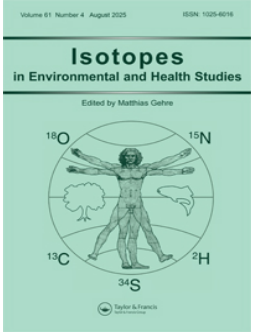

Isotopes in Environmental and Health Studies

ISSN: 1025-6016 (Print) 1477-2639 (Online) Journal homepage: www.tandfonline.com/journals/gieh20

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To cite this article: Ana B. Navarro, Rona A.R. McGill, Jason Newton, Claire J. Branston, Pablo Capilla-Lasheras, Luís F. Silveira & Davide M. Dominoni (19 Aug 2025): Urbanisation is associated with changes in stable isotopes across multiple trophic levels, Isotopes in Environmental and Health Studies, DOI: 10.1080/10256016.2025.2546139

To link to this article: https://doi.org/10.1080/10256016.2025.2546139

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https://doi.org/10.1080/10256016.2025.2546139

Urbanisation is associated with changes in stable isotopes across multiple trophic levels*

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ABSTRACT

The increase in urbanisation imposes important threats to biodiversity through habitat destruction, reduced availability of preferred food resources and higher pollution. To support future urban planning, it is necessary to gather more knowledge on how the ecology of organisms from different trophic levels varies across the urbanisation gradient. In our study, we employed carbon and nitrogen stable isotope analysis to investigate the relationship between increasing urbanisation and $\delta^{13}C$ and $\delta^{15}N$ values across a tri-trophic system of trees (birch and oak), invertebrates (aphids, other Hemiptera, and caterpillars) and a model avian species for urban ecology (the blue tit *Cyanistes caeruleus*). For the blue tits, we measured the isotopic niche to assess how urbanisation affect niche width at different life stages (adults and nestlings). We observed higher $\delta^{15}N$ values in all taxa in urban areas and $\delta^{13}C$ values were also higher in urban trees and blue tit nestlings. Exposure to increased air pollution in urban areas, mainly derived from anthropogenic NO_x gas emissions, is one of the main causes of the increase in $\delta^{15}N$ in urban organisms. Furthermore, in urban areas covered by impervious surfaces there is greater water scarcity in the soils, leading to physiological responses in plants that increase the $\delta^{13}C$ in leaves. We observed that the isotopic niche of urban blue tits is 4.5–18 times smaller in adults and nestlings, respectively, than that observed for forest individuals. Forest blue tits exhibit broader niches, likely reflecting a greater availability and diversity of optimal resources in less disturbed habitats. Conversely, urban blue tits exhibited narrower isotopic niches, suggesting an impact associated with lower diversity and abundance of profitable prey in urbanised habitats. Our study highlights that urbanisation can affect organismal physiology across different trophic levels in similar fashion.

ARTICLE HISTORY
Received 13 January 2025
Accepted 8 July 2025

KEYWORDS
Anthropogenic impacts; anthropogenic resources; land cover; trophic levels; SEA

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*Dedicated to the memory of Professor Keith Alan Hobson – Pioneer of isotope ecology.

Supplemental data for this article can be accessed online at https://doi.org/10.1080/10256016.2025.2546139.

1. Introduction

Urban areas are expanding worldwide, imposing a major threat to the survival of wild plants and animals and the maintenance of natural ecosystems [1]. The reduction of forests and other natural/semi-natural landscapes that accompany land use conversion to impervious surfaces in urban areas is usually associated with a decline in biodiversity [2]. In addition to leading to reduced biodiversity, urbanisation is also linked to greater biotic homogenisation [3–5], fundamentally altering the composition of ecological communities and the structure of ecosystems. These changes can have important ecological and behavioural repercussions in urban populations, such as altering foraging behaviour, diet composition and movement, which could all lead to altered trophic dynamics and, in extreme cases, break long-standing ecological relationships [6–8]. Considering that by 2050, 70 % of the human population is expected to live in urban areas [9], it is essential to gather more knowledge on the effects of urbanisation on trophic relationships and ecosystem functioning.

Stable isotope analysis is widely used in ecology as a useful tool for assessing how anthropogenic impacts influence trophic dynamics [10]. Stable isotopes of different elements occur naturally in ecosystems. Carbon and nitrogen are two of the most used elements in stable isotope ecology due to their large contribution to the chemical composition of biological samples [11]. Plants with different photosynthetic cycles (C_3, C_4 and CAM) characterise different types of habitats (e.g. urban, forest, savannah) and exhibit distinct ratios of carbon isotopes (^{12}C and ^{13}C). Since these different ratios in plants change within the food web, the carbon isotope value ($\delta^{13}C$) can be used in animal ecology to assess an organism's dietary and habitat use patterns [12]. Nitrogen isotope analysis is used for determining the diet and trophic position of organisms, since high trophic levels are enriched in their $\delta^{15}N$ values [12]. Together, carbon and nitrogen stable isotope analysis serve as a tool for evaluating changes in the ecology of species in urban environments, where human activities often alter the natural patterns of habitat and food resources [13,14]. For example, in blue tits (*Cyanistes caeruleus*), adults provide sub-optimal food resources (e.g. ladybirds, spiders and anthropogenic food provided at bird feeders) to their brood, and this was associated with an increased $\delta^{15}N$ values in nestlings [15]. In another study, ants have been found to add human food items to their diet, demonstrated through higher $\delta^{13}C$ values, which are typical of fast food made from corn and sugarcane [14].

Additionally, isotopic enrichment in urban organisms may be a consequence of CO_2 and NO_x gas emissions, derived from the combustion of fossil fuels and industrial activities, as well as the high rates of domestic and industrial sewage deposition in water resources [16,17]. The pollutants enter the metabolic pathways of plants and animals through their natural energy cycles, which means photosynthesis and diet, respectively [16,18]. Nevertheless, most studies using stable isotope analysis in the context of urban ecology have focused on a single trophic level and were limited in their spatial replication. To better understand the effects of urbanisation on ecosystems, and what specific environmental factors might modulate such effects, it is crucial to include organisms from different trophic levels [19] and increasing the number of sites sampled, for instance using urban gradients [20].

