban environments. The numbers above the bars indicate the total number of prey records. Chi-square analysis revealed significant differences in prey order preferences between species, with *V. analis* showing greater consumption of Coleoptera and Hymenoptera, particularly in urban areas, whereas *V. simillima* preferentially consumed Lepidoptera in nonurban environments.

ments, yet its overall diet composition shifted significantly between urban and nonurban sites.

Prey quality here refers to the net energy gain per unit handling time or risk (Stephens & Krebs 1986). Soft-bodied Lepidoptera larvae offer high nutritional returns with minimal processing costs, whereas adult Coleoptera and Hymenoptera often have chitinous exoskeletons or defensive traits that increase foraging cost or danger. Accessibility is also critical: urban environments may simplify vegetation structure through two complementary mechanisms: (1) reduced plant species richness and consequent homogenization of community composition and (2) uniform management practices (e.g., increased mowing frequency) that create structurally simplified, even-aged vegetation. This management-driven homogenization likely increases the visibility and accessibility of surface-active prey. For example, the urban development surrounding rice paddies has been shown to reduce both plant and butterfly species richness through more frequent mowing, thereby creating more open and predictable foraging patches (Uchida et al. 2018). This habitat simplification may partially explain *V. analis*'s observed shift toward more accessible prey under urban constraints.

These observations refine our understanding of generalist predator strategies. Generalism may entail not only broad taxonomic diets but also the ability or willingness to shift selectivity in response to prey availability. In this context, *V. simillima*'s generalism appears to be conservative and driven by prey-quality, whereas *V. analis* demonstrates greater behavioral flexibility. These distinctions help explain their divergent responses to urbanization.

4.3 Species-specific responses to prey turnover and trait filtering

Prey community turnover across urban–nonurban gradients was greater in *V. analis* than in *V. simillima*, with *V. simillima* showing relatively smaller compositional changes. NMDS and PERMANOVAs confirmed that *V. analis* shifted compositionally between habitats to a greater extent, and only urban environments yielded significant species-level dietary divergence (Fig. 5). This finding supports the idea that urbanization amplifies trophic niche differentiation by acting as a trait filter, selecting for behavioral, morphological, or ecological traits that confer resilience under stress (Williams et al. 2009; Díaz et al. 2013).

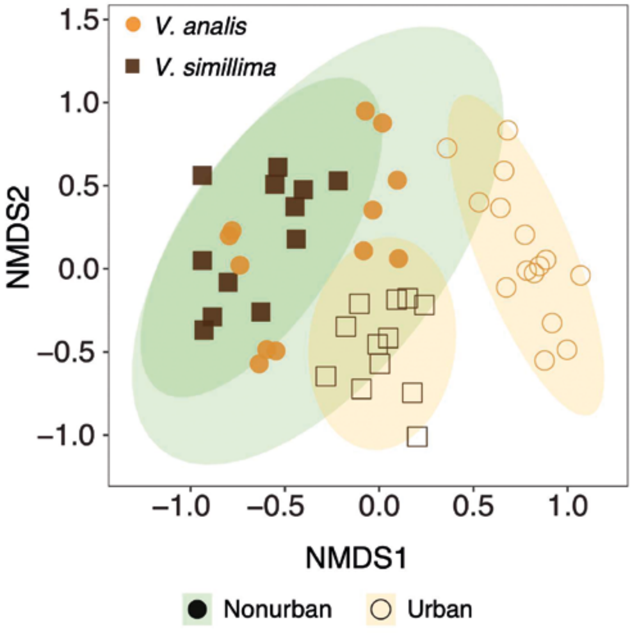

Fig. 5. Non-metric multidimensional scaling (NMDS) ordination of prey community composition on the basis of Bray–Curtis dissimilarities. Points represent individual larvae, with symbols indicating species (*V. analis* = circles, *V. simillima* = squares) and habitat type (nonurban = filled, urban = open). The ellipses show 95% confidence intervals around the group centroids. Stress value = 0.221. PERMANOVA revealed a significant species × habitat interaction ($F_{1,50} = 27.94$, $R^2 = 0.64$, $P < 0.001$), indicating divergent dietary responses to urbanization between the two hornet species.

Furthermore, the indicator taxa reflected these trends. Urban *V. analis* colonies were associated with human-adapted or synanthropic prey (species adapted to human environments), such as *Apis cerana* (eastern honeybee), *Polistes jokahamae* (a paper wasp species), and *Hermetia illucens* Linnaeus (Diptera: Stratiomyidae). In contrast, *V. simillima* in nonurban settings was linked to herbivorous and soft-bodied prey, including *Truljalia hibinonis* (a cricket species) and *Sphinx constricta* (Japanese privet hawk moth), suggesting a stronger reliance on natural, structurally complex habitats. These patterns reinforce the view that trait filtering under urbanization can reshape predator diets not only through prey loss but also via selection on the foraging traits of predators themselves.

4.4 Intraguild interactions and opportunistic behavior

A particularly intriguing result was the consistent detection of *V. simillima* DNA in *V. analis* larval guts, predominantly in urban colonies. While DNA metabarcoding cannot distinguish between active predation and scavenging, this finding raises the possibility of intraguild predation or resource cannibalism – interactions known to intensify under high competition or low prey availability (Polis et al. 1989). *V. analis* may engage in opportunistic foraging for larvae and pupae under urban pressure. This finding is consistent with its apparent dietary flexibility and could represent a behavioral adaptation to urban prey depletion.

