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RESEARCH ARTICLE

Beyond species loss: Community reshuffling shapes biodiversity along the urban–rural gradient

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Abstract

1. Urbanization is rapidly transforming landscapes and reshaping wildlife communities, yet it remains unclear whether assemblages along urban gradients are structured mainly by processes of selective species loss/colonization that generate nestedness or by species replacement that generates turnover; we tested these alternative mechanisms with a multi-taxa approach in a major European Metropolitan area.
2. Data from three vertebrate taxa characterized by contrasting ecological traits, dispersal ability and spatial scales of response—bats, small mammals and birds—were collected along an urbanization gradient in the Metropolitan City of Milan (Italy) and analysed to quantify beta diversity and its turnover and nestedness-resultant components. We disentangled environmental and spatial drivers of all beta components using distance-based redundancy analyses and variation partitioning. Finally, we evaluated whether community patterns were shaped by species-specific responses to environmental predictors and/or interspecific associations using Joint Species Distribution Models.
3. Beta diversity was high and consistently dominated by turnover across all taxa. Bat and small-mammal communities were primarily driven by environmental predictors, with bats additionally reflecting broad-scale spatial structuring of environmental conditions along the urban gradient, whereas small mammals responded mainly to local habitat characteristics. In birds, strong broad-scale spatial components also emerged. Consistently, community- and species-level analyses showed that bats mainly responded to a gradient of distance from the city centre associated with changes in vegetation structure; small mammals to urban-fabric characteristics and local habitat features such as grass cover; and birds to a broad-scale urbanization gradient and to a combination of green area extent, patch age, local landscape composition and vegetation features such as dead wood and fallen branches. Credible residual species associations were consistently absent across all taxa.

4. *Synthesis and applications*. Our results indicate that species turnover is a general ecological mechanism shaping vertebrate communities along the urban gradient, driven by taxon- and species-specific responses to environmental and spatial features. Community differences primarily reflect habitat suitability rather than dispersal limitation, highlighting the potential of targeted habitat restoration to support disturbance-sensitive species within urban landscapes. Such actions would help maintain a broader range of ecological niches and overall urban ecosystem functioning.

KEYWORDS
beta diversity, community analyses, habitat restoration, joint species distribution models, nestedness, species turnover, urbanization gradient

1 | INTRODUCTION

Urbanization is one of the most significant drivers of global environmental change, deeply affecting biodiversity and modifying the composition of wildlife assemblages (Petersen et al., 2022). The transformation of natural landscapes into urban areas leads to habitat loss, fragmentation and environmental alterations that exert selective pressures on species (Alberti, 2015; Mori, Di Lorenzo, et al., 2025). Some species may decline or disappear, while others persist or even thrive in urban environments, leading to substantial shifts in community structures. However, the ecological mechanisms underlying these compositional changes remain debated. Specifically, it is unclear whether urbanization primarily acts as a selective filter, leading to a progressive loss of species along the urbanization gradient (e.g. Fernández-Juricic, 2002; Jung & Kalko, 2011; Ślipiński et al., 2012), or whether it fosters species turnover, whereby species adapted to urban conditions replace those unable to persist (e.g. Ganci et al., 2022; Salomão et al., 2019; Starik et al., 2024).

If urbanization functions as a selective filter, urban wildlife assemblages would be structured in a nested manner, representing a subset of those found in less disturbed habitats (Bender et al., 2017; Dondina et al., 2022). In this scenario, species unable to withstand urban pressures would disappear as urbanization intensifies, leaving only the most tolerant behind (Ganci et al., 2022). Alternatively, if turnover predominates, community composition would shift along the gradient due to localized environmental conditions favouring certain species over others (Anderson et al., 2011; Baselga, 2010). In this case, urban communities would not simply be subsets of rural ones but would instead contain species especially suited to urban settings (Ganci et al., 2022). Understanding these processes is critical to underline how urbanization reshapes biodiversity and to develop conservation strategies in increasingly urbanized landscapes.

As most studies have focused on a single group, understanding differences in the simultaneous responses of multiple taxa to urbanization can provide valuable insights into the drivers of community composition shifts in human-dominated landscapes, which is necessary to predict and counteract the resulting disruption of ecosystem functioning and services (Weiskopf et al., 2024). A comparative multi-taxa approach can improve understanding of how urbanization shapes biodiversity. Community composition reflects the combined influence of local environmental conditions and spatial processes related to dispersal among habitat patches (Leibold & Chase, 2018). Because taxa differ in ecological requirements, spatial perception and movement strategies, they may respond differently to landscape transformation, with some taxa being mainly influenced by local habitat characteristics and others by landscape configuration and composition at broader spatial scales (Monadjem et al., 2023). A comparative multi-taxa approach can therefore help disentangle the extent to which responses to urbanization reflect general ecological mechanisms or taxon-specific processes.

In this study, we focused on three vertebrate groups—bats (Chiroptera), small terrestrial mammals (Eulipotyphla and Rodentia) and birds (Aves)—which represent key components of biodiversity within urban and peri-urban green spaces. These taxa are highly sensitive to habitat modifications and exhibit diverse ecological responses to urbanization, making them reliable bioindicators (Aronson et al., 2014; Dondina et al., 2025; Jung & Threlfall, 2016; Russo & Ancillotto, 2015; Sekercioglu, 2006). Bats, for instance, respond in species-specific ways to urbanization: some species benefit from urban roosting and foraging opportunities, while others suffer from habitat loss, artificial light pollution and reduced prey availability (Hale et al., 2012; Jung & Threlfall, 2016; Stone et al., 2015). Similarly, small terrestrial mammals display diverse adaptations to urbanization, with some species exploiting anthropogenic resources while others decline due to habitat loss and degradation (Dondina et al., 2025). Birds also show a wide range of responses to urbanization, with some species, such as generalist and synanthropic birds, thriving in cities, while others, particularly habitat specialists, suffer from habitat fragmentation and reduced nesting opportunities (Blair, 1996; Marzluff, 2017; McKinney, 2006). Importantly, these taxa differ markedly in movement strategies and dispersal ability, from highly mobile flying organisms (birds and bats) to less mobile terrestrial species (small mammals) and are therefore expected to respond to landscape structure at different spatial scales. This contrast strengthens the comparative framework of the study by

allowing evaluation of how taxa sharing a similar ecological context reorganize along urban gradients.

This study aims to investigate and compare the ecological mechanisms driving community composition changes in bats, small terrestrial mammals and birds along a gradient from natural to rural, peri-urban and urban areas in the Metropolitan City of Milan, Italy. By employing a multi-taxa approach, we sought to determine whether community changes result from selective extinction/colonization processes leading to nestedness patterns or from species turnover driven by localized environmental conditions, and whether the relative importance of these processes differs among taxa. We hypothesized that differences in movement ability among taxa would translate into contrasting patterns of community reorganization along the urban gradient, with turnover driven by species replacement along environmental gradients predominating in taxa with limited dispersal ability and stronger responses to local habitat characteristics (small mammals), and a greater contribution of nestedness and spatially structured drivers in highly mobile flying taxa responding to broader spatial gradients (bats and birds) (Soininen et al., 2018).

Specifically, this study aimed to: (i) identify the predominant pattern—nestedness or turnover—shaping community composition along the urbanization gradient, (ii) identify the environmental and spatial drivers of these patterns; (iii) examine whether community composition patterns are shaped by species-level responses to environmental drivers and/or interspecific associations; (iv) assess whether the relative importance of turnover, nestedness and their environmental and spatial drivers differs among taxa with contrasting ecology; and (v) identify implications for urban planning and habitat management aimed at promoting biodiversity persistence across the urban gradient.

