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RESEARCH ARTICLE

# Network dynamics revealed from eDNA highlight seasonal variation in urban mammal communities

**Jane Hallam** | **Nyeema C. Harris**

Applied Wildlife Ecology Lab, Yale School of the Environment, Yale University, New Haven, Connecticut, USA

**Correspondence**
Jane Hallam
Email: jane.hallam@yale.edu

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## Abstract

1. Urban ecosystems are expanding rapidly, significantly altering natural landscapes and impacting biodiversity.
2. Here we explore seasonal variation in mammal diversity using environmental DNA (eDNA) from soil samples collected during winter and summer across 21 urban parks in Detroit, Michigan. We estimated gamma (regional), alpha (local) and beta (compositional change) diversity to determine if seasonal shifts, reflecting winter scarcity and summer abundance in mammal community composition and human activity, could be detected using eDNA. We expected that larger parks would exhibit greater diversity and higher seasonal turnover, consistent with the species-area relationship (SAR) and hypothesised that increased summer resource availability would lead to decreased network density as species disperse more broadly.
3. We found that urban parks show subtle, park-specific changes in community composition influenced by both ecological and anthropogenic factors, with species including striped skunk, brown rat and groundhog responsible for the observed seasonal variation. Consistent with the SAR, larger parks supported higher species richness and diversity. Ecological network analysis, focusing on metrics such as clustering coefficient and network density, revealed a decrease in the overall connectivity and cohesiveness of species interactions from winter to summer, supporting our hypothesis of broader species dispersal during resource-rich periods. Notably, human DNA was prevalent in all parks, alongside detections of pig and cow eDNA, potentially reflecting human disturbance and anthropogenic food inputs.
4. Our findings underscore the efficacy of eDNA analysis in capturing urban mammal community dynamics, the impact of human activities on biodiversity and its potential as a valuable tool for urban ecological research. Ultimately, enhancing monitoring capacity aids in conservation and urban planning efforts that will promote human-wildlife coexistence and preserve the socio-ecological benefits stemming from biodiversity across cityscapes.

**KEYWORDS**
biodiversity, environmental DNA, human-wildlife interactions, mammal community dynamics, urban ecology, green spaces

## 1 | INTRODUCTION

Urbanization is a dominant global trend, with over half the world's population now living in cities (Derby Lewis et al., 2019). While urban areas comprise 2% of land in North America, cities are projected to continue expanding significantly in the coming decades (Simkin et al., 2022). By 2030, urban land cover is expected to increase by around 1.2 million square kilometres globally, with North America contributing a notable portion of this growth (Mahtta et al., 2022). This transformation replaces natural landscapes with built environments, altering biodiversity and ecosystem processes through habitat fragmentation, heat islands and road networks (Chen et al., 2020; Li et al., 2022). For mobile species, urban areas may function as refuges as well as hazards, a dichotomy influenced by the enhanced availability of food, predator release, and increased mortality risks (Bateman & Fleming, 2012; Fehlmann et al., 2021). The built environment often promotes species turnover, or the replacement of species within a community over time or space, favouring urban-adapted species while displacing specialists to less disturbed peripheral areas (Gallo et al., 2017). Over time, urban ecosystems can undergo biotic homogenization, wherein generalist species become dominant at the expense of community diversity (Alberti et al., 2020; Haight et al., 2023; Lowry et al., 2013). Such shifts in community composition can have significant implications for ecosystem stability and function (Hahs et al., 2023; McKinney, 2006).

Urbanization has been shown to simplify species co-occurrence networks, potentially reducing ecological redundancy and making communities more vulnerable to environmental disturbances (Pechlivanis et al., 2024). Anthropogenic disturbances can restructure mammal interaction networks, altering spatial co-occurrence patterns and modifying community dynamics (Curveira-Santos et al., 2024). Despite the challenges of coexistence with humans, green spaces in urban environments have the potential to act as oases for wildlife, supporting biodiversity and maintaining ecological connectivity (Ives et al., 2016; Rega-Brodsky et al., 2022). The species-area relationship (SAR) suggests that larger green spaces generally support higher species diversity due to the availability of more extensive habitats and resources (Kallimanis et al., 2008). As the area of a habitat increases, the number of species it can support typically increases logarithmically, making the size of urban green spaces a critical factor in preserving biodiversity (Beninde et al., 2015). Resource availability and human disturbance are key factors shaping species interactions and network structures in urban landscapes; within these spaces, species interaction networks may shift in response to seasonal fluctuations in resource abundance, human activity and habitat conditions, further influencing ecological dynamics (Gilbert et al., 2022).

Concurrent with shifts in community structure, seasonality also crucially shapes animal communities as dynamics in climate and food resources subsequently alter species interactions, distribution and behaviour (Porter et al., 2022; White & Hastings, 2020; Figure 1). However, urbanization may act as a buffer against seasonal extremes, reducing the effects of winter food scarcity and temperature fluctuations through the urban heat island effect and anthropogenic food sources (Filazzola et al., 2024; Marcacci et al., 2023; Vlaschenko et al., 2023). Anthropogenic subsidies influence hibernating species by altering resource availability. For example, warmer temperatures and increased access to human food have reduced black bear (*Ursus americanus*) hibernation periods in Colorado, USA, potentially escalating human-bear conflict and mortality (Johnson et al., 2018). Seasonality also shapes human activity, in turn modulating wildlife behaviour (Doherty et al., 2021). For example, human recreation during the day has led to black bear and coyote (*Canis latrans*) shifting their activity to nocturnal periods, while more sensitive species such as bobcats (*Lynx rufus*) reduced their habitat use near trails (Lewis et al., 2021). These patterns highlight how urban environments restructure species interactions, with potential consequences for ecological networks and community stability.

Tracking spatial and temporal dynamics is essential for understanding how habitat fragmentation, resource availability and human activity shape biodiversity in urban environments (Leveau et al., 2021; Malhotra et al., 2022). Monitoring methods such as camera traps, acoustic sensors and community science observations provide insights into species presence and activity (e.g. Gámez & Harris, 2021; Gibb et al., 2019; Pecorelli et al., 2019) and advanced technologies such as GPS tracking and satellite imagery provide further insights into movement patterns and habitat use across seasons (Brown et al., 2023; McDuie et al., 2019; Severson et al., 2021). However, these methods rely on direct detection and may underrepresent cryptic or nocturnal species, introducing biases related to body size, behaviour and detection probability (Newey et al., 2015). Environmental DNA (eDNA), which captures genetic material shed by organisms into their surroundings, provides an alternative approach to biodiversity monitoring, particularly in environments where direct observation is challenging (Clare et al., 2022; Hallam et al., 2021). Unlike camera traps, which primarily detect medium-to-large terrestrial mammals that pass within sensor range, eDNA enables the detection of a broader range of species, including smaller, elusive and nocturnal taxa that may not be captured by traditional survey methods (Leempoel et al., 2020). In urban ecosystems, where habitat fragmentation and anthropogenic pressures drive biodiversity patterns, eDNA can provide a powerful tool for detecting seasonal shifts in community structure that might be overlooked (Johnson et al., 2021).