Finally, the combined analysis of carbon and nitrogen at higher trophic levels, such as in secondary consumers, can also offer insights on the niche breadth of an organism, informing on variation in diet within and across habitats [21]. This type of analysis has been increasingly used to assess the effects of urbanisation on isotopic niche dynamics. Depending on the organism, high urbanisation may reduce isotopic niche width due to low availability of preferred resources in some species [22], but might also be associated with niche expansion in other species [23]. Limited access to preferred food in urban areas may drive animals to specialise in abundant and widespread anthropogenic food sources or, conversely, to adopt a highly opportunistic feeding strategy – thus increasing both within-individual diet diversity and between-individual diet variation, especially in a highly heterogeneous landscape. Indeed, the relationship between dietary niche breadth and isotopic niche breadth is not always straightforward. Isotopic niche width at the population level also depends on the dietary variation among individuals [24]. Generalist populations may show broader isotopic niches due to greater between-individual variation, whereas highly specialised populations, where individuals consume similar diets, tend to have narrower isotopic niches [25]. In urban habitats, where the presence of anthropogenic resources is widespread, individuals may converge on the same food sources, potentially narrowing the isotopic niche despite an overall generalist feeding strategy in the species level. Thus, whether urbanisation leads to niche expansion or contraction depends on both the diversity of available resources and the variation of individual diets within a population.

The aims of this study were to investigate how environmental metrics (e.g. NO_x pollution, tree cover, impervious surface) along an urban gradient affect the carbon and nitrogen isotopic values of a well-established tri-trophic system, including a common European urban passerine bird, the blue tit, the insects consumed by blue tits, and plants eaten by some of these insect species. We also seek to assess how urbanisation affects the niche dynamics of blue tits. We have previously shown that urban blue tit tissues are enriched with $\delta^{13}C$ and partly also with $\delta^{15}N$ (in nestlings but not adult birds) compared with forest conspecifics [15]. Moreover, isotopic niches were wider for urban adults but not nestlings compared to forest birds [15]. In this study, we extend these previous findings by (1) quantifying $\delta^{13}C$ and $\delta^{15}N$ values not only in bird but also in insects and plants to accomplish a multitrophic and ecosystemic approach; and (2) extending the spatial scale from three to 17 sites along an urbanisation gradient, allowing a more direct quantification of the environmental drivers of differences in isotopic signatures.

2. Materials and methods

2.1. Study areas

We conducted our study at 17 sites located along an urbanisation gradient between the city of Glasgow (coordinates: 55.869, −4.2851) and Loch Lomond and The Trossachs National Park (coordinates: 56.129, −4.6145), Scotland (Figure 1, Supplementary Table S1). We grouped the sites using a K-means clustering analysis, an unsupervised machine learning algorithm for partitioning a given dataset into a set of k groups, using the *cluster* and *factoextra* packages in R [26,27]. The Elbow method was employed to determine the optimal number of clusters. The clustering analysis of the sites was based on four environmental metrics (Supplementary Table S1). We used three land

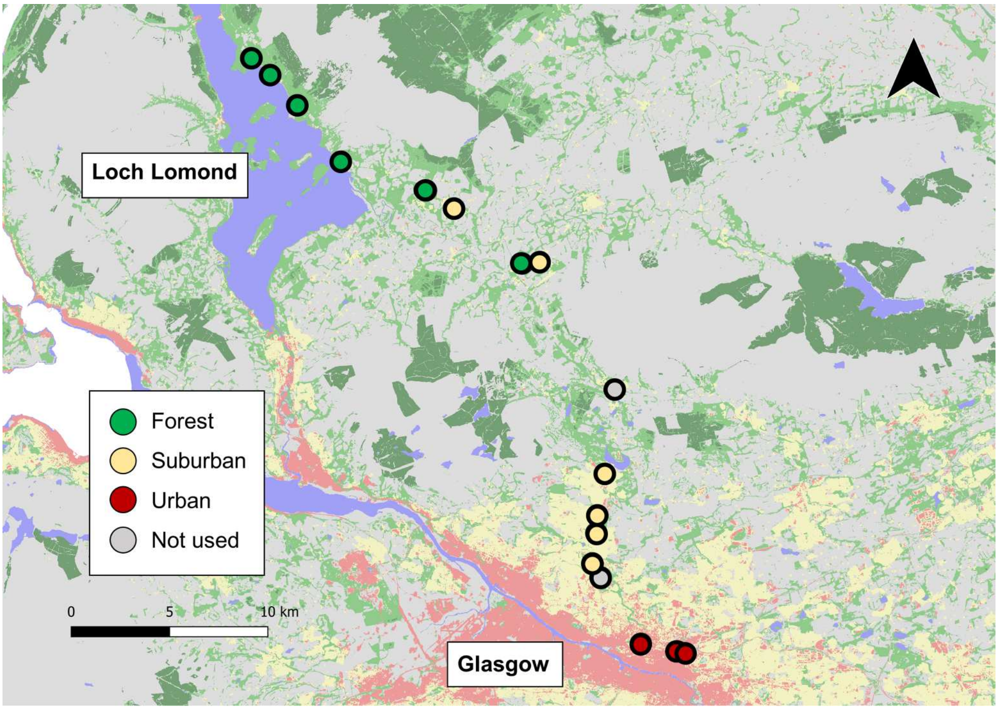

Figure 1. Location of the 17 sample sites within the urbanisation gradient between Glasgow and the Loch Lomond region, Scotland. The sites were grouped into forest (green circles), suburban (yellow circles), and urban habitats (red circles) through a cluster analysis, in which two sites were removed (grey circles) from the sampling universe after clustering. Background map shows the land cover classes obtained from UK Centre for Ecology & Hydrology (Marston et al. [28]), whereas the main classes used in our study represent urban (red), suburban (yellow) and forest (dark and pale green).

use metrics: % of broadleaved woodland (i.e. forest area), % of suburban area, and % of urban area obtained from the Land Cover Map 2021 10 m classified pixels, GB [28], and one metric that measured the average NO_x gas emission (tonnes/km^2) obtained from the UK National Atmospheric Emissions Inventory for 2021 [29]. We calculated these environmental metrics for each site using a 500 m buffer, with the size of the buffer based on the maximum distance used by breeding adult blue tits [30]. After the cluster analysis (Supplementary Table S2), we excluded one site from our analysis as it could not be grouped within the three categories of habitat type (forest, suburban, and urban) and one site from which we had only one sample of blood.