2 | MATERIALS AND METHODS

2.1 | Study area

The study was conducted in the Metropolitan City of Milan, located in the central-western Lombardy region (45°N, 9°E) (Figure 1). The Metropolitan City covers an area of approximately 1575 km^2 (https://www.geoportale.regione.lombardia.it/, current administrative boundaries) and spans the northern Po Plain. The Metropolitan City of Milan has a territorial structure consisting of a dense urban core surrounded by peri-urban and rural areas, with agricultural lands extending to the natural boundaries formed by the Ticino River to the west and the Adda River to the east (Figure 1). Remnants of floodplain forests, mainly oak-hornbeam forests dominated by pedunculate oak *Quercus robur* L. and European hornbeam *Carpinus betulus* L., are now confined to few protected areas, such as the Ticino Natural Park. According to the Agricultural and Forest Land Use data DUSAF 7.0 (ERSAF, 2021), the Metropolitan City of Milan consists of 48% agricultural areas, 40% urbanized areas, 10% natural

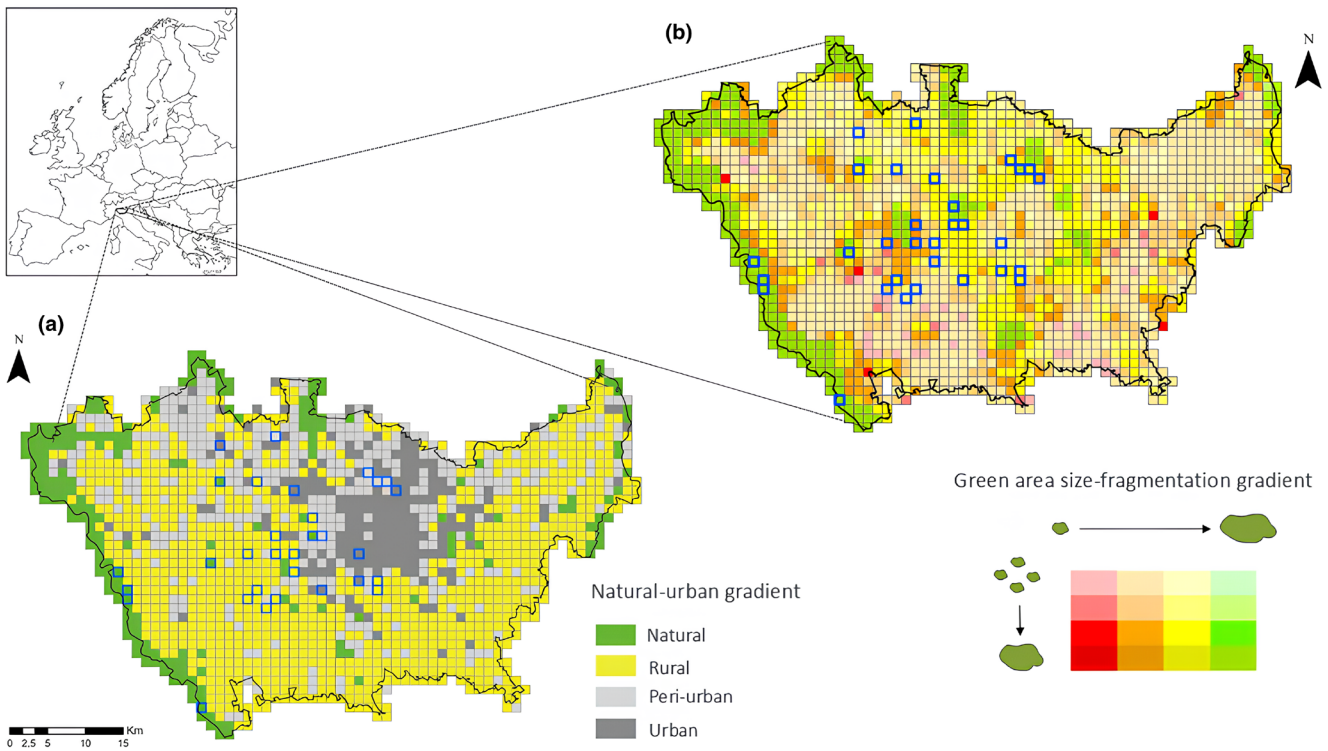

FIGURE 1 Sampling area and sampling design across the (a) natural–urban and (b) green area size-fragmentation gradients. Sample cells are represented in blue.

vegetation (forested areas and semi-natural environments), 0.5% wetlands and 1.5% water bodies.

2.2 | Sampling design

Sampling sites were selected along a composite gradient representing the natural–urban gradient and the green space availability and structure within the Metropolitan City of Milan. We overlaid a 1 × 1 km grid on the study area (EEA reference grid—Italy shapefile) and used the vectorial land use layer DUSAF 7.0 (ERSAF, 2021) to classify each cell into one of the following categories: natural (forests, grasslands and/or wetlands >25% of the cell), rural (discontinuous urban areas ≤25% and agricultural areas >25%), peri-urban (continuous urban areas ≤50% and discontinuous urban areas >25%) and urban (continuous urban areas or infrastructure >50%).

To account for the green space availability and structure we followed the standardized framework developed within the National Biodiversity Future Center (NBFC; Dondina et al., 2025). Specifically, the size and fragmentation of green areas within each 1 × 1 km grid cell were quantified using FRAGSTATS (McGarigal, 1995) based on the Land Cover and Land Use layers from the 2021 ISPRA National Maps (https://groupware.sinanet.isprambiente.it/uso-copertura-e-consumo-di-suolo/library/copertura-del-suolo). Based on green area size, determined as the total surface covered by all green spaces intersecting each 1 × 1 km^2 cell, grid cells were classified into four size categories: A (0.00–0.02 km^2), B (0.02–0.24 km^2), C (0.24–1.00 km^2) and D (>1.00 km^2). Fragmentation was assessed by computing the Aggregation Index (AI) of green areas within a 1.5 km radius circular buffer centred on each cell centroid. Cells were then assigned to one of four fragmentation categories: 1 (AI <67, highly fragmented), 2 (67 ≤ AI <73), 3 (73 ≤ AI <81) and 4 (AI >81, least fragmented). By combining size and fragmentation classifications, all grid cells in the study area were assigned to one of 16 possible categories (see Dondina et al., 2025; Viviano et al., 2024 for details).

We finally selected 31 sampling cells to maximize representativeness across the entire size-fragmentation gradient in order to capture the full variability of the study area in terms of green area availability and structure (Figure 1). Additionally, to encompass the entire natural–urban gradient, we constrained the selection of sampling cells to ensure representativeness of natural (7 sampling cells), rural (13 sampling cells), peri-urban (5 sampling cells) and urban (6 sampling cells) areas (Figure 1).

A random stratified sampling approach was then used to select two or three sampling plots within each 1 × 1 km sample cell, proportional to the size of the sampled green area. Overall, we selected 64 sampling plots, of which 53, 64 and 58 were surveyed for bats, small mammals and birds, respectively. The number of plots sampled for each taxon varied due to logistical constraints, mainly related to the difficulty of finding suitable sites to deploy Audiomoths or accessing sites during the early morning hours for bird surveys (e.g. public parks closed at night).

2.3 | Occurrence data

We focused on vertebrate taxa associated with woodland and tree-dominated habitats (bats, small terrestrial mammals and birds) to ensure ecological comparability across groups. Other tetrapods (e.g. reptiles and amphibians) were not included because they are often associated with more localized habitat types, such as xeric open areas or wetlands, whose spatial distribution is inherently patchy and less consistently structured by the urbanization gradient considered here. Similarly, invertebrates typically operate at different spatial scales and would require different sampling approaches, limiting comparability within the present multi-taxa framework.

2.3.1 | Bats

Data were collected through passive acoustic monitoring using Audiomoths model 1.2.0 updated to firmware version 1.9.3 (©OpenAcoustics, Southampton, UK). At each sampling plot, devices were mounted on vegetation at approximately 2 m above ground level and directed away from dense vegetation (Ancillotto et al., 2023). Data collection was conducted continuously for 3 h after the sunset (May: 8.30–11.30 PM, September: 8.00–11.00 PM) at a sampling rate of 384 kHz. Each recording lasted 60 s, followed by a 60-s pause. Each plot was surveyed during two separate surveys in May and September 2024, covering the seasonal peak of bat activity in the region. Sampling consisted of four consecutive nights of recording per sampling plot. Among the four sampling nights, the one with the best weather conditions (i.e. absence of precipitation and strong wind and minimum nighttime temperatures well above 10°C) was retained for bioacoustics analyses.