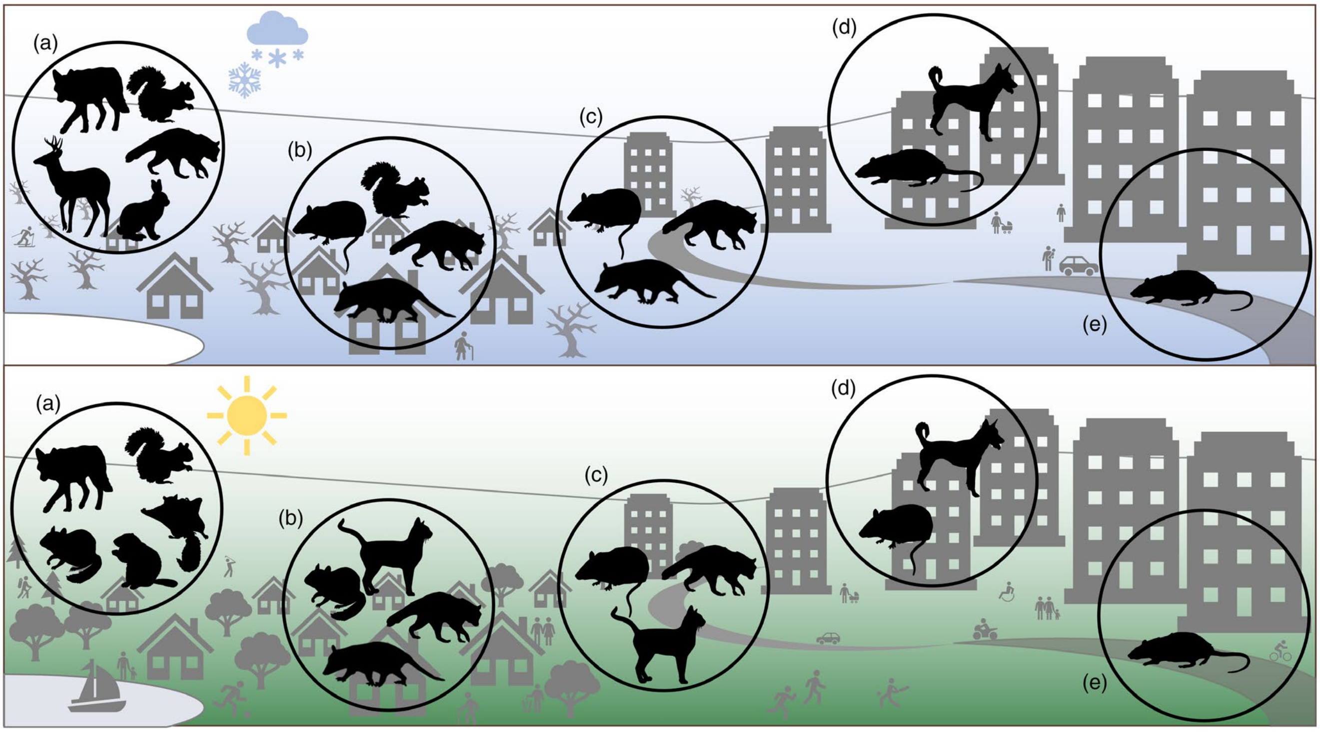

**FIGURE 1** Conceptual illustration depicting variation in animal diversity across different urban sites and between winter (top panel) and summer (bottom panel). Some species are widely distributed and can be detected across multiple sites regardless of the season, while others are rare or seasonally distinct. Alpha diversity decreases from left to right moving from more diverse large green spaces (a, b) to increasingly urban, smaller sites (d, e). As alpha diversity decreases, beta diversity transitions from being turnover-dominated to nestedness-dominated. High diversity sites (a, b) may support more specialist species whose presence is tied to seasonal resource availability. In contrast, less diverse sites (c–e) may exhibit a buffering effect of urbanization on seasonal change, resulting in more homogenized, urban-adapted wildlife communities.

Mammals play key ecological roles in urban environments, influencing ecosystems through prey regulation, seed dispersal and other functional interactions (Lacher et al., 2019; Russo et al., 2016), while also interacting with human populations through disease transmission, agricultural pest control and cultural significance (Ávila-Nájera et al., 2018; Hunold & Mazuchowski, 2020; Shultz et al., 2023). Understanding seasonal shifts in urban mammal diversity is critical for assessing how human activity and environmental changes influence wildlife populations. While research has examined urban species diversity, less attention has been given to the role of seasonality in structuring urban mammal communities, particularly in the context of species interactions and network dynamics. Species interactions shape ecological networks, and investigating how these relationships shift seasonally is key to understanding urban ecosystem resilience and function.

Here, we investigate seasonal variation in mammal diversity, community composition and interaction networks across urban parks. Using eDNA from soil samples collected during winter and summer across 21 urban parks in Detroit, Michigan (Figure 2), we address the following research questions: (1) How does seasonal variation influence mammal species richness (alpha diversity), community composition (beta diversity) and overall species pool (gamma diversity) in urban parks? (2) How do park characteristics, such as size and human activity, shape seasonal patterns in mammal communities? (3) How do species interaction networks differ between winter and summer, and what do these differences reveal about seasonal shifts in urban mammal ecology? We hypothesize that gamma diversity will remain stable across seasons, while alpha and beta diversity will fluctuate, reflecting species-specific seasonal behaviours. We expect larger parks to exhibit greater seasonal turnover, as habitat heterogeneity supports species with distinct seasonal activity patterns. Additionally, we anticipate seasonal changes in human-associated eDNA, reflecting park usage patterns and shifts in urban wildlife interactions. We predict seasonal shifts in mammal network structure driven by resource availability. In summer, increased resources may lead to greater dispersal, reducing degree centrality and network density. In winter, limited food and shelter may force species into closer proximity, increasing clustering and network density. We also expect humans and domestic species to play a central role in these networks, with higher co-occurrence with wildlife during summer when human activity in urban green spaces is greater. Our findings provide insights into how urban environments and seasonal dynamics shape biodiversity, informing conservation strategies and urban planning to support wildlife in cities.