Such interactions are rarely reported in social wasps but may become more prevalent under urban conditions where spatial overlap and resource limitation are pronounced. Future research using observational data or stable isotopic signatures may help disentangle the nature of these interactions and their fitness consequences.

4.5 Ecological implications and coexistence under urban pressure

The differential responses of *V. analis* and *V. simillima* suggest that sympatric predators may coexist through trophic partitioning along prey trait axes that are themselves filtered by the environmental context. Our findings challenge the classical view that competitive superiority directly translates into resource dominance under stress. Rather, adaptability in prey choice and behavioral flexibility may better predict predator success in novel environments.

These insights have implications for understanding coexistence mechanisms and community assembly under global change. They also demonstrate that even among ecologically similar predators, trait-based responses to urban stress can markedly diverge, potentially influencing the structure and stability of food webs. Moreover, the ecological roles of these hornets as predators, competitors, and providers of ecosystem disservices may shift with their diets, underscoring the value that trophic-level monitoring has in urban ecological assessments.

4.6 Methodological transferability and broader applications

The methodological framework developed here has broad applicability across landscape modification scenarios and predator guilds. DNA metabarcoding of predator diets has been effective for studying responses to various environmental changes, from agricultural systems to urban environments (Piñol et al. 2014). The spatial scale optimization approach, which systematically compares buffer radii to identify the most predictive scale, addresses a critical gap in landscape ecology studies, where scale selection is often arbitrary or based solely on species' maximum movement capacity rather than ecological relevance.

Our findings of trophic divergence under urbanization align with global patterns documented across diverse taxa and geographic regions. The urbanization predation paradox identified by Fischer et al. (2012), whereby predator numbers increase while predation rates decline in urban areas, provides a broader context for understanding the dietary shifts we observed. Meta-analyses have revealed consistent reductions in predation pressure along urban gradients (Eötvös et al. 2018), whereas studies from diverse geographic regions have demonstrated similar shifts in predator foraging strategies and prey selectivity (Rocha & Fellowes 2020).

The contrasting responses between *V. analis* and *V. simillima* mirror patterns observed in other predator guilds, where urbanization acts as a trait filter that selects for behavioral flexibility over competitive dominance, with specialist predators declining while generalists persist (Mata et al. 2017; Rocha & Fellowes 2020). Furthermore, the fine-scale effect of urbanization on foraging behavior that is documented here (500 m radius) corresponds to findings from diverse predator groups, including ground-dwelling arthropods, urban garden predators, and flower-visiting predators and parasitoids across multiple spatial scales (Corcos et al. 2019), suggesting that local habitat modifications universally constrain central-place foragers, regardless of their taxonomic affiliation or geographic location.

4.7 Limitations and future directions

While DNA metabarcoding offers broad taxonomic resolution, it does not measure prey biomass or distinguish between active predation and scavenging. The absence of parallel environmental prey surveys also limits our ability to disentangle preference from availability. Future studies integrating environmental DNA, behavioral observations, and stable isotope analyses could refine our understanding of foraging ecology and trophic plasticity under urbanization.

A notable limitation of our study is its focus on a single seasonal period during late summer and early autumn. Prey availability and composition likely fluctuate seasonally, potentially altering foraging strategies and dietary preferences throughout the active period of hornet colonies. Lepidopteran larvae, for example, show pronounced seasonal peaks that may influence the relative benefits of prey selectivity versus opportunism. Furthermore, hornet colonies have different nutritional requirements across their developmental cycles, from colony establishment to the reproductive phase. Future longitudinal studies spanning spring to autumn would provide valuable insights into how seasonal variation in prey communities interacts with urbanization effects to shape predator foraging behavior and resource partitioning across temporal scales.

Spatially replicated studies across regions and seasons that incorporate additional vespid species would help generalize our findings. Moreover, linking diet diversity to colony fitness outcomes could reveal how trophic responses scale to demographic consequences, which is a key question in predator ecology. This temporal dimension could reveal whether the observed trophic divergence between species is consistent year-round or represents a seasonal response to particular resource constraints.

5 Conclusion

In summary, our findings demonstrate that urbanization reduces prey richness and promotes dietary divergence in two sympatric hornet species, but the nature and magnitude of these effects differ markedly between species. *V. analis* exhibits greater compositional flexibility, potentially at the cost of prey quality, whereas *V. simillima* shows relatively smaller compositional changes in response to urbanization. These contrasting responses highlight the importance of trait filtering, prey quality, and behavioral flexibility for predator ecology in human-altered landscapes. Our results contribute to a growing body of evidence demonstrating that urbanization fundamentally alters trophic interactions through consistent mechanisms that operate across diverse ecosystems and taxonomic groups. The fine-scale spatial effects (500 m radius) and species-specific dietary responses documented here reflect broader patterns in the ways that environmental filtering shapes predator communities in human-modified landscapes. By integrating spatial habitat data with molecular diet analysis, our study not only advances the mechanistic understanding of hornet foraging ecology. It also provides a transferable framework for investigating how central-place foragers respond to landscape modifications across different biomes and urban contexts. This integrative approach contributes to a deeper understanding of trophic adaptation under urbanization, an increasingly vital insight in our rapidly changing world.

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