In addition to landscape metrics for clustering sites into habitat types, we also gathered remote sensing data on environmental variables from each site (Supplementary Table S3). We measured the following environmental variables: mean human population density (hereafter human density), mean percentage of impervious surface (hereafter impervious surface), mean percentage of tree cover density (hereafter tree density), and mean NO_x emission (hereafter NO_x emission). We calculated all of them with a buffer of 100 m, considering the restrictive influence of environmental effects on taxa of different sizes. The human density metric was obtained from the Data for Good website [31], the environmental metrics impervious surface and tree density were obtained from the Copernicus Land Monitoring Service [32], and the NO_x emission metric was obtained from the UK National Atmospheric Emissions Inventory for 2021 [29].

2.2. Biological sampling

We sampled birds, insects, and tree leaves between late April and late June 2023. For birds, we collected blood samples from a total of 77 nestlings and 47 adults of blue tits (*Cyanistes caeruleus*) across the urbanisation gradient. Nestlings were sampled between 10 and 12 days old and adults were caught directly in the nest box in June 2023. Blood was obtained by puncturing the wing vein with a 25- or 27-gauge needle, collected in a 70 µl capillary tube, and transferred into a glass vial for storage. Vials were stored in a −70 °C freezer and kept frozen until stable isotope analysis. Monitoring of bird nests was done in accordance with a NatureScot license to DMD (#207317). Bird blood sampling procedures were conducted by personal license holders performed in accordance with UK Home Office legislation, via a project license to DMD (#P6859F36E).

To sample invertebrates and plants, we selected six representative trees around the nest boxes used for bird sampling. We first identified each tree within a 15 m radius at each nest box. We then selected the three most common tree species at each site and used six representatives (two per species) from these three most common species for sampling invertebrates and leaves. However, as there was variation in tree composition along the gradient, we decided to focus only on species that were present at all habitat types. The selected tree species were oak (*Quercus* spp.) and birch (*Betula* spp.). Invertebrates were sampled using the branch beating method. Briefly, we selected a branch of the target tree that was within arm’s reach and was between 1 and 2 m long. We wrapped the branch in a 100 × 50 cm white plastic sack and shook it 30 times at a steady pace. We then identified each invertebrate collected to order level. We selected only the most representative orders that were Hemiptera and Lepidoptera larvae (hereafter ‘caterpillars’). Hemiptera were then further separated into Aphids and other Hemiptera as these were recorded separately during sampling. In May 2023, we collected a total of 42 fresh leaf samples and 71 invertebrate samples across the urbanisation gradient. Both leaves and invertebrates were immediately frozen at −70 °C until stable isotope analysis, although a subsample of caterpillars ($N = 31$) was stored in ethanol. Ethanol storage had no significant effect on the carbon isotope values as would be expected considering the chemical composition of ethanol (Supplementary Figure S1).

2.3. Stable isotope analysis

All samples were freeze-dried in the laboratory. After this procedure, the leaf samples were ground into powder in a Retsch MM301 Mixer Mill and the invertebrate samples were manually ground or used entirely, depending on the size of the individual. We individually weighed and transferred each of the samples of all taxa to tin capsules (5 × 3.5 mm) using approximately 1.3 mg of plant powder and 0.8 mg of blood and invertebrates, separately.

All the encapsulated samples were measured for carbon and nitrogen isotope composition on an Elemental Analyzer Vario-PYRO Cube interfaced to a Thermo Finnigan DELTA

PLUS XP continuous-flow isotope ratio mass spectrometer with every 10 samples separated by two laboratory standards, at the SUERC Stable Isotope Ecology Laboratory. The isotope values provided by the mass spectrometer are expressed in delta (δ) per mill (‰) notation, following the equation: δ^{13}C or δ^{15}N = [(Rsample/Rstandard) − 1], multiplied by a thousand, and where R is the corresponding ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$ of the sample.

2.4. Statistical analysis

To test for differences in δ^{13}C and δ^{15}N between habitat types (forest, suburban and urban), we ran full linear mixed effect models (LMMs) with habitat type, Julian day of the year and sex (only for adult blue tit samples) as explanatory variables, and site and nest box (only for blue tits) as random factors. We ran a single LMM for each of the four tissue types (adult blue tit blood, nestling blue tit blood, invertebrates and trees) using the *lme4* package in R [33]. Using the *anova* function in R, we compared the full models containing all explanatory variables with simpler LMMs that had only habitat type and sex (the latter only for adult blue tits) as explanatory variables while maintaining the same random factors. Since the full models were not significantly better at capturing the data (Supplementary Table S4), we favoured the simpler models. If the explanatory variable habitat type was found to be significant (P value < 0.05) in the simpler LMMs of each taxon, we conducted post hoc tests using the R package *emmeans* [34].

To assess whether variation in δ^{13}C and δ^{15}N may be related to specific environmental variables across the urbanisation gradient, we employed LMMs with the mean human population density, the mean percentage of impervious surface, the mean percentage of tree cover density, and the mean NO_x emission as explanatory variables after scaling them, and site and taxon as random factors. These explanatory variables are correlated to the landscape metrics employed in the cluster analysis (Supplementary Figure S2) and were chosen as proxies of urbanisation. Mean human population density can be representative of the availability of feeders to blue tits [35]. The mean percentage of impervious surface may be representative of the availability of soil for plants. The mean percentage of tree cover density is used as proxy for how 'natural' a site is [36]. Finally, the mean NO_x emission is used as a proxy for air pollution. To ensure that collinearity among explanatory variables did not bias model estimates, we calculated variance inflation factors (VIFs) for the fixed effects. However, given that each predictor reflects a complementary component of urbanisation, we chose to retain all variables in the model. If one or more explanatory variables had P value < 0.1, we ran random slope models for each of the explanatory variables separately (e.g. $\delta^{15}N \sim NO_x$ emission + (NO_x emission | Taxa)), a standard procedure used to capture the variability in the responses of hierarchical or grouped data to exploratory variables after fitting LMMs. Here, the random slope models were employed to test whether the effect of each environmental variable on isotopic value varied between distinct taxa, which would result in different slopes for each taxon, or whether the effect of the environmental variable on isotopic value was generalised across taxa, which would result in similar slopes.

To evaluate the isotopic niche width between blue tits from different habitats (forest, suburban and urban) and between adults and nestlings from the same habitat type, we used the *Stable Isotope Bayesian Ellipses* package in R (SIBER), which fits bivariate ellipses to stable isotope data using Bayesian inference [37]. To obtain the magnitude of the difference between the width of the compared isotopic niches, we calculated the probability of the posterior distributions being different from each other, where probabilities above 0.9 were considered as strong evidence for a difference between the niche widths, probabilities between 0.8–0.9 as moderate evidence, and below 0.8 there is no significant difference between niche widths.