Audio files were processed using Kaleidoscope software v5.3.8 (©Wildlife Acoustics, Inc., Maynard, MA) with a frequency range filter set between 8 and 190 kHz. This filtering step helped isolate bat calls from background noise. Species identification was performed using BatScope4 (Obrist & Boesch, 2018). The Europe MCP (Minimum Convex Polygon) database, based on GBIF distribution data, was used for species classification. Each audio file was divided into six 10-s sequences and processed through three sequential steps to standardize analysis and improve detection accuracy. First, calls were extracted using the Peak Search Cutter, which identifies signals based on spectral amplitude peaks. Then, 59 call parameters were measured, including signal directionality, duration and shape, with a high-pass filter at 5 kHz applied to eliminate low-frequency noise. Finally, calls were classified using seven machine learning classifiers (KNN, QDA, RF, SVM, KKNN, NN) implemented in BatScope4 (Obrist & Boesch, 2018), retaining only sequences with ≥95% confidence and agreement among at least four classifiers. All classifications were manually verified by inspecting spectrograms and comparing them with reference data from Dietz and

Kiefer (2016); during manual inspections, social calls were also used to confirm identification, whenever present (Pfalzer & Kusch, 2003).

Bat calls were identified to species or phonic groups, with some species (*Myotis* spp. and *Plecotus* spp.) grouped together due to overlapping acoustic characteristics.

2.3.2 | Small mammals

At each sampling plot, four PVC hair-tubes (40 cm in length, 5 cm in diameter) were deployed following a protocol adapted from Zozzoli et al. (2018). Double-sided adhesive tape, covering 8 cm on both inner tube sides, was used to collect hair samples. Two tubes were positioned at the base of tree trunks, and two were placed on branches at a height of approximately 1.5–2 meters, allowing detection of both ground-dwelling and arboreal small mammals. The tubes were baited with peanut butter and inspected monthly from June to November in both 2023 and 2024. Due to logistical constraints, 31 plots were surveyed in 2023 and 33 in 2024. During each visit, adhesive tapes were replaced and baits refreshed.

Collected tapes were examined under a stereomicroscope. Hairs were removed by applying a drop of vegetable oil, followed by sequential rinses in ethanol and water. Only hairs classified as GH1 or GH2 (Teerink, 2003) were used for species identification, following the dichotomous keys by Paolucci and Bon (2022). Hair length was measured with a stereomicroscope and graph paper. Cuticular patterns were analysed from casts made with transparent nail polish on microscope slides (De Marinis & Agnelli, 1993), while the medulla was examined directly after immersion in cedar oil. For cross-sectional analysis, hairs were mounted between strips of adhesive tape, and two 2 mm sections (taken from the proximal and distal ends of the shield) were excised with a scalpel. These were mounted perpendicularly on microscope slides for detailed morphological examination and classification.

It should be noted that small mammals were sampled in 2023–2024, whereas bats and birds were surveyed only in 2024. Because small mammals were surveyed using the same field protocol in both years, and given their low expected interannual turnover and the high sampling completeness achieved across taxa (see Results), this temporal discrepancy is unlikely to affect comparability.

2.3.3 | Birds

Data were collected through both passive and active acoustic monitoring techniques.

Passive acoustic data were collected using Audiomoths recording from 5.00 to 10.00 AM, and from 8.30 PM to 3.00 AM of the day after to detect nocturnal vocalizing species. Audiomoths were configured as described above for passive acoustic monitoring of bats. Data were recorded in one 4-day survey session in May. Data were analysed with BirdNET (Kahl et al., 2021; https://birdnet.cornell.edu) following a custom protocol (Pérez-Granados, 2023; Toenies & Rich, 2021) and BirdNET documentation. The procedure involved species identification using BirdNET-Analyser-GUI with default parameters and geographic filters, and verification of species with confidence >0.95, with manual checks for lower-confidence detections.

Active acoustic monitoring was developed following a standardized method based on the 10-min unlimited distance point count technique (Blondel, 1981). Each sampling plot was surveyed during two separate surveys in May and June 2024, covering the seasonal peak of breeding birds' activity in the region. Surveys were carried out from sunrise to 11.00 AM only in good weather conditions (Bani et al., 2019).

Data collected through passive and active monitoring techniques were integrated and a species was considered present at a site if it was detected by at least one of the two methods. The combination of passive and active monitoring enhanced detection probability and improved community composition estimates, which is essential for detailed analyses given that standard 10-min point counts typically record about 80%–90% of species (Ralph, 1999). The integration of methods was particularly beneficial in accounting for species vocalizing at different times of the day.

For each taxon—bats, small terrestrial mammals and birds—the data collected across the different surveys were aggregated at the plot level, with species considered present if detected in at least one survey and absent if not recorded in any of the surveys.

Ethical approval was not required for this study because no animal capture, handling or manipulative procedures subject to institutional or national regulations were performed. Field studies were conducted following the acquisition of all necessary licences and permits. Permission to carry out fieldwork was granted by the relevant municipal authorities and competent bodies.

2.4 | Environmental data

Each sampling plot was characterized based on the following set of environmental variables:

- Green area cover within the sampling cell: measure calculated both as a continuous variable and as a categorical variable with four levels (see the Sampling design section).
- Green area fragmentation degree within the sampling cell: measure calculated both as a continuous variable and as a categorical variable with four levels (see the Sampling design section).
- Distance from city centre: a continuous variable measured in meters.
- Age of the green patch in which the sampling plot was located: a categorical variable with five levels (4: year ≤ 1954, 3: 1955 ≤ year ≤ 1989, 2: 1990 ≤ year ≤ 1999, 1: 2000 ≤ year ≤ 2009, 0: year ≥ 2010). Patch age was obtained directly from site managers when available or reconstructed through interpretation of

historical orthophotos available through the Regione Lombardia Geoportal (https://www.geoportale.regione.lombardia.it).

- Habitat type: a categorical variable with four levels (woodland, hedgerow, reforestation, urban park) assigned in the field based on the dominant habitat characteristics of each site.
- Fractional cover of paved areas and lentic/lotic water bodies within a 100 m circular buffer around the plot: three continuous variables (source: DUSAF 7.0, ERSAF, 2021). A 100-m buffer was used as an ecologically meaningful scale to characterize land cover features around each plot, as it reflects the local range of less mobile taxa such as small mammals (typically 50–100 m; Ancillotto et al., 2025) while remaining appropriate for more mobile groups such as birds (Alba et al., 2025).
- Type of urban fabric in a 100 m buffer surrounding the plot: a categorical variable with four levels (central continuous fabric, peripheral continuous fabric, discontinuous fabric, industrial areas). This variable reflects differences in building density, infrastructure and land use composition (see Mori, Lazzeri, et al., 2025).
- Visually estimated tree cover in a 10 m and in a 100 m circular buffer around the plot: two continuous variables, estimated in the field by trained observers as the proportion of ground occupied by tree canopy within each buffer.
- Visually estimated average of tree height: a continuous variable, visually estimated in the field by trained observers as the average height of trees in each plot.
- Mean circumference of ten trees measured around the plot: a continuous variable.
- Tree layer composition: one categorical variable with seven levels (coniferous forest, mesophilic broadleaf forest, hygrophilous broadleaf forest, oak forest, plane tree forest, black locust forest and forests composed of other alien species). In each plot, we estimated the percentage cover of the three most abundant tree species and assigned the corresponding category based on their relative dominance (Ancillotto et al., 2025).
- Visually estimated shrub cover in a 10 m and in a 100 m circular buffer around the plot: two continuous variables, estimated in the field by trained observers as the proportion of ground occupied by shrubs within each buffer.
- Visually estimated average shrub height: a continuous variable, visually estimated in the field by trained observers as the average height of shrubs in each plot.
- Shrub layer composition: one categorical variable with five levels (native berry-bearing shrubs, Mediterranean scrub shrubs, hazel-dominated shrubs, brambles and alien shrubs). In each plot, we estimated the percentage cover of the three most abundant tree species and assigned the corresponding category based on their relative dominance (Ancillotto et al., 2025).
- Visually estimated grass cover in a 10 m and in a 100 m circular buffer around the plot: two continuous variables, estimated in the field by trained observers as the proportion of ground occupied by grasses within each buffer.
- Visually estimated average grass height: a continuous variable, visually estimated in the field by trained observers as the average height of grasses in each plot.
- Presence/absence of cavity trees, dead trees, fallen branches, clearings, water sources and rocks within a 50-m radius buffer around the plot: five categorical variables, each with two levels.