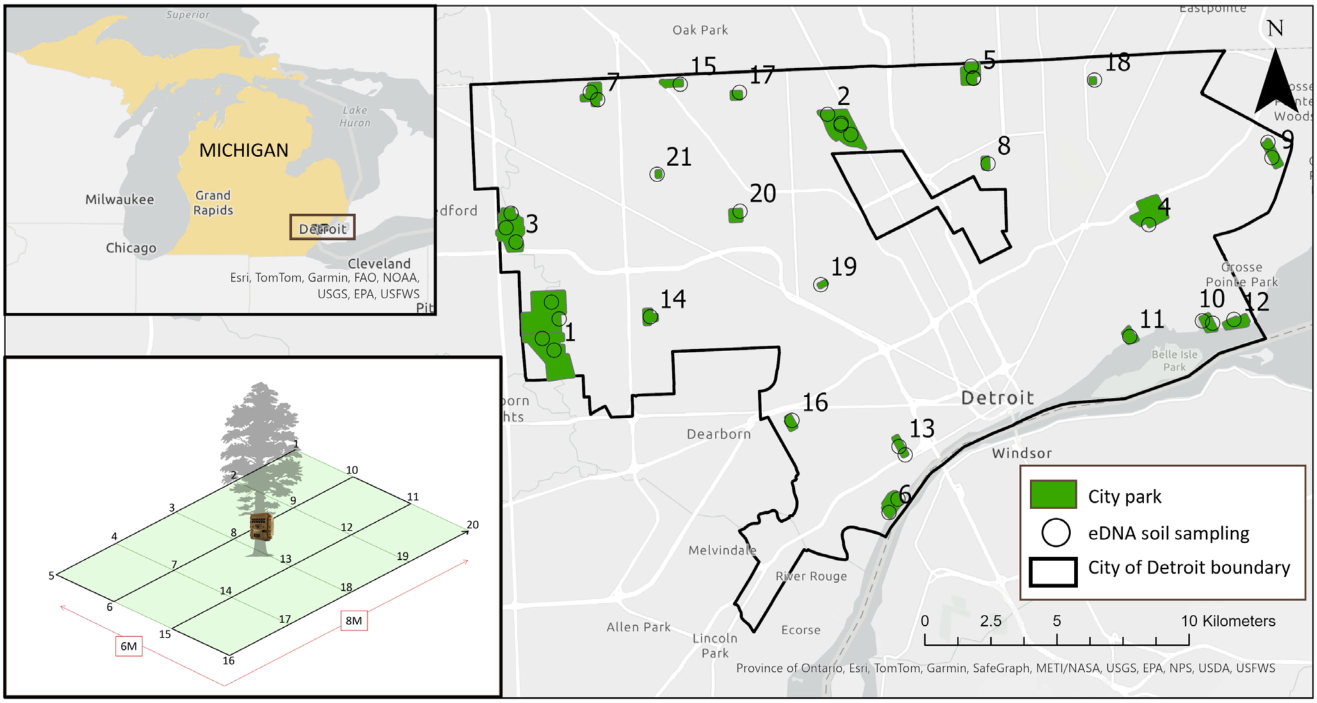

FIGURE 2 Study area- Detroit, Michigan. Shaded green areas represent the city parks where soil samples were collected for eDNA sampling in 2023 for this study. Diagram illustrates sample collection plan, where 20 subsamples of soil were collected along transects around a focal tree, covering 6 M by 8 M. Parks are numbered by size in descending order: (1) Rouge; (2) Palmer; (3) Eliza Howell; (4) Chandler; (5) Farwell; (6) Fort Wayne; (7) O'Hair; (8) Lasky; (9) Balduck; (10) Maheras; (11) Henderson; (12) Ford; (13) Clark; (14) Stoepel; (15) Comstock; (16) Romanowski; (17) Van Antwerp; (18) Marruso; (19) McCabe; (20) Butzel Playfield; (21) Fields. Mean monthly temperatures were 28°F (−2°C) [min 22°F (−6°C), max 35°F (2°C)] in February and 75°F (24°C) [min 66°F (19°C), max 84°F (29°C)] in July.

## 2 | METHODS

### 2.1 | Study area

We collected soil samples from 21 urban parks in Detroit, the largest city in Michigan (Figure 2). As the sampling was non-invasive, no animal ethics approval was required and a permit was not needed given the small amount of soil collection. These parks are included in a long-term camera trapping study and vary in size from ~1.6 to 480 ha. Additionally, the sampled parks vary in habitat, human visitation level, and degree of disturbance stemming from the presence of domestic pets (Gámez & Harris, 2021; Lima et al., 2021). In Detroit, the largest native carnivore present is the coyote, which co-occurs with grey foxes (*Urocyon cinereoargenteus*), red foxes (*Vulpes vulpes*), raccoons (*Procyon lotor*), Virginia opossums (*Didelphis virginiana*) and striped skunks (*Mephitis mephitis*). Herbivores such as white-tailed deer (*Odocoileus virginianus*) and Eastern cottontail rabbit (*Sylvilagus floridanus*) are present along with at least 15 species from the order Rodentia (iNaturalist, 2024). Located in southeastern Michigan, Detroit features vegetation typical of the southern deciduous forest region with tree species such as maples (*Acer* spp.), oaks (*Quercus* spp.), and American elm (*Ulmus americana*) as well as non-native ornamental shrubs and trees (Vankat, 1979). Detroit exhibits a temperate continental climate characterized by significant temperature differences, featuring warm to hot summers with temperatures ranging from 70 to 90°F (21–32°C), and cold winters with temperatures ranging from 20 to 35°F (−6 to 2°C), and an average snowfall of around 42 inches (~107 cm) (Loidi et al., 2022). Precipitation is relatively even throughout the year with slightly higher amounts in spring and summer influenced by the proximity to the Great Lakes (Oswald et al., 2012).

### 2.2 | eDNA sample collection and molecular workflow

We completed eDNA soil sampling throughout Detroit in February and July 2023, representing winter and summer seasons. During sampling soil, eDNA was collected at up to four sites per park with the number of sampling sites reflecting the park's size; for example, Palmer and Rouge parks, being larger, each had four sampling sites during the summer collection period. In total 32 soil samples were collected in winter and 33 in summer. At each site, we collected a total 200 mL of soil in 50 mL RNAse-free centrifuge tubes along four parallel transects around a focal tree and camera trap (Figure 2). Detection rates from soil eDNA have been shown to be higher when combining subsamples in a large grid rather from a single unique point (Andersen et al., 2012), and to minimize potential biases toward tree-associated taxa, soil was collected from an area extending up to 4 m away from the focal tree. As such, we collected 10mL of soil from the top layer up to 10cm of soil depth using trowels and spatulas from 20 points across the transects. To minimize contamination, disposable, single-use shoe covers were worn from approximately 5M from the camera trap to avoid tracking soil and DNA into the sampling area. We cleaned all utensils prior to each sampling site with bleach and then rinsed with distilled water. Samples were kept on ice in the field and then stored at −30°C until extraction.

We extracted DNA from the 65 soil samples in triplicate, yielding 195 eDNA extractions, using the Qiagen DNeasy Powersoil Pro Kit (Qiagen, USA). To amplify the eDNA, we used the MiMammal-U primer set, targeting a ~170bp fragment from the variable region of the 12S rRNA gene (Ushio et al., 2017). Each of the 195 eDNA samples were amplified in triplicate, and the resulting replicates pooled back together for each eDNA sample. (PCR conditions and optimisation are detailed in Supporting Information: Methods). One negative extraction control and one negative PCR control were amplified and sequenced with samples to monitor potential contamination. Library preparation and paired end sequencing (2×150bp, targeting 2 million reads per sample) was performed on an Illumina NovaSeq6000 at the Yale Center for Genome Analysis.

## 2.3 | Bioinformatics and statistical analyses

We employed a multistep process to analyse the resultant sequence data. First, we removed primer sequences from the demultiplexed data using *cutadapt v2.3* (Martin, 2011). We then used *DADA2* to perform quality filtering, assign amplicon sequence variants (ASVs), merge forward and reverse reads and remove chimeric sequences (Callahan et al., 2016). ASVs detected in the negative extraction and PCR controls were removed across all samples. For taxonomic assignment, we blasted the ASVs against a preformatted 'nt' blast database downloaded from NCBI, specifying vertebrate taxa IDs, using *BLAST+ v2.15* with a minimum 96% sequence similarity, with a minimum five database sequences per ASV returned. We sorted the BLAST results by highest percent sequence similarity and lowest e-value, with the top hit selected as the most likely taxonomic identity of each sequence. Read counts, representing the number of sequences attributed to each ASV, were recorded for further analysis. We consolidated the eDNA samples to camera site, and park level by summing the read counts of ASVs identified as species (or Genus) using the 'tidyverse' package (Wickham, 2019). Species not identified as mammals, which included 16 species in Class Aves, were excluded from further analyses, but domestic mammals and human detections were retained. Human ASVs detected in laboratory controls were removed from all samples during data processing to account for potential contamination introduced during laboratory work. This step ensures that downstream analyses reflect environmental signals rather than procedural artefacts. To assess sampling completeness, we used the 'iNEXT' package (Hsieh et al., 2016) to perform rarefaction based on frequency of detection across samples and calculated 95% confidence intervals through 100 bootstrap replications.

