

1 **Wildlife microbiomes and the city: A systematic review of urban**  
2 **impacts on wildlife bacterial communities**

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**26 Abstract**

27 This systematic review aims to investigate how urban living influences the microbiome of  
28 wildlife. We included all studies that compared the bacterial communities of non-human  
29 vertebrate wildlife living inside vs outside cities, and/or across an urbanisation gradient. We  
30 found that the effect of urban-living on bacterial diversity and community composition was  
31 not unidirectional: some studies found a positive association, others found a negative  
32 association, and some found no relationship. The definition of 'urban' was not specified in  
33 more than half of the studies, and when included was not consistent across studies; paired  
34 with limited site replication in many studies, these features could have obscured the impacts  
35 of urban living. Most studies also focused on urban-adapted wildlife species. Future studies  
36 that include clear definitions for 'urban' environments and good replication of land uses  
37 within contrasting environments would help clarify the impact of urban living on wildlife  
38 microbiomes and wildlife health.

39 Keyword: urban microbiome, urbanisation, urban wildlife, animal microbiome, bacterial  
40 community.

## 41 **Introduction**

42 Urban areas currently represent the fastest growing ecosystem on earth (MZ Levy *et al.*  
43 2014). Cities are expanding into wildlife's natural habitats, and animal species increasingly  
44 venture into these concrete jungles in search of resources (NSG Williams *et al.* 2006), with  
45 some having become well-adapted to urban life (AD Lewis *et al.* 2019). Thus, the urban  
46 environment is taking on an increasingly important role as new habitat and foraging ground  
47 for wildlife.

48 The urban ecosystem is complex, comprising many different kinds of landscapes – both built  
49 and natural (B Alizadeh & J Hitchmough 2019). There is a need for city designs that are  
50 beneficial for the health of city dwellers, non-human animals included. However, at present,  
51 wildlife remains little considered in urban designs, the majority of which usually incorporates  
52 ecological theories and considerations regarding vegetation (CAM Kay *et al.* 2022).  
53 Furthermore, much of the research on urban animals has been directed to the topic of human–  
54 wildlife conflict and risk management, rather than wildlife health (CD Soulsbury & PCL  
55 White 2016).

56 There is a small but growing body of literature on how cities affect wildlife community  
57 assemblages and wildlife health. Urbanisation is usually attributed with the decrease of  
58 wildlife species richness. However, some studies have found that this trend is not consistent,  
59 with varying relationships between urbanisation and diversity observed depending on wildlife  
60 taxonomy and the intensity of urbanisation. For example, a notable number of studies on  
61 invertebrates, and some on vertebrate species, have found an increase in species richness at  
62 moderate intensities of urbanisation, potentially due to an increase in habitat diversity with  
63 mosaic patterns of land use (M Mbiba *et al.* 2021; ML McKinney 2008).

64 The bacterial communities of urban animals have been gathering interest due to their  
65 importance for host health, and the potential for these communities to be influenced by the  
66 surrounding environment. In humans, microbiomes directly impact host health in many ways  
67 through immune and brain function, as well as energy storage and use (Jose C Clemente *et al.*  
68 2012; JK Nicholson *et al.* 2012). Presumably, microbiomes have similar strong influences on  
69 wildlife health, but the impacts of cities on wildlife bacterial assemblages, and any associated  
70 impacts on health, are unclear (AP Bernardo-Cravo *et al.* 2020; LJ Clarke *et al.* 2020)

71 There are evidence demonstrating the impact of living environments on animal microbiomes.  
72 The gut microbial diversity of mice responded rapidly to changes in living environment and  
73 was higher in mice that came into contact with non-sterile soil compared to sterile soil (D  
74 Zhou *et al.* 2018). The gut microbiota of baboons were more reliably predicted by the  
75 surrounding soil microbiome than by their genetics (LE Grieneisen *et al.* 2019). For urban  
76 living, the limited evidence seems to vary. For example, in one study, faecal microbiomes of  
77 domestic dogs growing up in large cities were found to be more diverse than microbiomes  
78 from dogs growing up in small cities and the countryside (Å Vilson *et al.* 2018). In contrast,  
79 another found that skin microbiomes of domestic dogs were overall more homogenous in  
80 cities, and urban dogs were more susceptible to allergic diseases than rural counterparts (J  
81 Lehtimäki *et al.* 2018). The mechanisms behind these differences are not clear.

82 For wildlife, living in cities might affect their bacterial communities directly by exposing  
83 them to different environmental microbial communities or indirectly by altering their  
84 foraging activities, stress hormone levels and immune function (CD Francis & JR Barber  
85 2013; K Birnie-Gauvin *et al.* 2016). To date, there has been no synthesis of the literature on  
86 urban environment-terrestrial vertebrate wildlife microbiome interactions. In this context, our  
87 goal is to understand how urban living might affect the microbiomes of wildlife. We do this

88 by reviewing the literature that compares the microbiomes of animals living in urban versus  
89 non-urban habitats, or along urbanisation gradients.

90 Specifically, this systematic review aims to:

91 (a) Investigate whether and how urban living affects the bacterial communities of terrestrial  
92 vertebrate wildlife.

93 (b) Explore the factors influencing the relationship between urban living and bacterial  
94 communities, such as level of urbanisation, land use, and/or wildlife species.

95 The review identifies current gaps in the literature, as well potential directions for future  
96 research to better understand the impact of urbanisation on wildlife microbial communities.