2.5 | Data analyses

To explore the effects of urbanization on the community composition of bats, small mammals and birds, we conducted a series of analyses to evaluate beta diversity patterns, environmental drivers and possible species associations with specific environmental conditions and/or other species.

2.5.1 | Assessment of sampling completeness

To assess sampling completeness, we used the *iNEXT* package (Hsieh et al., 2016) to estimate species richness (Hill number $q = 0$) based on incidence data. We generated sample coverage-based rarefaction/extrapolation curves to evaluate whether the sampling effort was sufficient to capture the majority of species within each taxonomic group.

2.5.2 | Beta diversity partitioning

We quantified beta diversity (βSOR) and partitioned it into turnover (βSIM) and nestedness-resultant (βSNE) components (Baselga, 2010) using the *betapart* R package (Baselga et al., 2018; Baselga & Orme, 2012). This allowed us to determine whether community differences across the urbanization gradient were primarily driven by species replacement (turnover) or by a progressive loss/gain of species that result in nested community patterns.

Detection probability was not explicitly accounted for in the analyses, which may be particularly relevant for small mammals and less vocal bird species. However, the high sampling completeness observed across taxa suggests that potential detection biases are unlikely to have substantially affected the main patterns of community dissimilarity along the urban gradient.

2.5.3 | Environmental drivers of beta diversity

To identify the main environmental and spatial factors shaping variation in community composition, we conducted distance-based redundancy analyses (db-RDA; Legendre & Anderson, 1999) using the *vegan* R package (Oksanen et al., 2026). We considered the overall Sørensen dissimilarity (βSOR) and its turnover (βSIM) and nestedness-resultant (βSNE) components (Baselga, 2010)

derived from the *betapart* analysis as response distance matrices. Environmental predictors were the set of environmental variables described in the Environmental data section. Prior to analysis, sites with no recorded species (bats: $N=3$; small mammals: $N=8$; birds: $N=0$) were excluded to avoid zero-sum rows in the community matrix. We scaled continuous variables and applied a variance inflation factor (VIF) calculated with the *vif* function in the *usdm* R package (Naimi et al., 2014). Categorical variables were converted into dummy variables, and VIF was computed directly on the set of predictors. A threshold of VIF < 10 was used to retain predictors, following Naimi et al. (2014). Spatial autocorrelation was modelled using distance-based Moran's eigenvector maps (MEM; Dray et al., 2006) computed from site coordinates (*adespatial* R package; Dray et al., 2026). dbMEM variables were derived from a truncated distance matrix based on the minimum spanning tree among sampling locations. Eigenvectors representing spatial structures at multiple scales were generated, and a subset of MEM variables was selected separately for each β-diversity component using forward selection with adjusted R^2 as stopping criterion (999 permutations).

For each β-component (βSOR, βSIM, βSNE), we quantified the relative contribution of environmental and spatial drivers by fitting a full db-RDA model including both environmental predictors and spatial MEMs, and then partitioning the explained variation into the pure environmental component, the pure spatial component and the spatially structured environmental variation. Model significance was tested with 999 permutations, and adjusted R^2 values were computed to quantify explanatory power. The significance of the pure environmental and spatial fractions was assessed using permutation tests (999 permutations) in partial db-RDA models.

To obtain a parsimonious set of significant predictors for each β-component (βSOR, βSIM and βSNE), we performed a forward selection procedure using the *ordiR2step* function in the *vegan* R package. This approach retained only variables that significantly improved model fit (based on adjusted R^2 and permutation tests, 999 permutations). The resulting set of predictors was then used in the final db-RDA models. Environmental predictors included in the selected models subsequently served as input variables for the species-level joint distribution models (see below).

2.5.4 | Species-level responses to environmental drivers and interspecific associations

To quantify species-specific responses to the environmental variables identified through the db-RDA analyses, separate Joint Species Distribution Models (JSDMs) were fitted for each taxonomic group (bats, small mammals and birds) using the Hierarchical Model of Species Community (HMSC) framework (Ovaskainen & Abrego, 2020) and the *Hmsc* package (Tikhonov et al., 2020). JSDMs integrate species-specific responses within a hierarchical framework that models shared environmental responses and residual interspecific associations beyond those explained by measured covariates (Ovaskainen & Abrego, 2020). This approach allowed assessing the response of individual species to the key environmental predictors driving community composition (Ovaskainen et al., 2017).

We modelled the incidence matrix (sites × species) using a hierarchical Bayesian framework with a probit link function and a Bernoulli error distribution. For each taxon, the environmental covariates included in the fixed-effects structure were those selected from the db-RDA analyses. To account for spatial autocorrelation and unmeasured environmental heterogeneity among sampling sites, we included a spatial random effect defined by site coordinates. This random level was specified with four latent factors (nf = 4), allowing spatially structured latent variables to capture residual species associations driven by proximity or other unmeasured environmental gradients. Default weakly informative priors implemented in HMSC were used for all model parameters. Posterior inference was conducted using Markov chain Monte Carlo (MCMC) sampling with two chains. For each chain, 1000 burn-in iterations were discarded, followed by 2000 retained samples saved every 10 iterations, resulting in a total of 30,000 MCMC iterations per chain. Convergence of the MCMC chains was assessed by computing the potential scale reduction factor (PSRF; Brooks & Gelman, 1998; Gelman & Rubin, 1992) and the effective sample size (ESS) for all model parameters, including fixed-effects (β) and latent-level parameters, namely the species-specific factor loadings (Λ) and the latent factor covariance matrix ($\boldsymbol{\Omega}$). Values of PSRF < 1.1 and ESS > 200 were considered indicative of adequate convergence. Posterior means and posterior support values (probability that $|\beta| > 0$) were extracted for each species–environment relationship. We interpreted relationships as credible when posterior support exceeded 0.95, indicating a consistent positive or negative response across chains.

Finally, we quantified residual associations among species by examining the posterior mean correlation matrix ($\boldsymbol{\Omega}$), which describes co-occurrence patterns not explained by environmental and spatial covariates. For each pair of species, we extracted the posterior mean correlation coefficient and the posterior support (probability of consistent sign across chains). Associations were interpreted as credible when the posterior support exceeded 0.95, reflecting strong evidence for positive (co-occurrence) or negative (segregation) residual correlation (Ovaskainen & Abrego, 2020).

All analyses were conducted in R (version 4.5.0) (R Core Team, 2025).

3 | RESULTS

3.1 | Assessment of sampling completeness

Overall, we detected nine species/group of species of bats, ten species/group of species of small mammals and 26 species of birds (Table S1). Sample completeness curves for all taxa approached an asymptote, indicating that the sampling effort was sufficient to capture nearly all species/group of species present in the study area (Figure S1). For bats, the observed species richness was nine, with an asymptotic estimate of 9.98 species (95% CI: 9.0–11.4), suggesting that

the sampling captured approximately 98% of the expected diversity. For small mammals, the observed and asymptotic richness were identical (10 species), indicating full completeness (sample coverage = 1). Similarly, for birds, both the observed and asymptotic richness were 26 species, confirming complete sampling (sample coverage = 1).

3.2 | Beta diversity partitioning

The beta diversity analysis for bats revealed a high overall dissimilarity (βSOR = 0.931), with species turnover (βSIM = 0.820) being the predominant driver of community variation across the urbanization gradient. The nestedness-resultant component (βSNE = 0.111) had a limited contribution to beta diversity.

For small mammals, overall beta diversity was also high (βSOR = 0.957), with turnover (βSIM = 0.909) being the primary driver of dissimilarity, while the nestedness-resultant component played a minimal role (βSNE = 0.048).

Bird communities exhibited a high level of beta diversity (βSOR = 0.895), predominantly driven by species turnover (βSIM = 0.830), with a lower contribution from the nestedness-resultant component (βSNE = 0.066).

3.3 | Environmental drivers of community composition

Distance-based redundancy analyses (db-RDA) identified distinct sets of environmental and spatial predictors influencing community composition across taxa. For all taxa, final models including both environmental and spatial components were significant, although the selected predictors and the amount and partitioning of explained variance varied among taxa (Table 1, Tables S2–S4).