Gamma diversity was investigated by consolidating eDNA detections across all parks and comparing them with research-grade mammal observations across Detroit, MI, recorded on iNaturalist. We compared eDNA detections with iNaturalist records from two timeframes: the sampling period (January 1st 2023 to July 13th 2023) and a broader, 5year window (July 14th 2018 to July 13th 2023). Presence-absence data were compiled, and McNemar's test was used to assess significant differences in detection probabilities between eDNA and iNaturalist records. Using the 'vegan' package (Oksanen et al., 2019), the eDNA samples were rarefied to the lowest read count to standardize species richness across different sampling efforts. Alpha diversity (species richness) was calculated at both sample level (with extraction triplicates pooled), and park level (with all sample sites within the park pooled). Analysis of variance (ANOVA) was used to test for differences in richness among parks, while Wilcoxon matched-pairs tests were used to assess seasonal differences in alpha diversity within parks.

Beta diversity in the form of the Sørensen dissimilarity index was computed to compare: eDNA detections within extraction replicates, sampling sites within parks, and parks within the Detroit study area. We used the 'betapart' package to calculate Sørensen dissimilarity, an index ranging from 0 to 1 with low values indicating high overlap in species composition and 1 indicating no shared species between communities. We also partitioned Sørensen dissimilarity into its two components: turnover (the substitution of some species in one community) and nestedness (when the species set of one community is a subset of the other; Baselga & Orme, 2012). Cluster dendrograms were generated based on the beta diversity values to visualize species composition differences across parks. We visualized park-level changes in community composition and abundance across the two seasons with the 'pheatmap' package (Kolde, 2019). To test the effects of season and park on community composition, we used non-metric multidimensional scaling (nMDS) to visualize the communities and tested for significant variation using ANOSIM and PERMANOVA.

We also assessed how park characteristics influenced mammal community structure. First, we calculated the proportion of human (*Homo sapiens*) sequencing reads per park as a proxy for anthropogenic disturbance to assess the influence of human presence on species diversity. We evaluated the influence of human DNA presence and park size on community composition using PERMANOVA and visualized their contribution to species assemblages with nMDS ordination. Second, we calculated the correlation coefficient between human DNA and detections of cow (*Bos taurus*) and pig (*Sus scrofa*) sequences in eDNA samples to explore potential associations between human presence and anthropogenic food sources. To assess the relationship between park size and biodiversity, we used linear regression models to examine correlations between park size and alpha diversity, Sørensen dissimilarity and its turnover and nestedness components. We used the 'mvabund' package to build multivariate generalized linear models (GLMs) and performed univariate tests to determine species-specific seasonal responses within parks. We expected seasonal variation to be context-dependent, influenced by park characteristics and surrounding landscapes (Wang et al., 2012). We also expected larger parks to host more diverse communities (higher alpha diversity) and exhibit greater seasonal turnover but lower nestedness, reflecting seasonal shifts in species presence.

Lastly, to investigate species co-occurrence patterns, we used the 'cooccur' package to identify species pairs with co-occurrences significantly higher than expected by chance (Griffith et al., 2016). For this analysis, co-occurrence was defined at the park level: a species pair was considered to co-occur if both were detected in at least one sample from the same park during a given season. We constructed presence-absence-based networks (to avoid biases related to PCR amplification variation) and computed key network metrics using the 'igraph' package to analyse seasonal changes in species interaction networks (Csárdi et al., 2024). We calculated degree centrality which computes the number of direct connections (edges) for each node and closeness centrality as the inverse of the average shortest path length from a node to all other nodes in the network. We also calculated the clustering coefficient which reflects the tendency of nodes to form tightly connected groups. We also calculated network density as the proportion of possible connections that are realized within the community. Metrics were computed separately for winter and summer networks (Martín González et al., 2010; Niquil et al., 2020). To compare these network metrics between seasons, we first assessed normality using the Shapiro–Wilk test. Based on these results, we applied paired Wilcoxon Signed-Rank tests to compare degree centrality and closeness centrality of species between seasons. All analyses were performed using R version 4.2.0 (R Core Team, 2021).

## 3 | RESULTS

### 3.1 | Variation across replicates and samples

We sequenced 195 eDNA soil samples collected from 21 parks in the Detroit Metro area, Michigan. Overall, 35.5 million reads were generated, with an average of 200 thousand per sample. We found a high degree of variation across sample replicates and parks, with an overall Sørensen dissimilarity index of 0.98 across the 195 samples. Balduck Park (Figure 2, number 9), a medium-sized park (22.7 ha) in eastern Detroit, had the lowest average dissimilarity among the eDNA samples (0.55), indicating that eDNA samples collected within this park were relatively similar in species composition. In contrast, Marruso Park (Figure 2, number 18), a small park (2.2 ha) in northeastern Detroit, displayed a high degree of dissimilarity (0.97), suggesting heterogeneity in species composition within the park. Due to the high variation, we decided to aggregate eDNA samples among replicates to obtain a more representative overview of the biodiversity present in each park.

### 3.2 | Gamma diversity

Of the 195 eDNA samples, 176 samples successfully yielded DNA sequences belonging to 23 mammal species including humans (Table 1). The species accumulation curves indicated that our eDNA sampling overall achieved 96% sampling coverage, demonstrating the effectiveness of our methodology in capturing the majority of the mammalian diversity present in the parks (Figure S1). The data from the eDNA samples per sampling location were pooled and provided species detection data from 65 sampled sites in total. Notably, human DNA was detected in 58 of the sampling sites (all parks), and when present accounted for on average one-third of the sequences of a sample. In addition, sequences from cattle and pig were detected in 6 and 2 samples (5 and 2 parks), respectively. Due to the nature of these species as food animals and their absence as direct occurrence, it is most likely the DNA originated from food waste or digested faecal material, or proximity to urban farms where livestock are present with potential for windborne transfer of DNA (Clare et al., 2022). We also detected several other domestic species; domestic dog (*Canis lupus familiaris*) DNA was present in 12 of the samples (10 parks), and domestic cat (*Felis catus*) in 13 samples (11 parks). For detailed results of the iNaturalist comparison, please refer to the Supporting Information: document.

Across the whole study area, 20 species were detected in winter and 20 in summer; however, there were differences in species detections between the seasons. Brown rat (*Rattus norvegicus*), pig and sequences identified to the order Rodentia were only detected in winter, while groundhog (*Marmota monax*), striped skunk and muskrat (*Ondatra zibethicus*) were only detected in summer (Table S2). The mean Sørensen dissimilarity for gamma diversity between seasons was 0.15, indicating a low level of dissimilarity that was entirely due to turnover. This suggests that the differences in gamma diversity between seasons are predominantly due to the presence of different species, rather than one season's community being a subset of the other's. This aligns with our hypothesis that dissimilarity is driven by the presence (or absence) of seasonal species.

### 3.3 | Alpha and beta diversity

The alpha diversity detected in parks varied significantly, ranging from 2 species at Butzel Playfield (a small, isolated 1.7 ha park) to 14 species detected at Eliza Howell Park (a large 101.2 ha park on the west border of Detroit, and part of a green corridor with Rouge Park) ($F_{(20,15)} = 2.02$, $p < 0.001$; Table S2). A paired analysis of alpha diversity by season showed no significant differences at individual park levels (Wilcoxon rank-sum test, $V = 28.5$, $p = 0.07$). Minimal seasonal impact on alpha diversity was observed, with nMDS visualizations indicating low dissimilarity between winter and summer communities (ANOSIM −0.003, $p = 0.5$). Season only accounted for 2% of the variation in community composition at the park level ($p = 0.5$). Overall, the parks exhibited varied responses in species richness to seasonal changes with no consistent trend observed (Figure 3).