97

## 98 **Search Strategy**

99 The Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols  
100 (PRISMA-P) were used as a guideline for collecting, extracting and synthesising results from  
101 peer-reviewed studies. The purpose was to critically assess articles that explored the changes  
102 in terrestrial wildlife microbiomes in relation to urban living. A thorough literature search  
103 was accomplished, then data were extracted from vetted articles and analysed.

104 On September 2022, articles were searched for using the databases Web of Science and  
105 Scopus without time limitation. Our search strategy combined keywords related to three  
106 components: wildlife, microbiome, and urban vs non-urban habitat. Terrestrial vertebrate  
107 wildlife species were targeted for this review; hence, we included .specific terms to capture  
108 key wildlife to class level such as: bird, avian, animal, mammal. For the bacterial

109 microbiomes, we used different synonyms of the term, as well as combinations of the word  
110 “community” and other relevant terms (e.g. ‘microbiot\*’ and ‘bacterial community’). For  
111 habitat, we used terms that denoted urban living and urbanisation, combined with terms to  
112 capture the types of comparisons we were aiming for: ‘urban’, ‘city’, versus non-urban, and  
113 urbanisation gradients. The searches used Boolean logic (‘and,’ ‘or’) to locate the appropriate  
114 articles in the two databases (Table S1.)

115

## 116 **Study selection**

117 Our inclusion criteria were as follows:

118 (a) Studies must compare animal bacterial communities in urban versus non-urban areas, or  
119 across an urbanisation gradient/scale.

120 (b) Studies must target unconfined terrestrial vertebrate wildlife, not domesticated, lab-raised  
121 or captive animals. Invertebrates were excluded as the relationship between invertebrates and  
122 urban soil microbiomes have been more extensively explored and reviewed previously (N  
123 Bray & K Wickings 2019); aquatic vertebrates were excluded as the gut microbiota of aquatic  
124 animals is more susceptible to change than that of terrestrial vertebrates, especially dietary  
125 changes, and thus are more appropriate as a separate review (E Ringø *et al.* 2016)

126 (c) Studies must examine the microbiome as a community (i.e. not specific species or  
127 antimicrobial resistant genes).

128 (d) Only peer-reviewed journal articles in English were included. Book chapters, conference  
129 abstracts, dissertations, editorials, case studies and opinion pieces were excluded.

130 Duplicates were removed from all retrieved data. One reviewer (HN) screened all titles and  
131 abstracts and excluded studies that did not meet the inclusion criteria. Studies that were  
132 included from this step then had their full text reviewed.

133 In the initial search, 870 records were identified from the two databases, and 654 remained  
134 after duplicates were removed. Following this step, the number of articles eligible from the  
135 title/abstract review process and selected for full-text screening was 41. One further article  
136 was excluded for not being written in English, therefore 40 papers proceeded to the full-text  
137 screening process. At this stage, 23 studies were eliminated as not meeting the criteria for the  
138 review, because they did not compare the targeted community(s) across urbanisation levels,  
139 did not focus on terrestrial vertebrate wildlife, and/or did not analyse the microbiome. The  
140 remaining 13 studies proceeded to the data extraction phase (Figure 1).

141

## 142 **The profile of studies**

143 The 13 studies were conducted in eight individual countries in Europe and the Americas  
144 (Table 1). All studies took place in the northern hemisphere. The USA had the largest number  
145 of studies (5/13, 38.5%), followed by Canada (4/13, 30.8%). All articles were published in  
146 the past four years.

147 Birds (Class: Aves) were the focus of nine studies (69%), among which seven targeted  
148 passerine birds (five sparrows, two tits). The remaining studies were on mammals (Class:  
149 Mammalia), for full details see Table 1.

150 Faecal sampling was the most common method for studying wildlife's bacterial assemblages  
151 (7/13, 53.8%), followed by cloacal swabs (4/13, 30.8%). Outer layer body tissues such as  
152 skin and feathers were the next most common (Table 1).

153

#### 154 **Assessment of urbanisation**

155 Most studies investigated the influence of urban living on wildlife bacterial communities by  
156 juxtaposing samples from one or more urban locations with samples from non-urban or  
157 'rural' areas (n = 11) (Table 2). Only two studies examined animal bacterial communities  
158 across an urban gradient.

159 Half the articles in this review (n = 6) clearly defined how they had classified sites as 'urban'  
160 vs 'non-urban' for analysis. As shown in Table 2, the metric used to delineate urban and rural  
161 areas for comparison was not consistent. Three studies used percent impervious surface area  
162 (ISA), two used percent built-up area (BUA), one used percent urban land cover and one used  
163 human population density (Table 2) to define areas considered urban and rural respectively.

164 As impervious surface area and built-up area are sometimes defined differently (Y Zhao & Z  
165 Zhu 2022), they were counted as separate categories for this review. Some studies used  
166 impervious surface area in combination with administrative boundaries to classify study sites  
167 into urban and rural categories. Ö Maraci *et al.* (2022) and JN Phillips, M Berlow & EP  
168 Derryberry (2018) used administrative limits as their main definition, but also included  
169 impervious surface area in their analysis.

170 The remaining studies did not specify how they classified 'urban' versus 'non-urban' for their  
171 sampling, and did not use a quantitative urbanisation metric for analysis.