The db-RDA models for bats were significant for all β-components. The proportion of explained variance (adjusted R^2—full) was moderate for both overall β-diversity and turnover and low for the nestedness-resultant component. Variation partitioning showed that, for all three components of beta diversity, only the pure environmental fraction had a significant effect. In contrast, the pure spatial component explained a much smaller proportion of variance and was not significant. For βSOR and βSIM, the shared environmental and spatial component also showed a relatively high explained variance. These results indicate that local environmental factors along with a spatially structured environmental variation were the main drivers of variation in β-diversity, while spatial structure alone had little explanatory power (Table 1). For βSOR ($F_{1,46} = 2.96$, $p = 0.015$) and βSIM ($F_{1,46} = 3.04$, $p = 0.017$), variation was mainly explained by mean tree circumference. The nestedness-resultant component (βSNE) was only weakly related to grass cover within the 100 m buffer ($F_{1,47} = 3.18$, $p = 0.044$). Predictors retained in the final models but not showing significant effects are reported in Table S2.

The db-RDA models for small mammals were significant for both overall β-diversity (βSOR) and turnover (βSIM), whereas no variables were retained for the nestedness-resultant component (βSNE) (Table 1). Both pure environmental and spatial components significantly contributed to βSOR and βSIM variations, with the environmental fraction slightly exceeding the spatial one in terms of explained variance, while the shared environmental and spatial component showed a negligible explained variance (Table 1). For βSOR, variation was mainly explained by a single spatial eigenvector (MEM3: $F_{1,51} = 3.01$, $p = 0.005$) reflecting the geographic clustering

TABLE 1 Summary of db-RDA results for bats, small mammals and birds, showing adjusted R^2 values for the full model, the pure environmental component (a), the pure spatial component (b) and the shared component (c), together with model significance (p-value) and the list of significant predictors selected for overall β-diversity (βSOR), species turnover (βSIM) and nestedness (βSNE).

		Adjusted R^2					
Taxon	**β-Component**	**Full**	**a**	**b**	**c**	***p*-value**	**Significant predictors**
Bats	βSOR	0.140	0.038*	0.002	0.102	0.003	Mean tree circumference
	βSIM	0.124	0.094*	0.015	0.129	0.001	Mean tree circumference
	βSNE	0.043	0.043*	0	0	0.041	Grass cover (100 m)
Small mammals	βSOR	0.081	0.063**	0.050**	0.005	0.001	MEM3, Urban fabric (peripheral continuous)
	βSIM	0.101	0.118**	0.069**	0.006	0.001	MEM3, Urban fabric (peripheral continuous), Mean tree circumference, Grass cover (100 m)
	βSNE	—	—	—	—	—	None selected
Birds	βSOR	0.211	0.087**	0.076**	0.161	0.001	MEM3, MEM6, Dead trees [Presence], MEM1
	βSIM	0.133	0.078**	0.094*	0.149	0.001	Patch age [4], MEM1, Dead trees [Presence]
	βSNE	0.335	0.167**	0.170**	0.165	0.001	MEM4, MEM3, Fallen branches [Presence], Mean tree height, Clearings [Presence]

Note: p-values are based on permutation tests (999 permutations). Asterisks indicate statistically significant pure environmental (a) and pure spatial (b) components based on permutation tests.

*$p < 0.05$. **$p < 0.01$.

of sampling sites within the central part of the Ticino Natural Park (Figure S2) and the peripheral continuous component of the urban fabric ($F_{1,51}=2.93$, $p=0.013$). For βSIM, significant predictors included MEM3 ($F_{1,51}=2.93$, $p=0.006$), the peripheral continuous component of the urban fabric ($F_{1,51}=2.51$, $p=0.020$), mean tree circumference ($F_{1,51}=2.32$, $p=0.037$) and grass cover within the 100 m buffer ($F_{1,51}=2.31$, $p=0.023$). Predictors retained in the final models but not showing significant effects are reported in Table S3.

The db-RDA models for birds were significant for all β-components (Table 1). The proportion of explained variance was relatively high for overall β-diversity and turnover and particularly high for the nestedness-resultant component. Variation partitioning indicated substantial and significant contributions of both pure environmental and spatial components, and their shared contribution, across all β-components (Table 1).

For βSOR, variation was mainly explained by spatial structure—MEM3 reflecting a north–south gradient ($F_{1,46}=2.31$, $p=0.007$), MEM6 reflecting a localized spatial structure ($F_{1,46}=2.03$, $p=0.018$) and MEM1 reflecting an east–west gradient ($F_{1,46}=1.80$, $p=0.043$, Figure S3)—and by vegetation structure, particularly the presence of dead trees ($F_{1,46}=1.92$, $p=0.033$). For βSIM, significant predictors included patch age (Patch age [4]; $F_{1,49}=2.06$, $p=0.014$), presence of dead trees ($F_{1,49}=1.90$, $p=0.029$) and the spatial components MEM1 reflecting the east–west gradient ($F_{1,49}=1.96$, $p=0.024$). Nestedness (βSNE) was strongly associated with spatial predictors—MEM4 ($F_{1,50}=6.08$, $p=0.004$) reflecting a geographic clustering of sampling sites located within the southern part of the Ticino Natural Park and MEM3 ($F_{1,50}=5.17$, $p=0.006$) reflecting the north–south gradient—the presence of clearings ($F_{1,50}=3.00$, $p=0.036$) and vegetation structure, including fallen branches ($F_{1,50}=5.78$, $p=0.007$) and mean tree height ($F_{1,50}=3.43$, $p=0.026$). Predictors retained in the final models but not showing significant effects are reported in Table S4.

3.4 | Species-level responses to environmental drivers and interspecific associations

For bats, convergence diagnostics indicated good MCMC performance, with all fixed-effect parameters showing PSRF <1.1 (maximum = 1.013) and effective sample sizes well above recommended thresholds (median ESS = 3161). Species-level responses showed several well-supported environmental effects (posterior support ≥0.95), with marked differences in both direction and magnitude among species (Figure 2a). Distance from the city centre was positively associated with *Nyctalus noctula* (support = 0.99) and *Vespertilio murinus* (0.98), whereas it exhibited a negative effect for *Hypsugo savii* (0.97). Grass cover within 100 m showed positive associations with *Cnephaeus serotinus* (0.96), *Nyctalus noctula* (0.98), *Pipistrellus nathusii/kuhlii* (0.97) and *Vespertilio murinus* (0.97). Mean tree circumference did not show credible effects for any species (all support <0.95).

No credible residual species associations were detected (posterior correlation support <0.57), suggesting that co-occurrence patterns were largely explained by environmental covariates and spatial latent structure.

For small mammals, convergence diagnostics indicated excellent MCMC performance, with all fixed-effect parameters showing PSRF <1.1 (maximum = 1.007) and high effective sample sizes (median ESS = 3105). The JSDM identified several well-supported species–environment relationships (posterior support ≥0.95), revealing substantial interspecific variability in response direction and magnitude (Figure 2b). The peripheral continuous component of the urban fabric showed a strong positive effect for *Erinaceus europaeus* (support = 0.98). At a local scale, grass cover within 100 m exhibited a positive effect for *Glis glis* (support = 0.95) and mean tree circumference showed a negative effect for *Erinaceus europaeus* (support = 0.97) and *Sciurus carolinensis* (support = 0.95).

No credible residual species associations were detected (all posterior support <0.95). A single negative association between *Muscardinus avellanarius* and *Sciurus carolinensis* showed relatively high support (correlation = −0.61; support = 0.91), although still below the credibility threshold, whereas all other pairwise associations showed substantially lower support.