**TABLE 1** Mammal species detected in Detroit via eDNA and iNaturalist observations from (1) 1 January 2023 and 13 July 2023 (the timeframe of eDNA collection), and (2) between 14 July 2018 and 13 July 2023 (a broader, 5 year timeframe).

| Order | Family | Scientific name | Common name | eDNA | iNaturalist survey window | iNaturalist 5-year window |
|---|---|---|---|---|---|---|
| Artiodactyla | Bovidae | *Bos taurus* | Cattle | X | | |
| | Cervidae | *Odocoileus virginianus* | White-tailed Deer | X | X | X |
| | Suidae | *Sus scrofa* | Pig | X | | |
| Carnivora | Canidae | *Cani lupus familiaris* | Domestic Dog | X | | X |
| | | *Canis latrans* | Coyote | X | X | X |
| | | *Vulpes vulpes* | Red Fox | | X | X |
| | Felidae | *Felis catus* | Domestic Cat | X | X | X |
| | Mephitidae | *Mephitis mephitis* | Striped Skunk | X | X | X |
| | Mustelidae | *Mustela nivalis* | Least Weasel | | | X |
| | Mustelidae | *Neogale vison* | American Mink | | X | X |
| | Procyonidae | *Procyon lotor* | Common Raccoon | X | X | X |
| Didelphimorphia | Didelphidae | *Didelphis virginiana* | Virginia Opossum | X | X | X |
| Eulipotyphla | Soricidae | *Blarina brevicauda* | Northern Short-tailed Shrew | | X | X |
| | | *Sorex cinereus* | Masked Shrew | | | X |
| | Talpidae | *Condylura cristata* | Star-nosed Mole | | | X |
| | | *Scalopus aquaticus* | Eastern Mole | | X | X |
| Lagomorpha | Leporidae | *Oryctolagus cuniculus* | European Rabbit | | X | X |
| | | *Sylvilagus floridanus* | Eastern Cottontail | X | X | X |
| Rodentia | Castoridae | *Castor canadensis* | American Beaver | | X | X |
| | Cricetidae | *Microtus montanus* | Montane Vole* | X | | |
| | | *Microtus pennsylvanicus* | Meadow Vole | | X | X |
| | | *Ondatra zibethicus* | Muskrat | X | X | X |
| | | *Peromyscus leucopus* | White-footed Mouse | X | X | X |
| | | *Peromyscus maniculatus* | Deer Mouse | X | X | X |
| | Muridae | *Mus musculus* | House Mouse | X | X | X |
| | | *Rattus norvegicus* | Brown Rat | X | | X |
| | Sciuridae | *Glaucomys volans* | Southern Flying Squirrel | X | X | X |
| | | *Ictidomys tridecemlineatus* | Thirteen-lined Ground Squirrel | | | X |
| | | *Marmota monax* | Groundhog | X | X | X |
| | | *Sciurus carolinensis* | Eastern Grey Squirrel | X | X | X |
| | | *Sciurus niger* | Fox Squirrel | X | X | X |
| | | *Tamias striatus* | Eastern Chipmunk | X | X | X |
| | | *Tamiasciurus hudsonicus* | American Red Squirrel | | X | X |
| | Zapodidae | *Zapus hudsonius* | Meadow Jumping Mouse | | | X |

*Note*: Montane vole is marked with an asterisk (*) to indicate it is not known to occur at this location.

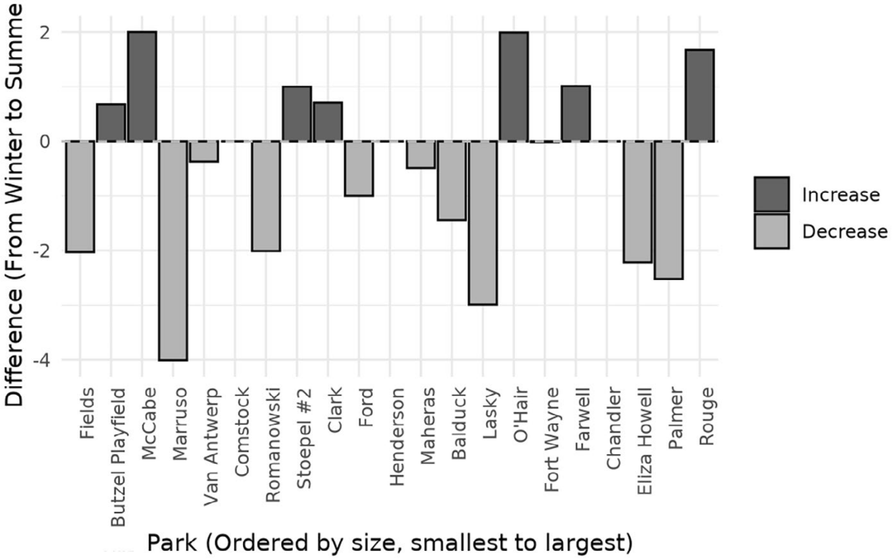

**FIGURE 3** Changes in alpha diversity (species richness) between winter and summer species detections from eDNA sampling of soil samples across 21 urban parks in Detroit, Michigan. Parks with increases in diversity are shown in dark grey, while those with decreased diversity are shown in light grey. Parks are ordered by size, from smallest to largest.

**TABLE 2** Rarefied alpha diversity (div) for the parks in the study across seasons, and total for the year.

| Map point | Park | Park size (hectares) | Winter div | Summer div | Year div | SIM | SNE | SOR |
|---|---|---|---|---|---|---|---|---|
| 1 | Rouge | 479.14 | 6.32 | 7.99 | 10 | 0.25 | 0 | 0.25 |
| 2 | Palmer | 119.78 | 9.62 | 7.1 | 11.64 | 0.25 | 0.08 | 0.33 |
| 3 | Eliza Howell | 101.17 | 11.21 | 8.99 | 13.99 | 0.22 | 0.11 | 0.33 |
| 4 | Chandler | 80.93 | 2 | 2 | 3 | 0.5 | 0 | 0.5 |
| 5 | Farwell | 36.42 | 4.01 | 5.02 | 6.17 | 0.2 | 0.07 | 0.27 |
| 6 | Fort Wayne | 33.58 | 8 | 7.99 | 10 | 0.25 | 0 | 0.25 |
| 7 | O'Hair | 31.56 | 5 | 6.99 | 8 | 0.2 | 0.13 | 0.33 |
| 8 | Lasky | 26.3 | 4.99 | 2 | 5 | 0 | 0.42 | 0.42 |
| 9 | Balduck | 22.66 | 3 | 1.56 | 3.96 | 0.5 | 0.09 | 0.6 |
| 10 | Maheras | 21.04 | 5.49 | 5 | 8.09 | 0.4 | 0.09 | 0.5 |
| 11 | Henderson | 14.44 | 1.01 | 1.01 | 2.25 | 1 | 0 | 1 |
| 12 | Ford | 13.75 | 3 | 2 | 3 | 0 | 0.2 | 0.2 |
| 13 | Clark | 12.14 | 3.29 | 4 | 5.42 | 0.5 | 0 | 0.5 |
| 14 | Stoepel #2 | 11.85 | 2 | 3 | 3 | 0 | 0.2 | 0.2 |
| 15 | Comstock | 10.52 | 3 | 3 | 4 | 0.33 | 0 | 0.33 |
| 16 | Romanowski | 10.52 | 4 | 1.99 | 5 | 0.5 | 0.16 | 0.66 |
| 17 | Van Antwerp | 7.24 | 3.39 | 3.02 | 6.62 | 0.6 | 0.03 | 0.63 |
| 18 | Marruso | 2.18 | 4.01 | 0 | 4.24 | NA | NA | 1 |
| 19 | McCabe | 2.06 | 1 | 3 | 3 | 0 | 0.5 | 0.5 |
| 20 | Butzel Playfield | 1.69 | 1 | 1.68 | 2 | 0 | 0.33 | 0.33 |
| 21 | Fields | 1.61 | 4.03 | 2 | 4.27 | 0 | 0.42 | 0.42 |

*Note*: Beta diversity is presented in the form of Sørensen dissimilarity index (SOR) and its components: turnover (SIM) and nestedness (SNE).