172 Given the potential for inter-site variability in multiple factors that can influence bacterial  
173 communities, including land use type, soil type and vegetation type (J Grierson *et al.* 2023),  
174 replication of sampling within urban and rural localities is an important component in a  
175 robust urban-rural comparison. However, amongst most studies included in this review,  
176 replication of sampling location was absent or poor. A number of studies did not provide  
177 details on how their sampling sites were selected to provide an optimum representation of the  
178 designated urban and non-urban areas, only mentioned that they sampled at different spots  
179 within urban/rural categories (A Gadau *et al.* 2019; CRA Stephens *et al.* 2021; MR Stothart,  
180 R Palme & AEM Newman 2019; R Gurbanov, U Kabaoğlu & T Yağcı 2022; A Nieto-  
181 Claudin *et al.* 2021). One collected samples opportunistically, then assigned urban or rural  
182 labels to them based on location of animal death (S Sugden *et al.* 2020).

183 Interestingly, a few studies compared bacterial communities across multiple land-use types  
184 along the urbanisation gradient; however not all land use types were assessed at all points of  
185 the gradient, hence they did not represent true replication. For example, Murray *et al.*  
186 (2020)'s chosen "park" sites were mostly highly urbanised landscapes (5/6 with >60% urban  
187 landcover), and their "wetland" sites mostly more "natural" landscapes (5/6 with <30% urban  
188 landcover), thus making theirs a possible wetland versus parks comparison, rather than  
189 urbanisation. Other examples of studies using different habitats to represent urban vs rural  
190 included Ö Maracı *et al.* (2022) (Table 2). The inconsistency makes it more difficult to  
191 separate the effect of urbanisation on the microbiomes, versus the effect of living at different  
192 types of habitats.

193

194 **The outcome of studies**

195 The studies included in this review returned inconsistent findings with respect to the effect of  
196 urbanisation on wildlife bacterial community composition and diversity. All studies  
197 employed 16S rRNA gene sequence analysis for identification of bacteria. The outcomes of  
198 all studies are summarised in Table 2, the key cross-study trends with respect to diversity and  
199 composition are summarised below.

#### 200 a) Diversity

201 Most studies investigated how urban living was associated with both alpha (within site) and  
202 beta (inter-site, within landscape unit) diversity of the microbiome (Table 2). Exceptions  
203 were MH Murray *et al.* (2020), which considered alpha diversity alone and MR Stothart, R  
204 Palme & AEM Newman (2019), which compared only beta diversity across urban and rural  
205 locations.

206 The studies included in this review measured alpha diversity by one or multiple metrics,  
207 ranging from total species richness (i.e. number of OTUs and ASVs) to indices combining  
208 richness and evenness. Alpha diversity measurements in microbiome studies must be  
209 interpreted with an understanding of the potential for biases to occur in the sequencing  
210 process, particularly with respect to measurements of relative abundance; for example the  
211 read capacity of sequencers can impact the number of reads returned for a particular OTU or  
212 ASV for a given sample (L Joos *et al.* 2020).

213 With this caveat, seven studies (53.8%) found a significant association between urban living  
214 and diversity of wildlife microbiomes. The direction of association was not consistent, with  
215 some finding an increase in diversity associated with urbanisation, some finding the opposite,  
216 and some not finding any difference (Table 2).

217 Some studies examined the relationship in finer details. MR Stothart & AEM Newman  
218 (2021) found an interaction effect of colour phenotype x environment on the diversity of  
219 eastern grey squirrel bacterial community. A Teyssier *et al.* (2020) found alpha diversity to  
220 be the same between urban and non-urban juvenile sparrows, but urban adult birds hosted  
221 less diverse communities than rural birds. A Teyssier *et al.* (2018) found local landscape had  
222 a stronger effect on the bacterial community with birds living in ‘green patches’ within  
223 urbanised landscapes having significantly higher alpha diversity than birds living in ‘highly  
224 urbanised local habitat surrounding by a rural landscape’.

225 b) Community composition.

226 Six studies reported significant differences in community composition associated with the  
227 degree of urbanisation, while three found no significant difference (Table 2). In some studies,  
228 differences in relative abundance were only detected at certain taxonomic levels (e.g.  
229 phylum, family or genus) (Table 2). For example, on phylum level, MH Murray *et al.* (2020)  
230 found urban land cover was associated with significantly lower relative abundance of  
231 Firmicutes and Cyanobacteria, and increased abundance of the Proteobacteria, TM7,  
232 Bacteroidetes, OP11, and TM6, but no significant differences were observed at genus level.  
233 In another example, some genera and families within the phylum Proteobacteria  
234 (*Pseudomonadaceae*, *Pseudomonadales*, *Williamsiaceae*, *Williamsia*, *Pseudomonas*) were  
235 more enriched in urban house sparrows, however no significant differences were observed at  
236 phylum level (A Gadau *et al.* 2019).

237 Notably, some OTUs belonging to potentially pathogenic microbial taxa were found to be  
238 more abundant in urban wildlife. For example, urban hosts exhibited higher abundances of  
239 OTUs belonging to the families Enterobacteriaceae (M Berlow, JN Phillips & EP Derryberry  
240 2021; Ö Maraci *et al.* 2022) and Campylobacteraceae (M Berlow, JN Phillips & EP

241 Derryberry 2021; JN Phillips, M Berlow & EP Derryberry 2018). The genus *Clostridium* was  
242 found in to be less abundant in urban coyotes (S Sugden *et al.* 2020), but more abundant in  
243 urban squirrels (MR Stothart & AEM Newman 2021) compared to their rural counterparts.