To ensure the accuracy of our results, considering that the sample size of the urban blue tits was smaller than desirable for the Bayesian niche model (adults = 6 individuals and nestlings = 9 individuals), we ran three additional niche analyses. Using Bayesian inference, we measured the niche width of blue tits by habitat without distinction by age (sample size = 15, satisfactory for an accurate estimate of the standard ellipse area; Jackson et al. [37]) and calculated the probability that the widths differed, in the same way as described above. Furthermore, without differentiation by life stage, we calculated the niche width of blue tits by habitat considering the mean values of δ^{13}C and δ^{15}N per nest box (Supplementary Table S5), even though the urban population continued to have a small sample size. In addition, we calculated the isotopic richness (IRic) metric for adult and nestling blue tits by habitat. This metric measures the amount of isotopic space filled by an organism, using a bootstrap procedure (1000 runs) to parameterise the sample size of all groups according to the group with the smallest sample size (i.e. adult blue tits, n = 6) [38].

3. Results

Nitrogen isotopic values (δ^{15}N) were significantly different among habitat types (Figure 2, Supplementary Table S6), especially for the comparison between the forest and urban habitats, where we observed an increase in the δ^{15}N of all taxa in the urban habitat in comparison to forest habitat (Table 1). The means of δ^{15}N between the two extremes of the gradient (forest and urban sites) showed a difference of 2.6–3.6 ‰ depending on the taxon, whereas suburban individuals in general showed intermediate values. Tree density and NO_x emission were negatively and positively related to δ^{15}N of all taxa, respectively, showing similar absolute estimate values (Supplementary Table S7). Random slope models indicated that the positive relationship between NO_x level and δ^{15}N was the same for all taxa, with very similar slope values (Figure 3, Supplementary Table S8). Conversely, the negative relationship between tree cover density and δ^{15}N differed between taxa, with stronger slopes at higher levels of the trophic chain (Figure 3, Supplementary Table S8).

Carbon isotopic values (δ^{13}C) in trees and blue tit nestlings were significantly higher in urban compared to forest habitats, whereas δ^{13}C values of invertebrates and blue tit adults did not vary across habitats (Figure 4, Table 1, Supplementary Table S6). δ^{13}C was negatively related to tree density, while positively related to NO_x emission and impervious surface for all taxa (Supplementary Table S7). Random slope models suggested that the slope of the relationship between δ^{13}C and environmental variables were shallower than those found for δ^{15}N in all taxa (Figure 3, Supplementary Table S8). The association of δ^{13}C with NO_x, impervious surface and tree density was stronger in trees than in insects and blue tits (Figure 3, Supplementary Table S8). VIF values in both models (δ^{13}C and δ^{15}N) ranged from 3.4 for NO_x emission to 7.1 for impervious

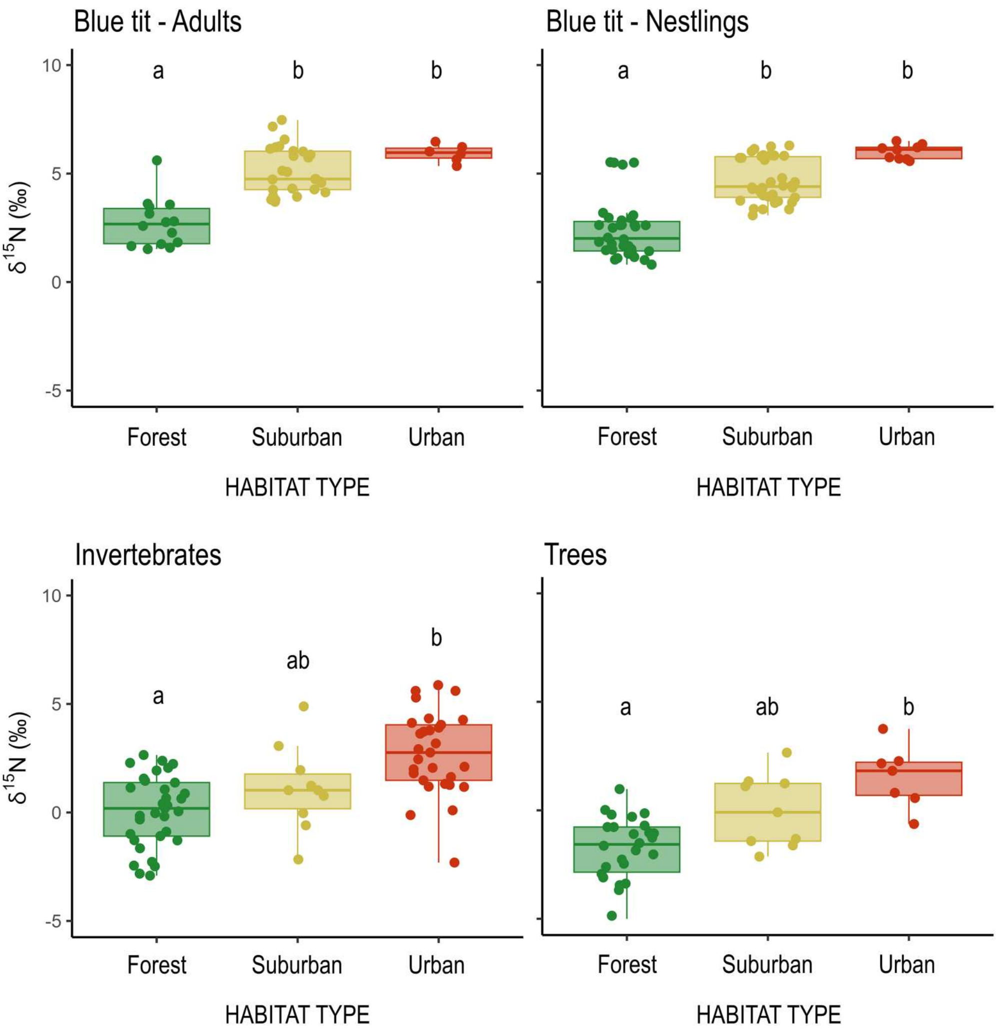

Figure 2. Nitrogen isotope values ($\delta^{15}N$) of blue tits (adults and nestlings), invertebrates and trees from different habitats (forest, suburban and urban). The letters above the boxplots represent the results of post hoc test, in which different letters are used to show significant differences in $\delta^{15}N$ between habitat types. The boxplots show the minimum, interquartile range, median and maximum values for each habitat type, and the dots represent individual data points.

surface, which are below the allowable maximum threshold of 10 [39,40]. Although some studies use a threshold below 2, we retained all variables in the models because each represents a distinct and theoretically relevant component of urbanisation. For instance, impervious surface and tree density capture different aspects of land cover, NO_x emission reflects air pollution, and human density proxies for anthropogenic food provisioning. These dimensions are partially correlated (Supplementary Figure S2), but not redundant, and their combined inclusion helps capture the multifaceted nature of urbanisation. Nonetheless, we recognise that moderate multicollinearity can inflate standard errors and may obscure the individual contribution of predictors, potentially conflating their effect sizes.