Similarly, we observed varied responses in the beta diversity among individual parks between seasons with the Sørensen dissimilarity index ranging from 0.2 to 1 (Table 2). Henderson Park, a 14.4-ha park in south Detroit, exhibited the largest dissimilarity between seasons attributed entirely to species turnover. In contrast, Stoepel Park, an 11.9-ha park in west Detroit, had the lowest dissimilarity between seasons attributed entirely to nestedness. Clustered dendrograms based on total Sørensen dissimilarity highlighted the variability of seasonal change for the individual parks and revealed parks with similar species compositions (Figure S3). Parks with visually denser plant and tree cover (based on field observations) such as Eliza Howell, O'Hair, Palmer and Rouge formed a distinct cluster, while smaller parks with more recreational opportunities and impervious surface such as Clark, Comstock and Lasky formed another subset.

To quantify the relationship between park size and diversity, we conducted linear regression analyses with total alpha diversity, Sørensen dissimilarity and its components turnover and nestedness. Linear regression analysis revealed a significant positive correlation between park size and the total alpha diversity ($\beta = 0.0154$, $R^2 = 0.23$, $p = 0.03$). This indicates that larger parks tend to host more diverse communities, consistent with our hypothesis that larger habitats provide more resources and niches to support a greater variety of species. However, we found no significant effect of park size on Sørensen dissimilarity ($\beta = -0.0006$, $R^2 = 0.08$, $p = 0.22$), turnover ($\beta = -0.00003$, $R^2 = 0.0002$, $p = 0.95$) or nestedness ($\beta = -0.0004$, $R^2 = 0.09$, $p = 0.20$) in regard to beta diversity.

### 3.4 | Community composition and human impact

Mammal communities were significantly different between parks ($R^2 = 0.58$, $p = 0.005$), following expectations that factors contributing to diversity are specific to individual park characteristics and likely the surrounding neighbourhood attributes. Carnivores (coyote, racoon, striped skunk and domestic cats and dogs) were detected in 17 of the 21 parks (Figure S4). Striped skunks were only detected during summer, while all other carnivores were detected in both seasons. Cats, dogs and raccoons were the most detected carnivores, while coyotes were detected in only three parks: Fort Wayne, Balduck and Maheras. The size of parks in this study ranged from 1.6 to 479 ha, but coyotes were only detected in parks larger than the median size of 14.4 ha. Larger parks such as Rouge, Eliza Howell, Farwell and Fort Wayne exhibited relatively stable carnivore detections between seasons, possibly reflecting the availability of more diverse habitats and resources allowing for the establishment of a home range. In contrast, smaller parks showed more variability in their detections.

We found a significant correlation between species composition and the proportion of human DNA recovered from the parks ($R^2 = 0.5022$, $p = 0.001$). We used heatmaps to visualize the species composition of individual parks during winter and summer and to group parks with similar species compositions (Figure S2). In winter, 10 parks all showed a predominantly high relative abundance of human detections compared with other species, and 4 other parks (O'Hair, Maheras, Fields and Lasky) were clustered due to their high relative abundance of domestic cat detections. However, the community composition shifted away from such high prevalences of human detections in the summer; although Rouge, Stoepel, Van Antwerp and Butzel remained clustered due to this detection. Dog detections were responsible for clustering Farwell, Clark, Fields and Romanowski, and Virginia opossum detections were the uniting theme at Fort Wayne, Henderson and Maheras Park. We conducted multivariate GLMs for each park to compare species composition across seasons and identified Balduck, Clark and Fort Wayne as having significantly different species compositions between seasons (see Table S3). Univariate testing revealed that season had a significant effect on certain species. For example, human detections decreased from winter to summer ($p = 0.043$) at Balduck Park. Eastern cottontail rabbit (detections decreased from winter to summer ($p = 0.05$) at Fort Wayne and fox squirrel (*Sciurus niger*) detections decreased from winter to summer ($p = 0.018$) at Clark.

Patterns of species co-occurrence also exhibited nuanced seasonal variation (Figure 4). In both winter and summer networks, Eastern grey squirrel (*Sciurus carolinensis*) and humans were central to the network structure, with the highest values of degree centrality (15 and 13 for winter and summer respectively). Domestic animals, such as dogs and cats, also showed significant connectivity. In winter, cats co-occurred with raccoons ($p = 0.001$) and white-tailed deer ($p = 0.008$) more often than expected. In summer, significant co-occurrences included dogs with mice, deer with fox squirrels and raccoons with grey squirrels ($p = 0.04$ for each). Overall, the mean degree centrality decreased from 6.1 to 4.7 (Wilcoxon signed-rant test: $p = 0.06$, $V = 72$), suggesting that species had fewer direct connections with others during the summer. However, house mice (*Mus musculus*) and flying squirrels (*Glaucomys volans*) showed an increase in degree centrality from winter to summer (Table S4). Clustering coefficient (0.9 to 0.86) and network density (0.32 to 0.25) both decreased, reflecting a more dispersed, less interconnected network in summer.

## 4 | DISCUSSION

Monitoring wildlife in urban environments is increasingly vital as environmental changes reshape habitat suitability and animal behaviour. Seasonal variation influences resource availability, species interactions and human-wildlife dynamics with shifts in temperature, vegetation and human activity impacting biodiversity, disease dynamics and urban ecosystem resilience. Our study highlights seasonal changes in mammal communities in a large US mid-western city, offering insights for urban planning and management. Understanding these temporal dynamics is crucial as they affect ecosystem functions, biodiversity resilience and human-wildlife coexistence. Our findings underscore the need for adaptive management strategies that account for seasonal shifts in mammal diversity.