For birds, convergence diagnostics indicated good MCMC performance, with all fixed-effect parameters showing PSRF <1.1 (maximum = 1.047) and effective sample sizes well above recommended thresholds (median ESS = 2154). The JSDM identified numerous well-supported species–environment relationships (posterior support ≥0.95), highlighting pronounced interspecific variability in environmental responses (Figure 2c). In terms of habitat composition and configuration, green area cover showed a positive effect for *Buteo buteo*, *Certhia brachydactyla*, *Dendrocopos major*, *Erithacus rubecula*, *Muscicapa striata*, *Oriolus oriolus*, *Poecile palustris*, *Sitta europaea* and *Troglodytes* (support = 0.96–1.00). Patch age showed multiple well-supported effects (support = 0.95–0.97): old patches ([4] year ≤ 1954; [3] 1955 ≤ year ≤ 1989) had a negative effect for *Erithacus rubecula*, *Fringilla coelebs*, *Muscicapa striata* and *Falco subbuteo*; while young patches ([1] 2000 ≤ year ≤ 2009) had a strong negative effect for *Sitta europaea* and *Troglodytes troglodytes*. The fractional cover of lotic water bodies within a 100 m circular buffer showed a negative effect for *Aegithalos caudatus*, *Erithacus rubecula*, *Phoenicurus phoenicurus* and *Regulus ignicapilla* (support = 0.96–1.00). Finally, the local occurrence of clearings showed a negative effect for *Erithacus rubecula*, *Luscinia megarhynchos*, *Oriolus oriolus* and *Regulus ignicapilla* (support = 0.96–0.97). In terms of vegetation structure, fallen branches showed widespread positive associations, with credible responses for *Aegithalos caudatus*, *Columba palumbus*, *Corvus cornix*, *Dendrocopos major*, *Fringilla coelebs*, *Muscicapa striata*, *Parus major*, *Sylvia atricapilla* and *Turdus merula* (support = 0.96–0.98); while the occurrence of dead trees showed a positive effect for *Luscinia megarhynchos* only (support = 0.98). Mean tree circumference showed a negative effect for *Aegithalos caudatus*, *Dendrocopos major*, *Luscinia megarhynchos*, *Muscicapa striata*, *Oriolus oriolus* and *Picus viridis*

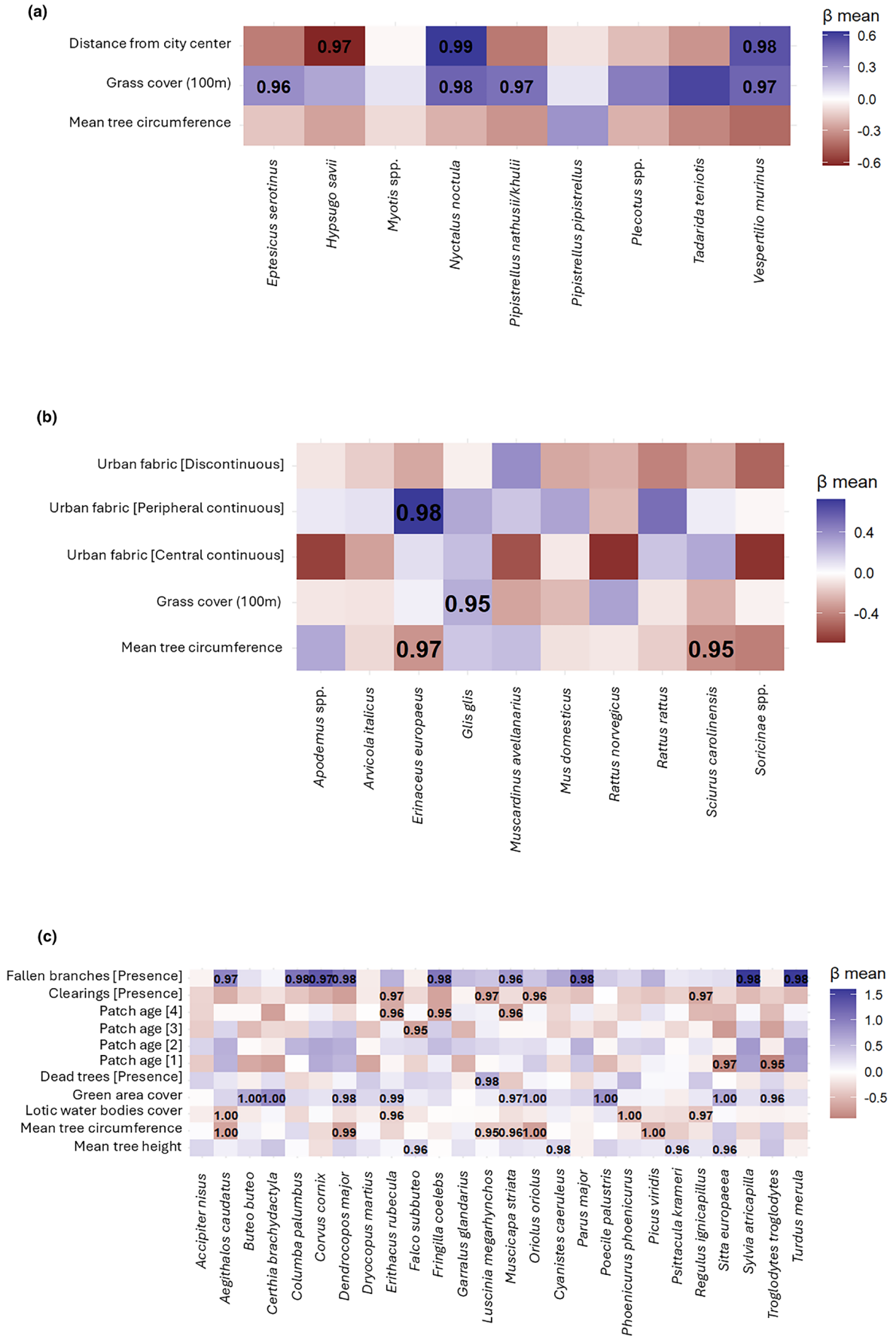

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FIGURE 2 Species-specific responses of bats (a), small mammals (b) and birds (c) to environmental predictors derived from the Joint Species Distribution Model. The heatmap shows posterior mean regression coefficients (β) for each species–environment relationship, with colour indicating effect direction (blue = positive, red = negative). Numbers represent posterior support values (probability that $|\beta| > 0$). Effects were interpreted as credible when posterior support ≥0.95. Empty cells indicate non-credible effects.

(support = 0.95–1.00). Finally, mean tree height showed a positive effect for *Falco subbuteo*, *Cyanistes caeruleus*, *Psittacula krameri* and *Sitta europaea* (support =0.96–0.98).

No credible residual species associations were detected (all posterior support <0.70), suggesting that co-occurrence patterns were largely explained by environmental predictors and spatial random effects.

4 | DISCUSSION

4.1 | Species replacement as the dominant mechanism

This study investigated how urbanization shapes vertebrate community composition along an urban–natural gradient in a major European metropolitan area using a comparative multi-taxa approach. Contrary to our expectations, community reorganization was consistently dominated by species turnover across all taxa, including highly mobile groups such as bats and birds. This indicates that differences in dispersal ability did not translate into fundamentally different compositional outcomes. Consistent with previous findings (Briones-Salas et al., 2024; Ganci et al., 2022), urban communities emerge as distinct assemblages characterized by the substitution of species along the urban gradient, rather than as depauperate subsets of more natural communities. Importantly, this pattern of species turnover does not indicate biotic homogenization, but rather a spatial reorganization of assemblages driven by changes in environmental conditions along the gradient. In this context, urbanization promotes the emergence of novel community configurations shaped by species-specific responses to habitat characteristics, rather than a simple convergence towards increasingly similar communities.

From a comparative perspective, our results indicate that species replacement represents a general ecological response of vertebrate communities to urbanization, while the relative importance of environmental and spatial drivers underlying these compositional changes remains taxon-specific.

4.2 | Taxon-specific environmental and spatial drivers and species-level responses

Although the turnover pattern we found was consistent across all taxa, the specific drivers influencing community composition varied, reflecting taxon-specific ecological responses. The redundancy analyses revealed that both environmental and spatial components contributed to explaining community patterns, but their relative importance—and the specific variables driving these patterns—differed among taxa.

4.2.1 | Bats

In bats, community changes along the urban gradient were primarily driven by environmental variation, with a substantial proportion of explained variance associated with spatially structured environmental components. This result highlights the importance of habitat characteristics associated with urbanization that vary systematically along the landscape. In particular, the prominence of mean tree circumference on overall and turnover-associated diversity (βSOR and βSIM) stressed the importance of vegetation maturity in determining how bat assemblages reorganize across the landscape. These structural features likely integrate multiple ecological functions—such as roost availability, microclimatic stability and prey support (Ancillotto et al., 2019; Mas et al., 2021)—therefore influencing both overall turnover and broader patterns of species replacement.