244 Urban wildlife were sometimes found to have higher abundances of some taxa that promote  
245 lipid metabolism and are beneficial to digestion such as Lachnospiraceae (S Sugden *et al.*  
246 2020; R Gurbanov, U Kabaoğlu & T Yağcı 2022; MR Stothart & AEM Newman 2021),  
247 Lactobacillaceae (A Teyssier *et al.* 2020; M Berlow, JN Phillips & EP Derryberry 2021; R  
248 Gurbanov, U Kabaoğlu & T Yağcı 2022) and *Sutterella* and *Parasutterella* (MR Stothart &  
249 AEM Newman 2021).

## 250 **Discussion**

251 Overall, our review found evidence suggesting that in some contexts, urbanisation may be  
252 impacting wildlife bacterial communities, both at phylum and lower taxonomic levels.

253 However, the nature, degree and direction of the effect was not consistent among studies, and  
254 the number of studies systematically investigating this topic remains small, increasing the  
255 difficulty of elucidating clear patterns and trends. In addition to the complexity of the  
256 influence of urbanisation on wildlife bacterial communities, our synthesis revealed several  
257 key findings with implications for ongoing wildlife microbiome research.

258 First, our synthesis suggests that specific bacterial taxa show varied responses to urbanisation  
259 that are associated with their ecological characteristics. For example, the literature reviewed  
260 here suggests an emerging pattern of urban wildlife having higher abundances of bacterial  
261 taxa associated with digestion and specific nutrient absorption needs. Specifically, taxa  
262 belonging to the phylum Firmicutes such as Lachnospiraceae and Lactobacillaceae are  
263 generally associated with diets that are high-fat and high-carbohydrate in human and mice (A  
264 Beam, E Clinger & L Hao 2021). Lactobacillaceae is a group of beneficial gut bacteria,  
265 which aid digestion of polysaccharides (U Huynh & ML Zastrow 2023), but a high  
266 concentration of some bacteria in this taxa has been linked to obesity in adult humans (CK  
267 Chakraborti 2015). And Lachnospiraceae is a beneficial group of bacteria, but high  
268 abundances of this taxa positively correlated with glucose and/or lipid metabolism, and is  
269 connected to a high-fat diet in mice and human (J Companys *et al.* 2021; M Vacca *et al.*  
270 2020). Similarly, *Parasutterella* and *Sutterella* of the phylum Proteobacteria were observed  
271 to be a strong feature of the microbiome of wild red squirrels supplemented with food with  
272 higher sugar and fat contents than their natural diet (KA Dill-McFarland *et al.* 2014), which  
273 might be the case with the urban squirrels in MR Stothart, R Palme & AEM Newman  
274 (2019)'s study. It could be hypothesised that the higher abundances of bacteria from these

275 groups is linked to urban wildlife having diets higher in fat and sugar, for example via  
276 consumption of human food waste, and this hypothesis could warrant further investigation.  
277 Our synthesis also reveals that bacterial taxa including potential pathogens were sometimes  
278 found to be more abundant in urban wildlife; for example, families such as  
279 Enterobacteriaceae, which includes *Salmonella* and *E. coli*. In birds, taxa within  
280 Enterobacteriaceae have been associated with dysbiosis and higher mortality and hatching  
281 failure in ostriches (E Videvall *et al.* 2020; T Knöbl, C Cappellete & MA Vigilato 2012), and  
282 the health burden of Enterobacteriaceae pathogens in humans remains high (XJ Lee *et al.*  
283 2021). Taxa from Campylobacteraceae were also found to be elevated in some urban wildlife;  
284 this group includes *Campylobacter sp.* and feather degrading bacteria. *Campylobacter sp.* is  
285 one of the most important contributors to the burden of foodborne disease in humans in high-  
286 income countries (B Devleeschauwer *et al.* 2017). However, *Clostridium*, which includes  
287 several pathogens including botulism and tetanus agents, was found to be both less  
288 abundance (S Sugden *et al.* 2020) and more abundant (MR Stothart & AEM Newman 2021)  
289 in urban wildlife compare to their rural counterparts. If urban wildlife does indeed harbour  
290 more pathogens, it may present concerns with respect to both wildlife health and disease  
291 transmission to human. While noting that this pattern was not uniform across all studies  
292 included in this review, which suggests a complex relationship, potentially dependent on  
293 wildlife species and environmental context, we suggest a need for further systematic work to  
294 clarify patterns of pathogens in urban wildlife bacterial communities, which would have  
295 implications on human health.

296 There was also a slight inconsistency in sequencing method. Both OTU and ASV-based  
297 methods are commonly used to classify sequences in microbiome studies: OTUs are usually  
298 defined as clusters that share at least 97% similarity in their genetic sequence (N-P Nguyen *et*

299 *al.* 2016), whereas ASV methods focus on detecting single nucleotide variants. This makes  
300 ASV taxonomic groupings more comparable across studies (H Maruyama *et al.* 2020);  
301 however, ASV methods are more complex and computationally intensive. The advantages of  
302 ASV over OTU approaches may depend on the context of the study: some studies have found  
303 ASV methods to be more sensitive in detecting bacterial strain differences, and OTU methods  
304 to inflate alpha diversity (M Chiarello *et al.* 2022), while other studies have found consistent  
305 results between both methods (S Moossavi *et al.* 2020). Regardless, the classification method  
306 can affect comparability of results across studies.