### 4.1 | Seasonal variation in mammal diversity

Seasonal shifts in species composition were evident, with high Sørensen dissimilarity index (0.98) observed across replicates, indicating significant spatial variability in species detections, which may result from localized movements, habitat preferences or differences in activity levels between seasons. While our sampling design, which pooled soil from multiple subsample points, aimed to mitigate this variability, it cannot fully account for the influence of animal behaviour and habitat heterogeneity on detection patterns. Recognizing this variability, we interpret our findings cautiously and emphasize the importance of replication and standardized

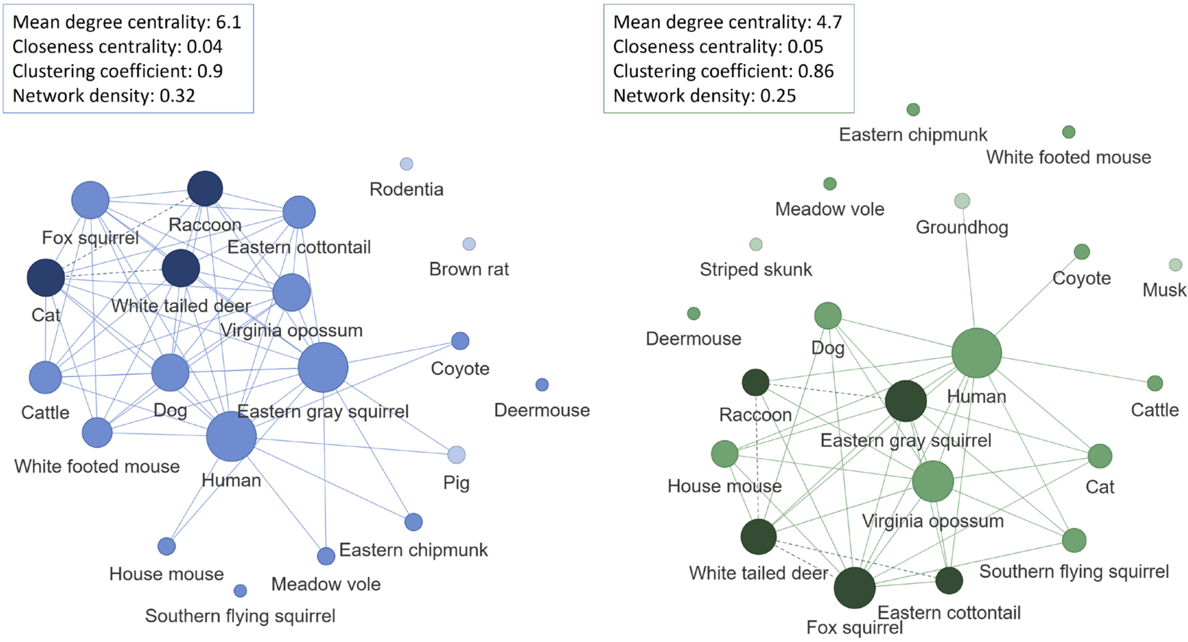

FIGURE 4 Species co-occurrence networks in winter (left) and summer (right). Nodes represent species, sized by degree centrality with colour indicating seasonal occurrence: Lighter nodes indicate species unique to that season, while dark nodes represent species with statistically significant co-occurrences. Edges represent co-occurrence relationships, where dashed edges indicate statistically significant co-occurrence ($p \le 0.05$), and solid edges indicate non-significant associations ($p > 0.05$). Isolated nodes indicate species that did not co-occur with others in the same park. Co-occurrence was defined at the park level. Layouts were generated using the Kamada-Kawai algorithm.

methodologies in reducing sampling biases (Buxton et al., 2021; Shirazi et al., 2021). Additionally, species turnover, rather than nestedness, was the primary driver of seasonal differences, suggesting that many species were detected in only one season. While this likely reflects true seasonal variation in species occupancy and activity, the persistence of DNA in soil could also influence detections (Guthrie et al., 2024). Winter-deposited DNA may remain detectable into the summer, potentially inflating estimates of species co-occurrence between seasons and we acknowledge that legacy DNA, particularly in cooler climates like Detroit, may still contribute to the observed differences in community composition (Guthrie et al., 2024; Valentin et al., 2021). Further research incorporating shorter sampling intervals or degradation experiments could help quantify DNA persistence and improve the resolution of seasonal comparisons in this system.

Despite these limitations, our study achieved 96% sampling coverage, indicating that our methodology effectively captured the majority of the mammalian diversity present. The observed seasonal shifts in mammal communities align with patterns documented in other urban environments, where factors such as temperature, resource distribution and human activity influence wildlife presence and movement. For example, anthropogenic subsidies, such as food waste and bird feeders can reduce seasonal fluctuations in resource availability, altering natural patterns of migration, hibernation and foraging behaviour (Crandall et al., 2024). Similarly, human recreation is often more frequent in warmer months, which can lead to shifts in species distributions, particularly for disturbance-sensitive species (Marion et al., 2020). These seasonal dynamics underscore the need for long-term monitoring to anticipate biodiversity changes under future climate and land-use scenarios. Understanding these patterns is crucial for designing urban green spaces that support wildlife and mitigate biodiversity loss. Additionally, our findings have implications for zoonotic disease transmission, as shifts in species distributions may influence pathogen-host interactions, a key concern in One Health frameworks (Gilbertson et al., 2023).

## 4.2 | Park size and mammal diversity

Community composition in urban parks can exhibit subtle and park-specific changes, as observed in our study. Seasonal shifts in temperature, food availability and breeding cycles, combined with localized factors such as vegetation density, water availability and habitat diversity, create cyclical patterns that influence species composition and lead to variation in presence and abundance between winter and summer (Johnson & Swan, 2014; Threlfall et al., 2017). We observed decreases in clustering coefficient and network density that suggest a more dispersed, less interconnected mammal community during summer. This shift likely reflects increased resource availability and habitat diversity, enabling broader species dispersal and reducing the formation of tightly connected groups. Additionally, parks in more urbanized areas of the city often experience higher levels of pollution, noise and human disturbance that can impact wildlife communities (Zhou & Chu, 2012). However, parks with more extensive natural habitat can mitigate these effects by providing more refuges for sensitive species (Beasley et al., 2023).

Larger parks in particular provide more diverse habitats, which are crucial for sustaining higher species richness compared to smaller, more fragmented green spaces (Beninde et al., 2015). For example, coyotes were detected only in parks larger than the median size of 14.4 ha, likely reflecting their preference for extensive territories with abundant resources. While our soil sampling focused on areas around focal trees, potential bias toward tree-associated taxa is expected to be minimal. Large mammals shed DNA broadly across their ranges through faeces, urine, skin cells, saliva and hair, leading to DNA accumulation across diverse substrates and reducing the influence of specific microhabitats like trees (Leempoel et al., 2020). Moreover, the sampled habitats exhibited varied environmental features, including underbrush vegetation, contributing to habitat heterogeneity. Eliza Howell Park, characterized by its large size, dense vegetation and connectivity to other green spaces, exhibited greater species richness and distinct community structure compared to more isolated parks like Butzel Playfield. These findings underscore the need to consider ecological variables, connectivity, and urban pressures when managing urban green spaces to support wildlife diversity. Larger parks play a critical role in maintaining urban biodiversity by offering essential refuges for wildlife in fragmented landscapes (Zellmer & Goto, 2022). This highlights the importance of urban planning policies that prioritize habitat corridors and green infrastructure to enhance species resilience. As urbanization continues, integrating biodiversity-sensitive designs into city planning can help mitigate habitat loss and promote species coexistence.