Table 1. Mean values of $\delta^{13}C$ and $\delta^{15}N$ for all taxa grouped by habitat type (forest, suburban and urban).

Taxon	Habitat	Sample size	$\delta^{13}C$ (mean ± sd)	$\delta^{15}N$ (mean ± sd)
Blue tits – Adults	Forest	14	−25.0 ‰ ± 0.4	2.7 ‰ ± 1.1
	Suburban	28	−24.9 ‰ ± 0.4	5.2 ‰ ± 1.1
	Urban	6	−24.9 ‰ ± 0.3	5.9 ‰ ± 0.4
Blue tits – Nestlings	Forest	34	−26.1 ‰ ± 0.4	2.4 ‰ ± 1.4
	Suburban	34	−25.4 ‰ ± 0.4	4.7 ‰ ± 1.0
	Urban	9	−25.4 ‰ ± 0.1	6.0 ‰ ± 0.3
Invertebrates	Forest	33	−28.4 ‰ ± 1.3	0.1 ‰ ± 1.6
	Suburban	9	−27.8 ‰ ± 1.2	1.1 ‰ ± 1.9
	Urban	29	−27.7 ‰ ± 1.1	2.7 ‰ ± 1.9
Trees	Forest	26	−31.7 ‰ ± 1.0	−1.8 ‰ ± 1.5
	Suburban	9	−30.8 ‰ ± 1.2	0.0 ‰ ± 1.7
	Urban	7	−30.4 ‰ ± 0.9	1.5 ‰ ± 1.4

The widths of the Bayesian standard ellipse areas (SEAb) of adult blue tits were significantly different between forest and urban individuals and between the suburban and urban individuals (Table 2 and Figure 5). The niches of adult blue tits in forest and suburban habitats are approximately 4.5 times wider than those in urban areas. This same pattern was observed for nestlings, wherein the forest niche was 18 times larger than urban niche. Unlike adults, nestling blue tits showed a significant difference in niche width between the forest and suburban groups (Table 2 and Figure 5). When comparing the isotopic niches between blue tits of different life stages from the same habitat, we observed that only suburban adults and nestlings had similar niche widths (Table 2 and Figure 5). In forest areas, the niche of nestlings is marginally larger than that of adults, whereas in urban habitat, the niche of adults is 3 times larger than that of nestlings (Table 2 and Figure 5).

The niche widths of blue tits in general, without distinction by life stages, was significantly different between habitats (all probabilities of SEAb between habitats being different were > 0.98). The forest blue tits niche was 1.8 times wider than that of the suburban population and approximately 9.0 times wider than that of the urban population (Figure 6). The difference in niche width between habitats was also significant when considering the mean values of $\delta^{13}C$ and $\delta^{15}N$ per nest box, especially when comparing urban blue tits (smallest niche) to those from other habitats (Supplementary Figure S3). The smaller niche width for urban blue tits (adults and nestlings) was also corroborated by the IRic metric (Supplementary Figure S4).

4. Discussion

Our results show that urbanisation alters the carbon and nitrogen isotopic values of organisms at three trophic levels in a terrestrial ecosystem. The $\delta^{15}N$ was significantly higher in trees, invertebrates, and birds in the urban habitat than in forest individuals. In addition, the isotopic niches of urban blue tits were significantly smaller than those of forest individuals, likely reflecting the impacts of urbanisation on the feeding ecology of an avian species widely distributed across different habitat types. Overall, our results corroborate the pervasive effects of urbanisation on the isotopic ecology of a well-established trophic network involving a common European urban passerine bird,