307 Another key finding of our synthesis was the need for greater rigour and consistency in  
308 urbanisation metrics. Defining and measuring urbanisation has been a long-running issue  
309 within the field of urban ecology; and ambiguity and inconsistency with definitions of urban  
310 spaces and gradients can lead to biases in our understanding (D Kendal *et al.* 2020). Similar to  
311 NE McIntyre, K Knowles-Yáñez & D Hope (2000), we found that many studies simply  
312 assumed what is 'urban', without clearly specifying why or how they had defined urban (and  
313 non-urban) landscapes, and few used a quantitative measure of urbanisation for analysis.  
314 Urban landscapes are complex mosaics with widely varying environmental variables (AK  
315 Hahs & MJ McDonnell 2006); using only administrative boundary to define cities is often  
316 insufficient to capture the socio- ecological characteristics of these places. Frameworks to  
317 define urbanisation with quantitative metrics have been proposed to increase comparability  
318 among independent studies (DM Theobald 2004), however only a small number of studies in  
319 this review utilised such approaches. Where quantification was used, the studies reviewed  
320 here used approaches based mainly on 1) physical geography: city boundaries, percentages of  
321 impervious surface area or build-up area and 2) demography: by human population at the  
322 areas.

323 The inconsistency in how urban areas were defined across the studies in this review may  
324 partly explain the inconsistent findings regarding the impacts of urbanisation on wildlife  
325 bacterial communities: we suggest that for this field of research, greater rigour and  
326 consistency in urban metrics would be of benefit. An additional factor underlying the  
327 inconsistency of trends may be the tendency for urban areas to comprise a mosaic of  
328 heterogeneous environments, making it difficult to quantify urban effects, as one study  
329 concluded that homogeneous environments (urbanised habitat in urban landscape or rural  
330 habitat in rural landscape) often harboured higher bacterial diversity compared to  
331 heterogeneous environments (A Teyssier *et al.* 2018). Some studies in this review found that  
332 urbanisation explained some variations in the composition of bacterial communities, but the  
333 relationship became weak or insignificant when other environmental factors were included in  
334 the models (MR Stothart, R Palme & AEM Newman 2019; MR Stothart & AEM Newman  
335 2021). We suggest that greater consideration of both local and landscape habitat factors  
336 would benefit this field of research, given clear evidence that both local and landscape  
337 habitats have been found to influence bacterial diversity (Z Han *et al.* 2021; K-O Bergman *et*  
338 *al.* 2008; M Cely-Santos & SM Philpott 2019; CT Callaghan *et al.* 2018). An example for  
339 this is A Teyssier *et al.* (2018)'s study, where birds living in very urbanised patches within  
340 rural landscapes had significantly lower bacterial diversity than birds from 'green patches'  
341 within urbanised landscapes. Future work should ensure local habitat variation is considered  
342 in study design.

343 With respect to administrative boundaries as a means of delineating urban from rural areas, it  
344 is generally considered that administrative boundaries are insufficient to capture the  
345 complexity and multitude of urban habitats (Ö Maraci *et al.* 2022). Administrative boundaries  
346 can also create ecological boundaries due to management differences between regions, but  
347 this may be context-dependant and hard to generalise across studies (CE Aslan *et al.* 2021).



348 Interestingly, one study in this review found that habitat type as defined by administrative  
349 limit was more influential to the bacterial community than percentages of impervious surface  
350 area (JN Phillips, M Berlow & EP Derryberry 2018). This further demonstrates the  
351 complexity of the structure and management of urban ecosystems, and the need for  
352 researchers to carefully consider how to define urban living and urbanisation for their  
353 research.

354 We also identified a need for greater rigour in replication in sampling of urban (and non-  
355 urban) habitats. Given the mosaic nature of urban (and rural) landscapes, sampling replication  
356 across land uses in each landscape category is key to support systematic examination of  
357 patterns, and adequate consideration of local landscape variation. Loosely defined replicates,  
358 or lack thereof, reduce the ability of studies to draw conclusions about the cause of any  
359 differences found between landscapes. Thoughtful sampling design that incorporates robust,  
360 independent replication, and which controls for key confounding factors, is important to  
361 support stronger conclusions about the cause of observed patterns. Many of the studies in this  
362 review did not have replicates within each of their urban and non-urban categories (CRA  
363 Stephens *et al.* 2021; M Fuirst *et al.* 2018; R Gurbanov, U Kabaoğlu & T Yağcı 2022) and/or  
364 did not incorporate systematic sampling of multiple land-use types and habitats in each  
365 landscape type. We suggest that replication and adequate consideration of local landscape  
366 heterogeneity should be key considerations for study design going forwards.

367 In this review, most studies focused on species known to be well adapted to urban  
368 environments, being either urban adapters (which make use of urban resources and habitats  
369 but remain largely dependent on natural resources), or urban exploiters, which are well-  
370 versed in navigating cities for food and shelter and can attain higher population density in  
371 urban spaces (N Winarni *et al.* 2022; ML McKinney 2006). There was a noticeable focus in  
372 studies of urban wildlife microbiome on avian species, especially passerine birds. Birds have