### 4.3 | Human influence on community composition

Urban parks serve as both recreational spaces for humans and critical habitats for wildlife. Human disturbances such as recreation, dog walking and littering can negatively impact wildlife, reshaping community composition by favouring generalist species while displacing more sensitive taxa (Beasley et al., 2023). These shifts have ecological consequences, potentially disrupting trophic interactions, disease dynamics and biodiversity stability in urban ecosystems (Gámez & Harris, 2021; Lima et al., 2021). Human DNA was the most frequently detected sequence in all parks, reflecting significant human activity and interactions with the urban wildlife ecosystem. Parks experience high foot traffic with visitors introducing DNA through food waste, animal products and faecal matter (Darling et al., 2021). Activities such as picnics, barbecues and recreational gatherings can contribute to the introduction of processed meat products into the environment (Crandall et al., 2024). While cooking can degrade DNA, studies have shown that detectable DNA fragments can persist even in highly processed meat products (Shokralla et al., 2015). The detection of cow and pig sequences likely stems from human or domestic animal waste or wildlife scavenging anthropogenic food sources—particularly in winter when natural resources are scarce (Henger et al., 2022). Although human eDNA remains detectable for extended periods, it has not been widely used as a proxy for urbanization (Antony Dass et al., 2022). It should be noted that human DNA in eDNA studies raises important ethical and legal considerations, including issues of privacy and consent, as highlighted in recent discussions (Doi & Kelly, 2023). In our study, we ensured that human DNA was treated generically, limiting analyses to species-level identification to avoid potential ethical concerns or privacy violations. Furthermore, we recognize the need for clear international guidelines to navigate the use of human eDNA responsibly, balancing the benefits of eDNA research with respect for ethical principles. While stringent contamination controls were implemented—such as removing human ASVs detected in lab controls—it remains challenging to fully separate human-derived signals from contamination. Our interpretation focuses on human eDNA as an ecological indicator of anthropogenic impacts, avoiding individual-level conclusions in line with ethical guidelines. Alongside humans, domestic species such as dogs and cats play central roles in the community network, influencing the structure and connectivity of species interactions (Herrera et al., 2022; Hughes & Macdonald, 2013; Ünal et al., 2019). Parks with higher human activity may attract adaptable generalist species, such as raccoons and squirrels, while deterring more disturbance-sensitive species (Parker & Nilon, 2012; Suraci et al., 2021). Our finding of a significant positive correlation between species composition and the proportion of human DNA in parks suggests that human disturbance amplifies shifts in community structure, consistent with prior research on urban wildlife dynamics (Gámez & Harris, 2021). Understanding the role of these species provides valuable insights into the resilience and stability of urban wildlife communities. While our study focused on mammals, incorporating data from other taxa—such as birds and invertebrates—would offer a more comprehensive picture of urban food webs, biodiversity and ecosystem health.

### 4.4 | Study limitations

While our study provides valuable insights into urban mammal diversity and seasonal dynamics, certain methodological and ecological factors introduce limitations that should be considered. We collected data over a single field season, limiting the robustness of winter vs. summer comparisons. Interannual variability in weather conditions, food availability and population dynamics may influence seasonal trends, highlighting the need for multi-year datasets to assess the consistency of these patterns and better understand long-term seasonal dynamics. While this study provides valuable insights into urban mammal diversity, methodological differences between eDNA and iNaturalist highlight inherent biases. Observational detections depend on observer effort, which may explain the absence of common species such as domestic dogs in iNaturalist records despite their known presence in parks. Similarly, the absence of brown rat observations in the iNaturalist data could be due to their cryptic nature or their status as an overlooked, ubiquitous species. The absence of 10 species in eDNA detections highlights opportunities for methodological refinement. The misidentification of montane vole as meadow vole, an expected species in the study area, likely resulted from genetic similarity and insufficient resolution of the amplified loci. Additional genetic markers could enhance species specificity and minimize cross-species misidentifications (Weitemier et al., 2021). Another challenge in this study is the differentiation between coyote and domestic dog sequences (Reese et al., 2020). Species-specific behaviours and habitat use likely influenced discrepancies between eDNA detection patterns and iNaturalist records. The eastern mole's subterranean lifestyle likely limited DNA deposition in surface soil, while American beavers, being primarily aquatic, are unlikely to leave detectable DNA in terrestrial soil. The European rabbit detection on iNaturalist was based on a single sighting, making its DNA presence unlikely. While eDNA can capture transient signals, such as those from cattle and pigs, iNaturalist relies on observer effort, potentially underrepresenting cryptic species. Future research could integrate complementary methods, such as camera trapping, to validate detections and assess landscape connectivity in urban wildlife communities.

## 4.5 | Future directions and conclusion

Understanding the composition and dynamics of urban wildlife communities is crucial for effective conservation planning (Des Roches et al., 2021). The presence of mammals in urban areas often leads to human-wildlife conflicts, including road mortality, property damage, direct interactions with people and domestic animals and potential zoonotic risks (Santini et al., 2019). The stable, year-round presence of core species, coupled with human activity, highlights the need for targeted management strategies that mitigate conflict and promote coexistence (Klees van Bommel et al., 2022). Strategies such as creating wildlife corridors, enhancing habitat complexity and implementing public education programs can raise awareness and foster coexistence between urban residents and wildlife (Basak et al., 2023). Urban green spaces provide essential habitat for wildlife, facilitating human-nature interactions and encouraging conservation interest (Basak et al., 2022). However, the role of urban areas in connecting or fragmenting ecosystems and their function as biodiversity reservoirs or ecological traps needs further investigation (Schnetler et al., 2021; Zuñiga-Palacios et al., 2021). Long-term monitoring of urban mammal populations can help identify critical periods when species are most vulnerable to human-wildlife conflicts while also shedding light on how individual species adapt to urban environments. As cities continue to expand, integrating eDNA into biodiversity monitoring programs could be crucial for conserving urban wildlife and maintaining ecological balance (Donald et al., 2021; Webster et al., 2020). A deeper understanding of these dynamics will inform conservation strategies and urban planning, ensuring that both wildlife and human needs are addressed.

## AUTHOR CONTRIBUTIONS

Nyeema C. Harris secured funding and resources. Jane Hallam and Nyeema C. Harris conceptualized the study and conducted the fieldwork. Jane Hallam performed the laboratory work and statistical analyses and drafted the initial manuscript. Nyeema C. Harris led the review and editing process. Both authors contributed to result interpretation and approved the final manuscript.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the Dryad Digital Repository at https://doi.org/10.5061/dryad.905qfttvb (Hallam & Harris, 2025).

## STATEMENT ON INCLUSION

This study was conducted in collaboration with Detroit Metro Parks, whose support was essential to the success of the fieldwork. Our diverse research team, led by two women of colour, actively engaged with local stakeholders to address regionally relevant ecological questions. By integrating varied perspectives, we aim to enrich ecological research and promote inclusivity, reflecting the journal's commitment to equitable and impactful science.

## ORCID

*Jane Hallam* https://orcid.org/0000-0003-3406-7054
*Nyeema C. Harris* https://orcid.org/0000-0001-5174-2205

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## SUPPORTING INFORMATION

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

**Table S1.** Richness, alpha diversity, and beta diversity were calculated for the detected communities, to investigate if there were statistical differences between samples PCRed with 25, 30, or 40 cycles.

**Table S2.** List of species detected by Park and season, bold indicates unique seasonal detection.

**Table S3.** Results from multivariate GLMs of parks community composition and season. Parks with statistically significant difference in community composition highlighted with (*).

**Table S4.** Network statistics (degree, closeness, clustering, and density) for each species detected, in the winter and summer networks.

**Figure S1.** Stacked bar chart depicting species with total proportions of DNA detected, at the 21 parks.

**Figure S2.** Heatmaps displaying the relative abundance of mammal species across the 21 parks for summer and winter.

**Figure S3.** Dendrograms representing the clustering of parks based on total Sørensen dissimilarity, turnover value, and nestedness value (L to R).

**Figure S4.** Bar chart focussing on carnivore detections across seasons and with parks organised in descending size.

**Supporting Information S2.** Methodological details, species detections by season, iNaturalist comparison, statistical results, and figures of detection patterns and network metrics. Master dataset of species detections from soil eDNA across 21 Detroit parks, including metadata, taxonomy, and read counts.

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