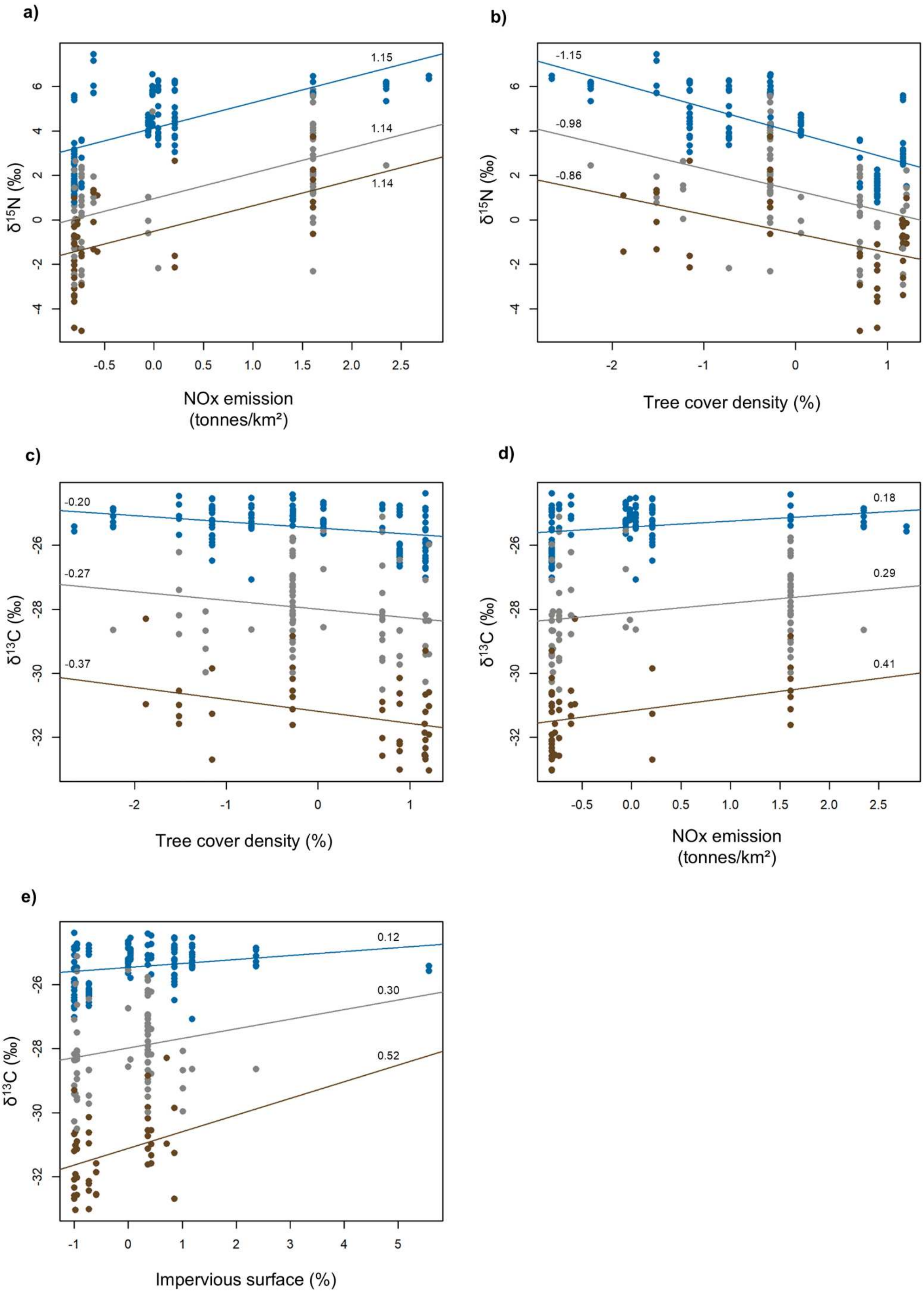

Figure 3. Random intercept and random slope models for the environmental variables that were significant in the LMMs (see Table S7). The slope values (shown in black text above each regression line) were used to evaluate the effect of the environmental variable on the isotope value of each taxon (regression lines: blue tits in blue, invertebrates in grey and trees in brown).

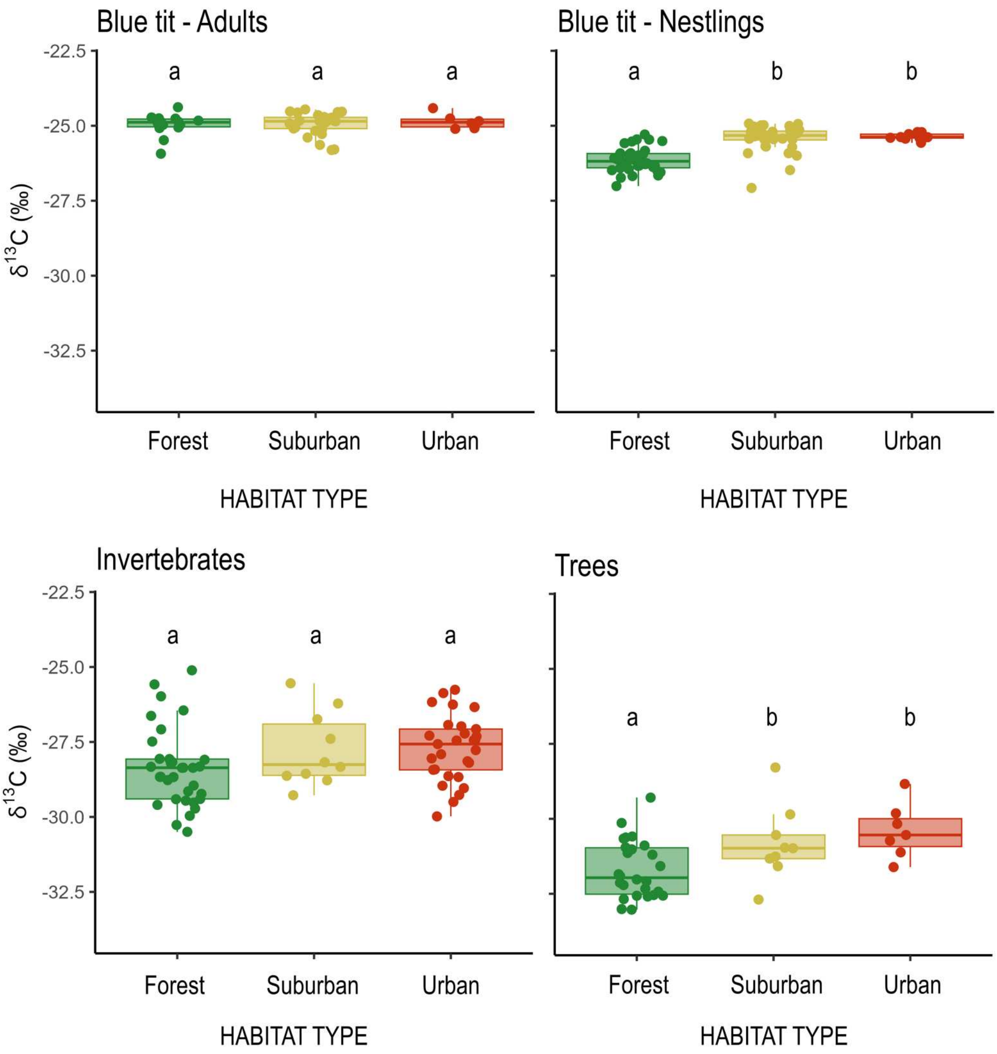

Figure 4. Carbon isotope values ($\delta^{13}C$) of blue tits (adults and nestlings), invertebrates and trees from different habitats (forest, suburban and urban). The letters above the boxplots represent the results of post hoc test, in which different letters are used to show significant differences in $\delta^{13}C$ between habitat types. The boxplots show the minimum, interquartile range, median and maximum values for each habitat type, and the dots represent individual data points.

the blue tit, the insects consumed by blue tits, and plants eaten by some of these insect species.