373 often been used to study effects of environmental changes such as urbanisation, especially  
374 passerines due to their omnipresence and small home range (J Ding *et al.* 2023). Small  
375 passerines featured in this review, such as great tit *Parus major*, chickadees *Poecile sp.* and  
376 house finch *Haemorhous mexicanus*, are urban adapters and are often the subjects of research  
377 examining how city living affects wildlife biology (C Isaksson 2018; J Donselaar *et al.* 2018).  
378 House sparrows (*Passer domesticus*) were the most represented species, likely due to their  
379 ubiquitous appearance in cities as an urban exploiter (B Mohring *et al.* 2021). The other non-  
380 passerine birds (gulls and ibises) are also known to be well able to utilise human resources in  
381 cities. Similarly, mammalian subjects of research such as rats, squirrels and coyotes were  
382 those that do well in urban settings and are able to coexist with human (PW Bateman & PA  
383 Fleming 2012; R Rimbach *et al.* 2022; KR Blasdell *et al.* 2022). All species are readily  
384 abundant at their local range. Urban avoiders were generally missing from studies. This is a  
385 trend across much ecological research, where common species are often targeted, as one risks  
386 lower data collecting availability and thus not being able to achieve rigorous statistical  
387 analysis (G Koertner *et al.* 2015; B Fancourt 2014). However, the consequence of wildlife  
388 microbiome research being driven by feasibility and convenience is that there is a knowledge  
389 gap on how the microbiome of urban avoiding species is affected by urban living and  
390 urbanisation, and if they play any role in their host's avoidance behaviour.

391 Beside the overall lack of clear definitions and sampling in urban areas, our review also  
392 suggests that a gap in how urban-living impacts microbiomes over time. Previously, a study  
393 found that, over a period of 18 days, community composition and abundance of magpie eggs  
394 and nests in urban versus rural populations exhibited different trends (S Lee *et al.* 2017).  
395 Among the studies included in this review, only A Teyssier *et al.* (2018) looked at time  
396 intervals, and they identified an interaction between season and urbanisation on the  
397 microbiome.

398 The findings of our review are limited by our usage of only two literature databases, although  
399 our approach complies with the minimum requirements set by the PRISMA guidelines.  
400 Secondly, the review only aimed for studies published in English. On this basis, we may not  
401 have identified the full range of literature in this field. And finally, the current relatively  
402 understudy state of this field of research limited what conclusion could be drawn with  
403 certainty. However, our study also has important strengths, being the first to examine the  
404 available literature on how urban living influenced the bacterial communities of wild animals,  
405 revealing some noticeable directions for expanding knowledge in this emerging field.

406 This review focused on the bacterial components of wildlife microbiome. Other components  
407 that have also been found to be influenced by urban living but are beyond the scope of the  
408 review are as fungi/yeast, virus and protozoa (S Lee *et al.* 2017; AM Fisher *et al.* 2023).  
409 Many wildlife carry fungal, viral and protozoan species that can infect human, increasing the  
410 burden of disease (N Akritidis 2011). Previously, antifungal resistance yeast species have  
411 been found in the gut microbiome of urban birds (MH Al-Yasiri *et al.* 2017), holding  
412 implications on both human's health as antifungal genes can be transferred to urban  
413 environment and then human, and animal's health as antifungal genes could have been  
414 transferred to them from living in urban environments. Loss of biodiversity, which  
415 urbanisation has been known to cause (A Kondratyeva *et al.* 2020), also increased the risk of  
416 zoonotic infection (CK Glidden *et al.* 2021). Thus, it is just important to investigate the  
417 impact of urbanisation on all components of wildlife microbiome for human and animals'  
418 health. Future reviews and studies can expand upon this aspect.

419

## 420 **Conclusions**

421 This review suggests that urban areas are complex habitats with many heterogeneities, which  
422 create complicated relationships between urban environments, wildlife hosts and wildlife

423 microbiomes. The research identified by this review reveals a number of differences between  
424 the microbiomes of animals habituating urban versus non-urban areas. However, patterns are  
425 complex, environment- and taxon-dependent. Our review also identified a range of  
426 improvements that could be made by future studies to generate more comprehensive results  
427 that are more comparable between studies. We suggest that in addition to greater replication  
428 and consideration of landscape heterogeneity, more rigour in definitions and metrics of  
429 urbanisation is key, with ecologically relevant metrics adopted such as human population, or  
430 percentage of built-up or impervious surface areas. There may also be benefit in expanding  
431 the range of species targeted for wildlife microbiome studies to include urban avoiders and  
432 exploring microbial taxa beyond bacteria. Greater clarity on the impacts of urban-living on  
433 wildlife microbiomes, and how this impacts wildlife health, could improve urban design and  
434 conservation of urban-dwelling wildlife.