The JSDM results further clarify how these environmental characteristics are structured along the urban gradient, particularly in relation to distance from the city centre. Species associated with woodlands and semi-natural habitats (e.g. *Nyctalus noctula*, *Vespertilio murinus*) showed positive responses to increasing distance from the city centre, reflecting their dependence on continuous habitat and mature forest elements typical of less urbanized contexts (Tzortzakaki et al., 2019). In contrast, urban-adapted species such as *Hypsugo savii* showed the opposite response, reflecting their ability to exploit dense built environments and artificial light foraging opportunities (Ancillotto et al., 2019; Azam et al., 2015; Russo & Ancillotto, 2015; Tomassini et al., 2014). The positive associations of several species—including *Cnephaeus serotinus*, *Nyctalus noctula*, *Pipistrellus nathusii/kuhlii* and *Vespertilio murinus*—with grass cover highlight the functional role of small open areas as foraging patches embedded within the urban matrix (Jung & Threlfall, 2016). Interestingly, although tree structure emerged as an important determinant of community-level β-diversity, no single species showed a strong response to this variable. This suggests that its influence operates cumulatively across species, shaping assemblages through subtle shifts rather than strong, species-specific reactions.

Finally, the absence of meaningful residual species associations indicates that once environmental and spatial variation are accounted for, interspecific interactions contribute little to community structure. However, the absence of residual correlations does not automatically imply that biological interactions are absent, as such interactions may not be detectable at the spatial scale of our sampling or may be absorbed by environmental predictors and spatial

random effects included in the models. More broadly, species co-occurrence patterns should not be interpreted as a direct proxy for ecological interactions (Blanchet et al., 2020).

4.2.2 | Small mammals

In small mammals, community reorganization along the urban gradient was driven by both pure environmental and spatial components. The spatial component was mainly associated with a single local scale spatial structure distinguishing sites ($N = 4$) located within the central part of the protected natural area (Ticino Natural Park) from those occurring in rural, peri-urban and urban contexts, indicating a marked compositional difference between these relatively undisturbed habitats and the rest of the gradient. Beyond this spatial contrast, variation in community composition was primarily explained by environmental characteristics associated with habitat structure and urban context. Overall β-diversity (βSOR) was shaped mainly by the type of urban fabric, with the peripheral continuous zone emerging as the principal driver. This intermediate portion of the urban gradient, where dense built areas transition into more heterogeneous peri-urban matrices, appears to host distinct small-mammal assemblages. The turnover component (βSIM) was influenced not only by urban-fabric context but also by local habitat features, including grass cover and tree structure, suggesting that species replacement in this group reflects a combination of the broader degree of urban continuity and fine-scale microhabitat features (Ancillotto et al., 2025; Dondina et al., 2025).

Species-level responses corroborate this interpretation and highlight the importance of taxon-specific ecological requirements. *Erinaceus europaeus*, a typical generalist species, responded positively to the peripheral continuous urban fabric, consistent with its known preference for structurally heterogeneous but moderately disturbed suburban environments (Baker & Harris, 2007; Turner et al., 2022). The recurring presence of this species in such areas contributes to making small-mammal assemblages in the peripheral urban zone distinct from those found in both the more natural outer landscape and the highly urbanized city core. Grass cover had a positive effect on *Glis glis*, likely because grass-rich plots in our study area represent semi-natural peri-urban or rural green areas with an associated arboreal component, where this species was consistently detected. *Erinaceus europaeus* and the invasive *Sciurus carolinensis* showed a negative response to mean tree circumference, suggesting an association with less structurally mature vegetation, possibly reflecting their ability to exploit managed green areas with simpler vertical structure (Pearce, 2017).

As for bats, the JSDM detected no credible residual species associations once environmental and spatial effects were accounted for, suggesting that interspecific interactions play a negligible role in shaping assemblages, consistent with previous evidence (Dondina et al., 2025). The weak negative trend between *Muscardinus avellanarius* and *Sciurus carolinensis* did not reach credibility and should be interpreted cautiously.

4.2.3 | Birds

Bird communities responded strongly to both environmental conditions and spatial variation. Across all β-components, key predictors were linked to multiple spatial components, habitat structure (e.g. dead trees, fallen branches and tree height), and to the anthropogenically driven history of forest patches, as captured by patch age. These variables reflect differences in vegetation complexity, management legacies and structural heterogeneity across the spatial gradient, which together shape how bird assemblages reorganize in urban and peri-urban landscapes.

Specifically, overall β-diversity was associated with spatial configuration, capturing broad-scale geographic gradients across the study area, including a north–south gradient (MEM3) and an east–west gradient (MEM1), as well as a more localized spatial structure linked to site-specific environmental characteristics (MEM6). The north–south gradient likely reflects differences in species pools associated with contrasting landscape contexts located south and north of the metropolitan area. Sites south of Milan are embedded within the agricultural landscape of the protected area Parco Agricolo Sud Milano and host assemblages influenced by rural and semi-natural lowland habitats, which also extend into the protected area Parco del Ticino to the west. Conversely, sites located north of the city are influenced by ecological conditions associated with the pre-Alpine foothills (Canedoli et al., 2017; Dondina et al., 2022). The highly urbanized core of Milan lies between these two contexts and likely contributes to maintaining differences in community composition by limiting the mixing of assemblages associated with these distinct regional landscapes. The east–west gradient broadly corresponds to the transition from natural and rural landscapes to increasingly urbanized contexts, reflecting spatially structured variation in habitat composition. In addition to spatial configuration, overall β-diversity was associated with the availability of dead trees, underscoring the importance of cavity-bearing structures and mature elements within forest patches.

Turnover was strongly associated with the main east–west spatial gradient, corresponding to the transition from natural and rural landscapes to increasingly peri-urban and urban contexts. Importantly, this spatially structured pattern was closely linked to variation in patch age and the availability of dead wood, two features typically associated with forest maturity. Along the gradient, structurally mature woodland patches located in the Parco del Ticino to the west are progressively replaced by residual forest patches in rural areas and by more recent reforestations in peri-urban and urban contexts. This indicates that species replacement is largely driven by spatially structured differences in forest developmental stages, whereby mature stands characterized by the presence of dead wood support assemblages that are progressively replaced by species associated with younger and more recently established woodland patches. Conversely, the nestedness component was associated with the north–south spatial gradient, indicating a progressive differentiation in community composition along this geographic gradient. In addition, the nestedness component was related to the occurrence of

clearings and to local vegetation features such as fallen branches and mean tree height, highlighting the role of habitat structural complexity in shaping the distribution of species subsets across the landscape.

Birds exhibited a wide range of species-specific responses. Forest-interior species (*Erithacus rubecula*, *Poecile palustris*, *Sitta europaea*, *Certhia brachydactyla* and *Troglodytes troglodytes*) showed positive associations with green area cover, a pattern that reflects the need for sufficiently large and weakly fragmented forest patches capable of maintaining true interior conditions, where the influence of edge-related degradation is reduced (Dondina et al., 2017). This is consistent with the well-documented sensitivity of interior species to habitat fragmentation and their dependence on extensive, structurally intact forest habitat (Matthews et al., 2014; McKinney, 2006; Van Dorp & Opdam, 1987). Within this group, *Sitta europaea*, a typical secondary cavity nester and *Troglodytes troglodytes*, which commonly nests in tree cavities (Barros-Leite & Mercival, 2024), showed negative associations with young forest patches, underscoring their reliance on stands that have undergone sufficient structural maturation to provide natural cavities, decaying wood and other microhabitat features essential for nesting and foraging (De Groot et al., 2021; Dondina et al., 2015). Conversely, *Erithacus rubecula* was negatively associated with the oldest patches, indicating that even within interior species there is a turnover related to forest-patch age, with different members of this group being favoured by different stages of structural development. These ecological preferences likely contribute to a replacement pattern along the urban gradient, whereby species strongly dependent on mature forest conditions—and generally recognized as urban avoiders, such as *Sitta europaea* and *Troglodytes troglodytes—are* supplanted by species better adapted to younger and anthropogenically influenced stands, typical of rural and peri-urban areas, such as *Erithacus rubecula* (Alba et al., 2025).