4.1. Effects of environmental metrics on carbon and nitrogen isotopic values

One of the main impacts caused by urbanisation is atmospheric pollution, which is generally derived from the emission of CO_2 and NO_x gases from the combustion of fossil fuels [41]. The input of such anthropogenic air pollutants directly interferes with the ecosystem cycles of elements such as carbon and nitrogen [42,43]. Exposure of plants to

Table 2. Comparison of niche widths (Bayesian standard ellipse areas; SEAb) between blue tits of different life stages (adults and nestlings) and between blue tits of the same life stage but from different habitats (forest, suburban and urban areas). The magnitude of the difference between the width of the compared isotopic niches was calculated by the probability of the SEAb posterior distributions being different from each other, where a value above 0.8 was considered significant.

	SEAb (‰^{2})		
Habitat	Adult	Nestling	Probability that SEAb between life stages are different
Forest	1.23	1.65	0.81
Suburban	1.23	1.35	0.62
Urban	0.27	0.09	0.98
Age	SEAb (‰^{2})		Probability that SEAb between habitats are different
	Forest	Suburban	
Adult	1.23	1.23	0.53
Nestling	1.65	1.35	0.83
	Forest	Urban	
Adult	1.23	0.27	0.99
Nestling	1.65	0.09	1.00
	Suburban	Urban	
Adult	1.23	0.27	0.99
Nestling	1.35	0.09	1.00

anthropogenic emissions shows that urbanisation can affect the base of the trophic chain through increased δ^{15}N values in plants from urban areas [17,44,45]. Due to anthropogenic interference in leaves and soils, animals that are part of the trophic chain and directly interact with plants also show an increase in δ^{15}N due to the effects of urbanisation [46]. Our results showed similar slope values for the relationship between NO_x level and δ^{15}N for all taxa, indicating a similar enrichment of ^{15}N in urban organisms across three trophic levels.

NO_x emission was not the only significant explanatory variable in our models. In fact, we observed that the reduction in tree cover density, one of the proxies that indicates a higher urbanisation rate, had a greater effect on the δ^{15}N values of blue tits than on other taxa and trophic levels. Trees, particularly native trees, are fundamental to sustain healthy invertebrate populations for passerine birds [47], and in urban areas there is a lower availability of tits' preferred food source (i.e. caterpillars) [15,48]. As a consequence, blue tit parents provision their nestlings with other food resources [48,49], likely altering the δ^{15}N values of urban individuals [15]. The pronounced increase in δ^{15}N in urban blue tits may also be due to the consumption of resources from feeders, usually of low nutritional composition (e.g. sunflower seeds and peanuts), or arthropods from higher trophic levels (e.g. spiders and ladybirds).

The δ^{13}C values were significantly different across habitats for trees and blue tit nestlings, particularly between forest and urban areas. Plants and soils in urban areas may have high δ^{13}C values as a physiological response to water scarcity, as impervious surfaces limit water infiltration into the soil [50,51]. We observed that the greatest effect (i.e. highest slope value) of the percentage of impervious surfaces was found for trees, corroborating that the limitation of water infiltration in urban areas may contribute to an increase of the δ^{13}C of plants. Previous studies have shown the trophic level that feeds on these ^{13}C-enriched plants may show enriched δ^{13}C values in urban populations, as observed in urban wild bees from Paris, France [46]. Nevertheless, in our study, urban invertebrates that directly consume ^{13}C-enriched plants did not show a significant

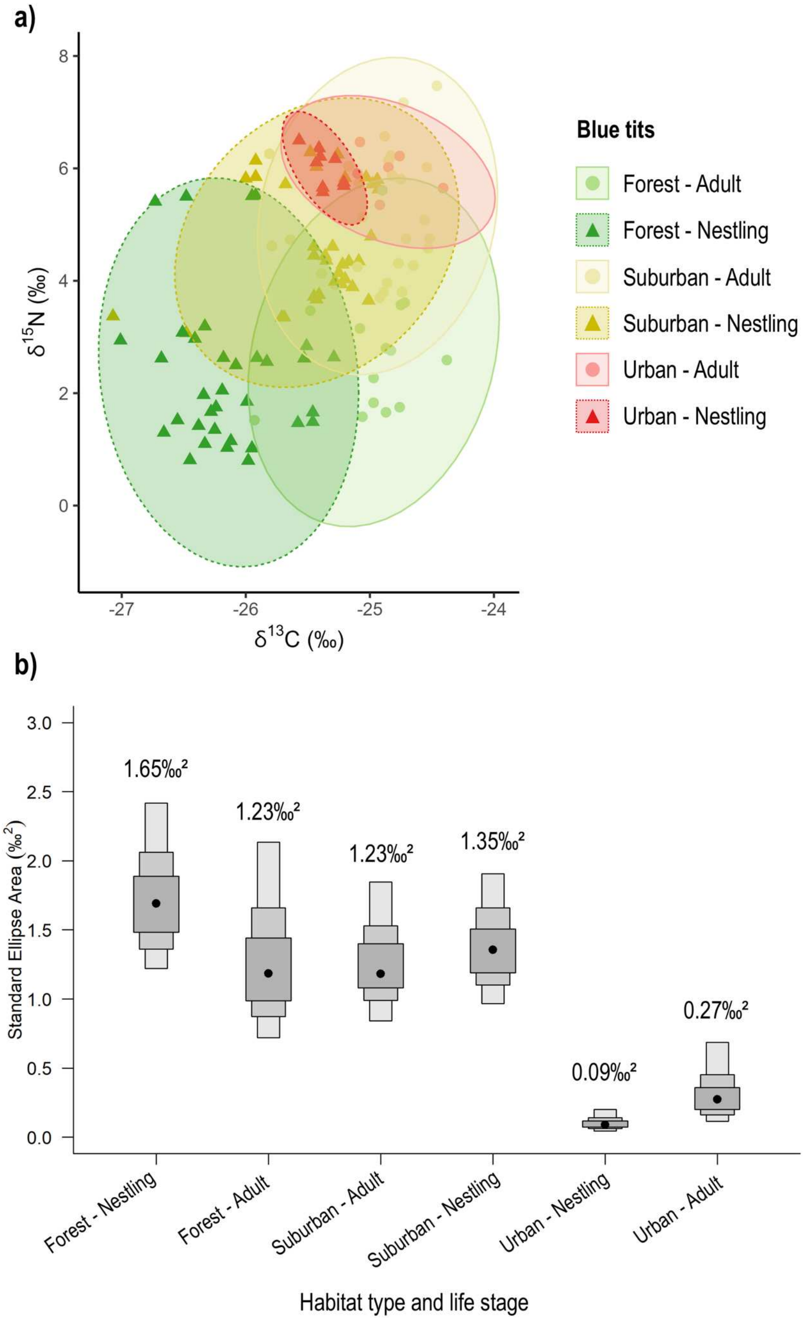

Figure 5. (a) Bayesian standard ellipse area (SEAb) of adult and nestling blue tits for each habitat type (forest, suburban and urban), wherein dots represent individual data; (b) density plot of Bayesian posterior estimates showing the mode of SEAb (black dot and the value at the top) and the 50, 75, and 95 % credibility intervals (grey boxes) for each group.

increase in δ^{13}C values. This weakens the hypothesis that higher δ^{13}C in blue tit nestlings results from reduced water availability in urban soils being transferred through the trophic chain.