#### 435 **Statements & Declarations**

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448

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659

**Table 1.** Key characteristics of studies included in this systematic review.

Study	Species	Wildlife	Tissue sampled	Sequenced region	Bacterial units	Country
Gadau <i>et al.</i> (2019)	House sparrows ( <i>Passer domesticus</i> )	Bird	Faeces	V4 region (515f/806f)	Phylum	USA
Stephens <i>et al.</i> (2021)	Mountain chickadees ( <i>Poecile gambeli</i> )	Bird	Nest and feathers	Noted as V4 region (341f/806r) - seem mistaken as this is the V3–V4 region	OTUs	Canada
Maraci <i>et al.</i> (2022)	Great tits ( <i>Parus major</i> )	Bird	Faeces	V3–V4 region	OTUs	Poland
Teyssier <i>et al.</i> (2020)	House sparrows ( <i>Passer domesticus</i> )	Bird	Cloacal wipe	V5–V6 region	OTUs	France
Berlow <i>et al.</i> (2021)	White-Crowned Sparrow ( <i>Zonotrichia leucophrys</i> )	Bird	Cloacal wipe	V4 region (515F/806R)	OTUs, ASVs	USA
Fuirst <i>et al.</i> (2018)	Larus gulls ( <i>Larus argentatus</i> )	Bird	Cloacal wipe	V3–V4 region (515F/806R)	ASVs	USA
Murray <i>et al.</i> (2020)	White ibises ( <i>Eudocimus albus</i> )	Bird	Faeces	V3–V4 region (341F/805R)	OTUs	USA
Teyssier <i>et al.</i> (2018)	House sparrows ( <i>Passer domesticus</i> )	Bird	Cloacal wipe	V5–V6 region (BACTB-F/BACTB-R)	OTUs	Belgium
Phillips <i>et al.</i> (2018)	Nuttall's white-crowned sparrow ( <i>Zonotrichia leucophrys nuttalli</i> )	Bird	Cloacal wipe	V4 region (515F/806R)	OTUs	USA
Stothart <i>et al.</i> (2019)	Eastern grey squirrel ( <i>Sciurus carolinensis</i> )	Mammal	Faeces	V4 region (515F/806R)	OTUs	Canada
Gurbanov <i>et al.</i> (2022)	Rat ( <i>Rattus rattus</i> )	Mammal	Faeces	V3–V4 regions (341F/805R)	OTUs	Turkey
Stothart & Newman (2021)	Eastern grey squirrel ( <i>Sciurus carolinensis</i> )	Mammal	Faeces	V4 region (515F/806R)	OTUs	Canada
Sugden <i>et al.</i> (2020)	Coyotes ( <i>Canis latrans</i> )	Mammal	Faeces	V4 region (515f/806R)	ASVs	Canada

**Table 2.** Summary of key findings regarding associations between degree of urbanisation and microbial communities of wildlife.

<b>Study</b>	<b>Diversity</b>	<b>Comparison</b>	<b>Sites (total)</b>	<b>Urban sites</b>	<b>Non-urban sites</b>	<b>Urban definition and/or metric</b>	<b>Results</b>
Gadau <i>et al.</i> (2019)	Not available	Urban vs rural	Not stated	Urban yards around university campus	Rural ranch properties	Not given.	No significant differences in the relative abundance of gut microbial taxa.
Stephens <i>et al.</i> (2021)	Alpha and beta diversity	Urban vs rural	2	Kamloops neighbourhoods	A nature park	Not given.	No significant differences in the relative abundance of dominant phyla. Urbanisation was related to a slight increase in community richness, but not alpha diversity.
Maraci <i>et al.</i> (2022)	Alpha and beta diversity	Urban mosaic	9	An office area, two residential areas, an urban forest, an urban park and two urban woodlands.	A natural forest and a peri-urban village	Administrative limits ISA	Phylogenetic diversity was lower in highly urbanised areas. Other alpha diversity metrics did not significantly differ while composition significantly differed.
Teyssier <i>et al.</i> (2020)	Alpha and beta diversity	Urban vs rural	6	3	3	BUA: urban sites had 100% urbanisation ratio (BUA:average home range)	Alpha diversity of juvenile birds showed no significant difference Alpha diversity of urban adult birds were significantly lower than rural. Community OTU composition was most explained by level of urbanisation.
Berlow <i>et al.</i> (2021)	Alpha and beta diversity	Noise gradient transect	10	7 noise transects	3 noise transects	Impervious surface area: increased percentages of impervious surface area and noise level in urban areas, more open areas in rural areas. Each transect was approximately 2 km long.	Bacterial richness higher in urban birds. Beta diversity of community membership differed more between urban and rural birds than beta diversity of community structure.
Furst <i>et al.</i> (2018)	Alpha and beta diversity	Urban vs other habitats	3	1 highly urbanised, 1 intermediate	1 least urbanised	Human population density (people/square mile)	Evenness was the highest at the least urbanised colony. No significant difference for other diversity metrics
Murray <i>et al.</i> (2020)	Alpha diversity	Urban land cover gradient	15			% of urban land cover. Five wetlands ranging 15-92%, two wildlife rehabilitation centres, a	Genus diversity did not correlate with urban land cover. Microbiome community

						landfill, a zoo, and six urban parks all <12%	composition was strongly associated with urban land cover.
Teyssier <i>et al.</i> (2018)	Alpha and beta diversity		18	6 urban 6 suburban	6 rurals	Percentage of BUA: 0–5% for “rural” plots, 5–10% for “suburban” plots, and >10% for “urban” plots	Landscape urbanisation did not significantly affect gut OTU richness, while the contrary was true for local urbanisation. Sparrows from more urbanised locations hosted fewer bacterial species. Strong influence of urbanisation x season on beta diversity.
Phillips <i>et al.</i> (2018)	Alpha and beta diversity	Urban vs rural	10	7	3	Administrative limits ISA	Urban birds had more diverse gut microbiomes than rural birds. Composition differed in urban and rural individuals. Beta diversity was most explained by interaction between habitat and territory impervious cover
Stothart <i>et al.</i> (2019)	Beta diversity	Forest vs urban	2	University campus	Small forest	Not given.	OTU differences could be explained by rural vs urban, but the relationship was weak to non-existent when taken into account with other factors
Gurbanov <i>et al.</i> (2022)	Alpha and beta diversity	Urban vs rural	2	Urban industrial areas	Cow farms	Not given.	Urban group had a larger bacterial richness and evenness. Composition was significantly different between groups.
Stothart & Newman (2021)	Alpha and beta diversity	Urban vs forest, intra urban	6	Urban university campuses	Forests	Definition of urban not given. Sampling was divided among three site pairs throughout southern Ontario. Site pairs were comprised of one within city limits and one nearby rural deciduous forest outside of city limits.	Urban squirrels had a greater abundance of a few genera and families. There was a colour phenotype x environment (urban/rural) effect on alpha diversity
Sugden <i>et al.</i> (2020)	Alpha and beta diversity	Urban vs surrounding peri-urban/rural	Opportunistic collection	Edmonton	Surrounding areas	Definition of urban not given. Samples were collected as roadkill, obtained from local fur trappers, or lethally managed. Coyotes were classified based on their location of death as either “urban” or “rural.”	Urban coyotes carried a more diverse bacterial communities with higher ASV richness. The nearest taxon index (NTI) was significantly lower in urban coyotes. Urban and rural coyote bacterial communities also significantly differed in overall community composition