Raptors showed contrasting patterns. *Buteo buteo* responded positively to green area cover, consistent with its reliance on semi-open habitats embedded within larger forest matrices and its known tendency to avoid highly fragmented woodland habitats for nesting (Butet et al., 2010). In contrast, *Falco subbuteo* showed associations with relatively young forest patches and taller trees, a pattern consistent with its documented use of heterogeneous landscapes that include structurally immature semi-natural stands, such as poplar plantations (Bogliani et al., 1994) widely distributed in the lowland rural landscapes surrounding the Metropolitan Area of Milan (Dondina et al., 2022; Tirozzi et al., 2026).

A broad group of generalist species showed heterogeneous responses characteristic of taxa capable of exploiting human-modified landscapes, yet still structured by specific habitat attributes. Green area cover prompted positive responses in several species across ecological groups, including a number of generalists, highlighting the broad functional value of extensive vegetated areas for foraging and breeding. Patch age played an important role on generalists, with older forest patches negatively affecting *Fringilla coelebs* and *Muscicapa striata*, indicating that highly mature stands are not uniformly suitable for flexible species. This pattern was reinforced by the negative responses to mean tree circumference observed in several species (*Aegithalos caudatus*, *Dendrocopos major*, *Luscinia megarhynchos*, *Muscicapa striata*, *Oriolus oriolus* and *Picus viridis*) further suggesting that structurally younger or less mature patches can offer more favourable conditions for a diverse set of woodland birds (Dondina et al., 2015). Taken together with the patterns observed for interior species, these results highlight patch age as a key driver of species turnover along the urban gradient. Mature stands in natural areas tend to support the most disturbance-sensitive interior species (e.g. *Sitta europaea*), which are progressively replaced by more tolerant interior taxa associated with younger forests (e.g. *Erithacus rubecula*) and ultimately by generalist species well adapted to young forest-patch typical of peri-urban and urban areas.

Generalist species showed other significant environmental preferences. Several species (*Aegithalos caudatus*, *Luscinia megarhynchos*, *Oriolus oriolus*, *Phoenicurus phoenicurus and Regulus ignicapilla*) showed negative responses to lotic water bodies and/or clearings (Figure 2c), suggesting that excessive openness or moisture-driven disturbance can reduce habitat suitability even for relatively tolerant woodland birds. Fine-scale habitat structure further refined species distributions. The presence of fallen branches had widespread positive effects, highlighting the importance of understory complexity for resource availability. Dead trees emerged as an important driver of community turnover at the assemblage level, but species-specific responses were more limited: among the species analysed, only *Luscinia megarhynchos* showed a credible positive response. Although a positive effect was expected for woodpeckers (Porro et al., 2021), no credible species-level response to dead trees was detected, suggesting that in this urban–peri-urban context dead wood influences community composition mainly through cumulative or context-dependent effects rather than through strong, species-specific dependencies.

Consistent with the other taxa, no credible residual species associations emerged, suggesting that once environmental and spatial structure are accounted for, interspecific interactions contribute minimally to community organization.

From a comparative multi-taxa perspective, the three groups differed in how environmental and spatial processes contributed to community reorganization along the urban gradient. Bats and small mammals were mainly influenced by environmental characteristics associated with urbanization. Consistent with our expectations, bat communities also reflected broad-scale spatial structuring of environmental conditions along the natural–urban gradient, whereas small mammals responded primarily to local habitat features and to the configuration of the urban fabric, highlighting the importance of local-scale environmental conditions for taxa with limited dispersal ability. In contrast, birds showed a stronger contribution of spatial components, with turnover associated with the main natural–urban gradient. These differences indicate that, although community changes along the urban gradient follow a common general pattern, the processes underlying species replacement remain taxon-specific and reflect differences in dispersal ability and ecological requirements. Across all groups, species-specific responses to habitat

structure and composition ultimately drive the observed community patterns, with important implications for biodiversity management in urban landscapes.

4.3 | Implications for urban biodiversity conservation

Our multi-taxa assessment demonstrates that urbanization reshapes vertebrate communities primarily through species turnover, reinforcing the view that urban ecosystems should not be conceptualized merely as depauperate versions of natural habitats but rather as distinct ecological systems in which novel community configurations emerge (Alberti, 2015; Briones-Salas et al., 2024). The biodiversity patterns along the investigated urban gradient should be interpreted as a community reshuffling, driven by species-specific environmental responses and by habitat features whose spatial configuration and structural development vary across the urban gradient.

Recognizing the distinctiveness of urban ecosystems, however, does not imply accepting biologically simplified communities dominated by urban-adapted species as inevitable. Instead, our results indicate that restoring or maintaining suitable habitat conditions within peri-urban or even urban areas can allow part of the biodiversity typical of more natural and rural contexts to persist within human-dominated landscapes. Importantly, the consistent absent nestedness signal suggests that community differences along the urban gradient are not primarily driven by dispersal limitation and by the consequent inability to colonize potentially suitable green areas isolated within an inhospitable urban matrix. This result indicates that the absences of sensitive species in urban and peri-urban green areas largely reflect the lack of suitable environmental conditions rather than barriers to dispersal. Consequently, these findings suggest that targeted habitat restoration within urban and peri-urban landscapes can create conditions under which colonization by disturbance-sensitive species is not precluded.

Urban and peri-urban landscapes could thus substantially increase their biodiversity by retaining or restoring the environmental conditions associated with disturbance-sensitive species alongside those already supporting generalist and urban-dwelling taxa. For birds, this particularly involves safeguarding large and structurally mature woodland patches and promoting features typical of late-successional stands—such as large cavity-bearing trees, standing dead wood and coarse woody debris on the ground. For bats and small mammals, our results highlight the importance of maintaining small open or grassy areas, alongside small woodland patches, which function as key foraging and movement elements embedded within the urban matrix. Integrating these habitat features into heterogeneous urban and peri-urban mosaics would allow sensitive species to persist where they would otherwise be replaced by urban-adapted taxa. In doing so, cities could promote a more dynamic form of turnover—not only across the urban–natural continuum but also within urban and peri-urban belts—supporting a wider spectrum of ecological niches and ultimately enhancing urban ecosystem functioning.

AUTHOR CONTRIBUTIONS

Olivia Dondina: Conceptualization, investigation, formal analysis, methodology, writing—original draft, data collection. Valerio Orioli: data curation, formal analysis, methodology, investigation, writing—review and editing, data collection. Pietro Tirozzi: Data curation, formal analysis, methodology, investigation, writing—review and editing, data collection. Andrea Viviano: Investigation, writing—review and editing. Emiliano Mori: Conceptualization, project administration, writing—review and editing. Matteo Celeste: Data curation, investigation, data collection. Leonardo Ancillotto: Conceptualization, investigation, writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

Authors certify that they have no affiliation with or involvement in any organization or entity with any financial or nonfinancial interest in the subject matter or materials discussed in this manuscript. Therefore, they have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

Data are available from the Mendeley Data repository via the following DOI: https://doi.org/10.17632/tv3ykds5yf.1 (Viviano et al., 2026).

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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. Bat, small mammal and bird species/group of species recorded across 53, 64 and 58 sampling plots, respectively, distributed along an urbanization gradient in the city of Milan (Italy).

Figure S1. Sample completeness curves (rarefaction/extrapolation) showing the relationship between sample coverage (y-axis) and sampling effort (number of sampling units; *x*-axis) for (a) bats, (b) small mammals and (c) birds. Solid lines represent interpolation (rarefaction) and dashed lines extrapolation. Shaded areas indicate 95% confidence intervals.

Figure S2. Spatial pattern represented by Moran's eigenvector map MEM3 retained in the db-RDA model for small mammals. Colours indicate the relative values of the spatial eigenvector across sampling sites. Coordinates are expressed in UTM projection.

Figure S3. Spatial pattern represented by Moran's eigenvector maps MEM1, MEM3, MEM4, MEM6 retained in the db-RDA models for birds. Colours indicate the relative values of the spatial eigenvector across sampling sites. Coordinates are expressed in UTM projection.

Table S2. Bats—Marginal effects (db-RDA selected models). *F*-values and *p*-values are based on permutation tests (999 permutations).

Table S3. Small mammals—Marginal effects (db-RDA selected models). *F*-values and *p*-values are based on permutation tests (999 permutations).

Table S4. Birds—Marginal effects (db-RDA selected models). *F*-values and *p*-values are based on permutation tests (999 permutations).

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