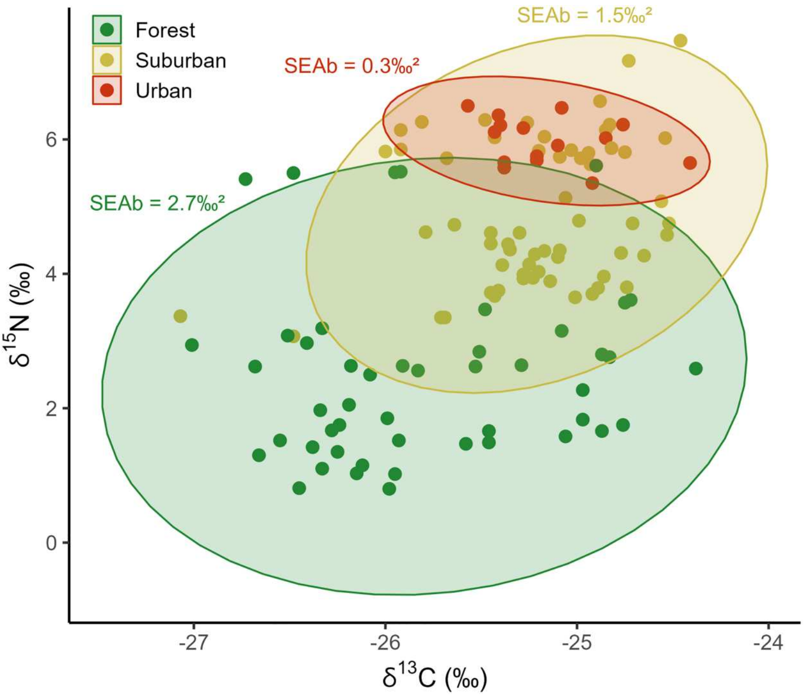

Figure 6. Niche widths (Bayesian standard ellipse areas; SEAb) of forest, suburban and urban blue tits without distinction by life stages.

4.2. Blue tits niche dynamics along an urbanisation gradient

In the urban habitat, we observed that the isotopic niche of blue tits was 4.5–18 times smaller than that of forest individuals, with this pattern being more pronounced in nestlings than in adults. This narrower isotopic niche suggests a more restricted use of available resources, likely reflecting limited availability and diversity of preferred food [52]. In addition, the low between-individual variation and similar isotopic values between individuals can also result in reduced isotopic niche breadth at the population level [25]. In contrast, forest environments offer greater diversity and availability of preferred food, which may support greater individual specialisation within the population. In this case, even if individuals are primarily consuming the preferred food resource, variation in the isotopic composition of those resources across broods can lead to a broader isotopic niche at the population level. Therefore, our results suggest that the broader niche observed in forest population may be driven by higher between-individual variation in diet. Additionally, the result showing that urban adult blue tits exhibited a broader isotopic niche than nestlings, in contrast to the forest population, may reflect a selective provisioning strategy by parents in urban habitats. While adults may exploit non-preferred or opportunistic anthropogenic food, they might prioritise preferred resources when feeding the nestlings, which is probably reflecting the narrower niche in urban nestlings. Such selectivity could be driven by the adults food choice preferences during nestlings development and the limited availability of preferred food in anthropogenic areas [53].

Our results for adult blue tits were contrary to those of Pollock et al. [15], which showed that the urban population had a wider niche width than forest individuals using the same methodology but at a smaller spatial scale. Nonetheless, the biological samples analyzed by Pollock et al. [15] were collected in an atypical year that was exceptionally poor in terms of blue tit reproductive success, which may have influenced this divergence between our results. In addition, we acknowledge that the narrower isotopic niche observed in the urban habitat may also reflect the smaller number of nest boxes sampled and close spatial proximity of urban sampling sites in our study, which may limit environmental and isotopic variability across sites and broods. We suggest that future studies should extend the temporal and spatial scale to decouple the specific effects of atypical years and site proximity. Thus, despite studies indicating that urbanisation leads to wider isotopic niches in urban-adapted animals [54,55], our study emphasises that the pervasive effect of urbanisation on the availability of preferred resources may actually reduce bird niches, at least in nestlings.

5. Conclusions

In this study, we showed that the impacts of urbanisation within food webs can be measured by comparing the isotopic values of species in different habitat types, including individuals from natural to completely urban habitats. By doing so, we showed that environmental metrics, such as NO_x emission, tree cover density and impervious surface, exert a significant influence on the isotopic values of distinct taxa across the trophic chain, which need to be taken into account when examining species dietary patterns between habitats. Considering that urban areas will expand in the future, it is essential to gather knowledge about the effects of urbanisation on trophic dynamics to mitigate the anthropogenic impacts on the ecology of species.

Acknowledgments

We respectfully honour the memory of Dr. Keith A. Hobson for his pioneering work in isotope ecology and ornithology. Dr. Hobson's studies inspired countless early career researchers, including ABN, who were captivated by this area of science.

We would like to thank all the field staff involved in the sampling and monitoring of the nest boxes. We thank Conor Haugh for processing and extracting some of the environmental variables that we used in the linear mixed effect models.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

ABN was supported by the São Paulo Research Foundation (FAPESP) under grants [2020/07619-0 and 2022/06154-9]. LFS was supported by the FAPESP under grant [2017/23548-2] and the National Council for Scientific and Technological Development (CNPq) under fellowship [302291/2016-6 and 308337/2019-0]. The stable isotope analysis was supported by a NERC grant to DMD [EK323-10/19 East Kilbride LSMSF]. The sample collection in the field was supported by the University of Glasgow.

Data availability statement

The data that support the findings of this study are available from the corresponding author, ABN, upon reasonable request.

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