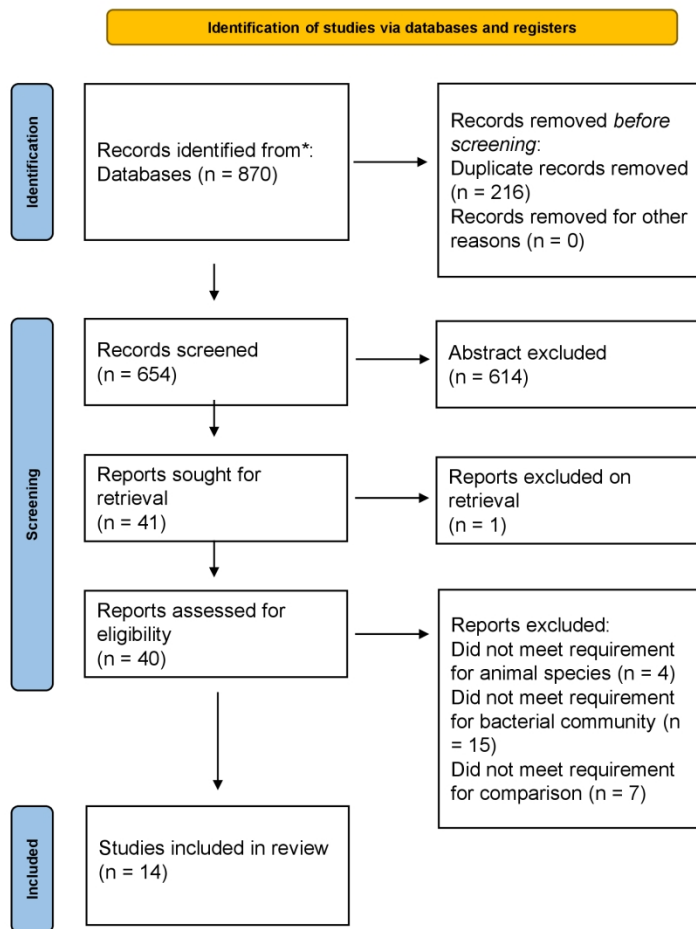


Figure 1. PRISMA flow diagram of study selection.

190x254mm (300 x 300 DPI)

Table S1. Search terms on the two databases.

Web of science	
Wildlife	bird OR animal OR reptil* OR wildlife OR mammal* OR vertebrate* OR avian OR synurb* OR avifauna
Microbiome	microbiome OR microbiot* OR microflor* OR ( microbial AND community ) OR symbiont* OR microbe*
Urban	*urban OR city OR urban* OR municipal* OR metropol*
Habitats	habitat* OR landuse OR land-use OR setting* OR gradients OR levels OR (land AND cover) OR (land AND use)
Scopus	
Wildlife	TITLE-ABS-KEY ( bird ) OR ( TITLE-ABS-KEY ( animal ) ) OR ( TITLE-ABS-KEY ( reptil* ) ) OR ( TITLE-ABS-KEY ( wildlife ) ) OR ( TITLE-ABS-KEY ( mammal* ) ) OR ( TITLE-ABS-KEY ( vertebrate* ) ) OR ( TITLE-ABS-KEY ( avian ) ) OR ( TITLE-ABS-KEY ( synurb* ) ) OR ( TITLE-ABS-KEY ( avifauna ) ) )
Microbiome	AND ( ( TITLE-ABS-KEY ( microbiome ) ) OR ( TITLE-ABS-KEY ( microbiot* ) ) OR ( TITLE-ABS-KEY ( microflor* ) ) OR ( TITLE-ABS-KEY ( microbial AND community ) ) OR ( TITLE-ABS-KEY ( symbiont* ) ) OR ( TITLE-ABS-KEY ( microbe* ) ) )
Urban	AND ( ( TITLE-ABS-KEY ( *urban ) ) OR ( TITLE-ABS-KEY ( city ) ) OR ( TITLE-ABS-KEY ( urban* ) ) OR ( TITLE-ABS-KEY ( municipal* ) ) OR ( TITLE-ABS-KEY ( metropol* ) ) )
Habitats	AND ( ( TITLE-ABS-KEY ( habitat* ) ) OR ( TITLE-ABS-KEY ( landuse ) ) OR ( TITLE-ABS-KEY ( land-use ) ) OR ( TITLE-ABS-KEY ( setting* ) ) OR ( TITLE-ABS-KEY ( gradients ) ) OR ( TITLE-ABS-KEY ( levels ) ) OR ( TITLE-ABS-KEY ( land AND cover ) ) OR ( TITLE-ABS-KEY ( land AND use ) )